Tagungsprogramm der

46. Jahrestagung der Deutschen Gesellschaft für Massenspektrometrie

10.-13. März 2013
Humboldt-Universität zu Berlin

HUMBOLDT-UNIVERSITÄT ZU BERLIN

Sonntag, der 10.03.13

ab 12:00 Uhr	Registrierung, Öffnung des Tagungsbüros	0‘101
	Workshops	
13:00 – 17:30 Uhr	**WS1**: Bildgebende Massenspektrometrie (Imaging MS) *B. Spengler, A. Römpp*	HS: 0‘307
14:00 – 17:30 Uhr	**WS2**: Ionenmobilitätsmassenspektrometrie (IMS-MS) *A. Springer, K. Pagel, M. Schlangen, I. Ebner, J. Benesh*	HS: 0‘110
14:30 – 17:30 Uhr	**WS3**: Open MS / MS-Informatik *K. Reinert, O. Kohlbacher*	HS: 0‘311
14:00 – 17:30 Uhr	**WS4**: Einführung in die anorganische Massenspektroskopie *N. Jakubowski, J. Vogl*	HS: 0‘313
14:00 – 17:30 Uhr	**WS5**: Biomedizinische Applikationen der Affinitätsmassen-spektrometrie *M. Glocker, M. Przybylski*	HS: 0‘310
18:00 – 18:30 Uhr	Eröffnung der Tagung	HS: 0‘115, 0‘110
18:30 – 19:30 Uhr	**Wolfgang-Paul-Vortrag** Klaus G. Heumann *Richtige Quantifizierungen durch ICP-MS: Von Elementspuren zu Molekül-verbindungen - eine persönliche Retrospektive*	HS: 0‘115, 0‘110
19:30 –22:00 Uhr	Empfangsbuffet	Foyer

Montag, der 11.03.13

8:45 – 9:30 Uhr	**Plenarvortrag PL1** Olivier Laprévote *Atmospheric Pressure Photo-Ionization, Reaction Mechanisms and Applications*	HS: 0‘115, 0‘110
9:30 – 10:30 Uhr	**Verleihung der Wolfgang-Paul-Studienpreise** Marko Haertelt, *Action Spectroscopy of Strongly Bound Clusters in the Gas Phase* Burkhard Butschke, *Mechanistic Studies on Metal-Mediated Bond-Activation Reactions in Gaseous Ions* Christine Wild, *Zellfreie Synthese von Stabilisotopen- und Selen-markierten Proteinstandards für die Absolute Quantifizierung*	HS: 0‘115, 0‘110
10:30 – 10:50 Uhr	Kaffeepause	Foyer
	Proteomics I	HS: 0‘115
10:50 – 11:10 Uhr	**Proteomics I.1** Hannes Hahne, Technische Universität München *Improved sensitivity in proteomics experiments using DMSO in nanoESI-LC-MS/MS*	
11:10 – 11:30 Uhr	**Proteomics I.2** Gunnar Schwarz, Humboldt-Universität zu Berlin *MeCAT – Recent developments and improvements in protein quantification using DOTA-based metal-chelate complexes harboring lanthanides*	
11:30 – 11:50 Uhr	**Proteomics I.3** Michael Zorn, Martin-Luther-Universität Halle/Wittenberg *Selective selC-Independent Selenocysteine Incorporation into Formate Dehydrogenases proven by Mass Spectrometry*	
11:50 – 12:10 Uhr	**Proteomics I.4** Marion Rohmer, Goethe-University Frankfurt *Neue Ansätze zur Charakterisierung von Biopharmazeutika mittels Massenspektrometrie*	
12:10 – 12:30 Uhr	**Proteomics I.5** Stefan Kalkhof, Helmholtz Centre for Environmental Research – UFZ *Quantitative proteomics reveals novel functions of osteoclast-associated receptor in STAT signaling and cell adhesion in human endothelial cells*	
	Organische Massenspektrometrie I	HS: 0‘110
10:50 – 11:10 Uhr	**Organische Massenspektrometrie I.1** Mario Thevis, Deutsche Sporthochschule Köln *Massenspektrometrische Bestimmung von Phosphodiesterase-4 Inhibitoren in der Dopinganalytik*	
11:10 – 11:30 Uhr	**Organische Massenspektrometrie I.2** Juliane Kleeblatt, University of Rostock *Determination of the photoionization properties of medically relevant substances*	
11:30 – 11:50 Uhr	**Organische Massenspektrometrie I.3** Stefan Neubauer, BOKU – Universität für Bodenkultur Wien *Quantitative Metabolomik; Evaluierung der Probenvorbereitung mit Hilfe eines $U^{13}C$ markierten Zellextraktes*	
11:50 – 12:10 Uhr	**Organische Massenspektrometrie I.4** Stefanie Pleik, Justus-Liebig-Universität Gießen *Crime Scene Investigations: New Method Developments for an Age Determination of Latent Fingerprints*	
12:10 – 12:30 Uhr	**Organische Massenspektrometrie I.5** Ales Svatos, MPI for Chemical Ecology *Surface analysis of bacteria*	

	Lipide & Kohlenhydrate I	KR: 0‘119
10:50 – 11:10 Uhr	**Lipide & Kohlenhydrate I.1** Julia Cuers, Technische Universität Braunschweig *ADVANCES IN THE OLIGOMER ANALYSIS OF NON-IONIC CELLULOSE ETHERS BY QUANTITATIVE (HPLC-) ESI-IT-MS*	
11:10 – 11:30 Uhr	**Lipide & Kohlenhydrate I.2** Chakravarthy Marella, National Centre for Biological Sciences, TIFR, Bangalore, India *Software Framework for Determining Lipidome Homologies*	
11:30 – 11:50 Uhr	**Lipide & Kohlenhydrate I.3** Thomas Piper, Deutsche Sporthochschule Köln *Wasserstoff – Isotopenverhältnis – Massenspektrometrie endogener urinärer Steroide als neue Methode in der Dopingkontrolle*	
11:50 – 12:10 Uhr	**Lipide & Kohlenhydrate I.4** Jürgen Schiller, Institute for Bee Researh Hohen Neuendorf *More than honey - A MALDI MS study of the lipids from bee spermatozoa*	
12:10 – 12:30 Uhr	**Lipide & Kohlenhydrate I.5** Cyrus Papan, Max Planck Institut für Zellbiologie und Genetik *Discovery of novel lipid classes from shotgun lipidomics mass spectra*	
12:30 – 14:00 Uhr	Mittagspause & Lunchseminare *Lunchseminar Bruker* *Lunchseminar Agilent* *Lunchseminar Knauer*	Foyer HS: 0‘110 HS: 0‘310 HS: 0‘311
14:00 – 14:45 Uhr	**Plenarvortrag PL2** Rebecca Jockusch *Fluorescence and FRET of Trapped, Mass-Selected Ions*	HS: 0‘115, 0‘110
	Organische Massenspektrometrie & Instrumentelle Methoden I.1	HS: 0‘115
14:45 – 15:05 Uhr	**Organische Massenspektrometrie & Instrumentelle Methoden I.1** Kai Schuhmann, Max Planck Institute of Molecular Cell Biology and Genetics *Towards accurate quantification of polyunsaturated glycerophospholipids by tandem mass spectrometry*	
15:05 – 15:25 Uhr	**Organische Massenspektrometrie & Instrumentelle Methoden I.2** Ana Isabel Gonzalez Florez, Fritz-Haber-Institut der Max-Planck-Gesellschaft *Catching biomolecular ions with liquid helium nanodroplets*	
15:25 – 15:45 Uhr	**Organische Massenspektrometrie & Instrumentelle Methoden I.3** Jürgen Burhenne, Universitätsklinikum Heidelberg *Quantifizierung femtomolarer Konzentrationen des CYP3A Substrats Midazolam und seines Hauptmetaboliten 1'-Hydroxymidazolam in humanem Plasma mittels UPLC gekoppelt zur Tandem-Massenspektrometrie*	
15:45 – 16:05 Uhr	Kaffeepause	Foyer
16:05 – 16:25 Uhr	**Organische Massenspektrometrie & Instrumentelle Methoden I.4** Agnieszka Kraj, Antec *Electrochemically Initiated Reactions Upfront MS - EC/MS an Unknown Panacea?*	HS: 0‘115
16:25 – 16:45 Uhr	**Organische Massenspektrometrie & Instrumentelle Methoden I.5** Ramona Heinke, Leibniz-Institut für Pflanzenbiochemie *Mass spectrometry-based metabolite profiling of crude fungal extracts*	

	Grundlagen der Massenspektrometrie I	HS: 0‘110
14:45 – 15:05 Uhr	**Grundlagen der Massenspektrometrie I.1** Ivy Hwee Lim, Max-Planck-Institut für Kohlenforschung *Insights to colloidal zeolite synthesis by mass spectrometry - ionic clusters and silicate fragments*	
15:05 – 15:25 Uhr	**Grundlagen der Massenspektrometrie I.2** Tino Jaschinski, Friedrich Schiller University Jena *Laser desorption/ionization mediated by bionanostructures from microalgae*	
15:25 – 15:45 Uhr	**Grundlagen der Massenspektrometrie I.3** Stephan Warnke, Fritz-Haber-Institut *Protein Structure in the Gas Phase - the Influence of Side-Chain Microsolvation*	
15:45 – 16:05 Uhr	Kaffeepause	Foyer
16:05 – 16:25 Uhr	**Grundlagen der Massenspektrometrie I.4** Axel Pramann, Physikalisch-Technische Bundesanstalt *Improvements combining IDMS and MC-ICP-MS for redefining Avogadro’s number*	HS: 0‘110
16:25 – 16:45 Uhr	**Grundlagen der Massenspektrometrie I.5** Diana Hofmann, Forschungszentrum Jülich GmbH *Haareis*	
	Instrumentelle Entwicklungen I	KR: 0‘119
14:45 – 15:05 Uhr	**Instrumentelle Entwicklungen I.1** Fiona Pachl, Technische Universität München *Characterization and optimization of a high field Orbitrap for proteome analysis*	
15:05 – 15:25 Uhr	**Instrumentelle Entwicklungen I.2** Mathias Müller, Thermo Fisher Scientific *Moving Orbitrap Instrumentation towards Intact Protein Analysis up to one Megadalton*	
15:25 – 15:45 Uhr	**Instrumentelle Entwicklungen I.3** Arndt Ingendoh, Bruker Daltonik GmbH *An Automated MS-based Screening Procedure for Clinical and Forensic Toxicology*	
15:45 – 16:05 Uhr	Kaffeepause	Foyer
16:05 – 16:25 Uhr	**Instrumentelle Entwicklungen I.4** Philipp Sulzer, IONICON Analytik GmbH *Proton Transfer Reaction and Selective Reagent Ionization - Mass Spectrometry (PTR/SRI-MS): Investigations of Toxic and Hazardous Substances*	KR: 0‘119
16:25 – 16:45 Uhr	**Instrumentelle Entwicklungen I.5** Ralf Zimmermann, Universität Rostock *Gaschromatographie gekoppelt mit ultraschneller hochauflösender TOF Massenspektrometrie (GC-HRTOF) oder ultrahochauflösender FT-Massenspektrometrie (GC-APCI-FTICR) zur Analyse hochkomplexer Mischungen*	
16:45 – 17:30 Uhr	**Plenarvortrag PL3** Peter Uwer *Das Higgsboson und das Geheimnis der Masse*	HS: 0‘115, 0‘110
17:30 – 21:00 Uhr	**Postersession I** (Poster mit geraden Nummern) mit Bier und Brezeln	1.OG

Dienstag, der 12.03.13

8:45 – 9:30 Uhr	**Plenarvortrag PL4** Michal Sharon *The Role of Mass Spectrometry in Structure Elucidation of Protein Complexes*	HS: 0‘115, 0‘110
9:30 – 10:30 Uhr	**Agilent-Research-Summer** Victoria Elsner *Comprehensive two-dimensional liquid chromatography (LCxLC) coupled with mass spectrometry for the analysis of fatty alcohol derivatives*	HS: 0‘115, 0‘110
10:30 – 10:50 Uhr	Kaffeepause	Foyer
10:50 – 12:30 Uhr	**Postersession II** (Poster mit ungeraden Nummern)	1.OG
12:30 – 14:00 Uhr	Mittagspause & Lunchseminare *Lunchseminar Thermo Fisher* *Lunchseminar Waters* *Lunchseminar ABSciex* *Lunchseminar Advion*	Foyer HS: 0‘110 HS: 0‘307 HS: 0‘310 HS: 0‘311
14:00 – 14:45 Uhr	**Plenarvortrag PL5** Michael Spiteller *Bioaktive Verbindungen aus tropischen und subtropischen Pflanzen und ihre assoziierten endophytischen Pilze*	HS: 0‘115, 0‘110
	Proteomics II	HS: 0‘115
14:45 – 15:05 Uhr	**Proteomics II.1** Michael O. Glocker, Proteome Center Rostock *Affinity-Mass Spectrometry and Immunoanalytical Characterization of Endogenous KRAB ZNF - TRIM28 Protein Complexes Isolated from a Human Cancer Cell Line*	
15:05 – 15:25 Uhr	**Proteomics II.2** Carla Schmidt, University of Oxford *Protein-Protein-Quervernetzung und native Massenspektrometrie der Chloroplasten-ATP-Synthase – Struktur, Funktion und Regulation eines transmembran-gebundenen Energieumwandlers*	
15:25 – 15:45 Uhr	**Proteomics II.3** Jens Pettelkau, Martin-Luther-Universität Halle/Wittenberg *Investigation of GCAP-2 dimerization by chemical cross-linking and ESI-LTQ-Orbitrap-MS*	
15:45 – 16:05 Uhr	Kaffeepause	Foyer
16:05 – 16:25 Uhr	**Proteomics II.4** Mahmoud Al-Majdoub, Proteome Center Rostock *Conformational epitope mapping by mass spectrometric and peptide chip analyses of a polyclonal antibody model serum directed against TRIM21 autoantigen*	HS: 0‘115
16:25 – 16:45 Uhr	**Proteomics II.5** Kathrin Lindner, University of Konstanz *Tracking down structures and conformational changes in the oligomerisation-aggregation of Parkinson’s Disease protein α-Synuclein using combined ion mobility- and affinity-MS*	

	Imaging I	HS: 0‘110
14:45 – 15:05 Uhr	**Imaging I.1** Anna C Crecelius, Friedrich-Schiller-University Jena *Correlating Raman and MALDI imaging*	
15:05 – 15:25 Uhr	**Imaging I.2** Dhaka Ram Bhandari, Justus Liebig University Giessen *High resolution AP-SMALDI MSI of plant and insect metabolites*	
15:25 – 15:45 Uhr	**Imaging I.3** Marcus Wurlitzer, Universitätsklinkum Hamburg-Eppendorf *A strategy for the identification of biomarkers in cancer tissues using MALDI mass spectrometry imaging and tissue microarrays*	
15:45 – 16:05 Uhr	Kaffeepause	Foyer
16:05 – 16:25 Uhr	**Imaging I.4** Dennis Trede, Steinbeis Innovation Center SCiLS Bremen *SCiLS Lab: software for analysis and interpretation in 2D and 3D MALDI-imaging*	HS: 0‘110
16:25 – 16:45 Uhr	**Imaging I.5** Uwe Karst, Universität Münster *Element-Bioimaging und Speziationsanalytik: Komplementäre Methoden für pharmazeutische Fragestellungen*	
	Lipide & Kohlenhydrate II	KR: 0‘119
14:45 – 15:05 Uhr	**Lipide & Kohlenhydrate II.1** Maria Fedorova, Universität Leipzig *Mass spectrometry as an analytical tool in oxolipidomics*	
15:05 – 15:25 Uhr	**Lipide & Kohlenhydrate II.2** Andrea Hahn, Christian-Albrechts-Universität zu Kiel *Unterscheidung Isomerer Oligosaccharide mittels Vis-PD*	
15:25 – 15:45 Uhr	**Lipide & Kohlenhydrate II.3** Clara Weigelt, University Medical Center Hamburg-Eppendorf *Brown Adipose Tissue Activation remodels the Lipidomic Landscape of Metabolically Active Tissues: A High Resolution UPLC-MS Fluxomic Approach*	
15:45 – 16:05 Uhr	Kaffeepause	Foyer
16:05 – 16:25 Uhr	**Lipide & Kohlenhydrate II.4** Christian Neusüß, Hochschule Aalen *CE-ESI TOF MS for the analysis of intact proteins: recent progress and the application towards the differentiation of EPO preparations*	KR: 0‘119
16:25 – 16:45 Uhr	**Lipide & Kohlenhydrate II.5** Andrej Frolov, Universität Leipzig *Mechanisms of advanced glycation studied in peptide-glucose systems under elevated temperatures*	
16:45 – 17:45 Uhr	**Jahreshauptversammlung der DGMS**	HS: 0‘115, 0‘110
ab 19:00 Uhr	**Konferenzdinner im CinemaxX am Potsdamer Platz**	

Mittwoch, der 13.03.13

8:45 – 9:30 Uhr	**Plenarvortrag PL6** Torsten Benter *Atmospheric Pressure Ionization: Shedding some light on the complex relationship between neutral analyte composition and recorded mass spectra*	HS: 0‘115, 0‘110
9:30 – 10:30 Uhr	**Preisverleihung Massenspektrometrie in den Biowissenschaften** John Cottrell, David Creasy & Jürgen Cox	HS: 0‘115, 0‘110
10:30 – 10:50 Uhr	Kaffeepause	Foyer
	Proteomics III	HS: 0‘115
10:50 – 11:10 Uhr	**Proteomics III.1** Robert Ahrends, Stanford Medical School *Quantitative functional proteomics reveals a multi-feedback system design for terminal differentiation*	
11:10 – 11:30 Uhr	**Proteomics III.2** Frederike Eggers, University of Konstanz *Identification of Carbohydrate-Contacting Peptides in Clinically Relevant Lectin by Proteolytic-Excision Mass Spectrometry*	
11:30 – 11:50 Uhr	**Proteomics III.3** Diana Hildebrand, University Medical Center Hamburg-Eppendorf *Detection of novel proteolytic Angiotensin I-processing activities in blood plasma by a mass spectrometry assisted enzyme screening system*	
11:50 – 12:10 Uhr	**Proteomics III.4** Tommy Hofmann, Helmholtz Gesellschaft für Umweltforschung UFZ *Analysis of the Interleukin-8/ Chondroitin sulfate complex by Hydrogen/Deuterium Exchange MS*	
12:10 – 12:30 Uhr	**Proteomics III.5** Sebastian Tittebrandt, DKFZ Heidelberg *Ambient ESI-HDX for Differential Analysis of Exchangeable Hydrogens in Small Molecules, Amino Acids and Peptides*	
	Organische Massenspektrometrie II	HS: 0‘110
10:50 – 11:10 Uhr	**Organische Massenspektrometrie II.1** Herbert Oberacher, Innsbruck Medical University *Highly efficient LC/MS of nucleic acids by proper selection of solvent additives*	
11:10 – 11:30 Uhr	**Organische Massenspektrometrie II.2** Esra Altuntas, Friedrich-Schiller-University Jena *“Polymeromics”: Detailed characterization of polymers via MS-based strategies*	
11:30 – 11:50 Uhr	**Organische Massenspektrometrie II.3** Nina Morgner, Oxford University *Investigation of the Hsp90 chaperone assembly, Hsp70 cycle and transfer of a client protein*	
11:50 – 12:10 Uhr	**Organische Massenspektrometrie II.4** Wibke Peters, LECO Instrumente GmbH *Characterization and Classification of Petroleum Crude Oils using High Resolution Time of Flight Mass Spectrometry*	
12:10 – 12:30 Uhr	**Organische Massenspektrometrie II.5** Konrad Koszinowski, Georg-August-Universität Göttingen *Charakterisierung hochreaktiver Metallorganyle durch ESI-Massenspektrometrie*	

	Instrumentelle Entwicklungen II	KR: 0‘119
10:50 – 11:10 Uhr	**Instrumentelle Entwicklungen II.1** Heiko Hayen, Bergische Universität Wuppertal *Vergleich der Matrixeffekte in der LC/MS-Pestizidanalytik mittels ESI, APCI und neuartiger plasmabasierter Ionisierung*	
11:10 – 11:30 Uhr	**Instrumentelle Entwicklungen II.2** Jacob Thomas Shelley, University of Münster *Tunable Desorption/Ionization Modes of Plasma-based Ambient Mass Spectrometry Sources*	
11:30 – 11:50 Uhr	**Instrumentelle Entwicklungen II.3** Arne Stindt, Bundesanstalt für Materialforschung und –prüfung *Atmosphärendruck-Ionisation levitierter Tröpfchen*	
11:50 – 12:10 Uhr	**Instrumentelle Entwicklungen II.4** Timo Dickel, GSI Darmstadt *Mobile Ultra-High Resolving Mass Spectrometer for in-situ Measurements*	
12:10 – 12:30 Uhr	**Instrumentelle Entwicklungen II.5** Alexander Pirkl, Universität Münster *Liquid AP-UV-MALDI Enables Stable Ion Yields of Multiply Charged Peptide and Protein Ions for Sensitive Analysis by Mass Spectrometry*	
12:30 – 13:00 Uhr	**Finale** Vorstellung des Tagungsortes 2014 Verleihung der Posterpreise Abschluss der DGMS 2013	HS: 0‘115, 0‘110

Proteomics I.1

Improved sensitivity in proteomics experiments using DMSO in nanoESI-LC-MS/MS

Hannes Hahne[1], Stefan K. Maier[1], Dominic Helm[1], Matthias Wilm[2], Bernhard Kuster[1]
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Einleitung

Dimethylsulfoxide (DMSO) has been reported as a supercharging reagent for peptides and proteins during nanoESI as well as leading to charge state coalescence which in turn improves signal intensity [1]. In the present study, we explore the benefit of low percentage additions of DMSO as an organic modifier in nanoESI-LC-MS/MS experiments for shotgun proteomics and attempt to rationalize the observed effects.

Methodik

A HeLa cell lysate was digested with trypsin using the filter-aided sample preparation (FASP) approach. Replicate analyses of digests with and without 5% DMSO in classical water/acetonitrile LC solvents were performed on an Eksigent nanoLC 1D+ ultra (Eksigent, CA) coupled to a LTQ Orbitrap XL (Thermo Fisher Scientific, Bremen). The mass spectrometer was equipped with a nanospray source (Proxeon, DK) using uncoated fused-silica PicoTip emitters (New Objective, MA) and the voltage was applied via a liquid junction. The instrument was operated in data-dependent acquisition mode. Raw data processing and peptide/protein identification was performed using Progenesis LC-MS 4.0 (Nonlinear Dynamics, UK) and Mascot 2.4.0 (Matrix Science, UK). Stalagmometric measurements of surface tension were performed with water as a reference.

Vorläufige Daten

The replicate nanoLC-MS/MS analysis of a tryptic FASP digest of HeLa cells on a LTQ Orbitrap XL mass spectrometer with and without DMSO as organic modifier revealed surprising effects. Most notably, the use of DMSO resulted in a 3-fold increase in precursor intensities, which, in turn, led to considerably shorter injection times (32ms vs. 16ms for MS/MS) and a higher data acquisition speed (10% more MS and MS/MS spectra). More importantly, the number of peptide and protein identifications increased by 15% and 20%, respectively. Similar results were obtained on other LC-MS/MS platforms equipped with different nanoESI sources including a Orbitrap Velos and a Synapt G2 QTOF instrument.

Interestingly, the commonly observed polysiloxane ions from ambient laboratory air were completely absent when DMSO was added to the LC solvent. Instead, numerous strongly mass deficient background ions arising from DMSO clusters were detected and, the *m/z* 401.92 species represents a viable lockmass alternative.

The astounding increase in measured peptide intensities cannot be explained by improved LC resolution as this was not significantly different between samples measured with and without DMSO. While charge state coalescence was observed in the presence of DMSO (11% instead of 19% of all peptides were observed in at least two charge states), it can also not explain the extent of the phenomenon.

Therefore, enhanced ionization efficiency during the nanoESI process is currently being investigated as an alternative explanation. The hypothesis is that DMSO as an amphiphilic molecule may change the physical properties of the ESI droplets, notably surface tension and density and, thus, may facilitate the formation of smaller initial droplets which according to electrospray theory would lead to more efficient ionization of peptides. This is consistent with surface tension measurements of different solvent compositions as well as with the altered nanoESI background.

Neuartige Aspekte

DMSO as organic modifier during LC-MS/MS increases peptide intensity and significantly improves protein identification in proteomic experiments

Referenzen

[1] Meyer et al. Charge state coalescence during electrospray ionization improves peptide identification by tandem mass spectrometry. J Am Soc Mass Spectrom. 2012; 23(8):1390-9

Proteomics I.2

MeCAT – Recent developments and improvements in protein quantification using DOTA-based metal-chelate complexes harboring lanthanides

Gunnar Schwarz[1,2], David Benda[1], Stefanie Ickert[1], René Becker[1], Sebastian Beck[1], Michael G. Weller[2], Michael W. Linscheid[1]
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Einleitung

In modern life science, the focus of proteomics nowadays turns from sole identification of proteins to their quantification, since the development of stable isotope labelling techniques including ICAT, iTRAQ and SILAC mass spectrometry has been shown to be capable of providing both qualitative and quantitative information. Furthermore, with the combination of high capacity separation techniques even complex mixtures can be analysed. We have developed MeCAT (Metal Coded Affinity Tag) labelling for quantification of peptides and proteins.[1-2] MeCAT uses chelate complexes of lanthanides for relative and absolute quantification with elemental mass spectrometry, while molecular mass spectrometry is used for sequence elucidation of peptides and proteins by tandem mass spectrometry.[1] Recently, we introduced a new improved reagent MeCAT-IA carrying an iodoacetamide moiety, which shows distinct advantages in labelling thiol groups in proteins and peptides over the originally applied MeCAT-MaI with maleinimide reactivity.[2] On the basis of MeCAT-IA we present further insights and developments, including further evidence of complete labelling on protein level and a comparison of different quantification strategies using elemental and molecular MS.

Methodik

Proteins were labeled using MeCAT-IA, which contains a cysteine-reactive group for quantitative labeling and an elemental tag harboring a lanthanide ion for quantification. Complex protein mixtures were separated by SDS-PAGE and digested subsequently. While identification of labeled proteins was achieved by nanoHPLC/ESI-MS and –MS/MS, for a comparison of quantification methods we applied direct ICP-MS, HPLC/ICP-MS, HPLC/ESI-MS and –MS/MS.

Vorläufige Daten

Although the intention of MeCAT is absolute quantification by applying elemental MS, relative quantification or comparison of different samples is often the first and most often simpler step in finding new targets for absolute quantification. Using fragmentation techniques (CID, ECD and IRMPD) unambiguous identification of labelled proteins on peptide level is still possible, as labelled peptides show similar fragmentation patterns in comparison to unlabelled peptides. Additionally characteristic fragments of the labelling group can be detected, thus, allowing differentiation between labelled and unlabelled peptides, as well as, screening for labelled peptides. Since differential labelling is possible by application of different metals, this offers the possibility of differential quantification with HPLC/ESI-MS. Here automated data analysis of MS/MS spectra is not only capable of identifying labelled proteins, but also of a relative quantification based on precursor ion or characteristic fragment signal intensities. Furthermore, a comparison of data generated by elemental MS showed no significant differences.

Neuartige Aspekte

Here we present how a combination of elemental and molecular MS can be used in a MeCAT based quantification workflow.

Referenzen

[1] R. Ahrends, S. Pieper, A. Kühn, H. Weisshoff, M. Hamester, T. Lindemann, C. Scheler, K. Lehmann, K. Taubner, M.W. Linscheid, A metal-coded affinity tag approach to quantitative proteomics, Mol. Cell. Proteomics, 2007, 6(11): 1907.;
[2] G. Schwarz, S. Beck, M.G. Weller, M.W. Linscheid, MeCAT - New iodoacetamide reagents for metal labeling of proteins and peptides, Anal. Bioanal. Chem., 2011, 401(4): 1203.;
[3] G. Schwarz, S. Beck, M.G. Weller and M.W. Linscheid, Comparison of the fragmentation behavior of differentially Metal coded affinity tag (MeCAT) labeled peptides, J. Mass Spectrom., 2012, 47(7), 885.

Proteomics I.3

Selective selC-Independent Selenocysteine Incorporation into Formate Dehydrogenases proven by Mass Spectrometry

Michael Zorn
Martin-Luther-Universität Halle/Wittenberg, Germany

Einleitung

The 21st amino acid is selenocysteine which is co-translationally incorporated at a specific position in the sequence of selenoproteins. The formate dehydrogenases (Fdh) Fdh-O, Fdh-N, and Fdh-H, are the only proteins in *Escherichia coli* that are known to incorporate selenocysteine [1,2]. Specific incorporation of selenocysteine is directed by an in-frame UGA codon in the mRNA encoding these enzymes. In order for the decoding to successfully outcompete translational termination, four *sel* gene products are required whose functions include the biosynthesis and incorporation of selenocysteine [2]. Here, we describe the simultaneous purification of Fdh-N and Fdh-O for the first time. Mass spectrometric analyses revealed an incorporation of selenocysteines at specific positions in Fdh-N and Fdh-O in addition to the known selenocysteine site.

Methodik

Native Fdh-N was purified from *E. coli* wild-type strain MC4100 grown in TGYEP-medium containing either 2 µM or 100 µM sodium selenite. Membrane fractions were solubilized, precipitated with ammonium sulfate, and the fraction obtained with 70% ammonium sulfate was subjected to three consecutive chromatographic steps comprised of a hydrophobic interaction, size-exclusion, and anion exchange chromatography (ÄKTA FPLC, GE Healthcare). Proteins from FPLC fractions were separated by SDS-PAGE, bands were excised and in-gel digested with trypsin. In-gel digested peptides were analyzed by LC/MS on an UltiMate Nano-HPLC system (LC Packings/Dionex) coupled to an LTQ-Orbitrap XL mass spectrometer (ThermoFisher Scientific) equipped with a nano-electrospray ionization source (Proxeon). Data analysis was performed using Proteome Discoverer 1.2 (Thermo Fisher Scientific) and Mascot (Matrixscience).

Vorläufige Daten

The respiratory Fdh-N and Fdh-O enzymes, along with nitrate reductase (Nar), were co-purified. SDS-PAGE analysis indicated the presence of all three subunits of Fdh-N and Fdh-O. Selenium-containing peptides were recognized in mass spectra based on their characteristic isotope patterns as selenium naturally occurs in five stable isotopes. Selenocysteine substitution sites were also indicated by the presence of dehydroalanines which originated from selenocysteines upon selenium loss during sample preparation [3]. Based on MS/MS experiments, we unambiguously identified both dehydroalanines as well as carbamidomethylated selenocysteines at specific positions in Fdh in addition to the UGA-specified selenocysteine residue. We did not exclusively find selenopeptides originating from the selenoproteins Fdh-O and Fdh-N, but also from NarG (or NarZ) and OmpA. The incorporation of selenocysteines into Fdhs did not seem to alter their enzymatic activity as indicated by the positive activity assay. Intriguingly, selenocysteine was incorporated at identical positions of both Fdh-N and -O, even if different growth conditions (2 µM vs. 100 µM Na selenite) were applied. Changing the growth temperature from 37°C to 22°C did not have any effect on selenocysteine incorporation. Selenocysteine substitution sites were also detected in NarG purified from an *selC* mutant. In order to rule out that the presence of selenocysteines is merely caused by an excess of selenium, we also considered the conversion of methionine into selenomethionine. Yet, no selenomethionine was observed during our analyses. The mass spectrometric analysis of two of the three selenoenzymes of *E. coli* presented here demonstrate that selenocysteine replaces particular cysteines in the proteins. Furthermore we show that selenocysteine and not selenomethionine is incorporated at excessive concentrations of selenite. Our results suggest that a degree of misincorporation of selenocysteine into enzymes through replacement of particular, non-essential cysteines, is tolerated and this might act as a buffering system to cope with excessive intracellular selenium.

Neuartige Aspekte

Confirmation of modification sites in fomate dehydrogenases of *E. coli*.

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Proteomics I.4

Neue Ansätze zur Charakterisierung von Biopharmazeutika mittels Massenspektrometrie

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Einleitung

Die Charakterisierung von Biopharmazeutika ist auch heute noch eine Herausforderung für die Analytik auf den verschiedenen Ebenen der Entwicklung und Produktion, da sich Protein-Biopharmazeutika durch eine sehr hohe Komplexität auszeichnen, die vor allem durch posttranslationale Modifikationen verursacht wird [1,2]. Der große Einfluss der Produktionsbedingungen auf das Endprodukt stellt hohe Anforderungen an die Produktion von von Biosimilars und erfordert eine strenge Kontrolle der Chargenvariabilität. Die Massenspektrometrie ist durch ihren hohen Informationsgehalt für die Erfassung dieser Komplexität sehr gut geeignet [3]. Ungeachtet dessen basieren viele etablierte Methoden zur Charakterisierung von Biopharmazeutika auf chromatographischen Trennungen mit UV- oder Fluoreszenzdetektion, denen meist eine aufwändige Probenvorbereitung mit nicht selten toxischen Reagenzien vorausgeht. Im Folgenden sollen neue Massenspektrometrie-basierte Protokolle auf ihre Anwendbarkeit zur Charakterisierung von Biopharmazeutika geprüft werden.

Methodik

Biopharmazeutika wie Cetuximab (Erbitux®, Merck Serono) und Lenograstim (Granocyte®, Chugai Pharma) wurden mittels verschiedener spezifischer (Trypsin), weniger spezifischer (Elastase, Chymotrypsin) und unspezifischer (Thermolysin, Pronase) Proteasen verdaut. Die Peptid- bzw. Glykopeptidmischungen wurden mittels MALDI-LTQ-Orbitrap-MS, nLC-MALDI-TOF/TOF-MS/MS oder nLC-ESI-Q-TOF-MS/MS analysiert. Wenn nötig wurde die Probe mittels C18- oder Graphit-Spin-Columns aufgereinigt. Die Quantifizierung der Produkte erfolgte mittels TMT-Labeling oder mittels alternativer Derivatisierungsprotokolle. Die Auswertung der Daten wurde mit Mascot, SimGlycan und selbst entwickelter Software durchgeführt.

Vorläufige Daten

Die Sequenzabdeckung konnte durch die Kombination aus den Ergebnissen der LC-MS/MS-Analysen verschiedener proteolytischer Verdaue stark erhöht werden. Dies konnte für LC-MALDI- wie für LC-ESI-Analysen gezeigt werden. Dabei ist eine hohe Massengenauigkeit für die Analyse der Peptidgemische aus weniger spezifischen Proteolysen sehr wichtig. Die Analyse von Pronase-Verdauprodukten erwies sich als besonders effizient für die Analytik der Glykosylierung [4]. Sowohl für N- als auch für O-glykosylierte Biopharmazeutika konnte dadurch ein komplettes Glykosylierungsprofil auch für Substanzen mit mehreren Glykosylierungsstellen erstellt werden. Die Protokolle wurden sowohl für Lösungsverdau als auch für den Verdau im Polyacrylamidgel optimiert. Dabei war die MALDI-Massenspektrometrie durch ihre relativ hohe Toleranz gegenüber Verunreinigungen und durch die Schnelligkeit in Kombination mit der hohen Auflösung eines Orbitrap-Massenanalysators sehr gut geeignet für die Analyse der Pronase-Verdaue. Die Reproduzierbarkeit des Verdaus und die Vergleichbarkeit zweier Chargen wurden mittels TMT-Labeling bestätigt. Außerdem wurden andere Derivatisierungsreagenzien auf ihre Anwendbarkeit für Quantifizierungsexperimente hin untersucht. So konnte gezeigt werden, dass untoxische, günstige Alternativen zu den etablierten Protokollen genutzt werden können. Anhand der gewonnen Daten kann der Einfluss der Datenauswertung und der Massengenauigkeit auf das Endergebnis verdeutlicht werden. Verglichen mit bereits etablierten Protokollen sind die vorgestellten Ansätze weniger aufwändig in der Probenpräparation und liefern reproduzierbare Ergebnisse mit Informationen über Glykosylierungsprofil und Glykosylierungsstelle aus nur einem Ansatz.

Neuartige Aspekte

Anwendung weniger spezifischer und unspezifischer Proteasen auf die Charakterisierung von Biopharmazeutika in Kombination mit unterschiedlichen massenspektrometrischen Methoden und Derivatisierungsprotokollen.

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Proteomics I.5

Quantitative proteomics reveals novel functions of osteoclast-associated receptor in STAT signaling and cell adhesion in human endothelial cells

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Einleitung

The osteoclast-associated receptor (OSCAR) has been identified as an important osteoimmunological mediator. In humans, it has been identified as an important receptor for osteoclast differentiation, cell activation, maturation of macrophages, monocytes and dendritic cells. Recently, Goettsch et. al could show that in human endothelial cells OSCAR is regulated by oxidized lipoproteins. However, the causative role and function of vascular OSCAR is unclear. Therefore, functional proteomics was used in combination with either a silencing or an overexpression strategy, cell fractionation and SILAC which gave us the ability to analyze the role of OSCAR in human endothelial cells.

Methodik

To determine cellular functions of OSCAR in human endothelial cells, we either silenced or overexpressed OSCAR in human microvascular endothelial cells (HMEC-1). For SILAC experiments cell lysates, membrane, cytosolic and nuclear fractions were prepared. After separation by 1D-SDS-PAGE the entire protein gel lanes were cut into slices and digested with trypsin. Protein identification and quantification was performed by nano-HPLC/nano-ESI-MS/MS using a NanoAcquity UPLC system (Waters) online-coupled to an LTQ-Orbitrap XL ETD (Thermo) by a chip-based nano-electrospray ion source (Advion). MaxQuant software (version 1.1.1.25) was used for relative quantification and regulated proteins were subjected to “Ingenuity Pathway Analysis” (Winter Release 2011, www.ingenuity.com) and “Transcription Factor Analysis”. For validation abundance and phosphorylation of selected proteins were verified by Western blots.

Vorläufige Daten

In an in-depth proteomic study using stable isotope labeling with amino acids in cell culture (SILAC) and analyzing different protein fractions (membrane, cytosol, nucleus) more than 4,975 unique proteins (FDR < 1%) were reproducibly quantified by nano-HPLC/nano-ESI-MS in OSCAR overexpressing or OSCAR deficient endothelial cells. Of these proteins, OSCAR overexpression modulated the expression of 145 proteins and OSCAR silencing regulated 110. These proteins were mainly involved in cellular proliferation, inflammatory response and cell-to-cell signaling.
Interestingly, OSCAR modulation reciprocally regulated signal transducer and activator of transcription 1 (STAT1) and 3 (STAT3). Thus, STAT1 and several interferon-induced proteins showed a clear inverse correlation to OSCAR expression, which was further verified by Western blot analysis. In contrast, it was found that OSCAR overexpression activated STAT3. Furthermore, OSCAR overexpression increased proteins involved in cell adhesion, which correlated with an increased adhesion of monocytes to the endothelium after OSCAR overexpression. In conclusion, using a comprehensive proteomic approach, endothelial cell-derived OSCAR was found to be involved in the STAT signaling pathway and to effect monocyte adhesion. This indicates a novel role of OSCAR in the vascular-immune cross-talk.

Neuartige Aspekte

Quantitative proteomics combined with protein knock-in and knock-out reveals function of a novel receptor in endothelial cells.

Proteomics II.1

Affinity-Mass Spectrometry and Immunoanalytical Characterization of Endogenous KRAB ZNF - TRIM28 Protein Complexes Isolated from a Human Cancer Cell Line

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Einleitung

The challenge in this project was to isolate endogenous low abundant transcription regulator proteins in their native complexes from human cancer cells and to characterize their interaction domains. To this end affinity – mass spectrometry methods were developed and combined with immunoanalytical approaches to determine interaction partners and binding domains, respectively. It was decided to use TRIM28 as bait in order to isolate KRAB zinc finger proteins (KRAB-ZNFs) in their bound state, i.e ready to interact with DNA via specific binding sites. The human genome encodes for approximately 400 KRAB-ZNFs [1] representing one of the largest families of transcriptional regulator proteins. TRIM28, most likely as trimers, is needed to mediate the repression of genes by tethering to DNA-bound KRAB-ZNFs and the following recruitment of chromatin modifying proteins or protein complexes [2, 3]. Here, we show that RBCC, a TRIM28 truncation mutant that contains one of the presumed protein-interaction-domains of TRIM28 interacts with (i) endogenous TRIM28 and (ii) that this complex binds KRAB-domain carrying proteins. Analyses of endogenous KRAB ZNF - TRIM28 protein complexes help to better understand the transcription repression machinery and its regulation on the molecular level.

Methodik

HeLa cells were transformed to express a TRIM28 truncation mutant, called RBCC, that contains one of the presumed protein-interaction-domains of TRIM28 in combination with a “OneStrep” tag. Extraction of proteins or protein complexes from cells was performed according to optimized non-denaturing extraction protocols. KRAB-ZNF - TRIM28 - RBCC complexes were purified by affinity chromatography using streptactin columns. Bound proteins / protein complexes were eluted either with desthiobiotin or with 1,10-diaza-phenanthrene and separated by SDS-PAGE. Protein bands were analyzed using MALDI-ToF-MS peptide mass fingerprinting. Identification results were confirmed by mass spectrometric sequencing of selected peptide ions on an Axima MALDI-QIT-ToF-MS^n [4]. Westen blot analysis using antibodies against TRIM28, RBCC, and KRAB-ZNFs, respectively, confirmed mass spectrometric results.

Vorläufige Daten

Endogenous KRAB-domain containing proteins, endogeous TRIM28 and other proteins like HP1gamma could be isolated from a human cancer cell line as protein complexes with RBCC used as bait. The most abundant protein captured through the tagged RBCC bait was the full-length endogenous TRIM28 that was present in comparable quantities. All other proteins were far less abundant indicating the existence of many different complexes that contain TRIM28. The list of isolated and identified KRAB-containing proteins encompassed 29 KRAB-ZNFs, POGK and CBX3 (HP1gamma), which were enriched by this method. These proteins were identified when eluting protein complexes bound to streptactin using desthiobiotin as elutant as des-thiobiotin replaces proteins or protein complexes that are attached to streptactin via a “OneStrep” tag [5].

In a second set of experiments, proteins were eluted from the streptactin columns using 1,10-diaza-phenanthrene as eluant. Binding of 1,10-diaza-phenanthrene to zinc atoms is known to occurr with high affinity and to result in protein conformational changes. Here, the adopted elution conditions released only such proteins that were bound to the affinity column via protein-protein interactions that rely on native zinc coordination. These experiments proved that KRAB ZNFs interact with the “RB-domain” only and that TRIM28 - RBCC trimers were kept intact through the “CC-domain”. Results were confirmed by Western blot analyses using antibodies against TRIM28, RBCC, and KRAB ZNFs, respectively, and enabled to dissect the N-terminal binding domains of TRIM28.

Our expression and affinity purification system allowed us to investigate effects on the TRIM28-dependent transcription machinery paving the way to the study of cellular transcription repression mechanisms on the molecular level.

Neuartige Aspekte

Identification of endogenous human transcription repressor protein complexes and characterization of TRIM28 interaction domains for the first time.

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Proteomics II.2

Protein-Protein-Quervernetzung und native Massenspektrometrie der Chloroplasten-ATP-Synthase – Struktur, Funktion und Regulation eines transmembran-gebundenen Energieumwandlers

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Einleitung

ATP-Synthasen sind Membran-gebundene Proteinkomplexe, welche Adenosintriphosphat verbrauchen oder herstellen und den pH-Wert der Zelle kontrollieren. Neueste Entwicklungen in der Massenspektrometrie haben es ermöglicht, solche Komplexe zu analysieren und somit ihren strukturellen Aufbau und damit auch funktionelle Aufgaben aufzuklären. Die Chloroplasten-ATP-Synthase (cATPase) stand häufig im Fokus struktureller Studien, jedoch konnte die intakte Struktur bis heute nicht ermittelt werden. Wir haben die cATPase mithilfe nativer Massenspektrometrie und mittels Protein-Protein-Quervernetzung untersucht. Dabei haben wir stabile Komplexe des intakten Proteinkomplexes und deren Dissoziationsprodukte detektiert und somit Informationen über die strukturelle Anordnung der Proteinuntereinheiten erhalten. Die Quervernetzung der cATPase gibt Aufschluss über Protein-Protein-Interaktionen auf atomarem Level. Außerdem haben wir posttranslationale Modifikationen (PTMs) der cATPase und deren Einfluss auf die Struktur und Regulation des Enzyms untersucht.

Methodik

Die cATPase wurde aus *Spinacia oleracea* (Spinat) aufgereinigt. MS- und Tandem-MS-Spektren wurden mit einem für den hohen Massenbereich modifiziertem Q-ToF II Massenspektrometer (Waters) [1] aufgenommen. Peak-Serien, exakte Massen und mögliche Zusammensetzungen der detektierten Proteinkomplexe wurden mithilfe der Software *Massign* [2] ermittelt. Für die Protein-Protein-Quervernetzung wurde deuteriertes (d4) und nicht-deuteriertes (d0) Bis[sulfosuccinimidyl]suberat (BS3) verwendet. Die quervernetzten Proteine wurden tryptisch gespalten und mittels nano-LC-MS/MS auf einem LTQ-Orbitrap XL Massenspektrometer (Thermo Scientific) analysiert. Potentielle quervernetzte Peptide wurden mithilfe der Software Massmatrix [3] ermittelt und manuell anhand des charakteristischen Peakmusters der MS-Spektren sowie anhand der Fragmentspektren verifiziert. Phosphorylierungsstellen der cATPase wurden nach Anreicherung der phosphorylierten Peptide mit Titandioxid und Analyse mittels LC-MSMS bestimmt.

Vorläufige Daten

Die Struktur der ATP-Synthasen ist universell und kann in zwei Domänen unterteilt werden: Die lösliche F_1-Domäne und die Membran-gebundene F_O-Domäne. Die Zusammensetzung, Stöchiometrie und Topologie der ATPasen ist jedoch unterschiedlich für verschiedene Spezies und Zellkompartimente. Atomare Strukturen wurden bisher nur durch Kombination einzelner Kristallstrukturen und Elektronenmikroskopiebilder erhalten. Neueste Entwicklungen in der Massenspektrometrie erlauben die Analyse Membran-gebundener Proteinkomplexe und ermöglichen somit die Untersuchung intakter ATPasen [4, 5]. Wir haben die cATPase aus Spinat aufgereinigt und konnten mittels nativer Massenspektrometrie intakte F_1F_O-Komplexe sowie F_1-Teilkomplexe detektieren. Eine Erhöhung der Aktivierungsenergie im Massenspektrometer führt zur Dissoziation dieser Komplexe und liefert Informationen über die strukturelle Anordnung der Proteinuntereinheiten. Außerdem konnten wir durch die Dissoziation der intakten F_1F_O-Komplexe Informationen über Membran-gebundenen Lipide und deren Stöchiometrie gewinnen. Um Protein-Protein-Bindungsstellen zu ermitteln haben wir die cATPase mit BS3 quervernetzt. Insgesamt wurden 869 Spektren quervernetzter Peptide identifiziert, aus welchen wir 32 Inter-Protein-Interaktionen ermitteln konnten. Unsere Quervernetzungsstrategie hat sich durch die bekannte Kristallstruktur des $\alpha_3\beta_3$-F_1-Komplexesbestätigt. Wir haben Homologie-Modelle von Proteinuntereinheiten unbekannter Struktur erstellt und diese anhand von Intra-Protein-Interaktionen begutachtet und verifiziert. Desweiteren konnten verschiedene Interaktionen regulativer Proteinuntereinheiten erfasst und somit anderen ATPasen ähnliche Regulationsmechanismen ermittelt werden. Da es keine Informationen über die PTMs der cATPase gibt, haben wir ihre Phosphorylierungsstellen bestimmt. Um den Einfluss dieser auf die Aktivität der cATPase zu untersuchen haben wir mit einer Phosphatase inkubiert und anschließend mittels nativer Massenspektrometrie analysiert. Wir konnten beobachten, daß die Dephosphorylierung zum Verlust gebundener Nukleotide führt und dass Protein-Interaktionen geschwächt wurden. Um dies genauer zu untersuchen, haben wir die native und die dephosphorylierte cATPase mit deuteriertem bzw nicht-deuteriertem BS3 quervernetzt, zu gleichen Mengen vereinigt und die identifizierten Quervernetzungen quantitativ mittels extrahierter Ionenchromatogramme analysiert. Unterschiede wurden hauptsächlich für Protein-Interaktionen des $\alpha b_3\beta_3$-F_1-Komplexes mit anderen Proteinuntereinheiten beobachtet. Die Phosphorylierung der cATPase spielt somit eine große Rolle für ihre Stabilität und Nukleotidbindung.

Neuartige Aspekte

Einfluss von Phosphorylierungsstellen auf die Struktur, Funktionsweise und Regulation der Chloroplasten-ATP-Synthase mittels Massenspektrometrie und Protein-Protein-Quervernetzung.

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Proteomics II.3

Investigation of GCAP-2 dimerization by chemical cross-linking and ESI-LTQ-Orbitrap-MS

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Einleitung

Guanylylcyclase-activating proteins (GCAPs) are important regulatory proteins within the retinal cells. GCAPs are hydrophobic, *N*-terminally myristoylated proteins containing four EF-hand motifs [1]. They regulate their target protein, the retinal guanylylcyclase (ROS-GC), in a calcium-dependent manner, which leads to an alternating cGMP-concentration within the cells and in consequence to a shift in membrane potential [2]. ROS-GCs are active in their homodimeric state [3, 4]. Previously, also a homodimeric state of isoform GCAP-2 was proposed to activate ROS-GC [5]. Whether dimerization of GCAP-2 is essential for ROS-GC activation still is a perception as no structural data of a GCAP-2 dimer have been published so far. We performed cross-linking studies in combination with ESI-LTQ-Orbitrap mass spectrometry to investigate GCAP-2 dimerization.

Methodik

GCAP-2 dimerization was investigated by size exclusion chromatography and analytical ultracentrifugation followed by cross-linking experiments with the homobifunctional amine-reactive cross-linker bis(sulfocuccinimidyl)glutarate (BS^2G) both in the presence and in the absence of Ca^{2+} ions. For differentiation between intramolecular (within one GCAP-2-molecule) and intermolecular (between two GCAP-2-molecules) cross-linking products, we applied a mixture of ^{14}N-GCAP-2 and ^{15}N-labeled GCAP-2 for BS^2G cross-linking to obtain characteristic isotope patterns. Cross-linking mixtures were separated by one-dimensional gel electrophoresis (SDS-PAGE). Gel bands corresponding to GCAP-2 dimers were excised and protein complexes were digested with trypsin and Glu-C. Peptide mixtures were analyzed by nano-HPLC (Dionex)/nano-electrospray ionization (ESI)-linear ion trap (LTQ)-Orbitrap-MS (LTQ-OrbitrapXL, ThermoFisherScientific). Cross-linking products were identified with StavroX and verified by manual reassignment of MS/MS signals.

Vorläufige Daten

Dimerization and the resulting structure of GCAP-2 dimer were studied by chemical cross-linking with BS^2G and high resolution mass spectrometry. One-dimensional gel electrophoresis revealed a similar propensity for GCAP-2 to form a dimer in the Ca^{2+}-bound and Ca^{2+}-free state, which contradicts previous findings [5]. By applying non-labeled and ^{15}N-labeled GCAP-2 we were able to differentiate between intra- and intermolecular cross-linked peptides. The resulting isotopic pattern, arising from combination of different cross-linked peptide species, namely, ^{14}N-peptide 1 with ^{14}N-peptide 2, ^{14}N-peptide 1 with ^{15}N-peptide 2, ^{15}N-peptide 1 with ^{14}N-peptide 2, and ^{15}N-peptide 1 with ^{15}N-peptide 2 alleviated data analysis. Only candidates were accepted, for which comprehensive annotation of signals was possible within a fragment ion mass spectrum of heteromeric (^{14}N and ^{15}N) cross-linked species. We were able to identify four distinct cross-linking sites within the GCAP-2 dimer in the Ca^{2+}-bound state and seven distinct cross-linking sites within the Ca^{2+}-free dimer. Three of these cross-linking sites are common to both states. Cross-linking experiments using isotope-labeled proteins and high resolution mass spectrometry followed by a detailed analysis of cross-linked products by MS/MS allowed to map interaction sites in the GCAP-2 dimer. Whether dimerization is important for ROS-GC activation in vivo is still a matter of debate.

Neuartige Aspekte

Investigation of dimerization by cross-linking using isotope-labeled proteins and high resolution mass spectrometry.

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Proteomics II.4

Conformational epitope mapping by mass spectrometric and peptide chip analyses of a polyclonal antibody model serum directed against TRIM21 autoantigen .

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Einleitung

TRIM21/Ro52 is an autoantigen associated with Sjøgren's syndrome (SS), systemic lupus erythematosus (SLE) [1], congenital heart block (CHB) and other autoimmune diseases. It triggers autoreactive antibody production (anti-TRIM21 Abs) in autoimmune patients [2]. Determination of the main epitopes on TRIM21 in SS and SLE patients is thought to be helpful for stratifying patients according to their epitope antibody recognition pattern (EAR) [3]. Mass spectrometric epitope mapping uses two main approaches called epitope excision [4] and epitope extraction [5]. Hitherto published reports show that these approaches work well when both, the (generally monoclonal) antibody and the antigen are well soluble in non-denaturing buffers. Typically, about 200 µg of antibody (ca. 1 nmol) are immobilized on a suitable column material and exposed in excess to the antigen (ca. 65 pmol) of interest [5]. In this presentation we demonstrate the development of a mass spectrometry (MS)-based epitope mapping procedure in combination with peptide chip analyses that works also with antigens that are insoluble in non-denaturing buffers consuming minute amounts of antigen (60 nmol) and antibody (13 pmol), respectively. A polyclonal anti-TRIM21 rabbit antibody serum is applied as a model serum for future patient analyses in order to set-up the system.

Methodik

rhTRIM21 that contained a His-tag was expressed in *E. coli* producing inclusion bodies. From these, rhTRIM21 was dissolved in 8 M urea buffer. 1D gel electrophoresis showed a major band. Next, rhTRIM21 was re-buffered into 6 M urea solution (pH 7.8). Protein concentrations ranged from 0.9 mg/ml to 1.3 mg/ml. In-solution digestion with Lys-C was performed under denaturing conditions. SDS-PAGE was used for separating rhTRIM21 peptides. Western blot analyses were performed after SDS PAGE using i) primary rabbit anti-TRIM21 antibodies (13 pmol) together with a secondary antibody labeled with 800 IRDy® as fluorescent dye (6 nmol), and ii) mouse anti-His-tag antibodies (67 nmol) in conjunction with secondary mouse antibodies labeled with 680 IRDy® (6 nmol), respectively.

Vorläufige Daten

SDS PAGE analysis of rhTRIM21 from inclusion bodies showed a strong band at 62.5 kDa. Mass spectrometry confirmed expression of the full-length protein. Digestion in-solution under denaturing conditions (6M urea) with Lys-C produced three bands (5.5, 8.5, and 10.5 kDa) by SDS PAGE separation. Western blot analyses showed that the upper band stained positive with the anti-His-tag Ab, the middle band was positive with anti-rhTRIM21 Ab, and the lowest band remained undecorated. Trypsin *in-gel* digestion was performed with all bands for amino acid sequence assignments. The herewith determined anti-rhTRIM21 epitope region encompassed 47 amino acids (aa216 - aa263); too long to call it the major epitope. Subsequently, truncated bands of rhTRIM21 from the expression experiment were analyzed after SDS PAGE separation. MS and Western blot analyses narrowed the epitope to 21 aa in length (aa242 - aa263). In total, antigen consumption was about 30 µg (57.6 nmol) and consumed primary antibody amount was ca. 13 pmol. Next, 25 suspectedly epitope-containing peptides each of 15 aa in length were synthesized and deposited on a peptide chip with a frame shift of 6 aa. Incubation with model serum confined the epitope to span the region from aa245 - aa259 (LQ-E*L*EKD*E*RE*Q*LRI*L*GE-KE), well in consistence with the MS-based analyses. Interestingly, 3D modeling of TRIM21 using “I-TASSER” suggested that the epitope region adopted an α-helical shape, posing the puzzle of identifying a presumably “sequential” epitope motif despite the presence of secondary structure features. Surface area calculations revealed that the best matching partial sequence to fulfill all primary and secondary structure requirements was the four amino acid spanning motif “L-E-Q-L” which is present in both, the sequential and the α-helical peptide conformation. Next we will apply the peptide chip platform for patient screening in order to identify subgroups of patients according to distinct epitope pattern.

Neuartige Aspekte

Analysis of a conformational epitope with difficult to solubilize antigens.

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Proteomics II.5

Tracking down structures and conformational changes in the oligomerisation-aggregation of Parkinson's Disease protein α-Synuclein using combined ion mobility- and affinity-MS

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Einleitung

A large variety of cellular processes are based on the formation and dynamics of multi- and supramolecular protein assemblies. Several neurodegenerative diseases are associated with the aggregation of misfolded proteins. The protein α-Synuclein (αSyn), a key protein in Parkinson´s disease, forms oligomers and aggregates to insoluble fibrils, the main components of the intracellular inclusions, Lewy bodies and neuritis. Details of conformational changes involved in aggregation are of great interest, but the chemical structures and reaction pathways of pathophysiological oligomers and/or aggregates are only poorly characterised at present. Combinations of HPLC with "soft-ionisation" MS or MS/MS are established, powerful techniques for MS-"bottom up" and -"top down" approaches, in the detection and identification of proteins, protein mixtures and/or proteolytically derived peptides [1]. However, the LC-MS approaches are often unsuitable for the direct analysis of reaction pathways and intermediates involved in aggregation processes. Accurate measurements of molecular weights as well as direct and simultaneous information about conformation and dynamics of protein folding [2] are of high interest. In our recent work, Combined MS methods, particularly ion mobility- and affinity-MS and HDXMS have been emerging as powerful tools to elucidate structure and dynamics of protein oligomersation-agregation.

Methodik

αSyn and its mutants were overexpressed in *E.coli* BL 21 (DE3) [pLys] strain and purified by centrifugation and RP-HPLC. For incubation studies in vitro, αSyn samples were reconstituted in NaP_i buffer (pH7.5) (c_{final}=30 µM) and incubated at 37°C and 600 rpm agitation. Pulsed HDX experiments were performed by mixing incubated samples and NaP_i in deuterated buffer, incubated on ice, and subsequently quenched. The quenched αSyn sample was digested on a pepsin column followed by LC-ESI-TOF-MS. From each peptide the deuterium uptake or peptide protection level was plotted vs time. For IM-MS experiments αSyn samples were ionized by nano-ESI, then the ejected ions passed the drift tube containing He as buffer gas and ions were detected by reflectron geometry TOF-MS [3].

Vorläufige Daten

Although it is known that the conversion of monomeric αSyn into fibrils (aggregates) is associated with the pathogenesis of Parkinson´s disease, the underlying molecular mechanism is only poorly understood. So far, the formation of toxic oligomeric intermediates has been observed; however, no detailed chemical structures of αSyn oligomers and their possible intermediates have been identified. The slow rate of formation and low concentration of aggregating intermediates could be major reasons for the failure of conventional mass spectrometric methods to detect and identify oligomers. We have recently reported the first identification of in vitro autoproteolytic fragments as oligomerisation products of αSyn using IM-MS [4]. Drift time profiles provided the analysis of different conformational states of αSyn and its mutants upon in vitro incubation. The assignment of the drift time and mass spectrometric data led to the conclusion that the short amino stretch from residues (70 - 75) is a key element regarding autoproteolytic degradation, and subsequent aggregation of αSyn. The combination of pulsed HDX and LC-MS analysis of on-line digested protein was used to study the properties of kinetic intermediates, addressing the question how the intrinsically disordered protein αSyn folds into compact and highly ordered conformation [5]. Hydrogen atoms in NH group of the protein backbone are "labile" and can undergo HD exchange if accessible to the solvent. Hence, unfolded regions of the protein will undergo more rapid exchange than folded regions. αSyn and its mutants were characterized by 10-15 kinetic data sets, enabling to interpret the folding mechanism of partial αSyn regions.

Neuartige Aspekte

Combined MS-analysis provides the basis for the detailed study of oligomerization/aggregation-pathways, and designing structures capable of inhibiting or modulating protein-aggregation.

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Proteomics III.1

Quantitative functional proteomics reveals a multi-feedback system design for terminal differentiation

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Einleitung

Cell differentiation is often described as a sequence of regulatory steps that end with a terminally differentiated state. However, control theory argues that interconnected multi-feedback systems are potentially better suited to create a robust bistable differentiation switch. Here we show that chemical and siRNA perturbations combined with selective reaction monitoring (SRM) enables a systematic identification of the molecular players and relevant feedback loops of nuclear regulators that drive differentiation. Markedly, we show that multiple synergistic feedback loops connect back to the transcription factor PPARg with different time delays to drive the terminal differentiation of adipocytes, a process critical for our understanding of obesity and metabolic diseases. Together, our study argues that the transition from pre-adipocytes to terminally differentiated adipocytes is an interconnected single module with multiple positive feedback loops rather than a series of sequential regulatory processes along the path to differentiation.

Methodik

Here we present a sensitive, quantitative approach which combines large-scale siRNA-mediated knockdown with highly-sensitive quantitative SRM that allows us to systematically, and on a large-scale, identify dynamic functional interactions in a protein network. By applying targeted proteomics using heavy isotope coded peptides key regulatory proteins are quantified during the time course of fat cell differentiation and perturbation experiments and functional relation between key players are revealed.

Vorläufige Daten

Applying this method to 36 proteins in the network controlling adipogenesis, we validated two previously identified feedback loops (C/EBPA, C/EBPB) and found 6 new dynamic regulatory connections with PPARG: two positive feedback loops (FABP4, C/EBPZ), one double negative feedback loop (FLNA), two upstream negative regulators (WDR5 and FOXC2), and one negatively-regulated downstream target (CRSP1). Our approach allowed us to determine the strength and timing of the feedback loops, and by using single-cell, immunofluorescence analysis, we could measure the impact of the different feedback loops on controlling the number of cells that differentiated.
The proteins that are in feedback loops with PPARG are of particular interest since these are the proteins working during differentiation which have the potential to be manipulated to allow for independent, parallel fine tuning of adipocyte cell number.
In the future, this method can be used to identify and understand feedback loops in other dynamic systems such as stem cell and neuronal precursor differentiation and thus is a valuable tool for cell signaling research.

Neuartige Aspekte

Combination of siRNA mediated knockdown for system wide perturbation with targeted proteomics revealed key players in differentiation signaling networks.

Proteomics III.2

Identification of Carbohydrate-Contacting Peptides in Clinically Relevant Lectin by Proteolytic-Excision Mass Spectrometry

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Einleitung

The emerging physiological significance of carbohydrate (glycan)-protein (lectin) interactions direct increasing attention to the structural analysis of this contact (1). Using human adhesion/growth-regulatory galectins as model, we have developed a robust and reliable approach to identify sequence stretches in contact to the ligand. Because of their biochemical properties, the characterisation of galectin- carbohydrate interactions and identification of binding sites are of crucial importance. In this study galectin-derived peptides, containing key amino acids for carbohydrate binding, were identified by proteolytic excision- mass spectrometry of the CRD domains (CREDEX-MS). The identified CRD-peptides were synthesized and their interaction with different carbohydrates were characterized by affinity- mass spectrometry.
Combination of proteolytic excision and high-resolution mass spectromentry yielded peptides reactive with cognate sugars, as ascertained by biosensor/cell assays.

Methodik

Experimentally, galectins were adsorbed to an affinity resin bearing cognate sugar and proteolytically digested. After washing out unbound galectin fragments, the fragments remaining on the column were eluted, and both fractions analysed by MALDI-TOF-MS. The identified CRD-peptides were synthesised by Fmoc-SPPS and their binding properties characterised by affinity-MS. The interactions of lactose with full-length galectins and with identified CRD-peptides were studied by SAW-bioaffinity-measurements and online-bioaffinity-coupling of SAW with ESI-MS. The lactose was immobilised on bioaffinity-sensor-chips in the form of a glycopeptide. SAW measurements were performed with an S-Sens K5 Biosensor instrument in PBS buffer and elution was performed with ACN : 0.1% TFA 2:1 and 10% acetic acid.

Vorläufige Daten

CREDEX-MS of galectins identified at least two carbohydrate-binding peptides for every galectin - in complete agreement with the binding sites of the crystal - or molecular modelling structures. All identified carbohydrate-binding peptides were synthesized and their affinity for lactose demonstrated by affinity-MS. To get more detailed information about the affinity and interaction kinetics of the CRD peptides and full galectins with lactose, SAW-bioaffinity measurements were performed. Some pmol Lactosyl-glycoprobe was immobilized on the SAW chip and dissociation constants of galectin peptides and galectins with lactose determined to be in the µM range. For a real-time study of carbohydrate-lectin interaction a newly developed online-coupling of SAW with ESI-Ion Trap MS was used. With this system the peptides interacting with on chip bound carbohydrate could be studied directly after the elution and to prove which peptides interact with the carbohydrate on the chip. This system was tested with synthetic CRD peptides and it was shown that online-coupling of the peptides was successful.
The presented results document the validity of the concept to obtain bioactive peptides from lectins (2). These bioinspired peptides are becoming objects of detailed structural analysis and inspire their tailoring, e.g. by incorporating non-natural amino acids, as medically useful mini-lectins or lectin blockers.

Neuartige Aspekte

SAW-bioaffinity analysis of carbohydrate peptide/protein interactions.
New clinically relevant carbohydrate epitopes.

Referenzen

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Proteomics III.3

Detection of novel proteolytic Angiotensin I-processing activities in blood plasma by a mass spectrometry assisted enzyme screening system

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Einleitung

Angiotensin I (AI), a 10 amino acid long peptidic prohormone is part of the renin-angiotensin system (RAS). It is generated through proteolytic cleavage of the precursor angiotensinogen by renin [1].AI can be further processed by different proteases like the angiotensin-converting-enzyme-1 which generates angiotensin II (AII), a strong vasoconstrictor, by removal of a C-terminal dipeptide from AI. In mammalian species, except humans, N-terminal processing of the precursor peptide AI was reported. We hypothesized that aminopeptidase-generated angiotensins bearing the same C-terminus as ANG-1-10 are also present in human plasma.
To investigate the generation of AI-derived angiotensin-peptides and the activity of AI processing proteases in plasma we established a mass spectrometry based enzyme assay (MES)[2] for the measurement of AI-processing protease activities.

Methodik

For the determination of AI-processing protease activities in complex protein fractions by the MES method the plasma proteins where covalently immobilized onto an affinity chromatography material. Unbound molecules like buffer components were removed by washing thus facilitating the following analysis by mass spectrometry.
The immobilized proteins are then incubated with AI, a reaction specific peptide comprising the potential cleavage site of the AII-generating protease and also of other AI-processing proteases. Aliquots are removed after defined incubation times and analysed by MALDI-MS or SRM-combined methods like LC-ESI ion trap-MS or LC-QQQ-MS. This enables the detection as well as the relative quantification of the angiotensin peptides necessary for the determination of AI-processing activities in plasma.

Vorläufige Daten

Immobilization of proteins stabilizes them because after immobilization plasma proteases are fixed and therefore can not degrade other proteins anymore. Furthermore incubation of immobilized proteins with a defined substrate can be carried out in a buffer of choice with regard to the following methods taken for the analysis of the reaction products.
The MALDI-MES is well suited for monitoring additional reaction products beside AII deriving from the substrate AI. In comparison to the MALDI-MES assay the SRM-ESI-MES method provides more reliable results concerning the quantification of the reaction products and consequently of the AI-processing protease activities in blood plasma.
Using the ESI- and MALDI-MES-assay we demonstrated the processing of AI by a second aminopeptidase-dependent cascade bypassing the known carboxypeptidase pathway of AI-processing in human plasma. We showed the generation of new angiotensin peptides, which we here describe for the first time, derived from aminopeptidase activity on AI in human plasma. Thus we widen the human classical circulating RAS.
By using various protease inhibitors, we showed that several different proteases are involved in the generation of these new angiotensin peptides which were characterized as bestatin insensitive metalloproteases as well as proteases of the serine/cysteine family.
In summary we identified several new C-terminal angiotensin peptides released from angiotensin-I by aminopeptidase. This gives new insights into the generation of angiotensin peptides and the understanding of the complex RAS.

Neuartige Aspekte

MALDI and ESI-based enzyme-screening-assays for analyzing angiotensin I-processing proteases. Mass spectrometric identification of unknown angiotensin peptides.

Referenzen

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Proteomics III.4

Analysis of the Interleukin-8/ Chondroitin sulfate complex by Hydrogen/Deuterium Exchange MS

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Einleitung

The recruitment of different chemokines and growth factors by glycosaminoglycans (GAGs) such as chondroitin sulfate or hyaloronan plays a critical key role in wound healing processes. Thus there is a special interest in the design of artificial extracellular matrixes (aECM) with improved properties concerning their interaction with common regulating proteins. A main goal is to gain new aECM formulations suitable as coatings for implants and capable to suppress harmful inflammation and support wound healing. Several studies focused on the structure analysis of the interaction interface between interleukin 8 (IL-8), a chemokine inducing chemotaxis of neutrophils, and different GAGs. Nonetheless standard techniques such as NMR or X-ray crystallography are highly time and material intensive and so far failed to solve protein complexes with native high molecular weight saccharides. In this work we combined Hydrogen-Deuterium-Exchange (HDX) with molecular modeling and docking experiments to show the ability of the method to obtain structure models of IL-8 in complex with tetrameric Chondroitin sulfate, which has been already solved by NMR, but also with native Chondroitin sulfate.

Methodik

Hyaloronic acid hexasaccharides (HA) had been purchased from Hyalose, L.L.C (Oklahoma), and used as supplied. Native chondroitin sulfate (CS_n) from bovine trachea was purchased from Sigma-Aldrich. Tetrameric Chondroitin sulfate (CS_4) was prepared by saccharolysis with hyaluronate lyase as descripted [1]. IL-8 was over expressed in *Escherichia coli*. For the HDX studies interleukin 8 and the GAGs had been exposed to deuterium isotopes in a 95 % D_2O low-salt buffer for four different exchange times with several replicates. After quenching samples were immediately denatured, desalted and measured by MALDI-TOF MS (Ultraflex III, Bruker Daltonik). All analyses were performed at least in 4 replicates. K_D-values have been determined by PLIMSTEX according Gross et al. 2005.

Vorläufige Daten

Binding interfaces of IL-8 and CS have been already explored by NMR (Pichert et al. 2011). Encouragingly, our results highly correlate to these data. We showed that HDX is capable of identifying binding surfaces in protein-GAG complexes with sufficient accuracy. As shown by Pichert et al. CS sulfated at position two and four bound with the helical binding area of the sequence positions 54-77 which was also observed with our analyses. Interestingly, we could find that native CS despite the different molecule size showed a similar binding behavior with a higher K_D-value. HDX proofed as valuable instrument for the analysis of protein-GAG complexes especially for the research of high molecular weight saccharides. We showed that binding surfaces and binding strength can be received from such experiments and that *in silico* models of those complexes are comparable to those of high resolution methods.

Neuartige Aspekte

Identifying of interaction surfaces of proteins in complex with native high molecular weight saccharides by Hydrogen-deuterium exchange.

Referenzen

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Proteomics III.5

Ambient ESI-HDX for Differential Analysis of Exchangeable Hydrogens in Small Molecules, Amino Acids and Peptides.

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Einleitung

In mass spectrometry the primary information of an analyte ion is the monoisotopic mass of the molecule and its fragment ions. The combination of mass spectrometric data with databases offers the possibility of analyte identification via comparison with known MS spectra or with probabilistic methods, respectively. Both methods use the natural isotopic composition of the analyte, if there is no covalent modification with stable isotopes present. Hydrogen deuterium exchange (HDX) reactions allow to study the exchange of hydrogen bound to a heteroatom like N, O or S by the mass shift introduced incorporation of deuterium.
So far HDX has been successfully used in the analysis of protein structures, binding affinities, spatial structures and reaction mechanisms. In our work we show that the combination of complete and differential HDX increase the information content in the analysis of small molecules, peptide and proteins.
The complete exchange of labile Hs gives the number of exchangeable Hs [1]. This is an important information for unknown compound identification or for decisions between alternate structures that differ in their number of exchangeable Hs. With differential HDX we can show that N-H bound nitrogen present in different chemical form exhibit different exchange efficiencies.

Methodik

For the differential and complete HDX we established the following ESI-based Methods:
Soft exchange gas-phase exchange conditions were realized in an open nanoESI source using the combination H_2O solvent and (hot) D_2O gas for in-exchange of D, or the reverse arrangement for out-exchange of D. Enhanced exchange conditions were realized in the ESI source of an LCQ Deca plus system. Here the combination H_2O solvent and D_2O-saturated nitrogen stream was used for D in-exchange and the reverse arrangement for D out-exchange.

Vorläufige Daten

With the combination of these different HDX methods a variety of molecular information is accessible in particular about the chemical nature of heteroatom-bound hydrogen present in the analyte. O-bound Hs exchange very well whereas the N bound Hs are inaccessible via the soft exchange method. Using enhanced exchange conditions, selective labeling within the group of N-bound hydrogens can be observed. Amino groups in peptides are exchanging whereas side chain and backbone amides, as well as guanidine hydrogens are mostly not exchanged. Furthermore, also for peptides and proteins, differentiation between amine and amide-bound hydrogen Hs is possible. For some analytes such as benzamidine, unusual, bimodal HDX patterns were observed for single exchangeable protons using soft HDX conditions, which we ascribe to the presence of different protonation sites or to different conformations. Finally complete HDX is demonstrated for small molecules, peptides and proteins, which gives access to the total number of exchangeable Hs. The maximum number of exchanged hydrogens in a single molecule was 227 for human hemoglobin alpha.

Neuartige Aspekte

Differentiation between N bound hydrogens and complete HDX for peptides and proteins

Referenzen

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Organische Massenspektrometrie I.1

Massenspektrometrische Bestimmung von Phosphodiesterase-4 Inhibitoren in der Dopinganalytik

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Einleitung

Im Rahmen der Suche nach den Wirkmechanismen des Resveratrols und seiner Eigenschaft Kalorienrestriktion zu imitieren wurden umfangreiche Studien insbesondere hinsichtlich der so genannten Sirtuine unternommen. Kürzlich wurde in diesem Zusammenhang der Phosphodiesterase-4 (PDE4)- inhibierende Effekt des Resveratrols entdeckt und als fehlendes Puzzle-Teil im ungelösten Rätsel der lebensverlängernden Wirkung und verbesserten Fettverwertung bezeichnet. [1, 2] Es konnte gezeigt werden, dass PDE4-Inhibition zu verstärkter Mitochondrien-Biosynthese und Fett-Utilisation führt und nicht zuletzt auch die körperliche Leistungsfähigkeit (von Labortieren) um bis zu 40% verbesserte. [2] Obwohl in erster Linie zu therapeutischen Zwecken hergestellt und untersucht [3, 4] stellen synthetische PDE4-Inhibitoren wie Rolipram, Roflumilast, und Cilomilast somit eine neue Herausforderung auch für die Dopinganalytik dar. Zum eindeutigen Nachweis der Therapeutika und deren Stoffwechselprodukten wurden Studien zum massenspektrometrischen Verhalten unter ESI/CID Bedingungen durchgeführt, *in vitro* generierte Metabolite charakterisiert und anschließend ein Flüssigkeitschromatographie-Tandem-Massenspektrometrie basiertes Nachweisverfahren etabliert und validiert, um proaktiv gegen einen potentiellen Missbrauch dieser Substanzklasse im Sport vorzugehen.

Methodik

Die zu untersuchenden PDE4-Inhibitoren wurden zunächst unverändert mittels hochauflösender/hochakkurater (Tandem)Massenspektrometrie studiert und Dissoziationsmuster der Analyten postuliert und zugeordnet. Mit Hilfe der erhaltenen Elementarzusammensetzung der Produkt-Ionen, H/D-Austausch und MS^n-Experimenten konnten plausible Strukturen vorgeschlagen werden, die zur Charakterisierung möglicher Phase-I-Metabolite und deren Modifikationen herangezogen wurden. Diese Metabolite wurden *in vitro* mit humanen Lebermikrosomen hergestellt [5] und anschließend flüssigkeitschromatographisch-massenspektrometrisch identifiziert sowie stoffwechsel-bedingte Veränderungen (Hydroxylierungen, Dealkylierungen, Oxidationen, etc.) lokalisiert. Schließlich wurden Rolipram, Roflumilast und Cilomilast sowie deren Hauptmetabolite in eine existierende LC-MS/MS Multi-Analyten-Methode implementiert und deren Charakteristika (wie z.B. Nachweisgrenze und Spezifität) bestimmt. Proof-of-Concept der Methodik wurde anschließend durch eine Urinprobe eines Patienten erhalten, der aufgrund einer COPD-Erkrankung Roflumilast zu Therapiezwecken einnimmt.

Vorläufige Daten

Drei PDE4-Inhibitoren wurden detaillierten Studien zum Fragmentierungsverhalten nach Elektrospray Ionisation und kollisionsinduzierter Dissoziation mittels hochauflösender und hochakkurater Tandem Massenspektrometrie unterzogen. Das Pyrrolidinon-Cyclopentyloxy-Derivat Rolipram generiert ausgehend vom protonierten Molekül m/z 276 durch Verlust von Cyclopenten ein typisches Produkt-Ion bei m/z 208, welches anschließend unter Umlagerung des Pyrrolidinon-Rests Ammoniak freisetzt und somit m/z 191 produziert. Dieses kann daraufhin Kohlenmonoxid und Methanol abspalten, so dass das intensivste Produkt-Ion des Analyten mit m/z 131 entsteht. Diese Zuordnung der Fragment-Ionen zu Substrukturen des Roliprams erlaubt die Lokalisation von metabolischen Veränderungen, die im durchgeführten in vitro Versuch in erster Linie Dealkylierungen (Entfernung des Cyclopentyl-Rests bzw. Entfernung einer Methyl-Gruppe) und Hydroxylierungen der Cyclopentyl- und Pyrrolidinon-Einheiten zugeschrieben werden konnten. Trotz struktureller Verwandtschaft mit Rolipram verhält sich Cilomilast deutlich verschieden unter vergleichbaren analytischen Bedingungen. Die Präsenz einer Carboxyl-Funktion scheint die Ionisationseigenschaften substantiell zu beeinflussen, so dass eine wesentlich geringere Protonenaffinität aber auch lediglich moderate Azidität vorliegt. Nach Protonierung eliminiert Cilomilast ähnlich Rolipram zunächst Cyclopenten, was von einem Verlust von HCN gefolgt zum intensivsten Produkt-Ion des Spektrums bei m/z 249 führt. Die weiterführende Abspaltung von Ameisensäure trägt zur Bildung von m/z 203 bei, so dass charakteristische Bruchstücke des Analyten zur eindeutigen Analytik bzw. Metabolitenidentifizierung vorliegen. Ein wesentliches Stoffwechselprodukt der *in vitro* Inkubationen ist das Hydroxylierungsprodukt des Cyclopentyl-Rests. Roflumilast unterscheidet sich strukturell etwas deutlicher von den vorhergenannten Analyten durch umfangreiche Halogenierung. Roflumilast generiert im Wesentlichen zwei Produkt-Ionen bei m/z 241 und 187, die einer Amid-Spaltung und einer anschließenden Elimination von Methylencyclopropan zugeschrieben werden konnten. Aufgrund der Anwesenheit eines dichlorierten Pyridin-Rests ist nahezu ausschließlich ein Metabolit bekannt, der als N-Oxid beschrieben und in der vorliegenden Studie anhand der verfügbaren massenspektrometrischen Daten bestätigt werden kann.
Mit Hilfe dieser Informationen konnte eine Nachweismethode der Dopinganalytik bezüglich neuer Zielanalyten erweitert und Nachweisgrenzen von 1-5 ng/mL aus Urinproben erzielt werden.

Neuartige Aspekte

Erstmalige Untersuchung des massenspektrometrischen Verhaltens von PDE4-Inhibitoren und deren Phase-I-Metaboliten; Erweiterung des analytischen Spektrums der Präventiven Dopingforschung

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Organische Massenspektrometrie I.2

Determination of the photoionization properties of medically relevant substances

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Einleitung

In medicine it is of interest to measure medically relevant substances in breath gas. Therefore, we want to use photoionization time-of-flight mass spectrometry (PI-TOFMS), which represents a sensitive, selective and soft ionizing method for analyzing organic trace components in complex mixtures. Before measuring substances (amantadine, diazepam, dimethyltryptamine, etomidate, ketamine, mescaline, methadone and propofol) in breath gas, it is essential to determine the photoionization properties [ionization energies (IEs), appearance energies (AEs) of fragments, VUV (vacuum ultraviolet) spectra and REMPI spectra].

Methodik

For the first part of the measurements single photon ionization (SPI) [1], which is based on the absorption of one VUV-photon ($\lambda < 180$ nm), was utilized. The VUV photons were generated by synchrotron radiation from BESSY II synchrotron facility in Berlin. The spectra were recorded in the energy range of 7.1 to 11.9 eV (174.6 to 104.2 nm) [2]. Additionally, some of the mentioned compounds were ionized by (1+1)-resonance enhanced multiphoton ionization (REMPI) [3]. The spectra were recorded by a continuous scan in the energy range between 3.6 and 5.7 eV (345 to 218 nm) using a tunable optical parametric oscillator (OPO) laser system [4].

Vorläufige Daten

At the BESSY II synchrotron facility the ionization energies and appearance energies of the corresponding fragment(s) were determined for seven substances: amantadine (IE 8.3 eV), diazepam (IE 8.2 eV), dimethyltryptamine (IE 7.5 eV), etomidate (IE 8.2 eV), ketamine (IE 8.0 eV), mescaline (IE 8.0 eV) and methadone (IE 8.6 eV). These data and the VUV and REMPI spectra give an overview of the photoionization properties of the measured compounds. It was determinable which VUV or UV wavelengths are suitable for an analytical detection of the target compounds by SPI-MS or REMPI-MS. The next step was to couple the photoionization time-of-flight mass spectrometer with needle trap device (NTD) as preconcentration technique to analyze the very low concentrations (ppt) of the medically relevant substances in breath gas. For example, propofol was already measured successfully in the breath gas of ventilated pigs using NTD-REMPI-TOFMS.

Neuartige Aspekte

Ionization energies, appearance energies of the fragments, VUV and REMPI spectra of the investigated medically relevant substances are hitherto unknown.

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Organische Massenspektrometrie I.3

Quantitative Metabolomik; Evaluierung der Probenvorbereitung mit Hilfe eines $U^{13}C$ markierten Zellextraktes

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Einleitung

Quantitative Metabolomik eröffnet neue Wege im Bereich der biotechnologischen Prozessoptimierung und beruht auf der massespektrometrischen Messung intrazellulärer Stoffwechselprodukte. Die Erfassung des zentralen Kohlenstoffkreislaufs stellt höchste Anforderungen an die Analytik aufgrund der großen chemischen Diversität der Metaboliten, der teils sehr geringen Konzentrationen und aufgrund von Stabilitätsproblemen in einer komplexen und reaktiven Probenmatrix. Zum Einsatz kommen hochenwickelte Methoden der Flüssigkeitschromatographie und der Massenspektrometrie. Im Besonderen muss jedoch auf die richtige Probennahme und Probenaufbereitung geachtet werden, denn es gilt den Stoffwechsel abrupt zu stoppen, den metabolischen Zustand der Zelle unverfälscht zu erhalten und das Zellinnere selektiv und ohne Verluste der Analyse zuzuführen.

Methodik

Diese Arbeit beschreibt die Probenaufbereitung und Analyse, wie sie an der BOKU für die Hefe *Pichia pastoris* optimiert wurde [1][2].Der Stoffwechsel der Zellen wird bei der Probennahme durch kaltes Methanol gestoppt („Quenching“), extrazelluläre Flüssigkeit wird entfernt und die Zellen werden mittels kochendem Ethanol aufgeschlossen („Extraktion“). Die Probe wird in wässriger Form zweier orthogonaler Trennmethoden zugeführt (Umkehrphasenchromatographie und Hydrophile Interaktionschromatographie) und mit einem Triple Quadrupol Massenspektrometer im multiplen Reaktionsmodus gemessen. Außerdem wird ein eigens hergestellten isotopenmarkierten ($U^{13}C$) Zellextrakt als internenen Standards zur Quantifizierung sowie als Werkzeug zur Evaluierung der Probenvorbereitung eingesetzt [1]. Im Speziellen wurden die Wiederfindungsraten der einzelnen Probenvorbereitungsschritte für über 20 Metabolite ermittelt.

Vorläufige Daten

Die von uns optimierte Methode ist im Stande über 50 Metabolite, darunter Aminosäuren, organische Säuren, Nukleotide und Vitamine, durch Eisatz zweier komplemenärer chromatographischer Methoden simultan zu quantifizieren. Die zuverlässige und genaue Quantifizierung aus Zellen in der Phase ihres Wachstums erfordert ausgefeilte Techniken zur Probennahme, Probenvorbereitung und Analyse. Kritische Punkte sind hier das Quenching und die Extraktion. Zur Korrektur von Extraktionsverlusten und Matrixeffekten hat sich die Isotopenverdünnungsanalyse in der Metabolomik durchgesetzt [3][4]. In der vorgestellten Methode wird ein selbst hergestellter isotopenmarkierter Zellextrakt verwendet. Zudem wurde der isotopenmarkierte Zellextrakt zur Bestimmung von Wiederfindungsraten sowohl einzelner Probenvorbereitungsschritte als auch der gesamten Probenvorbereitung eingesetzt. Die hiermit für über 20 Metaboliten gewonnenen Daten zeigen Wiederfindungsraten der gesamten Probenvorbereitung von 60 % bis 100 % mit Wiederholpräzisionen von unter 10 %. Weiters wurden diese Daten zur Berechnung der Messunsicherheit herangezogen. Anhand von drei exemplarischen Substanzen wurden Messunsicherheitsbudgets für den Bereich der Probenvorbereitung erstellt [1][5]. Im idealen Fall (einfach zu extrahierender Metabolit) wurde eine Gesamtmessunsicherheit von 12% ermittelt. Es konnte gezeigt werden, dass die Extraktionseffizienz und die Extraktionswiederfindung den überwiegenden Anteil an der Gesamtmessunsicherheit ausmachen.

Neuartige Aspekte

Im Bereich der quantitativen Metabolomik wurde estmals ein umfassendes Messunsicherheitsbudget erstellt, welches die maßgeblichen Punkte dieser komplexen Thematik aufzeigt.

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Organische Massenspektrometrie I.4

Crime Scene Investigations: New Method Developments for an Age Determination of Latent Fingerprints

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Einleitung

The analysis of the aging behaviour of latent fingerprints is a major challenge in forensic sciences and has the capability to be of high evidentiary value in future criminal investigations. Information about the age of a collected fingerprint should help to determine the date when the fingerprint was left behind on a crime scene and should give more information about the offender. Due to the very small amounts of fingerprint ingredients, mass spectrometry is a suitable technique providing the required low limits of detection. The aim of the described studies is the development of a validated analytical method for age determination of fingerprints. Analytical investigations were performed to detect substances in fingerprints which are useable for an age determination.

Methodik

For aging experiments, test fingerprints were placed on a piece of aluminium foil (1.5 x 1.5 cm). After a certain time samples were extracted with dichloromethane and derivatized with MSTFA (N-Methyl-N-(trimethylsilyl)trifluoracetamide). These samples were analyzed by GC/MS in splitless mode and with electron impact ionization to identify the volatile components in fingerprints and their degradation products.

Vorläufige Daten

Investigations were focused on the identification of components and their corresponding degradation products. Aging curves for selected compounds were recorded. The obtained results allowed us to elucidate the decomposition mechanisms of detected compounds. Besides several saturated and monounsaturated fatty acids the highly unsaturated hydrocarbon compound squalene and the steroid cholesterol were found to be present in fingerprint residues, being promising target molecules for the age determination of fingerprints. Their aging behaviours differ in their degradation kinetics. Degradation products were identified, giving useful hints on the degradation pathways. To ensure the compatibility of the age determination method with conventional fingerprint collection methods, crime scene powder and fingerprint lifters were analyzed by GC/MS. Additional investigations were made to ensure the conservation of fingerprint samples after preservation of evidence. The basic analytical concept for the application of a GC/MS method in forensic sciences for the age determination of fingerprints will be described. Several targets for the age determination were found and the compatibility with evidence recovery techniques was assured. According to these results an age determination up to 30 days appears possible. Depending on their degradation rates some compounds enable a short-time age determination up to a few days while other compounds enable a long-time age determination up to a few weeks.

Neuartige Aspekte

Novel aspects are the identification of compounds which can be useable for the age determination of fingerprints.

Organische Massenspektrometrie I.5

Surface analysis of bacteria

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Einleitung

Antibiotics are indispensable agents for human live. To combat resistant pathogenic bacteria there is always medical need to explore new antibiotic substances. The classic way to search for antibiotics involved the extraction, chromatographic separation and subsequent analysis of bacterial natural products using mass spectrometric and other structural analysis techniques. The results were, however, disappointing. In contrast, liquid extraction surface analysis (LESA) using Triversa Nanomate technology is a promising and highly reproducible extraction infusion technique based on an automated chip-based nanoelectrospray ionization. Recently we have developed a method for LESA/Orbitrop screening of antibiotics directly from bacterial colony surface[1], here we present new applications of this method.

Methodik

Liquid extraction surface analysis using Triversa Nanomate technology is coupled with the high mass accuracy and resolution available on LTQ-OrbitrapXL tandem mass spectrometer. Using diverse solvent mixtures natural products produced by bacteria was screened directly from bacterial colony surface.

Vorläufige Daten

The first obtained data suggest that this technique can be a powerful tool for antibiotic discovery and other microbiological approaches, e.g. investigations of signal molecules at surfaces directly between different microorganisms. Known antibiotics were correctly detected with high mass accuracy (<4 ppm) and structurally characterized using tandem mass spectra. For intense ions partial characterization of unknowns was assisted using FT-BLAST method[2].

Neuartige Aspekte

High throughput bacterial screening using LESA technology and LTQ-Orbitrap XL was use to study bacterial natural products..

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Organische Massenspektrometrie II.1

Highly efficient LC/MS of nucleic acids by proper selection of solvent additives

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Einleitung

LC/MS represents a commonly applied technique in nucleic acids research [1]. Applications include the characterisation of synthetic and modified oligonucleotides, metabolic profiling of nucleic acids therapeutics as well as typing of genetic markers. Of utmost importance for efficient LC/MS analysis is the proper choice of solvent additives. The mobile phase composition influences chromatographic and mass spectrometric performance. A wealth of solvent additives has been presented. Commonly applied additives include triethylammonium acetate (TEAA) [2], cyclohexyldimethylammonium acetate (CycHDMAA) [3] and triethylamine/hexafluoroisopropanol (TEA-HFIP) [4]. The aim of this presentation is to provide guidance for the selection of the most convenient solvent additive for common applications of LC/MS in nucleic acids research.

Methodik

Oligonucleotides with lengths of 12-51 nucleotides were used as test samples. ESI-MS of nucleic acids was accomplished on a QqTOF instrument (QStar XL, AB Sciex) in negative ion mode. Solvent additives tested included mixtures of either acetonitrile or methanol with 2.3 and 10 mM CycHDMAA (pH 8.4), 2.3 mM CycHDMAA (pH 7.0), 2.3 mM TEAA (pH 7.0) and 2.3 mM TEA/400 mM HFIP (pH 7.0). Direct infusion experiments as well as LC/MS experiments were used to assess the performance of the different solvent additives applied in terms of detection sensitivity, mass spectral quality and chromatographic retention. Chromatographic separations were accomplished on 60 × 0.2 mm i.d. monolithic capillary columns at 70 °C.

Vorläufige Daten

We have evaluated the suitability of different solvent additives for LC/MS of nucleic acids. The nucleic acids analyzed included small synthetic oligonucleotides, a therapeutic oligonucleotide as well as a very large oligonucleotide mimicking a genetic marker. The mobile phase additives tested included CycHDMAA, TEAA and TEA-HFIP at concentrations and pH-values commonly applied in LC/MS of nucleic acids. Direct infusion experiments as well as LC/MS experiments were used to assess performance differences. The parameters tested included spectral quality, limits of detection, and chromatographic performance. The most important results of our study can be summarized as follows: (1) Highest signal intensities and lowest limits of detection can be reached with TEA-HFIP. This solvent is, therefore, particularly useful for the quantification of therapeutic oligonucleotides using single ion monitoring or selected reaction monitoring. (2) TEA-HFIP most efficiently denatures oligonucleotides during chromatographic separation, which reduces the effect of oligonucleotide sequence and secondary structure on the chromatographic elution order. (3) Spectra generated from nucleic acids dissolved in TEAA or CycHDMAA show the most abundant signals at low charge states. The charge state distribution can be shifted to higher charge states and lower m/z-values by adding HFIP. (4) Extensive formation of non-covalent adducts with metal ions is observed with TEA-HFIP, which severely hampers the molecular mass measurement of very large oligonucleitides. Accordingly, for genotyping applications the use of CycHDMAA is recommended.

Neuartige Aspekte

We present guidance for the proper selection of mobile phase additives in LC/MS of nucleic acids.

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Organische Massenspektrometrie II.2

"Polymeromics": Detailed characterization of polymers via MS-based strategies

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Einleitung

Mass spectrometry (MS) is the most versatile and comprehensive method in OMICS sciences. The applications of MS and tandem MS (MS/MS) provide sequence information of the full complement of biological samples. Nowadays, the control of polymer sequences and their accurate characterization is one of the significant challenges of current polymer science. MS-based techniques play a crucial role in the characterization of synthetic polymers to satisfy the need for an absolute method. In this contribution, the utilization of MS-based strategies (especially tandem MS) together with special software tools was shown on the way to sequential analysis of polymers "Polymeromics" [1-2].

Methodik

Various polymer samples were analyzed by using different mass spectrometers such as ESI-Q-TOF, APCI-Q-TOF and MALDI-TOF/TOF MS. The mass spectrometers were operating in the positive ion mode. For the MS/MS experiments, selections of the certain precursor ions were performed to obtain further structural information about polymer samples. PLUMS software tool was utilized for the automated evaluation of MS/MS data of homopolymers. The software assists to identify fragmentation series of homopolymers by determining sum formula for the monomer repeating unit and start- and end-groups of the fragments. Further knowledge of the polymer class or the fragmentation mechanism is not required.

Vorläufige Daten

A number of polymer classes were investigated *via* MS/MS analysis. Several mass spectrometry instruments (ESI-Q-TOF, APCI-Q-TOF and MALDI-TOF MS) were employed to elucidate the fragmentation pathways of these polymers which are synthesized *via* different polymerization techniques such as atom transfer radical polymerization (ATRP), nitroxide-mediated polymerization (NMP), the reversible addition-fragmentation chain transfer (RAFT) process and cationic ring-opening polymerization (CROP). MS-based strategies allowed a relatively precise examination of the investigated polymeric structures. MS/MS experiments were performed upon precursor ions of interest in order to gain further structural information from the resulting fragmentation patterns and to understand the fragmentation mechanism of these polymers. The obtained information provided the necessary knowledge for the structural elucidation of various synthetic polymer classes.
MS/MS offer accurate structural information from intricate macromolecular structures; however, it produces vast amount of data to interpret. In order to overcome the issues related with the interpretation of complicated MS/MS data, PLUMS software was developed. With this approach, it is possible to gain further insights into the polymer composition by straightforward and rapid interpretation of tandem mass spectra. This kind of software development studies for synthetic polymers will certainly increase the usage of MS-based approaches to characterize synthetic polymers, as already seen in the fields of proteomics, metabolomics, genomics and glycomics. They will be part of the recently introduced research field "Polymeromics".

Neuartige Aspekte

Detailed characterization of various synthetic polymer classes *via* MS-based strategies was shown on the way to "Polymeromics".

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Organische Massenspektrometrie II.3

Investigation of the Hsp90 chaperone assembly, Hsp70 cycle and transfer of a client protein

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Einleitung

The heat shock protein Hsp90 is a chaperone, whose activation and regulation depends on a range of co-chaperones. The Hsp90 chaperones main functions are support of client maturation or stabilisation of a specific conformation of client proteins.
We have established a mass spectrometry (MS) based method to monitor the development of heterogeneous systems, such as the formation of the Hsp90 complex out of the constituting proteins.
Mass spectrometry is ideally suited to monitor the parallel steps in the assembly process of a multicomponent system. All participating complexes can be investigated simultaneously without need for labelling or uncertainty about the oligomerization state.

Methodik

This enabled us to assign the key steps in the Hsp90 assembly process and identify binding processes that occur independently as well as those that present a rate limiting step [1].
We can follow the complexes assembly depending on time or concentration and analyse the spectra with the software *Mass*ign, which allows us to extract the component spectra. These allow us a quantitative analysis of the mass spectra. The concentrations of all components are numerically modelled, according to the expected reaction pathways, by minimizing the deviation of theoretical and experimentally determined concentrations. This enables extraction of K_Ds of all participating partial reactions within the assembly network.

Vorläufige Daten

For the 14 component system of the assembling Hsp90 chaperone complex we determined a self-consistent set of 10K_D values [1]. The assembly of the functional chaperone is the prerequisite for acquiring a client protein. The next steps in our investigation are the binding events of the client protein GR (glucocorticoid receptor) in the Hsp70 cycle, the transfer to the Hsp90 complex and further processing, assisted by additional co-chaperones.
We analysed the binding events of the GR in the Hsp70 cycle and then joined this system with the parallel assembling Hsp90 complex. We followed the key steps in the overall assembly / disassembly process, including the observation of previously unknown complexes. We could determine the catalytic role of Hsp40 not only for client binding onto the Hsp70 but for client handover between the Hsp70 and the Hsp90 cycle.
We can show that restrictive binding processes allow only correctly preassembled chaperone precursors to proceed to the functional complex.

Neuartige Aspekte

Monitoring chaperone assembly, including all simultaneously occurring pathways, reveals mechanisms of client interaction.

Referenzen

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Organische Massenspektrometrie II.4

Characterization and Classification of Petroleum Crude Oils using High Resolution Time of Flight Mass Spectrometry

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Einleitung

Crude oil samples from different sources and having different quality chemical attributes are examined by high performance time of flight mass spectrometry. The data are evaluated using software specifically designed to derive key chemical attributes from the high resolution data. Data are acquired using electrospray ionization by direct infusion or flow injection at a variety of resolving powers on FTMS and high resolution (R=100,000) time of flight (HRT) mass spectrometers.
These data are used to classify the crude oil samples based on their O, N and S content and double bond equivalents as well other measurements of significance to petroleum processing. The ability to glean significant descriptors of crude oil pertinent to source identification and processing quality is demonstrated. The data were acquired on a time of flight mass spectrometer using acquisition times of less than 5 minutes per sample with a simple sample preparation. The information content of significance to petroleum researchers and engineers that is retained at the various resolving powers is compared and contrasted with better than 90% of the information content being retained on the HRT compared with 400,000 resolving power on the FTMS.

Methodik

Crude Oil samples are diluted in a mixture of toulene and methanol to a concentration of 0.7-2 mg/ml. The sample is directly infused using a syringe pump at low flow rates. In positive mode, no further additives to facilitate ionization are added, in negative mode, 1% ammonia in H_2O was used. Instrumentation is the LECO Citius LC-HRT (R=100,000) and a 7.2 T FTMS (R = 100,000 - 400,000).

Vorläufige Daten

The High Resolution TOF MS data show sufficient resolution and mass accuracy to routinely perform fingerprint analysis of the polar composition of crude oils and distlillates. Performance tests show a mass accuracy at about 1 ppm, and a stable resolving power of ca. 100.000 with a slight increase with m/z. This increase in R_P makes the 100,000 at m/z 400 on the HRT overall comparable to the R_P of 200,000 on the FTMS (at m/z 400).
The resolving power is sufficient to correctly resolve most isobaric interferences, e.g. C1H2 x N1 (12.6 mDa). In the lower mass range, sulfur characterization is possible demonstrated by the resolution of C3 x SH4 isobars (3.4 ppm).
Using electrospray ionization, the results focus on polar components. Molecular formulas are correctly assigned, and heteroatom class composition can be calculated. This is done via the HRT integrated software and specific software solutions (PetroOrg) for the work up of petroleum data. Unsaturation levels calculated as double bond equivalents (DBE) and carbon number are visualized as classical geochemical plots.
For the assignment of heteroatom classes and the analysis of the DBE level, both the FT-ICR and HRT MS data show comparable results, as well in positive as in negative ionization mode.
With electrospray ionization, the HRT offers an accurate, sensitive, fast and cost-effective platform for most routine petroleomic MS studies.

Neuartige Aspekte

HR-TOFMS petroleum characterization brings an effective alternative to FT technology by nearly indistinguishable information content between the two.

Organische Massenspektrometrie II.5

Charakterisierung hochreaktiver Metallorganyle durch ESI-Massenspektrometrie

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Einleitung

Organometallverbindungen wie Organocuprate, Zinkorganyle oder Grignard-Reagenzien sind aufgrund ihrer hohen Reaktivität von überragender Bedeutung für die organische Synthese. In Lösung liegen diese Reagenzien in komplexen Gleichgewichten vor, die bis heute nur unzureichend verstanden sind. Als komplementäre Methode zu konventionellen analytischen Verfahren wie der NMR-Spektroskopie bietet sich die Elektrosprayionisierungs (ESI)-Massenspektrometrie an, die insbesondere Einblicke in das Aggregationsverhalten ermöglichen sollte. Frühe Arbeiten von Lipshutz et al. an Organocupraten zeigten das Potential dieses Ansatzes auf, blieben aber wegen der Hydrolyseempfindlichkeit und Thermolabilität dieser Verbindungen auf wenige Beispiele limitiert. [1]

Methodik

Lösungen der zu untersuchenden Metallorganyle ($c \approx 10 - 50$ mmol L^{-1}) in trockenem Tetrahydrofuran oder Diethylether werden über eine gasdichte Spritze in die Quelle eines ESI-Massenspektrometers (Quadrupolionenfalle HCT oder Multipol-Gerät TSQ 7000) gegeben. Die durch ESI unter besonders milden Bedingungen in die Gasphase überführten Ionen werden durch Bestimmung ihrer m/z-Werte und durch MS^n-Experimente charakterisiert.

Vorläufige Daten

Unter sorgfältig optimierten Bedingungen lassen sich hochreaktive und -empfindliche Organocuprat [2,3]- ,Organozinkat [4]-, Organoaluminat [5]- und Organomagnesat-Anionen in intakter Form mittels ESI-Massenspektrometrie nachweisen. Neben einkernigen Komplexen wie CuR_2^- (R = Alkyl, Aryl) können dabei auch die entsprechenden mehrkernigen Spezies wie $Li_2Cu_3R_6^-$ beobachtet werden. Variation der Konzentration, des Lösungsmittels, der Gegenionen und der Organylreste erlaubt es, Trends im Aggregationsverhalten der Metallorganyle zu bestimmen und mit Ergebnissen von elektrischen Leifähigkeitsmessungen (Konduktometrie) und NMR-spektroskopischen Experimenten zu vergleichen. Dabei zeigen sich weitgehende Übereinstimmungen. Gasphasen-Untersuchungen an massenselektierten Organometallat-Ionen gestatten darüber hinaus die Charakterisierung ihrer mikroskopischen Reaktivität, was in Lösung wegen des Vorliegens komplexer Gleichgewichte unmöglich ist. Erste Arbeiten haben hier die Reaktivität verschiedener Organometallate gegenüber Wasser und anderen Brønsted-Säuren bestimmt. Die erhaltenen Ergebnisse tragen zu einem besseren Verständnis der molekularen Struktur der untersuchten Metallorganyle bei und können so langfristig helfen, metallorganische Reagenzien und Katalysatoren gezielt zu optimieren.

Neuartige Aspekte

1.) Beobachtung hochreaktiver Metallorganyle durch ESI-Massenspektrometrie
2.) ESI-Massenspektrometrie relativ konzentrierter Lösungen ($c \approx 10 - 50$ mmol L^{-1})
3.) Kombination ESI-Massenspektrometrie/Konduktometrie

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Lipide & Kohlenhydrate I.1

ADVANCES IN THE OLIGOMER ANALYSIS OF NON-IONIC CELLULOSE ETHERS BY QUANTITATIVE (HPLC-) ESI-IT-MS

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Einleitung

Mass spectrometry with soft ionization methods like electrospray ionization (ESI) is a powerful tool for the determination of the substituent distribution in non-ionic cellulose ethers like methyl cellulose.[1,2] For a better understanding of the relationship of the substituent pattern and the physicochemical properties of the cellulose ethers, it is important to obtain reliable data about the substituent distribution in the glucosyl units and along the polymer chain as well.
Since polysaccharide derivatives are complex mixtures no sequencing is required, but the determination of probabilities of various substituent profiles in oligomeric domains. These profiles can be determined by mass spectrometry of partially degraded polymers. To overcome discrimination effects caused by different polarity and coordination properties for sodium, methyl celluloses (MC) are permethylated with methyl iodide-d_3. Furthermore, labeling of the reducing end with a permanently charged tag [3,4] was studied and HPLC-ESI-MS was compared with syringe pump infusion.

Methodik

Perdeuteromethylation of the sample was performed with MeI-d_3 and powdered NaOH according to Ciucanu and Kerek.[5] After sample cleanup by dialysis and freeze-drying the sample is depolymerized by acid catalyzed hydrolysis with trifluoroacetic acid at 120 °C for 15-45 minutes.
The reducing ends of oligomers are labeled in with *m*-aminobenzoic acid (mABA) by reductive amination using 2-picolin borane for reduction. After dilution the samples are applied to the mass spectrometer (Bruker, HCT Ultra ETDII) either by direct infusion with a syringe or by HPLC.

Vorläufige Daten

Compared to sodium adducts cellooligomers labeled with mABA can be measured in the more selective negative ion mode when the carboxyl group is deprotonated. Moreover, mABA enables UV-detection of the oligomers and a simultaneous quantification using HPLC-ESI-MS.
Derivatization of the cellooligomers with mABA shows a clear improvement of the experimentally determined substituent distribution compared to the measurement of sodium adducts. Moreover, the combination of HPLC and ESI MS allows the adjustment of measurement parameters during the experiment and therefore reaching optimum conditions for every oligomer and avoiding competition amongst and suppression of analytes.
With this labeling strategy in combination with HPLC-ESI-MS under optimized measurement conditions, the relative quantification of the methyl pattern in perdeuteromethylated MC is possible for monomers (DP1) up to decamers (DP10).

Neuartige Aspekte

Automated determination of the substituent pattern of methyl cellulose up to decamers by HPLC-ESI-MS is possible.

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Lipide & Kohlenhydrate I.2

Software Framework for Determining Lipidome Homologies

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Einleitung

Modern mass spectrometric approaches enable us to characterizes lipidomes consisting hundreds of molecular species. In this context several initiatives have delivered insights into the developmental and tissue specificity of lipidomes [1-3].
Depending on their biosynthetic ability drosophila, C. elegans, yeast and mammals exhibit membrane systems which can fulfil the functional requirements to act as neuronal, epithelial or secretory tissue. These widely accepted model organisms exhibit unique molecuar species in their lipidomes which cannot be addressed from a perspective of changing quantities of shared molecules.
To utilize the systematic analysis of biological models for our understanding of cellular mebrane organization an lipid homeostasis we developed a metrics system which can help in analogy to proteome, transcriptome and genome analysis to define motifs and domains in a lipidome. To this end, we suggest an informatic method to determine the homolgy between lipidomes.

Methodik

We represent all molecules of a lipidome as SMILES strings. SMILIGN, Smith-Waterman and Levenshtein algorithm are used to calculate distances of all components of a lipidome. On the distance matrix Principal Component Analysis are performed to represent a lipidome in two- and three-dimensional maps. For each molecule in a lipidome unique coordinates are assigned within the drawn chemical space. To determine the similarity of two lipidome (A->B) the euclidian distance of the nearest neighbor between all lipids (A) and (B) is computed. For the set of all eucledian distances the mean value is calculated. The process is repeated in reversed order of the lipidomes (B->A) and the higher average value is utilized as Lipidome Overlap Score (LOS).

Vorläufige Daten

We tested several cheminformatics approaches to compute realistic structural similarities of lipids. Here we could show that only methods based upon alignment paradigms are capable to sufficiently differentiate the vast complexity of isomeric structures in a lipidomes. To this end we applied SMILIGN (a in-house developed method), Smith-Waterman and Levenshtein algorithm to calculate distance matrices of all components of a lipidome.
We applied our workflow on the LIPIDMAPS structure database [4] computing a chemical space for thousands of molecules regaining a structure resembling the curated nomenclature of lipid classes and groups. Next we tested the approach on 8 lipidomes reported by Ejsing CS et al. for three mutant strains (with defective fatty acid elongation), the wild-type yeast strain and two culturing temperatures (24C and 37C). In this well defined system we can cluster accurately the phenotypical impact of the mutations and the temperature shifts using the LOS as metric.

Neuartige Aspekte

Informatics framework to determine homologies between large molecular ensembles with focus on comparing lipidomes.

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Lipide & Kohlenhydrate I.3

Wasserstoff – Isotopenverhältnis – Massenspektrometrie endogener urinärer Steroide als neue Methode in der Dopingkontrolle

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Einleitung

Aufgrund ihrer anabolen und leistungssteigernden Wirkung sind Steroide nach wie vor die am häufigsten nachgewiesene Substanzklasse in der Dopinanalytik [1]. Besonders problematisch sind in diesem Zusammenhang Steroide, die auch vom Körper synthetisiert werden können und somit ubiquitär sind. Bekanntester Vertreter ist hier das Testosteron, bei dem weder seine absolute Konzentration im Urin noch sein Konzentrationsverhältnis zum inaktiven Isomer Epitestosteron einen definitiven Beweis für die verbotene Applikation von Testosteron darstellen. Einzig im Verhältnis der stabilen Isotope gibt es nachweisbare Unterschiede zwischen körpereigenem und körperfremdem Testosteron. Während dieser Unterschied für die stabilen Isotope des Kohlenstoffs bereits hinreichend untersucht worden ist [2,3], gibt es nun erste Ergebnisse zum $^{2}H/^{1}H$ Verhältnis, die die Praktikabilität und den Nutzen dieser Methode im Bereich der Dopingkontrolle eindeutig belegen.

Methodik

Um die für die Gaschromatographie/Pyrolyse/Isotopenverhältnis – Massenspektrometrie notwendige Aufreinigung der urinären Steroide zu erreichen, wurde eine aufwendige Methode entwickelt, deren Kernstück die Isolierung einzelner Steroide mittels Hochdruck – Flüssigkeitschromatographie ist [4,5]. So ließen sich 10 Steroide aus der Konjugatfraktion der Glucuronide und 6 aus der der Sulfate isolieren und vermessen. Untersucht wurde zunächst eine Referenzpopulation bestehend aus 67 Frauen und Männern um die natürliche Bandbreite der vorkommenden Wasserstoffisotopenverhältnisse zu bestimmen und um die Berechnung von für die Dopingkontrolle relevanter Grenzwerte zu ermöglichen. Die Anwendbarkeit dieser Limits wurden mit Hilfe eines Testosteron – Ausscheidungsversuches bestätigt und anschließend auf reale Dopingkontrollproben angewendet. Des Weiteren wurde der Einfluß von Änderungen im Deuteriumanteil des Trinkwassers auf das entsprechende Isotopenverhältnis der Steroide an zwei Probanden untersucht.

Vorläufige Daten

Die Untersuchungen der Referenzpopulation zeigten, daß die absoluten $^{2}H/^{1}H$ Verhältnisse aller Steroide im Bereich von –320 bis –220 ‰ lagen. Isoliert für sich betrachtet, deckt jedes einzelne Steroid eine Bandbreite von ca. 50 ‰ ab. Wie schon für Kohlenstoffisotope gezeigt werden konnte [3], weisen unterschiedliche Steroide auch signifikant unterschiedliche Isotopenverhältnisse im Urin auf. Dies, betrachtet in bezug auf die Tatsache, daß alle Steroide denselben endogenen Präkursor Cholesterol teilen, deutet darauf hin, daß die enzymatischen und chemischen Umsetzungen im Körper nicht frei von Isotopeneffekten ablaufen. Die hieraus resultierenden interindividuellen Unterschiede erwiesen sich als klein genug um verläßliche Grenzwerte für die gesamte Population festlegen zu können. Im Dopingbereich ist insbesondere der sogenannte Δ – Wert, gebildet aus dem $^{2}H/^{1}H$ eines endogenen Referenzsteroids minus des $^{2}H/^{1}H$ eines Zielanalyten, von Bedeutung. Als endogene Referenz eignen sich solche Steroide, die durch die Applikation von z.B. Testosteron nicht beeinflußt werden, Zielanalyten hingegen sind das applizierte Steroid selbst oder seine Metabolite. Nach der Einnahme von Testosteron zeigten sich diese Δ – Werte für Testosteron selbst und für die meisten seiner Stoffwechselprodukte über die Grenzwerte hinaus beeinflußt, in einem Ausmaß, daß der Nachweisbarkeit mittels Kohlenstoffisotopie nicht nachsteht. Würde die Veränderung der Isotopensignatur des Trinkwassers unterschiedlich schnell von unterschiedlichen Steroiden reflektiert werden, würde sich also das $^{2}H/^{1}H$ eines Zielanalyten schneller ändern als das einer endogenen Referenz, so bestände die Gefahr, eine falsch positives Ergebnis zu erhalten. Der Δ – Wert könnte das Limit überschreiten, ohne das eine verbotene Applikation stattgefunden hat. Um ein solches Szenario auszuschließen, wurde das Trinkwasser um ca. 300 ‰ für 2 Wochen angereichert. Schon nach wenigen Tagen zeigt sich eine deutliche Veränderung in den urinären Steroiden, die den drastischen Eingriff in das $^{2}H/^{1}H$ des Wassers jedoch nur zum Teil abbilden. Eine Überschreitung der festgelegten Grenzwerte ist zu keinem Zeitpunkt des Experimentes festgestellt worden.

Neuartige Aspekte

Zum ersten Mal wurden die Wasserstoffisotopenverhältnisse von Steroiden eingehend untersucht und die Relevanz dieser neuen Methode für die Dopinganalytik bestimmt.

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Lipide & Kohlenhydrate I.4

More than honey - A MALDI MS study of the lipids from bee spermatozoa

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Einleitung

Bees are extremely important insects. Their importance does not only result from their production of honey and other products but much more from their relevance in the pollination of plants: it is estimated that without bees about 1/3 of human food would be lost. Therefore, the worldwide reduction of the bee population is a serious problem and the reasons need to be investigated in more detail. The reproduction of bees is very different from mammalians: Bee queens undertake mating flights shortly after emergence and store the semen received from the drones for the rest of their lives in a special body chamber, the “spermatheca” [1]. Astonishingly, spermatozoa remain fertile for several years under these conditions. There are two possible explanations: (a) the spermatheca may exert conditions that prevent sperm oxidation and/or (b) the bee sperm itself may withstand oxidation due to its specific (membrane) composition. We have analyzed the (phospho)lipid composition of bee spermatozoa and compared these data with mammalian sperm. It will be shown that the composition of bee sperm is very simple and comprises almost exclusively moderately unsaturated fatty acyl residues. This makes bee sperm less susceptible to oxidation than the highly unsaturated lipids of mammalian sperm.

Methodik

All chemicals, solvents, and the applied MALDI matrices (9-aminoacridine (9-AA) and 2,5-dihydroxybenzoic acid (DHB)) were obtained in the highest commercially available purity from Sigma-Aldrich (Taufkirchen, Germany). Phospholipid standards were from AVANTI Polar Lipids (Alabaster, AL, USA) and used as supplied. TLC/MALDI analysis was performed as recently described [2,3].
DHB was either used as 0.5 M solution in methanol or as a solution of 100 mg/ml in acetonitrile/water (1:1, v/v), while 9-AA was used in a concentration of 10 mg/ml in isopropanol/acetonitrile (60/40, v/v)) [4]. Lipid extracts from spermatozoa were obtained according to the Bligh & Dyer method. Positive and negative ion spectra were recorded on an Autoflex workstation (Bruker Daltonics, Bremen) in the reflector mode as previously described [2].

Vorläufige Daten

The phospholipids of human and almost all mammalian spermatozoa [5] are characterized by significant amounts of highly unsaturated fatty acyl residues, particularly docosahexaenoic acid (22:6). Diacyl compounds predominate in human sperm, while ether lipids are more abundant in several animal sperm. The presence of both highly unsaturated fatty acids and alkenyl lipids (plasmalogens) makes spermatozoa very susceptible to oxidation. Additionally, the introduction of double bonds into the alkyl chain is an energy-consuming process. On the other hand, the presence of many double bonds makes the sperm membrane very “soft” and it is commonly accepted that this is an important prerequisite for successful fertilization.
In contrast, the lipid composition of the bee sperm is very simple. Beside minor amounts of glycerophosphorylethanolamine (GPE) and sphingomyelin (SM), glycerophosphorylcholine (GPC) is most abundant and comprises about 85% of the total phospholipids. The GPC fraction consists almost exclusively of GPC $18{:}0_{alkenyl}/18{:}1_{acyl}$ and GPC $18{:}0_{alkyl}/18{:}1_{acyl}$ that were identified by positive ion MALDI-TOF MS in combination with thin-layer chromatography (TLC) and verified by digestion with phospholipase A_2 (PLA_2) and exposure to HCl fumes. As the lipid amounts from the bee sperm are very small, 9-AA is the matrix of choice, because this matrix provides higher sensitivity and is not affected by the presence of impurities such as plasticizers from plastic material. Additionally, sperm stored in the spermatheca for many months was also investigated and, surprisingly, no oxidation products at all were detectable, while the expected oxidation products (lysophosphatidylcholine and formyl-lysophosphatidylcholine) were found when the bee sperm was exposed to air ex vivo. Therefore, the spermatheca must contain powerful but so far non-identified antioxidative systems. It is also unknown why mammalians rely on highly unsaturated lipids while bee sperm gives evidence that less unsaturated lipids are sufficient to generate offspring.

Neuartige Aspekte

This is the first MS study of the phospholipids of bee spermatozoa. The longevity of bee sperm was partly explained.

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Lipide & Kohlenhydrate I.5

Discovery of novel lipid classes from shotgun lipidomics mass spectra

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Einleitung

Lipids comprise of a wide array of different classes, and new types of lipids are still being identified. However, novel lipids are usually only discovered by chance or by targeted approaches. In order to systematically search for new lipid classes, we are developing a discovery approach based on the computational querying of spectral data from shotgun mass spectrometric measurements of total lipid extracts by our LipidXplorer software. A property of a shotgun spectra dataset is that the structural information of each analyte of a complex sample is present in the collection of fragment spectra. Thus, any unknown lipid should be represented in this collection and, in principle, be detectable by an appropriately designed LipidXplorer query. In an initial experiment we tested this idea on extracts of the nematode C. elegans. Samples from Dauer stages and L3 larva of the nematode C.elegans were extracted and measured. Computational analysis of the MS data revealed a putative lyso-marado lipid, a new variant of the marado lipid class.

Methodik

Crude lipid extracts from C.elegans larvae were infused via nanoflow ESI (Advion Triversa) into a quadrupole-orbitrap hybrid mass spectrometer (QExactive, Thermo Fisher Scientific). Full-MS and MS/MS-spectra with the precursor ions isolation window of 1 Da are collected over 10 minutes using a targeted MS/MS (t-MS2) method in conjunction with an inclusion list covering the entire mass range of interest. In this way, every peak in the spectrum is subjected to fragmentation. Spectra are then processed using the LipidXplorer software[1].

Vorläufige Daten

Finding novel lipids by mass spectrometry is difficult when the structure is entirely novel, because unknown fragments are not easily assigned to chemical structures. However, novel lipids in most cases comprise of known building blocks (i.e.fatty acids, glycerol, carbohydrates, etc.) or modifications of known lipids like actetylations or hydroxylations. Such novel lipids variants are not accessible by data bases, yet they are identifiable because they generate fragments according to known fragmentation rules. With the LipidXplorer software, we can search a complete shotgun data set for the presence of novel fragment combinations or modifications. This is possible because LipidXplorer does not rely on querying a known data base but rather uses a molecular fragmentation query language to probe shotgun spectra for arbitrary sum compositions defined by the experimenter, independently of the instrument used. To test this idea, we measured crude lipid extracts from the nematode C. elegans for novel classes related to marado lipids [2] by shotgun lipidomics in a targeted MSMS experiment, in which all masses between 500 and 1000 Da were fragmented with an isolation window of 1 Da. Marado lipids are 6,6' di-O-acetyl threhalose glycolipids which upon fragmentation generate two acetyl fragments and one fragment from the threhalose moiety. We hypothesized that there could exist other lipid classes related to this basic structure which may contain modifications on the trehalose or fatty acid residues, and designed LipidXplorer queries to search for known sugar and fatty acid modifications. The queries uncovered a previously unreported lyso-marado lipid consisting of trehalose with a single acetyl moiety. Importantly, no other variants were found, suggesting the specificity of this observation. This result opens a perspective for further systematic screens for other novel lipid classes and it should be noted that this approach can be used to screen other previously acquired shotgun data sets.

Neuartige Aspekte

Unbiased shotgun screening for new lipid classes.

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Lipide & Kohlenhydrate II.1

Mass spectrometry as an analytical tool in oxolipidomics.

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Einleitung

Oxidative stress, defined as imbalance between reactive oxygen species (ROS) production and their elimination by antioxidant defense system, was linked to the pathogenesis of over 200 human diseases. At the molecular level the accumulation of ROS yields elevated concentrations of oxidized proteins, lipids and nucleic acids. This oxidation leads to the loss of functional activity, autoimmunity and inflammatory responses. Lipids, due to the presence of (poly-) unsaturated fatty acids (PUFA), are a major target of oxidative stress. Lipid perxoidation generates multiple compounds characterized by high cellular toxicity due to the presence of chemically active functional groups. These lipid peroxidation products (LPP) contain reactive carbonyl groups, which can modify nucleophiles in proteins, nucleic acids and other lipids. Identification and quantification of all oxidized products is important not only in predictive diagnostics, but also for understanding the molecular mechanisms of oxidative stress in the context of related pathologies. Mass spectrometry (MS) is the only technique that can detect and identify oxidatively modified biomolecules with the prospect of a high-throughput screening. Here we present a new MS-based platform used to study carbonyl stress resulting from lipid peroxidation.

Methodik

Different LPP classes were identified by new shotgun and LC-MS- (/MS-) based lipidomics methods. Carbonylated LPP were identified after derivatization with 7-(diethylamino)coumarin-3-carbohydrazide (CHH) using specific neutral loss or reporter ion scans on ESI-LTQ-Orbitrap- and ESI-Iontrap-MS. Acquired CID spectra allowed to determine the structures of most oxidized compounds.

Vorläufige Daten

Several classes of LPP were studied *in vitro* and *in vivo* using optimized shotgun and LC-MS-based protocols: low molecular weight (LMW; e.g. malondialdehyde and hydroxynonenal), high molecular weight LPP (HMW; e.g. 1-palmitoyl-2-(9-oxononenoyl)-*sn*-glycero-3-phosphoethanolamine), and oxysterols (e.g. 3-hydroxy-5-oxo-5,6-secocholestan-6-al). CHH derivatization was applied to study carbonylated LPP in a mixture of oxidized phospholipids (PL) from six different classes. CHH specifically derivatized reactive carbonyl-groups (i.e. aldehydes and ketons) in all classes of LPP, independent from their molecular weight or structure (PL class). The high hydrophobicity and superior ionization efficiency of CHH allowed the simultaneous extraction and detection of all LPP in positive ion mode ESI-MS/MS independent of their chemical properties. Based on our new analytical strategy we were able to identify more than 200 compounds in different *in vitro* oxidized PUFA and PL using high resolution shotgun lipidomics. In case of oxysterols, consecutive derivatization of carbonyl and hydroxyl groups was employed prior MS detection which allowed to distinguish oxidized isobaric compounds. Further on, a LC-MS based protocol was established for the simultaneous detection of LMW- and HMW-LPP from a mixture of oxidized PL based by reversed-phase chromatography. The separation relied on the length of the oxidized fatty acid chain. Additionally, several LPP (e.g. hydroxyhexanal and hydroxynonenal) were quantified in plasma samples after CHH-derivatization using RPC-MS.

Neuartige Aspekte

Oxolipidomics analysis of different LPP allows multi-targeted biomarker screening in plasma samples from patients with various oxidative stress related disorders.

Lipide & Kohlenhydrate II.2

Unterscheidung Isomerer Oligosaccharide mittels Vis-PD

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Einleitung

Die Massenspektrometrie ist, aufgrund der häufig geringen Probenmengen, in der Kohlenhydratanalytik eine grundlegende Methode.Durch ihre strukturelle Vielfalt ist die Identifizierung von komplexen Oligosacchariden aufwendig und erfordert eine Fragmentierung der Analyte, dies gilt besonders für isomere Strukturen.
Verschiedene Fragmentierungsmethoden wurden entwickelt und verwendet, wobei Photodissoziationsmethoden (PD-Methoden), wie z.B. die Infrarot Multiphotonendissoziation (IRMPD), an Bedeutung gewonnen haben. Auch die Vakuum-Ultraviolett-PD (VUVPD) [1] und Ultraviolett-PD (UVPD) [2] wurden erfolgreich zur Fragmentierung von Oligosacchariden eingesetzt.
In unserer Arbeit zeigen wir, dass unter Verwendung von geeigneten chromophoren Gruppen eine Fragmentierung von Maltopentaose und der isomeren Oligosaccharide *lacto*-N-Fucopentaose I und II (LNFP I und LNFP II) mittels Vis PD durchgeführt werden kann. Des Weiteren wurde das Fragmentierungsmuster durch Verwendung verschiedener Adduktionen und Ladungszustände variiert.

Methodik

Die Experimente wurden mit einem APEX Qe FT-ICR-Massenspektrometer mit einer Apollo II Combi Quelle, ESI / MALDI-Version durchgeführt (Bruker Daltonics). Die Fragmentierung fand der ICR Infinity Cell statt. Für stoßinduzierte Fragmentierungen wurde Argon als Stoßgas verwendet, VIS-PD Experimente wurden mit einem Argon Ionen-Laser Innova 70c (Coherent) in Multi- und Single-Line-Modus durchgeführt. Im Single-Line-Modus wurden, abhängig von dem verwendetem Label, die Wellenlängen 457 nm, 488 nm und 514 nm verwendet.

Vorläufige Daten

Maltopentaose wurde mit verschiedenen chromophoren Gruppen derivatisiert und mittels VisMPD fragmentiert. Dabei wiesen die Rhodamin110- und 2-Aminoazotoluolderivate die besten Fragmentierungseigenschaften auf. Im positiven Ionenmodus wurden fast ausschließlich Y-Typ Fragmente beobachtet, wohingegen im negativen Ionenmodus B-, C-Typ Fragmente und auch Ringbruchfragmente (A-Typ) beobachtet wurden.
Die Rhodamin110-Derivate der isomeren Oligosaccharide LNFP I und LNFP II wurden mittels CID und VISMPD fragmentiert.
Die protonierten Derivate der Isomere konnten mit den verwendeten Fragmentierungsmethoden im positiven nicht, und im negativen Ionenmodus nur anhand weniger Fragmente unterschieden werden. Die Fragmentierungsmuster konnten unter Verwendung von verschiedenen Addukt-Ionen variiert werden. Es wurden die Addukt-Ionen des Typs $[M+X]^+$, $[M+H+X]^{2+}$ und $[M+2X]^{2+}$ (Y = Li, Na oder K) fragmentiert, wobei insbesondere die Ionenspezies $[M+H+X]^{2+}$ eine große Fragmentvielfalt bot.

Neuartige Aspekte

VisMPD von Oligosacchariden unter Verwendung verschiedener chromophorer Gruppen und unterschiedlicher Addukt-Ionen.

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Lipide & Kohlenhydrate II.3

Brown Adipose Tissue Activation remodels the Lipidomic Landscape of Metabolically Active Tissues: A High Resolution UPLC-MS Fluxomic Approach

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Einleitung

Excess lipid accumulation in metabolically active tissue is contributing to the development of metabolic diseases. As the natural function of brown adipose tissue (BAT) is to produce heat by burning glucose and fatty acids to defend the body against cold [1], BAT activation is a promising route to treat obesity and associated disorders. Previously, our laboratory has shown that BAT activation lowers blood lipids by accelerating plasma triglyceride clearance into BAT [2]. However, cellular metabolic homeostasis and lipotoxicity is not only controlled by the amount of lipids but rather by the complex composition of lipids. Here, we characterize the systemic changes in lipid metabolism after BAT activation by investigating the static and kinetic lipidomic and fluxomic landscape of tissues and circulating plasma lipids.

Methodik

Wild-type C57BL/6J mice were treated with vehicle or beta3-adrenergic agonist CL316,243 (CL) for 4 weeks to induce BAT activation. For the fluxomics approach D_2O was administered via intraperitoneal injection following D_2O addition to the drinking water for 100 h [3]. Plasma lipoprotein profiling was performed using fast performance liquid chromatography. Lipoprotein fractions, white adipose tissue (WAT)-, BAT- and liver- samples were extracted using the method of Folch [4]. Lipidomic analysis were conducted on a Dionex3000 UPLC (Column: Kinetex C18, 150 x 2.1 mm; 1.7 µm (Phenomenex)) coupled to an ESI-UHR-Q-TOF mass spectrometer (maXis, Bruker Daltonik). Data were acquired using high-resolution, full scan MS and collisional induced dissociation (CID) fragmentation. The D_2O-content of plasma water was determined via GC-MS [5].

Vorläufige Daten

In BAT and WAT, the steady state lipidomic landscape revealed that CL treatment leads to a shift in the fatty acid composition in main lipid classes from polyunsaturated fatty acids towards monounsaturated fatty acids. In contrast, no sizeable alterations were observed in liver. Interestingly, although plasma lipoproteins are produced in the liver, their fatty acid signature was very similar to BAT and WAT (but not liver). Using systemic dynamic isotope lipid labeling we found that these changes in the lipidomic landscape are caused by an induction of *de novo* lipogenesis (DNL), the biosynthesis of fatty acids from non-lipid precursors, exclusively in BAT and WAT.
In summary, BAT activation rapidly exchanges exogenous by endogenous fatty acid species in BAT, WAT and in circulating plasma lipoproteins. We conclude that activated BAT controls not only the amount of lipids but, moreover, also dominates the composition of circulating plasma lipids, conferring presumably beneficial effects on metabolic health. Using an unbiased lipidomic approach, we have identified BAT as a master regulator of lipid metabolism in metabolically active tissues.

Neuartige Aspekte

Kinetic measurement of lipid metabolism after BAT activation.
Plasma lipid-composition is controlled by BAT instead of liver after BAT activation.

Referenzen

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Lipide & Kohlenhydrate II.4

CE-ESI TOF MS for the analysis of intact proteins: recent progress and the application towards the differentiation of EPO preparations

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Einleitung

CE/MS is gaining importance as an analytical technique especially for the identification and characterization of intact proteins [1]. This is due to the combination of the selectivity of CE, i.e. the ability to separate even large molecules based on charge-to-size-ratio, and the powerful detection by mass spectrometry leading to an accurate mass of even numerous non-separated molecules. Thus, CE/MS has been used frequently in the last years for the analysis of intact proteins especially in biopharmaceutical applications [2].

Methodik

CE experiments were performed on an Agilent CE instrument using capillaries with an ID of 50 µm and a length of approximately 60 cm. UltraTrol™ low normal (LN) (derivatized polyacrylamide from Target Discovery) have been used for dynamic precoating. The CE-MS coupling was carried out via electrospray ionisation (ESI) using a commercial CE-ESI-MS triple-tube interface (Agilent). A micrOTOFQ or maXis quadrupole time-of-flight mass spectrometer (Bruker Daltonik) was used. The ESI sprayer was grounded while the transfer capillary was kept at a constant voltage of -4500 V (positive ion polarity mode). The nebulizer gas (nitrogen) pressure was set to 0.2 bar. The ion optics were optimized to the highest possible intensity in the mass range of m/z 700 – 3000.

Vorläufige Daten

A CE/MS method has been optimized and validated with respect to the general application for small intact protein analysis in biopharmaceutical applications. The soluble acrylamide-based coating results in efficient and reproducible separation (migration times mean RSD = 1.4%; peak areas mean RSD = 12.3%) using an optimized rinsing and re-coating procedure [3]. An extensive principal component analysis revealed that the presented method is robust and useful for the relative quantitation of proteins and protein isoforms.
Isotopic resolution can be achieved for the 30kDa protein Erythropoietin (EPO) using a TOF instrument with long flight path [4]. The potential of this information will be discussed as well as the achieved mass accuracy.
Based on the distribution of major glycoforms taking sialilation, antennarity, and acetylation into account various preparations of EPO were compared. Using Principal Component Analysis all preparations, including those with small differences (different batches) could be differentiated based on the first three PCs [5].
This example exhibits the power of the presented method. In this way it will contribute to the improved characterization of a large variety of intact proteins in the biomedical and pharmaceutical area including the analysis of antibodies, including the differentiation of different preparations as well as possible counterfeits.

Neuartige Aspekte

Validierte CE/MS-Methode für intakte Proteine; Chemometrie zur Unterscheidung von Isoformen von Glykoproteinen basierend auf Daten von intakten Proteinen

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Lipide & Kohlenhydrate II.5

Mechanisms of advanced glycation studied in peptide-glucose systems under elevated temperatures

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Einleitung

During thermal treatment of meals reducing sugars react with proteins in course of Maillard reaction. Oxidative degradation of the resulting Amadori compounds under elevated temperatures is accompanied by formation of various carbohydrate and dicarbonyl intermediates and yields advanced glycation end-products (AGEs). Though these substances are known for their toxic and pro-inflammatory effect in mammals [1], the pathways and mechanisms of particular AGE formation are still not completely characterized.

Methodik

To estimate the impact of Amadori product degradation in formation of AGEs, synthetic model peptide Ac-AKASASFL-NH_2 was incubated at 95°C in 100 mmol/L phosphate buffer with equimolar amounts of *D*-glucose in parallel to corresponding Amadori peptide in glucose-free medium for up to 4 h. Samples were analyzed by GC-MS (carbohydrate quantification) and RP-HPLC-ESI-QqTOF-MS (quantification of peptide glycation products).

Vorläufige Daten

A GC-MS method for simultaneous quantification of 15 carbohydrates in the presence of high phosphate concentrations was established. The method relied on SPE-based depletion of phosphate followed with methoximation and trimethylslylation of the resulting oximes [2]. Glucose was degraded by 85 % and 93 % in absence and presence of peptide after 2 h incubation, respectively. In contrast, the Amadori peptide was completely degraded within 30 min under the same conditions. Arabinose was the main glucose degradation product, whereas the Amadori peptide yielded mainly erythrose at 95 °C. The Amadori product demonstrated an essential potential for AGE formation, yielding considerably higher amounts of N^{ε}-(carboxymethyl)lysine in comparison to *D*-glucose. The obtained results in this study indicated the glycoxidation pathway as main CML formation pathway under the studied conditions.

Neuartige Aspekte

A new analytical platform to evaluate AGE formation was established. Fructosamine is the main precursor of AGEs under elevated temperatures.

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Instrumentelle Entwicklungen I.1

Characterization and optimization of a high field Orbitrap for proteome analysis

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Einleitung

Mass spectrometry-based proteomics is heavily driven by advances in high resolution instruments with improved performance. Recently, a novel version of the Orbitrap family, the Orbitrap Elite, was introduced with improvements in three main areas, the ion transfer optics, a compact high field Orbitrap and an enhanced Fourier Transform algorithm for signal deconvolution. This allows an MS/MS acquisition speed up to 12 Hz in the dual cell linear ion trap and 6 Hz in the Orbitrap. In this study, data-dependent acquisition methods for proteome analysis were characterized and optimized for several types of proteomic samples.

Methodik

BSA, E. coli and HeLa digests were analyzed in various combinations of dilution series and replicates. Mass spectrometry was performed on an Orbitrap Elite mass spectrometer coupled to an Eksigent nanoLC 1 D+ ultra system. Several acquisition methods were designed to address diverse aspects of proteomic analyses like sensitivity, acquisition speed, mass accuracy and general data quality. Protein identification and quantification was performed by MaxQuant.

Vorläufige Daten

The over-all sensitivity of the instrument was tested by a dilution series from 100 zmol to 100amol of a commercial BSA digest in HCD mode where even the lowest concentration produced a 5:1 signal to noise precursor ion and generated identifiable fragment spectra.
Basic measurement parameters were optimized using 1 ug E.coli digest showing that low target values and a high number of precursors for fragmentation achieve best numbers of peptide and protein identifications.
For the qualitative comparison of scan modes for complex samples, a dilution series containing 10 ng to 1 µg of FASP digested HeLa lysates was prepared. All dilution steps were analyzed by a 60 min LC gradient using HCD mode and several CID scan speeds. Identification rates were highest in HCD mode (56% at 1ug) while both CID scan rates achieved similar identification rates around 31%. The difference in identification rates can be assigned to the higher quality of HCD scan data for which the high mass accuracy can be used in the database search and better discriminate true identifications from decoy hits. Although the identification rates were lower, improved sequencing speed of the CID methods acquired in average 36% more MS/MS spectra.
Moreover, the relative performance gain compared to the previous Orbitrap generation was analyzed. Elite HCD measurements identified 1675 proteins (8679 unique peptides) within 60 minutes which is an increase of 40% over the Orbitrap Velos. The faster ‘CID rapid’ scan type of the Elite instrument proved to be superior for low sample quantities and achieved >30% more identifications compared to the ‘CID normal’ scan type of the Velos Orbitrap.

Neuartige Aspekte

In depth characterization of data-dependent acquisition methods of a hybrid ion trap high field Orbitrap for proteome analysis

Referenzen

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Instrumentelle Entwicklungen I.2

Moving Orbitrap Instrumentation towards Intact Protein Analysis up to one Megadalton

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Einleitung

With the introduction of the compact highfield Orbitrap mass analyzer, topdown experiments became feasible at LC time scale if the experimental parameters are chosen appropriately. Minor modifications on an iontrap-FTMS hybrid instrument and a benchtop orbitrap instrument enable improved performance for topdown analysis of intact proteins and protein complexes.
Depending on protein molecular weight (MW), different instrument modifications are applied. Especially the trapping pressure is depending on the MW of ions.
Up to MWs of 30 kDa, topdown MS/MS using electron transfer dissociation (ETD) and higher energy collisional induced dissociation (HCD) is possible for deep protein sequencing and characterization. Nitrogen flow reduction in the HCD cell increases Orbitrap vacuum quality and thus reveals isotopically resolved MS data up to MWs of 50 kDa.
At MWs higher than 50 kDa, using short transients becomes more important. Orbitrap Elite with a mass range up to m/z 4,000 is used for the analysis of denatured proteins with high charge numbers. Under native MS conditions with lower charge numbers, Exactive Plus instruments using an extended mass range up to m/z 40,000 allow analysis of protein complexes up to one megadalton.

Methodik

All HCD and ETD topdown measurements were done on a modified Thermo Fisher Scientific Orbitrap Elite ETD instrument (Thermo Fisher Scientific, Bremen, Germany). A modified Exactive Plus Orbitrap mass spectrometer using an extended mass range (Thermo Fisher Scientific, Bremen, Germany) was used for native MS studies of intact antibodies and large protein complexes. Gas pressure was adjusted by an electronic switching valve. Standard proteins were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Vorläufige Daten

Middle down approaches using an Orbitrap Elite instrument enable screening of intact proteins up to 30 kDa and result in isotopically resolved selected ion monitoring (SIM) data and MS/MS data with extended resolution power up to 480,000 at m/z 400.
For the analysis of molecules lower than 50 kDa, reduction of gas load in the C-trap/HCD collision cell allows pressure reduction in the Orbitrap mass analyzer and hence reduces decay of transients of intact proteins. On the other hand, lowered pressure in the C-trap/HCD collision cell results in undesired reduction of trapping efficiency in the C-trap.
Isotopic resolution in *online* LC/MS analysis up to around 50 kDa is achieved. This increases signal-to-noise in spectra of heavier proteins such as antibodies and improves analysis of their glycosylation status.
Orbitrap Elite instruments with a mass range up to m/z 4000 enable analysis of intact antibodies (150 kDa) under denatured conditions.
Using native ionization conditions, noncovalent protein/protein and protein/ligand interactions are maintained. Increased pressure in the C-trap and using Argon or Xenon as cooling gas improve trapping of ions larger than m/z 10,000. A modified Exactive Plus instrument with an extended mass range up to m/z 40,000 was used thus allowing analysis of large scale protein complexes up to 1 megadalton. These modifications open up new applications such as monitoring antibody-antigen binding and studying the assembly of large protein complexes.

Neuartige Aspekte

Modifications to a standard iontrap-FTMS hybrid instrument and a benchtop orbitrap instrument enable improved intact protein analysis.

Instrumentelle Entwicklungen I.3

An Automated MS-based Screening Procedure for Clinical and Forensic Toxicology

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Einleitung

Screening applications in clinical and forensic environments require rapid and unambiguous results that can be generated even by unskilled users. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) combined with library search is an emerging screening technology in these fields. It provides more valuable information than LC-UV detection while covering a broader and in some respect complementary range of analytes when compared to GC-MS. This study focuses on applying and challenging a robust and easy-to-use Ion-Trap-based solution for the detection and identification of common drugs, drugs of abuse and their metabolites in shortest time as possible in a fully automated environment.

Methodik

Serum sample preparation was performed according to a liquid-liquid extraction (LLE) protocol. For chromatographic separation a fast reversed phase LC separation (11 min full duty cycle) was used. An amaZon speed ion trap MS was used to generate a spectral library of currently 830 compounds based on retention time MS, MS^2 and MS^3 information. Data evaluation and reporting was carried out by an automated spectral library search algorithm.

Vorläufige Daten

The automated screening approach revealed a high reproducibility of correct identifications and an overall high transferability between different laboratories. In conclusion the presented screening method offers a fast and reliable routine identification tool for clinical and forensic analysis. A subset of 200 compounds were defined and spiked into human serum matrix at 3 different concentration levels (low therapeutic, therapeutic and elevated). All compounds were combined into mixtures containing 5-10 substances each and then analyzed. Commonly used or miss-used benzodiazepines like diazepam could be successfully detected and identified at low therapeutic doses (low ng/ml range). Also low-dose benzodiazepines like flunitrazepam could be detected and identified in spiked serum samples. The results show statistical evaluation of the data listing identifications at different concentration levels, false positive and false negative rates. The presented library screening method offers a fast, reliable and sensitive procedure for clinical and forensic analysis. The automated screening approach revealed a high reproducibility of correct identifications and an overall high transferability between different laboratories. The combination of MS^2/MS^3 spectra and retention time allows identification of drugs and metabolites at therapeutic levels and offers a fast and reliable routine identification tool for clinical and forensic analysis

Neuartige Aspekte

Easy-to-use Ion-Trap-based solution for the detection and identification of common drugs, drugs of abuse in shortest time in fully automated environment.

Instrumentelle Entwicklungen I.4

Proton Transfer Reaction and Selective Reagent Ionization - Mass Spectrometry (PTR/SRI-MS): Investigations of Toxic and Hazardous Substances

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Einleitung

Proton Transfer Reaction - Mass Spectrometry (PTR-MS) technology, which is well established in various fields of application (e.g. environmental chemistry, food and flavor science, medical research, etc.; compare [1]), underwent two major advancements in the recent past: the combination with high sensitivity and high mass resolution time-of-flight mass spectrometers and the possibility of switching the reagent ions, i.e. thus enhancing PTR-MS to Selective Reagent Ionization - Mass Spectrometry (SRI-MS). Here, we will present (partly unpublished) experimental data focused on the detection and identification of toxic and hazardous substances (such as toxic industrial compounds (TICs), chemical warfare agents (CWAs), explosives and designer drugs) illustrating the benefits of these advancements for the established applications, as well as providing proof-of-principle for new applications.

Methodik

In conventional PTR-MS water vapor is transformed to H_3O^+ in a sophisticated ion source. The high selectivity of this process makes the use of a mass filter between the ion source and the adjacent drift tube, where chemical ionization of the trace compounds takes place, obsolete. In 2012 [2] we presented a novel way of operating such a PTR-MS instrument, that permits the selection of various reagent ions including H_3O^+, NO^+, O_2^+, Xe^+ and Kr^+, respectively, thus enabling SRI-MS with a slightly modified PTR-MS instrument. For the present studies we utilized quadrupole based PTR-QMS, as well as time-of-flight based PTR-TOFMS instruments equipped with this feature, demonstrating the advantages and disadvantages of both technologies.

Vorläufige Daten

As the known advantages of PTR-MS (no sample treatment, below 100 ms response time, real-time quantification, etc.) seem ideal for the detection of toxic and hazardous substances, we already started in 2009 a series of investigations on those compounds [3] which is still ongoing [4-5]. Here we present a short review of these studies, including the unambiguous identification of explosives utilizing the reduced electric field strength in the drift tube as an additional data dimension and a prototype for detection of TICs and CWAs in air supply systems of buildings developed within the framework of the FP7 project “SPIRIT”.
In addition and in comparison unpublished PTR/SRI-MS results of novel “legal highs” (i.e. recreational drugs that are not affected by drug laws; e.g. ethylphenidate, 5-MeO-DALT, synthacaine, etc.) will be presented. It turns out that although these substances were purchased at rather obscure internet vendors, they are surprisingly pure and that they can be easily identified due to characteristic fragmentation behavior. Furthermore, with SRI-MS operated in Kr^+ mode it is now also possible to detect toxic gases, such as e.g. CO. We present data on engine exhaust measurements proving that the mass resolution of the PTR-TOFMS instrument is sufficient to separate CO^+ (m/z 27.994) from the isobar N_2^+ (m/z 28.006) and CO_2^+ (m/z 43.989) from the isobar N_2O^+ (m/z 44.001).
In order to give an outlook of the performance of an improved PTR-TOFMS instrument we investigated different gas standards containing certified concentrations of trace gases. With a high mass-resolution PTR-TOFMS we achieved a sensitivity of 500 cps/ppbv (at m/z 181; protonated trichlorobenzene) and a linearity in the instrumental response down to 200 ppqv.
We want to gratefully acknowledge financial support by the FFG, Wien, and the EC, Brussels, via the FP7 projects “PTR-TOF” (GA 218065), “SPIRIT” (GA 242319) and “;PIMMS” (GA 287382).

Neuartige Aspekte

We introduce a novel method for threat agent detection utilizing an improved PTR/SRI-MS instrument.

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Instrumentelle Entwicklungen I.5

Gaschromatographie gekoppelt mit ultraschneller hochauflösender TOF Massenspektrometrie (GC-HRTOF) oder ultrahochauflösender FT-Massenspektrometrie (GC-APCI-FTICR) zur Analyse hochkomplexer Mischungen

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Einleitung

Hochauflösende Massenspektrometer (R > 30.000) wie Orbitrap- oder FT-ICRMS- Massenspektrometer werden überwiegend mit ESI- oder MALDI-basierten Ionenquellen zur Analyse größere Moleküle verwendet. Mit der steigenden Bedeutung z.B. der „metabolomics“ und der petrochemischen Anwendungen rücken neuerdings kleinere, verdampfbare Moleküle wieder mehr in das Interesse der Analytischen Chemie. Neue Entwicklungen in der Gaschromatographie (z.B. „comprehensive“ zweidimensionale Gaschromatographie, GCxGC), Flugzeitmassenspektrometrie (Multireflektions-TOF) und Einlass-/Ionisationstechnik (z.B. APCI, APLI) ermöglichen nun die Erreichung höchster Selektivitäten durch Kopplung von hoch- oder ultrahochauflösender MS mit hochauflösender Gaschromatographie.
Interessant für die Strukturaufklärung über die Exakte Masse ist einerseits die Verwendung ultrahochauflösender Massenspektrometer wie FT-ICRMS mit weicher Ionisation. Allerdings sind hier nur geringe Aufnahmeraten möglich (~ 1Hz).
Die Multireflektions-TOF Technik hingegen erlaubt Aufnahmeraten von bis zu 200 Hz bei einer Auflösung R = 50.000. Damit wird erstmals eine auflösungsoptimierte Gaschromatographie und Verwendung von spektraler Dekonvolution und sogar „comprehensive“ zweidimensionale Gaschromatographie (GCxGC) möglich. Weiterhin kann die schnelle TOF Technologie auch zur on-line Verfolgung von Prozessen eingesetzt werden. Für viele Anwendungen reichen hier Auflösungen um 5000

Methodik

Zur Untersuchung komplexer Proben aus den Arbeitsbereichen Forensik und Petrochemie wurde einerseits ein ultrahoch massenauflösendes GC-MS System verwendet. Im Detail handelt es sich um eine Kopplung von Kapillargaschromatographie über ein APCI Interface an ein 7T FT-ICR (Bruker GmbH, Bremen, D).
Weiterhin wurden die Proben mit einer Kopplung von Gaschromatographie sowie „comprehensive“ zweidimensionaler Gaschromatographie (GCxGC, experimentell) an ein Multireflektions-TOF-MS System mit Elektronenionisation (Leco Inc., St. Joseph, USA; R=50.000) untersucht.
Für massenspektrometrische Studien an Prozessen (Direkteinlass) kam ein klassische oa-Reflektron (Tofwerk, Thun, CH) zum Einsatz.

Vorläufige Daten

Die GC-APCI-FTICR Methode erlaubt die Bestimmung der Summenformeln von petrochemischen Produkten oder Pflanzenmetaboliten in Extrakten von Drogen. Die relativ langsame Aufnahmefrequenz verhindert allerdings eine volle Optimierung der gaschromatographischen Trennung. Die weiche Ionisation durch APCI ist vorteilhaft für die Anwendung, allerdings werden bestimmte Substanzklassen unterdrückt.
Das HR-TOF mit Elektroionisation erlaubt eine hohe Aufnahmefrequenz, die eine direkte Verwendung für die „comprehensive“ zweidimensionale Gaschromatographie (GCxGC) ermöglicht. Die Auswertung von zweidimensionalen GCxGC-Chromatogrammen kann über ein sog. Scripting Verfahren erfolgen [1], bei dem die (Retentionszeit-)Positionen sowie charakteristische Fragmente für die Zuordnung von Substanzklassen verwendet werden (z.B. 57, 71m/z – Alkane; 60 m/z Alkansäuren, etc.). Die hochauflösende TOFMS erlaubt nun den neuen Ansatz, die exakte Masse von charakteristischen Fragmenten zu verwenden um „gefilterte“ zweidimensionale GCxGC Chromatogramme zu erstellen. Die hohe Selektivität der Auswertung wird am Beispiel von petrochemischen Proben demonstriert.

Neuartige Aspekte

Verwendung von hoch-/ultrahoch-auflösender TOFMS/FTICRMS als Detektor für die Gaschromatographie: Forensische und petrochemische Anwendungen sowie neue Datenauswertungskonzepte

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Instrumentelle Entwicklungen II.1

Vergleich der Matrixeffekte in der LC/MS-Pestizidanalytik mittels ESI, APCI und neuartiger plasmabasierter Ionisierung

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Einleitung

Die LC/MS-Kopplung hat sich in den letzten Jahren rasant entwickelt und Selektivität und Sensitivität konnten wesentlich verbessert werden. Die Quantifizierung leidet jedoch noch immer unter den Einschränkungen der sog. Matrixeffekte, beispielsweise bei der Rückstandsanalyse von Pestiziden in Lebensmittelproben. Am häufigsten kommt dabei die Electrospray-Ionisierung (ESI) zum Einsatz, und oftmals wird Ionensuppression beobachtet. Dagegen treten Matrixeffekte bei der Chemischen Ionisierung bei Atmosphärendruck (APCI) seltener auf [1].
Die LC/MS-Kopplung mittels dielektrisch-behinderter Mikroplasma-Ionisierung (Dielec-tric Barrier Discharge Microplasma-Ionization, DBDI) ist eine neue Technik, die auf einem Helium Plasma-Jet basiert [2]. Damit lassen sich Substanzen mit sehr unterschiedlichen physikochemischen Eigenschaften effizient ionisieren [3]. Der Ionisierungsmechanismus der DBDI zeigt Parallelen zur APCI, und folglich wurden im Vergleich zu ESI ebenfalls geringere Matrixeffekte antizipiert.

Methodik

Zur Ionisierung wurde ein kaltes dielektrisch-behinderte Helium-Mikroplasma bei Atmosphärendruck (DBDI) [2] verwendet. Ein detaillierter Vergleich der Matrixeffekte bei der Rückstandsanalyse von 43 verschiedenen Pestiziden wurde mit ESI und APCI in den drei Lebensmittelmatrices Apfel, Orange und Tomate durchgeführt. Die komplexen Extrakte wurden nach QuEChERS-Aufarbeitung (Akronym für Quick, Easy, Cheap, Effective, Rugged and Safe) [4] erhalten.

Vorläufige Daten

Die DBDI hat sich als alternative Ionisierungstechnik in der LC/MS-Multi-komponentenbestimmung von Pestizidrückständen in komplexen Lebensmittelproben (Apfel, Orange, Tomate) erwiesen. Zudem wurde beobachtet, dass ESI vor allem durch Ionensuppression gekennzeichnet ist und sowohl APCI als auch DBDI vornehmlich durch Signalerhöhung. Ein vertieftes Verständnis dieser Effekte ist insofern bedeutsam, dass es bei Konzentrationen nahe der festgesetzten Rückstandshöchstmengen zu falsch-positiven Befunden durch Signalerhöhung bzw. zu falsch-negativen Befunden durch Ionensuppression kommen kann.

Neuartige Aspekte

Einsatz der DBDI zur Multikomponentenbestimmung von Pestizidrückständen in komplexen Lebensmittelproben und Vergleich der Matrixeffekte mit ESI und APCI.

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Instrumentelle Entwicklungen II.2

Tunable Desorption/Ionization Modes of Plasma-based Ambient Mass Spectrometry Sources

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Einleitung

The ideal analytical method is one that would quickly provide a comprehensive assessment of species (e.g., type, structure, amount, etc.) in a sample through a manner that is minimally harmful to the sample and can be performed *in situ*. While this ideal currently seems far from reality, recent efforts in the design and application of ionization sources for mass spectrometry have demonstrated many of these attributes to be achievable. In such ambient mass spectrometry experiments, the source extracts or desorbs molecules from a sample surface, softly ionizes the species resulting in one mass-spectral peak per analyte, and transfers these ions into a mass spectrometer. The separation power and sensitivity of the mass spectrometer is used to resolve individual components in a complex mixture. The identity of detected molecules is commonly determined through the use of high mass accuracy and/or tandem mass spectrometry; however, this approach often comes at the expense of time and money. Two other problems that plague this analysis method are matrix effects and a limited range of detectable analytes, which are ultimately dictated by the fundamental properties of the desorption/ionization sources. Here, different operation modes of plasma-based sources are investigated in detail.

Methodik

Two plasma-based ambient ionization sources, the low-temperature plasma (LTP) probe and the flowing atmospheric-pressure afterglow (FAPA), were characterized through optical and mass spectrometric means. . The spatial distribution of reactive species created in both the discharge and the afterglow regions of the source could be recorded with the use of a spatially selective optical detection system. These spatially resolved emission profiles yielded insight into the origin of impurities such as H_2O, N_2, and O_2 important in mass spectrometric analyses. Rotational temperatures were subsequently used to determine the major ionization mechanisms of N_2. Lastly, optical and mass spectrometric measurements were used to uncover variable desorption and ionization mechanisms that exist for these plasma-based sources.

Vorläufige Daten

The LTP is an ideal source for measuring spatially resolved emission spectra as the discharge and open-atmosphere afterglow can be monitored simultaneously. Interestingly it was found, by monitoring OH emission, that a substantial amount of water in the sample introduction region originates from the gas supply rather than diffusional penetration of atmospheric water vapor into the plasma. In contrast, it was determined that most of the N_2, N_2^+ and atomic oxygen emission was due to ambient N_2 and O_2 diffusing into the discharge. It was also found that the spatial location of the N_2^+ emission maximum correlated well with a second maximum from atomic helium emission in the open atmosphere. This spatial correlation was found to be due to a charge-transfer reaction between He_2^+ and N_2. Thus, it is believed He_2^+ acts as a carrier to transfer energy from the discharge to the afterglow region in the open atmosphere.
Mass spectrometric analyses have revealed that some species are liberated into the gas phase through mechanisms other than thermal desorption, which is often believed to be the main desorption pathway for these plasma-based sources. Currently we believe certain analytes, which have extremely low vapor pressures, can undergo a chemical-sputtering process where a reaction with excited plasma species leads to liberation of molecules from a surface.
Finally, additional operational modes of plasma-based sources were explored to provide improved and alternative utility of these ambient ionization sources. Such tuned desorption/ionization was realized for both the FAPA and LTP probe through the above experiments. These fundamental findings were then used to develop different source designs and/or operating modes, which enhance identification and quantification of analytes.

Neuartige Aspekte

Experimental evidence that demonstrates additional and tunable reagent-ion formation pathways and desorption mechanisms exist for plasma-based ambient ionization sources.

Instrumentelle Entwicklungen II.3

Atmosphärendruck-Ionisation levitierter Tröpfchen

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Einleitung

Die Ultraschalllevitation stellt ein wertvolles Werkzeug der analytischen Chemie und besonders der Mikrofluidik dar. Sie erlaubt die kontaktlose Handhabung von Tröpfchen im Mikrolitermaßstab. Zusätzlich zu den großen Vorteilen, die sich aus dem geringen Probenbedarf ergeben, können so Verunreinigungen durch Kontakt mit dem Reaktorgefäß vermieden werden. Darüber hinaus werden mögliche Adhäsions- und Nukleations-Prozesse an Gefäßwänden verhindert. Während optische Spektroskopie und Röntgenbeugung in der Ultraschalllevitation bereits etablierte Methoden darstellen, befindet sich die Kopplung an massenspektrometrische Methoden noch in ihrer Anfangsphase.

Methodik

Mittels IR-Laser-Strahlung wurden kleine Mengen der levitierten Tröpfchen verdampft. Das gebildete Aerosol konnte anschließend direkt mittels eines Atmosphärendruck-Massenspektrometers ohne zusätzlichen Ionisationsschritt untersucht werden. Die so erhaltenen Massenspektren zeigen Signale protonierter Moleküle, jedoch konnte keine Fragmentierung beobachtet werden.Die Tröpfchen wurden hierzu in einer selbstgebauten Ultraschallfalle direkt vor der Einlasskapillare eines Flugzeitmassenspektrometers positioniert. Der Verdampfungsprozess wurde zusätzlich mit Hochgeschwindigkeits-Schattenabbildung untersucht.

Vorläufige Daten

Mit Hilfe der beschriebenen Methode können aus levitierten Tröpfchen direkt Ionen erzeigt werden, wobei die Abwesenheit von Fragmenten auf eine weiche Ionisierung hindeutet. Aufgrund der geringen Einzelphotonen-Energie der IR-Strahlung ist eine elektronische Anregung oder sogar eine Photoionisation auszuschließen. Außerdem zeigen die Signale protonierter Analyten bzw. deren Cluster eine starke pH-Abhängigkeit, was auf einen statistischen Ionisationsmechanismus wie dem Lucky-Survivor-Modell[1] hindeutet.

Neuartige Aspekte

Es wurde eine direkte Ionisationsmethode für levitierte Tröpfchen entwickelt, die neben der praktischen Anwendung zum besseren Verständnis klassischer Ionisationsmethoden beiträgt.

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Instrumentelle Entwicklungen II.4

Mobile Ultra-High Resolving Mass Spectrometer for in-situ Measurements

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Einleitung

In-situ mass spectrometry with real-time analysis of samples is a rising field and offers new insights and applications. So far existing mobile MS are restricted to moderate resolving power. Multiple-reflection time-of-flight mass spectrometers (MR-TOF-MS) allow for a mass resolving power exceeding 100,000 and sub ppm accuracy[1,2,3]. Thus they allow to resolve isobars and enable to accurately determine the composition and structure of large bio-molecules. MR-TOF-MS are used in physics and have recently been installed at different accelerator facilities. They are employed as isobar separators with high ion capacity and/or mass spectrometers with ultra-high resolving power and accuracy for experiments with exotic (very short-lived) nuclei. On the basis of the MR-TOF-MS, for the SuperFRS at the FAIR facility in Darmstadt [4,5], a mobile MR-TOF-MS has been developed at Justus-Liebig-University Giessen as part of the LOEWE-Schwerpunkt AmbiProbe.

Methodik

The mobile MR-TOF-MS consists of an atmospheric pressure interface for various atmospheric ion sources, an RFQ beam preparation system (mass filter, ion cooler, ion trap), a time-of-flight analyzer and a micro-channel plate detector. Inside the analyzer the ions are reflected up to several hundred times. Thus their flight path is increased by orders of magnitude compared to convential TOF-MS and ultra-high mass resolving power is achieved in a compact system. The complete instrument is mounted into a single frame with a total volume of only 0.8 m^3. A further reduction in size and weight of the device is readily possible to fulfill the needs of specialized future applications.

Vorläufige Daten

The basis for the mobile MR-TOF-MS, the MR-TOF-MS of the FRS Ion Catcher Experiment at GSI, Germany, achieved ultra-high resolving power (600,000), transmission efficiency (>50%), mass accuracy (~0.1ppm), ion capacity (>10^6 ions/s) and repetition rates (>100Hz). But due to its size (~4m^3) and weigth (~1t) the system is not suited for in-situ measurements. Hence a smaller and robust MR-TOF-MS for field application has been built. It can be brought to the measurment sight and is operational within few minutes. Besides a power supply no supplemental infrastructure is needed. It provides a mass resolving power larger than 100,000, repetition rates of up to 1 kHz and is equipped with an atmospheric pressure inlet. Thus, high performance in-situ measurements are possible without the need of any sample preparation. Envisaged applications are in environment sciences (e. g. to monitor the water and air quality at hot spots) and in medicine (e. g. to distinguish between healthy and cancerous tissue during surgery in less than a second). A performance characterization of the system and first results with atmospheric ion sources will be reported. Special novel operation modes will be explained, such as tandem MS with ultra-high resolution precursor selection.

Neuartige Aspekte

Ultra-High Resolving Mass Spectrometer compatible with atmospheric ion sources for in-situ applications

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Instrumentelle Entwicklungen II.5

Liquid AP-UV-MALDI Enables Stable Ion Yields of Multiply Charged Peptide and Protein Ions for Sensitive Analysis by Mass Spectrometry

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Einleitung

(Nano)ESI and MALDI are the two predominantly used soft ionization techniques for the MS analysis of peptides and proteins. One key feature of ESI is that it readily generates multiply charged ions. This enables the analysis of high mass analytes on mass spectrometers with limited m/z range and improves the sensitivity in tandem MS experiments. Recently, we showed that highly charged peptide ions can also be generated by atmospheric pressure (AP-)MALDI if a glycerol matrix is used in combination with an IR-laser for IR-MALDI-MS [1]. Here, we report progress in achieving high and prolonged yields of multiply charged peptide and protein ions using liquid UV-MALDI matrices, similar to the ones previously reported [2], and an AP ion source that is equipped with a heatable ion transfer tube which can be used at variable elevated temperatures of up to 400 °C. In contrast to the IR-MALDI work and other successively reported approaches [4], an analytical sensitivity in the 10 fmol range and stable ion signals over several thousands of laser pulses applied on an analyte/matrix-drop are obtained under typical MALDI fluence conditions. These new possibilities – reported in more detail in [3] – can have substantial consequences for current high-throughput proteomics applications.

Methodik

Mass spectra were acquired on an orthogonal TOF mass spectrometer (QSTAR pulsar i, AB Sciex) using an in-house built AP UV-MALDI ion source, that was adapted from a previously reported design [5]. A capillary tube that is heated by a coil heater served for ion collection and to assist ionization. Samples were irradiated with a frequency-tripled Nd:YAG laser (λ=355nm, τ=10ns, 10Hz) using fluences in the 200–2000 J/m^2 range. Liquid matrices were based on either 2,5-dihydroxybenzoic acid (DHB) or alpha-cyano-4-hydroxycinnamic acid (CHCA) mixed with glycerol and/or triethylamine in various concentrations. Peptide and protein reference compounds served as analytes.

Vorläufige Daten

In previous papers, several benefits of liquid UV-MALDI matrices, e.g. for the highly accurate analyte quantitation, were demonstrated [3]. While predominantly singly-charged peptide ions are produced from the matrices if standard MS configurations are used, this picture changes dramatically when ionization is achieved in the new AP ion source. Under optimized conditions doubly and triply charged peptide ions form the base peaks in the mass spectra even for small mass analytes such as MK-bradykinin. Making use of these multiply charged species, an improved CID tandem MS analysis is achieved. Higher mass analytes such as insulin and myoglobin are detected with narrow charge states that for the applied experimental conditions centered around 4-5 and 8-9, respectively. Relative signal ratios between multiply and singly charged peptides show a strong dependence on the temperature of the inlet capillary and peak at about 200-250 °C for the set-up used in these experiments. Also the choice of matrix compound influences the charge state distribution. For example, a CHCA-based matrix resulted in the detection of mostly doubly charged melittin ions, while spectra from a DHB-based one are almost exclusively comprised of triply and quadruply charged ion signals. We foresee that the current sensitivity limit (50 fmol for the analysis of reference peptides) will be in the near future decreased by using further optimized ion source designs and more current mass spectrometers.

Neuartige Aspekte

High and stable yields of multiply charged peptide ions are obtained by liquid UV-AP-MALDI mass spectrometry

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Organische Massenspektrometrie & Instrumentelle Entwicklungen I.1

Towards accurate quantification of polyunsaturated glycerophospholipids by tandem mass spectrometry

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Einleitung

Fragmentation of lipid ions in mass spectrometry is induced by collision induced dissociation (CID). In bottom-up lipidomics this fragments are utilized to specify the identity and the quantity of lipid analytes. Anions of fatty acid moieties are abundant structure specific fragments of glycerophospholipids. While carboxylate anions of saturated fatty acids are stable, anions of polyunsaturated acids undergo facile fragmentation via poorly understood pathways. Here we report on systematic investigation of the yield and pathways of fragmentation of lipids with polyunsaturated fatty acid moieties and present solutions for their accurate und unbiased absolute quantification.

Methodik

For systematic evaluation of the fragmentation characteristics we synthesized a series of glycerophospholipids comprising all essential saturated and unsaturated fatty acids as well as relevant isomers. Phosphatidylcholine diacyl (PC) and ether (PC-O) species were synthesized starting from lyso PC 16:0 and lyso PC-O 16:0 and the corresponding fatty acid chloride.
Collision induced dissociation experiments of the lipid analytes were performed on a LTQ Orbitrap Velos equipped with Ion Max ESI source (both Thermo Scientific). HCD FT MS/MS(-) spectra were acquired under normalized collision energies (nCE) within 0-120% in triplicates. The intensities of relevant fragments were extracted using XCalibur software v2.2 (Thermo Scientific).

Vorläufige Daten

Fragmentation of several of formate adducts of PC and PC-O species suggested that the recovery of carboxylate anion fragments was strongly CE dependent. Specifically, the higher the number of double bounds (DB) in the acyl chain and the closer they are located to the carboxyl group, the lower the recovery of the corresponding carboxylate anions at nCE > 55%. In contrast to saturated moieties, carboxylate anions of unsaturated moieties produced specific CO2 loss and multiple products of cleavages of carbon – carbon bonds. Their intensities are difficult to account for the accurate quantification.
We envisioned two approaches to amend the quantification deficiency:
First, we could reduce the fragmentation of polyunsaturated carboxylate anions by reducing nCE values. The breakdown curves acquired for different species intersect at approx. 45% for phosphatidylcholine and -ethanolamine species. For phosphatidylinositol corresponding curves intersect at ca. 60% nCE suggesting that a separate acquisition at elevated CE is required. This approach is also not applicable to lysoglycerophospholipids and triacylglycerols.
Alternatively, we built a mathematical model accounting for response differences depending on the total number of DB and the their distance to the carboxyl group, which allowed the linearization of response differences with the abundance correlation factor > 0.95.

Neuartige Aspekte

Accurate quantification of polyunsaturated glycerophospholipids by tandem mass spectrometry

Organische Massenspektrometrie & Instrumentelle Entwicklungen I.2

Catching biomolecular ions with liquid helium nanodroplets

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Einleitung

To understand the function of biological molecules, it is necessary to study their structure and dynamics. For this, spectroscopic experiments on well defined, cold, isolated biomolecules have shown to be useful. Helium droplets are an ideal matrix for the spectroscopic study of molecules since the droplets are isothermal at 0.38 K, superfluid, only weakly interacting with the embedded species and transparent over a wide spectral range from the far IR to the deep UV [1]. Here, we report on the use of liquid helium droplets as nano-cryostats for the investigation of mass/charge selected biomolecular ions.

Methodik

We have built up an experimental setup [2] that allows the doping of mass/charge selected ions in helium droplets. In the experiment, the biological molecules are brought into the gas phase via electrospray ionization. They are then mass-to-charge selected using a quadrupole mass spectrometer, and accumulated and stored in a linear ion trap. The trapped ions are picked up by helium droplets generated with a pulsed nozzle, and since the kinetic energy of the droplets is larger than the longitudinal trapping potential, the ion doped helium droplets can escape the trap. Further downstream, the charged droplets are investigated and detected.

Vorläufige Daten

In our experiment, we have successfully doped helium droplets with mass-to-charge selected ions from single amino acids to proteins as large as Cytochrome C (approx. 12000 amu). We have characterized the size of the doped droplets, which range from tens of thousands to several million [3] helium atoms depending on the source temperature and on the size of the captured ion. The ion embedded in a helium droplet can be ejected upon photoexcitation. Using a UV laser we have investigated the ejection efficiency of the ion with respect to the size of the helium droplet, and it is observed that the ejection efficiency increases when the droplets become smaller. Monitoring the ejected ion as a function of the excitation wavelength can be used to obtain the optical spectrum of the investigated species. We have used a UV/VIS laser to obtain the electronic spectra of molecules such as protonated tryptophan and protonated tryptamine as well as the porphyrine complex hemin. Currently, we are investigating larger peptides and an update of those systems will be discussed as well. In the near future, the experimental setup will be used to measure IR spectra of ions embedded in liquid helium droplets using the FHI free electron laser.

Neuartige Aspekte

Measurement of electronic spectra of biomolecular ions embedded in helium droplets.

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Quantifizierung femtomolarer Konzentrationen des CYP3A Substrats Midazolam und seines Hauptmetaboliten 1'-Hydroxymidazolam in humanem Plasma mittels UPLC gekoppelt zur Tandem-Massenspektrometrie

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Einleitung

Das Benzodiazepin Midazolam ist eine Testsubstanz zur Bestimmung der Aktivität des Cytochroms P450 3A (CYP3A) im Menschen (Phänotypisierung). CYP3A ist das dominante Enzym beim Metabolismus von Arzneiwirkstoffen und spielt aufgrund von Enzym-Inhibition und –Induktion durch andere Arzneistoffe eine bedeutende Rolle beim Entstehen von Arzneimittelwechselwirkungen. Zur Bestimmung der CYP3A-Aktivität sind sedative Plasmakonzentrationen weder notwendig noch erwünscht und die Gabe sehr niedriger Midazolam-Dosen (Microdosing) vorteilhaft. Zu diesem Zweck wurde eine hochempfindliche Methodik zur Quantifizierung von Midazolam und 1'-Hydroxymidazolam in Humanplasma im femtomolaren Bereich basierend auf Festphasenextraktion zur Probenvorbereitung und Ultra-Performance Liquid Chromatography (UPLC) gekoppelt zur Tandem-Massenspektrometrie (UPLC/MS/MS) entwickelt und gemäß FDA-Guideline validiert [1,2]. Diese Methode ermöglicht es erstmals nach Gabe von mikro- bis nanogramm Midazolam-Dosen noch vollständige Pharmakokinetik-Profile aufzuzeichnen [1,3].

Methodik

Midazolam und 1'Hydroxymidazolam wurde aus humanen Plasmaproben (0,25 mL) mittels 96-well-Festphasenextraktion (µ-Elution) basierend auf Kationenaustauscher (MCX-Material) extrahiert. Anschließend wurden die Extrakte (150µL) innerhalb von 2 min auf einer Waters BEH UPLC-Säule (BEH C18 1,7 µm) mit einem schnellen Gradient (Ammoniumformiat-Puffer gegen Acetonitril) chromatographiert. Zur Quantifizierung der Analyten wurden isotopenmarkierte interne Standards verwendet $^{2}H_5$-Midazolam, $^{13}C_3$-1'-Hydroxymidazolam). Der UPLC-Eluent wurde direkt in die Elektrospray-Quelle des Massenspektrometers geleitet und die Analyte sowie die internen Standards ionisiert. Die positiven Parent Ions wurden entsprechend ihrer intensivsten Massenübergänge (Parent Ion → Produkt Ion) im Multiple Reaction Monitoring Mode detektiert (Quantifier). Zusätzliche Massenübergänge wurden für die beiden Analyte zur Bestätigungsanalyse detektiert (Qualifier). Jeder einzelne Analysenschritt wurde hinsichtlich höchster Empfindlichkeit optimiert [1].

Vorläufige Daten

Die Wiederfindungsraten (Recovery) für beide Analyten nach Festphasenextraktion lagen zwischen 75 und 92%. Lower limits of quantification waren 50 fg/mL (Midazolam) und 250 fg/mL (1'-Hydroxymidazolam) im Kalibrierbereich von 0,05 bis 250 pg/mL (Midazolam) und 0,25 bis 125 pg/mL (1'-Hydroxymidazolam) mit Korrelationskoeffizienten >0,99. Within-Batch und Batch-to-Batch Präzision für beide Analyten war <14% bzw. <12%. Mit einer Bestimmungsgrenze von 50 fg/mL ist die beschriebene Methodik bei weitem die empfindlichste publizierte Quantifizierungsmethodik für Midazolam. Die Methodik wurde erfolgreich in einer klinischen Nanodose-Studie mit 12 gesunden Probanden eingesetzt [3]. Die dabei verabreichten Dosen pro Proband variierten von 100 ng bis 3 mg in acht Dosisstufen, wobei reguläre Dosen zur Sedierung von Patienten bei 3 mg liegen. In allen Dosisstufen konnten pharmakokinetische Profile von mindestens 4 Stunden (100 ng Dosis) bis 24 Stunden aufgenommen werden und C_{max} und AUC (Area und the Curve) stiegen linear an. Insgesamt betrachtet waren die Midazolam Kinetiken linear über einen Konzentrationsbereich, der den Faktor 30.000 umfasst.
Zusammenfassend kann bestätigt werden, dass die moderne Analysentechnik basierend auf UPLC/MS/MS, und beispielhaft an Midazolam gezeigt, neue Forschungsbereiche der klinischen Pharmakologie eröffnet. Ultraempfindliche Messmethoden erlauben es zukünftig biologisch hoch aktive Wirkstoffe (z.B. Chemotherapeutika) im Rahmen von Microdosing oder sogar Nanodosing in niedrigsten, nicht toxischen Dosen an gesunden, freiwilligen Probanden zu testen und pharmakokinetische Daten zu erheben.

Neuartige Aspekte

Hochempfindliche Methodik basierend auf modernster UPLC/MS/MS-Technik zur femtomolaren Midazolam-Quantifizierung in Humanplasma. Erlaubt die Aufnahme von Pharmakokinetik-Profilen nach Gabe von nanogramm-Midazolam-Dosen.

Referenzen

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Organische Massenspektrometrie & Instrumentelle Entwicklungen I.4

Electrochemically Initiated Reactions Upfront MS - EC/MS an Unknown Panacea?

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Einleitung

Electrochemistry (EC) in combination with mass spectrometry creates a powerful platform to simulate various oxidation and reduction processes in life sciences. Electrochemistry is a complementary technique to traditional *in vivo* or *in vitro* metabolism studies, and delivers the oxidative metabolic fingerprint of a (drug) molecule in a very short time [1, 2]. Furthermore, protein/peptide cleavage, disulfide bonds reduction and covalent drug-protein binding can be performed in the electrochemical cell.
Currently, the application of electrochemistry combined with mass spectrometry has been extended towards new areas such as protein chemistry [3], forensic toxicology, metabolite synthesis, environmental degradation of xenobiotics (pollutants) [4], etc.

Methodik

An analytical and preparative electrochemical cell (Antec, The Netherlands) equipped with a glassy carbon or conductive diamond (MD) working electrodes were used for the oxidation of drugs. The cell potential was ramped to 2000 mV (glassy carbon) or to 3000 mV (MD). The outlet of the cell was connected to the ESI-MS. Depending on the type of compounds typically 2 - 20 µM solutions were pumped through the electrochemical cell at a flow rate of 10 -50 µL/min. For the formation of GSH adducts, 50 µM GSH solution was added after the cell. For disulfide bond reduction experiments in proteins/peptides, newly designed Titanium based electrodes were used.

Vorläufige Daten

In this presentation we will review the potential of EC/MS to simulate various reactions that take place in nature. Oxidative drug metabolism is a major field where electrochemistry coupled on-line or off-line mass spectrometry has been successfully used as a biomimetic tool. The possibility of electrochemical synthesis of (reactive) metabolites in a short period of time is another important application area that is gaining increasing attention.
As example, the easy and fast electrochemical conversion of Amodiaquine into its major phase I metabolites will be presented, using analytical and preparative EC cells. In a second step Glutathione (GSH) is added to the electrochemically generated metabolites to form the appropriate GSH-metabolite adducts, mimicking phase II reactions. All known adducts were successfully formed and identified with MS. Furthermore, we present a highly efficient method for metabolite synthesis by applying square-wave potential pulses to the working electrode.
Furthermore, we will show the application of on-line EC/MS in protein chemistry. Disulfide bonds are one of the most important post-translational modifications in proteins. Therefore, a novel method for the reduction of the disulfide bonds in biologically active peptides and proteins based on electrochemistry (EC) will be presented. Our method is purely instrumental (push button) and does not use any harsh reducing agents such as DTT.
Hyphenation of electrochemistry and mass spectrometry delivers selective and sensitive detection and allows for fast and unambiguous identification of all reaction products generated in the electrochemical cell. In this lecture latest data will be shown illustrating the tremendous power of Electrochemistry/MS as a real panacea to mimic nature's REDOX reactions.

Neuartige Aspekte

Electrochemistry as universal reaction technique upfront MS.

Referenzen

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Organische Massenspektrometrie & Instrumentelle Entwicklungen I.5

Mass spectrometry-based metabolite profiling of crude fungal extracts

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Einleitung

Compounds important for the chemosystematic organization of Boletales such as *Suillus* species are terpenyl quinones, cyclopentenones, pulvinic acid derivates, grevillins, prenylated phenols and prenylated benzoquinones. The distribution of grevillins and pulvinic acids can be used to distinguish between three different types of pigmentation within the genus *Suillus*. [1]
Several metabolites were investigated by liquid chromatography electrospray tandem mass spectrometry [2]. Furthermore, the metabolite profiling of *Suillus* species was carried out using rapid ultra-performance liquid chromatography-electrospray ionization mass spectrometry (UPLC/ESI-MS) including tandem mass spectrometric methods and direct injection electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR-MS).

Methodik

The ESI ion trap mass spectra were recorded on a LCQ Deca XP MAX (Thermo) coupled with a Waters ACQUITY UPLC system (BEH C8 1.7 µm, 1.0x100 mm, gradient system: water/acetonitrile each containing 0.2% CH_3COOH). The high-resolution ESI mass spectra were obtained from a Bruker Apex III FTICR mass spectrometer (Bruker Daltonics) equipped with an Infinity™ cell, a 7.0-T superconducting magnet (Bruker), a radio frequency-only hexapole ion guide and an external APOLLO electrospray ion source (Agilent). The reference compounds analyzed were kindly provided by Prof. Dr. Dr. h.c. Wolfgang Steglich and co-workers (Ludwig-Maximilians-University, Munich).
Lyophilized fruit bodies or cultures of fungi were extracted thrice with ethylacetate under ultrasonication. Voucher specimens are deposited at the IPB Halle, Germany.

Vorläufige Daten

Detailed studies of the fragmentation pattern of different prenylated and non-prenylated fungal secondary metabolites were carried out by using an ion trap system. The mass spectral behavior of the prenylated benzoquinones and phenols under negative ion electrospray conditions allowed a characterization and identification of these compounds. The loss of the whole side chain as well as characteristic neutral losses of the isoprene units provides useful information for their structural features. Therefore, these findings represent an appropriate basis for metabolite profiling investigations of such compounds by combining UPLC/MS(MS).
The metabolite profiling of *Suillus* species was carried out using UPLC/ESI-MS and ESI-FTICR-MS. The obtained mass spectral data were applied to a classification of several fungal species of *Suillus* by a principal component analysis (PCA).

Neuartige Aspekte

Metabolite profiling and classification of several fungal species of *Suillus* using different mass spectrometric methods

Referenzen

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Imaging I.1

Correlating Raman and MALDI imaging

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Einleitung

Matrix-assisted laser desorption/ionization (MALDI) imaging is on the way to become a mainstream analytical technique in life science related areas. [1] It is commonly used to monitor endogenous or administered compounds, such as lipids, metabolites, peptides, proteins, and xenobiotics, within biological tissues. The aim of the present contribution is to present for the first time the combination of MALDI and Raman imaging to link the molecular content of the tissue obtained by MALDI imaging with the non-invasiveness of Raman spectroscopy. In the present example we analyzed lipids by MALDI imaging and obtained information of proteins by interpreting the corresponding Raman spectra.

Methodik

A 10 µm mouse brain section was cut on a cryostat and transferred to a precooled conductive ITO-coated glass slide. Raman spectra were acquired using a Confocal Raman Microscope Model CRM alpha 300R (WITec, Ulm, Germany) with a HeNe laser at 633 nm. The sample was scanned through the laser focus in a raster pattern point by point stepping modus. Spectra were collected at a 25 µm grid. For the following MALDI imaging analysis of lipids the common matrix alpha-CHCA was applied onto the tissue section using the ImagePrep station (Bruker Daltonics) following a standard protocol. The MALDI imaging analysis was performed on an Ultraflex III MALDI-TOF/TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) using a resolution of 75 µm.

Vorläufige Daten

The correlation of Raman and MALDI imaging is shown in the current contribution exemplary on a mouse brain cryosection. By performing a reliable and robust registration methodology of the Raman and MALDI scans every point on the MALDI grid can be described by a MALDI spectrum. In the present example the *m/z* peak at 799 is highly concentrated in the grey matter, while the peak at *m/z* 703 is localized in the white matter. Both peaks can be attributed to phospholipids. [2] From the corresponding Raman spectra can be seen that a higher CH-stretching peak is obtained where the MALDI peak at *m/z* 799 is high and the one at *m/z* 703 is low. One step further, by applying the Raman data to a multivariate calibration model a certain MALDI signal can be translated to a complex Raman fingerprint. For example the white matter presented by a strong MALDI signal of *m/z* 703 shows high Raman signals at 1170, 1255 and 1662 cm^{-1}, which can be assigned to protein vibrations. [3-4] Therefore, it can be concluded that this region is reach in the protein content. Hence, by combining both imaging technologies further complementary information can be obtained, as presented. In the future this methodology should enable the search for MALDI peaks under *in-vivo* conditions.

Neuartige Aspekte

Combining Raman and MALDI imaging

Referenzen

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Imaging I.2

High resolution AP-SMALDI MSI of plant and insect metabolites

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Einleitung

Mass spectrometry imaging (MSI) has gained significant attention due to its capability of studying topographic distributions of molecules in complex biological systems. The technique has developed to become a promising tool for clinical investigations and biomolecular research [1-2]. Herein, we present mass spectrometry images generated from plant and insect tissues that reveal spatial distributions of metabolites with high resolution in mass and space (HR^2). MSI is able to visualize the distribution of metabolites with high spatial resolution aiding to explain mechanisms of complex biological processes.

Methodik

An Atmospheric-Pressure Scanning Microprobe Matrix-Assisted Laser Desorption/Ionization (SMALDI) ion source (TransMIT GmbH, Giessen, Germany) for imaging was used for ion generation. Sections from plants and insects were obtained using a cryotome after optimizing various preparation techniques, such as carboxymethyl cellulose (CMC) and tragacanth gum embedding for creating uniform sections. Common shaving blades were used for root samples, alternatively. A dedicated home-built pneumatic sprayer was employed for matrix deposition. The SMALDI source was operated with a nitrogen laser at 337 nm wavelength with 60 Hz repetition rate. The source was coupled to an orbital trapping mass analyzer (Exactive or Q Exactive mass spectrometer, Thermo Fisher Scientific GmbH, Bremen) set to a mass resolving power of 50,000 to 140,000 at m/z = 200.

Vorläufige Daten

The home-built software package 'Mirion' was used to generate mass spectral images from the raw files obtained from the mass spectrometer. All measurements including mass spectra from 5 µm pixels were acquired with a mass accuracy of 2 ppm (root mean square). Molecular images were generated with a bin width of $\Delta m/z = 0.01$. Images from seeds (rape and wheat), roots (rice and wheat) and insects *Paederus* and *Harmonia*) were generated at 5 to 25 µm spatial resolution. For rapeseed at 10 µm pixel size, it was possible to image an ion at m/z = 496.24 (cyclic spermidine conjugate) that was only found in the germinating region of the seed. For wheat seeds, several different metabolites (amino acids, carbohydrates, phospholipids, polyphenol glycosides, phosphocholine, phosphoglycerol, triglycerides etc.) were imaged which were located in different regions of the seed. Whole insect imaging of *Paederus riparius* was performed at 10 µm spatial resolution and the defensive compound pederin was observed with high mass accuracy as sodium adduct at m/z = 526.29, as well as its metabolite pseudopederin. Similarly for *Harmonia axyridis*, MS images were generated using the signal of the resistance-related substance harmonine (m/z = 283.31). In addition the signals of its metabolites were detected in different regions of the insect.

Neuartige Aspekte

High resolution in mass and space method for understanding metabolites distributions in plants and insects.

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Imaging I.3

A strategy for the identification of biomarkers in cancer tissues using MALDI mass spectrometry imaging and tissue microarrays

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Einleitung

In cancer research, the discovery of new diagnostic or prognostic markers for the detection of malignant cells in biopsies and tissue sections is an important and challenging aim. Mass spectrometry imaging (MSI) yields information about the spatial distribution of a large number of biomolecules in a single experiment. In combination with tissue microarrays (TMAs), which allow for the parallel and fast analysis of hundreds of patient samples under identical analytical conditions [1], MALDI MSI is a powerful tool for biomarker discovery [2]. We demonstrate a combined approach using MALDI mass spectrometry imaging applied to a TMA, high-resolution FT-ICR MS and LC tandem MS to discover and identify potential biomarkers in cancer tissues.

Methodik

MALDI mass spectrometry imaging was applied to sections of a formalin-fixed, paraffin-embedded (FFPE) TMA containing needle cores of prostate tumors of 700 patients for which clinical follow-up data were available. TMA sections were tryptically digested on-tissue by spraying a trypsin solution directly onto the slide. MALDI-TOF imaging results were statistically correlated to clinico-pathological features of the tumors.
An exact mass of each m/z signal of interest was obtained by MALDI-FT-ICR mass spectrometry of the tissue microarray. To identify the peptides underlying the m/z signals, a tissue extract was obtained from the tumor area of a tissue block which was represented in the TMA. Proteins were tryptically digested and analyzed by LC-MALDI-TOFTOF and LC-ESI-QTOF tandem mass spectrometry.

Vorläufige Daten

Statistical analysis of the MALDI imaging data of the tissue microarray revealed associations of four signals with clinico-pathological features that indicate a favorable tumor type, i.e. early pT stage, low Gleason grade or low Ki67 labeling index (Ki67LI). One signal was related to a high Ki67LI. A total of seven signals was related to the ERG status of the prostate tumors. The presence of another signal indicated prolonged recurrence-free survival of the patients.
For all signals of interest, an exact mass was obtained by high-resolution FT-ICR mass spectrometry of the same TMA section. Proteins were extracted from tissues represented in the TMA and various sample preparation steps were performed to obtain high-quality fragment spectra in the MS/MS experiments. Several peptides underlying the m/z signals found in the imaging experiment were identified either by MALDI-TOFTOF or ESI-QTOF tandem mass spectrometry. Comparing the precursor masses in the MS/MS experiments with the exact mass obtained by FT-ICR MS, the identified peptides could be assigned to the specific m/z signals with high confidence.
The combination of these methods is a powerful strategy for not only discovering signals in tissues which correlate to clinical features, but also reveal the identity of the underlying peptides for further examination and validation.

Neuartige Aspekte

A high-throughput strategy for biomarker discovery involving MALDI imaging of tissue microarrays, high-resolution FT-ICR mass spectrometry and LC-MS/MS.

Referenzen

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Imaging I.4

SCiLS Lab: software for analysis and interpretation in 2D and 3D MALDI-imaging

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Einleitung

MALDI-imaging is an emerging spatially-resolved mass spectrometric technique which can obtain spatial distribution of hundreds of molecules in a thin tissue section directly from the sample surface. Presently, data mining of a MALDI-imaging dataset, which consists of thousands of individual mass-to-charge (m/z) values, is mostly done manually and this represents a serious bottleneck in the data analysis workflow. We present our commercially available software SCiLS Lab for visualization and analysis of 2D and 3D MALDI-imaging data. For construction of a 3D MALDI-imaging dataset from consecutive 2D MALDI-imaging datasets of individual serial sections, a semiautomatic registration algorithm is available which optimally aligns the 2D datasets based on the optical images of the serial sections. Furthermore, in the software several computational pipelines for mining, analysis, and interpretation of 2D and 3D MALDI-imaging datasets are available. The computational methods are grouped according to the tasks they perform: firstly, pre-processing of spectra, then unsupervised data mining methods for preliminary data examination, then supervised classification for biomarker discovery. We apply methods from all three groups to a 2D and a 3D dataset.

Methodik

For unsupervised data mining an algorithm for spatial segmentation is presented. It gives a first overview of the dataset [1]. In order to reduce significant spectrum-to-spectrum variation, we propose to use edge-preserving image denoising prior to segmentation [2]. For supervised data analysis the Receiver Operating Characteristics (ROC) analysis is introduced to automatically discover discriminating peaks of two different groups [3]. For creating a 3D MALDI-imaging dataset the software takes several 2D datasets as an input, each corresponding to a consecutive slice. Then, it creates a 3D dataset with a spectrum assigned to each point (x,y,z). For mining large 3D data, we propose using the spatial segmentation approach with the efficient clustering method called bisecting k-means [4].

Vorläufige Daten

We demonstrate the algorithmic range of the software SCiLS Lab on a 3D MALDI-imaging dataset of the central part of a mouse kidney. Using the PAXgene fixation (which is compatible with magnetic resonance imaging), and paraffin embedding, in total we analyzed 122 serial sections of 3.5 µm thickness on an autoflex speed LRF MALDI-TOF mass spectrometer (Bruker Daltonik). The lateral resolution was set to 50 µm and spectra were acquired in the mass range of 2-20 kDa. The complete dataset including all sections was comprised of 2 million spectra, and was of size of 200 GB.A typical MALDI-imaging study results in a set of ions of interest, which are visualized as m/z-images corresponding to their m/z-values. Based on the mouse kidney dataset we show how spatial segmentation as well as ROC analysis can be used to automatically find such ions of interest which discriminate different tissue types or tissue states. At first, we apply methods to one of the 122 serial sections by doing a two-dimensional data analysis. Secondly, we build the 3D MALDI-imaging dataset by assembling the 122 consecutive slices. We show how the computational methods can be applied to the three-dimensional dataset. We discuss visualization of found m/z-images. For facilitated interpretation of 3D distribution of ions, we evaluate m/z isosurfaces providing simplified visualization.

Neuartige Aspekte

SCiLS Lab is the software for visualization and analysis of 2D and 3D MALDI-imaging data released in March 2013.

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Imaging I.5

Element-Bioimaging und Speziationsanalytik: Komplementäre Methoden für pharmazeutische Fragestellungen

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Einleitung

Die Speziationsanalytik beschäftigt sich mit der Bestimmung von Metallen und anderen Heteroelementen in ihren verschiedenen Oxidationsstufen und Bindungsformen. Hintergrund ist die Tatsache, dass die Effekte und Eigenschaften einer Metallverbindung nicht durch das Metall selbst, sondern durch seine entsprechenden Spezies bestimmt werden. Aus diesem Grund ist eine Gesamt-Elementanalytik des entsprechenden Metalls nicht ausreichend, um Konzentrations-Wirkungs-Beziehungen herstellen zu können.

Methodik

Im Bereich der Pharmazie werden überraschend viele metallhaltige Verbindungen für verschiedene Anwendungen eingesetzt. Hierzu zählen Platinkomplexe als Zytostatika, Gadoliniumkomplexe als Kontrastmittel für die Kernspintomographie und Quecksilber- sowie Silberspezies als Bakterizide. Weiterhin werden viele Metalle und Legierungen in Form von Biomaterialien, beispielsweise als Implantate, eingesetzt. Um die Aufnahme-mechanismen, Transportwege und den Metabolismus von Metallspezies im menschlichen und tierischen Organismus vollständiger zu verstehen, werden Methoden auf Basis der HPLC mit komplementär eingesetzter Elektrospray-Massenspektrometrie (ESI-MS) und induktiv gekoppelter Plasma-Massenspektrometrie (ICP-MS) entwickelt. Hierbei dient die ESI-MS zur Identifizierung, die ICP-MS zur Quantifizierung der Spezies, wobei insbesondere die Möglichkeit der ICP-MS zur substanz-unabhängigen Quantifizierung genutzt wird.

Vorläufige Daten

Komplementäre ortsaufgelöste Daten über die Konzentration der Metalle in biologischen Proben, beispielsweise in Gewebe-Dünnschnitten, werden unter Verwendung der Laserablations-ICP-MS erhalten. Hier stellt die Entwicklung von Methoden zur Quantifizierung eine besondere Herausforderung dar. In diesem Beitrag werden daher die zugrundeliegenden pharmazeutischen und medizinischen Fragestellungen vorgestellt und es werden analytisch-chemische Problemlösungen auf Basis von Kopplungstechniken zur Identifizierung und Quantifizierung der Metallspezies anhand experimenteller Daten diskutiert. Die teils massiven Nebenwirkungen von Cisplatin (Nephrotoxizität, Ototoxizität, Reproduktionstoxizität) sind Gegenstand zahlreicher Untersuchungen. In dieser Arbeit wurden zum einen die entstehenden Platinspezies in Zellkulturen identifiziert, zum anderen wurde Platin ortsaufgelöst in Proben aus Tierversuchen bestimmt. Hierzu wurden die Gewebe in Polymere eingebettet. Nach Erstellung der Dünnschnitte wurde durch Einsatz von Elementstandards aus demselben Polymermaterial extern kalibriert. Es wurde gezeigt, dass durch die Einbettung das weitgehend proteingebundene Platin nicht aus der Probe entfernt wird. Auf diese Weise konnte die Platinkonzentration in Nieren, Hoden und Cochlea von Mäusen quantifiziert werden. Es konnte gezeigt werden, dass die Platinkonzentration erwartungsgemäß mit steigender Wartezeit nach der Applikation des Pharmazeutikums sinkt, dass aber auch nach mehreren Tagen noch erhebliche Konzentrationen vorhanden waren. Erste Daten an Maushoden deuten an, dass offenbar die Blut-Testis-Schranke bei den Versuchstieren nicht durch Cisplatin durchbrochen wird.

Neuartige Aspekte

Die Kombination aus Element-Bioimaging und Speziationsanalytik erlaubt die Gewinnung wertvoller Daten über das Verhalten von Metallopharmazeutka im Organismus

Grundlagen der Massenspektrometrie I.1

Insights to colloidal zeolite synthesis by mass spectrometry - ionic clusters and silicate fragments

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Einleitung

Zeolites are microporous crystalline material largely based on silicates. They are commonly made up of corner sharing TO_4-tetrahedra and possess regular channels and cavities, facilitating important applications such as catalysis, adsorption and chemical separation. The final physical and chemical properties of zeolites are dependent on their framework and the types and distribution of heteroelements. These factors are highly influenced by the formation process and scientific interests are mainly focused on understanding this process.
ESI-MS was developed for the monitoring of nucleation process in clear solution zeolite syntheses. [1] As syntheses proceed, structural species corresponding to framework of final materials were observed, providing interesting information on the formation process.
Instead of clear solution synthesis, more commonly, colloidal based synthesis were employed in most zeolite preparation. Mechanistic studies were performed by different analytical techniques, either looking into the direct chemical environment of atoms in the structure or on structures and sizes of the particles involved. Detailed evolution of species in silicate-1 colloidal synthesis was analyzed by a combination of ^{29}Si NMR, dynamic light scattering and small-angle X-ray scattering. [2] Complementing these techniques for the first time, ESI-MS was used to provide further insights into silicate-1 colloidal synthesis.

Methodik

The synthetic mixture was prepared by drop wise addition of tetraethyl orthosilicate (TEOS) into an aqueous solution containing tetrapropylammonium hydroxide (TPAOH) with the molar composition TEOS:TPAOH:H_2O of 25:9:400. The mixture was allowed to stir for 24 hours followed by heating in a polypropylene bottle at 95˚C. Samples were taken over time, filtered through 450 nm syringe filter and introduced into the mass spectrometer by direct infusion. All mass spectrometric analyses were carried out on Waters Micromass ZMD 2000 equipped with an electrospray ion source.

Vorläufige Daten

Comparing spectra of different samples taken over time, a full range of silicate species developed rapidly and remains relatively constant with time. This is in agreement with analysis made by ^{29}Si NMR. [2] However, by manipulating the cone voltages (CV) of the instrument, different degree of fragmentation was induced, providing further insights into molecular species present in the synthesis mixture.
It was observed that the increase in CV resulted in the formation of species obtained by gas phase removal of water molecules. Species with larger mass to charge (m/z) value also became more prominent at higher CV, suggesting a certain degree of internal fragmentation occurring in the sample. Clusters of silicate species with tetrapropylammonium cations were also detected at certain CV.
As a comparison to other analytical techniques, ESI-MS provides a rapid overview of the types of silicate species present in the solution and small changes can be easily detected. By altering measurement parameters, more details of the larger species are obtainable.
Work is in progress to understand the emergence of these species and the effect of instrument parameters on silicate speciation studies.

Neuartige Aspekte

This is the first attempt to investigate colloidal-based zeolite synthesis using ESI-MS in comparison with other analytical techniques

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Grundlagen der Massenspektrometrie I.1

Laser desorption/ionization mediated by bionanostructures from microalgae

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Einleitung

Since early days of Laser Desorption/Ionization Mass Spectrometry (LDI-MS) organic matrices are the state-of-the-art ionization mediators. Interfering matrix-related signals complicate the use of this powerful technique for low molecular compounds, especially in the mass to charge range up to 600. Matrix-free approaches based on synthetic nanostructured surfaces tried to overcome this problem. Desorption/ionization on silicon (DIOS) or nanostructure initiator mass spectrometry (NIMS) shows powerful results regarding sensitivity and resolution.

Methodik

Here we introduce nanostructured cell wall preparations as alternative to elaborate synthetic surfaces. These easily accessible biological materials were prepared from micrialgal cultures using a robust clean-up procedure. This includes the oxidation with sodium hypochlorite and several washing steps with a mixture of acetonitrile and water. Cell wall preparations were pipetted on stainless-steel targets and added molecules afterwards ionized in common MALDI sources. We compared our results with established MALDI matrices in positive and negative ionization mode.

Vorläufige Daten

It was possible to successfully ionize analyte molecules of different compound classes. The ionization of PEG600, D-sphingosine and raffinose was mediated by nanostructured cell wall preparations of the two different microalgae species *Thalassiosira pseudonana* diatom) and *Prorocentrum minimum* (dinoflagellate) despite the fact that these two species have a completely different chemically and morphologic cell wall architecture. Furthermore, without any changes in the sample preparation protocol, we could detect stearic acid in the negative ionization mode.
Commercial available celite is a suitable alternative to microalgae shell preparations. This powder contains fossilzed diatom shells and therefore delivers functional bionanostructures. As a proof of principle experiment, characteristic ingredients of fresh coffee were ionized with the help of nanostructures from celite powder. Among others, caffeine was detected in the positive and typical fatty acids in the negative mode.
The achieved detection limits were comparable to those in established MALDI experiments.

Neuartige Aspekte

Bionanostructure-enhanced-ionization is a suitable alternative to known matrix-free LDI-MS approaches with a broad range of ionizable compound classes.

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Grundlagen der Massenspektrometrie I.1

Protein Structure in the Gas Phase - the Influence of Side-Chain Microsolvation

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Einleitung

Today, the gentle electrospray ionization (ESI) technique is routinely applied to characterize peptides and proteins via mass spectrometry (MS). However, how much of a protein's structures is retained after ionization and transfer into the gas phase is still not fully understood. In this context, it is widely accepted that the repulsion between equal charges is one of the major determinants for the protein's structure: low charge states typically adopt compact, native-like conformations, while high charge states tend to unfold into more extended structures. Intermediate charge states can show a multitude of coexisting conformations. [1] Here we show that not only the direct effect of Coulomb repulsions but also the much more subtle coordination of charged side chains onto the protein backbone can severely influence the conformation of proteins in the absence of solvent. [2]

Methodik

In order to study the impact of side chain-backbone coordination on the structure of a gas-phase protein, we noncovalently attached different amounts of crown ether (18-crown-6) to the charged lysine side chains of the protein cytochrome C. The resulting complexes were analyzed via ion mobility spectrometry (IMS) using a commercially available traveling wave IMS apparatus (Waters, Synapt G2-S). In addition, absolute collision cross section (CCS) were determined using an in-house constructed drift tube IMS apparatus that follows the design described in [3].

Vorläufige Daten

Independent of the charge state, complexes with up to five crown ethers attached to the lysine side chains of cytochrome C can be observed at gentle ionization conditions. Ions of low as well as high charge states essentially retain their shape upon crown ether complexation. Intermediate charges states, however, undergo an unusually distinct compaction upon binding to the crown ether, which has not been observed previously. This rather counter-intuitive effect can be explained to occur predominantly as a result of two competing types of microsolvation. In the absence of crown ether, uncapped protonated side chains rapidly collapse onto the protein backbone where they coordinate to carbonyl groups that are otherwise involved in vital H-bonds. Once the side chain is solvated with crown ether, however, the structure-forming H-bonds are less affected and a more compact, native-like conformation is retained. In a more general context this implies that crown ether can solvate gas-phase proteins in a similar way to water molecules is the condensed phase.

Neuartige Aspekte

Crown ether can solvate charged side chains of gas-phase protein ions similarly to water in the condensed phase.

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Grundlagen der Massenspektrometrie I.1

Improvements combining IDMS and MC-ICP-MS for redefining Avogadro's number

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Einleitung

The isotopic composition of silicon in an ultra pure Si crystal enriched in ^{28}Si higher than 99.995 % has been determined using a combination of a modified isotope dilution mass spectrometry (IDMS) technique and a multicollector-ICP-mass spectrometer (MC-ICP-MS) in three different alkaline matrices. Depending on the matrix concentration and –type, hidden molecular interferences have been detected and removed. The relative measurement uncertainty of the molar mass M of silicon was $u_{rel}(M) = 8 \cdot 10^{-9}$ [1]. This is a precondition of the redetermination of the Avogadro constant N_A with a relative uncertainty in the lower 10^{-8} range aiming at a possible new definition of the SI unit kilogram [2,3].

Methodik

A *Neptune*™ MC-ICP-MS was used to determine the molar mass of the silicon crystal material by isotope ratio measurements. Using modified IDMS by measuring mainly isotope ratios ^{30}Si/^{29}Si yields smaller uncertainties than the usual determination of isotope ratios related to the most abundant ^{28}Si. Si crystals were dissolved in a single chemical step using aqueous NaOH with 100 % recovery. The detection of broad interferences due to sodium-induced scattering in the context of increasing the MS transmission finally required the use of another strong sodium-free alkaline solvent. Mass bias effects were corrected for by calibration factors K, which were determined experimentally [4].

Vorläufige Daten

By increasing the MS transmission, a hidden interference on the $^{30}Si^+$ signal (using the C($^{29}Si^+$) and H3 ($^{30}Si^+$) Faraday cups of the Neptune) was uncovered when using Si solutions based on aqueous NaOH (w(NaOH) > 0.001 g/g). They were visible occurred in aqueous NaOH blank solutions (e. g. w(NaOH) = 0.25 g/g) of unusually high concentrations [5]. The isotope ratio ^{29}Si/^{30}Si can thus be influenced by up to 20 %. When working with KOH solutions, no interferences appeared. This interference was detected on all cups except the center cup which can be explained by the fact that large concentrations of sodium produce strong ion scattering in the location of the MS detectors. Due to a geometrical shielding, no interference could be detected in the central Faraday cup. It was concluded that for improved molar mass determination of Si sodium-free solutions should be used.
Considering these findings and improvements, the molar mass of the silicon crystal material enriched in ^{28}Si "Si28") is M("Si28") = 27.97697027 g/mol with a relative associated combined uncertainty of $u_{rel}(M$("Si28")) = $8 \cdot 10^{-9}$. The metrological quality of the results was underpinned by setting up uncertainty budgets according to the "Guide to the Expression of Uncertainty in Measurement" (GUM).

Neuartige Aspekte

isotopic abundance of silicon crystals using MC-ICP-MS with lowest uncertainty, detection and removal of concentration-dependent interferences

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Grundlagen der Massenspektrometrie I.1

Haareis

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Einleitung

Haareis ist ein ungewöhnliches Naturphänomen. Im Gegensatz zu Reifnadeln, die aus atmosphärischem Wasser entstehen und deren weiterer Zuwachs jeweils an der Spitze stattfindet, tritt bei Temperaturen knapp unter dem Gefrierpunkt (-4°C…0°C) Haareis an der Basis morschen, nassen Laubholzes aus. Die haarähnlichen, flexiblen, linear angeordneten Strukturen können bis zu 10 cm Länge erreichen, ohne jedoch die für Raureif typischen Verästelungen auszubilden. Es wird vermutet, dass die Haareisbildung durch ein im Holz vorkommendes Pilzmyzel verursacht wird.
Vereinzelt wurden Springschwänze (winteraktive Insekten) auf Haareis entdeckt. Steigt die Temperatur über den Schmelzpunkt, entstehen aus den einzelnen Eishaaren perlkettenartig angeordnete, bräunlich gefärbte Wassertröpfchen, die durch schwach sichtbare Fäden verbunden bleiben. Aus den Beobachtungen resultiert die Fragestellung, ob Haareis möglicherweise organische Verbindungen enthält.

Methodik

Filtrierte und unfiltrierte Schmelzwasserproben von Haareis sowie zum Vergleich von Schnee wurden zunächst auf ihren TOC- bzw. TN-Gehalt geprüft. Die qualitative Charakterisierung des Stickstoffs wurde durch eine anschließende ionenchromatographische Analyse erreicht.
Mit verschiedenen (massenspektrometrischen) Methoden, meist gekoppelt mit jeweils geeigneten Separationstechniken, wurden die Proben zunächst als Screening auf Organika verschiedenster Polarität und Molekulargewicht geprüft: (Head space)-GC-MS, Kapillarelektrophorese, UPLC-MS und FTICR-MS.
Nach ersten Hinweisen auf die enthaltene Substanzklasse der Fulvinsäuren erfolgten die finalen Analysen nach Entsalzung und Aufkonzentrierung der Proben mittels SPE (C-18 Material) erneut mittels ESI-FTICR-MS, abweichend von den vorherigen Messungen als gemittelte Spektren aus 6 Scans mit jeweils 50 Transienten unter einer Auflösung von 400.000 (bei m/z=400 Da).

Vorläufige Daten

Die Proben enthalten mit 235 mg/l TOC eine relevante Fracht an Organik. Im Gegensatz dazu kann nur 11 mg/l Gesamt-Stickstoff nachgewiesen werden, 70 % davon liegt zudem anorganisch als Ammonium vor.
Zunächst vermutete Stoffwechselprodukte wie niedermolekulare Organika, Carbonsäuren, Peptide oder Lipide konnten nicht nachgewiesen werden. Erst eine UPLC-MS-Analyse des Haareis-Schmelzwassers an einem Triple-Quadrupol-Massenspektrometer zeigte ein ein (unaufgelöstes) Chromatogramm, gemittelte Spektren aus verschiedenen Bereichen des Chromatogramms zeigten eine vergleichbare, breite Verteilung von Peaks über einen breiten Molekulargewichtsbereich (100-650 Da) mit bevorzugt intensiveren, ungeradzahligen Peaks. Ein solches Pattern erinnert stark an gelösten organischen Kohlenstoff (*dissolved organic carbon*, DOC), der u.a. aus terrestrischen & marinen Wässern, Bodenauszügen bzw. Aerosolproben bekannt ist. Da die Schnee-Referenzprobe kein solches Chromatogramm zeigt, kann eine aerosolbürtige Herkunft/ Kontamination ausgeschlossen werden.
Erneute Messungen mit ESI-FTICR-MS unter modifizierten Aufnahmebedingungen zeigen im Haareis-Schmelzwasser ein für DOC typisches Spektrum. Ausschnitte zeigen intensive Pattern von CHO-Verbindungen und deren zugehörige ^{13}C-Isotopenpeaks, dagegen nur wenige, schwache Signale stickstoff- und/ oder schwefelhaltiger Komponenten.
Die komplette Charakterisierung solch komplexer Spektren erfolgt mit einem selbst entwickelten Kalkulationsprogramm auf SciLab-Basis. Die automatisch generierten Summenformeln werden zunächst exemplarisch überprüft und anschließend in eine Excel-Tabelle zur weiteren Auswertung/ grafischen Darstellung z.B. als Kendrick- bzw. Van Krevelen Diagramme übertragen. Die erhaltenen van Krevelen-Diagramme (als zweidimensionale Darstellung H/C über O/C) zur Charakterisierung von Polarität und Aromatizität der organischen Komponenten werden mit den aus der Literatur [1,2] bekannten Bereichen für verschiedene (biopoymere) Verbindungen unterlegt - im Ergebnis können die Signale überwiegend Lignin zugeordnet werden.
Als Ergebnis wird postuliert, dass das Pilz-Myzel offensichtlich die bevorzugt in den Holzstrahlen vorhandenen Nährstoffe (Kohlenhydrate, Fette) aktiv verstoffwechselt, das schwerer abbaubare Lignin der Zellwände bleibt zunächst übrig und bildet die organische Komponente in Form sehr kleiner, aber zahlreicher Kristallisationskeime, die unter entsprechenden Wetterbedingungen zur Ausbildung von Haareis führen.

Neuartige Aspekte

Haareis wurde erstmals organisch-chemisch untersucht, wobei als Hauptinhaltsstoff Lignin nachgewiesen werden konnte.

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Purification and 3D-Structural Characterization of the N-terminal Region of p53

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Einleitung

The tumor suppressor p53 acts as a DNA sequence specific transcription factor which induces or represses a wide range of specific target genes involved in cell cycle control, senescense and apoptosis in response to genotoxic stress. P53 exerts its function as the guardian of the genome via a complex interplay of independently folded and intrinsically disordered domains. Intracellular levels of p53 and its function are regulated by factors like post-translational modifications, degradation, and interactions with a broad palette of other proteins [1]. Here we describe the production, purification, and characterization of an N-terminal fragment of p53, (Np53, comprising amino acids 1-292 of human p53 (wt)) Structural mass spectrometry analysis indicated that this fragment assembles into tetramers even though it does not contain the carboxy-terminal tetramerization domain. This implies that other p53 domains contribute to tetramer formation in addition to the known tetramerization domain. By applying the cross-linking approach we aim to map the intramolecular binding sites within Np53.

Methodik

Np53 was subcloned into a pET-21a (+) expression vector (Novagen) for overexpression in *E. coli* BL21 (DE3) following a published protocol [2]. The resulting plasmid was provided by the Weizmann Institute, Israel. The protein was purified by affinity chromatography (HisTrap FF, GE Healthcare) and size exclusion chromatography (Superdex 75 HiLoad 16/600, GE Healthcare). The purified Np53 was characterized via peptide mass fingerprint analysis after tryptic digestion. Initial cross-linking experiments were carried out with 10 µM Np53 (in 20 mM HEPES, pH 7.2) using the cross-linker $BS^2G\text{-}d_0$ in a protein-to-cross-linker molar ratio of 1:100. Samples were proteolyzed with trypsin and the resulting peptide mixtures were analyzed by nano-HPLC (RP C-18 column, 75 µm x 250 mm, Dionex Corporation)/nano-ESI-LTQ-Orbitrap-MS/MS (Thermo Fisher Scientific).

Vorläufige Daten

Np53 has been successfully produced using the T7-promoter-based *E. coli* expression system and purified using a two-step strategy. The initial immobilized ion metal affinity chromatography step (IMAC) with a linear imidazole elution gradient was sufficient to separate Np53 from the majority of soluble *E.coli* proteins. The following size exclusion chromatography step with a separation range from 3,000 to 70,000 Da removed these impurities which had bound nonspecifically to the IMAC column. Np53 was characterized by MALDI-TOF-MS operating in linear and positive ionization mode and peptide mass fingerprint analysis after tryptic digestion by nano-HPLC/nano-ESI-LTQ-Orbitrap-MS/MS resulting in sequence coverage of 94 percent. The successfully purified p53 variant will be used for identification of intra- and intermolecular interaction sites within the C-terminally truncated p53 variant by chemical cross-linking followed by a proteolytic digestion and nano-HPLC/nano-ESI-LTQ-Orbitrap-MS/MS. Preliminary experiments gave a hint, that Np53 might form homotetramers although the tetramerization domain (residues 325-356) is missing. For cross-linking experiments a mixture of unlabeled and stable isotope-labeled (i.e. deuterated) cross-linker $BS^2G\text{-}d_0$ /d_4 will be used, which allows a facilitated identification of cross-linked sites based on their characteristic isotope patterns [3]. In order to discriminate between intra- and intermolecular cross-links, a mixture of ^{15}N-labeled Np53 and unlabeled Np53 in a ratio of 1:3 will be used. Initial cross-linking experiments with Np53 only yielded intramolecular cross-links such as a cross-link between Lys164 and Lys292. Therefore, we are focusing our investigations on the intramolecular interactions of Np53. These investigations could provide further information about the interplay between folded and intrinsically unstructured domains of p53.

Neuartige Aspekte

3D-Structural Analysis of the N-terminal Domain of p53

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MeCAT – Relative quantification using HPLC/ESI-MS and software assisted data analysis

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Einleitung

In modern life science, the focus of proteomics nowadays turns from sole identification of proteins to their quantification. The development of stable isotope labelling techniques including ICAT, ITRAQ and SILAC yielded capable methods that are able to provide both qualitative and quantitative information. Furthermore, with the combination of high capacity separation techniques even complex mixtures can be analysed. The use of lanthanides in combination with MeCAT (Metal Coded Affinity Tag) for protein quantitation has also been introduced. [1-2] MeCAT uses chelate complexes of lanthanides for relative and absolute quantification with elemental mass spectrometry, while molecular mass spectrometry is used for sequence elucidation of peptides and proteins by tandem mass spectrometry. Here we present recent progress in performing MeCAT analysis on peptide level using HPLC/ESI-MS and software assisted data analysis.

Methodik

Proteins are labeled with MeCAT-IA. Mixtures of samples and standards were either proteolysed direct in solution or in gel after SDS-PAGE prior to analysis by HPLC/ESI-MS and -MS/MS, applying CID and IRMPD fragmentation. Data analysis was performed using Proteome Discoverer Software (Thermo Fisher, Version 1.3).

Vorläufige Daten

Differential labelling of proteins is achieved by application of different metals within the DOTA complex and offers the possibility of relative quantification by HPLC/ESI-MS and –MS/MS analysis. Using a combination of CID and IRMPD fragmentation, unambiguous identification of labelled proteins on peptide level is possible, as labelled peptides show similar fragmentation patterns in comparison to unlabelled peptides.[3] Additionally characteristic fragments of the labelling group can be detected, thus allowing differentiation between labelled and unlabelled peptides, as well as, screening for labelled peptides. Relative quantification is possible due to different intensities of peptide precursor or of characteristic fragments after IRPMD fragmentation.

Neuartige Aspekte

Here we present proceedings of software assisted analysis containing identification and relative quantification of MeCAT labeled proteins.

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MeCAT – Comparing Quantification methods based on Ovalbumin in Hen Egg White

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Einleitung

In modern life science, the focus of proteomics nowadays turns from sole identification of proteins to their quantification, since the development of stable isotope labelling techniques including ICAT, iTRAQ and SILAC mass spectrometry has been shown to be capable of providing both qualitative and quantitative information. Furthermore, with the combination of high capacity separation techniques even complex mixtures can be analysed. We have recently developed MeCAT (Metal Coded Affinity Tag) as a further quantification methodology. [1-2]
MeCAT uses chelate complexes of lanthanides for relative and absolute quantification with elemental mass spectrometry, while molecular mass spectrometry is used for sequence elucidation of peptides and proteins by tandem mass spectrometry. Here we present a comparison of protein quantification methods using MeCAT-IA in a biological matrix.

Methodik

Lyophilized hen egg whites from different sources and ovalbumin as internal standard were labeled differentially using MeCAT-IA. For absolute quantification, proteins were separated using SDS-PAGE. Spots were excised and mineralized. Quantification was performed using ICP-MS and external calibration with lanthanides standard salt solutions. For relative quantification, we performed in gel proteolysis and HPLC/ICP-MS and HPLC/ESI-MS/MS.

Vorläufige Daten

The absolute quantification of ovalbumin metal concentration in mineralized gel spots was determined by ICP-MS using external calibration with standard salt solutions. This yielded in the exact determination of the content of ovalbumin in hen egg whites. To compensate for analyte losses during sample processing, also commercially available ovalbumin was used as internal standard. Beside quantification on protein level, we also performed quantification on peptide level, using HPLC/ICP-MS, HPLC/ESI-MS and -MS/MS relative to an internal standard. No significant differences in the results using different methods were found.

Neuartige Aspekte

We were able to quantify ovalbumin in hen egg white absolute and relative to an internal standard applying MeCAT-IA.

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Characterization of protein kinase D complexes at the Trans-Golgi network

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Einleitung

Protein kinase D (PKD) is a serine-threonine kinase comprising three isoforms, which is involved in various cellular functions, such as cell proliferation, stress and immune response, and protein transport from the Trans-Golgi network (TGN) to the plasma membrane. In particular, PKD2 mediates the fission of transport vesicles at the TGN via the interaction of its cysteine-rich subdomains C1a and C1b with diacylglycerol (DAG) and the ADP ribosylation factor 1 (ARF1) [1]. Yet, the detailed constitution of the *in vivo* PKD2-ARF1 complex and its interface regions are still a matter of debate. Also, interaction partners of PKD at the TGN have to be identified. To address these issues we apply *in vitro* and *in vivo* cross-linking experiments and ESI-Orbitrap mass spectrometry.

Methodik

Chemical cross-linking will be carried out with the heterobifunctional cross-linker Sulfo-SBED[1]. Purified GST-PKD2 will be incubated with Sulfo-SBED to conduct amine-reactive labeling. After removal of non-reacted Sulfo-SBED, labeled GST-PKD2 will be incubated with TGN proteins and photo-reactive cross-linking is applied via exposure to UV light (365 nm).
For *in vivo* cross-linking the photo-amino acids photo-leucine and photo-methionine will be incorporated into GST-PKD2. Photo-reactive cross-linking will be induced by irradiating the cells with UV light (365 nm).
In both approaches, analysis of cross-linked products will be performed by enzymatic digestion and nano-HPLC/nano-ESI-Orbitrap mass spectrometry. [1] Sulfo-*N*-hydroxysuccinimidyl-2-(6-[biotinamido]-2-(p-azido benzamido)-hexanoamido) ethyl-1,3'-dithioproprionate

Vorläufige Daten

As the detailed knowledge of protein-protein interactions is the key for a better understanding of biological processes the results of our study will gain insight into how cellular protein transport is organized at the TGN. In particular, the elucidation of PKD2-ARF1 interaction as well as the identification of further interaction partners of PKD2 will contribute to a precise perception of the underlying mechanisms of carrier vesicle formation.
Obtaining structural data of PKD2 complexes is of utmost importance. So far, a crucial role in the context of ARF1 interaction has been shown for the proline at position 275 of PKD2 by a cell biological approach [1]. This observation will be underlined by 3D-structural data derived from cross-linking experiments. Thus, mapping of interface regions between PKD2 and ARF1 as well as the identification of additional PKD2 interaction partners will help to trace functions and mechanisms of complex formation at the TGN back to structural characteristics.

Neuartige Aspekte

Identification of novel PKD2 interaction partners, elucidation of PKD2-ARF1 complex and mapping of respective interface regions

Referenzen

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Charakterisierung des Proteoms der aktiven Zone der murinen Präsynapse durch Fraktionierung bei erhöhtem pH-Wert und nLC-ESI-MS/MS

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Einleitung

Das Proteom der aktiven Zone der Präsynapse ist zwar nur von geringer bis mittlerer Komplexität, stellt aber aufgrund der großen Anzahl an Membranproteinen besondere Anforderungen an die analytischen Methoden [1].
Das Ziel dieser Arbeit ist die Kombination verschiedener Fraktionierungstechniken und massenspektrometrischer Methoden basierend auf ESI-MS/MS zur möglichst vollständigen Charakterisierung des Proteoms der aktiven Zone der murinen Präsynapse.

Methodik

Die Präparation der aktiven Zone erfolgte wie bereits beschrieben [2]. Um Detergenzien im resultierenden Eluat zu vermeiden, wurde für die LC-basierten Methoden das Protokoll dahingehend adaptiert, dass die mit den Membranen assoziierten Proteine durch Ultraschall statt Detergenzien herausgelöst wurden.
Die Fraktionierung des Eluats erfolgte zum einen mittels SDS-PAGE, der sich ein tryptischer In-Gel-Verdau und eine LC-Trennung anschloss; desweiteren wurde ein tryptischer Lösungs-Verdau durchgeführt, dem eine Fraktionierung bei pH 9 über C18-SpinColumns (Thermo Scientific) und eine LC-Trennung (Easy nLC II, Thermo Scientific) bei pH 2 folgten. In beiden Fällen erfolgte die Detektion mithilfe eines ESI micrOTOF-Massenspektrometers (Bruker Daltonics).
Die Datenbanksuche wurde über Mascot 2.4. (Matrixscience, London) und die weitere Datenprozessierung mittels eines im Institut entwickelten Programms durchgeführt.

Vorläufige Daten

Es konnte gezeigt werden, dass trotz der veränderten Elutionsbedingungen ohne Detergenzien nach der Immunopräzipitation ähnliche Resultate im Vergleich zu den ursprünglichen Bedingungen erreicht werden konnten: In der GeLC-MS/MS-basierten Methode konnten 93 Proteine durch 1021 Peptide identifiziert werden, verglichen mit 863 Peptiden und 84 identifizierten Proteinen aus einem tryptischen Lösungsverdau, der mittels nLC-ESI-MS/MS analysiert wurde. Durch eine einfache Fraktionierung der Peptide des Lösungsverdau bei alkalischem pH-Wert mittels C18-SpinColumns konnte die Zahl der identifizierten Peptide auf 2805 und die Zahl der identifizierten Proteine auf 141 erhöht werden.
Neben der verbesserten Charakterisierung der aktiven Zone der murinen Präsynapse konnte der zeitliche Aufwand der Analyse durch die direkte Zugänglichkeit der Probe nach dem Lösungsverdau im Vergleich zur GeLC-MS/MS-Methode weiterhin wesentlich reduziert werden.

Neuartige Aspekte

Die Analyse der aktiven Zone der murinen Präsynapse konnte ohne Verwendung von Detergenzien unmittelbar über nLC-ESI-MS/MS durchgeführt werden.

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Lagerungsbedingte Veränderungen des cytosolischen Erythrozyten-Proteoms: Analyse mittels 2D-DIGE und Orbitrap MS

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Einleitung

Während der *ex vivo* Lagerung von Erythrozyten-Konzentraten treten an den Zellen morphologische und biochemische Veränderungen auf, die in der Transfusionsmedizin unter dem Begriff „erythrozytäre Lagerungsschäden“ zusammengefasst werden [1]. Sie beinhalten sowohl die Bildung von Echinozyten und Membranverluste durch Vesikulation, als auch die Depletion von ATP und 2,3-Bisphosphoglycerat oder die Oxidation von Membranbestandteilen [2]. Auch wenn diese Veränderungen zum Teil reversibel sind, können sie langfristig zu einer verminderten Funktionalität und Überlebensfähigkeit transfundierter Erythrozyten führen. Derzeit wird offen diskutiert, inwieweit die Transfusion „alter“ Blutprodukte mit der Komplikations- und Mortalitätsrate von Blutempfängern in Zusammenhang steht [3]. Ziel dieser Studie war es nun, die lagerungsbedingten Veränderungen des cytosolischen Erythrozyten-Proteoms mittels 2D-DIGE zu untersuchen, um charakteristische Protein-Muster und potentielle Marker-Proteine für die Feststellung von erythrozytären Lagerungsschäden zu identifizieren.

Methodik

Für die Untersuchung der lagerungsbedingten Veränderungen des cytosolischen Erythrozyten-Proteoms wurden 12 leukozytendepletierte Erythrozyten-Konzentrate in PAGGS-M Lagerungslösung unter Blutbank-Standardbedingungen bei 4 °C gelagert und zu festgelegten Zeitpunkten von 0, 7, 14, 28 und 42 Tagen mit Hilfe eines validierten Aufarbeitungsprotokolls analysiert [4]. Die gewaschenen Zellen wurden lysiert und zur Abreicherung des enthaltenen cytosolischen Hämoglobins mit einer speziellen Depletions-Matrix behandelt. Die aufgereinigten Proteine wurden anschließend mit den Fluoreszenzfarbstoffen Cy2, Cy3 oder Cy5 gelabeled und auf 18-cm IPGs (pH 4-7) und 10 % Tris-Glycin Acrylamid Gelen aufgetrennt. Die Auswertung der Gele erfolgte mit Hilfe einer geeigneten Software und signifikant veränderte Proteine wurden mittels Trypsin-Verdau und LC-MS/MS identifiziert. Einzelne ausgewählte Marker-Proteine wurden schließlich mittels Western Blotting bestätigt und validiert.

Vorläufige Daten

Die statistische Auswertung der Gele mittels Student’s T-Test, Varianzanalyse und PCA ergab eine ansteigende Anzahl signifikant veränderter Spot (Faktor -1,6 bis 1,4) mit zunehmender Lagerungsdauer: Während nach 7 und 14 Tagen Lagerung jeweils nur zwei Spots veränderte Volumina aufwiesen, waren nach 28 Tagen insgesamt neun Spots signifikant verändert. Nach 42 Tagen zeigten schließlich 25 Spots signifikante Veränderungen ihrer Volumina. Eine zusätzliche False-Discovery-Korrektur der *p*-Werte reduzierte die Anzahl der signifikant veränderten Spots auf jeweils null nach 7 und 14 Tagen, zwei nach 28 Tagen und 14 nach 42 Tagen *ex vivo* Lagerung. Trypsinverdau und LC-MS/MS Analyse führten zur Identifizierung zahlreicher charakteristischer Bestandteile des Erythrozyten-Cytosols wie beispielsweise Peroxiredoxin 2 und Beta Aktin. Aus den insgesamt 18 identifizierten Proteinen (9 nach FDR-Korrektur) wurden schließlich Beta Aktin, Transglutaminase 2 und das Kupfer-Chaperon für die Superoxid Dismutase als potentielle Marker-Proteine für erythrozytäre Lagerungsschäden ausgewählt und erfolgreich mittels Western Blotting validiert [5]. Die Ergebnisse dieser Studie tragen somit nicht zur zum Verständnis der molekularen Vorgänge bei der Entstehung von erythrozytären Lagerungsschäden bei, sondern können auch als Grundlage für die Entwicklung eines Screening-Assays dienen, vom dem sowohl Transfusionsmedizin, als auch präventive Dopingforschung im Hinblick auf den Nachweis von autologem Blutdoping profitieren würden.

Neuartige Aspekte

Analyse der lagerungsbedingten Veränderungen des Erythrozyten-Proteoms mittels 2D DIGE auf Ebene des Cytosols, Identifizierung neuer potentieller Marker-Proteine und charakteristischer Proteinmuster

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Increasing the Multiplexing of High Resolution Targeted Peptide Quantification Assays - Scheduled MRMHR Workflow on the TripleTOF® Systems

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Einleitung

Targeted quantitative proteomics has become a key tool in proteomics research and is driven by the well-established sensitivity and selectivity attributes of multiple reaction monitoring (MRM)[1]. As more extensive protein panels need to be monitored in a targeted way across multiple samples, higher MRM multiplexing is becoming essential for throughput.
The speed and sensitivity of MS/MS acquisition on the TripleTOF® Systems have enabled a high resolution targeted quantification strategy. This strategy enables a degree of selectivity using high resolution that cannot be reached using standard triple quadrupole based instruments.
Scheduled MRM^{HR} workflow, now available will be assessed for the degree of multiplexing possible and its impact on quantitative reproducibility.

Methodik

A Mixture of protein digests of BSA, Myoglobin and Beta-Galactosidase as well as E.coli protein digest were used for this analysis. Separation of protein digests was performed on an Eksigent NanoLC-Ultra® 2D System and cHiPLC® system. The MS analysis was performed on a TripleTOF® 5600 system using both the MRM^{HR} Workflow and Scheduled MRM^{HR} Workflow. Peptide identification on the MS/MS collected on the *E.coli* sample was performed using ProteinPilot™ Software 4.0. Skyline Software was used to select Proteins and peptides for analysis and MRM^{HR} workflow data was acquired and processed to determine peptide retention times. Final Scheduled MRM^{HR} workflow acquisition methods and MultiQuant™ Software quantitation methods were created simultaneously by Skyline. Final data processing was performed using MultiQuant™ Software.

Vorläufige Daten

High resolution targeted assays can provide higher specificity for some peptides in complex biological samples and now with the ability to time-scheduled acquisition, high multiplexing can be achieved. TripleTOF® Systems have the MS/MS sensitivity and speed to perform MRM-like analysis. Post-acquisition extraction of fragment ions from the high resolution TOF MS/MS data allows for high specificity MRM-like data (MRM^{HR} Workflow) to be obtained.
Scheduled MRM^{HR} Workflow extends the multiplexing possible with this high resolution workflow, maintaining quantitative robustness. In the multiplexing test on standard protein digests, up to 800 precursors were analyzed in a single method and high reproducibility was observed. This reproducibility translated into the complex biological sample test, where 130 peptides were monitored in E.coli with 90% of the transition peak areas showing reproducibility better than 20%.

Neuartige Aspekte

TripleTOF® System using Scheduled MRM^{HR} enables MRM like multiplexing and robustness, but with higher selectivity for targeted quantitative workflows.

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Using Differential Mobility Separation to Differentiate and Localize Sites

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Einleitung

Post-translational modifications (PTMs) of proteins play an important role in many biological processes. Mass spectrometry is an important tool for the characterization and especially the localization of sites of PTMs within a protein using high resolved MSMS spectra. However, for mixtures of isobaric peptides with PTMs on different residues of the same peptide sequence it is hard to clearly identify the correct site of modification. Isobaric peptides are in most cases absolutely coeluting in chromatography and show of course no differences in the precursor mass, so in many cases MSMS spectra alone are not enough, an additional dimension of separation is necessary. Here we present differential mobility separation to differentiate and localize sites of post-translational modifications of peptides.

Methodik

A total of ten synthetic peptides with various modifications were obtained from GL Biochem (Shanghai, China). Peptides with the same amino acid sequence but different modification sites / types were mixed as one sample, for a total of 5 different samples. Samples were analyzed using the Eksigent ekspert™ microLC 200 (Eksigent, USA), coupled with an AB SCIEX QTRAP® 5500 system equipped with the SelexION™ Technology for differential mobility separations. Results were analyzed using PeakView® Software.

Vorläufige Daten

The SelexION™ Technology is a differential mobility separation (DMS) device that was installed on the QTRAP® 5500 system, attaching in front of the curtain plate. This device was used to separate isobaric peptides, which only differ by the site of modification of the same amino acid sequence. Three different post-translational modifications were tested: Phosphorylation, methylation and acetylation. After on-column tuning of the compensation voltage (CoV) it was possible in all three examples to separate the isobaric variants of the modified peptides.
Differential Mobility Separation (DMS) provides an orthogonal level of selectivity by separating components based on their chemical properties and mobility. Full scan MS/MS can be obtained at the unique compensation voltage for each peptide isoform to achieve a clean spectrum and confident modification site localization.

Neuartige Aspekte

Differential mobility separation of isobaric peptides.

Referenzen

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Labeling of Proteins and Peptides via their Glycan Structures

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Einleitung

The development of methods for accurate protein quantitation is currently one of the most challenging and rapidly changing areas of proteomics. One of the most promising approaches for absolute and relative quantification is the metal labeling of proteins via the thiol groups of included cysteines. This method is well established for the quantification of proteins if the number of free thiol groups is known. Nevertheless, this method holds some limitations. Firstly, it cannot be applied to proteins without cysteines. Secondly, for an exact quantification the number of cysteines has to be known. Especially for antibodies the precise amino acid sequence is often unknown. Additionally, the labeling degree of a protein is mostly less than 100 % and often varies which makes a quantification via Metal-Coded Affinity Tag labeling (MeCAT) nearly impossible. Thus, in this study our aim is to develop a method for the metal labeling of an alternative functional site. For this purpose glycan structures of glycoproteins seem to be very promising because the knowledge of the precise structure neither of the amino acid sequence nor of the glycan itself is not necessarily needed. In this study we use the glycoprotein Erythropoietin (Epoetin, EPO) as a model compound.

Methodik

Firstly, for the labeling of EPO a partially deglycosylation is necessary. That is carried out by various enzymes called exoglycosidases [1]. This partially deglycosylated EPO is labeled via a so-called “Click-IT™ O-GlcNAc Enzymatic Labeling System“ [Invitrogen™]. Then, EPO is azide labeled by UDP-GalNAz using a special mutant of glycosyltransferases β4-Gal-T1-Y289L (EC 1.4.1.38) [2]. Finally, EPO is labeled using MeCAT-Lanthanide-Label via Click-Chemistry [3]. Any step of the whole procedure is analyzed via nano-HPLC/nano-ESI FT ICR MS/MS (Agilent 1100 Series/Thermo Scientific LTQ FT Ultra Hybrid). Thus, it is necessary to digest EPO with a protease like Trypsin (EC 3.4.21.4), Chymotrypsin (EC 3.4.21.1) or Glutamylendopeptidase (EC 3.4.21.19) to get glycopeptides. Finally, EPO can be quantified by using ICP-MS (Thermo Scientific ELEMENT XR).

Vorläufige Daten

Prior to use EPO was purified via an Amicon® Ultra centrifugal filter device by Millipore™, reduced with dithiothreitol (DTT), alkylated with iodacetamide (IAA) and purified once again [4]. For the detection by standard C18-HPLC-MS/MS it is necessary to digest the modified glycoprotein to get smaller glycopeptides containing glycan structures at N24, N38, N83 and S126. Unfortunately these glycan structures are very inhomogeneous which means that the amount of each single glycopeptide is strongly limited. Thus, we used PNGase F for a complete deglycosylation of EPO and digested it with Trypsin, Chymotrypsin and Glutamylendopeptidase (Glu C), respectively. We found that Glu C gives best results concerning the size and availability of the glycopeptides.
Partially deglycosylation was carried out in two ways: 1) by various enzymes (exoglycosidases) - Neuraminidase (EC 3.2.1.18), β-*N*-Acetylglucosaminidase (EC 3.2.1.96), β-Galactosidase (EC 3.2.1.23), and Endo F3 (EC 3.2.1.96) [1] consecutively or as a mixture and 2) by deGlycIT™ Micro Spin columns containing IgGZERO™ covalently coupled to agarose beads [GENOVIS AB]. No partially deglycosylated glycopeptides could be identified via nano-HPLC/nano-ESI FT ICR MS/MS investigating the digests of the partially deglycosylated EPO using Trypsin or Chymotrypsin. A digestion by Glu C lead to the identification of two of the three expected partially deglycosylated N-glycopeptides generated by the mixture of exoglycosidases.
The next step is the labeling of these partially deglycosylated glycopeptides with UDP-GalNAz using the “Click-IT™ O-GlcNAc Enzymatic Labeling System“ [Invitrogen™]. Afterwards an easy labeling with MeCAT via Click-Chemistry could be possible [2].

Neuartige Aspekte

This study shows an alternative method of quantitative proteomics via metal labeling of glycan structures of proteins and peptides.

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Development of a sensitive, robust and automated protein analysis method for intact cell MALDI mass spectrometry biotyping

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Einleitung

Since the seminal discovery [1] that species-specific protein fingerprints of intact bacterial cells obtained by matrix assisted laser desorption/ionization (MALDI) mass spectrometry (MS) have the potential for identification and classification of microorganisms, intact cell (IC)- MALDI MS has rapidly evolved into a fast, specific, automatable and well-characterized analytical tool, with a broad application in clinical and environmental microbiology [2]. It has recently also been applied to the investigation of mammalian cells including primary blood cells and culture cells [3, 4]. However, few automated procedures suitable for higher throughput and little analytical standardization of mammalian biotyping approaches have been reported so far. Here, we present a novel sensitive and automated method for the characterization of primary as well as cell culture cell lines [5].

Methodik

Sample preparation for IC-MALDI cell analysis highlights through simplicity and lack of expensive reagents. In a first step, frozen cells are resuspended in ddH_2O to obtain a cell concentration of 5,000 cells/µL. Next, cells are mixed and disrupted by thorough pipetting. Fifteen microliters of the suspension are mixed with 15 µL SA [38 mg/mL in water/ACN/TFA 40/60/0.2] and one µL of the matrix-analyte mix is applied on the top of the thin SA layer using the dried droplet preparation (double layer).

Vorläufige Daten

The generation of specific cell fingerprints is dependent on the precise adjustment of MALDI matrices, solvent acidity and cell counts. We demonstrate that intact cell mass spectrometric signatures of different cell lines start to resemble each other at higher trifluoroacetic acid (TFA) concentrations and that therefore low concentrations of TFA in the matrix solution are preferred. We present a novel sensitive automated method that robustly classifies as few as 250 cells per spot. Automatically acquired cell fingerprints from cultured and primary cells show high technical (R>0.95) and biological reproducibility (R=0.83–0.96), with a median peak variance below 12 %. Further, we show that *in vitro* differentiation of HL-60 cells into a neutrophil-like phenotype can be rapidly and robustly monitored. We applied the optimized method for global analysis of person-to-person differences in mass spectral signatures of intact human polymorphonuclear neutrophils and monocytes obtained from healthy volunteers. The cell populations were distinct with a high specificity as shown by principal component analysis (PCA) and unsupervised hierarchical cluster analysis (HCA).

Neuartige Aspekte

Robust, automated, sensitive IC-MALDI MS method aided by multivariate statistical evaluation methods, could become a complementary method for clinical research.

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Sample Displacement Chromatography as a method for sample preparation for mass spectrometry of proteins

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Einleitung

Complex protein mixtures still remain an analytical challenge in mass spectrometry analysis. Top-down mass spectrometry of proteins, in which intact proteins are analyzed, is a useful method, especially in the comprehensive characterization of protein species (isoforms). However, up to now, the protein fraction must be relatively homogenous for yielding appropriate mass spectrometric results from a protein species. Here we present a fast, simple and inexpensive method for the separation of complex protein mixtures, based on sample displacement batch chromatography (SDC). Chromatography in sample displacement mode relies on the competition of the sample molecules among each other towards the binding sites at the stationary phase. The sample displacement effect can be utilized for the separation of molecules by using segmented columns in series. After sample loading, the proteins adsorb to the column segments in order of their affinity. The protein with the highest affinity towards the stationary phase is located on the first column segment, the protein with the lowest affinity is adsorbed on the last column segment [1, 2, 3].

Methodik

Anion-exchange chromatographic material (Fractogel-TMAE) was used for fractionation of human plasma proteins (Cohn fraction IV-4) by SDC. To determine optimal parameters for the purification, a buffer screening was performed. For SDC the sample was loaded to 15 column segments in series. The single segment consists of chromatography material suspended in a buffer. After application of the sample to all column segments, the chromatographic material of the individual segments was washed and the absorbed proteins were eluted from the stationary phase of each segment. The resulting fractions from the screening experiments were monitored by SDS-PAGE electrophoresis. Selected SDS-PAGE bands were digested by trypsin and identified by an LC-ESI-ion trap system.

Vorläufige Daten

SDC fractionations with a sample loading buffer with a pH of 7 was determined as most efficient for the separation of Cohn IV-4 plasma proteins, since the plasma proteins were well separated and distributed to all segments. By analysis of selected SDS-PAGE bands with LC-ESI-MS/MS (ion-trap) the efficacy of the SDC separation was confirmed [4].
The results clearly demonstrate the advantage of SDC as a tool for protein separation prior to mass spectrometric analysis. Since SDC guarantees fractions with high protein concentrations it is well suited as sample preparation method for top-down mass spectrometry.

Neuartige Aspekte

Sample displacement chromatography offers the opportunity for efficient separation of complex protein mixtures without the need of expensive HPLC equipment.

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Combinatorial Use of Electrostatic Repulsion-Hydrophilic Interaction Chromatography (ERLIC) and Strong Cation Exchange (SCX) Chromatography for In-Depth Phosphoproteome Analysis

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Einleitung

In large-scale phosphoproteomics studies, fractionation by strong cation exchange (SCX) or electrostatic repulsion-hydrophilic interaction chromatography (ERLIC) is commonly used to reduce sample complexity, fractionate phosphopeptides from their unmodified counterparts, and increase the dynamic range for phosphopeptide identification. However, these procedures do not succeed to separate, both singly and multiply phosphorylated peptides due to their inverse physicochemical characteristics. Hence, depending on the chosen method only one of the two peptide classes can be efficiently separated.

Methodik

SCX−ERLIC Chromatography
ERLIC−SCX Chromatography
TiO2 Chromatography

Vorläufige Daten

Compared to single step fractionations by SCX, the combinatorial strategies, SCX−ERLIC and ERLIC−SCX, yield up to 48% more phosphopeptide identifications as well as a strong increase in the number of detected multiphosphorylated peptides. Phosphopeptides identified in two subsequent, complementary fractionations had little overlap (5%) indicating that ERLIC and SCX are orthogonal methods ideally suited for in-depth phosphoproteome studies.

Neuartige Aspekte

In this study, we present the benefits of combining complementary fractionation methods for phosphopeptide analysis.

Referenzen

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Protein cross-linking by the use of azides linker substances: Efficiency and side reactions

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Einleitung

Cross-linking in combination with mass spectrometric (MS) analysis is popular for the investigation of both the tertiary structure of proteins and their interactions with other molecular species. In order to enrich the cross-linked peptides we became interested in azide compounds, namely Sulfo-SBED. This heterobifunctional molecule links primary amines and nearly any protein functional group. The cross-linking reaction is induced by UV-light irradiation, whereby the highly reactive nitrene intermediate derived from the azide functional group can attach unspecifically to protein moieties by addition or insertion [1]. Unsatisfied with the efficiency of the linking reaction with proteins, we reacted standard peptides with the linker and looked also at simplified azide chemicals using MS/MS experiments.

Methodik

Cross-linking of neurotensin (100 pmol/µL) with sulfo-SBED (1 mM) was performed in phosphate buffer (0.1 M, pH 7.2). The reaction mixture (1:5) was incubated in the dark for 1 h and then irradiated with UV-light (365 nm) in a BioLink (Vilber Lourmat) apparatus. The UV-light intensity was varied from 1-8 J/cm^2. In order to ensure efficient reaction, experiments were conducted at 8 J/cm^2. One aliquot was not further treated. The other aliquots were reduced or reduced and alkylated. Prior to MS measurement the reaction mixtures were desalted with µC$_{18}$-ZipTip® pipette tips (Millipore). Samples were analyzed using capHPLC / ESI-QIT- (Agilent HP1100; Esquire3000, Bruker Daltonics), nanoUPLC / Q-TOF- (nanoAcquity, Q-TOF Premier) and MALDI-TOF-MS (MALDI micro MX; both Waters Corp.).

Vorläufige Daten

Using Sulfo-SBED, the formation of expected intra- and intermolecular links as well as single peptides containing a linker molecule with NHS hydrolyzed and the azide moiety still present or reacted accompanied by a loss of N_2 was reported [2,3]. Oxidation was also observed and it was located at the biotin group using MALDI-TOF-MS [2]. When we pre-separated the modified standard peptides and analyzed them by MS/MS, results did not seem to agree with this hypothesis. Other authors did not suggest an oxidation site when fragmentation data were not available [3]. We observed the formation of two molecular species of the cross-linker itself. The NHS-hydrolyzed linker reacted with water on the azide moiety (*m*/*z* 671.2) in agreement with [4]. However, we find the additional incorporation of one more oxygen (*m*/*z* 687.2). This second signal was typically of lower intensity due to immediate abundant water loss (*m*/*z* 669.2). The effect was similarly observed in the peptides. It could be excluded that the biotin moiety was oxidized, because the biotin marker ion (*m*/*z* 227.1) [5] was detected. The reaction of azide with water can easily be mistaken for the oxidation of sulfur in the biotin group [2], because the molecular masses are identical. This is also true, e.g., for the masses of the supposedly oxidized intramolecular peptide and the peptide, where the linker is bound only to lysine, but the azide reacted (- 2N + 2H 2O) and water is lost. Since often only mass and no fragmentation data are available for modified peptides, the blocking of the reactive azide moiety by reaction with buffer components presents an important detail to consider for both method optimization and data interpretation.

Neuartige Aspekte

Characterization of side products and thus improved data interpretation. Clarification of assignment ambiguities for peaks of similar/equal mass.

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Mass spectrometric investigation of the effect of proteasome inhibition on endothelial cells using iTRAQ

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Einleitung

Factors involved in the production or degradation of proteins represent a potential target in anticancer treatment. A crucial role in intracellular protein degradation plays the ubiquitin-proteasome-system which is important for apoptosis, transcription, angiogenesis, and cell cycle [1]. An inhibition of the proteasome hampers the degradation of proteins and therefore influences homoestasis, cell proliferation, and important signalling pathways, e.g. of NF-κB [2]. Bortezomib is the first proteasome inhibitor approved by the FDA for the treatment of newly diagnosed and refractory multiple myeloma and mantle cell lymphoma. Valentiner et al. showed that bortezomib reduced the formation of pulmonary metastases in a metastatic xenograft model [3]. Here, the effect of bortezomib on pulmonary endothelial cells is investigated through an iTRAQ-based proteomic approach.

Methodik

Human pulmonary microvascular endothelial cells (HPMEC) were incubated with 20 nM Bortezomib or its carrier solution as control for 1 h or 24 h followed by treatment with 10 ng/ml TNF-α or its carrier solution for 4 h. Afterwards, cells were harvested and lysed. 100 µg of protein per sample were tryptically digested and labelled with iTRAQ 8-plex reagents. Labelled peptides were separated by isoelectric focusing (OFFGEL, Agilent Technologies). Mass spectrometric measurements were done on an ESI-QTOF (Premier, Waters) equipped with an UPLC system (Acquity, Waters). Raw data were processed with PLGS 2.5.2 (Waters). Identification and quantification were performed with the OpenMS software using the search engines OMSSA and X!Tandem and the quantification tool iTRAQ analyzer 1.9.0.

Vorläufige Daten

In total, 940 proteins were identified in this experiment. Bortezomib-regulated proteins were filtered that might participate in metastatic processes. By the iTRAQ approach, the majority of proteins were identified as being down-regulated upon bortezomib treatment whereas a small number of proteins was identified as being up-regulated.Two bortezomib-regulated proteins will be discussed in more detail. Caveolin-1 was identified as being down-regulated in bortezomib-treated HPMEC. Podar et al. demonstrated that bortezomib inhibits caveolin-1 expression in multiple myeloma cells as well as VEGF-triggered tyrosine phosphorylation of caveolin-1 in human umbilical vascular endothelial cells [4]. Consequently, migration in both cell lines was decreased. Caveolin-1 is essential for caveolae formation. Caveolae are transport vesicles that are in contact with the blood and can transport molecules across the endothelium. Caveolin-1 and Caveolae are described as potential therapeutic targets in multiple myeloma [4]. This result is in accordance with Podar et al. and is one example demonstrating the plausibility of the applied iTRAQ-based quantitative proteomic approach. Clathrin heavy chain 1 is another protein which was down-regulated. Clathrin is the major protein of coated pits and vesicles. It is involved in microtubules-induced focal adhesion disassembly. The dynamic assembly and disassembly of focal adhesions plays a central role in cell migration which is a fundamental process in cancer metastasis [5]. In summary, the iTRAQ approach revealed promising and novel potential protein targets of bortezomib. Nevertheless, the candidates require further investigations by orthogonal techniques for validation.

Neuartige Aspekte

Investigation of the effect of the proteasome inhibitor bortezomib on HPMEC with a proteomic approach in view of cancer metastasis.

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Peptide Secondary Structure Studied by Mass Spectrometry

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Einleitung

The secondary structure of short peptides can be elusive to commonly applied methods – either due to the simultaneous presence of several conformations, the requirement of high peptide concentrations or simply because they cannot be properly assigned. Examples are β-hairpins or ‑turns, whose CD signals are often ambiguous and sometimes indistinguishable from those of other β-structures. Moreover, these structurally related signals are often of low intensity and easily masked by aromatic contributions.
Previously, diazirine labeled, so-called photo-amino acids, were developed to covalently link protein complexes within living cells in preparation of pull-down measurements [1]. Despite being originally designed with no subsequent mass spectrometric analysis in mind, photoamino acids are particularly suited for our general strategy to obtain structural information of proteins based on distance constrains from mass spectrometric characterization of chemically or photoactivated cross-linking products [2].
In our study, we used photoactivated cross-linking of peptides with photo-leucine incorporated followed by mass spectrometric detection and characterization of the cross-linking products to show reverse turn structure formation of peptides in solution. This initial approach allowed a qualitative differentiation between stably and transiently formed reverse turns as well as a semiquantitative measurement of complex-forming peptides [3].

Methodik

Buffered solutions of photoamino acids and labeled peptides (ThermoFisher Scientific) were subjected to UV-A radiation in a home-built device, proteolytically digested (peptides) and analyzed by LC/nanoESI-MS and -MS/MS using an analytical HPLC (Agilent 1200 for photoamino acids) or nanoHPLC (Ultimate Plus for peptides) system coupled to an LTQ-Orbitrap XL hybrid mass spectrometer (ThermoFisher Scientific) equipped with a nanoelectrospray ionization source (Proxeon). Precursor ions were typically subjected to CID fragmentation, occasionally to HCD fragmentation and fragment ions were analyzed in the orbitrap. Potential cross-linked products were identified using the in-house software StavroX [4] and verified by manual inspection of the spectra. Far-UV-CD spectra were recorded on a J-810 spectropolarimeter (Jasco).

Vorläufige Daten

Our first peptides were designed to display reverse turns or β-hairpins. As was confirmed by far UV CD spectroscopy, such structures were indeed formed, although to different extents. As photoactivation of pure photoamino acids as well as of labeled peptides yields internal reaction products (alkenes), a proteolytic cleavage step was included in order to discern (linear) alkenes from (circularized) cross-linking reaction products. Furthermore, this created new termini, which greatly enhanced the potential to exactly identify the interacting residues by tandem MS. The presence of “backbone bends” within these peptides was proven by identification of intramolecular cross-linking products between their N- and C-termini.
The stability of the interaction, i.e. the relative abundance of peptides in turn conformation in solution was estimated as follows: First, there was a reasonable concurrence between supposed strength of the intramolecular interaction and the appearance of precursor ion and fragment ion spectra representing the respective cross-linking products. Second, peptides displaying well-defined turns produced singular cross-linked product, whereas fluctuating turns resulted in a more dispersed product pattern.
Since even quantitative mass spectrometric techniques like multiple reaction monitoring were of no use in the quantitative evaluation of our proteolytically digested peptides, a new set of peptides was designed. Due to their inherent structural properties, they were competent to form homodimers. These homodimers were readily identified and characterized by tandem MS. The distribution of homodimeric, i.e. cross-linked, relative to monomeric species was deduced from the relative abundance of their precursor ions in the total ion current chromatograms. The application of classical enzyme kinetic methods of evaluation finally produced kinetic models of the structurally induced complex formation.

Neuartige Aspekte

Application of incorporated photoamino acids to study secondary structures in isolated peptides

Referenzen

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Enhanced Peptide/Protein Characterization Using Electrochemically Assisted S-S Bond Reduction

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Einleitung

A new method for the electrochemically-assisted reduction of disulfide bonds in peptides and proteins followed by on-line mass spectrometric detection is presented. The method is based on a square-wave potential pulse for fast and stable reduction of the disulfide bonds. Efficient reduction is achieved by making use of a specially developed Titanium based working electrode. The method is purely instrumental (push button) and does not use any harsh reducing agents such as DTT. The application of a simple electrochemically-based method creates new opportunities for the determination of disulfide bond arrangements and efficient peptides and proteins identification using top- and/or mid- down strategies.

Methodik

In infusion mode experiments, typically 2 - 20 µM solutions of the target compound in 1% formic acid /acetonitrile (90/10, v/v) were pumped into the electrochemical (EC) cell at a flow rate of 50 µL/min and the outlet of the cell was directly connected to the ESI-MS.
In flow injection experiments, the sample was introduced in 0.1% formic acid and 10% acetonitrile using 5µL injection loop. The mobile phase consisted of 0.1% formic acid and 25% acetonitrile. 1% formic acid was mixed with the mobile phase prior the electrochemical cell. The total flow rate was 100µL/min. The cell was operating in pulse mode to reduce the compounds of interest.

Vorläufige Daten

In this poster, we present electrochemically (EC) assisted reduction of biologically active peptides and proteins containing disulfide bonds followed by on-line mass spectrometric detection. A specially developed Titanium working electrode was used for the reduction of disulfide bonds. The unique properties of the working electrode allow for complete reduction of all disulfide bonds of the tested proteins and peptides. Furthermore, a special electrochemical method based on square-wave potential pulses was developed and optimized for efficient and stable reduction.
Insulin, a small protein of 5733 Da containing 3 disulfide bridges was used as model protein for system evaluation. The results are compared with the previous approach exploiting a conductive diamond working electrode. Reduction of other peptides and proteins such as somatostatin with one disulfide bond (1638 Da) and α-lactalbumin with four bonds (14 178 Da) will be shown to demonstrate the power of electrochemical S-S bond reduction.
The effects of different experimental parameters such as potential, flow rate, mobile phase composition, were tested and resulted in an optimized protocol for the electrochemical reduction of disulfide bonds. Furthermore, we show data on long term stability and repeatability based on flow injection analysis.
On somatostatin we demonstrate that backbone cleavage by CID is substantially enhanced in comparison to intact peptide, producing many more structurally informative fragment ions. Only the CID of somatostatin with reduced S-S bond leads to unambiguous identification of the sequence of the tested peptide.
The electrochemical cell can be positioned before or after the HPLC separation, i.e., in an EC/LC/MS or LC/EC/MS set-up, resulting in a fully automated platform for fast characterization of S-S bonds in protein/peptide based biopharmaceuticals.

Neuartige Aspekte

Efficient reduction of disulfide bonds using a novel electrochemical approach based on a new electrode type and square wave pulses.

Determination of oxidized cysteines in peptides/proteins

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Einleitung

Cysteines' thiols are well-known to undergo intracellular oxidation through reactive oxygen species (ROS) such as hydrogen peroxide. The oxidation of cysteines plays an important role as a redox control in oxidative stress and post-translational modifications and can indicate disease states or cell damage. The oxidation of cysteines lead to several products including sulfenic acid (R-SOH), sulfinic acid ($R\text{-}SO_2H$) and sulfonic acid ($R\text{-}SO_3H$).The reversible sulfenic acid formation (R-SOH) is a post-translational modification involved in protein regulation. The stabilization of the transient sulfenic acid should allow better understanding of many redox-mediated intracellular events that would be of interest in fields like cancer and cell biology [1,2]. Dimedone derivatives (cyclic diketone) are a known reagent that reacts selectively with sulfenic acid and has been used in some molecular mass spectrometry-based applications [3,4]. The development of a metal-containing ligand that has the dimedone moiety will allow the use of ICP-MS as detector. The superb sensitivity of ICP-MS compared to molecular mass spectrometry could lead to unprecedented qualitative and quantitative information pertaining to the sulfenic acid formation.

Methodik

In vitro oxidation of cysteines was studied using standard peptides and hydrogen peroxide as oxidizing agent. The reactivity with the commercially available dimedone derivative was studied using nanoHPLC-ESI-FTICR-MS after incubation of the samples at physiological pH and temperature.
On the other hand the synthesis of the metal-containing dimedone reagent was conducted through the reaction of t-Butyl-protected DOTA-alkyne and dimedone-azide using Click chemistry where Cu(I) was used as catalyst.
The synthesis of the label implies two major steps: a step that includes the alkyne-azide interaction via Click chemistry and another step includes deprotection of DOTA and then metallation with a lanthanide metal.
The reaction of the synthesized sulfenic acid probe was tested with the H_2O_2-oxidised standard peptide.

Vorläufige Daten

In vitro oxidation of cysteines using H_2O_2 and dimedone revealed that different forms of oxidized thiol are formed and that the yield of the sulfenic acid form increases as the concentration of oxidizing agent decreases within the experimental limit. The maximum sulfenate-dimedone yield was 70% when the dimedone concentration was 20 mM and the cysteine:H_2O_2 ratio was 1:5.
The different synthetic approaches revealed the poor reproducibility and the poor yield when DOTA-alkyne is used in its protected form due to the complexation of Cu by DOTA which should affect the catalytic role of Cu in Click reaction.
In an attempt to have a better control on Cu, we proposed a scheme in which DOTA is deprotected and metallated before Click reaction. Having DOTA in a complexed form will prevent Cu from making DOTA-Cu complex and thus DOTA will not affect the Cu-based catalysis in Click reaction.
After successful Click reaction with a good yield we developed an HPLC method that allowed the separation of the target compound with good resolution. Fractions were collected and analyzed. Analysis of the fractions proved the identity and purity of the peaks.
The proposed ligand was tested with oxidized cysteines of peptides/proteins and proved to be reactive and specific.Selectivity of dimedone to oxidised thiol allows the development of a simple, sensitive and straightforward ICP-MS-based method that enables the determination of oxidised thiol content of peptides/proteins.

Neuartige Aspekte

Sensitive determination of oxidized cysteines in peptides/proteins

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Label-free quantitative proteomic assessment of stress-responses in Candida albicans using mobility assisted data independent LC-MS

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Einleitung

The fungal pathogen *Candida albicans* survives in humans most commonly as a commensal organism in the flora of the gastrointestinal and urogenital tracts, occasionally causing opportunistic infections, such as thrush. However, in subjects that are immunocompromised, *C. albicans* can cause severe, life-threatening infections. Stress adaptation is critical for the pathogenicity of *C. albicans* and studied in detail at the genomic level to try to assess the mechanisms for host-defence implemented as adaptations to the environments encountered. Previous studies have exposed *C. albicans* to osmotic, oxidative and heavy metal stress conditions. Here we use liquid chromatography coupled with ion mobility assisted mass spectrometry in order to gain a greater understanding and measure of the salt-stress response of *C. albicans* at the proteomic level.

Methodik

This study investigated the proteomic changes between *C. albicans* grown under normal conditions and those experiencing salt-stress in the growth media. Three biological replicates of each condition were proteolysed with trypsin and the resulting peptides separated over a 90 minute linear reversed-phase nanoscale LC gradient. Data were acquired using an ion mobility data independent HDMS^E approach, whereby the collision energy was switched between low and elevated energy state during alternate scans and associate precursor and product ions correlated by means of retention and drift time alignment. The acquired data were processed using ProteinLynx Global SERVER and searched against a *C. albicans* database. Normalized label-free quantitation results were generated using Progenesis software.

Vorläufige Daten

Six *C. albican* tryptic digest samples (three based on normal conditions and three subjected to salt-stress) had 10 fmol/µl phosphorylase B digestion standard added and were analysed in triplicate, allowing for protein abundance levels to be achieved. The material loaded on column for these experiments was 100 ng. Resulting data were processed using a dedicated data independent search algorithm, which correlates the precursors and fragments prior to being searching against the *C. albicans* database. The search results revealed more than 1500 protein identifications per run. Quantitative results were achieved over a wide dynamic range using the three most intense peptides of the internal standard protein to determine a response factor for quantification of other proteins in the sample. The data was interrogated further using Progenesis LC-MS software between the two biological conditions. Data interpretation has returned confident expression ratios for over 1000 proteins and indicates that enzymes involved in glycerol metabolism and heat-shock proteins are some of the most highly up-regulated proteins during the salt-stress in *C. albicans*. Low abundance regulated proteins identified in the salt-stress samples were targeted in a separate acquisition on a high sensitive Q-Tof platform. A comparison of the correlation variance (CV) and fold change between the two groups will be presented.

Neuartige Aspekte

Mobility assisted data independent label-free LC-MS is used to quantify C.albicans which have been exposed to normal and salt-stress conditions.

Enhancing Quantitative Precision in Complex “Systems” utilizing a Highly Selective and Orthogonal Analytical Workflow

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Einleitung

Is there enough peak capacity in the applied workflow to insure that labeled variants do not interfere? Quantitative proteomics label-free or labeled is based on the area ratio of matched pairs. The accuracy of quantitative change is limited to how uniquely each ion is measured. Described is workflow utilizing an ion detection algorithm which assigns a purity value to each detected ion. Purity is determined by comparing each ions calculated half-heights (t_r, mass, t_d) to the mean the dataset. Enhanced accuracy is obtained by controlling which ions are utilized in determining the quantitative change. Given that product ions of matched pairs are present in the same elevated-energy spectra allows for additional ion statistics for accurately ascertaining quantitative change.

Methodik

PC3 cells were grown at 37°C in L-arginine, L-lysine depleted DMEN media supplemented with 10% dialyzed fetal bovine serum and L-[$^{13}C_6$,$^{15}N_4$]-arginine, L-[$^{13}C_6$,$^{15}N_4$]-lysine for the “heavy” and L$^{12}C_6$,$^{14}N_4$]-arginine, L-[$^{12}C_6$,$^{14}N_4$]-lysine for the “light”. ~50 µg of each sample was reduced and alkylated both individually and as 1:1 mixtures. Triplicate 1 µL injections of ~100 ng were separated over 120 minutes on a 1.7 µm T3 column operated at 400 nL/min at a mass resolving power of 25k (FWHM) with and without Ion Mobility Separation (IMS). Resulting datasets were processed and searched using the identical parameter settings. Ion interference rates were determined utilizing the ions' mass and chromatographic peak widths at half-height.

Vorläufige Daten

Analysis of the "light" and "heavy" sample sets produced on average 148k (+/- 9%) low-energy ions without IMS and 178k (+/- 12%) with despite a 40% reduction in signal intensity afforded by the IMS separation. Querying the two data sets at +/-10ppm and 15 seconds resulted in ~32k common ion detections. Many of these ions were traced back to keratins and the growth media. The degree of ion interference was determined by querying the "light" ion lists with pseudo "heavy" *m/z* values at a mass tolerance reflective of the mass resolving power with retention-time tolerances of 15 and 30 seconds. For IMS a tolerance of +/- 1 drift bin was also employed. As expected the interference rate reduced from ~18.6% to 3.4% at 15 seconds and 27.9% to 5.3% at 30 with IMS. Plotting retention-time *v* interference reflected a direct relationship to ion flux. A similar relationship was demonstrated when comparing the effective mass resolution to retention-time. To ascertain which ions should be used for quantification a unique identifier was created for each peptide and its replication rate determined. A 0.5% peptide error rate was employed. The mass, retention and drift times for all injections were compared outliers removed and each validated sequence was annotated with its' average mass, retention and drift time. The resulting list was then matched back to each replicates' ion list to fill in any holes where ions were measured albeit not identified. At this point the algorithm has the area of every isotope of every charge-state to every parent peptide. Ion selection is then determined utilizing the calculated purity scores and the associated area ratios calculated. Limiting the ion selection reduced the average co-efficient of variations from 20% to 8% with the best reductions at the lower concentration levels.

Neuartige Aspekte

Use of ion purity measurements for enhancing quantitative accuracy and precision in stable isotope labeled complex "systems"

HR/AM Targeted Peptide Quantitation: A Unique Combination of High Selectivity, High Sensitivity and High Throughput

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Einleitung

Quantitative proteomics enables the identification of large number of protein candidates, which display biologically interesting dynamics on a global scale in the early discovery phase. Targeted MS approach, in particular selected reaction monitoring (SRM), has become the preferred platform for quantitatively analyzing tens to hundreds of peptide candidates across large number of samples, either for understanding of signaling regulation or for verification and selection of potential clinical biomarkers.

Methodik

High-resolution, accurate-mass (HR/AM) MS methods provide an attractive alternative for targeted peptide analyses. High resolution on an Orbitrap™-based mass spectrometer can resolve peptide species differing in mass by as little as 5 ppm, providing high selectivity. The combination of accurate mass, isotope pattern recognition and elution time provides confident confirmation of targets even in highly complex mixtures such as human serum or plasma [1].

Vorläufige Daten

In this study, the Q Exactive™, a true high resolution and accurate mass (HR/AM) benchtop mass spectrometer, was evaluated for targeted quantitation of proteins in human plasma using short 10 min gradients aiming at high throughput. The dynamic range and LOD/LOQ of different HR/AM data acquisition strategies were evaluated and compared: High resolution Full scan, multiplexed targeted selected ion monitoring (msx tSIM), and the targeted MSMS approach applying fragmentation via HCD (tHCD).

Neuartige Aspekte

Peptide Quantitation using high resolution/accurate mass methods from complex matrices combined with new acquisition strategies on the Orbitrap platfrom

Referenzen

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Absolute quantification of Amadori peptides in human plasma as potential diabetes type 2 biomarkers

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Einleitung

Glycation is one of the most common non-enzymatic posttranslational modifications formed by the reaction of reducing sugars and amino groups in proteins. Resulting Amadori compounds can undergo oxidative degradation yielding advanced glycation end-products (AGEs), known as markers of diabetes. As AGEs are heterogeneous and characteristic for advanced stages of disease, Amadori products appear to be more suitable for early diagnostics and therapy control. Quantification of glycation sites in selected plasma proteins, however, requires new sensitive analytical techniques.

Methodik

Plasma samples obtained from five diabetes type 2 patients and control subjects were digested with trypsin. Glycated peptides were enriched by *m*-aminophenylboronic acid (*m*-APBA) affinity chromatography and separated by RP-HPLC coupled online with ESI-QqQ-MS/MS operating in MRM-mode. Glycated peptides were quantified by internal standardization.

Vorläufige Daten

The quantification of prospective marker peptides [1] was established with a LC-ESI-QqQ-MS/MS (MRM)-method by using authentic und internal (stable isotope-labeled) synthetic peptide standards. This included the optimization of MS parameters for these analytes. Sample preparation relied on *m*-APBA affinity chromatography and solid phase extraction (SPE) was optimized in terms of recovery and precision. Thereby Amadori peptides were quantified in digests obtained from the plasma of diabetes patients and healthy individuals. Glycation rates at several human serum albumin (HSA) sites were significantly ($p < 0,05$) higher in patients, indicating that the corresponding Amadori peptides might represent promising type 2 diabetes biomarkers.

Neuartige Aspekte

The method to identify novel peptide biomarkers for type 2 diabetes mellitus was established.

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High Reproducibility Targeted Quantitation at Highest Multiplexing MS/MSALL with SWATH™ Acquisition on the TripleTOF™ 5600 System

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Einleitung

Recent QqTOF innovations providing high speed acquisition of high resolution MS/MS spectra have enabled a data independent acquisition strategy. In this workflow the Q1 quadrupole is stepped at 25 amu window increments across the mass range of interest. The transmitted ions are fragmented and the resulting fragments are analyzed in the TOF MS Analyzer. This is done in a looped fashion with an LC compatible cycle time, such that MS/MS spectra are acquired on every peptide in the sample. Because the fragment ions are collected at high resolution, high quality XICs can be generated post-acquisition to produce the MRM-like data. The utility of this workflow for highly multiplexed targeted quantification in plasma samples and other complex proteomes will be evaluated.

Methodik

The samples were analyzed using the Eksigent nanoLC-Ultra® 2D System combined with the cHiPLC®-nanoflex system in Trap-Elute mode. Replicate injections were run to assess reproducibility, using ~500 ng of sample on column. Eluant from the column was sprayed using the Nanospray® Source into a TripleTOF™ 5600 system (AB SCIEX). The acquisition method was an MS/MS^{ALL} with SWATH™ Acquisition method, where Q1 was scanned from 400-1000 m/z in 25 Da window steps. A protein ID experiment was done and searched with ProteinPilot® Software to create a spectral library of the proteins and peptides in the sample. All data were processed using the MS/MS^{ALL} with SWATH™ Acquisition MicroApp in PeakView® Software from these spectral library.

Vorläufige Daten

The data independent acquisition strategy, MS/MS^{ALL} with SWATH™ Acquisition, provides a comprehensive analysis of complex proteomes, providing full scan high resolution MS/MS on all peptides eluting off the column, within the detection dynamic range. It is effectively a permanent record, a digital archive of everything in the detectable dynamic range of the sample, that can be re-interrogated over and over again, for different proteins and peptides as new hypothesis. Depleted plasma and a range of other proteomes were analyzed using replicate analysis to understand the quantitative performance of this technique. All samples are collected using the same generic acquisition method, then analyzed post-acquisition for the peptide targets of interest. The datafile is therefore an archive of MS/MS of all analytes which enable the retrospective *in silico* interrogation for any targets after acquisition. High resolution MS/MS provides higher resolution XICs with reduced chance of interferences, providing quantitative performance comparable to triple quadrupole quantitation, but with much higher multiplexing.

Neuartige Aspekte

SWATH™ is a data independent acquisition strategy, which provides highest multiplexing with MRM-like quantitation quality.

Referenzen

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Analysis of cross-linked peptides by online 2D-nano-HPLC/nano-ESI-LTQ-Orbitrap-MS/MS

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Einleitung

In functional proteomics studies, chemical cross-linking has emerged as a strategy for the identification of specific interactions between proteins and their binding partners, such as proteins, fatty acids, or nucleic acids. The cross-linked complex is enzymatically digested and cross-linked products are analyzed by LC/MS/MS. In order to increase the amount of structural information from one cross-linking experiment, enrichment of cross-linked species has been performed by strong cation exchange chromatography [1] and affinity chromatography by using trifunctional cross-linkers with an additional biotin moiety [2]. In case biotinylated cross-linkers are used, monolithic affinity columns with immobilized monomeric avidin (MACMAs) have proven highly efficient for a rapid and efficient enrichment of cross-linked products [3].

Methodik

Monolithic affinity columns with immobilized monomeric avidin (ID 100 µm, 20 cm length) were prepared as described recently [4]. A 20 cm-monolithic affinity column served as first dimension and a 75*µ*m ID C18 reversed phase separation column (Dionex) as second dimension in an *online* 2D-nano-HPLC system (Ultimate, Dionex). The HPLC system was *online* coupled to an LTQ-Orbitrap mass spectrometer (ThermoFisher Scientific) interfaced with a nano-ESI source (Proxeon). Proteins were cross-linked using a novel trifunctional cross-linker with two amine-reactive groups and biotin as an affinity label. Prior to 2D-LC/MS/MS analysis, cross-linked proteins were *in-solution* digested with trypsin. Cross-linked products were identified with StavroX 2.0.6 [5].

Vorläufige Daten

The analysis of cross-linked peptides by the 2D-nano-HPLC/nano-ESI-LTQ-Orbitrap-MS/MS setup was optimized with chemically cross-linked, digested cytochrome *c* as a model substrate. After affinity enrichment using the biotin group of the cross-linker, five cross-linked products were successfully identified with the 2D-LC/MS/MS setup. MS/MS data were successfully employed to narrow down potentially cross-linked amino acids. Unambiguous identification of cross-linked amino acids was not possible in all cases due to the presence of several lysine clusters in cytochrome *c*.
The optimized enrichment method was used for the analysis of a complex between calmodulin and a peptide derived from the skeletal muscle myosin light chain kinase (skMLCK). Three intermolecular cross-links were identified after analysis with the 2D-nano-LC/nano-ESI-MS/MS setup. The cross-link distances, cross-linked amino acids, and the orientation of the peptide in the complex with calmodulin are in agreement with results that have previously been obtained using alternative amine- and photo-reactive cross-linkers (Schaks et al., unpublished data).

Neuartige Aspekte

A fully automated system for affinity enrichment, separation and identification of cross-linked peptides is presented.

Referenzen

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Solid-Phase Based Partial Reduction and Alkylation Improves the Assignment of Disulfide Linkages of Closely Spaced Cysteine Residues

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Einleitung

A typical MS-based approach for the assignment of protein disulfide linkages involves endoprotease cleavage of peptide backbone under non-reducing conditions to form species that ideally contain only one disulfide bond prior to MS/MS identification. However, unambiguous disulfide linkage assignment for species containing multiple disulfide linkages, particularly those with closely spaced cysteine residues in sequence, can be very challenging. Therefore, we developed a solid-phase based partial reduction and alkylation strategy for disulfide linked proteins followed by proteolytic cleavage, using two proteases simultaneously, and nanoESI-LTQ-Orbitrap MS/MS analysis [1] and applied it for the assignment of disulfide linkages of the membrane-proximal domain (MPD) of the metallo-protease ADAM 17.

Methodik

Partial reduction of RP-HPLC purified recombinant ADAM 17 MPD was carried out by incubating ADAM 17 MPD shortly with TECP under low pH (pH< 3, 65°C); the reaction was stopped by rapid alkylation of reduced cysteine residues with concentrated IAA at neutral pH on a µC18 Ziptip. The partially reduced and alkylated ADAM 17 MPD eluted from Ziptip was diluted with Tris-HCl buffer to adjust pH to 7.0 prior to enzymatic digestion with trypsin and Glu-C at 37°C for overnight. The resulting peptides were subjected to IP-RP-HPLC coupling online to nanoESI-LTQ-Orbitrap Velos MS for peptide separation, identification and disulfide bonds assignment.

Vorläufige Daten

Determination of accurate intact protein molecular weight by Orbitrap MS indicated that all ten cysteine residues in ADAM 17 MPD were involved in the formation of five internal disulfide bonds. Due to the presence of several closely spaced and adjacent cysteines as well as rigid disulfide bonds, direct proteolysis of the native ADAM 17 MPD with either trypsin or GluC-protease only delivered few large peptides that were not suitable for disulfide bond assignment. To increase the accessibility of proteolytic sites of the ADAM 17 MPD, we developed and applied a solid-phase based method to partially reduce disulfide bonds under controlled conditions followed by protection of newly formed reduced cysteine residues by rapid alkylation to prevent disulfide rearrangement. Intact protein LC-MS analysis of proteins showed a number of different reduced/alkylated species including fully reduced alkylated forms as well as species with one to four reduced disulfide bonds. These proteins were further subjected to proteolytic cleavage using both trypsin and Glu-C; the peptides were separated by IP-RP-HPLC and analyzed by nanoESI Orbitrap MS/MS. Two types of collision activation, collision-induced dissociation (CID) and high-energy collision dissociation (HCD) were applied to interrogate the peptide sequences and to assign disulfide linkages by manual spectral interpretation. Five disulfide linkages of the ADAM 17 MPD were unambiguously identified; C_{582}-C_{604}, C_{591}-C_{611}, C_{593}-C_{603}, C_{600}-C_{635}, and C_{630}-C_{641}.

Neuartige Aspekte

Small scale (µg range) solid-phase based partial reduction and alkylation for protein disulfide linkages assignment

Referenzen

[1] Michalek M, Sönnichsen FD, Wechselberger R, Dingley AJ, Hung CW, Kopp A, Wienk H, Simanski M, Herbst R, Lorenzen I, Marciano-Cabral F, Gelhaus C, Gutsmann T, Tholey A, Grötzinger J, Leippe M. 2013, Nat Chem Biol. 91(1):37-42

Adsorption von Plasmaproteinen auf überzogenen und nicht überzogenen PLGA-(Polyglykolmilchsäure)-Nanopartikeln

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Einleitung

Nanopartikel als Arzneifreisetzungssystem können eine Verbesserung der Medikamentenwirkung bewirken. So kann dieses Freisetzungssystem verwendet werden, um ungünstige Pharmakokinetik, schlechte Löslichkeit oder geringe Zellaufnahme vorteilhaft zu beeinflussen. Besonders bei der Behandlung von soliden Tumoren kann durch Medikamente, die an die bioabbaubaren Nanopartikel gebunden sind, eine verbesserte Akkumulation dieser Arzneistoffe im Tumor erreicht werden. Nanopartikel in Plasma adsorbieren unmittelbar einige der darin enthaltenen Proteine. Die angelagerte Proteinschicht wird als Korona bezeichnet. Des Weiteren wird der gebildete Nanopartikel-Plasmaprotein-Komplex vom Retikuloendothelialen System (RES), insbesondere von den Makrophagen des mononukleären-phagozytären Systems (MPS) als Fremdkörper erkannt. Dabei spielen Proteine eine Rolle, die die Aufnahme dieser Komplexe in die Zellen des RES und MPS beschleunigen, die Opsonine. Andererseits gibt es Dysopsonine, die diese Aufnahme verlangsamen. Aus Veröffentlichungen können einige allgemeine Trends abgeleitet werden[1]: Neutrale Teilchen zeigen deutlich langsamere Opsonisierung als geladene Teilchen. Hydrophobe Partikel werden schneller als hydrophile opsonisiert. Da die Oberflächeneigenschaften eine so große Rolle spielen, kann durch gezielte Modifizierung eine verlängerte Blutzirkulation erreicht werden. Dieser Modifizierung und der Adsorption bestimmter Proteine aus der Gruppe der Apolipoproteine wird eine Schlüsselrolle in der Überwindung der Blut-Hirn-Schranke (BHS) zugeschrieben[2,3]. In dieser Arbeit wurde die Zusammensetzung der Korona an modifizierten und unmodifizierten PLGA-Nanopartikeln untersucht.

Methodik

Die PLGA-Nanopartikel wurden mit dem Doppel-Emulsions/Evaporations-Verfahren hergestellt. Die Nanopartikel werden in Plasma für einen definierten Zeitraum inkubiert. Die Probe wird zunächst zentrifugiert und der Überstand entfernt. Dann werden die Partikel wiederholt gewaschen, zentrifugiert und der Überstand wird entfernt. Der PLGA-Partikel-Protein-Komplex wird reduziert, alkyliert und mit Trypsin verdaut. Die anschließende Peptidtrennung wurde mit einem NanoLC-System (Proxeon Biosystems, Odense) durchgeführt, gekoppelt an einen MALDI-Spotter (Sunchrom, Friedrichsdorf) zur Off-line Messung mit einem 4800 MALDI-TOF/TOF (AB Sciex, Darmstadt). Es wurden C18-Partikel (Sigma-Aldrich, München) oder Poros R2-Partikel (AB Sciex, Darmstadt) in selbst gepackten Säulen verwendet.

Vorläufige Daten

Mit dem Poros R2 Material konnte eine bessere Reproduzierbarkeit ohne Selektivitätsverlust beobachtet werden. Die Selektivität war teilweise für Poros R2 (Polystyren–Divinylbenzen) höher, allerdings war dafür vermutlich die Möglichkeit, mit diesem Materials ein bestimmtes Detergenz effektiver zu entfernen, verantwortlich. Auch hinsichtlich einer Quantifizierung mit Hilfe des N-reaktiven Reagenz iTRAQ zeigte Poros R2 sich überlegen. So konnte beispielsweise auf die übliche SCX-Trennung zur Entsalzung vor einer Umkehrphasenchromatographie verzichtet werden. Das Poros R2 Material zeigte hier ebenfalls keinen Selektivitätsverlust. Für beide Materialien, C18 und Poros R2, konnte die bisher berichtete Zusammensetzung der Korona bestätigt werden. Ebenso konnte die Anwesenheit von Proteinen, die wahrscheinlich der Schlüssel zur Überwindung der BHS für unmodifizierte sowie für modifizierte PLGA-Nanopartikel sind, auf beiden Systemen nachgewiesen werden. Die Quantifizierung der einzelnen Proteine auf den Nanopartikeln ist derzeit noch nicht abgeschlossen.

Neuartige Aspekte

Vereinfachte Methodik der Qualifizierung und Quantifizierung von Plasmaproteinen, die an PLGA-Nanopartikeln adsorbiert vorliegen.

Referenzen

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Identification of rare protein modifications by LC-MS/MS

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Einleitung

Unaccounted protein modifications may influence the confidence of protein identification, reduce protein sequence coverage and affect the quantification. As a compromise between identification confidence and modifications variability, a very limited set of protein modifications is considered during stringent database searches. Here we present a de-novo sequence prediction-based approach that can be used in unbiased searches for rare or unexpected modifications. A case study carried on milk proteins revealed unexpected S- and T-formylation of the kappa casein.

Methodik

GeLC-MS/MS was performed on a LTQ Orbitrap Velos mass spectrometer (Thermo Fisher Scientific). Database searches were performed by MASCOT (Matrix Science) against comprehensive protein database (NCBI). De-novo sequence prediction were performed by PepNovo software [1], the resulting sequences were screened by error tolerant pattern search using an in-house script Mass difference calculated between assigned by script modified precursor and the corresponding unmodified sequence was used in second pass search against customized database. Peptide abundances were quantified by Progenesis (Nonlinear Dynamics).

Vorläufige Daten

We screened for rare modifications that occurred in close proximity to the chymosin-cleavage site HPHPHLSFMAIPPK of the major milk protein kappa-casein, which triggers milk curdling. Apart from anticipated oxidation of methionine, a mass difference of 27.99 Da was revealed by the script, which might correspond to the formyl moiety. Using it as a variable modification in database searches revealed that in all major milk proteins formylation affected three amino acid residues: formylation of internal serine and threonine residues likely happened in-vivo [2], whereas N-terminal formylation of tryptic peptides is a sample preparation artifact.

Neuartige Aspekte

Unbiased screen for protein modifications

Referenzen

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Sensitive and specific detection of lysine carboxymethylation and carboxyethylation peptide products in vitro and in vivo

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Einleitung

Glycation is a non-enzymatic post-translational modification of protein amino groups with reducing sugars. Resulting early products are involved in oxidation reactions yielding advanced glycation end-products (AGEs) [1]. The lysine-derived AGEs N^{ε}-(carboxymethyl)-(CML) and N^{ε}-(carboxyethyl)lysine (CEL) are recognized markers of arteriosclerosis, diabetes mellitus and ageing [2]. Despite their high diagnostic value and importance for the understanding of disease mechanisms, the protein targets of advanced glycation and exact modification sites are mostly unknown.

Methodik

Synthetic AGE-containing model peptides and tryptic digests obtained from *in vitro*-glycated human serum albumin (glycHSA) and pooled diabetic plasma samples were analyzed by ESI-QqTOF-, -QqQ-, and -QqLIT-MS/MS in product ion and precursor ion scanning experiments. The *m/z* values detected by precursor ion scanning were fragmented in targeted RP-UPLC-LTQ-Orbitrap-MS/MS experiments and AGE-modified peptides were identified by both database searching and manual interpretation.

Vorläufige Daten

The dominant modification-specific signals in the tandem mass spectra of model peptides were annotated as protonated carboxymethylated *m/z* 142.1 and 187.1) and carboxyethylated (*m/z* 156.1 and 201.1) derivatives of pipecolic acid and α-amino-ε-caprolactam, respectively. Based on these signals, sensitive and selective precursor ion scanning methods to detect correspondingly modified peptides in enzymatic protein digests were established and applied to tryptic digests of glycHSA and plasma. Thereby, 11 modification sites were unambiguously identified in glycHSA, while 16 glycated peptides representing 14 proteins were identified in a diabetic plasma digest.

Neuartige Aspekte

A method for sensitive detection of CML- and CEL-containing peptides in human plasma was established.

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MeCAT – Analysis of complete labeled proteins by ESI-MS

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Einleitung

As part of proteomics, the need for reliable protein quantification methods steadily increases. Thereby, the use of stable isotopes for labeling including ICAT and iTRAQ mass spectrometry (MS) is used progressively. Labels are based on a chemical labeling reaction of the proteins or peptides with a specific reagent. Mass differences of differently labeled samples are then detected by MS to get qualitative as well as quantitative information. As a further quantification methodology, we developed MeCAT (Metal Coded Affinity Tagging).[1 - 2] MeCAT uses chelate complexes of lanthanides for relative and absolute quantification. For the later elemental mass spectrometry is employed. Lately, we introduced the new MeCAT-IA reagent, carrying an iodoacetamide moiety that shows some distinct advantages over the previously used MeCAT-Mal with maleinimide reactivity.[2]

Methodik

Model proteins are labeled with the MeCAT-reagent, which contains a cysteine-reactive group for quantitative labeling and an elemental tag, loaded with a lanthanide ion for quantification. For separating labeled proteins from other sample components a HPLC was used online to electrospray ionization (ESI)-MS. Besides, fragmentation experiments with labeled proteins were applied, including infrared multi photon dissociation (IRMPD) and higher-energy C-trap dissociation (HCD). Relative quantification was performed with differentially labeled samples using ESI-MS, ESI-MS/MS and ICP-MS as reference.

Vorläufige Daten

The investigated model proteins cover a mass range of about 14 to 67 kDa in the unlabeled state and about 20 to 92 kDa in the completely labeled state. The completeness of the labeling was examined by using substoichiometric quantities of the labeling reagent with regard to the existing thiol groups of cysteine's residues in the proteins. The obtained spectra were compared with those at which the labeling reagent was used in excess. It was shown that all four different model proteins could be labeled completely. Hence, an important requirement for reliable quantification of proteins was fulfilled and that certain labeled proteins can be used as an internal standard for the relative quantification by MeCAT-IA. In further experiments, the fragmentation behavior of labeled proteins was investigated. For that reason, the fragmentation techniques IRMPD and HCD were applied. In addition, several differentially labeled proteins were detected simultaneously in order to verify the possibility of relative quantification. Lanthanides being used for the differential labeling were europium, terbium, holmium, thulium and lutetium. All five differentially labeled proteins could be isolated and detected simultaneously for fragmentation. These results can be used for determining the concentration of proteins or peptides relatively to a labeled internal standard protein with known concentration in ESI-MS/MS-analysis. The results of the relative quantification were confirmed by ICP-MS experiments.

Neuartige Aspekte

A complete labeling reaction for different model proteins and different workflows for protein quantification with MeCAT.

Referenzen

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Production of Intact Full-length Protein Standards for Quantitative Proteomics. Standards for human plasma Tf, Gpx3 and SEPP1

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Einleitung

Mass spectrometry is the most powerful technique to study proteomes providing both qualitative and quantitative data. Absolute quantification is a more challenging task compared to relative quantification because quantified standards are required. The optimal strategy in this context is the use of intact, stable isotope labeled proteins as internal standards.
In the presented work, production and characterization of intact protein standards for human serotransferrin (Tf) and human plasma selenoproteins (glutathione peroxidise 3, GPx3 and selenoprotein P, SEPP1) has been demonstrated. Selenoproteins play an important role as enzymes and serotransferrin can be a marker in "iron-out-of-balance" disorders. Standards presented here will help to establish novel, validated and traceable analytical methods for quantification of human plasma Tf, GPx3 and SEPP1.

Methodik

Entry clones containing coding sequence of proteins to be expressed were obtained from in-house Gateway repository or from Genecopoeia (THP Medical Products, Austria). Conventional and Gateway cloning strategy was used to produce plasmids to be used as DNA templates for cell-free *E. coli* protein expression. A continuous-exchange cell-free *E. coli* expression protein system (5PRIME, Germany) was used to produce stable isotope and SeMet labeled protein standards. Protein purification was achieved using electro-elution technique (Electro-eluter Model 422, Bio-Rad). In-gel protein tryptic digestion was performed according to a standard protocol. LC-MS/MS analyses were performed using a UPLC-system (Waters, Milford, USA) connected to an LTQ-Orbitrap XL instrument (Thermo, Bremen, Germany). Statistical data evaluation was performed in SigmaPlot applying one way ANOVA-Holm-Sidak test.

Vorläufige Daten

Standard protein production is achieved by cell-free synthesis, which allows simultaneously incorporation of selenomethionine (SeMet) and of selected stable isotope ^{13}C, ^{15}N) labeled amino acids. Standard protein quantification is performed by ICP-MS and selenium quantification which was demonstrated previously as RISQ concept [1]. This strategy allows for highly accurate protein standard quantification due to the exclusive selenium presence in RISQ protein standards, however, the stability of RISQ protein standards might be influenced by the objectionable reactivity of selenium. Therefore, to further develop the RISQ protein concept, we generated Se-quantified but Se-free standard proteins. For this purpose we introduced the two types of label into two separately expressed proteins. The Se-containing protein is quantified by ICP-MS and is then used as standard for quantification of the purely stable isotope labeled standard by LC-ESI/MS. The latter then represents the final Se-free protein to be used as internal standard. The extra quantification step by LC-ESI/MS can be performed with a relative standard deviation around 1 % [2]. This new concept is demonstrated for production of an intact standard for human serotransferrin labeled with [$^{13}C_9$,^{15}N]-Phe (F+10). Standard preparation and purification, its characterization and purity control are described in detail.
Due to high reactivity of selenocysteine (Sec) and very complex *in vivo* incorporation mechanism of Sec as building block of selenoproteins, in our strategy we produced selenocysteine-free SEPP1 and GPx3 standards. All Sec coding codons (UGA) of coding sequence of SEPP1 and GPx3 proteins were site-mutated to Cys coding codons (UGC). Such prepared vectors were used as DNA templates for cell-free protein synthesis. During protein synthesis ^{13}C and/or ^{15}N labeled amino acids and SeMet were introduced into protein structures allowing for quantification of the newly synthesized full-length proteins by ICP-MS via Se detection and subsequent use of them as internal standards for LC-ESI-MS analyses.

Neuartige Aspekte

Novel standards will allow to establish novel validated analytical methods for quantification of human plasma Tf, GPx3 and SEPP1

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Pharmacokinetic study of apidaecin-derivatives: A LC-MS/MS based quantification

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Einleitung

As more and more bacterial strains are resistant against approved antibiotics the development of new antimicrobial agents is increasingly important. Promising new antimicrobial peptides (AMPs) from different organisms, investigated as a class of lead compounds by many research groups. The insect-derived proline-rich AMP apidaecin 1b, was optimized for pharmaceutical applications by modification of the native sequence to improve its serum stability and activity against Gram-negative bacteria in our group. [1, 2] Thus four sequences (Api88, Api134, Api137, and Api155) showed improved properties as well as promising efficacies in murine infection models, with a high safety margin. To study their pharmacokinetic and -dynamic in mouse models, a method to quantify apidaecin-derived AMPs in plasma, urine and organ homogenates is described using reversed phase liquid chromatography/tandem mass spectrometry (RP-HPLC-MS/MS).

Methodik

Peptides were synthesized by automated synthesis using Fmoc/HOBT strategy with subsequent manual, *N*-terminal guanidation. The apidaecin-derivatives (i.e. Api88, Api134, Api137, and Api155) were separated by RP-HPLC from matrix components and selectively detected and quantified on-line by MS/MS with multiple reaction monitoring (MRM) on a 4000 QTRAP®. To achieve best results, the sample preparation prior to analysis was optimized in respect of selectivity, matrix effects and recovery. The method was internally standardized by stable isotope-labeled apidaecin derivatives, containing $^{13}C_5$,^{15}N-Pro in position 16, which provides a mass shift of 6 Da.

Vorläufige Daten

At the beginning, separation of peptides from the biological matrices by RP-HPLC was optimized providing short analysis times based on a five minute gradient at 55°C. The MRM relied always on the triply charged precursor ion, the most intense fragment ion and the two most intense fragment ions with higher *m/z*-values than the precursor ion. The MS-parameters were optimized for all MRM transitions to obtain the best sensitivities. To increase the intensity of triply charged precursor ions an eluent system with pH 3.0 was used. The most dominant fragment ions were formed by consecutive losses of dimethylamine cleaved from the *N*-terminal *N*,*N*,*N*',*N*'-tetramethylguanidino group, yielding specific mass losses of 30.05 *m/z* units relative to the $[M+3H]^{3+}$ precursor ion. To analyze pharmacokinetics in blood, mouse plasma with lithium heparin as anticoagulant gave best analytical results. Plasma as well as organ homogenates were prepared by solid phase extraction. To achieve highest sensitivities, polypropylene tubes and inserts were used for storing and processing of the basic peptides. The established RP-HPLC-MS/MS approaches of the four AMPs provided limits of detection and quantification of 5 and 10 ng/mL, respectively, with a linear dynamic range of 3 orders of magnitude. Finally the method was utilized for a pharmacokinetic study with Api 88 treated mice.

Neuartige Aspekte

The first method for quantification of antimicrobial apidaecin-derivatives in biological samples is shown and utilized for a pharmacokinetic study.

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Label-Free Phosphopeptide Quantitation and Occupancy Determination from a Single Protein Using LC-MALDI

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Einleitung

Protein phosphorylation has gained much attention in proteomics. Most work focuses on establishing catalogues of phosphopeptides in a proteome of interest and determining whether a certain peptide is phosphorylated or not, but this research stops short of checking for the occupancy of individual residues while verifying multiple phosphorylation sites. Here, we describe a new approach for the quantitative assessment of phosphorylation occupancy at various previously known target sites within a single isolated protein resembling the situation after immunoprecipitation of a protein in question.

Methodik

The protein KAPCA_HUMAN, containing 10 known phosphorylation sites, was used to quantitatively determine the occupancy of all sites. After EDTA-removal, the sample was trypsinized and split into two aliquots. One aliquot was enzymatically de-phosphorylated in contrast to the other aliquot, so that a native (N) and a dephosphorylated (D) peptide aliquot were obtained. Aliquots were analyzed in replicates by nanoLC-MALDI-TOF/TOF for peptide identification and the label-free quantification of the relative abundance of the free, unphosphorylated peptides. The intensity of the D-peptides was assumed to be 100 % free peptide. By subtracting the lower intensity of the N- peptides (1-x), the occupancy x [%] could be determined.

Vorläufige Daten

KAPCA_HUMAN contains 10 phosphorylation sites, 8 partial and 2 stoichiometric. For the determination of the occupancy, 3 replicate LC runs of 1 pmol of each the native (N) and the dephosphorylated (D) tryptic digest were done. MALDI-MS/MS and Mascot search provided peptide sequence verification. The native sample yielded pairs of phosphorylated and non-phosphorylated peptides with the same sequence. Unique to this method, only the non-phosphorylated peptides were used for the occupancy determination.
As expected, the peptides containing the stoichiometrically phosphorylated residues were detected with 100 % phosphorylation. Three other sites were non-stoichiometrically phosphorylated at Ser10 (93 %), Ser139 (37 %), and Ser259 (23 %). For 5 residues, phosphorylation was not detectable, implicating that the degree of phosphorylation at these 5 sites was less than 2 %. The coefficients of variation (CVs) of the label-free dephosphorylation-LC-MALDI method are within a range of 10 to 30 % in agreement with established and cost intensive isotope labeled reference methods.

Neuartige Aspekte

We describe a new approach for the quantitative assessment of phosphorylation occupancy at various previously known target sites

Determination of core fucosylation in glycopeptides of monoclonal antibodies

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Einleitung

Fucosylation is a glycan modification which affects glycoprotein confirmation and function. α-1,6 fucosylation of the chitobiose core found on N-linked glycans plays an important role in physiological processes. Involvement of core fucosylation has been shown in pathological processes like cancer and described as biomarker for liver cancer malignity. Here we describe a fast and reliable approach to unambiguously determine core fucosylation directly on the glycopeptides in tryptic digests by MALDI-TOF/TOF and ESI-IT.

Methodik

Standard glycoproteins like asialofetuin, horseradish peroxidase and a panel of therapeutic and standard monoclonal antibodies (e.g., MOPC21 from Sigma) were reduced, alkylated and trypsinized. Tryptic peptides and glycopeptides were separated by RP-LC and fractions were analyzed by ESI-IT-MS and MALDI-TOF/TOF-MS. An algorithm for N-linked glycopeptide analysis automatically classified MS/MS spectra taking the specific fragmentation patterns of MALDI and ESI CID into account, clearly distinguishing glycopeptides with or without core fucose. Subsequent protein and glycan database searches provided glycan structures and peptide sequences of the respective glycopeptides.

Vorläufige Daten

In ESI-IT, the fragment mass of the peptide including the first GlucNAc of the chitobiose is detected in the majority of cases of N-glycopeptides. In addition to this YY or Y fragment (for core (non-)fucosylated N-glycans), the ZZ fragment of the peptide & GlucNAc, the Z fragment of peptide & GlucNAc & fucose and the Y fragment of the peptide & 2 GlucNAc & fucose are observed..
This information was used to adapt the glycoprotein analysis software by extending lists of fragmentation patterns to distinguish different glycan structures. That capacity enabled to identify the high glycan micro heterogeneity at HC-Asn294 in murine IgG1 MOPC21. Core fucosylation was found to be a common modification in more than 50 % of the identified N-glycans. The main glycan in MOPC21 is G0F1 (GlucNAc4 Man3 Fuc1), followed by the same structure lacking the core fucose (G0).

Neuartige Aspekte

Automatic fragmentation pattern based detection of core fucosylation of N-linked glycopeptides in LC-MS/MS datasets.

Purification of a Lactose-specific Lectin from the Invertebrate Lamellidens corrianus and Elucidation of N-linked Glycan Structure.

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Einleitung

Lectins acting as carbohydrate recognition determinants in diverse biological processes are widely used in a broad range of applications including the detection, isolation and structural studies of glycoconjugates due to their specific sugar-binding properties. An increasing amount of lectins with diverse sugar specificities have been isolated so far giving rise to new possible applications [1]. Representing proteins and glycoproteins, the lectin structures on protein and glycan level have been intensively studied to gain an insight into the complexity of glycosylation in various species. These include *N*-linked glycans in simple organisms differing from the traditional classification of *N*-linked glycans into oligomannosidic-, complex- and hybrid type based on glycan structures found in vertebrates. Containing a multitude of modifications of the core regions and unusual antennae *N*-linked glycans in simple organisms are of special interest [2]. Thus, the freshwater mollusc *Lamellidens corrianus* which is widespread in India and representing a common alimentary source was studied with respect to the presence of a lactose-specific lectin. In the present contribution we communicate the purification and structural characterization of the above-mentioned lectin with special focus on the elucidation of the unusual *N*-glycan structure present in the analysed protein.

Methodik

In the present study a lactose-specific lectin was isolated from the crude extracts of the invertebrate *Lamellidens corrianus* by use of ammonium sulfate precipitation (80 %) and subsequent purification by affinity chromatography on divinyl sulfone activated Sepharose-6B gel covalently linked to lactose. The lectin obtained was analysed by SDS-PAGE under reducing conditions and distinct bands were cut and separately subjected to tryptic in-gel digestion. Proteolytic peptides were desalted by SPE (Zip-Tip C_{18}, Millipore) and submitted to mass spectrometric analyses. Structural elucidation of glycosylation was performed using nanoESI Q-ToF MS and low-energy CID experiments.

Vorläufige Daten

SDS-PAGE of the affinity purified lactose-specific lectin performed under reducing conditions revealed three distinct bands corresponding to molecular weights of 28, 30 and 32 kDa, respectively. Each of the three bands was submitted to proteolytic digestion using trypsin and derived peptides were analysed by MS and MS/MS experiments. A glycopeptide species giving rise to doubly charged ions with *m/z* 1202.15 was identified in the digest of the 30 kDa subunit. Evaluation of the low energy CID spectrum of these precursor ions revealed the presence of an *N*-glycan modification which has not been reported for a protein from a bivalvia species, so far. Representing a paucimannosidic-like glycan structure, the mannosylchitobiosyl core region (Manα1–6(Manα1–3)Manβ1–4GlcNAcβ1–4GlcNAcβ1) is modified by the addition of xylose attached to the β-Man residue. The *N*-glycan is lacking antennal GlcNAc and comprises three mannose residues, of which the two terminal hexoses are methylated. The latter modification has already been reported in bacteria, fungi, algae, and plants and is limited in the animal kingdom to worms and molluscs but has not been found in mammals [3]. Furthermore, an unusual modification by a fucose linked to the distal HexNAc of the chitobiose unit was found. This rare type of fucosylation has only been reported for glycoproteins found in the nematode *Haemonchus contortu*s [4] and the arthropod *Manduca sexta* [5]. As the *N*-linked glycans in simple organisms including invertebrates represent complicated and uncommon structures it is likely that many more types of modification of the core and antennal region remain to be discovered.

Neuartige Aspekte

Structural characterization of unusual *N*-glycan structure found in a lactose-specific lectin from the invertebrate *Lamellidens corrianus*.

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The Precision of Heavy-Light Peptide Ratios measured by MALDI-TOF Mass Spectrometry and its Application to LC-Free SISCAPA Protein Quantification

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Einleitung

The most critical, validation step in translating candidate protein markers into clinical diagnostics requires accurate protein and peptide quantitation in large sample sets. NanoLC/MRM provides precise relative quantitation of proteotypic signature peptides representing clinically interesting proteins. However, nanoLC inhibits the use of this approach in clinical settings. SISCAPA (Stable Isotope Standards and Capture by Anti-Peptide Antibodies) are introduced and combined with MALDI-TOF as an LC-free method for high throughput protein quantitation.

Methodik

Six pairs of synthetic proteotypic peptides were used in 2 forms: unlabeled (L) and labeled (H) in the C-terminal K or R residue. Peptides were proteotypic for human proteins such as thyroglobulin, PCI, HER2/neu, HE-4.. Peptides from 10 mM stocks were prepared for mixtures at varying L:H ratios across 11-point calibration series. Dilutions were prepared in 96-MTP using a liquid-handling robot (Bravo, Agilent) and spotted on AnchorChip MALDI targets (Bruker) in quadruplicate. The SISCAPA assays utilized Invitrogen´s protein G dynabeads, rabbit monoclonal antibodies selected for high peptide affinity and human plasma with titrated levels of spiked peptides. MALDI-MS spectra were acquired on an autoflex speed.

Vorläufige Daten

The precision of peptide quantitation by MALDI-TOF was explored using 6 pairs of proteotypic peptides (L) and same-sequence stable isotope labeled synthetic internal standards (H). These were combined in dilution curves spanning up to 2,047-fold ratios. Coefficients of variation (CV) of L:H peak area ratios were examined across 4 replicate MALDI spots per sample. Averaged across 11 points of a 100-fold dilution curve and over all 6 peptides, the overall CV was 2.5% at 11 fmol total (light + heavy) of each peptide applied per spot. The average CV at near-equivalence (the center of the dilution curve) for the six peptides was 1.0%. Response curves across the 100-fold range could be closely modeled by a power law fit giving R^2 values > 0.998.

Neuartige Aspekte

SISCAPA are introduced and combined with MALDI-TOF as an LC-free method for high throughput protein quantitation.

Quantitative phosphoproteome analysis of apoptotic Jurkat cells

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Einleitung

Apoptosis plays an important role in multicellular organisms as it is involved in modeling of tissue structures, elimination of mutated and damaged cells and homeostasis. Hence, a dysfunction can lead to severe diseases, such as cancer, autoimmune diseases or neurodegenerative disorders. Furthermore, in bioreactor cultivations of animal cells apoptosis negatively affects the production of biopharmaceuticals.
As protein phosphorylations are of great importance for apoptosis regulation and induction, the elucidation of changes in the phosphoproteome would lead to a better understanding of apoptosis-related diseases and new solutions for handling animal cells in bioreactor cultivations. Here, we present a comprehensive approach combining SILAC and complementary ESI-MS techniques for identification and quantification of apoptosis-involved protein phosphorylations.

Methodik

Cultivation of Jurkat ACC 282 cells was carried out in chemically-defined, serum-free medium. Three of six replicates were grown in heavy medium, containing stable isotope labeled lysine and arginine, and induced for apoptosis with 100 µM etoposide while the other three replicates, grown in light medium, were left untreated. 4h after apoptosis induction cells from treated and untreated cultures were harvested and mixed. Extracted total protein was fractionated by SDS-PAGE. In the next step the fractions were enriched for phosphopeptides via IMAC and TiO2, respectively. The samples were finally analyzed by nanoLC coupled to an ESI(CID/ETD)-ion trap and an ESI-UHR-TOF, respectively.

Vorläufige Daten

After apoptosis induction in Jurkat cells with 100 µM etoposide strong effects were seen in cell growth of the cultures. 81h after induction only 18% of the cells in the treated culture remained alive compared to 93% in the untreated culture.
The differential phosphoproteome analysis of Jurkat ACC 282 cells induced for apoptosis led to the identification of 1029 phosphoproteins. Regulated phosphorylations sites could be found for 105 phosphoproteins of which 71 are clearly associated with DNA damage, apoptosis, or cell cycle/growth.

Neuartige Aspekte

Better understanding of apoptosis-related diseases and new solutions for handling animal cells in bioreactor cultivations

Mass spectrometric analysis of post-translational modifications of free light chains in patients with multiple myeloma and AL-amyloidosis

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Einleitung

Multiple myeloma (MM) and AL-amyloidosis are pathologic conditions which belong to group of disorders designated as monoclonal gammopathies. These are associated with an increased excretion of free light chains (FLC) of immunoglobulins. The monoclonal light chains either circulate in the body fluids or deposit as amyloid in various organs of the patients. What structural phenomenon of FLCs makes them prone to aggregate and in certain cases finally deposit in a respective form is still unknown. It has been hypothesized that structural features e.g. sequence alterations in the variable region or post-translational modifications (PTMs), present throughout the LC, may be responsible for these processes [1-5].
Given the potential importance of PTMs in MM and AL-amyloidosis, characterization of six pathological human FLCs by means of mass spectrometry has been undertaken in this study in order to identify the proteomic changes related with these diseases.

Methodik

The FLCs were from bone marrow of patients suffering from MM and AL-amyloidosis or from MM only featuring the overproduction of a monoclonal κ-chain. The analysis focused on κ1-light chains because this is the most common single amyloidogenic LC subfamily. The target proteins were isolated by means of immunoprecipitation using specific antibodies against FLC covalently bound to the chromatographic material. The isolated light chains were purified by SDS-PAGE. Liquid chromatography - electrospray ionization tandem MS analysis (LTQ-Orbitrap Velos, Thermofisher) and matrix-assisted laser desorption/ionization (MALDI-TOF, Autoflex, Bruker) mass spectrometry, in combination with multiple complementary enzymatic digestions (trypsin, chymotrypsin, Glu-C, thermolysin, elastase), were used to verify the amino acid sequences and identify and locate post-translational modifications in the free light chains.

Vorläufige Daten

For all six patients, amino acid sequences of the free light chains could be identified by PCR. The predicted primary structure was verified through MS/MS-LTQ-Orbitrap sequencing. The obtained sequence coverage was between 83-100%.
To date observed post-translational modifications include oxidation of methionine and tryptophan or N-terminal acetylation of aspartic acid. Other modifications are under investigation. The individual impact of these modifications to pathology of diseases from the group of monoclonal gammopathies is still unknown. However, it has been hypothesized that several of these structural features may be markers of the process of amyloidogenesis [1-5] and are worth of detailed exploration. Their potential pathological impact remains the scope of further studies.
In some cases, a concomitant component of the isolated light chains was a co-precipitating protein from the group of apolipoproteins. These proteins are known to be a common constituent of amyloid deposits or to interact with the amyloidogenic proteins.

Neuartige Aspekte

Bone marrow light chains were characterized regarding their PTMs. So far, *Met*- and *Trp*-oxidation and N-terminal *Asp*-acetylation were identified.

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Tryptischer Verdau von PVDF-Membran gebundenem Protein

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Einleitung

Der Verdau im Polyacrylamid-Gel ist nicht immer gut reproduzierbar und erfordert im Vergleich zum Lösungsverdau eine höhere Mindestmenge an Protein. Eine Alternative ist der Verdau auf einer Blot-Membran. Ein weiterer Vorteil - besonders in Hinblick auf die nachfolgende Analyse der Peptide mittels MALDI-MS - ist die Möglichkeit, Detergenzien wie SDS leicht auszuwaschen [1]. Um die Adsorption des Verdauenzyms an die Membranoberfläche zu verhindern, wird diese in einigen Protokollen zuvor mit Polyvinylpyrrolidon-40, Tween 80, Triton X-100, Zwittergent 3-16 oder Octylglucopyranosid abgesättigt [2]. Es wurde berichtet, dass eine Ein-Schritt-Methode mit 1% reduziertem Triton X-100, 10% Acetonitril ACN und 100mM Tris pH 8,0 als Verdaupuffer unter Zeitersparnis die gleichen Ausbeuten liefert [3]. Die Konzentration an Trypsin ist bei kleinen Mengen Protein (fmol-Bereich) von ausschlaggebender Bedeutung, da besonders beim Membranverdau die Interaktionshäufigkeit wegen der großen Oberfläche zusätzlich reduziert wird. Gleichzeitig wird dieses Konzentrationsfenster vom Selbstverdau des Enzyms nach oben hin begrenzt. Diese Arbeit soll dazu beitragen, eine robuste Methode für den Membranverdau zu finden, die auch im niedrigen, realistischen Konzentrationsbereich gute Ergebnisse liefert.

Methodik

Um herauszufinden, ab welcher Konzentration eine der Methoden an ihre Grenzen stößt, wurde eine Verdünnungsreihe von 1000 bis 10 ng BSA hergestellt. Das Protein wurde im Dot-Blot-Verfahren auf 28,3mm² große Immun-Blot PVDF-Membranscheiben von Bio Rad aufgetragen. Diese wurden nach dem Trocknen entweder mit Rotiblock der Firma Carl Roth oder mit 0,5% PVP-40 Lösung inkubiert oder blieben für die Ein-Schritt-Methode unbehandelt. Der Verdau wurde mit variierenden Trypsin-Konzentrationen bei 37°C für 18 Stunden in 20 µl 10% ACN/25mM Ambic-Puffer durchgeführt, im Falle der Ein-Schritt-Methode unter Zusatz von Octylglycosid. Der Verdau wurde mit TFA abgestoppt, die Probe mit α-Cyano-4-Hydroxyzimtsäure gespottet und an einem MALDI LTQ Orbitrap XL-MS von Thermo Scientific gemessen.

Vorläufige Daten

Ein zusätzlicher Elutionsschritt der wie oben beschrieben behandelten Membranen mit ACN erwies sich als störanfällig, da hierbei häufig Signale von Verunreinigungen (Polymer aus der Membran oder aus der Rotiblock-Lösung) zu verzeichnen waren. Auch das Aufkonzentrieren der Proben durch Einrotieren großvolumiger Verdauansätze (200µl) bei 40°C brachte keine Verbesserung gegenüber den direkt gemessenen Spektren der Verdaulösung. Die niedrigste Menge an BSA, die noch gute Peptid-Spektren lieferte, konnte mit der Ein-Schritt-Methode erreicht werden und liegt im Schnitt bei nur wenigen hundert fmol. Die bisherigen Membranverdau-Protokolle konnten damit für den fmol-Konzentrationsbereich optimiert werden und sind für das hier verwendete Modellprotein BSA vergleichbar mit dem entsprechenden Lösungsverdau, bieten aber gleichzeitig den Brückenschlag zur SDS-PAGE.

Neuartige Aspekte

Die bisherigen Membranverdau-Protokolle wurden auf ihre Eignung für den fmol-Bereich hin untersucht.

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Evaluation of targeted proteomic approaches on a Q-TOF system

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Einleitung

Mass spectrometry-based quantitative proteomics is a popular tool for biomarker discovery. Advances in the speed of separation of proteomics samples and the MS data quality also enable the subsequent validation of discovered protein biomarkers in complex biological matrices by targeted approaches. Typically, MRM approaches are applied for validation tasks. Nevertheless, those methods require detailed target knowledge and do not allow the detection of unknown in addition. We describe here targeted proteomics approaches using the high resolution and accuracy achievable with an Ultrahigh Resolution (UHR) Q-TOF system. Different data acquisition methods are used for targeted quantitative proteomics, namely MS-based acquisition, broad band collision induced dissociation (CID), and middle band CID.

Methodik

Target protein quantification is done in a complex *E. coli* background (500ng) spiked with different amounts of a proteomic standard, which spans a concentration range of 6 decades in total (UPS-2, Sigma). Protein identification information, including m/z values and retention times, used as targets for quantification is obtained out of the equimolar proteomic standard (UPS-1, Sigma) containing the same set of proteins as the dynamic range mixture. Tryptic peptides are separated on a nanoUHPLC system (RSLCnano, Dionex) and data acquisition is done using an Ultrahigh Resolution (UHR)-TOF (maXis impact, Bruker) equipped with a CaptiveSpray ionization MS source.

Vorläufige Daten

Peptide targets for quantitative proteomics are obtained out of an equimolar proteomic standard by a data-dependent MS/MS approach. Results of different identification runs are combined and reveal successful identification of all 48 proteins. Creation of high resolution extracted ion chromatograms used for quantification is done based on the information of identified peptides with regard to m/z values and retention. Quantification of the target peptides spiked into *E. coli* background based on pure MS data acquisition reveals reliable results of targeted proteomics. In total 28 proteins were significantly regulated ($p<0.05$) demonstrating a high quantification efficiency from 500 fmol down to the amolar range. Results of targeted proteomics approaches using broad band and middle band CID will not only provide quantitative but also qualitative data in single runs. Quantification efficiency with regard to the coverage of a high dynamic range of these methods will be compared to the pure MS-based acquisition.

Neuartige Aspekte

Targeted proteomics approaches using the high resolution and accuracy achievable with an Ultrahigh Resolution (UHR) Q-TOF system

Mass Spectrometric Identification of Metastasis Protein Ezrin in Sentinel Lymph Nodes of Breast Cancer Patients Reveals Two Subgroups

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Einleitung

The reason for most cancer deaths are not primary tumors but distant metastases. In breast cancer patients sentinel lymph node biopsy is routinely applied to determine whether tumors had begun to spread. Because axillary lymph node status is an important clinical indicator of prognosis for survival, complete axillary lymph node dissection is recommended in cases of metastasized sentinel lymph node(s) [1]. Metastasis potential of tumor cells is crucially influenced by processes of cytoskeleton remodeling and cell adhesion. In particular, ezrin is known to participate in metastasis of breast cancer cells and its expression is estrogen regulated [2]. Ezrin functions as crosslinker between membrane and cytoskeleton. It is involved in cell adhesion, motility, and morphogenesis as well as signaling pathways such as Rho GTPase signal transduction [3]. We investigated ezrin expression in breast tumors [4,5] and in metastasized axillary lymph nodes of breast cancer patients by mass spectrometric proteome analysis. In addition to ezrin expression determination, structural characterization showed truncations of ezrin by in-depth mass spectrometric analysis. Structural characterization of involved proteins is of need for a basic understanding of the molecular and cellular processes in metastasis, for advancement of diagnostic applications, and to suggest potential new therapeutic targets.

Methodik

Primary tumors and metastasized sentinel lymph nodes were taken from breast cancer patients. Clinical parameters such as tumor grade, nodal and receptor status were provided for all patients. Protein extracts were generated after pathological examination and subjected to 2D gel electrophoresis (500 mg, pH 3-10 NL). Two gels per sample were run. Coomassie stained gels were analyzed with Progenesis PG200, Version 2006 (Nonlinear Dynamics Ltd). Protein spots of interest were excised, tryptically digested and peptide mixtures were analyzed with a Reflex III MALDI ToF mass spectrometer (Bruker Daltonik) for protein identification and sequence coverage analysis. Additionally, ezrin was detected by Western blotting using a monoclonal antibody against the C-terminal region (aa 362-585) (Pierce Biotechnology).

Vorläufige Daten

Ezrin was successfully identified in well-defined spots in 2D gels generated from tissue of primary breast tumors as well as from metastasized sentinel lymph nodes of breast cancer patients using peptide mass fingerprinting. Typically 2D gels showed about 1000 spots from which ca. 10% have been found differentially regulated, i.e. more than two-fold difference in spot abundances. In-depth MS and MS/MS measurements revealed that ezrin was present as full-length protein and as N-terminally truncated protein derivatives in both, primary tumor and metastasized sentinel lymph node samples. These results were confirmed by Western blotting of 1D- and 2D gels from the same protein extracts and staining with monoclonal anti ezrin antibody. We found that full length ezrin, migrating at about 80 kDa, was present in all samples in nearly equal amounts. Additionally, we found truncated ezrin forms migrating at about 60 kDa (covering about 500 aa in the C-terminal protein part) and 45 kDa (corresponding to about 400 aa of the C-terminal region), respectively. This finding opens the way to study molecular pathways leading to ezrin truncation in breast cancer and in sentinel lymph node metastasis of breast cancer patients, respectively, posing the question of its functional significance. Most interestingly, densitometric analysis of the 60 kDa bands from Western blot analyses allowed a statistically significant differentiation of the sentinel lymph node samples into two groups, enabling to correlate these sub-grouping with clinical parameters. Also, a stronger presence of the 60 kDa band seems to correlate with a lesser abundance of the 45 kDa band and vice versa. Detailed protein structure investigations of metastasis proteins are a prerequisite for the basic understanding of molecular and cellular processes crucial for metastasis and for the identification of key-markers for diagnostics and prognostics or future therapeutic targets in clinical applications.

Neuartige Aspekte

A truncated form of metastasis protein ezrin differentiates sentinel lymph node samples of breast cancer patients into two subgroups.

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Exploring the dynamic range of label-free mass spectrometric quantification in complex samples

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Einleitung

The proteome is characterized by its high complexity and wide range of analyte concentrations which are constantly in flux due to developmental and stress-related processes. Thus, discovery proteomics approaches are required which are completely unbiased and cover the complete proteome.
Accurate quantification of differently expressed proteins is still challenging and relies heavily on a stable analytical platform. Due to advances in instrumentation it is possible to perform quantification without the need for labeling based on the comparison of ion intensities delivering quantitative information for a theoretically unlimited number of samples.
In this study we will investigate the quantitative performance of label-free approaches with a focus on dynamic range.

Methodik

Tryptic peptides were separated on a nanoUHPLC system (U3000 RSLCnano, Dionex) using a 120min gradient and data for label-free quantification were acquired with an Ultrahigh Resolution (UHR) Q-TOF system (maXis impact, Bruker Daltonics) equipped with a CaptiveSpray ionization MS source. A proteomic standard of 48 proteins spanning a concentration range of 6 decades in total (UPS-2 standard, Sigma) was used to determine the dynamic range for label-free quantification. This standard was spiked at two different concentrations (250 fmol to .5 amol and 500 fmol to 5 amol) into a highly complex *E. coli* background (500ng) mimicking the complexity typically found in biological samples. The label-free quantitation workflow was based on signal intensities from LC-MS runs for quantification.

Vorläufige Daten

The determination of the dynamic range of label-free quantification was performed for a standard protein mixture spiked at 2 different concentrations into a constant *E. coli* background. The experimental setup covered concentration ranges from 500fmol down to 2.5amol. Separation of tryptic peptides on a nanoLC system coupled to UHR-TOF detection showed excellent retention time and signal intensity stability for five replicates per concentration, both being a prerequisite for label-free quantification studies. Quantitative information was obtained from MS signal intensities. Signals originating from the same peptide, e.g. different charge stages and isotopes were combined and used for quantification. Results show almost no variation for E.coli background, whereas regulation ratios were determined for the standard peptides spiked into the complex background. In total 27 proteins from the UPS2 mixture were detected as significantly regulated ($p<0.05$). Very accurate quantification was obtained down to the low fmol range. Results also show the label-free approach to be capable of providing the correct quantification of peptides in the amol range. The high dynamic range of this approach allows in-depth studies of quantitative changes in biological systems. Therefore the method will be applied to determine the effect of sodium butyrate treatment of Chinese hamster ovary cells.

Neuartige Aspekte

We will investigate the quantitative performance of label-free approaches with a focus on dynamic range

HPTLC als alternative Trennmethode zur Identifizierung von Proteinen und Peptiden mit der Massenspektrometrie

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Einleitung

Die Dünnschichtchromatographie ist ein lange bekanntes Trennverfahren, welches aufgrund mangelnder Leistungsfähigkeit jedoch bisher wenig Anwendung in der modernen Hochleistungsanalytik fand. Gegenwärtig wird diese Methode in Form der HPTLC (*High performance thin layer chromatography*) aber zunehmend als hochempfindliche, präzise und schnelle Technik mit Anwendungspotenzial in verschiedenen analytischen Bereichen eingesetzt. [1] Durch Automatisierung und Verbesserung der Sorbentien konnte eine Steigerung der Trennleistung und eine Erhöhung der Präzision sowie der Reproduzierbarkeit erreicht werden. Ein großer Vorteil dieses Verfahrens liegt in der Möglichkeit der simultanen Analyse sowie der vielfältigen Detektions- und Kopplungsmöglichkeiten. Dabei bieten sich Möglichkeiten der massenspektrometrischen Kopplung, insbesondere der HPTLC-MALDI-MS, der HPTLC-nanoESI-MS oder der HPTLC-DESI-MS an. [2, 3] Daher kann die HPTLC eine vielversprechende Alternative zu herkömmlichen HPLC-Trennungen in der Protein- und Peptidanalyse darstellen.

Methodik

Die Identifizierung von Proteinen wurde modellhaft mit Peptiden des tryptisch verdauten Myoglobins durchgeführt. Die Trennung der Peptide erfolgte mittels HPTLC unter Verwendung von Kieselgelplatten und einem Laufmittelgemisch (2-Butanol/Pyridin/Ammoniak/Wasser). Die Peptide wurden nach der Auftrennung mittels UV-Detektion sowie gegebenenfalls mittels verschiedener Derivatisierungsreagenzien (Fluorescamin oder Ninhydrin) visualisiert (TLC Visualizer, CAMAG Chemie-Erzeugnisse & Adsorptionstechnik AG & Co. GmbH, Berlin). Die massenspektrometrische Analyse erfolgte per MALDI-TOF(/TOF)-MS (ultrafleXtreme, Bruker Daltonik GmbH, Bremen) nach Beschichtung der Platten mit Dihydroxybenzoesäure als Matrix oder per nanoESI-Ionenfallen-Massenspektrometrie (amaZon ETD, Bruker) über die *liquid extraction surface analysis*-Methodik (LESA^{TM}) unter Verwendung des TriVersa Nanomate® (Advion, USA). Beim LESA-Verfahren werden die Peptide durch einen Tropfen des Extraktionslösungsmittels direkt von der Plattenoberfläche nach einem Dosier-/Ansaugzyklus extrahiert.

Vorläufige Daten

Während bislang gezeigt werden konnte, dass eine Analyse von Standardpeptiden mittels HPTLC-MALDI-TOF-MS möglich ist [3], erfolgte im Rahmen dieser Arbeit vergleichend eine Identifizierung von tryptisch erzeugten Peptiden mittels HPTLC-MALDI-MS und HPTLC-nanoESI-MS. Hierfür wurden die Parameter: stationäre Phase (einschließlich der Trennleistung), Detektierbarkeit und Nachweisgrenze beider Methoden getestet. Im Anschluss an die Trennung mittels HPTLC ist es sowohl mit der ESI- als auch mit der MALDI-Kopplung möglich, tryptische Peptide zu analysieren und durch MS/MS-Spektren Sequenzinformation für die Identifizierung unbekannter Proteine zu erhalten. Die Nachweisempfindlichkeit der HPLC-MS-Messungen kann dabei noch nicht ganz erreicht werden. Es ergeben sich aber einige Vorteile gegenüber herkömmlichen LC-MS-Messungen; dazu gehören die simultane Auftrennung mehrerer Proben, die Verwendung nicht-LC-MS-geeigneter Lösungsmittel oder Lösungsmittelgemische, sowie die Derivatisierung der Analyten. Die Analyse von tryptischen Peptiden steht dabei noch ganz am Anfang ihrer Entwicklung und eröffnet ein weites Feld von Optimierungsparametern, um die Trennleistung und die Nachweisempfindlichkeit weiter zu verbessern.

Neuartige Aspekte

HTPLC-MS-Kopplung zur Analyse von Peptidgemischen.

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Affinity and structural characterisation of Abeta-autoantibdies

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Einleitung

Alzheimer's disease is one of the most common disorders with a progressive neuro-degeneration characterized by the abnormal accumulation of Abeta peptides in the extra-cellular space between neurons, forming amyloid plaques. At present, the amyloidogenesis process and causality is not understood, aswell as the Abeta peptide clearance from the brain in the healthy patients. Abeta-autoantibodies levels and functionality may explain the abeta peptide removal from the brain. Design of future immunotherapies for the treatment of Alzheimer's disease must take into consideration Abeta-autoantibodies - Abeta specific interaction and structural particularities of Abeta-autoantibodies.

Methodik

Previous work performed in our laboratory led to the identification of the epitope in Abeta(21-37). In this study, a complex analytical approach was used for the elucidation of the binding site of Abeta-autoantibodies, especially the core of the epitope. There were employed techniques such as: affinity chromatography, affinity- mass spectrometry, biosensor, biosensor- mass spectrometry coupling. For the structural analysis of Abeta autoantibodies, due to the high number of chain variants showed in 2D gel electrophoresis, a multiple stage strategy was employed, involving separation of the light and heavy chains, Edman sequencing of the light and heavy chain N-terminus, and parallel bottom up experiments with different proteolytic enzymes.

Vorläufige Daten

The results of the affinity experiments showed that the overlapped fragment present in all the affinity studies is Abeta(25-28). Our study showed that the epitope core Abeta(25-28) is not large enough or structured to bind by itself to Abeta- autoantibodies, a larger more rigid structure is needed, hence the affinity preference for a larger fragment of Abeta. The structural studies showed a high variability in the light chain N-terminus, and a dominant heavy chain. Both light chain and heavy chain were subjected to digestion with trypsin, chymotrypsin and LysC on parallel batches. The proteolytic peptides were isolated by analytical HPLC to reduce the complexity of the samples and then analysed by LC/MS/MS and/or Edman sequencing to obtain comprehensive information about their primary structure. All the results were pulled and sequences were compiled manually. We report here examples of complete sequences of both light and heavy chains.

Neuartige Aspekte

Abeta-autoantibodies show a high affinity for turn Abeta region, confirmed by their primary structure.

Referenzen

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Beta-casein phosphorylation in 4000-years old dairy products

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Einleitung

Phosphorylation of caseins, the major milk proteins plays a key role in formation of milk micellular structures and calcium retention, and is a quality marker in dairy industry [1,2,3]. We quantitatively compared the phosphorylation pattern of beta casein in contemporary dairy products and thousands years old products recovered in archeological excavations in China.

Methodik

Contemporary dairy products were either self-made or purchased locally: archeological samples were excavated from several Early Bronze Age sites in China. Proteins were extracted from ca. 15mg dried material by 10% SDS in 63mM TrisHCl and 10mM DTT) and then separated on 1D-SDS gel electrophoresis. GeLC MS-MS analysis was carried on a LTQ Orbitrap Velos mass spectrometer (Thermo Fisher Scientific). Database searches were performed by MASCOT against a customized cattle casein database without enzyme specificity restrictions and considering variable modifications specific to protein aging [4]. Label-free quantification was performed by integrating chromatographic peak areas of matched peptides by Progenesis (Nonlinear Dynamics). The relative abundance of phosphorylated sites was calculated as a ratio between intensities of corresponding phosphorylated and unphosphorylated peptides.

Vorläufige Daten

We compared the phosphorylation abundance of serine at position 50 of beta-casein [P02666] in fresh milk, dairy products (commercial and traditionally self-made kefirs and cheese) and 4000 years samples excavated in China. Over 94% of the corresponding peptides were phosphorylated in fresh milk and dairy products. Chymosin no acidic curdling of milk casein as well as fermentation by yeast and *Lactobacillus* species did not alter the phosphorylation profile. However, the phosphorylation rate of the same sites in ancient dairy samples did not exceed 11%, suggesting their slow age-dependent degradation.

Neuartige Aspekte

Phosphorylation of beta-casein is the aging marker for dairy products.

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Label-free STAT5A/B Isoform Ratio Determination by LC-MS

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Einleitung

The occurrence of genetically encoded protein isoforms is one principle by which the diversity of the proteome is increased. In general, protein isoforms share large identical sequence regions mixed with regions exhibiting minor differences extending over one or two amino acids or more extended sequence regions. Quantitative protein isoform analysis is of interest since protein isoforms may exhibit specific functions or may undergo specific covalent modifications. Methods for isoform-specific analysis of proteins mostly rely on immunological or mass spectrometric procedures. However, it is still challenging to obtain reliable quantitative results with these methods. In this work, we validate mass spectrometric relative isoform quantification by using reference mixtures of protein isoforms produced by cell-free synthesis and ICP-MS and show the characterization of the isoform-specificity of antibodies by mass spectrometric methods.

Methodik

STAT5A human and STAT5B human was prepared by cell-free synthesis, RISQ protein standards and selenium quantification by ICP-MS. Using these standards, samples with defined STAT5A/B isoform ratio were prepared. Quantitative STAT5A/B isoform analysis was performed by a combination of tryptic digestion and LC-ESI-MS/MS (UPLC, Waters and LTQ-Orbitrap, Thermo). Quantitative data evaluation was performed both manually and using MaxQuant. For immunoprecipitation we applied standard procedures using the STAT5 antibodies L-20, C-17, and N-20 (Santa Cruz). The corresponding blocking peptides were sequenced using nanoESI-QTOF-MS/MS.

Vorläufige Daten

Label-free relative quantification of STAT5A/B by LC-ESI-MS/MS revealed that the A/B ratios in our STAT5 isoform reference mixture were satisfactorily reproduced. Manual and MaxQuant-supported quantitative data were not significantly different. By applying this procedure to STAT5 fractions obtained by immunoprecipitation of identical cell lysates with three different antibodies, we found characteristic differences in the STAT5A/B ratio. Two antibodies were found to exhibit high specificity to either the A or B form, whereas one antibody showed an intermediate A/B ratio of 1:3. The experimentally observed antibody isoform specificities showed a good correlation to the degree of identity between blocking peptide and sequences of STAT5A and STAT5B.
The true STAT5A/B ratio was obtained by using immunoprecipitation by a 1:1 mixture of the two isoform-specific antibodies, recognizing only the A or B form, respectively. In this way, the STAT5A/B ratio in BAF3 cells was found to be about 2:1. This shows that the encoding of STAT5A and B by a single gene each does not transform into a 1:1 ratio of the corresponding proteins. Using this result, the antibody which is selective for both isoforms of STAT5 could be quantitatively characterized to be 85 % B-specific and 15 % A-specific. Additional data for STAT5A/B isoform abundances were generated with this methodology and cell-specific isoform distribution data were observed.

Neuartige Aspekte

Relative isoform quantification of STAT5A/B in cells by LC-MS & antibody isoform specificity characterization is described.

Cell-Free Synthesis of Stable Isotope and Selenium Labeled Protein Standards for Absolute Quantification

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Einleitung

Regulation of cellular processes is often achieved by quantitative changes of the proteome. Expression and modification of proteins can be followed by mass spectrometry (MS) in a highly specific manner. For this purpose, stable isotope labeling is often used, as for instance in SILAC [1], FLEXIQuant [2], or in the RISQ protein standards [3]. The latter standards carry two types of labeling, which are selenomethionine (SeMet) and selected stable isotope labeled amino acids containing ^{13}C and ^{15}N. This study reports on the use of RISQ, RSQ, and RIQ protein standards for quantification of cellular STAT5. The family members of STAT proteins are components of the JAK/STAT pathway of signal transduction, which is abnormally active in many forms of cancer.

Methodik

Using cell-free protein synthesis, standards containing (SeMet,[$^{13}C_6$]-Lys,[$^{13}C_6$,$^{15}N_4$]-Arg)-STAT5 (= RISQ STAT5), (SeMet)-STAT5 (= RSQ STAT5), and ([$^{13}C_6$]-Lys,[$^{13}C_6$,$^{15}N_4$]-Arg)-STAT5 (= RIQ STAT5) were prepared. Expression vectors were generated using the Gateway system. The corresponding plasmids contained the STAT5 genes and an additional His-tag. Purification of standards was achieved by Ni^{2+}-affinity chromatography, dialysis, and the combination of SDS-PAGE and electroelution. RISQ- and RSQ- standards containing SeMet were quantified by inductively-coupled plasma ionization (ICP)-MS and selenium detection. Standards containing stable isotope labeled amino acids were used as standards for LC-electrospray ionization (ESI)-MS analyses. RIQ standards had to be quantified by a two-step procedure. First, the RSQ protein was quantified by ICP-MS, which then was used for quantification of the RIQ protein by LC-ESI-MS.

Vorläufige Daten

Cell-free synthesis of STAT5 proteins was performed using a batch system and a continuous-exchange cell-free (CECF) system. The latter system delivered an increased protein yield. SeMet, [$^{13}C_6$]-lysine and [$^{13}C_6$,$^{15}N_4$]-arginine were successfully incorporated into the protein standards which was confirmed by LC-MS/MS analysis. These proteins are named RISQ standards (for **R**ecombinant **I**sotope Labeled and **S**elenium **Q**uantified). For purification of standards the combination of SDS-PAGE and electroelution proved to be most successful. ICP-MS and selenium detection resulted in a concentration of 170 ± 9 fmol/µl for RISQ-STAT5B human, and of 88 ± 5 fmol/µl for RSQ STAT5A mouse (Lys, Arg, SeMet), respectively. Using the latter standard and LC-ESI-MS, a concentration of 883 ± 88 fmol/µl was determined for RIQ STAT5A mouse (Lys+6, Arg+10, Met). The two-step concept for production of RIQ secondary standards has the advantage that the final standard does not contain SeMet, but that its quantification is still based on Se-quantification of the RSQ primary standard. In quantification of a RIQ standard versus the target protein, all methionine-containing peptide pairs can be included into the quantification. Finally, using the RIQ STAT5A mouse standard (Lys+6, Arg+10, Met), cellular STAT5A mouse was quantified in BAF_3EpoR-cells at 250.000 ± 40.000 copies per cell. For this cell line the determined STAT5A copy number is within the expected range. The MS data show a reduced error margin compared to existing data based on quantitative immunoblots.

Neuartige Aspekte

Improved stable isotope labeled STAT5 family protein standards for absolute quantification in cell lysates

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ToF-MS for the analysis of intact proteins from natural product biosynthetic pathways

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Einleitung

Microbial natural products play an important role for the discovery of novel pharmaceutical lead structures.[1,2] Many of these secondary metabolites are synthesized by non-ribosomal peptide synthetases (NRPSs) and polyketide synthases (PKSs); both being large multi-modular biosynthesis enzymes that may reach several 100 kDa in size.[3,4] Within these systems each module is responsible for one elongation step during biosynthesis. Individual modules consist of enzymatic domains catalyzing a distinct biochemical transformation, e.g. transfer of an extender unit to a carrier domain. Here we describe the detection of recombinantly expressed NRPS domains and modules in apo and holo state, as well as loaded with extender units of different kinds, resulting in analytes ranging from 70 to 180 kDa in size.

Methodik

An LC-MS method for intact protein analysis was established based on a Dionex Ultimate 3000 RSLC system coupled to a Q-ToF-MS (Bruker maXis 4G) using positive ESI-mode. A C8 column with 3.6 µm core shell particles was used at elevated temperature of 45 °C. Recombinantly expressed proteins were enriched by open column affinity chromatography prior to LC-MS. An initial chromatographic run was mandatory for a good MS signal since the proteins of interest are highly susceptible to precipitation upon attempts of buffer exchange. The chromatographic approach also enabled injections of samples from in vitro assays without further workup.

Vorläufige Daten

Along with the biochemical analysis of molecular mechanisms underlying NRPS and PKS assembly lines, several MS-based approaches emerged. The aim is usually the detection of biosynthetic intermediates and extender units which are covalently attached to a phosphopantetheinyl moiety (Ppant) of a carrier domain (PCP or ACP). Most reported procedures include full or partial digestion of the proteins.[5] However, these methods demand for very high peptide coverage as one must find the peptide of interest amongst all other peptides.
Using intact proteins can in principle overcome this problem, but requires careful method development. In this study, we detected several NRPS modules and domains and distinguished easily between apo and holo forms. Moreover, we also checked whether we can detect biosynthesis intermediates while being attached to a protein. An NRPS-related two-domain protein (A-PCP, 76 kDa) was detected in apo form, holo form, as well as loaded with different substrates (extender units) with errors of less than 2 ppm for the deconvoluted masses. We thereby unambiguously verified the modules active state as well as its ability to make use of different, non-natural substrates. By offering different extender units to the module it was also possible to determine in vitro the preferred substrates of the enzyme. Establishing this methodology covered sample preparation, optimization of the LC
conditions and thoroughly tuning of MS parameters. Especially the influence of His-Tag modifications as well as charge state modulation turned out to be of importance when measuring the proteins of interest.
In conclusion, the presented Q-ToF-MS approach is a suitable method for the characterization of recombinantly expressed NRPS-related proteins with increased information content beyond SDS-PAGE.

Neuartige Aspekte

Straight forward TOF-MS method for detection of biosynthetic intermediates while covalently attached to intact biosynthetic proteins.

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Development of a new proteomics approach by direct MALDI-MS analysis of dry polyacrylamide gels using stain-free protein detection

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Einleitung

Gel electrophoretic separation using protein visualization by staining procedures such as Coomassie Brilliant Blue, followed by spot picking, destaing, proteolitic digestion and MALDI-MS analysis has been a widely established method in mass spectrometric proteomics. However, significant drawbacks and limitations of this approach arise from the high background in the mass spectrometric analysis. A new approach for MALDI-MS protein analysis has been recently described by the Laboratory of Analytical Chemistry, University of Konstanz using the LaVision BioTec stain-free gelreader for electrophoretic separation and visualization based on native protein fluorescence [1].

Methodik

In this study we report the MALDI-MS identification of (i) intact proteins, directly from dry SDS-PAGE gels, and (ii) peptide fragments resulted from proteolitic digestion of gel separated proteins. For the identification of intact proteins, the gels were dried using cellophane foil, affixed in plastic frames; the dry gel was attached to the MALDI target, matrix was added, and the analysis was performed. For peptide mass fingerprinting, the protein containing gels were dried over night in open air, allowed to reswell in a trypsine containing buffer, digested for 8h at 37° C, re-dried using cellophane and analysed by MALDI-MS. The matrix solution was applied on the dry gels using the MALDI spotter SunCollect from SunChrom.

Vorläufige Daten

The protein visualisation using the BioAnalyzer from LaVision BioTec is based on the native flurescence of proteins in UV domain, caused by the aromatic amino acids. The identified intact proteins were ß-lactoglobulin I, ß-lactoglobulin II and lysozyme from donkey milk. Different peptides were identified from BSA containing gels which were digested with trypsine and analysed directly by MALDI-MS, allowing the peptide fingerprinting of the protein. As the whole approach involving digestion proved to be difficult to perform on whole gels, several adjustments were made to the procedure, the most important being the fixation of the protein bands with TCA and the use of low percentage glycerol buffer for the second drying step. The new method proves the feasibility of MALDI analysis directly from gels, and the integration of native fluorescence protein detection in MALDI sources will provide a high-throughput tool for proteomics.

Neuartige Aspekte

The new proteomics approach allows direct analysis of proteins and peptides directly from dry-gels, visualised by protein native fluorescence.

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High resolution MALDI imaging of tryptic peptides after on-tissue digestion

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Einleitung

Mass spectrometry imaging is a method of scanning a sample of interest and generating an image of the intensity distribution of a specific analyte signal. Spatial distributions of proteins can be evaluated to identify potential markers for pathological processes. The direct detection and identification of intact proteins in MS imaging is difficult due to a limited mass range and fragmentation efficiency. On-tissue digestion of proteins and detection of resulting peptides can overcome some of these limitations. Here we present new approaches for on-tissue tryptic digestion which is currently one of the most active fields in MS imaging. We focus on optimizing the spatial resolution and reliability of peptide identification. Practical issues of sample preparation and data handling will be discussed. Even though experiments are partially based on home-built instrumentation, a large part of the presented concepts and approaches is generally applicable.

Methodik

A series of ethanol/water washing steps was applied to tissue sections for fixation and to remove salts and lipids. Subsequently trypsin solution (0.05 µg/µL in NH_4HCO_3 buffer) was deposited (15 cycles à 2-10 µl) on tissue with a home-built spraying device [1][2]. The matrix dihydroxybenzoic acid (30 mg/mL in acetone/H_2O, 1:1, v/v with 0.1% TFA) was applied using the same device. An atmospheric pressure scanning microprobe matrix assisted laser desorption (AP-SMALDI) ion source was used for imaging [3]. The source was coupled to a Q Exactive orbital trapping mass spectrometer (Thermo Fisher Scientific GmbH, Bremen).

Vorläufige Daten

Tryptic peptides were identified by matching imaged m/z peaks to peptides which were identified in complementary LC-MS/MS measurements of homogenized tissue sections (adjacent to the imaged section). This procedure was based on accurate mass measurements (< 3 ppm RMS) and provides a confident identification of tryptic peptides. Peptide peaks (at *m/z* 700) were detected on-tissue with a mass resolution of up to R = 80000 under imaging conditions. This high mass resolution improves the specificity of the spatial distribution information by reducing interferences with neighbouring m/z peaks.
Human gastric cancer tissue was imaged at 50 µm spatial resolution. Numerous peptides with distinct spatial distributions were identified. This data can be used to investigate intratumor heterogeneity on a molecular level. A whole body section of an infant mouse was imaged at a spatial resolution of 50 µm. Tryptic peptides were identified which were specifically located in individual organs such as brain, liver and muscles. This measurement was divided in four data files which comprise a total of 230000 spectra (corresponding to more than 40 GB of data). A coronal mouse brain section was imaged at a pixel size of 25 µm. The resulting ion images of tryptic peptides showed excellent correlation with histological examination (myelin and H&E staining). This measurement constitutes a significant improvement in spatial resolution for tryptic peptides compared to our previous studies (50 µm) [1] and to published results (150 µm).
The optimization of on-tissue digestion will be continued in the framework of a comparison study of the EU-funded COST Action (European Cooperation in Science and Technology) „Mass Spectrometry Imaging: New Tools for Healthcare Research" (BM1104) which will be briefly discussed.

Neuartige Aspekte

Reliable identification of tryptic peptides in MS imaging with a spatial resolution of 25 µm

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Der Variantenreichtum von CapG - ein Potpourri an Proteinmodifikationen

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Einleitung

Actin-bindende Proteine wie das CapG (gCap39, Makrophagen Capping Protein, Mbh1) spielen eine Rolle bei der Invasion und Metastasierung eines Tumors. Tumore wie Glioblastome und Melanome, die häufig Metastasen bilden, weisen eine erhöhte CapG-Expression auf. Ein bedeutender Faktor ist dabei offenbar die subzelluläre Lokalisation von CapG. Es wird ein direkter kausaler Zusammenhang zwischen einer erhöhten Expression von CapG und dem Metastasierungs- bzw. Invasionspotential vermutet. Interessanterweise ist die Expression von CapG in Mamma- und Pankreaskarzinomzellen durch mehrere Proteinvarianten unterschiedlicher isoelektrischer Punkte geprägt. Es stellt sich daher die Frage, welche Modifikationen zu diesen Varianten führen, und welchen potentiellen Einfluss sie auf die Lokalisation von CapG haben.

Methodik

Es werden vergleichende Analysen von nativem CapG aus Karzinomzellen (Mamma- und Pankreaszellen), sowie überexprimiertem CapG aus *Pichia pastoris* durchgeführt. Nach Zellaufschluss erfolgt eine Anreicherung von CapG mittels Hydrophobizitäts-Interaktions Chromatographie (HIC) über eine Phenylsepharose-Matrix. Neben dem Gesamtzellextrakt werden auch subzelluläre Fraktionen (Cytosol und Kern) untersucht. Die anschließende zwei-dimensionale Gelelektrophorese (2-DE) erfolgt in immobilisierten pH-Gradienten (pH 4-7, 18 cm). Proteinmuster werden mit einer hochsensitiven Coomassie-Färbung [1] sowie einer phosphospezifischen Färbung visualisiert. Die massenspektrometrischen Analysen erfolgen an einem QqTof Instrument.

Vorläufige Daten

Mittels 2-DE kann CapG in bis zu zehn Spot-Isoformen aufgetrennt werden. Zum einen konnte eine Arginin- (R_{335}) und eine Histidinvariante (H_{335}) nachgewiesen werden, deren Austausch auf einem Single-nucleotid-Polymorphism (SNP rs6886) zu basieren scheint. Diese Aminosäurevarianz ist im 2-DE Gel charakteristisch als Doppelspot-Muster mit minimaler Molekulargewichtsänderung dargestellt. Die Analysen der Perlenketten-Spots haben ergeben, dass die geringfügigen Änderungen im isoelektrischen Punkt zum einen auf N-terminale Modifikationen (Acetylierung+Oxidation), limitierte Proteolyse von 3, 4 bzw. 6 Aminosäuren, und möglicherweise Phosphorylierung (S_{337}) zurückzuführen sind. Trotz dieses Potpourris an Modifikationen konnten wir bisher noch nicht alle Spotvarianten von CapG charakterisieren. Ausstehende Analysen vor allem Kompartiment-spezifischer Diversitäten sollen Aufschluss über das Metastasierungs- bzw. Invasionspotential der modifizierten CapG-Varianten geben.

Neuartige Aspekte

Perlenkettenmuster im 2-DE Gel nicht ausschließlich bedingt durch Proteinphosphorylierung

Referenzen

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Identification of new protein coding sequences and signal peptidase cleavage sites of Helicobacter pylori strain 26695 by proteogenomics

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Einleitung

Conventional proteomic studies almost exclusively rely on correct annotation of protein coding genes which are deposited in freely available databases like UniProt or NCBI. However protein coding gene annotation is usually solely based on gene-finding software like IMG, RAST, Glimmer, or GeneMark. The annotation accuracy of these tools is limited which inevitably leads to missing or incorrect protein annotations such as erroneously determination of gene boundaries. Furthermore it is even harder to additionally correctly predict signal peptides. Proteogenomics is a technique able to detect missing protein annotations as well as prediction errors. In a proteogenomics approach the existing database of the investigated organism is combined with a six-frame translation of the genome. A proteomic dataset which should be as complete as possible is searched against this combined database. Peptide matches which are unique to the six-frame translation are used to determine errors in the database or to identify novel protein coding genes. Here, we made a proteogenomics study of *Helicobacter pylori* strain 26695. This major human pathogen inhabits the gastric mucosa of about 50% of the world's population. In recent years many proteomic studies were carried out on *H. pylori*. However, this is the first proteogenomics study of this organism.

Methodik

Cell lysates of two biological replicates were either subjected to SDS-PAGE or to SEC. 20 gel fractions were digested with trypsin. Four SEC fractions below 25 kDa were subjected to in-solution digestion separately with trypsin, LysC and AspN. Each fraction was analyzed on a nanoUPLC coupled to an Orbitrap XL mass spectrometer. Mascot and X!Tandem were searched against a reverse concatenated NCBI database of *H. pylori* strain 26695 combined with a six-frame translation of the genome (FDR < 1%). An external dataset of Jungblut et al. [1] was analyzed identically. Peptide identifications unique to the six-frame translation were further investigated by their genomic locations using the UCSC genome browser. Signal peptidase cleavage sites were identified by semi-proteolytic database searches.

Vorläufige Daten

The database search revealed overall 1115 identifications of proteins annotated by NCBI which represents 71% of the proteome of *H. pylori* strain 26695. The SEC approach offered 24 additional proteins to the GeLC-MS approach which were all below 17 kDa. With our dataset, we were able to identify four new protein annotations which were previously missing in the NCBI protein annotations. A ferrous iron transporter protein similar to other *H. pylori* strains was identified in the intergenic region between HP0585 and HP0586. The other three novel protein annotations of HP0744, HP0619 and HP0058 were previously missing due DNA sequencing errors which resulted in frame shifts. Medigue *et al.* [2] made a comparative genome study which showed inconstancies of these regions in different *H. pylori* strains. Furthermore, we could unambiguously correct annotations of six different proteins which were a result of wrong translation start assignment or DNA sequencing errors. For signal peptidase cleavage site annotation, we searched Mascot and X!Tandem with semi-proteolytic specificity. This means that only one term of a peptide has to be cleaved specifically by the applied protease. Non-specific cleaved protein N-termini were supposed to be cleaved by signal peptidases. Overall signal peptidase cleavage sites of 72 proteins could be identified. Signal peptidases of gram negative bacteria have a more or less conserved recognition sequence at the -1 and -3 positions relative to the cleavage site. Our data shows that the predominant signal peptidase recognition sequence is LXA for *H. pylori*. In conclusion, we could correct six protein annotations, identify four new proteins and detect signal peptide cleavage sites for 72 proteins. We show that protein databases are partly erroneously even for a well-studied organism like *H. pylori*. Therefore we highly recommend the application of proteogenomics in new sequencing projects to generate more accurate databases for proteomic studies.

Neuartige Aspekte

Our proteogenomics workflow combines database construction, proteomic and genomic data analysis for improvement of protein annotations and signal peptide identification.

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ERLIC – an efficient in-depth analysis strategy for simultaneous analysis of proteome and phosphoproteome of human cells via multidimensional LC-MS-based approaches

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Einleitung

Proteins and their modifications are key players of many biological processes. Because of its role as a molecular on/off-switch for enzymatic activity, phosphorylation is by far the most studied post translational protein modification (PTM). Thanks to advances in liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), it is now possible to detect and monitor phosphorylation in biological samples. However, due to its substoichiometric nature, there is the need for selective enrichment prior LC-MS/MS analysis when analyzing complex mixtures. Besides offline affinity chromatography, various HPLC-based techniques like IEX or HILIC have been used in the past decades targeting either the higher hydrophilicity (HILIC) or the reduced net charge (IEX) of phosphopeptides [1]. Here we describe the use of a mixed-mode chromatography, termed electrostatic repulsion hydrophilic interaction liquid chromatography (ERLIC) that combines HILIC and IEX to retain net charge-reduced, polar compounds like phosphopeptides after tryptic digest of whole cell protein fractions.

Methodik

ERLIC separation of tryptic HeLa peptides was performed using a 150 x 1.0 mm PolyWAX^{TM} LP column (PolyLC Inc., Columbia, MD). An Ultimate Classic HPLC system (LC Packings, NL) was used in the first dimension. Sodium methylphosphonate (solvent A) and triethylammonium phosphate (TEAP) (solvent B) buffers were prepared as stock solutions at a pH of 2.0 [2]. The fractionation was carried out within a short gradient of 0 – 100 % B in 5 min.
For desalting the ERLIC fractions, porous graphitic carbon (PGC), reversed phase (RP) and polyHYDROXYETHYL A (HILIC) solid phase extraction (SPE) materials were used in various combinations. After desalting, the peptides were identified via online nano-LC MS/MS.

Vorläufige Daten

In this work we prove evidence of the applicability of ERLIC for bottom up proteome and phosphoproteome analysis of HeLa cells with only 200 µg starting material per replicate. ERLIC is not only efficient in separating phosphopeptides from nonphosphorylated ones, but also distinguishes between different phosphopeptide species. We describe the combinatorial use of different SPE materials like PGC, RP and HILIC for desalting of ERLIC fractions with respect to phosphorylation state and coverage of different subsets of the phosphoproteome.
Hence, we were able to identify more than 20,000 peptides, of which 2,000 contained at least one phosphorylation site, proving the high potential of the used techniques. Additionally, we demonstrate that ERLIC is able to separate phosphopeptides by their phosphorylation state.

Neuartige Aspekte

Obtained results can now be used for setting multidimensional approaches like ERLIC-Hypercarb-LC-MS/MS or ERLIC-HILIC-RP-MS/MS for online fractionation, desalting and analysis

Referenzen

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Novel approach for the labeling of biopolymers with DOTA complexes using click chemistry

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Einleitung

The quantitative determination of bio-molecules is an interesting topic with direct application to proteomics. In the last years, our group contributed to this challenge by developing metal-coded affinity tag (MeCAT) labels which target cysteine groups of proteins and peptides (1) and allow elemental and molecular mass spectrometry monitoring. In this regard, new labels are being studied trying to improve the labeling efficiency and reduce the steric hindrance.

Methodik

For the purpose mentioned, a new labeling procedure based on click chemistry (2) were tested. At first, the thiol group of cysteine was modified with a small residue containing a terminal alkyne. After that, without any washing step, the metal-harboring DOTA-azide complexes were introduced via click chemistry.

Vorläufige Daten

The labeling efficiency of the methodology was optimized using standard peptides and then applied to bovine serum albumin (BSA) digests. For all the tested standard peptides complete labeling was demonstrated. In the case of the digested BSA (25 cysteine-containing peptides), at least 88% coverage of the cysteinyl sequence was achieved based on this two-step labeling strategy. DV**C**K and **CC**AADDK cannot be detected, neither unlabeled nor labeled. In the case of E**CC**HGDLLE**C**ADDR, it can be found not totally labeled, with only one metal label or fully labeled. The rest of the peptides were found totally labeled. It is worth to mention that peptides with two adjacent cysteine residues (**CC**TKPESER, **CC**TESLVNR, E**CC**DKPLLEK, ETYGDMAD**CC**EK, YNGVFQE**CC**QAEDK, EYEATLEE**CC**AK) were totally labeled. Therefore, the lower steric hindrance using this click chemistry-based approach seems to enhance the labeling efficiency. Moreover, this strategy avoids diastereomeric products, improving the results obtained with the maleimide MeCAT. Fragmentation experiments were conducted with satisfactory peptide identifications as well as the identification of a typical reporter ion from the label.

Neuartige Aspekte

High efficiency and robustness of labeling biopolymers based on Click Chemistry have proved to be the main advantages

Referenzen

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Molecular Characterization of Neuronal Protein Aggregates by Mass Spectrometry-based footprinting methods

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Einleitung

Aging is a major risk factor for the neurodegenerative disorders of Alzheimer's disease (AD) and Parkinson's disease (PD), and the number of people with these conditions is increasing rapidly. Formation and accumulation of fibrillar plaques of ß-amyloid peptide (Aß) and α-synuclein (Syn) in brain have been recognized as characteristics of AD and PD. Although circular dichroism, atomic force microscopy, electron microscopy, light scattering were directed at understanding these molecules, a detailed understanding of protein aggregation remains to be achieved. Understanding how these proteins assemble will provide new targets for the development of aggregation modifiers that could potentially limit their toxicity. In our work, we are investigating two aggregating proteins, Aß and synuclein, as model systems around which we will develop MS strategies.

Methodik

Various protein footprinting strategies were employed to study oligomerization of Aß and synuclein: high resolution MS in combination with affinity-biosensor system, H/D amide exchange, and FPOP (fast photochemical oxidation of proteins). Recently, a new method for determination of protein-ligand interaction was developed in our laboratory by direct coupling of surface acoustic wave biosensor with MS. This method enables the determination of the binding stoichiometry and affinity of synuclein-abeta peptides interactions. FPOP is a chemical footprinting method whereby exposed amino-acid residues are covalently labeled by oxidation with hydroxyl radicals produced by the photolysis of hydrogen peroxide. Modified residues can be detected by standard trypsin proteolysis followed by LC/MS/MS, providing information about solvent accessibility of various residues of the protein.

Vorläufige Daten

The oligomers were prepared from chemically synthesized $A\beta_{WT}$(1-40) and recombinant human alpha-synuclein (1-140) monomers by using different protocols for which we changed several factors that influence the oligomerization; examples are peptide/ protein concentration, salt nature/content, temperature, pH. By determining the global incorporation of deuteriums, we found that 29.4 ± 0.8 of the 35 backbone amide hydrogens in Aβ(1-40) exchange with deuteriums when the monomeric peptide was suspended in 95% D_2O for 60 min. Another option for measuring the incorporation of deuterium in monomeric Aβ(1-40) peptide was by analysis at the peptide level. For this, we employed pepsin, the most common protease used in H/D exchange-MS. The mass spectrometric data showed that ~8 deuteriums were incorporated in the Abeta (1-19) pepsin fragment and ~14 deuteriums were incorporated in the Aβ (20-34) pepsin fragment. The C terminal part (35-40) was not found as being exchanged. To obtain the oligomeric conformation, Aβ(1-40) peptide was used right after dissolution in HFIP (hexafluoro-isopropanol) followed by immediate dilution with PBS to a concentration of 20 µM. The aggregation reaction was performed at room temperature under magnetic stirring. After 7 h of incubation, we observed that Abeta (1-40) still exchanges a maximum of ~ 21 hydrogens and no significant change occurred within 24 hours of incubation. Interestingly, after 48 h to 7 days, the number of deuteriums incorporated in Aβ oligomers decreased. More importantly, we observed several oligomeric states (~2.5 to 4.2 deuteriums). The first H/DX exchange results for synuclein aggregation are not consistent with the literature that synuclein do not aggregates itself, this process is more complex and might be influenced *in vivo* by several biological processes such as contribution of metal ions or even Aβ peptides.

Neuartige Aspekte

Mass spectrometry-based footprinting methods we present here are important tools to study complex processes like aggregation at amino acids level.

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Mass spectrometric based methods for rigorous characterization of antibody-antigen interactions.

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Einleitung

The nitration of tyrosine residues represents an oxidative post-translation modification in proteins which occur in physiological conditions and in pathophysiological processes associated with oxidative stress such as diabetes, chronic hepatitis, atherosclerosis, Alzheimer's and Parkinson disease, asthma and lung disease. There are different *in vivo* pathways for tyrosine nitration, from which peroxynitrite and heme peroxidase are the most common mechanisms. Methods used for characterization of protein nitration include using antibodies against 3-NT and immunoanalytical methods such as immunoaffinityanalysis by ELISA, imunoprecipiation and Western blot. Using MS in identification of tyrosine nitration sites, major problems may occur because of the low nitration levels and the instability of these proteins under conditions of MS. Using the combination of affinity and MS methods, selective proteolytic digestion and the surface acoustic wave (SAW) has been successfully applied in determination of biding affinities and specificities of the nitrated PCS peptides to a monoclonal anti-3NT-antibodies (to the structural characterization and molecular identification). SAW technology in comparison with immuno-analytical techniques is more useful because of the direct and rapid determination of association/dissociation constants with small sample amounts.

Methodik

SAW-ESI-MS. A solution of 300 nM antibody was immobilized on the SAM, and remaining NHS groups were blocked with ethanolamine (pH 8.5). A solution of peptide mixture was added. Elution was carried out with glycine buffer and transfer into the ESI source. An ion trap mass spectrometer was used for on-line SAW-ESI-MS. Determination of dissociation constants was performed after covalent immobilization by plotting the pseudo first-order kinetic constant versus concentration, and applying linear regression.
Proteolytic Excision and Affinity-MS Methods. Affinity-MS analysis was performed with 10 µg antigen peptides in PBS buffer bound to the antibody column. Unbound peptides were removed by washing with PBS, and the immune complex dissociated by acidifiation. The elution fractions were collected and analysed by MS.

Vorläufige Daten

In prostacyclin synthase (PCS), a specific nitration by peroxynitrite has been identified at the tyrosine-430 residue near the catalytic center, which inactivates the enzyme. Since the nitrated PCS showed high affinity to the 3-NT antibody, were synthesized by SPPS using Fmoc protection, Tyr-430 and other Tyr residues. The peptides were purified by preparative HPLC and characterized by nano-ESI-FTICR MS. ESI-mass spectra of tyrosine-nitrated peptides provide most abundant intact multiply protonated molecular ions without any detectable fragmentation. Binding affinities of the tyrosine-nitrated and non-nitrated PCS peptides were initially characterized by affinity-MS, by affinity binding of peptides on the anti-3NT antibody column and subsequent MS analysis of eluted peptides. A significantly higher abundance in the affinity eluate was found for the Tyr-430 nitrated (H^{424}GKRLKNY^{430}(NO_2)SLPWGA^{436}-OH). The mass spectrum of the mixture of the eluted peptides showed that the $[M + 2H]^{2+}$ ion of the Tyr-430-nitrated peptide had the highest abundance, suggesting higher affinity to the nitrotyrosine-antibody, while the elution of a mixture of Tyr-430-nitrated and non-nitrated peptides provided only ions of the nitrated peptide. This result suggested an effect of the amino acid sequence adjacent to the Tyr-nitration site on the binding affinity to the antibody; thus peptide H^{424}GKRLKNY^{430}(NO_2)SLPWGA^{436}-OH contained basic amino acids *N*-terminal to the nitration site, while the other peptide contained neutral and acidic residues. SAW-Determinations of dissociation constants were performed with the SAM-immobilized peptides, and provided a KD of ~60 nM for the Tyr-430 nitrated peptide, while other nitrated peptides showed significantly lower affinities, consistent with a specific epitope motif for Tyr-nitrated sequences with adjacent basic residues.

Neuartige Aspekte

New mass spectrometric - bioaffinity methods were developed for a rigorous characterization of the antigen-antibody binding.

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Plants under stress: A novel approach for the absolute quantification of partially N-labeled proteins and the simultaneous determination 15N-incorporation

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Einleitung

For many ecological studies it is of major interest to follow nitrogen flux within an organism by pulse-labeling with ^{15}N, and to quantify the amounts of specific proteins in the same tissue. However, such studies have not been possible in the past due to the lack of suitable methods. Here, we demonstrate that a novel algorithm for the calculation of ^{15}N incorporation combined with an approach for absolute quantification of proteins based on LC-MS^E analysis (Silva et al. 2006) enables studying plant stress under ecologically relevant growth conditions (Ullmann-Zeunert et al 2012).

Methodik

Total soluble proteins were extracted from leaves of coyote tobacco (*N. attenuata*), spiked with a universal, unlabeled standard (bovine serum albumin), digested and analysed by LC-MS^E (Silva et al. 2006). Leaves were either labeled with a known amount of ^{15}N to validate our approach for the estimation of the ^{15}N incorporation, or pulse-labeled with ^{15}N resulting in unknown amounts of labeling. The absolute quantity of each protein of interest was calculated based on the relation between the average of the robust estimations of total ion count of the three most intense peptides of internal standard and the three most intense peptides of the protein of interest.

Vorläufige Daten

We demonstrate the reliability of protein identification and quantitation in a complex mixture by spiking known amounts of phosphorylase b into our protein extracts. Nicotiana attenuata plants grown in hydroponic culture at different known concentrations of ^{15}N-labeled nitrate, were used to further evaluate the procedure, and the small and large subunits of ribulose-1,5-bisphosphate-carboxylase/oxygenase (RuBisCO) and RuBisCO activase 2 were used as model proteins. The studied proteins showed an overall dynamic range of quantitation of about 1.5 orders of magnitude. Technical and biological measurements were highly reproducible independent of the time-point of measurement. ^{15}N-incorporation calculated by our new algorithm did not deviate from the theoretical values more than 1.0 atomic percent (At%). A time-course analysis after applying a ^{15}N-pulse to a realistic ecological set-up by using soil-grown plants demonstrated that proteins with unknown labeling can be quantified. Additionally, we calculated the changes in the absolute amounts of a protein which plays an important role in plant-herbivore interactions with and without herbivore treatment. Our results clearly show that we now have a tool at hand that allows for in-depth quantitative proteomics and ^{15}N flux analyses of the metabolic dynamics elicited during plant stress.

Neuartige Aspekte

Novel algorithm for absolute quantification of labeled proteins in complex plant extracts

Referenzen

[1] Ullmann-Zeunert et al. 2012;
[2] Silva et al. 2006

A tandem MS study of peptides derivatized with a thiourea-reagent designed for chemical cross-linking

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Einleitung

Recently, urea- and thiourea-based reagents have been introduced to chemical cross-linking for the elucidation of protein structures by means of tandem MS [1-4]. These cross-linking reagents dissociate preferentially upon collision activation making them potentially interesting for protein structure elucidation by MS/MS. Critical examination of the initial experimental results with the thiourea cross-linking reagent lead us to elongate the compound by a glycine moiety at each end to eradicate ambiguities in its fragmentation behavior. The revised thiourea-reagent was condensed to model amines (propyl- and cyclohexylamine), peptides (substance P, LHRH) and a protein (lysozyme) and the products were analyzed by ESI-MS^n. The results document an interesting competition of fragmentation pathways.

Methodik

Propyl- and cyclohexylamine (220 µM) were used 1:1 (v/v) with the cross-linker NHS-Gly-B-Tu-B-Gly-NHS and analyzed by ESI-LTQ-Orbitrap-MS/MS (Thermo Fisher Scientific). Cross-linking reactions with substance P, LHRH or lysozyme (10 µM each) were carried out in 20 µM HEPES (pH 7.5) using protein-to-cross-linker molar ratios of 1:50, 1:100 and 1:200 and different reaction times. The results of the peptide cross-linking reactions were analyzed by MALDI-TOF/TOF-MS/MS (Ultraflex III, Bruker Daltonik) and offline-nano-ESI-LTQ-Orbitrap-MS^n (Thermo Fisher Scientific) and as well as by 1D-SDS-PAGE and linear MALDI-TOF-MS for lysozyme. Additionally, lysozyme samples were proteolyzed with trypsin and subsequently analyzed by offline nano-HPLC (RP C-18 column, 75 µm x 150 mm, Dionex)/MALDI-TOF/TOF-MS/MS and online nano-HPLC (RP C-18 column, 75 µm x 250 mm, Dionex)/nano-ESI-LTQ-Orbitrap-MS/MS.

Vorläufige Daten

The design of the revised thiourea reagent for chemical cross-linking is based on the original symmetrical thiourea cross-linker [1]. Similar to that initial reagent, a central thiourea moiety (Tu) is flanked by two aminobutyric acids (B), now condensed to an additional glycine residue (Gly) on each side, which is *C*-terminally activated for reaction with amine functionalities as NHS-ester (NHS-Gly-B-Tu-B-Gly-NHS) [1].
In a previous study, we thoroughly examined the fragmentation patterns of a set of representative thiourea- and urea-based model compounds to understand the fundamentals of their behavior upon CID [5]. The computations confirm experimental findings suggesting that the migration of the ionizing proton upon collision activation is in all cases energetically most costly. Subsequent fragmentation reactions governing the product ion ratios are apparently sensitive to subtle influences [5]. In line with this we find that the thiourea-based model compounds show a reversed product ion distribution [5] compared to the symmetrical thiourea cross-linker when attached to simple amines (cyclohexyl- & propyl-amine): In the former, former ones the thiourea moiety decomposes, whereas in the latter ones cleavage of the adjacent amide bond via a nucleophilic attack of the thiourea sulfur dominates.
In a second set of experiments, the peptides LHRH and substance P were derivatized with NHS-Gly-B-Tu-B-Gly-NHS and analyzed by MALDI-TOF/TOF-MS/MS and offline nano-ESI-LTQ-Orbitrap-MS^n. For both peptides “dead-end” cross-links were found. However, the tandem MS results document that the fragmentation of the thiourea group is dominating the CID patterns.
For lysozyme, six different tryptic peptides with partially hydrolyzed cross-linker and one interpeptidal cross-linking product were detected by online nano-HPLC/nano-ESI-LTQ-Orbitrap-MS/MS. Similar to the tandem-MS results obtained for substance P and LHRH, mainly the thiourea group decomposes in MS^2 product ion spectra, but with different rates dependening on the modified peptide and its corresponding charge state.

Neuartige Aspekte

A tandem MS study of peptides derivatized with a new thiourea-reagent for chemical cross-linking.

Referenzen

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A tandem MS study of peptides derivatized with a thiourea-reagent designed for chemical cross-linking

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Einleitung

Recently, urea- and thiourea-based reagents have been introduced to chemical cross-linking for the elucidation of protein structures by means of tandem MS [1-4]. These cross-linking reagents dissociate preferentially upon collision activation making them potentially interesting for protein structure elucidation by MS/MS. Critical examination of the initial experimental results with the thiourea cross-linking reagent lead us to elongate the compound by a glycine moiety at each end to eradicate ambiguities in its fragmentation behavior. The revised thiourea-reagent was condensed to model amines (propyl- and cyclohexylamine), peptides (substance P, LHRH) and a protein (lysozyme) and the products were analyzed by ESI-MS^n. The results document an interesting competition of fragmentation pathways.

Methodik

Propyl- and cyclohexylamine (220 µM) were used 1:1 (v/v) with the cross-linker NHS-Gly-B-Tu-B-Gly-NHS and analyzed by ESI-LTQ-Orbitrap-MS/MS (Thermo Fisher Scientific). Cross-linking reactions with substance P, LHRH or lysozyme (10 µM each) were carried out in 20 µM HEPES (pH 7.5) using protein-to-cross-linker molar ratios of 1:50, 1:100 and 1:200 and different reaction times. The results of the peptide cross-linking reactions were analyzed by MALDI-TOF/TOF-MS/MS (Ultraflex III, Bruker Daltonik) and offline-nano-ESI-LTQ-Orbitrap-MS^n (Thermo Fisher Scientific) and as well as by 1D-SDS-PAGE and linear MALDI-TOF-MS for lysozyme. Additionally, lysozyme samples were proteolyzed with trypsin and subsequently analyzed by offline nano-HPLC (RP C-18 column, 75 µm x 150 mm, Dionex)/MALDI-TOF/TOF-MS/MS and online nano-HPLC (RP C-18 column, 75 µm x 250 mm, Dionex)/nano-ESI-LTQ-Orbitrap-MS/MS.

Vorläufige Daten

The design of the revised thiourea reagent for chemical cross-linking is based on the original symmetrical thiourea cross-linker [1]. Similar to that initial reagent, a central thiourea moiety (Tu) is flanked by two aminobutyric acids (B), now condensed to an additional glycine residue (Gly) on each side, which is *C*-terminally activated for reaction with amine functionalities as NHS-ester (NHS-Gly-B-Tu-B-Gly-NHS) [1].
In a previous study, we thoroughly examined the fragmentation patterns of a set of representative thiourea- and urea-based model compounds to understand the fundamentals of their behavior upon CID [5]. The computations confirm experimental findings suggesting that the migration of the ionizing proton upon collision activation is in all cases energetically most costly. Subsequent fragmentation reactions governing the product ion ratios are apparently sensitive to subtle influences [5]. In line with this we find that the thiourea-based model compounds show a reversed product ion distribution [5] compared to the symmetrical thiourea cross-linker when attached to simple amines (cyclohexyl- & propyl-amine): In the former, former ones the thiourea moiety decomposes, whereas in the latter ones cleavage of the adjacent amide bond via a nucleophilic attack of the thiourea sulfur dominates.
In a second set of experiments, the peptides LHRH and substance P were derivatized with NHS-Gly-B-Tu-B-Gly-NHS and analyzed by MALDI-TOF/TOF-MS/MS and offline nano-ESI-LTQ-Orbitrap-MS^n. For both peptides “dead-end” cross-links were found. However, the tandem MS results document that the fragmentation of the thiourea group is dominating the CID patterns.
For lysozyme, six different tryptic peptides with partially hydrolyzed cross-linker and one interpeptidal cross-linking product were detected by online nano-HPLC/nano-ESI-LTQ-Orbitrap-MS/MS. Similar to the tandem-MS results obtained for substance P and LHRH, mainly the thiourea group decomposes in MS^2 product ion spectra, but with different rates dependening on the modified peptide and its corresponding charge state.

Neuartige Aspekte

A tandem MS study of peptides derivatized with a new thiourea-reagent for chemical cross-linking.

Referenzen

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Investigation of Non-Conserved Ca2+/Calmodulin Binding Sites in Munc13 Proteins by Cross-Linking and ESI-Orbitrap Mass Spectrometry

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Einleitung

Munc13 proteins are important presynaptic regulators, which are essential for synaptic vesicle priming and adaptive synaptic mechanisms. The calcium-dependent interaction of Munc13 with calmodulin (CaM) was recently found to link the changes in residual calcium concentrations to presynaptic vesicle priming and short-term plasticity [1]. Previous cross-linking and photo-affinity labeling (PAL) experiments [2] showed binding of peptides derived from the homologous Munc13 variants, Munc13-1 and ubMunc13-2, to CaM via a 1-5-8 binding motif in an antiparallel orientation. Here, we used the amine/photoreactive cross-linker *N*-succinimidyl-*p*-benzoyldihydrocinnamate (SBC) to extend these results and confirm the same binding mode for the other Munc13 isoforms, bMunc13-2 and Munc13-3, despite the lack of sequence homology between their Ca^{2+}/CaM binding sites [3].

Methodik

Reaction mixtures for CaM labeling with cross-linker (SBC, 200 µM) contained 10 µM CaM and a Ca^{2+}/chelator (EGTA) buffer system ($[Ca^{2+}]_{free}$ 30 nM) in 20 mM HEPES buffer (pH 7.4). CaM was allowed to react with the amine-reactive site of SBC for 30 min. Excess cross-linker was quenched with 20 mM NH_4HCO_3 and removed by microfiltration. After adding the respective Munc13 peptide (10 µM), cross-linking reaction mixtures were irradiated with UV light (365 nm, irradiation energies 4000 and 8000 mJ/cm^2) to induce photo-cross-linking. The mixtures were separated by gel electrophoresis, bands of interest were *in-gel* digested, cross-linked peptides were separated by nano-HPLC (Ultimate 3000, Dionex), and analyzed by nano-ESI-LTQ-Orbitrap-MS/MS (LTQ-OrbitrapXL, ThermoFisher Scientific). Data analysis was conducted with StavroX [4].

Vorläufige Daten

In agreement with previous experiments we found products of the CaM labeling reaction, in which the amine-reactive site of SBC (*N*-hydroxysuccinimide ester) had reacted with lysines at the surface of CaM, i.e., lysines 21, 30, 75, 77 and 94. After irradiating the reaction mixtures of CaM and Munc13 peptides with UV light, we identified several different intra- and intermolecular cross-links, in which the benzophenone group of SBC had reacted with different amino acids of the Munc13 peptide (alanine, leucine, methionine, lysine). We created models of each CaM/Munc13 peptide complex using the distance information from present cross-linking and previous PAL experiments. The CaM/NO synthase peptide complex served as basis for modeling the complex as the CaM binding motif of NO synthase is highly similar to that of Munc13 peptides. Cross-linking experiments confirm previous data [2] and deliver insights into the CaM interaction site of all four Munc13 isoforms under investigation. The CaM/Munc13 peptide complexes exhibit similar structures showing that Munc13 peptides bind to CaM in an antiparallel orientation [3]. In contrast to a recently published NMR structure with longer Munc13 peptides [5], the conformation of CaM within the complex was found to be more compact suggesting that the length of the Munc13 peptide determines the conformation of CaM in the complex. Currently, we are extending our cross-linking studies to larger Munc13 domains to gain further insights into the binding interfaces of the respective Munc13 proteins. First results of these cross-linking experiments seem to confirm the data obtained with short Munc13 peptides.

Neuartige Aspekte

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Low-Temperature Plasma Ambient Desorption/Ionization High-Resolution Mass Spectrometry: Fundamental Study of the Ionization Characteristics Compared to ESI and APCI

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Einleitung

Low-temperature plasma (LTP) probe high-resolution mass spectrometry (HR-MS) is an attractive method for the direct analysis of complex samples. Desorption and ionization of analytes is performed at the sample surface in the ambient environment. Benefits of the method include high sensitivity and mass accuracy and little to no sample pretreatment. In the present study, the ionization behavior of selected compound families was investigated in LTP-HR-MS and directly compared to electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI).

Methodik

The home-built LTP probe is based on a design published earlier [1]. In principle, a dielectric barrier discharge is formed in helium by applying a high-voltage, alternating current waveform between two electrodes separated by a glass tube. The LTP was coupled to a high-resolution Orbitrap mass spectrometer. Several model analytes of different compound families were studied. Ionization efficiencies for amides, amines, aldehydes, imides, polycyclic aromatic hydrocarbons (PAHs), ionic species, a nucleoside, and a pharmaceutical were determined and directly compared to ESI and APCI.

Vorläufige Daten

In general, LTP can be considered to be a relatively soft ionization method because little fragmentation of model compounds was observed. Further, it was found that LTP shows comparable ionization characteristics to APCI for amides, amines, and aldehydes. A benefit of LTP over conventional methods is the possibility to successfully ionize PAHs and imides. Here, the studied model compounds could be detected by neither APCI nor ESI. LTP can be classified to be an attractive method for the ionization of low molecular weight compounds over a relatively wide polarity range.

Neuartige Aspekte

Ionization characteristics of LTP, comparison of the ionization behavior of LTP, ESI, and APCI.

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Strategy for Optimizing Low-Temperature Plasma Ambient Ionization for High-Resolution Mass Spectrometry: Design of Experiments

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Einleitung

Low-temperature plasma (LTP) probe is an attractive non-thermal plasma source for the direct mass spectrometric analysis of complex samples. Desorption and ionization of analytes is performed at the sample surface in the ambient environment. For example, LTP ionization was used successfully to detect explosives, drugs, and pesticides directly on the target.

Methodik

In this study, we coupled a home-built LTP probe [1] to a high-resolution mass spectrometer with Orbitrap detector (Exactive HCD) and developed a software-aided method to optimize the overall performance.

Vorläufige Daten

Specifically, optimization of the ionization source parameters was performed with a design of experiments (DOE) approach. DOE allows screening for and optimization of multiple parameters in an organized fashion. Interplay of different parameters can be easily explored and optimized. Here, a variety of parameters such as the geometry of the ion source, positioning of the probe, and operating parameters were optimized for best analyte signal intensities. With this approach, the dynamic range for, e.g., 4-acetamidothiophenol could be extended and the limit of detection was improved by approx. a factor of 10.

Neuartige Aspekte

Methodic optimization of LTP operating parameters for ambient desorption/ionization mass spectrometry with design of experiments.

Referenzen

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Amino acid analysis in biological samples by LC-MS/MS

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Einleitung

In plant science, determination and quantification of amino acids is needed in a large number of experiments as amino acids are primary metabolites in plants that play a major role in plant physiology. Genomic and metabolomic experimental set-ups require a high-throuput in amino acid analysis.

Methodik

A LC-MS/MS method was developed for the direct analysis of amino acids in plant extracts or bacteria media cultures. Chromatography was performed on a reversed phase UPLC column. The liquid chromatography was coupled to an API 3200 tandem mass spectrometer (Applied Biosystems, Darmstadt, Germany) equipped with a Turbospray ion source operated in positive ionization mode. All samples were spiked with ^{13}C, ^{15}N labeled amino acids (algal amino acids 13C, 15N, Isotec, Miamisburg, US). Individual amino acids in the sample were quantified by the respective 13C, 15N labeled amino acid internal standard. Bacteria media samples were analysed in negative ionization mode on LC-MS/MS after derivatisation with 9-fluorenylmethoxy-carbonyl chloride.

Vorläufige Daten

Plant samples were extracted with methanol and were diluted 1:10 or 1:20 in water for direct LC-MS/MS analysis. The method was successfully applied to samples originating from leaf, root, and seed samples from Arabidopsis thaliana, Populus nigra, Zea mays, and Nicotiana attenuata.
The same direct LC-MS/MS analysis was applied to the analysis of amino acids of bacteria media cultures grown in MMAB medium. However, it turned out that the high concentrations of the media components (i.e. ammonium chloride, fructose) led to a strong quenching effect in the ionisation of the mass spectrometer due to coelution of a number of amino acids with the media components. Therefore, we mixed the samples with internal standards amino acid mix (13C-, 15N-labelled amino acids) and first derivatized the amino acids with 9-fluorenylmethoxy-carbonyl chloride (FMOC-Cl) in order to convert them into less polar derivatives. After 5 minutes of reaction, hexane was added to stop the reaction and to remove excess FMOC-Cl reagent. The derivatized samples were analysed on an LC-MS/MS system (API 3200 tandem mass spectrometer, Applied Biosystems, Germany) operated in negative ionization mode. The instrument parameters were optimized by infusion experiments with pure FMOC-derivatized amino acid standards. This approach allowed the quantification of the amino acids in the media without interference of media components.
Both, the direct amino acid analyis in the plant extracts as well as the analysis after derivatization with FMOC-Cl for bacteria media samples can be achieved within 7 minutes in a 96 well plate format which allows high-throughput analyis (capacity around 200 samples per day).

Neuartige Aspekte

high throughput amino acid analysis of plant samples and bacteria media samples

Isotope-dilution UPLC-ESI-MS/MS quantification of nucleoside adducts originating from 1-methoxy-3-indolylmethyl glucosinolate in human urine after consumption of broccoli

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Einleitung

1-Methoxy-3-indolylmethyl (1-MIM) glucosinolate was recently identified as a potent genotoxicant in bacteria and mammalian cells [1]. 1-MIM glucosinolate forms DNA adducts *in vitro* in the presence of the plant enzyme myrosinase and in the large intestine of mice after activation by enzymes of gut bacteria [1-3]. As described earlier, we have identified N^2-(1-MIM)-dGuo and N^6-(1-MIM)-dAdo as the predominant DNA adducts formed. Moreover, we developed a sensitive MRM quantification method for these 2´-deoxyribonucleoside adducts based on isotope-dilution UPLC-ESI-MS/MS [3] suited for *in vitro* and invasive *in vivo* studies. Humans are exposed to high amounts of 1-MIM glucosinolate by consumption of *Brassica* vegetables. Broccoli, in particular, contains up to 80 µg/g fresh weight 1-MIM glucosinolate [4]. Therefore, the nutrition-related exposure of humans to 1-MIM glucosinolate is usually much higher compared to that of known foodborne genotoxicants such as benzo[*a*]pyrene and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP). The aim of the present study was to develop a non-invasive human biomarker which can be used for screening the exposure to electrophilic metabolites of 1-MIM glucosinolate. In this context, we extended the analysis to the accordant ribonucleoside adducts, N^2-(1-MIM)-Guo and N^6-(1-MIM)-Ado, and modified it for usage with human urine samples.

Methodik

Liquid-liquid extraction of 1-MIM-adducted nucleosides from human urine was optimized using ^{15}N-labeled internal adduct standards. To ensure quantitative conversion of possible precursors into nucleosides within the urine matrix, an enzymatic hydrolysis of 1-MIM-adducted DNA and RNA prior to extraction was tested. The extracts of 2-ml urine samples were evaporated to dryness, redissolved in 50 µl methanol and subjected to UPLC-ESI-MS/MS analysis using a Xevo TQ MS. The detection of three mass transitions for each compound enabled the simultaneous and unambiguous detection of unlabeled and labeled N^2-(1-MIM)-dGuo, N^6-(1-MIM)-dAdo, N^2-(1-MIM)-Guo and N^6-(1-MIM)-Ado. Optimized LC conditions allowed a separation of the analytes within 5 min. We applied this new analytical approach to an intervention study, in which four volunteers consumed raw broccoli.

Vorläufige Daten

Taken into account both recovery rates and matrix influences, ethyl acetate (among seven organic solvents tested) most effectively extracted the 1-MIM-adducted nucleosides from human urine. The enzymatic digestion of modified DNA and RNA was unaffected by the urine matrix. We found substantial amounts of all four adducts analyzed in the urine of each study participant. The identity of 3 out of 4 adducts was additionally confirmed recording product ion spectra. Cumulatively excreted amounts ranged from 0.2-5.9 nmol for the individual adducts. The time course of the urinary adducts was similar for all volunteers. N^6-(1-MIM)-Ado was the most and N^2-(1-MIM)-dGuo the least abundant adduct detected. The analysis of these compounds exemplifies the necessity of stable-isotopic labeled internal standards and at least three mass transitions for unambiguous MS/MS detection as both compounds possess the same molecular weight ($[M + H]^+$: *m/z* 427.2) and two identical mass transitions: *m/z* 427.2 > 160.1 (loss of the nucleoside) and *m/z* 427.2 > 145.1 (loss of the nucleoside and an additional methyl group). Using ^{15}N-labeled internal standards for retention time comparison in assistance with the third mass transition *m/z* 427.2 > 311.1: loss of deoxyribose for N^2-(1-MIM)-dGuo and *m/z* 427.2 > 264.0: secession of 1-methoxyindole and an additional hydroxyl group of the ribose moiety for N^6-(1-MIM)-Ado) the differentiation of both analytes is facilitated. Since we have obtained similar adduct levels using a sample preparation with or without enzymatic digestion prior to liquid-liquid extraction, we conclude that 1-MIM adducts excreted with the urine are quantitatively present as nucleosides. In summary, the developed MS method is a specific and sensitive procedure suited for the detection of urinary 1-MIM-adducted nucleosides. In order to evaluate whether this method can be used as biomarker of human exposure to the genotoxicant 1-MIM glucosinolate further studies with more subjects are being conducted.

Neuartige Aspekte

Identification and quantification of urinary 2´-deoxyribonucleoside and ribonucleoside adducts originating from metabolites of the *Brassica* constituent 1-methoxy-3-indolylmethyl glucosinolate in humans.

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Massenspektrometrischer Nachweis von Gendoping mit small interfering (si)-RNA

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Einleitung

Einzig RNA verfügt unter den derzeit bekannten Biomolekülen exklusiv die Eigenschaft genetische Information zu speichern und biologische Prozesse zu katalysieren. Dies macht diese Substanzklasse höchst interessant für die Entwicklung von neuen Medikamenten. [1] Leider ist damit auch einem möglichen Missbrauch zur Leistungssteigerung im Sport Vorschub geleistet. Die Manipulation von biologischen Prozessen mit Hilfe von si-RNA (oder auch anderen Oligonukleotiden) stellt dabei eine neue Dimension dar, die von der Welt Anti-Doping Agentur als Gendoping klassifiziert worden ist. [2] Dabei ist si-RNA selbst nicht Protein-codierend, aber greift als RNA-Interferenz hoch effizient und gezielt in die Proteinbiosynthese ein. Dabei ist es möglich einzelne Proteine nicht zur Ausbildung kommen zu lassen (gene silencing). In der vorliegenden Studie ist Myostatin, ein Schlüsselprotein für das Muskelwachstum, als Zielprotein gewählt worden. Eine mögliche si-RNA-Variante (doppelsträngig, 2x21 Nukleotide (nt)) mit verschiedenen chemischen Modifikationen, die die Pharmacokinetik verbessern soll, stellt dabei die zu untersuchende Modellsubstanz dar. Als chemische Modifikationen sind in dieser Studie Phosphorothioate, 2`F-OH-, 2`Methyl-Nukleotide und „locked nucleid acids" berücksichtigt.

Methodik

Zwei unterschiedliche si-RNA Varianten (als Blocker gegen die Myostatin-Gen mRNA) wurden in therapeutischer Dosis (1mg/kg) Ratten appliziert und anschließend Blut und Urin gesammelt. Nach unterschiedlichen Probenvorbereitungen wurden die Proben mit Hilfe von Flüssigkeitschromatographie (UPLC, C18-RP) und hochauflösender Massenspektrometrie (HRMS, Quadrupole / Orbitrap-Analysator) im negativen Ionisierungsmodus analysiert. Als Puffersystem für die LC wird 15 mM Ammoniumacetat (pH 7.5) und Acetonitril verwendet. Dabei wurde die RNA sowohl intakt, als auch nach Hydrolyse (basisch und enzymatisch) gemessen und charakterisiert. Zusätzlich wurden die Proben auch elektrophoretisch (denaturierendes Polyacrylamid-Gel) getrennt, aus dem Gel enzymatisch mit Ribonukleasen hydrolysiert und mit UPLC-MS/MS massenspektrometrisch analysiert. Hierzu wurde mittels HRMS eine datenabhängige Analyse (Motiv für Produktionenexperiment: Mehrfachladung im negativen Modus) durchgeführt.

Vorläufige Daten

Die Analyse der gesammelten Proben zeigte schnell, dass die Urinproben im Gegensatz zum Plasma, deutlich höhere Mengen der applizierten si-RNA (oder deren Metabolite) enthielten. Mit Hilfe der HRMS konnten hier verschiedene Metaboliten über die akkurate Masse als intakte, mehrfach negativ geladene Moleküle identifiziert werden. Diese Metaboliten sind hauptsächlich durch einen Abbau über Endo- oder Exonukleasen im Körper entstanden und stellen dementsprechend sequenzverkürzte RNA-Bruchstücke dar. Durch Analyse (Produktionenexperimente) der basischen Hydrolyseprodukte konnte eindeutig nachgewiesen werden, das diese si-RNA-Metabolite xenobiotischer Herkunft sind (Phosphorothioate, 2`-F-Nukleotide etc.). [3] Auch die gelelektrophoretische Analyse bestätigte die MS-Ergebnisse und durch den „In-Gel-Verdau" der RNA mit spezifischen Ribonukleasen konnte eine MS-basierte Sequenzierung mit hoher Sequenzabdeckung (im Sinne eines „RNomics"-Ansatzes) erreicht werden. [4, 5] Dies gilt für den Sense- und den Antisense-Strang gleichermaßen. Für die Chromatographie wurde sowohl für die intakten Oligonukleotide (bis 21 nt) als auch für die Hydrolyseprodukte (2-10 nt) auf klassische Ionenpaarreagenzien (üblicherweise TEA, HFIP etc.) verzichtet (s.o.) und trotzdem ein akzeptables Retentionsverhalten beobachtet.

Neuartige Aspekte

Gendoping mit Oligonukleotiden in der Dopinganalytik, „RNomics" mittels PAGE und LC-HRMS ohne Ionenpaarreagenzien

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Massenspektrometrische Untersuchung von SIRT1 Aktivator SRT1720 und 4 Modellaktivatoren sowie deren in vitro und in vivo Metaboliten

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Einleitung

Das Enzym Sirtuin1 steht aufgrund seiner wichtigen Rollen in zahlreichen metabolischen Prozessen im Fokus vieler medizinischer Studien. Zur Behandlung von verschiedenen Stoffwechselerkrankungen (z.B. Typ 2 *Diabetes mellitus*) werden derzeit zahlreiche SIRT1 Aktivatoren (z.B. SRT2104) auf ihre Wirksamkeit und Verträglichkeit in klinischen Studien untersucht. [1] SIRT1 Aktivatoren haben eine gesteigerte Deacetylierung von Sirtuin1 Substraten (z.B. FOXO1 und PGC1-alpha) zur Folge.[2, 3] Diese spielen unter anderem eine wichtige Rolle im Zucker- und Fettsäurestoffwechsel und dem damit verbundenen Energiehaushalt der Zelle. Folglich sind SIRT1 Aktivatoren potentielle Mittel zur Verbesserung von physikalischen Leistungen im Sport. [4] Die Untersuchung des Fragmentierungsverhaltens zum Nachweis von SIRT1 Aktivatoren und ihrer Metaboliten mittels LC/MS aus Blut- und Urinproben ist ein Ziel der präventiven Anti-Dopingforschung.

Methodik

Es wurden 4 verschiedene SIRT1 Aktivatoren analog zum bekannten SRT1720 in einer 7-stufigen Synthese dargestellt. Anschließend wurden mit einem *in vitro* Assay (humane Leber-Enzyme) so wie mit einem *in vivo* Rattenmodell Phase I und Phase II Metaboliten generiert. Die Auftrennung der Metaboliten erfolgte mittels HPLC. Zur Untersuchung des Fragmentierungsverhaltens der Ausgangssubstanzen und aller erhaltenen Metaboliten wurde positive Elektronenspray-Ionisation und CID Fragmentierung in niedrig- und hochauflösender Tandem-Massenspektrometrie verwendet. Desweiteren wurden MS^3-Experimente durchgeführt. Die Unterscheidung von Oxiden und Hydroxiden erfolgte mit Hilfe von D_2O/H_2O-Austausch Experimenten. Anhand dieser Daten wurden mittels charakteristischer Ionenübergänge, detektiert durch eine multiple reaction monitoring Methode und zwei synthetisierten, D_8-markierten Standards, Screening-Methoden für SIRT1 Aktivatoren und mögliche Metaboliten aus Plasma und Urin Proben entwickelt.

Vorläufige Daten

Aktuell werden neben dem in der Literatur viel beschriebenen SRT1720 zahlreiche weitere SIRT1 Aktivatoren (z.B. SRT2104) in klinischen Studien untersucht von denen die genaue Strukturformel immer noch unbekannt ist. Desweiteren ist zu erwarten, dass zukünftig neue SIRT1 Aktivatoren durch Variation von Substituenten am Grundgerüst mit einer verbesserten Wirksamkeit, Verträglichkeit und Verweildauer im Organismus, entwickelt werden. Aus diesem Grund wurden im ersten Teil dieser Arbeit vier Modell-SIRT1 Aktivatoren (Strukturanaloga zu SRT1720) synthetisiert. Anschließend wurde das Fragmentierungsverhalten sowohl für das SRT1720, $[M+H]^+$ bei *m/z* 470, als auch für alle vier synthetisierten Modellaktivatoren untersucht und die für diese Strukturklasse charakteristischen Produktionen wie z.B. *m/z* 254 nach Abspaltung beider Substituenten oder dem Methylen-piperazinium Kation bei *m/z* 99 beschrieben. Aufgrund dieser Daten wurde eine LC/MS Screening-Methode für diese SIRT1 Aktivatoren aus Blutplasma-Proben mittels charakteristischer Ionenübergänge (z.B. für SRT1720 $[M+H]^+$ bei *m/z* 486à384) und eines synthetisierten D_8-SRT1720-Standards entwickelt und unter Berücksichtigung von Spezifität, Nachweisgrenze (0.1-1 ng/mL), Wiederfindung (90-98 %) und Präzision (2-18 %) validiert. [5] Im zweiten Teil wurden in einem *in vitro* Assay und einem *in vivo* Rattenmodell mögliche Metaboliten generiert, mittels LC/MS charakterisiert und deren Fragmentierungsverhalten untersucht. Dabei wurden ausschließlich Oxid- und Hydroxid-Metaboliten gefunden (z.B. für SRT1720 fünf Metaboliten $[M+H]^+$ bei *m/z* 486) Zur genauen Charakterisierung mittels ^1H NMR wurde außerdem ein Hydroxy-Metabolit des SRT1720 synthetisiert. Resultierend aus diesen Daten wurde eine LC/MS Screening-Methode für diese SIRT1 Aktivatoren und deren Metaboliten mittels charakteristischer Ionenübergänge und zwei synthetisierten D_8-Standards (D_8-SRT1720 und D_8-SRT1720-Hydroxy-Metabolit) entwickelt. Die Ergebnisse dieser Arbeit beschreiben das grundstrukturabhängige Fragmentierungsverhalten von möglichen SIRT1 Aktivatoren und deren Metaboliten und geben eine Idee für mögliches Metaboliten im humanen Organismus. Desweiteren konnte eine Doping-Kontroll-Nachweismethode sowohl aus Blut als auch aus Urin entwickelt und validiert werden.

Neuartige Aspekte

Fragmentierungsverhalten von möglichen SIRT1 Aktivatoren und deren Phase I und II Metaboliten sowie eine LC/MS-Nachweismethode aus Blut und Urin.

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Quantifizierung des erythrozytären Metaboliten AICAR-PO4 als Langzeitparameter zum Nachweis der missbräuchlichen Anwendung im Leistungssport

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Einleitung

Vorausgehende Studien haben gezeigt, dass Agonisten der AMPK-Signalkaskade, zu denen auch das oral verfügbare 5-Amino-4-imidazolcarboxyamid-ribonucleosid (AICAR) zählt, eine ausdauersteigernde Wirkung aufweisen und somit als potentielle Dopingsubstanzen in den Fokus missbräuchlicher Anwendungen rücken [1]. Nach intravenöser Applikation begrenzt die hohe renale Clearance ein sicheres Detektionsfenster von urinärem AICAR auf ca. 10 Stunden [2]. Die Tatsache, dass AICAR mehr oder weniger stark in den Erythrozyten phosphoryliert wird, erhöht die Wahrscheinlichkeit einer möglichen Langzeitdetektion auch noch nach ca. 10 Tagen, da die akkumulierte Menge an AICAR-PO_4 in direkter Relation zur Lebensdauer dieser steht [3]. Ziel dieser Studie war eine erste Erhebung und Validierung endogener AICAR-PO_4 Referenzwerte gesunder Leistungssportler mit anschließenden in-vitro Experimenten zur Pharmakokinetik und gemeinsamer Auswertung per LC/MS-MS.

Methodik

Zur Ermittlung repräsentativer, endogener AICAR-PO_4-Konzentrationen wurden Erythrozyten-Lysate (aq.) von 100 gesunden Sportlern mit einem vorher synthetisierten internen Standard [$^{15}N_4$]-AICAR-PO_4 versetzt und durch darauffolgende Ultrafiltration (MW cut-off 3kDa) gereinigt. Das Filtrat wurde per Vakuumzentrifuge eingeengt, mit 50 µl Wasser erneut aufgenommen und mittels LC/MS-MS analysiert (Applied Biosystems API 4000 QTRAP Massenspektrometer, Foster City, CA, USA). Dabei wurde der Analyt über ein 5mM Ammoniumacetat-Puffer [pH 3,5] / Acetonitril-Gemisch per Gradientenelution bei Raumtemperatur aufgetrennt. Als stationäre Phase diente eine Phenomenex Gemini C6-Phenyl-RP-Säule(4.6×150 mm, 3 µm Partikelgröße, Aschaffenburg, Deutschland). Die externe Kalibrierung erfolgte mit Hilfe einer künstlichen Matrix (Octaplas®). Über eine Validierung mit den Parametern Präzision (interday/intraday), Richtigkeit, Nachweisgrenze, Wiederfindung, Stabilität und Ionen-Suppression wurde die Eignung dieser Quantifizierungsmethode überprüft.

Vorläufige Daten

Die Quantifizierung der lysierten Proben zeigte eine endogene AICAR-PO_4 Durchschnittskonzentration von 203,69 [ng/ml Matrix] mit einer Standardabweichung von 184,95 [ng/ml]. Dabei folgte die Konzentration der Proben einer log-Normalverteilung. Die Nachweisgrenze, mit einem geforderten Signal-Rausch-Verhältnis > 3, liegt bei 10 ng/ml. Ein negativer Einfluss durch mögliche Adsorptionseffekte mit nachfolgendem Verlust des Analyten konnte durch eine Wiederfindungsrate von 89% widerlegt werden. Da eine natürliche AICAR-PO_4-freie Matrix nicht zur Verfügung stand, wurde die Richtigkeit der Methode mit dem Vergleich einer Standard Addition zu einer externen Kalibrierung mit künstlicher Matrix validiert, um mögliche systematische Fehler ausschließen zu können. Dabei betrug die Richtigkeit 94%. Die Präzision (intraday/interday) wurde an drei aufeinander folgenden Tagen für drei verschiedene Konzentrationsbereiche (endogener Analyt, 200 ng/ml, 500 ng/ml) durch die Hinzugabe entsprechender Mengen AICAR-PO_4 und internem Standard ermittelt und lag bei < 20%. Matrix Effekte wurden zur entsprechenden Retentionszeit beobachtet und sind durch den stabil-isotopenmarkierten ISTD kompensiert. Die Überprüfung auf Stabilität des Analyten in Gegenwart von Vollblut ist derzeit noch in Bearbeitung. Die Erfassung valider, endogener AICAR-PO_4 Referenzwerte gesunder Leistungssportler bildet die Basis für weitere zukünftige Untersuchungen, mit dem Hintergedanken kritische Blutspiegel von AICAR-PO_4 über einen langfristigen Zeitraum effizienter detektieren zu können und somit dem Risiko missbräuchlicher Anwendungen vorzubeugen.

Neuartige Aspekte

Erhebung valider, endogener AICAR-PO_4 Konzentrationen in Erythrozyten-Lysaten mittels LC/MS; Ermittlung eines sicheren Detektionsfensters und Rückschlüsse auf die Möglichkeit einer Langzeitdetektion

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Characterization of a Cisplatin-DNA-Antibody and its Corresponding Antibody-Antigen-Complexes by Mass Spectrometry Under Native Conditions

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Einleitung

Since antibodies have been increasingly used in the diagnostic and therapeutic medicine, new analytical tools for their detection and characterization are getting more and more important. In recent years, especially soft ionization techniques of mass spectrometry like electrospray ionization (ESI) were improved for the detection of high masses. This allows the analysis of precise molecular masses of large native proteins like antibodies without any fragmentation. In order to understand structural aspects of native non-covalent complexes the analysis of a whole antibody-antigen-complex is a big challenge and first results are presented in this work.

Methodik

In this study we analyzed the monoclonal rat antibody R-C18 specific for Cisplatin induced DNA adducts which was developed by Thomale et al. [1] The detection of this antibody in its native state was successfully performed by a modified nano-ESI time of flight mass spectrometer (ToF-MS). [2]

Vorläufige Daten

The correct mass of the unmodified antibody was determined to 144 kDa. As a model for the antigen we used synthetic oligonucleotides with known base sequences to produce the specific platinated DNA antigens. Oligomers contain 50 bases and were analyzed as single and double strands. The sequences of these 50mers was chosen that only one of the single strands contains two adjacent guanines to give the supposed binding pattern G*G*-cisPt(NH3)2 after incubation with Cisplatin. As a result we could detect the unmodified and the platinated DNA oligomers using mass spectrometry in positive and negative mode. The whole antibody-antigen-complex using these synthetic platinated oligomers could be successfully measured in positive mode. Surprisingly, the antibody binds not only to the platinated synthetic 50mer double strand but also to each of the corresponding platinated single strands. That means that the binding pattern of this special antibody might neither be restricted to DNA double strands, nor to the until now assumed binding pattern of G*G* **intra**strand Cisplatin adducts.
In conclusion we show the successful detection of antibodies and their antibody-antigen-complexes under native conditions via nano-ESI-High Mass-Q-ToF-MS.

Neuartige Aspekte

In future it could be possible to determine the precise epitope-binding structure of DNA antigens detected by diagnostic relevant antibodies.

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Experimental Evidence and Characterization of Cobalt(I) Complexes Relevant in Regioselective Diels-Alder Reactions. A Gas Phase Study.

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Einleitung

In situ-formed cobalt(I) complexes are proposed to act as catalytically active species in numerous transition metal catalyzed transformations such as cycloaddition reactions of olefins, 1,3-dienes and alkynes. [1] One important example in modern organic synthesis is the highly regioselective *Diels-Alder* reaction utilizing cobalt catalysts generated from [$CoBr_2$(ligand)] in the presence of ZnI_2 and Zn. Following this protocol the *para*-substituted product is formed when the ligand = dppe (1,2-bis(diphenylphosphino)ethane) while the respective *meta*-product is obtained with the ligand = imine ((*E*)-2,4,6-Trimethyl-*N*-(pyridin-2-ylmethylene)aniline). [2] Reducing conditions provided by the addition of ZnI_2 and Zn are essential for the reaction, tentatively to form a catalytically active cobalt(I) species *in situ*. [2] Electrospray ionization (ESI) combined with tandem-MS proved to be a promising approach in the analysis of transition metal catalysts as even labile and transient species can be studied directly out of complex reaction mixtures. [3] Furthermore, the intrinsic reactivity of isolated transition metal complexes towards substrates can be examined in the gas phase using ion/molecule reactions. [4,5] For this purpose, neutral reagents can be introduced into the buffer gas flow of quadrupole ion trap mass spectrometers. [4,5] We now report experimental results on cobalt (II) and (I) complex ions potentially relevant for the regioselective *Diels-Alder* reaction in combination with theoretical investigations.

Methodik

ESI-MS^n experiments and exact ion mass measurements of precursor and product ions were conducted on a LTQ Orbitrap XL instrument (*Thermo Fisher*, Bremen, Germany). Gas phase ion/molecule reactions were performed in the LTQ part of this LTQ Orbitrap XL instrument equipped with a modified buffer gas flow inlet allowing the quantity-controlled introduction of neutral reagents, such as isoprene or phenylacetylene, into the LTQ helium supply, a setup similar to the ones proposed by Gronert and O'Hair. [4,5] To study the kinetics of the catalyst-substrate-complex formation cobalt(II) and cobalt(I) precursor ions encountered active collisions with gaseous neutral reagents in the LTQ and the extend of catalyst-substrate-adduct formation was monitored at varying activation times (0.3 - 10000 ms).

Vorläufige Daten

The (+)ESI-MS spectra of acetonitrile solutions (10^{-5} M) of [$CoBr_2$(dppe)] and [$CoBr_2$(imine)] showed prominent signals referring to the ionic cobalt(II) species $[CoBr(dppe)]^+$ and $[CoBr(imine)]^+$,respectively. After addition of Zn and ZnI_2 to a reaction mixture of each cobalt(II) complex in THF under inert gas atmosphere the respective cobalt(I) species $[Co(I)(dppe)]^+$ and $[Co(I)(imine)]^+$ aside from cationic zinc organyls were detected by (+)ESI-MS. The identity of both cobalt(I) and cobalt(II) complex ions could be clarified by exact ion mass measurements and also by MS^2-product ion experiments exhibiting characteristic product ions. To examine intrinsic reactivities of the $[Co(I)(ligand)]^+$ and $[Co(II)Br(ligand)]^+$ species towards the *Diels-Alder*-substrates isoprene and phenylacetylene gas phase ion/molecule reactions at varying reaction times have been performed in the LTQ part of a LTQ Orbitrap XL instrument modified as described in literature. [4,5] It is possible to measure the flow rates of helium and of the neutral reagents and by that determine the resulting concentration of neutral reagent in the buffer gas atmosphere of the LTQ. The performed ion/molecule reactions clearly indicate the greater affinity of cobalt(I) species towards isoprene and phenylacetylene compared to cobalt(II) complex ions which is in line with DFT calculations of the respective cobalt species and the ion/molecule reactions. This finding is strong analytical evidence that cobalt(I) species are the actual active catalysts in solution phase *Diels-Alder* reactions. [1]

Neuartige Aspekte

First characterization of $[Co(I)(ligand)]^+$ species relevant in regioselective *Diels-Alder* reactions by ion/molecule reactions, MS^n and theoretical investigations.

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Massenspektrometrische Analyse beschlagnahmter Produkte mit Verdacht auf dopingrelevante Inhaltsstoffe mittels HPLC-(HR)MS, GC-(HR)MS und 1D-Gelelektrophorese-UPLC-MSn

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Einleitung

Das Streben nach sportlichem Erfolg ist leider allzu oft mit der missbräuchlichen Einnahme von leistungssteigernden Substanzen wie z.B. Steroiden, Antiestrogenen, Wachstumshormonen, Stimulanzien und anderem mehr verbunden, die eigentlich als Wirkstoffe zur Therapie von Erkrankungen entwickelt und als Medikamente auf den Markt gebracht werden.[1] Um dem Missbrauch und den damit einhergehenden möglichen gesundheitlichen Schäden Einhalt zu gebieten, wird von der Welt-Anti-Doping-Agentur (WADA) eine jährlich aktualisierte Liste mit verbotenen Substanzen und Methoden veröffentlicht, die für Spitzenathleten unterschiedlichster Sportverbände verpflichtend ist.[2] Diese Athleten werden in einem Kontrollsystem regelmäßig auf die Einhaltung der Verbote mit leistungsfähigen qualitativen und quantitativen analytischen GC-(HR)MS und HPLC-(HR)MS Methoden überprüft. Neben dem Spitzensport ist auch der Breitensport vom Missbrauch leistungssteigernder Substanzen betroffen, hier werden die Sportler jedoch nicht in einem Kontrollsystem erfasst. Dieser Umstand öffnet einen Schwarzmarkt auf dem originale und gefälschte Medikamente, sowie in Untergrundlaboratorien hergestellte Arzneien illegal vertrieben werden. Die aufgrund des Verdachtes eines Verstoßes gegen das Arzneimittelgesetz von Polizei und Zoll beschlagnahmten Produkte (Kapseln, Pulver, Lyophilisate, Cremes, sowie ölige und wässrige Lösungen) bieten somit die Möglichkeit die Doperszene zu beobachten und auf neue Entwicklungen frühzeitig reagieren zu können. Seit 2010 wurden u.a. im Rahmen der Europäischen Beobachtungsstelle für neue Dopingsubstanzen (EuMoCEDA) 185 Produkte analysiert.[3]

Methodik

Mittels Hochleistungsflüssigkeits-Chromatographie/hochauflösender Massenspektrometrie (HPLC-HRMS) werden die Wirkstoffe im *full-scan* und Produkt-Ionenscan anhand substanz-spezifischer Fragmentierungswege und Ionen qualitativ und quantitativ bestimmt. Beinhaltete Analyte sind anabole Wirkstoffe, Stimulanzien, Wachstumsfaktoren allgemein, natürliche und synthetische Insuline, IGF-1 und synthetische Analoga sowie Wachstumshormon-Releasing Faktoren. Für Analysen mittels Gas-Chromatographie/(hochauflösender) Massenspektrometrie (GC-(HR)MS) werden Aliquote derivatisiert, im *full-scan*-Modus vermessen und mit Referenzsubstanzen und/oder Referenzdatenbanken qualitativ und quantitativ bestimmt. Beinhaltete Analyte sind hier primär anabole Wirkstoffe, Stimulanzien, Beta-2-Agonisten und Narkotika. Zur Analyse von Peptiden und Proteinen werden Aliquote mittels Polyacrylamid-Gelelektrophorese aufgetrennt und durch proteinspezifische Färbung gekennzeichnet. Durch *bottom-up* Analytik wird die Identität der Substanz mit nanoFlüssigkeitschromatographie / Tandem Massenspektrometrie belegt. Beinhaltete Analyte sind humanes Wachstumshormon (hGH), Wachstumsfaktoren allgemein (z.B. FGF, MGF, etc.), verschiedene Erythropoietine (EPO) und Wachstumshormon-Releasing Faktoren.

Vorläufige Daten

Es wurden insgesamt 185 konfiszierte und vom Schwarzmarkt bezogene Produkte auf ihren Inhalt hin analysiert. Die Produkte enthalten 44 unterschiedliche Wirkstoffe, von denen 38 eine Dopingrelevanz besitzen. Mit einem Anteil von über 85% nimmt die Stoffklasse der Steroide und ihrer Abkömmlinge den größten Teil ein, hier wurden vor Allem Testosteron, Trenbolon und Nandrolon frei und als Ester identifiziert. Die Gruppe der Wachstumshormone hat einen Anteil von über 10%, hier sind das Protein hGH (*human Growth Hormone*) und die Peptide der GHRPs (*Growth Hormone Releasing Peptide*) zu nennen. Einen kleineren Anteil von 3% haben die Stimulanzien Koffein und Synephrin sowie die Antiestrogene Clomifen und Tamoxifen. Zu den Analyten ohne Dopingrelevanz gehören virilisierende und dermatologisch wirkende Stoffe, die die Nebenwirkungen der Steroidapplikation, wie z.B. Verlust der Libido und Hautreaktionen entgegenwirken sollen.Im Besonderen ist auf die Befunde in 20 Produkten mit nicht-deklariertem 17-Methyltestosteron hinzuweisen, da die Konsumenten sich aufgrund der lebertoxischen Wirkung dieses Stoffes unwissentlich einem erhöhten Gesundheitsrisiko aussetzen. Mit Hilfe von LC-MS^3- und GC-QToF-HRMS-Experimenten konnten des Weiteren die Strukturen von bislang in der Dopinganalytik unbekannten Steroidderivaten ermittelt werden: zum Einen ein D-Ring-methyliertes Stenbolon-Derivat und zum Anderen 5α-Androstano-(2,3-c)-Furazan-17β-Tetrahydropyranol.

Neuartige Aspekte

Beobachtung von Entwicklungen in der Dopingszene; Erweiterung des analytischen Spektrums der Präventiven Dopingforschung

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GC-ICP-MS-Optimierungen zum Nachweis von Tributylzinn in Realwasserproben entsprechend der EU-Wasserrahmenrichtlinie „WFD 2000/06/EG“

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Einleitung

Die weite Verbreitung von Organozinnverbindungen, die z.B. als Pestizide, Antifoulingfarben und als Licht-Stabilisatoren in der PVC-Herstellung genutzt werden, führt zu einem beträchtlichen Eintrag in verschiedenste Ökosysteme. Der öffentliche Fokus liegt vor allem auf der Belastung durch das toxische und östrogenwirksame Tributylzinn (TBT) und seinen Metaboliten. Aber auch andere Substanzen wie die Phenylzinnverbindungen zeigen starke biozide Effekte, die auf aquatische Lebewesen wie Fische, Algen und Muscheln wirken und zu Veränderungen in deren Organismus bis hin zum Absterben führen können (1,2). Die im Jahre 2000 erlassene Europäische Wasserrahmenrichtlinie (WFD 2000/06/EG) hat das Ziel die aquatischen Ökosysteme und Grundwässer einheitlich zu überwachen. Aber auch die Verbesserung der Gewässer und deren nachhaltige Nutzung sind festgelegt. Im Projekt “Traceable measurements for monitoring critical pollutants under the European Water Framework Directive” des European Metrology Research Programmes (EMRP) sollen quantitative Methoden für prioritäre Stoffe wie für den Umweltschadstoff TBT entwickelt werden. Für diesen Analyten wird eine sehr geringe Bestimmungsgrenze von 0.06 ng/L gefordert. Um diese erreichen zu können ist eine nachweisempfindliche Analysenmethode notwendig. Die GC-ICP-MS bietet die Möglichkeit, Umweltschadstoffe in Spuren detektieren zu können. Zudem hat sich dieses Verfahren in den letzten Jahren zunehmend als Methode der Wahl für die Organozinnanalytik in wässrigen Proben entwickelt (2).

Methodik

Die Optimierungen von GC- und auch ICP-MS-Parametern sind unerlässlich um effektive und nachweisempfindliche Experimente durchzuführen. Zahlreiche Plasma-, Linsen-, und Quadrupoleinstellungen wurden untersucht und optimiert um die höchste Empfindlichkeit für verschiedenen Organozinnverbindungen zu erhalten. Dafür wurden die Empfindlichkeiten für Zinn und die eines Tune-Gases unter variierten Parametern bestimmt und verglichen. Auch GC-Einstellungen wie beispielsweise die Probenaufgabetemperatur wurden optimiert um eine gute und gleichmäßige Empfindlichkeit für die Analyten zu erreichen. Um die Empfindlichkeit des Systems zu evaluieren wurde verschiedenen Wassermatrices mit TBT dotiert und unter optimierten Methodenbedingungen aufgearbeitet und analysiert.

Vorläufige Daten

Die Empfindlichkeit der Analyse von Tributylzinn mittels GC-ICP-MS zeigt innerhalb und zwischen Messtagen große Schwankungen. Tägliche Optimierung ist notwendig um die bestmöglichsten Ergebnisse zu erzielen. Für die GC-ICP-MS-Kopplung wird zum Tunen eine Mischung aus Xenon und Helium genutzt. Die optimalen Messeinstellungen dieses Tunegases müssen nicht äquivalent mit denen der Zinnspezies sein. Daher wurden ICP-Parameter variiert und die Intensitäten von Xenon (m/z=129) und die Peakfläche des Ethyltributylzinn (m/z=120) gemessen und deren Verläufe verglichen. Als optimale Plasmastärke wurde 500 W bestimmt. Die Plasmaparameter Sample Depth und RF-Matching zeigen nur geringe Abhängigkeiten für Zinn. Weiteren Plasmaparameter wie Torch-Positionen und Carrier Gas zeigen zwischen Zinn und Xenon sehr gute Übereinstimmungen der Variationsverläufen. Auch die Einstellungen der Ionenlinsen wurden untersucht. Für die beiden Extraktionslinsen konnten wiederholt gute Übereinstimmungen bestimmt werden. Für den Octapole Bias gibt es keine gute Übereinstimmung zwischen Zinn und Xenon. Da es bei einer Vielzahl von Parametern zu einer guten Übereinstimmung von Xenon und Zinn kommt, wird Xenon weiterhin als Tunegas verwendet. Die Ethyltributylzinnlösung wird aber weiter messtäglich injiziert um die Messempfindlichkeit für den Analyten zu überprüfen. Bei der Optimierung der GC-Parameter wurde die Probenaufgabetemperatur untersucht. Im Allgemeinen wird eine Temperatur von 250 °C gewählt. Arbeitet man hingegen mit einem temperierbaren Aufgabesystem und einer Starttemperatur von 60 °C, können sowohl TBT als auch die Metaboliten Monobutylzinn und Dibutylzinn empfindlich analysiert werden. Mit den optimierten GC-ICP-MS-Parametren werden unterschiedliche 1L-Wasserproben mit einem TBT-Gehalt von 1ng kg^{-1} als Sn untersucht. In der Milli-Q-Wasser-Probe, welche weder Salze noch Huminstoffe enthält kann TBT 10-fach höher als in einer Trinkwasserprobe, welche einen natürlichen HS-Gehalt von ca. 4 mg L^{-1} aufweist, nachgewiesen werden. Vergleicht man Trinkwasser und eine Huminsäure-dotierte Trinkwasserprobe wurde nochmals eine Abnahme um einen Faktor von 10 beobachtet. Die Optimierung könnte den Nachweis im Ultraspurenbereich von TBT in natürlichen Gewässern im Rahmen der Wasserrichtlinie ermöglichen.

Neuartige Aspekte

Systematische Optimierung der ICP-Parameter in Abhängigkeit von Sn-Empfindlichkeit
Untersuchung verschiedenster Wassermatrices in Hinblick auf WFD-Problematik

Referenzen

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Direct experimental evidence and characterization of enamine radical cation species as key intermediates in SOMO catalysis using ESI-MS

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Einleitung

Enantioselective organocatalysis is a major research topic in organic chemistry. [1] SOMO catalysis was introduced by the group of David MacMillan and has obtained large interest as a new and powerful version of amine mediated, enantioselective organocatalysis which includes radical steps initiated by a one-electron oxidation. [2] ESI mass spectrometry has proven its avail as an excellent tool for the investigation of reaction mechanisms [3] and the detection of short-lived, reactive intermediate species such as radical cations in solution.[4] The key intermediate enamine radical cation has been postulated for SOMO catalysis but had not been observed directly until it we could detect it by ESI mass spectrometry combined with an online microreactor technique. Four typical MacMillan imidazolidinone organocatalysts were chosen for detailed study of their reaction behavior.

Methodik

ESI spectra were recorded on a Bruker Apex IV FT-ICR and a micrOTOF-Q mass spectrometer equipped with an Apollo ESI source. Fragmentation experiments were carried out using ISCID and CID techniques with argon as collision gas. Enamines were obtained by mixing solutions of the respective catalyst TFA-salt and phenylacetaldehyde solutions in 1:20 ratio. Chemical oxidation was performed *in situ* using the one-electron oxidant Tris(p-bromophenyl)aminiumhexachloroantimonate. Electrochemical oxidation can be done by using a combination of a ReactorCell and a ROXY potentiostat (Antec, Leyden) which is directly coupled to the ESI source. All signal assignments were confirmed by exact mass measurements.

Vorläufige Daten

We have identified enamine radical cation species in the case of four commonly used imidazolidin-4-one organocatalysts using chemical oxidation so far. Subsequent reactions of the enamine radicals with e.g. styrene could be observed in our experiments as well. [5] Furthermore, studies of the gas-phase fragmentation behavior of the catalysts, the enamines and the enamine radical cations were carried out to elucidate and compare the intrinsic properties of the compounds. Adequate intensities could be reached for all enamines and radical cations which enabled us to perform IRMPD MS/MS experiments. Loss of benzyl or butyl is detected in case of all radical cations resulting in extremely stabilized iminium ions. Comparison of the fragmentation behaviour of all four radical cations, enamines and pure catalysts will help us to elucidate the intrinsic properties of these species involved in SOMO catalysis.
Currently, the project is extended towards establishing electrochemical oxidation online coupled to the MS to replace the chemical oxidant which causes a great number of experimental inconveniences, particularly many unwanted signals resulting from partly decomposed oxidant molecules. First results prove the potential adaptability of the used electrochemical cell for our aims. Ongoing work focuses on the optimization of oxidation conditions to reach high signal intensities for the enamine radical cations which will enable us to perform MS/MS experiments and further mechanistic studies.

Neuartige Aspekte

Detailed characterization of MacMillan imidazolidinone reaction behaviours via mass spectrometry in combination with online microreactor techniques and electrochemistry/MS-coupling.

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Imidazolidin-4-one Organocatalysts in the Gasphase

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Einleitung

Imidazolidin-4-ones have succeeded as readily accessible[1] and very powerful organocatalysts which enable high enantioselectivities both for iminium- and enamine-based catalysis[2] including highly interesting connections with photo-redox [3] and with radical reactions [4] (SOMO catalysis): Adding a one-electron oxidant to an enamine catalysis reaction mixture should give an enamine radical cation which further reacts by typical radical reaction pathways. We recently reported the first direct detection of such an enamine radical cation key intermediate by ESI-MS.[5] During our work on reaction mechanisms in enamine and SOMO catalysis, we felt the need for a profound mass-spectrometric characterization of the respective organocatalysts. Thus, four typical imidazolidin-4-one organocatalysts were chosen for a detailed study of their gas-phase fragmentation behaviour.

Methodik

ESI spectra were recorded on a Bruker Apex IV FT-ICR and a micrOTOF-Q mass spectrometer equipped with an Apollo ESI source. Fragmentation experiments were carried out using ISCID and CID techniques with argon as collision gas.
EI spectra were recorded on a Thermo Finnigan MAT 95 XL sector instrument. All signal assignments were confirmed by exact mass measurements.

Vorläufige Daten

Three competing ring cleavages have been identified to dominate the CID spectra of protonated closed-shell $[M+H]^+$ imidazolidin-4-ones. Structures for the product ions are proposed based on MS^3 fragmentation spectra and deuterium labeling. The respective $[M+Na]^+$ fragmentation spectra are hard to record due to extensive loss of Na^+. Nevertheless, they reveal some interesting differences compared to the protonated species, loss of a benzyl radical in particular. Expectedly, alpha cleavages start the fragmentation cascades observed in the EI spectra, which have been followed with the aid of metastable fragmentation ("daughter ion") spectra. Some of the most abundant iminium fragment ions are present in the ESI-CID spectra as well. They thus represent the most viable fragmentation pathways for imidazolidin-4-ones irrespective of the ionization method.

Neuartige Aspekte

Detailed mass spectrometric characterization of imidazolidin-4-one organocatalysts using EI and ESI

Referenzen

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On-line Coupling of Electrochemistry to LC/ESI-MS as Approach for Protein Labeling

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Einleitung

Electrochemistry (EC) has proven to be a valuable tool for the simulation of oxidative drug metabolism. The formation of reactive quinoidic metabolites via dehydrogenation leads to a Michael-type addition of these electrophilic structures to nucleophilic compounds. Therefore, modification of proteins and peptides that contain the amino acid cysteine can easily be achieved due to the nucleophilic nature of their free thiol groups. In this poster, we present an approach for the electrochemical generation of reactive intermediates and their subsequent coupling with free thiol groups in peptides and proteins.

Methodik

The hyphenation of an electrochemical cell to electrospray ionization mass spectrometry (ESI-MS) enables the direct detection of the generated oxidation products. Applying a potential ramp from 0 - 2500 mV and the presentation of the data in form of three dimensional mass voltammograms provides a broad overview on the generated oxidation products. Furthermore, a fast and efficient optimization of the EC and MS conditions is possible for each compound.
An extension of the instrumental set-up by means of liquid chromatography (LC) enables the on-line generation, separation and identification of modified proteins. By employing a switching valve for the injection, the EC oxidation and the LC separation conditions can be optimized independently regarding the flow rate, solvent composition and pH.

Vorläufige Daten

The oxidation of the two phenolic species phenol and acetaminophen to their corresponding quinoidic structures was accomplished with an electrochemical thin-layer cell, equipped with a boron-doped diamond (BDD) working electrode. By adding the protein solution to the effluent of the EC cell via a T-piece, the on-line generation of modified proteins was obtained. In order to separate and identify the protein adducts, EC was coupled to LC/ESI-MS.
Labeling of free thiol groups in cysteine moieties enables the determination of cysteine containing proteins. Thus, the identification of the proteins is simplified. Differential labeling of the cysteine moieties with the isotope labeled and native form of a generated oxidation product results in a mass shift between the labeled biomolecules. Based on this mass shift, the labeled molecules can be identified.
In this work, phenol and acetaminophen were electrochemically oxidized and the obtained benzoquinone and *N*-acetyl-*p*-benzoquinoneimine (NAPQI) were added on-line to the tripeptide glutathione in order to figure out the optimal conditions for the generation of reactive intermediates. Afterwards, the small and structural homogeneous model protein β-lactoglobulin A (β-LGA), carbonic anhydrase I, which is highly abundant in red blood cells, and the larger human serum albumin (HSA) were incubated with the reactive intermediates. Both, the native as well as the corresponding isotope labeled form of the compounds ($^{13}C_6$-phenol and D_4-acetaminophen) were oxidized and allowed to react with the biomolecules. Hence, a mass shift can be observed, which can be traced back to the heavier, labeled species of the oxidized compounds.

Neuartige Aspekte

On-line EC/LC/ESI-MS allows labeling and subsequent identification of cysteine containing peptides and proteins.

Identifizierung von Verunreinigungen in organischen Solarzellen und deren Einfluss auf die Solarzellenperformance am Beispiel eines Merocyanin-Farbstoffes

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Einleitung

Mercyaninfarbstoffe werden seit wenigen Jahren in organischen Solarzellen als Elektronendonorkomponente eingesetzt [1]. Die Reinheit der verwendeten Materialien sowie mögliche Degradationsprozesse während des Betriebs können eine entscheidende Rolle für die Effizienz und Lebensdauer der Zellen spielen. Der Einfluss hängt dabei sehr stark von der Struktur der Verunreinigung und insbesondere von der Lage des HOMO und LUMO der Materialien ab. Die Energieniveaus bestimmen dabei die Bildung von Exzitonen, die Trennung von Ladungsträgern sowie den Ladungsträgertransport zu den Elektroden und damit die Erzeugung elektrischer Energie. Im Rahmen dieser Studie wurde der Merocyanin-Farbstoff HB194 [2,3] untersucht, Verunreinigungen massenspektrometrisch identifiziert und deren Einfluss auf die Solarzellen getestet.

Methodik

Der Farbstoff HB194 wurde synthetisiert [2], und unterschiedliche Aufreinigungsstufen der Synthese wurden mittels LC-UV auf ihre Reinheit untersucht. HB194 besteht aus einer 2-(3-Oxo-indan-1-ylidene)-malononitril-Akzeptoreinheit und einem 1,1,2-Trimethyl-1,2,5,6-tetrahydro-4H-pyrrolo[3,2,1-ji]chinolin-Donor, die über eine Ethylen-Brücke verbunden sind. Nebenprodukte wurden mittels hochauflösender/hochakkurater Massenspektrometrie und auch mittels NMR-Spektroskopie identifiziert und zudem mittels cyclischer Voltammetrie (CV) elektrochemisch charakterisiert, um die HOMO und LUMO der Substanzen zu bestimmen. Ein hydroxyliertes Nebenprodukt wurde mit präparativer HPLC isoliert und angereichert, um den spezifischen Einfluss dieser Verunreinigung auf die elektrischen Charakteristika der Solarzellen zu untersuchen.

Vorläufige Daten

Unterschiedliche Chargen von HB194 wurden nach verschiedenen Aufreinigungsstufen (Reinheit von 97.3-99.9%) für Solarzellen verwendet, welche eine deutliche Effizienzsteigerung (PCE=power conversion efficiency) in Abhängigkeit von der Reinheit von HB194 zeigten. Die massenspektrometrische Identifizierung der im HB194 gefundenen Nebenprodukte der Synthese ergab u.a. ein hydroxyliertes, sowie eine chloriertes Derivat von HB194, letzteres vermutlich entstanden durch Verwendung von chlorierten Lösungsmitteln während der Aufreinigung. Desweiteren konnten eine aus zwei Akzeptorkomponenten bestehende Verunreinigung sowie die Kombination zweier Akzeptoreinheiten mit einem Donor identifiziert werden. Die Analyse von HB194 (in Acetonitril oder Methanol) mittels +ESI-Orbitrap MS ergab größtenteils das Radikalkation mit der akkuraten Masse 403.1681 u ($C_{27}H_{21}ON_3$, 0.5 ppm). Die bevorzugte Bildung des Radikalkations [4] spiegelt dabei die Funktion von HB194 in der Solarzelle als Lochleiter wider. Die Verunreinigungen wurden mittels akkurater Masse und MS^n Experimenten identifiziert. Die hydroxylierte Variante zeigte dabei sowohl ein protoniertes Quasimolekülion mit *m/z* 420.1709 ($C_{27}H_{22}O_2N_3$, 0.6 ppm), als auch eine Spezies mit *m/z* 418.1557, deren Bildung noch aufgeklärt werden muss ($C_{27}H_{20}O_2N_3$, 1.5 ppm). Die MS/MS Spektren lieferten charakteristische Fragmente im Wesentlichen der Donoreinheit, deren eindeutige Zuordnung noch durch Vergleich mit strukturell ähnlichen Merocyanin-Farbstoffen bestätigt wird. Die CV-Messungen zeigten, dass die hydroxylierte Variante als Lochfalle wirken und dadurch die Solarzellenperformance negativ beeinflussen könnte. Die selektive Zugabe dieser Verunreinigung zeigte eine Verschlechterung der Solarzellen bereits ab 0.05% Verunreinigung. Diese Studie zeigt, dass schon kleinste Verunreinigungen in organischen Solarzellen zu einer Beeinträchtigung führen können und somit die Aufreinigung und Reinheitsüberprüfung ein kritischer Parameter bei der Herstellung organisch elektronischer Bauteile sind. Die LC-MS Analyse und massenspektrometrische Untersuchung von Ausgangssubstanzen sowie von neuen und gealterten Zellen kann in Zukunft ein wichtiger Teil der Forschung und Bauteilherstellung in der organischen Elektronik werden.

Neuartige Aspekte

LC-MS^n zur Reinheitskontrolle von organischen Solarzellen und Leuchtdioden (OLEDs)

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Kinetic Gas-Phase Studies on the Reaction of Ferracyclobutadienes with Alkynes

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Einleitung

Metallacyclobutadienes belong to an intriguing class of organometallic compounds, which are mostly known as intermediates in alkyne metathesis. Only a small number of compounds could be synthesized and characterized so far, as these compounds are usually very reactive. In 2002 the group of Filippou has developed a novel method for the synthesis of a new class of metallacyclobutadienes containing iron [1]. An interesting aspect of the isolated, closed-shell 16VE ferracyclobutadienes of the general formula $[Fe(CO)_{3-n}L_n(C_3R_3)]^+$ (n = 0 - 3; L = CO, phosphane, isonitrile, nitrile; R = NMe_2, NEt_2) and the products $[FeL_n(C_3R_3)]^+$ (n = 0 - 2) generated thereof by ligand dissociation, is their isolobal analogy with the fragments $[Fe(CO)_{4-m}]$ (m = 0 - 2). This suggests, that the ions $[FeL_n(C_3R_3)]^+$ (n = 0 - 2) may exist in a single or triplet electronic state, which in turn is expected to have a strong effect on their reactivity.

Methodik

The metal complexes were studied on an FT-ICR with ESI as the ionisation method of choice, as it allows the analysis of the unfragmented ion due to it softness. The intact 16 VE ferracyclobutadiene cations $[Fe(CO)_{3-n}L_n(C_3R_3)]^+$ were fragmented by either CID or IRMPD to obtain the highly unsaturated species $[FeL_n(C_3R_3]^+$ through ligand expulsion. To study the kinetic of the reaction between the unsaturated species and small alkynes two methods were applied. We started with introducing the reaction gas (alkyne) as a background gas via a leak valve into the ICR-cell, but as the reaction already took place during isolation and thermalisation of the ion, the alkyne had to be introduced at the end of preparation via a pulse valve.

Vorläufige Daten

The fragmentation of the saturated ferracyclobutadiene cations through either CID or IRMPD resulting in their unsaturated compounds allowed the determination of the relative bond dissociation energies. They range from 3 to 5 kJ which is confirmed by quantum chemical calculations. The analysis of the gas phase reactions under single-collision conditions turned out to be very challenging, as the coordinately unsaturated ferracyclobutadienes react extremely fast and are involved in complex reactant/product equilibria.To first analyze the reaction of the saturated ferracyclobutadiene cations and their unsaturated species, the compounds were isolated, thermalised while the reaction gas (ethyne, propyne, 1-butyne) was introduced as a background gas via a leak valve. With this method unsaturated 15 ferracyclobutadienes were analyzed but even though results seemed to be consistent on the first view they were not reproducible. This led to a change in the experimental set up, where the reaction gas (ethyne, propyne, 1-butyne) was no longer present as a background gas during all the preparation steps but only introduced into the cell for the pure reaction via a pulse valve. The reaction time was changed through the pulse length. The exemplary analysis of the ion $[Fe(PMe_3)(C_3(NMe_2)_3)]^+$ shows that the small singlet-triplet gap provides us with two reactive species, which do react in two completely different ways and time scales.
Species one adds only 1-butyne in the gas-phase leading to an equilibrium. Species two on the other hand does not display the direct addition of 1-butyne, but reacts with the alkyne under loss of 1-3 H_2 molecules. Modulation of the obtained experimental results with the least square method provided us with a kinetic model about the reaction rate constants of species one and two. Species one forms with a reaction rate of 80 about 4 times slower as species two with a reaction rate of 335.

Neuartige Aspekte

Ferracyclobutadienes posses small singulet-triplet gaps, shown by their individual reactivity with alkynes in the gas-phase.

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Determination of cyanobacterial microcystin fingerprints from blue-green algae

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Einleitung

Microcystins (MCs) are cyclic peptides produced by blue-green algae. They have received increasing attention in recent years because of their toxicity in humans, animals and plants. Cyanobacterial blooms in water reservoirs and lakes used as drinking water sources and recreational areas pose a high risk to human health. Consequently, the World Health Organization (WHO) has published a provisional guideline value of 1 µg/l for MC-LR in drinking water. The primary aims of this study were the development and implementation of a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for trace analysis of microcystins in water samples as well as in cyanobacterial cultures as well as the study of the diagnostic potential of microcystin fingerprints to derive environmental biomarkers.

Methodik

Cyanobacterial cell material was obtained from the Department of Clinical and Toxicological Analysis, University of São Paulo, Brazil, including lyophilized *Microcystis aeruginos*a and genetically modified *Microcystis sp* strains. The latter does not produce microcystins and was used to prepare blank matrix for ion suppression experiments. The samples were cultivated for 30 days on ASM-1 media at 25°C, irradiance of 1100 lux and a photoperiod of 12h/12 h light/ dark. Microcystins were isolated from lyophilized algal material by ultrasonication, centrifugation and filtering, followed by solid-phase extraction. Separations were carried out using HPLC on a reversed-phase C-18 column. The mobile phase was a gradient of water and acetonitrile. An electrospray quadrupole linear ion trap (QqLIT) tandem MS (5500 QTRAP) was used LC-MS/MS.

Vorläufige Daten

In this study, the mass spectrometric behavior of six selected microcystins by electrospray ionization (ESI) tandem mass spectrometry (MS/MS) was studied [1]. Chromatographic separation of the microcystins followed by multiple reaction monitoring (MRM) analysis was applied for quantification of the compounds in natural extracts. The presentation will highlight aspects of the electrospray ionization behavior of the toxins, as different factors significantly influenced the charge state distributions (multiply-charged ions, adducts, structure dependent relative ionization efficiencies). MRM analyses were conducted using a set of diagnostic product ions. The effect of biological matrix on ESI response of the microcystins was studied using post-column infusion and post-extraction spike methods. Post-column infusion gave no clear indication of matrix effects, while comparison of responses of microcystin standards to microcystins in blank matrix, however, showed suppression of 12-46% for the six representative microcystins. Additionally, the feasibility of the method for application to metabolomics techniques was investigated by comparing two cyanobacterial cultures grown under different conditions, to detect changes in the relative distribution of the microcystins. This feasibility aspect of the work was undertaken to discover diagnostic environmental biomarkers from the microcystin fingerprints in algal cultures. Currently, additional cyanobacterial cultures are grown under different conditions (temperature, pH, light and nutrient input) and toxin profiles will be measured for correlation with phenotype. Finally, it was shown that several, not yet fully identified microcystin variants were produced by *M. aeruginosa* in our experiments. The consistency of the product ion spectra of these variants with the known microcystin toxins substantiated their biogenetic link to the microcystin family.

Neuartige Aspekte

Use of microcystin fingerprints (chemotypes) as diagnostic markers for bloom formation

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Laserspektroskopische Untersuchung an heterosubstituierten halogenbenzolen mittels REMPI- und MATI-Spektroskopie

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Einleitung

Ionenspektroskopie (mit Startpunkt im Neutralmolekül) ist eine essentielle Methode zur Untersuchung der vibronischen Strukutur von molekularen ionischen Spezies und angeregten Neutralen. Theoretische Konzepte zur Kopplung von elektronischen und Vibrationszuständen lassen sich durch spektroskopische Untersuchungen vibronischer Zustände validieren. Die vibronische Struktur des ersten angeregten Zustands (S_1) wurde mit Hilfe der Resonance Enhanced Multi Photon Ionization (REMPI) Spektroskopie untersucht, der ionische Grundzustand (D_0) mit Hilfe der Mass Analyzed Threshold Ionization Spektroskopie. Bei der Analyse der REMPI-Spektren richtet sich ein besonderer Fokus auf starke Aktivitäten formal symmetrieverbotener Moden und möglicher Schwingungsprogressionen und Kombinationsbanden. In den MATI-Spektren wird besonders auf Abweichungen von der Δv=0 Vorzugsregel [1] geachtet. Diese Phänomene können wichtige Hinweise auf vibronische Kopplungen und/oder Geometrieverzerrungen bei der Anregung bzw. bei der Ionisierung sein. Substitution eines oder mehrerer Wasserstoffatome im Benzolring führt zu einer signifikanten Anderung der Elektronendichteverteilung. Vorangegangene Arbeiten haben gezeigt, dass bestimmte Substitutionsmuster am Benzolring zu einer durch vibronische Kopplung verstärkten Aktivität von formal symmetrieverbotenen Schwingungsmoden führen [2]. Das Ziel der aktuellen Untersuchungen ist es, den Einfluss der Fluorsubstitution in Halogenbenzolen zu analysieren. Die experimentellen Ergebnisse werden durch quantenchemische Rechnungen gestüzt.

Methodik

Das Experiment besteht aus einem mit einstufiger Ionenquelle und Reflektron ausgerüstetem Time of Flight (ToF)-Massenspektrometer und zwei separat (355 nm, Nd:YAG) gepumpten, durchstimmbaren Farbstofflasern. Die Dye-Wellenlänge beider Laser wird in einem BBO-I frequenzverdoppelt. Der Aufbau lässt somit Zwei-Farben-Experimente zu. Ein Uberschall Molekularstrahl aus Probenmolekülen und Seed-Gas (Argon) wird durch ein gepulstes Jet-Ventil in die Ionenquelle expandiert. Im REMPI-Modus werden die gebildeten Photoionen direkt in das ToF beschleunigt. Im MATI-Modus hingegen wird im Anschluss an die Laserionisation ein schwaches elektrisches Feld angelegt, das die prompt gebildeten Ionen von den Rydberg-Neutralen diskriminiert. Schließlich wird ein Hochspannungspuls angelegt, der die Rydberg-Neutralen ionisiert. Die nach ihrem m/z-Verhältnis separierten Ionen werden durch einen MCP-Detektor erfasst. Das Ionensignal wird von einem digitalen Oszilloskop ausgelesen.

Vorläufige Daten

Die oben genannten Untersuchungen lieferten sehr genaue Werte für die elektronische Anregungsenergie (AE) und Ionisierungsenergie (IE) von 1,3-Dichlor-2-fluorbenzol. Desweiteren konnte erstmals eine detailierte Analyse der Schwingungsstruktur durchgeführt werden. Das REMPI-Spektrum zeigt eine ungewöhnlich hohe Aktivität einer niederfrequenten out-of-plane-mode ($17b^2$). MATI-Spektren über verschiedene Moden zeigen eine Verletzung der Δv=0 Vorzugsregel. Außerdem lässt sich im MATI-Spektrum uber die 17b-Mode eine kurze, dreigliedrige Progression der Mode 17b mit einem Shift des Frank-Condon-Maximums zu kleineren Schwingungsquantenzahlen beobachten. Während die Rechnungen für den elektronischen und ionischen Grundzustand eine planare Struktur zeigen, legen die Rechnungen für den angregten Zustand eine verzerrte, nicht planare Struktur nahe.

Neuartige Aspekte

Geometrieänderung bei Anregung und Ionisation

Referenzen

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Untersuchung des Fragmentierungsverhaltens von n-Alkyl-substituierten Anilinderivaten mittels FT-ICR- Massenspektrometrie

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Einleitung

Aufbauend auf die Beobachtungen von Peters [1] bei der Untersuchung der Zerfallsprozesse von verschiedenen Rhodaminen und den dabei entstehenden Radikalen wurden verschiedene Anilinderivate mittels FT-ICR-Massenspektrometrie untersucht. Ausgehend von den käuflich zu erwerbenden Anilinen mit einer Dimethyl bzw. Ethylseitenkette wurden die Modellsysteme durch verschiedene Synthesen erweitert und modifiziert, um weitere Hinweise auf die Zerfallsprozesse zu bekommen. Bei der Beobachtung der entstehenden Fragmente wurde der Schwerpunkt auf die Fragmente der Aminoseitengruppe gelegt, da dies die einzige Möglichkeit zur Bildung der beobachteten Radikale ist. Zur genaueren Untersuchung der entstehenden Fragmente wurden die Moleküle modifiziert und das entstehende Fragment mittels MS^3 analysiert.
Zusätzlich wurde der Einfluss einer fixierten Ladung am Molekül auf die entstehenden Fragmente untersucht.

Methodik

Die Experimente wurden an einem 9,4 T FT-ICR Massenspektrometer (*Bruker Daltonics*, Bremen) durchgeführt. Zur Ionisation der unterschiedlichen Proben wurde die Elektrospray Ionisation verwendet. Die Fragmentierung der untersuchten Substanzen wurde mittels CID in der ICR-Zelle unter Nutzung von Argon als Stoßgas durchgeführt. Bei den MS^3 Experimenten wurde zusätzlich mit einem Quadropol vor der Zelle fragmentiert und die entstehenden Fragmente anschließend in der ICR-Zelle mittels CID fragmentiert.
Die verwendeten Moleküle N,N-Dimethyl-4-(2-Pyridin-4-ylvenyl-)Anilin sowie N,N-Diethyl-4-(2-Pyridin-4-ylvinyl-)Anilin wurden durch Hydrierung und Einführung bzw. Eliminierung einer Methylgruppe modifiziert.

Vorläufige Daten

Bei der Fragmentierung der Ausgangsverbindung sowie der modifizierten Derivate mit einem durchkonjugierten aromatischen System sind neben dem erwarteten Fragment eines Methans (-16 Da) auch ein Fragment eines Methyls (-15 Da) zu erkennen. Bei der Untersuchung der hydrierten Derivate mit einer Dimethylaminoseitengruppe zeigt sich als einziges Fragment eine Bindungspaltung zwischen den beiden konjugierten Systemen. Bei der Untersuchung der hierbei entstehenden Fragmente mittels MS^3 konnte nur eine Abspaltung eines Methans (-16 Da) beobachtet werden. Bei den Untersuchungen an N,N-Diethyl-4-(2-Pyridin-4-ylvinyl-)Anilin sowie den Derivaten konnte bei allen Molekülen eine Abspaltung eines Propans (-44 Da) beobachtet werden. Bei den durchkonjugieren Substanzen konnten verschiedene radikalische Fragmente (-15, 29, 43 Da) beobachtet werden. Bei allen verwendeten Derivaten mit einer fixierten positiven Ladung konnten gleiche Fragmentierungsmuster wie bei den durchkonjugierten Systemen beobachtet werden. Die Entstehung der beobachteten Fragmente kann über zwei verschiedene Wege erfolgen, denkbar sind ein konzertierter Mechanismus oder die Bildung von Radikalen. Der Reaktionsverlauf über Radikale ist durch das häufige Auftreten von radikalischen Fragmenten nicht auszuschließen, würde aber eine Verletzung der even-electron-rule bedeuten. Der Reaktionsverlauf über ein Intermediat ist ebenfalls nicht auszuschließen, kann aber mit der verwendeten Messtechnik nicht nachgewiesen werden.

Neuartige Aspekte

CID-Fragmentierung verschiedener Anilinderivate unter ESI-Bedingungen

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Untersuchung der Ammoniak Clusterbildung von 1,2-Diolen im CI-Prozess

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Einleitung

Bei der Untersuchung von Estriolderivaten zeigten sich in den CI-Massenspektren mit Ammoniak als Reaktandgas, Cluster der Masse [M+18]. Aufgrund der hohen thermischen Energie, die unter CI-Bedingungen vorherrscht, ist eine Clusterbildung nicht erwartet worden. Zudem zeigten sich die Cluster nicht bei den Estradiolderivaten. Es sollte nun untersucht werden, was für ein Cluster vorliegt und wozu dieser zerfällt. Hierzu wurde zusätzlich 1,2-Cyclopentadiol als Modellsystem untersucht.

Methodik

Verwendet wurde ein ZAB-2F der Firma Vacuum Generators mit inverser Nier-Johnson Geometrie. Die mittels Chemischer Ionisation bei 200 eV generierten Ionen wurden in einem Magnetfeld aufgetrennt und anschließend in einem E-Feld analysiert. Während für die MIKE-Spektren das B-Feld konstant bleibt und nur das E-Feld variiert wird, müssen für den linked-scan beide Felder in konstanter Abhängigkeit verändert werden. Für die Stoßaktivierung im zweiten Feld freien Raum wurde Argon als Stoßgas verwendet.
Die notwendige computergestützte Signaldetektion und Ansteuerung wurde mittels eines LabView-Programms realisiert.

Vorläufige Daten

Die Massenspektren der Estriolether (Estriol-3-X-ether, mit X= Methyl, Ethyl, Propyl, Benzyl) zeigten im CI-Massenspektrum einen Peak bei [M+18]. Die Untersuchung dieses Peaks mittels MIKE-Spektrometrie, zeigte eine Abspaltung von 17 Da. Die Untersuchung des [MH^+] mittels B^2*E-linked-scan zeigte einen Vorläufer bei [MH^++17].
Diese Ergebnisse lassen auf mehrere mögliche Clusterarten schließen. Eine Möglichkeit wäre ein [MH^++NH_3] Cluster, welcher unter Abspaltung des Ammoniak das [MH^+] bildet. Die zweite Möglichkeit wäre ein [$M+NH_4^+$] Cluster, welcher nach Protonierung einer Hydroxygruppe ein neutrales Ammoniakmolekül abspalten würde. Weiterhin wäre ein verbrückender Cluster der Art [$M+H^+$+NH_3] denkbar. Welcher der möglichen Cluster hier vorliegt, lässt sich anhand der gegebenen Daten nicht abschließend klären. Aufgrund der im CI-Prozess stattfindenden Reaktionen wird aber von einem [$M+NH_4^+$] ausgegangen.
Diese Theorie wird gestützt von den Untersuchungen am Modellsystem 1,2-Cyclopentadiol.
Die CI-Massenspektren des cis-1,2-Cyclopentadiol und des trans-1,2-Cyclopentadiol zeigen ein Signal bei [M+18], allerdings kein [MH^+].
Die Untersuchung mittels MIKES des [M+18] von trans-1,2-Cyclopentadiol zeigt nach Stoßaktivierung im zweiten Feld freien Raum einen Peak bei m/z 18, was der Abspaltung des Molekülions als Neutralteilchens entspricht. Dies deutet auf die Bildung eines sehr stabilen [$M+NH_4^+$] Clusters hin, welcher unter Stoßaktivierung in das Molekülion und ein Ammoniummolekül zerfällt.

Neuartige Aspekte

Cluster im CI-Prozess bei 1,2-Diolen.

Accurate mass determination and structural elucidation of components in one-pot cascade reactions using a multifunctional organocatalyst

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Einleitung

In the last few years, organocatalysis has emerged as a new catalytic methods based on metal-free organic molecules. In many cases, these small compounds give rise to extremely high enantioselectivity. Usually the reactions can be performed under an aerobic atmosphere with non-anhydrous solvents. The catalysts can be easily synthesized in both enantiomerically pure forms and are often more stable than enzymes or other bioorganic catalysts. A new concept of multifunctional organocatalysts have been introduced where catalytic moieties are connected to a peptide backbone. This work includes a multi-reaction sequence ideally catalyzed by only one multifunctional catalyst in a one-pot synthesis. Herein, we show how important accurate mass determination and structure elucidation by ESI-MS and ESI-MS^n on a high resolving mass spectrometer is for the determination of chemical mechanisms.

Methodik

The mechanistic studies of different one-pot cascade reactions using multifunctional organocatalysts were accomplished using a research-type LTQ-Orbitrap Elite mass spectrometer (Thermo Scientific, Bremen, Germany). This ultra-high resolution instrument allows determining not only the reaction intermediates accurately by using ESI-MS also to make accurate structural elucidation of these intermediates by ESI-MS^n. The first reaction is the acylation of *meso*-1,2-cyclohexane diol, using acetic acid anhydride and catalyst **A**, followed by the oxidation step using *m*-CPBA as cooxidant. The second example is an epoxidation of alkenes using catalyst **B** and hydrogenperoxid, N,N'-Diisopropylcarbodiimid followed with epoxide-opining and mono-acylation of the corresponding alcohol with acetic acid anhydride. Different techniques were applied (e.g. online-monitoring reaction) to study individually steps, intermediates and side products of this reaction.

Vorläufige Daten

The focus of this study was the detection of reaction intermediates and the investigation of the behaviour of the different substrates and the catalyst in each reaction step, especially with such tough oxidation condition. One important point that needed to be investigated was the influence of the temperature on the reaction because unexpectedly the oxidation reaction was slower and showed a lower yield at room temperature comparing to 10 °C or even 0 °C which showed the best results. The reaction was fully studied, the important intermediates were detected and structurally confirmed by using MS^n experiments and especially the reason for the temperature effect was determined. All relevant data will be shown and a proposed mechanistic cycle will be reported.

Neuartige Aspekte

Accurate mass determination and structure elucidation by ESI-MS(MS^n) on a high resolving mass spectrometer for the determination of chemical mechanisms.

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Kombinierte Ionisation und Oxidation sowie Fragmentierung von Analyten durch funktionalisierte Matrizes in der UV-MALDI Massenspektrometrie

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Einleitung

Massenspektrometrische Ansätze zur Strukturaufklärung von Analyten beinhalten überwiegend physikalische Maßnahmen wie eine Vorläuferionen-Selektion mit nachfolgender Analytfragmentierung durch Kollisionsgas und Nachweis der gebildeten Fragment-Ionen, die eng an die jeweiligen instrumentellen Ausstattungen gekoppelt sind und nicht an beliebigen Systemen reproduziert werden können. Chemische Fragmentierungsansätze wie eine Fragmentierung in der Ionenquelle (*in-source decay*, ISD) beinhalten hingegen Einschränkungen durch mangelnde Vorläuferionen-Selektion oder fehlende MS^x-Fragmentierungsmöglichkeiten, ermöglichen jedoch geräteunabhängige Fragmentierungen sowie die Möglichkeit der Strukturaufklärung auch mit Massenspektrometern älterer Bauart. Während bei bisherigen chemischen Analyt-Fragmentierungen mittels MALDI diese durch Übertragung von Wasserstoffradikalen von der Matrix auf die Analyte induziert werden, wird in dieser Arbeit eine Matrix vorgestellt, die primär Wasserstoffradikale von Analyten abstrahiert. Die daraus resultierenden Fragmentierungen und Oxidationen werden an unterschiedlichen Stoffklassen detailliert vorgestellt.

Methodik

Um die Matrix derart zu modifizieren, dass sie bei Interaktion mit Analyten zu einer effektiven Abstraktion von Wasserstoff- und Hydroxylradikalen befähigt ist, wurde ein α-Cyanzimtsäurederivat mit zueinander orthoständiger Hydroxyl- und Nitrogruppe synthetisiert, das durch intramolekulare Wasserstoffbrückenbindung von der Hydroxyl- zur Nitrogruppe die bekannte photochemische Fragmentierung der Nitrogruppe um die Möglichkeit einer Wasserstoffumlagerung von der benachbarten Hydroxylgruppe ergänzt. Ausgewählt wurde die Verbindung α-Cyan-3-hydroxy-4-nitrozimtsäure (3-OH-4-NO_2CCA), die anhand einer Knoevenagel-Kondensation [1] aus Cyanessigsäure und 3-Hydroxy-4-nitrobenzaldehyd dargestellt wurde. Als Analyte wurden verschiedene Stoffklassen eingesetzt, darunter Peptide (Substanz P), Lipide (1,2-Dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamin und 1,2-Dipalmitoyl-*sn*-glycero-3-phosphatidylcholin), Oligosaccharide (Lacto-*N*-Fucopentaose III) und Polyethylenglykole (PEG-2000). MS- und MS/MS-Experimente wurden u.a. an einem Triple Quadrupol-oTOF-MS (QStar Pulsar I) oder einem Bruker Autoflex durchgeführt.

Vorläufige Daten

Aus der gewählten Substituentenanordnung resultiert nach Studium der Matrix-MS und -MS/MS-Eigenspektren die Möglichkeit einer Hydroxylradikal- sowie Wasserstoffradikalabspaltung, die bei Anwesenheit von Analyten zu deren Gasphasenoxidation, oxidativen Dehydrogenierung und/oder Fragmentierung führen kann. So können entsprechende Funktionen von Peptiden oxidiert werden, wie beispielsweise die Thioethergruppe des *C*-terminalen Methionins von Substanz P, die sehr viel stärker zum Sulfoxid oxidiert wird als bei Verwendung anderer Matrizes wie HCCA oder DHB. Zusätzlich weisen Peptide eine bis mehrere stark ausgeprägte Abspaltungen von molekularem Wasserstoff auf, wobei in schwächerer Ausprägung auch der Verlust von atomarem Wasserstoff als Zwischenstufe detektiert werden kann. Die Abspaltung von molekularem Wasserstoff ist nicht nur auf Peptide beschränkt, sondern kann auch bei anderen Stoffklassen wie Lipiden und Oligosacchariden mit deutlicher Intensität detektiert werden. Fragmentierungen der oxidativ dehydrogenierten Verbindungen zeigen, dass die Wasserstoff-Abspaltung(en) annähernd gleichverteilt an abstrahierbaren Funktionen auftritt und eine zumeist nur schwach ausgeprägte Spezifität z.B. bei Lysinen unter Ausbildung von Iminen vorliegt. Des Weiteren erfolgt die Gasphasenoxidation an den Alkyl-Seitenketten von Phospholipiden unter Ausbildung von C=C-Doppelbindungen, wie bei den Fragmentierungsexperimenten des untersuchten Phosphatidylethanolamins sowie -cholins gezeigt wird. Polyether wie Polyethylenglykole (PEGs) können sehr leicht gebrochen werden. Durch Addition von Hydroxyl-Radikalen an den Bruchstellen werden so zum einen verkürzte PEGs gebildet, während durch Addition oder Abstraktion von Wasserstoffen jedoch auch Alkyl- oder Vinylseitengruppen resultieren können. Interessanterweise konnte bei der hier vorgestellten Matrix 3-OH-4-NO_2CCA trotz Beteiligung von Wasserstoffradikalen keine verstärkte ISD-Fragmentierung festgestellt werden. In diesem Beitrag werden die durch diese spezielle Matrix verursachten Oxidationen und Fragmentierungen unterschiedlicher Stoffklassen vorgestellt und die zugrunde liegenden Matrixreaktionen näher diskutiert.

Neuartige Aspekte

Es wird eine neue Matrixverbindung vorgestellt, die eine Vielzahl verschiedener Stoffklassen effektiv in der Gasphase oxidiert und Strukturanalysen erleichtern könnte.

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Characterization of soluble organic matter in samples related to oil sand-processing by Fourier transform-ion cyclotron resonance-mass spectrometry (FT-ICR-MS)

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Einleitung

Crude oils are highly complex mixtures of organic compounds, which show a huge variety in their elemental compositions and chemical structures. Besides hydrocarbons, oils contain variable but sometimes very high amounts of heteroatom-containing compounds (nitrogen, sulfur and oxygen (NSO) compounds). These heteroatomic compounds can provide geochemical information about geological origin, thermal maturity or microbial degradation. To understand the influence of heteroatom-containing constituents on the properties and quality of fossil fuels, their isolation from the hydrocarbon fractions and subsequent identification is an important challenge for industry and scientists. Fourier transform-ion cyclotron resonance-mass spectrometry in connection with specific ionization methods is a powerful tool for the detailed characterization of complex mixtures of such heteroatomic and high molecular weight compounds occurring in fossil organic matter.

Methodik

The analyzed sample set covers Canadian oil sand samples, oil containing leftovers from oil sand production (mature fine tailings, MFT) and samples from recultivated sites (tailings sands, TS) in the oil sands mining area in Alberta. The *n*-hexane-soluble fractions (maltenes) were separated from the *n*-hexane-insoluble fraction (asphaltenes) and have been analysed by direct infusion atmospheric pressure ionization coupled to ultrahigh resolution FT-ICR-MS. Electrospray ionization (ESI) mass spectra were acquired in the negative and positive ion mode using a solarix FT-ICR mass spectrometer equipped with a 12 Tesla refrigerated superconducting magnet (Bruker Daltonik GmbH).

Vorläufige Daten

The three different sample types (oil sands, MFT and TS) were compared regarding the chemical composition of the polar constituents. As expected, compounds that contain only oxygen and no other heteroatoms were most abundant in the negative-ion ESI mass spectra of all samples. Among this class O_2-compounds were most frequently detected. The oil sand samples also showed quite high amounts of N_1-compounds, while the MFT samples showed higher abundances of S_1O_2-compounds. Overall, the analytical data reveal that the chemical composition of polar compounds within oil sand samples and the mature fine tailings are fairly similar. Even higher abundances of oxygen-containing compounds and lower intensities of nitrogen- and sulphur-containing compounds were found for the maltene fractions of the tailings sand on reclamation sites.
This contribution will present a detailed molecular-level characterization of different sample types related to oil sand processing and provide a comparison of compositional information obtained by negative- versus positive-ion ESI mass spectrometry.

Neuartige Aspekte

Changes of organic matter composition over a complete oil sand mining and recultivation process.

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Quantitative Analysis of Chloramphenicol in Milk using a Novel QqQ

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Einleitung

Chloramphenicol is a broad spectrum antibiotic first employed in 1949. Although inexpensive, chloramphenicol is no longer recommended as a first line treatment in humans because of the potential for adverse effects and interactions. Bone marrow suppression resulting in non-regenerative anemia or potentially fatal idiosyncratic aplastic anemia has been reported. In addition, chloramphenicol is a potent noncompetitive microsomal enzyme inhibitor and can affect the metabolism of other drugs. Chloramphenicol is also commercialized for veterinary applications, but its use in food animals is restricted or prohibited in many countries because of the potential for adverse effects in humans. European Union decision 2003/181/EC states that any detected level of chloramphenicol in milk is prohibited, and methods for chloramphenicol detection must meet or exceed the Minimum Required Performance Level (MRPL) of 0.3 ppb.

Methodik

Chloramphenicol (98%) and Chloramphenicol-d5 (100 µg/mL in acetonitrile) was purchased. The blank milk matrix was prepared according to the method of Cronly. 1 mL acetonitrile was added to 0.5 g whole milk in a 2-mL centrifuge tube and vortexed for 30 seconds. 0.25 g NaCl was added and shaken. The slurry was centrifuged at 12000 rpm for 10 min. The top organic layer was transferred to another centrifuge tube and evaporated to dryness on an Eppendorf VacuumConcentrator at 45 °C. The extract was reconstituted in 0.5 mL initial mobile phase and filtered through a 0.2 um PVDF syringe filter. Samples were separated with a Bruker Advance UHPLC on ACE column, coupled to a Bruker EVOQ Elite LC/QqQ System.

Vorläufige Daten

A 6-level matrix matched calibration curve from 0.020 to 1.0 ppb was generated with each level analyzed in triplicate. Excellent linearity was obtained with R^2=0.9980 (weighting factor =1/X). In the current analysis, the ion ratios were found consistent (<10% RSD) from 0.05 ppb to 1 ppb levels. The ion ratios at 0.02 ppb level were out of range due to matrix contribution to the quantitation MRM (320.9>152). However, after correction with matrix blank, the ion ratios are consistent within 15% of the higher levels.

Neuartige Aspekte

Quantitation of Chloramphenicol down to 0.002 ppb level

Bioanalysis of Dermorphin, an Opioid Peptide in Equine Urine

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Einleitung

Dermorphin is an opioid hepta-peptide derived from the skin secretions of South American frogs (*Phyllomedusa sauvagei*). It is 30-to-40 times more potent an analgesic than morphine and is unique because it contains Dalanine, a property that makes it highly resistant to protease mediated degradation. While d-amino acid peptides are sought after for their low immunogenic response and in peptidomimetic drug design, it was dermorphin's use in horse racing that catapulted this opioid peptide from obscurity into the headlines. The pain-killing properties of dermorphin were used to drive race horse performance beyond normal physical limits, often injuring the animal. As a result, dermorphin is now a class I prohibited substance as decreed by the Association of Racing Commissioners International (RCI) Model Rules. The analysis of dermorphin in equine plasma is a challenging assay because of the associated complex matrix and lack of a stable labeled internal standard.

Methodik

After solid phase extraction cleanup, samples were separated with a Bruker Advance UHPLC on C18 column (ACE 3µ, C18, 100x2.1mm), coupled to a Bruker EVOQ Elite LC/QqQ System.

Vorläufige Daten

Accurate quantification of dermorphin in equine plasma between the range of 0.5-1000ng/mL in equine plasma was easily achieved on the EVOQ Elite. The calibration curve had a response factor relative standard deviation of 12% RSD. The response factors for the QC's had a RSD of 7% RSD. The ion source on the EVOQ Elite easily met the LLOQ requirements for dermorphin in equine plasma without the use of a divert valve.

Neuartige Aspekte

Met the LLOQ requirements for dermorphin in equine plasma without the use of a divert valve.

Clenbuterol in Equine Plasma – A Study on Ion Source Robustness & High Sensitivity

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Einleitung

Sustained high sensitivity and robust performance are crucial for success in a laboratory doing quantitative analysis. To test the ion source design of a novel LC/QqQ, horse plasma was used in a dilute and shoot manner, without incorporation of an in-line divert valve. The ion source was exposed to all matrix eluting off the column, from every sample injection. Since the liquid eluent is evaporated using nebulization via heated nitrogen gas during the electrospray process, salts, lipids, sugars, proteins, and other contaminants are liable to coalesce within the ion source, causing sensitivity degradation over time. If the gas flow dynamics within the ion source are carefully studied, and the design avoids areas of coalescence, cold spots, and eliminates gas recirculation, using a gas entrainment exhaust system can dramatically improve the robustness of the ion source, while maintaining high sensitivity performance. Crashed equine plasma was chosen as the matrix for the dilute-and-shoot experiment.

Methodik

Equine plasma was crashed using cold acidified acetonitrile (3:1 v/v). The samples were centrifuged for 10mins and the supernatant dissolved 1:1 v/v with mobile phase A. Samples were separated with a Bruker Advance UHPLC on a ACE C18 column (3µ, 100x2.1mm) , coupled to a Bruker EVOQ Elite LC/QqQ System. 500 injections were constantly running for over 48hrs (the weekend). Clenbuterol was injected (dilute-and-shoot) without the use of a divert valve. Four calibration curves were analyzed, distributed at the beginning, two bracketing the middle, and one at the end of the series.

Vorläufige Daten

The success criterion was to achieve <10% relative standard deviation (%RSD) for the response factor for the four calibration curves dispersed within 500 horse plasma samples. Any decrease in sensitivity over the 48 h injections would have been reflected by a higher %RSD response factor. The %RSD of the response factors of the four calibration curves was 4.38%, which is well within the acceptance criteria of validated bioanalytical methods. The Active Exhaust system of the ion source evacuates the ion source housing by a slight pressure differential thereby eliminating recirculation of nebulized gases and contaminants. The large exhaust opening ensures rapid removal of the nebulized components and keeps the area in front of the ion sampling orifice clean.

Neuartige Aspekte

A Study on Ion Source Robustness & High Sensitivity

Derivatisierung- unverzichtbares Werkzeug für die massenspektrometrische Strukturaufklärung

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Einleitung

In der forensischen analytischen Chemie müssen sehr häufig Verbindungen aus den Bereichen Betäubungsmittel, Arzneimittel, Doping und Life-Style massenspektrometrisch analysiert werden, die nicht in kommerziell zugänglichen massenspektrometrischen Spektrenbibliotheken enthalten sind. Dabei handelt es in der Mehrzahl um niedermolekulare, polare Substanzen, für die die massenspektrometrische Strukturaufklärung in Kombination mit Derivatisierungtechniken zu einem unverzichtbaren Werkzeug geworden ist. *N*-Methyl-N-trimethylsilyltrifluoracetamid (MSTFA) [1] ist ein weitverbreitetes Derivatisierungsmittel in der GC/MS-Analytik, das zur Bildung von Trimethylsilyl (TMS)-Derivaten führt. Ein großer Vorteil dieses Reagenzes ist die Reaktionsfähigkeit mit vielen reaktiven Gruppen wie Aldehyden Alkoholen, primären und sekundären Aminen, Ketonen (bei Keto-Enol-Tautomerie), Säuren und Thiolen ohne dass die Reaktionsbedingungen verändert werden müssen. Im Gegensatz dazu werden nur sehr selten isotopenmarkierte Formen der Derivatisierungsmittel zusätzlich zur Strukturaufklärung eingesetzt. Die zusätzliche Verwendung von deuteriertem MSTFA (MSTFA-D_9) [2] liefert weitere wichtige Informationen zur Molmasse der untersuchten Verbindung und zur Struktur der beobachteten Fragmentionen. Die Auswertestrategie und die Vorteile der kombinierten Verwendung beider Formen der verwendeten Derivatisierungsmittel werden vorgestellt.

Methodik

Zur Herstellung der TMS-Derivate werden die Analyte in einem aprotischen Lösemittel gelöst und mit einem Überschuss der Derivatisierungsmittel MSTFA bzw. MSTFA-D_9 versetzt. Zur quantitativen Umsetzung können zusätzlich Katalysatoren zugegeben werden. Alternativ können MSTFA und MSTFA-D_9 auch direkt als Lösemittel eingesetzt werden, weil beide Derivatisierungsmittel hervorragende Lösungseigenschaften sowohl für die polaren Edukte als auch für die Derivate besitzen. Danach werden die Probengemische für 20 Minuten auf 70 °C erhitzt und können direkt oder nach Verdünnung mit einem aprotischen Lösungsmittel in das GC/MS-System injiziert werden. Ein Überschuss des Derivatisierungsmittels stört nicht den Nachweis, sondern führt im Gegenteil zu einer Absättigung reaktiver Stellen im GC-System, was zu einer Verbesserung der Nachweisgrenzen beiträgt. Für die gaschromatographische Trennung sind unpolare oder leicht polare GC-Säulen geeignet.

Vorläufige Daten

In den Elektronenstoß-Massenspektren der Derivate geben Fragmentionen der TMS-Gruppe einen schnellen Überblick, bei welchen Retentionszeiten TMS-Derivate von der Trennsäule eluieren. Ein besonderer Vorteil dieser Derivatisierungstechnik ist, dass bei komplexen Substanzgemischen die TMS- und TMS-D9-Derivate nur in einen Abstand von wenigen Sekunden von der Trennsäule eluieren. Die Molmassen der untersuchten Verbindungen können durch das gleichzeitige Auftreten des Molekülradikalkations und einer Methylgruppenabspaltung ($M^{+\cdot}$-15) der TMS-Derivate in nahezu allen Fällen bestimmt werden. Die Bestätigung erfolgt mit Hilfe der deuterierten TMS-Derivate. Dabei beträgt der Massenunterschied zwischen den deuterierten und nicht-deuterierten Verbindungen für das Molekülradikalkation neun Masseneinheiten und bei den Signalen der Methylgruppenabspaltung nur sechs Masseneinheiten. Zusätzlich treten substanzklassenspezifische Fragmentionen auf, die die in den untersuchten Analyten enthaltenen funktionellen Gruppen belegen. In diesem Zusammenhang sind vollständige Umsetzungen der Ausgangsverbindungen zu den TMS-Derivaten, wie sie z. B. für Quantifizierungen erforderlich sind, für Strukturaufklärung nicht erforderlich, weil unterschiedliche Silylierungsstufen in einem Chromatogramm eine leichtere Bestimmung der vorliegenden funktionellen Gruppen zulassen. Im Gegensatz zu Alkoholen und Säuren werden primäre und sekundäre Amine selten vollständig ohne Katalysator umgesetzt. Ketone, bei denen eine Keto-Enol-Tautomerie möglich ist, können mit MSTFA bis zu vier Derivate bilden. Sehr charakteristische Fragmentionen werden in den Massenspektren von Aldehyden beobachtet, die eine direkte Zuordnung zu dieser Substanzklasse zulassen.
Die Derivatisierungen von unbekannten Verbindungen mit MSTFA und MSTFA-D_9 erhöht deutlich die Zuverlässigkeit des entwickelten Strukturvorschlages, wenn die Massenspektren auf einfachen Massenspektrometern aufgenommen wurden. Bei Verwendung weiterer massenspektrometrischer Methoden wie MSn-Untersuchungen und exakter Massenbestimmung kann die Leistungsfähigkeit für die Strukturaufklärung nochmals deutlich gesteigert werden.

Neuartige Aspekte

Die Zuverlässigkeit von entwickelten Strukturvorschlägen wird durch die kombinierte Verwendung von MSTFA und deuteriertem MSTFA deutlich verbessert.

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LC-Q-TOF based metabolic profiling of dansylated metabolite to study yeast arginine biosynthesis deletion mutants

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Einleitung

Yeast has been an import model system for biological studies because of its simplicity, easy of manipulation and the availability of strains with individual gene deletions. In this work, a non-targeted LC-Q-TOF based approach was used to profile the metabolism of two deletion mutants in the arginine biosynthesis pathway to identify metabolic changes.
To enable this metabolic profiling work using standard C_{18} chromatographic separation, the extracted amino acids metabolites were dansylated. Additionally, dansylation often leads to an enhancement of the electrospray ionization signal intensities.

Methodik

Statistical analysis of wild type versus the gene deletion mutants using PCA and t-test approaches revealed an accumulation of several metabolites. Elemental formulae for these compounds were determined using SmartFormula3D by evaluating the MS and associated MS/MS data. Molecular formulae were queried against public access databases to derive candidate chemical structures. Correlating these database matches with information contained in MS/MS spectra enabled the confirmation of particular structural hypotheses using the FragmentExplorer. The characteristic reporter fragment ion (m/z 170; $C_{12}H_{12}N$) for the dansylated compounds facilitated this work by limiting the candidate structures to classes of compound which can be derivatised by dansylation.

Vorläufige Daten

Using this approach an accumulation of argininosuccinic acid and N-acetylornithine could be observed in arg4 mutant yeast cells accompanied with a decrease in relative amounts of aspartate. N-acetylornithine had a higher abundance in the arg7 deletion mutant compared to yeast wild type cells. These metabolic profiling data agreed with the hypothesis that there would be an accumulation of a precursor upstream of the blocked enzyme.
Further, unexpected, but highly statistically significant changes in the metabolic profile were determined which were not associated with arginine biosynthetic pathway. This represents the power of untargeted metabolic profiling as such changes might not be detected if a targeted approach were to be used.

Neuartige Aspekte

Profiling the metabolism of two deletion mutants in the arginine biosynthesis pathway to identify metabolic changes.

Vergleich der MS-Fragmentierungsmuster von Umweltchemikalien bei Verwendung von hochauflösenden Massenspektrometern

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Einleitung

Mit Hilfe von hochauflösenden Massenspektrometern (HR-MS) können zum einen über die Messung der exakten Masse die Elementzusammensetzung und zum anderen über die Fragmentierungsmuster (MS^n) Informationen über Strukturelemente gewonnen werden. Zur Identifizierung unbekannter Verbindungen sind Spektren-Datenbanken hilfreich, welche die MS-Informationen (exakte Masse, Fragmentierungsmuster) sammeln und dann für Recherchen nutzbar machen. Eine Voraussetzung dafür ist die Reproduzierbarkeit und Vergleichbarkeit der Spektren unterschiedlicher HR-MS-Systeme.
In dieser Studie wurden die ermittelten exakten Massen und die Fragmen-tierungsmuster von LTQ-Orbitrap MS, FT-ICR MS und Q-ToF-MS verglichen, um zu prüfen, inwieweit die Grundvorrausetzungen für geräteübergreifende MS-Datenbanken gegeben sind. Zum anderen sollte untersucht werden, welches MS-System in Bezug auf Vergleichbarkeit und Reproduzierbarkeit der Ergebnisse am ehesten für eine Spektren-Datenbank geeignet erscheint.

Methodik

Insgesamt wurden 25 umweltrelevante Chemikalien (199 Da - 444 Da; Arzneistoffe, Biozide, Industriechemikalien, Hormone, Biotransformationsprodukte) mittels Direktinjektion an drei verschiedenen MS-Systemen (LTQ-Orbitrap MS, FT-ICR MS und Q-ToF-MS) mit insgesamt fünf unterschiedlichen Fragmentierungs-techniken (*c*ollision *i*nduced *d*issociation: $CID^{Orbitrap,\ FT\text{-}ICR\ und\ ToF}$; *h*igher energy *c*ollisional *d*issociation: HCD und *i*nfra*r*ed *m*ulti*p*hoton *d*issociation: IRMPD) untersucht. Aus jedem Spektrum wird eine bestimmte Anzahl Fragmente erhalten. Die Vergleichbarkeit von beispielsweise $CID^{Orbitrap}$ mit IRMPD ergibt sich dann durch die Anzahl der übereinstimmenden Fragmente in beiden Spektren derselben Substanz dividiert durch die Summe aller Fragmente beider Spektren abzüglich der Anzahl der Übereinstimmungen. Auf diesem Weg wurde die Auswertung offline in Excel für alle 25 Substanzen sowie die drei Systeme mit unterschiedlichen Fragmentierungsarten durchgeführt.

Vorläufige Daten

Die Fragmentierungsmuster an den drei verwendeten MS-Systemen waren für die meisten der 25 untersuchten Substanzen nicht oder nur bedingt vergleichbar. Dies bedeutet, dass eine Spektrendatenbank, die von allen drei MS-Systemen gespeist würde, nur eine begrenzte Aussagekraft hat. Beispielsweise variierte das Fragmen-tierungs-verhalten des Antibiotikums Tetracyclin im Positivmodus in Abhängigkeit von der Fragmentierungsart und der - energie. Wird $CID^{Orbitrap}$ direkt mit HCD verglichen, wurden je nach Fragmentierungsenergie qualitative Übereinstimmungen von lediglich 0-20% erreicht (ohne Berücksichtigung der Intensitäten). Die höchste Übereinstimmung betrug 43% zwischen $CID^{Orbitrap}$ und CID^{ToF}. Chemisch zeigt sich, dass von drei entstehenden Fragmenten bei $CID^{Orbitrap}$ ein Produktion (Abspaltung von NH_3) nicht am FT-ICR MS zu sehen ist. Dort sieht man das Fragment, welches aus einer Abspaltung von Ammoniak und Wasser entsteht. Die unspezifische alleinige Abspaltung von Wasser kann mit sämtlichen hier genannten Methoden erfasst werden. Die weiteren Fragmente, die mit $CID^{FT\text{-}ICR,ToF}$, HCD und IRMPD zu erkennen sind, ermöglichen zum Teil deutlich mehr Einblick in die Struktur des Ausgangsions, da sie neben den eben erwähnten unspezifischen auch charakteristische Abspaltungen zeigen. Um möglichst viel Information über das zu dem Zeitpunkt unbekannte Ausgangmolekül zu erhalten, ist es notwendig, viele und spezifische Fragmente zu erzeugen, wie es hier z.B. im Fall von Tetracyclin mit $CID^{Orbitrap}$ nicht gelungen ist. Im Hinblick auf die Entwicklung von Datenbanken z.B. für die Identifizierung von unbekannten Substanzen deuten die bisher erzielten Ergebnisse darauf hin, dass Tetracyclin bei Verwendung unterschiedlicher MS-Systeme möglicherweise nicht identifiziert worden wäre. Derzeit wird überprüft, welches der drei MS-Systeme sich am besten eignet um a) reproduzierbare MS-Fragmentierungen und b) möglichst viele Strukturinformationen zu erhalten.

Neuartige Aspekte

Vergleich der Fragmentierungsmuster dreier hochauflösender Massenspektrometer zur Identifizierung und Strukturaufklärung kleiner organischer Moleküle.

Ultrafast Statistical Profiling: FTMS based profiling of myxobacteria natural products for rapid detection and identification of marker compounds

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Einleitung

Myxobacteria represent an important source of novel natural products exhibiting a wide range of biological activities. Some of these so-called secondary metabolites are investigated as potential leads for novel drugs. Traditional approaches to discovering natural products mainly employ bioassays and activity-guided isolation from different myxobacterial isolates, but genomics-based strategies become increasingly successful to reveal additional compounds. These "metabolome-mining" approaches hold great promise for uncovering novel secondary metabolites from myxobacterial strains, as the number of known compounds identified to date is often significantly lower than expected from genome sequence information.

Methodik

Currently, successfully established methods are based on LC-QTOF-MS analysis requiring about 20 min analysis times per sample. Facing several thousand myxobacterial strain isolates as well as numerous genetic knock out mutant strains available to analyze for the presence of novel secondary metabolites, analysis time becomes a bottleneck.

Vorläufige Daten

In this proof of concept study we analyzed several metabolite extracts from genetic knock-out mutants by ultra-high resolution FT-ICR direct infusion measurements, requiring only about 1 min / sample to measure. Principal Component Analysis (PCA) of the acquired MS spectra showed a clustering of the samples according to the bacterial genetic background, i.e. wild type and mutants could be differentiated. PCA loadings pointed to compounds responsible for this differentiation. By measuring these regions of interest in continuous accumulation of selected ions (CASI) mode, the sensitivity and resolution of these mass ranges could be enhanced significantly. The instrument resolving power of R>750.000 provided isotopic fine structure information. This enabled to literally “read out” the correct elemental composition for the target compounds. We have demonstrated for myxoprincomide, a recently discovered myxobacterial secondary metabolite, that this approach enables an unequivocal molecular formula generation for a compound with MW >1000. The ultra high resolution of an FT-ICR instrument enables for the first time an elemental composition determination for a molecular weight range where mass accuracy of existing mass spectrometers would be not enough for an unequivocal decision.
The presented “Ultrafast Statistical Profiling” workflow enables a rapid profiling of complex metabolite extracts and identification of relevant marker compounds by making use of the ultrahigh resolution and the wide dynamic range provided by the FT-ICR technology – addressing two of the major bottleneck in metabolomics, sample throughput and compound identification.

Neuartige Aspekte

The workflow enables a profiling of complex metabolite extracts and identification of relevant marker compounds by making use of the FT-ICR.

Improved Method for Characterization of Phenolic Compounds from Rosemary Extracts using ESI-Qq-TOF MS

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Einleitung

Rosemary (*Rosmarinus officinalis* L.) is a shrub traditionally used as culinary spice, as well as in folk medicine, being a greatly valued medicinal herb. In fact, this plant exerts a great number of pharmacological activities. Most of these observed effects are linked to its phenolic content. Recently there has been a growing interest in the use of natural antioxidants in the food industry as a preservation method. Due to its high antioxidant capacity, rosemary turned out being one of the most used natural antioxidant because of its value for preservation and its beneficial effects mentioned above.

Methodik

This work comprehensively characterizes the bioactive secondary metabolites present in two rosemary extracts obtained by SFE (CO_2 at 150 bar) and PLE (H_20 at 200º C).
A sensitive analytical method for phenolic compounds has been developed using high resolution chromatography and ESI-Qq-TOF mass spectrometry. The extracts were analysed by both positive and negative ionization modes for comprehensive characterization. Modern ESI-Qq-TOF-MS instrumentation provides high mass resolution and mass accuracy in combination with isotopic pattern information, for both MS full scan and MS/MS spectra thus providing an established method for molecular formula determination and confirmation.

Vorläufige Daten

The presented methodology proved to be an effective tool for the separation of a wide range of phenolic compounds in rosemary extracts: phenolic diterpenes (carnosol, carnosic acid, rosmadial, rosmanol and its isomers epirosmanol/epiisorosmanol), flavonoids (apigenin, genkwanin, salvigenin, ladanein, cirsiliol), phenolic acids (artepillin C), among other classes of phenolic compounds such as rosmarinic acid. The results of this study were in good agreement with prior characterization of rosemary or other species of the Lamiaceae family (Borrás-Linares *et al,* J. Chromatography A, 1218 (2011) 7682-7690). In this work, the analysis time and the flow rate were optimized without affecting the chromatographic resolution. Using the methodology that will be presented, we were able to find new compounds which were not detected previously in these extracts such as cirsiliol, 5-methoxy-6,7-methylenedioxyflavone and 12-O-methylcarnosol.

Neuartige Aspekte

The methodology proved to be an effective tool for the separation of a wide range of phenolic compounds in rosemary.

MASS SPECTRAL STRUCTURE ELUCIDATION OF PRODUCTS FROM THE OXIDATION OF SULFONAMIDES BY BIRNESSITE

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Einleitung

Sulfonamides are a class of antibiotic pharmaceuticals which are widely used in livestock production. They reach agricultural soils and the aquatic environment mainly through the use of manure or directly through grazing livestock. To assess the potential risks of sulfonamides released into the environment a comprehensive understanding of their transport [1] and fate is required. Bio- [2] and photodegradation [3] have been recognized as possible transformation pathways of sulfonamides in soil and water. Manganese oxides like birnessite are common oxidants in soil and were used for the oxidative degradation of sulfadiazine in aqueous solution [4]. Recently the products of MnO_2-mediated sulfonamide transformation have been reported [5].

Methodik

Here we present the oxidative degradation of various sulfonamides (sulfadiazine, sulfamethazine, sulfamethoxazole) in the presence of birnessite. Reaction rates were followed by HPLC-UV and oxidation products were identified by high resolution Fourier transformation ion cyclotron resonance mass spectrometry (FTICR-MS) and liquid chromatography-tandem mass spectrometry (LC-MS-MS).

Vorläufige Daten

The obtained products included oxidized sulfonamides, SO_2 extrusion products and dimeric products.

Neuartige Aspekte

Identification of new oxidation products from the reaction of sulfonamides with a MnO_2 mineral.

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The impact of high-resolution MS and NMR techniques on the discovery

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Einleitung

Myxobacteria represent an important source of novel natural products exhibiting a wide range of biological activities. Some of these so-called secondary metabolites are investigated as potential leads for novel drugs. Traditional approaches to discovering natural products mainly employ bioassays and activity-guided isolation, but genomics-based strategies become increasingly successful to reveal additional compounds. These "metabolome-mining" approaches hold great promise for uncovering novel secondary metabolites from myxobacterial strains, as the number of known compounds identified to date is often significantly lower than expected from genome sequence information. Their discovery enables characterization of the underlying biosynthetic pathways and at the same time chances are good to find compounds with potent biological activity.

Methodik

The important key for the rapid identification of known compound classes is the ability to generate a partial (or even complete) structure based on MS and NMR information as early as possible in the course of the discovery workflow. LC-SPE-NMR/MS is an important tool to achieve this objective. After initial separation of a myxobacterial extract obtained from small scale fermentation into crude fractions by size-exclusion chromatography, analytical HPLC with MS monitoring was used to track fractions of interest. Candidate peaks were then trapped on SPE cartridges and transferred via a Cryofit flowprobe to the NMR system using deuterated solvent. The acquisition of 1D and 2D NMR spectra sets from small scales was achieved by applying multiple solvent suppression pulse programs.

Vorläufige Daten

Combining NMR data with elemental composition information for precursor and fragment ions obtained from accurate mass MS measurements enabled the rapid identification of known and novel structural scaffolds. Thus, a well-founded decision whether to include a candidate compound into the labor-intensive up scaling, fermentation and purification pipeline can be rapidly made.
Here we show that UHPLC-coupled UHR-TOF mass spectrometry and LC-NMR represent complementary analytical tools for the discovery of novel natural products, efficient dereplication, comprehensive mining of metaboloms and rapid structure elucidation of novel metabolites.

Neuartige Aspekte

UHPLC-coupled UHR-TOF mass spectrometry and LC-NMR represent complementary analytical tools for the discovery of novel natural products.

Identification of Glutathione Conjugates by Liquid Chromatography-High Resolution Time of Flight Mass Spectrometry and High Performance Fragment Ion Analysis

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Einleitung

New chemical entities (NCEs) have to be tested for their potential to be converted into electrophilic substances by metabolic enzymes of the human body. Normally, electrophilic metabolites are detoxified by conjugation with glutathione (GSH; g-L-Glutamyl-L-cysteinylglycine), a tripeptide which binds to electrophilic metabolites via the thiol group of cysteine. The identification of GSH conjugates can be performed with liquid chromatography mass spectrometry using neutral loss experiments (-129; pyroglutamine). However, since NCEs are often converted into several metabolites, the clear identification of GSH conjugates is still a challenge. In this contribution, we applied high resolution mass spectrometry for the identification of GSH conjugates of clozapine (CLP) and acetaminophen (AAP), well known drugs which are converted to reactive metabolites in the human liver by cytochrome P450 enzymes.

Methodik

Samples resulting from incubations were cleaned by SPE, prior to MS analysis. The samples were analyzed by an UHPLC system interfaced to a LECO Citius high resolution time-of-flight mass spectrometer, operating the ESI source in positive ion mode. Pulsed post-source collision induced dissociation was used to acquire alternating precursor and product ion spectra that were recorded as separated data channels.

Vorläufige Daten

Glutathione conjugations give a common loss corresponding to the pyroglutamic acid moiety,which can be monitored. Neutral loss scanning using a triple quadrupole mass spectrometer is a widespread technique for screening the GSH adducts. However, the NL scanning results in the appearance of many false positive peaks. Therefore the orginal LC-QQQ method was transferred to an UHPLC-high resolution TOF system, minimizing the analysis time significantly. High resolution MS was performed using a multi-reflecting time-of-flight MS (MR-TOFMS). MR-TOFMS technology can exhibit mass independent and acquisition rate-independent resolving power greater than 50,000 and sub-ppm mass accuracy. The acquired accurate mass data were employed for empirical formula generation to aid in the confirmation of GSH adducts and the elimination of false positives. The utility of post-source CID allows the generation of precursor and product ion sprectra by pulsing between low and high energy in a post-source controlled pressure region. The data from each energy state are collected independently. Precursor and fragment ion chromatograms were extrac- ted from these channels. The results show that accurate mass information for precursor and fragment ions using cpmprehensive CID that UHPLC/high resolution TOF MS is a good alternative for screening reactive metabolites.

Neuartige Aspekte

High Resolution Time of Flight Mass Spectrometry aids in the confirmation of GSH adducts and the elimination of false positives.

Bestimmung von Gallensäuren mittels HPLC-MS/MS

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Einleitung

Der menschliche Körper scheidet täglich etwa 1 g Cholesterin in Form von Gallensäuren aus, welche auf diesem Weg in das Abwasser gelangen. Da in Kläranlagen diese Gallensäuren nur teilweise zurückgehalten und abgebaut werden, gelangt ein Teil von ihnen in das Oberflächenwasser und kann dort als potentieller anthropogener Marker genutzt werden. Da Gallensäuren keine chromophoren Gruppen im Molekül aufweisen, bietet sich nach einer HPLC-Trennung die Massenspektrometrie als Detektionsmethode an.

Methodik

Aufgrund der Carboxylgruppe im Molekül lassen sich die verschiedenen Gallensäuren sehr effizient mit Elektrospray (ESI) im negativen Modus ionisieren. Bei der Ionisation wird nahezu ausschließlich das $[M-H]^-$ - Quasimolekülion gebildet [1]. Nur bei einigen wenigen der untersuchten Gallensäuren lassen sich auch Fragmentionen nachweisen. Diese $[M-H]^-$ - Quasimolekülionen sind so stabil, dass sie auch beim Einsatz der Tandem-Massenspektrometrie nach Stoßaktivierung in der Kollisionszelle nicht zerfallen. Ein selektiver und empfindlicher Nachweis im Spurenbereich mittels MS/MS ist auf diesem Wege nicht möglich.
Wenn man als Laufmittel für die HPLC jedoch ein Wasser/Methanol/Ammoniumacetat/Essigsäure – Puffergemisch verwendet, werden neben den $[M-H]^-$ - Quasimolekülionen auch $[M-H+CH_3COOH]^-$ - Adduktionen gebildet. Diese Adduktionen spalten in der Kollisionszelle wieder Essigsäure ab, so dass man den entsprechenden Zerfallsprozess mittels MS/MS registrieren kann.

Vorläufige Daten

Werden die Gallensäuren ausschließlich über ihre $[M-H]^-$ - Quasimolekülionen im MID-Modus („multiple ion detection“) detektiert, lassen sich hier mittels HPLC/MS Nachweisgrenzen von etwa 100 µg/L erreichen. Diese Nachweisgrenzen variieren sehr stark in Abhängigkeit von der Probenmatrix und dem durch das HPLC-System verursachten Signaluntergrund (Säulenbluten…). Da bei Oberflächenwasserproben die erwarteten Analytkonzentrationen unterhalb von 10 µg/L liegen, ist die Nachweisempfindlichkeit dieser Methode dafür nicht ausreichend.
Mit der spezifischen MS/MS-Detektierung des Zerfalls der $[M-H+CH_3COOH]^-$ - Adduktionen zu den $[M-H]^-$ - Quasimolekülionen können ohne vorhergehende Anreicherung der Oberflächenwasserproben Nachweisgrenzen von 0,2-1 µg/L erreicht werden. Unter Nutzung von Anreicherungsverfahren wie zum Beispiel der SPE lässt sich die Gesamtempfindlichkeit des Verfahrens noch weiter steigern.

Neuartige Aspekte

Gallensäuren lassen sich mittels HPLC-MS/MS über Adduktionenfragmentierung sehr empfindlich mit Nachweisgrenzen unter 1µg/L in Wasserproben bestimmen.

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Fluorierte Pyridine – Anionen- π-Wechselwirkungen?

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Einleitung

ESI-FTICR-Massenspektrometrie ist ein nützliches Werkzeug zur Analyse von supramolekularen Strukturen. Sie bietet die Möglichkeit, auch schwache, nicht-kovalente Wechselwirkungen, wie Wasserstoffbrückenbindungen, π-π-Wechselwirkungen und ionische Wechselwirkungen, zu untersuchen. Seit einiger Zeit werden besonders Wechselwirkungen von Anionen mit aromatischen Systemen (Anionen- π-Wechselwirkungen) erforscht, welche zum Design von Anionenrezeptoren genutzt werden können.[1] Aufgrund der negativen Ladungsdichte der π-Systeme, wurde lange Zeit von einer repulsiven Natur dieser Wechselwirkungen ausgegangen. Durch einige theoretische Studien konnte jedoch bereits gezeigt werden, dass durch geeignete elektronenziehende Substituenten eine anziehende Wechselwirkung zwischen Anion und Aromat bestehen kann.[2] Experimentelle Nachweise, besonders in der Gasphase, sind nach wie vor selten.[3]
In dieser Arbeit wurden verschiedene fluorierte Pyridine auf Komplexbildung mit Anionen in der Gasphase untersucht.

Methodik

Die Experimente wurden mit einem IonSpec QFT 7 FTICR-Massenspektrometer durchgeführt, welches mit einem 7 T Magneten ausgestattet ist. Zum Nachweis der Komplexe wurden die entsprechenden Peaks isoliert und anschließend in der ICR-Zelle mittels Kollisionsinduzierter Dissoziation (CID) fragmentiert.
Zur Ermittlung der relativen Bindungsstärke der Anion-Pyridin-Komplexe wurden Konkurrenzexperimente durchgeführt. Dabei befanden sich in der Messlösung zwei verschiedene Komplexe, deren Isolation und Fragmentierung somit unter gleichen Bedingungen stattfand. Gaspuls und Anregungsenergie wurden bei allen Messungen konstant gehalten.

Vorläufige Daten

Bei den zu untersuchenden Verbindungen handelte es sich um organische aromatische Moleküle, die sich in Anzahl und Position der Fluorsubstituenten unterscheiden. Neben der Bindung von Halogenen wurde auch die Bindung von mehratomigen Anionen sowie die Bindung zum Dianion Indigokarmin untersucht.
Es konnte gezeigt werden, dass verschiedene Unterstrukturen keine der Anionen binden, während andere, vor allem perfluorierte Strukturen, fast ausschließlich mit den Halogenen Komplexe bilden. Für die qualitative Bindungsstärke der unterschiedlichen Halogene konnte ein Trend erstellt werden. Ebenso war es von einigen Komplexen möglich, relative Bindungsenergien zu ermitteln. Außerdem wurde nachgewiesen, dass einige Strukturen an das Dianion Indigokarmin binden.

Neuartige Aspekte

Experimenteller Nachweis von Anion-π-Wechselwirkungen in der Gasphase.

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Comparing the performance of tandem mass spectral libraries used in small molecular identification

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Einleitung

Small molecule identification is an important field of application for bioorganic MS. One competent approach involves fast and automated matching of mass spectra to spectral collections. Nevertheless, the development of reliable, robust and transferable tandem mass spectral libraries was found to be challenging. Tandem mass spectra turned out to be very much dependent on experimental settings and showed significant variability in terms of relative ion abundances and spectral content among instruments. Different strategies have been developed to solve that problem. This study is aimed to evaluate the usefulness of these approaches by comparing the performance of representative tandem mass spectral libraries.

Methodik

The tandem mass spectral libraries tested included the “Wiley Registry of Tandem Mass Spectral Data, MSforID” (Wiley Registry MSMS, www.msforid.com), the MS/MS collection of the “NIST/NIH/EPA Mass Spectral Library 2011” (www.nist.gov/srd/nist1a.cfm#) and the Weinmann library (www.chemicalsoft.de/MSMS_QTrap/MSMS_QTrap-index.php). The MSforID Search program or the NIST MS Search program 2.0g was used for matching. In addition to the reference spectra, spectra acquired on multiple instruments, extracted from literature ore generated in silico were used as test sets. The results of more than 100,000 database queries were statistically evaluated to assess the performances of the libraries in terms of sensitivity, specificity and robustness.

Vorläufige Daten

We have assessed and compared the performance of three important tandem mass spectral libraries. Positive and negative control samples were used to determine sensitivity and specificity of the libraries tested. Sample spectra were acquired on various instrumental platforms or generated in silico. Statistical evaluation of the search results revealed that overall best performance was provided by the Wiley Registry MSMS, which is attributable to the systematic coverage of compound-specific breakdown curves by multiple reference spectra as well as the mass accuracy and resolution provided by QqTOF instrument used to develop the library. Sensitivity for instance was found to be >95% even for test sets acquired on tandem-in-time instruments or generated by up-front CID. Accordingly, we consider the Wiley Registry MSMS as standard model for creating tandem mass spectral libraries for small molecular identification.

Neuartige Aspekte

This study brings important conclusions on the usefulness of different library designs to the attention of practitioners in the field.

LC-MS/MS characterization of PTAD-derivatized carotenoids

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Einleitung

Carotenoides are naturally occurring tetraterpenes with antioxidant activities [1], protecting cells and tissue from oxidative damage [2]. Carotenoids, in particular β-carotenes, are limited in their physicochemical diversity, particularly with respect to functional groups that allow ionization by chemical ionization under ESI or APCI conditions in positive or negative modes. Xanthophylls contain additional oxygen atoms, which sometimes allow attachment of a cation in the gas phase by APCI. This process, however, usually exhibits only limited efficiency. Therefore, the goal of this study was to introduce chemical functions into the carotenoid molecules via derivatization reactions that readily ionize by ESI or APCI. As carotenoids exhibit common conjugated double bonds, these groups were targeted for derivatization by a Diels-Alder reaction with PTAD.

Methodik

Standard solutions of β-carotene and canthaxanthine were derivatized with PTAD in a Diels-Alder reaction. Reaction conditions were chosen such that a complete derivatization was accomplished without considerable loss of the desired products by further reactions. The reaction was carried out with varying concentrations of PTAD, ranging from 2-fold excess up to 30-fold excess. The resulting reaction mixtures were characterized by LC-DAD-MS/MS using a 3D ion trap mass spectrometer.

Vorläufige Daten

The analytical system was initially optimized with respect to obtaining maximum sensitivity for carotenoid detection. For this purpose, DAD, APCI-MS, and ESI-MS responses were compared under different experimental conditions. β-carotene was determined most efficiently as a radical cation under ESI conditions, whereas canthaxanthin also produced protonated molecules under APCI. The yield of the derivatization reaction for the carotenoids was determined to be 99.7 %. This number was based on the conversion of the precursor compounds. For two-fold excess of PTAD, the resulting reaction mixtures exhibited up to three PTAD moieties attached to the precursor molecule. Interestingly, the addition of PTAD did not result in formation of protonated analytes under ESI in these cases. While mono-substituted β-carotene was still predominantly ionized as a radical cation, the di- and tri-substituted products favored alkali adduct formation. Under the same conditions, canthaxanthin, as a less reactive dien, yielded mono- and di-substituted products, both of which were detected solely as cation adducts. From the chromatographic peak shapes and mass spectrometric data, we concluded formation of significant amounts of stereo-isomers, the identity of which is not known yet. Upon increasing the amount of PTAD, the distribution of products shifted to higher substitution levels. Using a 30-fold excess delivered tetra-substituted carotenoids almost exclusively. This finding is in agreement with data published on PTAD derivatized vitamin A [3], where di-substituted products could only be observed when one of the double bonds remained intact. Preliminary MS/MS data on di-substituted β-carotene and canthaxanthin showed similar dissociation behavior than vitamin D/PTAD derivatives [3]. Fragmentation patterns changed for higher substituted products and we are currently investigating the dissociation behaviors of these products using accurate mass measurements.

Neuartige Aspekte

Novel method for enhanced mass spectral analysis of carotenoids

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Derivatisierung für die verbesserte massenspektrometrische Strukturaufklärung unbekannter organischer Verbindungen in der rp-HPLC-ESI-MS/MS-Analytik

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Einleitung

In den letzten Jahren wird das Kriminaltechnische Institut vermehrt beauftragt, chemische Verbindungen zu untersuchen, die nicht in kommerziell verfügbaren massenspektro-metrischen Datenbanken vorliegen. Diese Substanzen stammen meist aus den Bereichen Arznei-, Doping- und Betäubungsmittel. Trennung, Nachweis und Strukturaufklärung dieser niedermolekularen, hydrophilen Verbindungen mittels rp-HPLC-MS sind jedoch erschwert. Bei der chromatographischen Trennung weisen diese entweder eine sehr geringe Retentionszeit oder kaum Retentionszeitunterschiede auf. Die anschließende massenspektrometrische Strukturaufklärung erfordert bei unbekannten, nicht in Datenbanken enthaltenen, Verbindungen prinzipiell eine manuelle Auswertung und Interpretation der Massenspektren. Die in der Struktur vorliegenden reaktiven, funktionellen Gruppen (meist Hydroxyl- und Aminogruppen) führen jedoch häufig zu Umlagerungsreaktionen und können zu einer Fehlinterpretation der Massenspektren führen. Durch Derivatisierung kann die Hydrophobizität der Moleküle erhöht und Umlagerungsreaktionen unterdrückt werden. Es wurden unterschiedliche Derivatisierungsmittel auf deren Eignung in rp-HPLC-ESI-MS/MS-Analytik geprüft. Im Vordergrund stand die Suche nach einem Derivatisierungsmittel, das ohne aufwendige Probenpräparation möglichst alle reaktiven Strukturelemente (hauptsächlich Hydroxyl- und Aminogruppen) in unbekannten organischen Verbindungen während eines Reaktionsschrittes umsetzt.

Methodik

Die Derivatisierungen wurden so durchgeführt, dass die Verbindungen als Festsubstanzen mit dem Derivatisierungsmittel überschichtet wurden. Für die Untersuchungen wurden die Derivatisierungsmittel Benzoylchlorid, Acetanhydrid, 9-Fluorenylmethylchloroformat und Phenylhydrazin mit den Analytsubstanzen Ephedrin/Pseudoephedrin (Stereoisomere), Melatonin, Meskalin/Methoxamin (Konstitutionsisomere) sowie Testosteron umgesetzt. In Abhängigkeit vom Derivatisierungsmittel wurden unterschiedliche Reaktionstemperaturen und Reaktionszeiten verwendet. Zum Teil wurden zusätzlich Katalysatoren eingesetzt. Die Reaktionsgemische wurden nach der Desaktivierung des überschüssigen Reagenzes mit Wasser und Zugabe eines geeigneten Lösungsmittels ohne weitere Probenaufarbeitung auf die rp-HPLC-Säule injiziert. Anschließend wurden die Proben mittels eines hochauflösenden ESI-Massenspektrometers vermessen. Das am besten geeignete Derivatisierungsmittel und die entwickelte Messmethode wurden qualitativ validiert. Bei der Ermittlung der Nachweisgrenzen, Richtigkeit, Selektivität und Spezifität wurden auch unterschiedliche Mengen des Derivatisierungsmittels und unterschiedliche Lösungsmittelzusammensetzungen berücksichtigt.

Vorläufige Daten

Generell zeigen alle mit ESI-MS nachweisbaren Reaktionsprodukte deutliche Retentionszeiterhöhungen bei der rp-HPLC-Trennung. Die Umsetzung mit 9-Fluorenylmethoxycarbonylchlorid führt zu *N*-substituierten Derivaten, während *O*-substituierte Derivate nicht auftreten. Für Derivatisierungen mit Acetanhydrid sind in Abhängigkeit von den vorliegenden funktionellen Gruppen unterschiedliche Probenpräparationen erforderlich. Beide Derivatisierungen führen zu übersichtlichen, unkomplizierten Massenspektren, jedoch ohne zusätzliche spezifische Fragmentierungen für die Strukturaufklärung zu bieten. Mithilfe von Benzoylchlorid reagieren sowohl Amino- als auch Hydroxylfunktionen in einem Präparationsschritt simultan zu Produkten, die hydrophober sind als die Edukte. Aus den Massenspektren benzoylierter Verbindungen kann eindeutig zwischen Sauerstoff- und Stickstoff-Substitutionen unterschieden werden: Die Abspaltung von Benzoesäure ist der direkte Hinweis auf eine O-Substitution, die Abspaltung von Benzamid und die Bildung von Benzoylkationen weisen auf eine N-Derivatisierung hin. Generell werden Umlagerungen stark unterdrückt; allerdings können durch sie bei Benzamid-Derivaten wertvolle zusätzliche Informationen für die Strukturaufklärung gewonnen werden. Primäre Amine spalten Isocyansäure (HNCO) und Methanimin (CH_2NH) ab. Sekundäre Amine (N-Methylaminogruppen) eliminieren Methylisocyanat (CH_3NCO) und Methylamin (CH_3NH_2). Die aufgrund der sehr guten, den Zielsetzungen entsprechenden Ergebnissen durchgeführte qualitative Validierung der Benzoylierung zeigte, dass das System selektiv, spezifisch und richtig arbeitet. Die Nachweisgrenzen der Analytsubstanzen im Probengemisch liegen alle im sub-Pikomol-Bereich. Benzoylierung bietet daher einen wertvollen Beitrag zum hochempfindlichen Nachweis und zur Identifizierung unbekannter organischer Substanzen.

Neuartige Aspekte

Die Verwendung von Derivatisierungsmitteln ist ein wertvolles Werkzeug für die Strukturaufklärung unbekannter Verbindungen und für die Aufklärung von Fragmentierungsreaktionen.

New superbasic matrices for low-mass MALDI-MS

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Einleitung

Matrix assisted laser desorption/ionisation mass spectrometry (MALDI-MS) is a powerful technique for analyzing large biomolecules like peptides, proteins and sugars, as it requires relatively little sample preparation and allows a lot of samples to be analyzed in a single run. To extend the scope of possible analytes to compounds with m/z < 600, new MALDI-matrices were developed in our lab which do not show interfering signals in the low mass region, as it is common for conventional matrices. A key feature of these new matrices, is their ability to ionize the analytes during crystal formation on the MALDI-plate prior to desorption. This occurs via proton exchange from the analytes to the superbasic matrix as a result of a simple acid/base reaction between analyte and matrix. The superbasic 1,8-Bis(dimethylamino)-naphthalen (DMAN, pK_a = 12,1) [1] is an excellent matrix for this method giving mass-spectra without any matrix derived signals, when an approximate 1:1 molar ratio of analyte and DMAN is used. [2]
The aim of this study is to develop new matrices with even better ionization properties to not only improve the limit of detection but also to extend the scope of possible analytes towards weaker acidic analytes e.g. amino acids.

Methodik

Taking the structure of DMAN as starting point, several new matrices were synthesized. These were then screened for their potential as MAILD-matrices with compounds and metabolites with varying degree of acidity e.g. fatty acids, citric acid and trifluoroacetic acid.

Vorläufige Daten

Screening revealed two superbasic matrices with superior ionization capacity to DMAN. In contrast to DMAN, these matrices do not require a 1:1 molar ratio of analyte and matrix for optimal ionization. Both have the potential to be used in metabolomical studies of acidic analytes.

Neuartige Aspekte

New matrix-compounds for low mass MALDI-MS in negative mode with improved ionization properties.

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1,14-diaza[5]helicene: A novel MALDI matrix for the analysis of acidic metabolites

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Einleitung

Although MALDI-MS has proven to be a powerful tool in the analysis of large biomolecules, extending this technique towards small molecule (< 500 Da) analysis appers to be challenging, as conventional MALDI matrices produce large number of interfering peaks at low mass region. DMAN (1,8-bis(dimethylamino)naphthalene), a super base belongs to a class of compounds called proton sponges was shown to be well suited for the negative mode MALDI-MS analysis of acidic metabolites, obviating the problem of low mass region interferrences. These findings, thus indicate the potential of MALDI-TOF technique for the analysis of low molecular weight compounds at physiologically relevant concentrations [1]. As some azahelicenes are also displaying high basicity [2], [3], it would be interesting to study the effectiveness of those azahelicenes as novel MALDI matrices for the analysis of fatty acids and organic acids in wide range of samples.

Methodik

Twelve azahelicenes were screened interms of achieved sensitivity, ion-lessness and suitability for the analysis of anions with the use of weakly acidic low molecular weight acids (tartaric acid, stearic acid, palmitic acid and absicic acid) . Based on the preliminary observations, the promising candidate, 1,14-diaza[5]helicene was further tested to study its suitability to detect organic acids in fruit juices (apple juice, orange juice) / wine and to determine its applicability for the analysis of diverse compounds of natural origin. Furthermore, specific parameters (pK_a, proton affinity ,UV absorbance) were measured and calculated to predict its mechanism of action as a MALDI matrix.

Vorläufige Daten

Out of the tested helicenes, 1,14-diaza[5]helicene appeared as a potential candidate, which could be used to detect analytes at picomole concentrations in the negative mode MALDI-MS. Clear signals for acid anions were observed for the tested acids with suppression of matrix-related ions in the low mass region while clear signals for deprotonated organic acids were detected in the tested fruit juices and wine samples. This ability could be attributed to its structure and high basicity as this molecule is comprised of an extended π conguated system with two nitrogen atoms which are arranged in similar manner as in DMAN although the hydrophobic shielding is absent. With this arrangement, it might be possible to stabilize the proton released from the acidic analyte by formation of N---H----N bond, eventhough 1,14-diaza[5]helicene is reported to be a kinetically active proton sponge [4]. At the moment, the experiments are underway to absolutely quantify the organic acids in fruit juice and wine samples using isotopically-labelled standards in order to ascertain its suitability as a novel MALDI matrix.

Neuartige Aspekte

Development of novel super-basic matrices facillitate small molecule analysis via MALDI-MS

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MS-based Natural Products Discovery supported by Myxobase

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Einleitung

Myxobacteria represent a promising source of biologically active natural products exhibiting significant potential as pharmaceutical leads [1]. Bioinformatic analysis of myxobacterial genomes for the presence of natural product biosynthetic pathways reveals the overwhelming potential of myxobacteria for the production of secondary metabolites. However, only a fraction of the in silico predicted compounds have been identified to date. We use high-resolution LC-MS to underpin our efforts to discover novel secondary metabolites from a large number of myxobacterial strains [2-4]. Here, we describe the implementation of MS-based tools into the Myxobase screening framework. These functions connect tightly to routine analytical workflows and enable us to efficiently utilize the vast amount of analytical data generated in the course of our screening campaign.

Methodik

Extracts from cultivations of more than 1500 myxobacterial strains were analyzed by separation of the complex samples on a RP-C18 column under UPLC conditions and coupling to an UHR Q-ToF mass spectrometer (Bruker maXis 4G) using positive ESI. Dereplication was performed using a targeted screening library generated from Myxobase, and analytical performance evaluation was carried out in order to qualify our LC-MS system for comprehensive metabolomics measurements covering an extended timeframe. Specialized import/export tools were developed for interfacing to instrument software. Moreover, functions were implemented to enable the storage and mining of feature-extracted secondary metabolome datasets inside Myxobase, and to manage mass spectral data from HR-MS/MS measurements.

Vorläufige Daten

We use a comprehensive metabolomics-based setup employing high-resolution LC-MS for our analysis of myxobacterial metabolite profiles, as this approach allows us to apply both targeted queries and unbiased statistical treatment to the same data. In addition to condensed target screening reports, the full complement of molecular features detected from an extract under investigation is deposited in Myxobase and serves as an in-silico representation of the secondary metabolome of the respective myxobacterial strain under standardized conditions. With these data at hand, we are able to identify alternative producers exhibiting improved yield for newly discovered metabolites, which is an important feature for the successful production of a novel natural product at increased scale. Moreover, we demonstrate how statistical mining of feature-extracted secondary metabolomes can facilitate the discovery of novel myxobacterial natural products. Following the definition of hypothetical candidate compounds (m/z-retention time pairs), the coverage of tandem-MS measurements can be significantly increased using targeted SPL-based (scheduled precursor list) analysis. In contrast to auto-MS/MS analysis, this method ensures that fragment spectra are preferentially recorded for potentially novel secondary metabolites (rather than for the multitude of highly abundant matrix compounds). We consider this approach as a crucial prerequisite for the success of future HR-MS/MS-based dereplication and identification workflows [5]. As preparation for extensive MS/MS-based analysis, we developed an efficient schema for dealing with high-resolution tandem-MS spectra within the Myxobase platform.

Neuartige Aspekte

Developing a database system to support natural products discovery from myxobacteria, with integration for secondary metabolomics and mass spectral data.

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Dynamik zweikerniger NHC-Gold(I)-Komplexe in der Gasphase

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Einleitung

Gold(I)-Komplexe mit N-heterocyclischen Carbenen als Liganden haben in den letzten Jahren vielversprechende Anwendungen gefunden, in so unterschiedlichen Bereichen wie der homogenen Katalyse, als lumineszente Materialien, bis hin zur medizinischen Chemie. Während die NHC-Liganden den Komplexen außergewöhnliche Stabilität verleihen und eine große Vielfalt an Derivaten zugänglich werden lassen, sind es aurophile Wechselwirkungen zwischen zwei oder mehreren Gold(I)-Atomen, die unter anderem hohe Fluroreszenz-Quantenausbeuten zur Folge haben. Wir haben eine systematische Reihe zweikerniger kationischer NHC-Gold(I)-Komplexe mit flexiblen Alkyllinkern unterschiedlicher Kettenlänge synthetisiert und deren Konformationen und Verhalten in Abhängigkeit von der Linkerlänge untersucht: Im Festkörper mittels Kristallstrukturanalyse, in Lösung mittels NMR-, UV/Vis- und Fluroreszenz-Spektroskopie, und schließlich in der Gasphase durch ESI-MS/MS und Ionen-Mobilitäts-MS.

Methodik

ESI-Spektren wurden mit einem Bruker Apex IV FT-ICR und einem micrOTOF-Q Massenspektrometer aufgenommen. Fragmentierungen wurden durch IRMPD und CID induziert, als Kollisionsgas diente Argon. Alle Signalzuordnungen wurden durch exakte Massenbestimmungen bestätigt. Ionen-Mobilitätsmassenspektren wurden an einem Waters Synapt G2 HDMS aufgenommen.

Vorläufige Daten

Alle untersuchten zweikernigen NHC-Gold(I)-Komplexe lassen sich durch ESI mit sehr hoher Sensitivität nachweisen. Die dikationischen Molekülionen fragmentieren unabhängig von der Linkerlänge durch symmetrische Spaltung in zwei identische einkernige Komplexe, erst im Anschluss bekommen andere Fragmentierungswege (z.B. Verlust der Seitenketten) Bedeutung. IM-MS-Spektren zeigen, dass sich die Molekülionen in Konformere unterschiedlicher Größe auftrennen lassen, im Einklang mit dem Verhalten in Lösung, das insbesondere durch VT-NMR-Spektren beobachtet werden kann. Auch im Festkörper werden u.a. abhängig vom Gegenion zurückgefaltete geschlossene und gestreckte offene Konformere gefunden. Letztere könnten im Inneren des Rings Gastmoleküle binden. Dies wird durch ESI-MS- und- NMR-Studien mit Serin und Cystein überprüft.

Neuartige Aspekte

Eine systematische Studie zum Verhalten neuer flexibler zweikerniger NHC-Gold(I)-Komplexe in der Gasphase im Vergleich mit Ergebnissen aus Lösung und Festkörper.

Metabolomics enabeled identification of infochemicals

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Einleitung

Chemically mediated interactions between organisms have a substantial impact on processes in our environment. Signal molecules can influence simple interactions between individuals from one species (pheromones) but also influence an entire ecosystem structure. Advances in chemical ecology have often been closely linked to advances in analytical chemistry techniques since only with information about the structure of the active metabolites the mechanism of signal chemistry can be understood. One recent development is the use of metabolomics to support the identification of so-called infochemicals. Metabolomics techniques have the potential to provide a substantial short cut in the otherwise often tedious bioassay-guided fractionation approach to active principles.

Methodik

We introduce comparative GC/MS and LC/MS based metabolomics techniques that support the elucidation of active metabolites released by microalgae. We developed routines that allow the combined evaluation of bioassays and of complex exo- or endometabolomes. In combination these techniques point towards candidate metabolites that are further elucidated and tested.

Vorläufige Daten

We show that a multi-stage mating process in the pennate diatom *Seminavis robusta* is under the control of the size-dependent production and reception of signal molecules [1]. These induce reciprocal mate perception, cell cycle arrest and cell attraction. Using the comparative metabolomics approach, we identify the attraction pheromone di-L-prolyl diketopiperazine as the first diatom pheromone. Further we use this methodological approach to elucidate the metabolite that is active in allelopathic interactions within diatom dominated biofilms [2,3].

Neuartige Aspekte

Metabolomics enableld search for infochemicals provides a substantial shortcut compared to traditional bioassay guided structure elucidation.

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Monitoring Conformational Changes in PPARα by Photo-Affinity Labeling and Orbitrap Mass Spectrometry

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Einleitung

Chemical cross-linking combined with an enzymatic digestion and mass spectrometric analysis of the reaction products has evolved into an alternative strategy to identify protein-protein and protein-ligand interactions. Peroxisome proliferator-activated receptors (PPARs) belong to a subfamily of the nuclear receptors that are involved in energy and metabolic processes. One subtype, PPARα, plays a crucial role in lipid metabolism and presents an important target for designing antidyslipidemic drugs for the treatment of the metabolic syndrome [1]. Conformational changes in PPARα upon ligand binding were investigated by photo-affinity labeling (PAL) studies combined with mass spectrometry.

Methodik

PPARα variants were purified on an ÄKTA FPLC system (GE Healthcare) using 1ml-HisTrapFF and 1ml-HiTrap Q HP columns. Protein expression and purification was monitored by 1D-gel electrophoresis (SDS-PAGE). After *in-solution* or *in-gel* digestion, cross-link reaction mixtures were analyzed by nanoHPLC/nano-ESI-MS/MS using a nano-HPLC system (Dionex, RP C18 column 75 µm x 250 mm) coupled to an LTQ-Orbitrap XL mass spectrometer (Thermo Fisher Scientific) equipped with a nano-ESI source (Proxeon). Cross-links were identified with the *in-house* Software StavroX [2], checked manually, and visualized by PyMol.

Vorläufige Daten

Expression und purification of PPARα variants was optimized to obtain maximum protein yields. For PAL studies, the photoreactive amino acid *para*-benzoylphenylalanine (Bpa) was incorporated at specific positions in the ligand binding domain of PPARα [3]. The integration of Bpa instead of Leu-258 and Phe-273 was confirmed by MS/MS. Both PPARα variants were employed for PAL studies in the absence and presence of the PPARα antagonist GW6471 complementing previous studies [4]. In the absence of GW6471, a higher number of cross-linked products were identified in both PPARα variants than in the presence of GW6471 verifying that a specific conformation is stabilized. Several cross-links were found in the Bpa-258 variant confirming that the omega loop is highly flexible. By a detailed inspection of MS/MS data, we were able to confirm mixed cross-linked species between Bpa-258 and Phe-218 / Arg-226 and Ala-233 / Pro-238. Further experiments with additional ligands are currently conducted to monitor conformational changes in PPARα after ligand binding.

Neuartige Aspekte

Bpa, photo-affinity labeling PPARalpha

Referenzen

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sequencing of copolymers using mass spectrometry

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Einleitung

Synthetic polymers are of a great interest in medicinal-drug delivery systems; however, this necessitates smart polymers to achieve the goal. Thus, the sequencing of polymers has started to elucidate the detailed structure of polymers, which was initially performed with proteins and peptides. Firstly, this kind of sequencing approach has been applied to homopolymers by the determination of the chain length and fragmentation series.

Methodik

A statistical model was proposed for the polymerization process and to reduce the model fitting to high-dimensional numerical optimization and NP-hard combinatorial problems from MALDI-ToF MS and MS/MS spectra.

Vorläufige Daten

Synthetic polymers result in more complex spectra in comparison to peptides due to these polydisperse natures, which include different chain lengths and sequences. In 2007, Thalassinos *et al.* presented a software for the determination of end groups in homopolymer MS/MS spectra. Furthermore in 2011, Baumgärtel *et al.* reported on a software for *de novo* identification of fragmentation mechanisms of homopolymers. Copolymers have an even more complex spectra in comparison to homopolymers. Therefore, a new method is established to provide the analysis of linear copolymers, which is independent of their composition and polymer class.
Quantification of the abundance of every linear copolymer chain after optimization of certain parameters has been performed. Currently, the synthesis of copolymer libraries and additional experiments are conducted to demonstrate the plausibility of the computational results. The libraries will also help to explore the feasibility limits of the method. Furthermore, chemical questions will be correlated to a statistical model and supported by interpretable visualizations.

Neuartige Aspekte

The use of MALDI -ToF mass spectrometry to sequence copolymers.

Comparing DDP (dried droplet preparation) and GSP (graphite supporte preparation) using complex peptide mixtures from elastase digests in MALDI-MS

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Einleitung

Using the DDP (dried droplet preparation) in MALDI-MS for quantification might result in poor shot to shot and spot to spot reproducibility mostly due to crystallization heterogeneity. Therefore a new sample preparation protocol was developed to reduce these limitations. The new GSP (graphite supported preparation) protocol was tested with different standard peptides with good results on signal intensities and signal reproducibility using MALDI-MS [1]. However, if the beneficial effects of GSP are maintained for enzymatic digests of proteins still needed to be proven. Therefore the less specific protease elastase and various standard proteins were used to generate a wide range of different peptides [2]. These digests were measured using the normal DDP and the new GSP protocol and the results were compared with regard to different peptide properties and their influence on signal intensity and reproducibility.

Methodik

All spectra were acquired on an AB Sciex 4800 MALDI TOF/TOF (AB Sciex, Darmstadt, Germany). The used matrix CHCA (α-cyano-4-hydroxycinnamic acid), the graphite powder and all chemical reagents as well as the standard peptides and proteins were from Sigma Aldrich (Sigma Aldrich, Munich, Germany). The protease elastase is from USB (USB Europe GmbH) and the digestion of the proteins was performed using an in house standard protocol. After digestion the samples were dried in a speedvac and resolubilized to the final concentration of 100 ng/µl referring to the digested protein. The surface characteristics of the MALDI target were changed in an easy way by wiping the graphite powder over the surface using a commercial cotton Q-Tip [1].

Vorläufige Daten

For statistical analysis all digests were measured from 6 individual preparations and compared with regard to different peptide properties and their influence on signal intensity and reproducibility. As already reported in [1] the GSP protocol leads to lower coefficient of variation (CV) and higher signal intensities for a mixture of standard peptides. When, however, inspecting the complete data set from elastase digests of the standard proteins myoglobin, ß-casein, alcohol dehydrogenase, ovalbumin, serum albumin and apotransferrin a more diverse picture results. Although in the majority of the inspected proteins the GSP protocol is clearly superior compared to the DDP with respect to the signal CV, the digests of ADH and apotransferrin are inferior in this aspect. With regard to peptide MW there is a trend towards higher signal intensities for peptides above 2 kDa for the GSP protocol and a benefit for the DDP for peptides below 2 kDa. With respect to the peptide pI there is a trend towards improved CV´s for peptides above a pI of 5 for the GSP protocol, but no uniform tendency when looking at the signal intensities. With regard to the GRAVY score [3] it became apparent that the intensities of very hydrophilic peptides (GRAVY score below -1.1) are higher for the DDP, but improved CV´s for more hydrophobic peptides (GRAVY score above -0.8) are generally found for the GSP protocol. The results show that the GSP protocol can be used as a well suited and easily applicable alternative with some but only minor losses in performance, if the quantification of peptides is the major goal. The latter is facilitated by an improved CV or higher signal intensities.

Neuartige Aspekte

Deeper insight of the GSP protocol for a better reproducibility of MALDI-MS spectra using elastase digests.

Referenzen

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Preparation of a Primary Reference Material for Magnesium Isotope Ratio Determination at Highest Metrological Level; Determination of Magnesium Atomic Weight

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Einleitung

Achieving and improving comparability of measurement results is of critical significance in the field of isotope ratio mass spectrometry. In order to achieve comparability in routine applications, δ-reference materials are used in field laboratories. However, no such material exists for magnesium [1]; a solid material from NIST that is often used instead has shown to be inhomogeneous with respect to its isotope ratio, so that it is inappropriate to serve as anchor point for the δ-scale. With the current project, we aim at developing new magnesium isotope reference material solutions for the δ-scale, and to characterize them with respect to their absolute isotope ratio based on a primary calibration with the three purified natural magnesium isotopes (^{24}Mg, ^{25}Mg, ^{26}Mg). The activities are embedded into the new project SIB09 ("Primary Standards for Challenging Elements") funded by the European Metrology Research Programme [2].

Methodik

We use an existing high-vacuum sublimation apparatus (base pressure < 10^{-7} mbar) to purify magnesium inside vitreous carbon crucibles. Starting materials are commercial samples of a) natural magnesium (Alfa Aesar, "99 + %"), and b) isotopically enriched materials of all natural isotopes (^{24}Mg, ^{25}Mg and ^{26}Mg; Oak Ridge National Lab; isotopic enrichment > 90 %). The evaporation profiles of magnesium and the impurities are followed during sublimation by online quadrupole mass spectrometry (Pfeiffer Vacuum, Prisma, QMA200). The obtained materials are first characterized with respect to the purification factors using glow-discharge mass spectrometry (Thermo Fisher Scientific, Element GD). Calibrated multi-collector ICP-MS (Thermo Fisher Scientific, Neptune Plus) is then used for the characterization of the isotope ratios in samples of natural magnesium, and to certify the new isotope reference material.

Vorläufige Daten

We are currently evaluating and improving our method to purify natural magnesium from its impurities with a target level of metallic impurities of below 1 mg/kg in high vacuum (< 1×10^{-6} mbar) at sublimation temperatures between 510 and 560 °C and collector temperatures between 350 and 450 °C. Characterization of the purification factors, the maximal possible purification and their dependence on parameters such as material loading, sublimation vessel dimensions, sublimation temperature and sublimation rate are currently being conducted based on the purity measured by GDMS. First tests have shown that purifications of more than 99 % can be achieved for the high-boiling components in three purification steps [3]. Further tests using even lower sublimation temperatures, or more purification steps are running. The most critical issue in the purification is the separation of magnesium from the low-boiling components K and Zn, and the separation of components of high abundance (Fe, Mn, Pb). Collection efficiencies are excellent (>> 99 %).
After achieving the targeted purification with natural magnesium, the same purification protocol will be applied to the chemically impure enriched isotopes. Then, precisely defined standard solutions of highest metrological quality will be prepared from the purified materials under full gravimetric control, and will be used as a basis for the preparation of mixtures to be used for the calibration of the MC-ICP-MS instrument. This calibration will be used as a basis for the precise and accurate determination of the atomic weight of terrestrial magnesium, and for the characterization of candidate materials for new isotope ratio reference materials.

Neuartige Aspekte

SI-Traceable Magnesium Isotope Reference Material

Referenzen

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Data independent MALDI Imaging ion mobility acquisition for the visualisation and identification of lipids directly from a single tissue section

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Einleitung

Advances in MS enabled the simultaneous analysis of a wide range of lipids and the rise of lipidomics. However, the spatial localization of lipids within tissue microstructures is often lost during the process of lipid extraction. Recently, MS imaging allowed visualizing lipid species in entire tissue section. Structural identification is traditionally performed after processing the untargeted MS imaging data, followed by further manual acquisitions either on the same or consecutive sections.
A data independent MALDI imaging acquisition method is presented, where MS and MS/MS information are acquired within the same experiment, without any precursor selection. Post-acquisition, precursors and fragments are correlated on the basis of ionic drift in the gas phase, which is further refined utilizing their spatial distributions.

Methodik

Data were acquired using a MALDI SYNAPT G2 instrument with tri-wave ion guide optics to separate ions according to their gas phase mobility.
Within the same MALDI imaging experiment, the mass spectrometer was set to apply alternate collision energies to the transfer cell between low energy and an elevated collision energy ramp, with the latter inducing lipid fragmentation. As fragmentation occurs post-ion mobility separation, the precursors at low energy share drift time with their associated fragments from the elevated energy scan.
The dataset was subsequently processed using High Definition Imaging (HDI) MALDI software for enhanced image analysis. Drift time alignment and spatial correlation of the data from both low and elevated energy functions were carried out with the same platform.

Vorläufige Daten

Proof-of-principal experiments have been carried out using a thin section of rat brain, produced using a cryotome and deposited on standard microscope slides. CHCA matrix was applied evenly to the sample in several coats using a SunCollect nebulising spray device.
The MALDI imaging experiment was designed such that adjacent pixels had low and elevated CE applied. Using new HDI MALDI software, both elevated and low energy functions of the MALDI imaging data were independently processed using the Apex 3D detection algorithm.
The two types of ion images were then visualised within the Analysis tab of the HDI software, with the low energy function displaying lipid precursor ion images and the elevated energy function lipid fragmentation profiles. During image and data processing, the low and elevated data streams were drift time aligned and further correlated based on their spatial distribution for supplementary refinement.
Further work will be conducted with HDI generated output (i.e. pkl file) for the structural identification of multiple lipids from MALDI imaging experiment.

Neuartige Aspekte

Single MALDI imaging experiment methodology for lipid characterisation using a data independent method based on orthogonal IMS and spatial correlation.

Integration of MALDI MSI into Plant Physiological Questions – Metabolite Distributions during Grain Development in Barley

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Einleitung

MALDI MS *Imaging* approach, meanwhile widely used in medical and pharmaceutical research, was applied to elucidate metabolite distributions in barley grains. The study of grain development is of outmost importance as cereals serve as a major staple food. During first two weeks after pollination massive changes of kernel structure occur, including evolving and diminishing of tissues. Also, physiological processes having an impact on changes of kernel chemical composition take place. In this study, different time points representing major developmental stages were analyzed for metabolite distribution patterns in a spatial and developmental depending manner. MALDI MSI technique was found to be highly advantageous for unraveling candidate compounds showing highly specific accumulations in different tissue types. These data were the basis for further targeted analysis and integration into the physiological context of grain development.
The presentation will introduce our untargeted MALDI MSI approach together with subsequent targeted analyses of candidate molecules. Selected examples of candidate compounds showing developmental specific changes are presented and integration of MALDI MSI data into biological context of grain development will be discussed.

Methodik

MALDI MSI was carried out using longitudinal and cross sections of barley grains from different developmental stages. Preliminary experiments revealed DHB matrix as best suited in this biological context [1]. Candidate *m/z* values were selected by comparison of MSI runs from different developmental stages and grain regions. DHB background signals were used as internal standard to elucidate developmental changes of selected signals across various MALDI MSI measurements. Identification of *m/z* values constitutes a major challenge. MS/MS analyses as well as on tissue hydrolysis of carbohydrates helped to classify many compounds. Targeted analyses regarding further identification and quantification using dissected seed material were performed by means of LC-MS, GC-MS and LC coupled to electrochemical detection.

Vorläufige Daten

During grain development individual polar lipids were found to show high tissue specificity. Among them, several were found to be developmentally regulated. Reallocations during development were also found for specific sugar species. These MALDI MSI results were complemented by targeted analysis of proteins and transcripts revealing new insights into grain physiology, particular into endosperm development and composition.

Neuartige Aspekte

Application of MALDI MSI to plant tissues
Elucidation of metabolite distributions during development
Direct identification of compounds from tissue sections

Referenzen

[1] Peukert et al., Spatially resolved analysis of small molecules by matrix-assisted laser desorption/ionization mass spectrometric imaging (MALDI-MSI). New Phytologist, 2012

MALDI-Imaging in der Hautforschung

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Einleitung

MALDI-Imaging als innovatives, bildgebendes Verfahren hat sich im letzten Jahrzehnt als eine kaum noch wegzudenkende Messmethode etabliert. Die Zahl an unterschiedlichen Anwendungen steigt weiterhin, insbesondere im Bereich der Identifizierung von Markermolekülen und der medizinischen Diagnostik. Trotzallem gibt es bisher nur wenige Publikationen zu dem Thema MALDI-Imaging und ex-vivo Humanhaut [1-3]. Dabei ist die Lokalisierung von endogenen und exogenen Substanzen in intakten Gewebeschnitten und damit die Untersuchung von biologischen Prozessen direkt im Gewebe eine bedeutende und herausfordernde Aufgabe im Bereich der Hautforschung. Der Fokus dieser Arbeit liegt zum einen auf einem MALDI-Imaging basierten Vergleich verschiedener Hautzustände (gesunde Haut vs. Haut mit problematischem Zustand). Hierbei ist insbesondere die Arbeit auf Protein-Ebene von Bedeutung. Die Anwendung beschränkt sich dabei nicht nur auf frisch eingefrorene Proben, sondern auch auf formalin-fixierte paraffin-eingebettete Tissue Microarrays. Neben der Abbildung solcher endogenen Hautsubstanzen stellt auch die direkte Lokalisierung von exogenen (Wirk-) Stoffen nach erfolgten Penetrationsexperimenten in ex-vivo-Haut ein Ziel dar. Grundsätzlich kann die Penetrationstiefe und der Penetrationsweg von (kosmetischen oder pharmazeutischen) Wirkstoffen mittels MALDI-Imaging abgebildet werden.

Methodik

(i) Abbildung von endogenen, kleinen Molekülen; Zur Lokalisierung von kleinen Molekülen in Hautgewebeschnitten wurde 9-AA als Matrix eingesetzt. Eine Identifizierung der Signale erfolgte mittels PSD-MALDI.

(ii) Abbildung von endogenen Proteinen; Ein „spatial proteomics approach“ wurde auf verschiedene zu vergleichende Hautzustände angewendet. Dabei wurden zwei aufeinanderfolgende Schnitte einem auf-Gewebe-Verdau mit Trypsin unterzogen. Während der erste Schnitt mit α-CCA überschichtet und mittels MALDI-Imaging vermessen wurde, wurden die tryptischen Peptide von dem zweiten Schnitt eluiert und anschließend mittels nano-LC-MS/MS identifiziert. Eine Korrelation der entsprechenden Ergebnisse kombiniert Lokalisierung und Identifizierung.

(iii) Abbildung exogener Substanzen; Entsprechende Penetrationen wurden an *ex-vivo* Schweinehaut in sogenannten Franz´schen Diffusionszellen durchgeführt. Anschließend wurden Stanzen entnommen, Schnitte generiert und klassische MALDI-Imaging-Messungen durchgeführt.

Vorläufige Daten

(i) Abbildung von endogenen, kleinen Molekülen; Es konnten Verteilungen für eine Vielzahl an Substanzen erhalten werden: u.a. für Cholesterolsulfat, ATP, ADP, Phosphatidylinositol C38:4.

(ii) Abbildung von endogenen Proteinen; Vor der Durchführung von MALDI-Imaging-Untersuchungen auf Proteinebene ist es empfehlenswert, Salze, Lipide und Phospholipide weitestgehend zu entfernen. In der Literatur sind hierzu eine Vielzahl an unterschiedlichen auf-Gewebe-Waschprotokollen beschrieben. Grundsätzliche, vergleichende Untersuchungen hinsichtlich eines Einflusses der Waschprotokolle auf den Gesamtproteingehalt und ebenso auf die Qualität der MALDI-Spektren fehlen jedoch. Die vergleichende Proteingehaltsbestimmung mittels BCA-Assay (bicinchoninic acid assay) lieferte Proteinverluste in einem Bereich von 17-38%, wobei der Proteingehalt von ungewaschenen Hautschnitten auf 100% gesetzt wurden. Unsere Ergebnisse zeigen im Weiteren den deutlichen Einfluss von Waschprotokollen auf die Anzahl an detektierbaren Massensignalen. Diese Untersuchungen wurden an Eluaten von Hautschnitten nach einem auf-Gewebe-Verdau durchgeführt.
MALDI-Imaging wurde im vorliegenden Fall sowohl auf Hautgewebeproben angewendet, die in Carboxymethylcellulose als auch in einem Polyethylenglykol enthaltenen Medium eingebettet waren. Zudem wurde MALDI-Imaging auf FFPE Tissue Microarrays angewendet.
Mittels MALDI-Imaging konnten molekulare Unterschiede zwischen verschiedenen Hautzuständen herausgearbeitet werden. Die Identifizierung einzelner potentieller Markersubstanzen erfolgte mit Hilfe des „spatial proteomics approach“.

(iii) Abbildung von exogenen Substanzen; Die Verteilung von Nilrot (Modelsubstanz) wurde nach ex-vivo Penetrationsexperimenten in Schweinehaut mittels MALDI-Imaging bestimmt. Dabei konnte Nilrot im Bereich eines Haarfollikels lokalisiert werden, welches für eine Penetration entlang des Haares spricht. Da Nilrot fluoreszenzaktiv ist, konnte dieses Ergebnis mit Hilfe einer fluoreszenzmikroskopischen Aufnahme bestätigt werden.

Neuartige Aspekte

Einfluss von auf-Gewebe-Waschprotokollen auf den Proteingehalt; Anwendung von MALDI-Imaging auf verschiedene Hautzustände; Abbildung exogener Substanzen nach ex-vivo Penetrationsexperimenten an Schweinehaut

Referenzen

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Separationseffekte in ‚dried droplet' Probenpräparationen für die MALDI Massenspektrometrie von synthetischen Polymeren

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Einleitung

Auf Grund ihrer Einfachheit und Schnelligkeit stellt die "dried droplet' Tropfenmethode die am weitesten verbreitete Technik zur MALDI-Probenpräparation dar. Dabei werden entweder einige hundert nL der gelösten Probe und der Matrix nacheinander (Sandwich-Methode) oder die Mischung beider Lösungen zusammen auf ein Target aufgetragen. Bei vielen derartig hergestellten Probenspots geht die Verdampfung des Lösemittels mit einer Ringbildung einher. Dies kann zu einer Separation zwischen Matrix und Polymer bzw. zwischen Polymeren unterschiedlicher Kettenlänge führen.

Methodik

Zum Messen der Separationseffekt wurde MALDI-MS Imaging in Kombination mit einer "dried droplet" Probenpräparation verwendet. Das Ziel dieser Arbeit ist durch die Aufklärung der wesentlichen Entmischungsparameter zuverlässigere MALDI-ToF-MS Ergebnisse (z.B. zur Bestimmung der Molmassen und -verteilungen von Polymeren) zu erhalten. Des Weiteren sollen erste Modelle zur Darstellung dieser Effekte entwickelt werden.

Vorläufige Daten

Erste Versuche zeigten, dass mit steigender Temperatur die Analyten weiter an die Grenze des Spots wandern und somit die Breite des Ringes abnimmt. Neben der Temperatur kann auch die Struktur des Polymeren, das Lösemittel sowie andere physikalisch-chemische Parameter (z.B.: Diffusion, Kapillarkräfte) einen Einfluss auf die Entmischung haben.

Neuartige Aspekte

Es wurde erstmals MALDI-MS Imaging zur Untersuchung der Separationseffekte verwendet.

Referenzen

Comparison of tumor containing RGB-fluorescence marked cells with microscopy and MALDI mass spectrometry imaging.

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Einleitung

Mass spectrometry imaging (MSI) represents a novel promising technology for characterization of spatial protein distribution in tissue samples. We combined MSI with the novel method of red-green-blue (RGB) marking which allows labelling individual cells with highly specific colour codes. Thereby, it enables clonal cell tracking after injection in a mouse during tumor formation, dissemination and metastasis [1].
The aim of this study was to analyze liver tissue from immune-deficient mice (*scid*-mice) containing RGB-marked metastases from human tumor cells with MALDI-MSI and fluorescence microscopy and to clarify if the combination of MALDI-MSI and RGB is helpful for cancer research.

Methodik

RGB-marked BON1 carcinoid tumor cells were transplanted into spleens of *scid*-mice to engraft in the liver. After 5‑6 weeks mice were sacrificed and livers were dissected [2].
In preparation for the MALDI-MSI analysis 6 µm thick frozen tissue sections from the mouse liver were treated with sinapinic acid as a matrix.
Liver tissue sections containing various different coloured and non-coloured fluorescent cells were analyzed by MALDI-MSI (autoflex speed, Bruker Daltonik) with a resolution of 160 µm. Signal intensities of the spectra were then compared with fluorescence images of the RGB marked cells as well as hematoxilin-eosin (H & E) staining images of consecutive liver sections.

Vorläufige Daten

The signals of the MALDI-MSI measurement were compared with the corresponding areas on the tissue section. All tumor areas showed m/z signals which allow a clear differentiation between human tumor tissue and mouse liver tissue. Notably, a signal with m/z=10090 Da was detected in all 17 tumor areas of the tissue section.
In the lentiviral transduction of Bon1 cells about 90 % of the cells show RGB fluorescence. The intensity of fluorescent protein expression depends on several factors, such as vector copy number [1]. As a result some of the clones show only low or even no fluorescence. Therefore MALDI-MSI complements RGB marking very well because with MALDI-MSI in all metastasis areas m/z markers were identified which are distinct in comparison to mouse tissue.
RGB marking allows a much better differentiation of tumor cells than conventional H & E staining. In combination with MALDI-MSI the RGB approach is an additional proof for the presence of metastases and therefore increases the confidence of identifying metastatic cells correctly. Furthermore it adds an additional bias for the interpretation of mass spectrometric data, since the different fluorescent colours indicate different tumor genomes.
Combination of both methods is a good proof for the detection of tumor cells in tissue, as we can demonstrate with our results.

Neuartige Aspekte

Combination of RGB marking of cancer cells with MALDI-MSI.

Referenzen

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[2] Weber K, Thomaschewski M, Benten D, Fehse B: RGB marking with lentiviral vectors for multicolor clonal cell tracking. Nature Protocols, 839 - 849, 2012

Quantitative Analyse phosphorylierter Proteins mittels LA-ICP-MS

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Einleitung

Die reversible Proteinphosphorylierung wird als eine der wichtigsten posttranslationalen Modifikation erachtet. Sie ist in die Proteinregulierung aber auch die Signalweiterleitung involviert und ist dabei selbst von der Zellaktivität und einwirkenden Umweltfaktoren abhängig. Da Beeinträchtigungen der Signalweiterleitung oft mit Krankheiten, wie etwa Krebs oder Diabetes, in Verbindung stehen, stellt die Analyse und Quantifizierung phosphorylierter Proteine eine wichtige Aufgabe dar [1]. Neben der Proteinmenge ist dabei auch der Phosphorylierungsgrad, das Verhältnis von Protein zu Phosphoprotein, wichtig zur Charakterisierung von Krankheiten oder Stressfaktoren.
Die Kombination von Gelelektrophorese (GE) und Laserablation (LA) bietet eine effiziente Methode zur Proteinauftrennung und anschließenden Untersuchung mittels Massenspektrometrie mit induktiv gekoppeltem Plasma (ICP-MS) [2, 3]. Die Verwendung von Western Blot zur Übertragung der Proteine auf Membranen vor der LA-ICP-MS ermöglicht dabei Strategien wie Immunoassays [4]. Da die direkte Detektion von Phosphor aufgrund seiner hohen Ionisierungsenergie und isobarer polyatomarer Interferenzen erschwert ist, müssen alternative Detektionsmethoden für die Proteinphosphorylierung gefunden werden.

Methodik

Mittels eindimensionaler Natriumdodecylsulfat-Polyacrylamidgelelektrophorese (SDS-PAGE) wurden verschiedene phosphorylierte und unphosphorylierte Proteine aufgetrennt und anschließend zum Teil auf Membranen übertragen. Nach „Anfärben“ im Gel oder auf der Membran mit metallhaltigen Stains und Trocken wurden die Gelproben mittels LA-ICP-MS untersucht
Um den Phosphorylierungsgrad bestimmter, biologisch relevanter Proteine ermitteln zu können, wurden protein- bzw. phosphoproteinspezifische Antikörper metallmarkiert und in Multipleximmunoassays nebeneinander angewendet. Die Proben, Lysate menschlicher Caco-2 Darmzellen, wurden zuvor unter unterschiedlichen Bedingungen mit dem karzinogenen Benzo[a]pyren inkubiert. Nach SDS-PAGE und Western Blot auf PVDF-Membranen wurden diese ebenso wie die Gelproben analysiert.

Vorläufige Daten

Die Untersuchung der Standardproteine mittels Staining und LA-ICP-MS erlaubte es uns, bis zu 1 µg phosphoryliertem Protein pro Probe zu detektieren. Die hier vorgestellten Ergebnisse zeigen dabei eine gute Übereinstimmung zwischen der aufgetragenen Proteinmenge und der gemessen Signalintensität des Staining-Metalls. Bei der Analyse der Zellinkubation mittels Immunoassay und LA-ICP-MS konnten wir Veränderungen im Phosphorylierungsgrad des signalübertragenden Proteins ERK1 aufzeigen. So nimmt mit steigender Konzentration des Benzo[α]pyrens der Phosphorylierungsgrad des Proteins ab.
Metallhaltige Färbemethoden eröffnen in Verbindung mit der LA-ICP-MS einen einfachen und schnellen Weg für die Detektion und Analyse phosphorylierter Proteine. Metallmarkierte Antiphosphoproteinantikörper können für die Analyse spezifischer, phosphorylierter Proteine eingesetzt werden. Die Duplexmethode, die gleichzeitige Verwendung von Protein- als auch der Phosphoproteinantikörper, wurde von uns erfolgreich zur Bestimmung des Phosphorylierungsgrades eingesetzt. Das ICP-MS als Multielementmethode wird in zukünftigen Untersuchungen dazu genutzt, um unterschiedliche Phosphorylierungsstellen durch weitere metallmarkierte Antikörper mittels Multiplexstrategie gleichzeitig zu untersuchen.

Neuartige Aspekte

Metallhaltige Stains bzw. metallmarkierte Antikörper wurden zur Ausarbeitung von Quantifizierungsstrategien für die Proteinphosphorylierung bzw. Ermittlung des Phosphorylierungsgrad mittels LA-ICP-MS eingesetzt.

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Entwicklung und Anwendung einer neuartigen internen Standardisierung zur Verbesserung der Bildauflösung von immunhistologischen Gewebeschnitten beim Laserablation-ICP-MS Imaging

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Einleitung

Die Immunhistologie ist eine etablierte diagnostische Methode in der Medizin, um in Gewebeschnitten Tumorzellen zu identifizieren und zu klassifizieren, die krankheitsrelevante Proteine (Antigene) exprimieren. Die Antigene werden über spezifische Antikörper, die bisher meist mit einem Fluoreszenzfarbstoff markiert sind, im Einzelnachweis detektiert. Die Verwendung von MeCAT (Metal Coded Affinity Tags) [1,2] markierten Antikörpern ermöglicht den gleichzeitigen Multiplex Nachweis verschiedener Antigene in einer einzigen Analyse. Jeder eingesetzte Antigen-spezifische Antikörper wird mit einem anderen Lanthanoid markiert und die Gewebeschnitte anschließend mit allen MeCAT-markierten Antikörpern inkubiert. Das Auslesen der immunhistologischen Gewebeschnitte erfolgt durch das bildgebende Verfahren der Laserablation-ICP-MS (LA-ICP-MS), wodurch die verschiedenen MeCAT Metalle und damit verschiedenen Antigene parallel hoch sensitiv im Gewebeschnitt ortsaufgelöst detektiert und differenziert werden können.
Die besonderen Vorteile der ICP-MS sind [3]:

- Ein weiter linearer-dynamischer Messbereich (bis zu neun Größenordnungen).
- Außergewöhnliche NWG (> 0,1-1 ppt).
- Matrixunabhängige Quantifizierung.
- Multielement-Analytik, die es erlaubt die meisten Elemente des Periodensystems zu quantifizieren.

Zur Standardisierung von LA-ICP-MS Imaging-Analysen entwickelten wir eine neue Methode, bei der ein interner Standard zur Kompensation von gerätespezifischen Schwankungen bei der LA-ICP-MS Detektion von Gewebeschnitten eingesetzt wird. Am Beispiel von Formalin-fixierten und Paraffin-eingebetteten (FFPE) Mammakarzinom Gewebeschnitten und eines validierten tumorspezifischen Antikörper, der in der routinemäßigen immunhistologischen Färbung [4] eingesetzt wird, werden die Vorteile präsentiert.

Methodik

FFPE Brustgewebeschnitte wurden mit einem MeCAT modifizierten tumorspezifische Antikörper inkubiert, anschließend iodiert, mit einer Indium versetzten Tinte bedruckt und mittels LA-ICP-MS detektiert. Um eine unspezifische Bindung des modifizierten Antikörpers nachzuweisen, erfolgte die Positiv-Kontrolle an hoch tumorösen Brustgewebe, hingegen wurde die negative Kontrolle an nicht tumorösen Brustgewebe durchgeführt. Der positive und negative Befund des Gewebes wurde zuvor über eine immunhistochemische Färbung (IHC) validiert. Die Detektion der Gewebeschnitte erfolgte mittels LA-ICP-MS mit einer Laserspotgröße von 50 µm und einer Laserscangeschwindigkeit von 50 µm/s unter Verwendung von Iod und Indium als interne Standards, um diese miteinander vergleichen zu können

Vorläufige Daten

Beim Imaging mittels LA-ICP-MS wird der Gewebeschnitt Spur für Spur abgetragen und das entstandene Probenaerosol mit Hilfe eines Trägergases ins ICP-MS transportiert, dort atomisiert, ionisiert und schließlich detektiert. Die vorgestellte Arbeit zeigt die Anwendung eines herkömmlichen Tintenstrahldruckers zur homogenen Aufbringung eines internen Standards auf einen Formalin-fixierten und in Paraffin eingebetteten Gewebeschnitt, der auf einen Glasobjektträger immobilisiert wurde. Es konnte gezeigt werden, dass die mit Indium dotierte Tinte nahezu homogen aufgebracht wurde. Die Anwendung von MeCAT als Markierungsreagenz für die Modifikation von Antikörpern wurde bereits für LA-ICP-MS basierte Westernblot Immunoassays etabliert [5]. In unserer Forschungsarbeit wird ein MeCAT markierter Antikörper erstmals erfolgreich für die immunhistochemische Färbung von Gewebeschnitten eingesetzt. Die guten Signalintensitäten ermöglichen eine Verbesserung der Ortsauflösung von 200 auf 50 µm. Für diesen Auflösungsbereich wurde das neue Verfahren für das Aufbringen eines internen Indium-Standards mittels Tintenstrahldrucker mit einer etablierten Methode, der Iodierung [6], verglichen. Auf Grund der guten Signalintensitäten war es uns möglich die Ortsauflösung durch Verkleinerung des Laserspots auf 50 µm zu verbessern und trotz geringerer Metallmenge, infolge einer kleineren Ablationsfläche des Lasers, reproduzierbare Signale weit oberhalb des Rauschsignals zu erreichen. Es konnte eine signifikante Verbesserung der Bildauflösung zum einen durch die Verringerung der Laserspotgröße und Laserscangeschwindigkeit, aber vor allem durch die homogene Aufbringung des internen Standards mittels Tintenstrahldrucker erzielt werden. Unter den von uns optimierten Laser Bedingungen (Laserspotgröße: 50 µm, Laserscangeschwindigkeit: 50 µm/s) ist die Iodierung von Gewebeschnitten nicht mehr als konventioneller interner Standard zur Korrektur der Gewebeschichtdicke geeignet. Bei der Iodierung handelt es sich um eine chemische Modifikation an aromatischen Resten von Aminosäuren (z.B. Thyrosin), so dass die Iodierung Strukturinformationen sowie die Proteindichte der Gewebeschnitte widerspiegelt. Durch die Kombination der Markierungsreagenzien MeCAT und der neuartigen Applikation des internen Standards Indium konnten die Signal-zu-Rausch-Verhältnisse signifikant verbessert werden.

Neuartige Aspekte

Die Entwicklung einer internen Standardisierungs- Methode zur Korrektur geräte spezifischer Schwankungen der LA-ICP-MS bei der bildgebenen Analyse von Gewebeschnitten.

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MALDI Mass Spectrometry Imaging of Bone Tissue

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Einleitung

MALDI mass spectrometry imaging (MSI) is a valuable technique for rapid and direct spatial analysis of biomolecules in biological tissues. Large numbers of biomolecules can be detected and localized without having any knowledge about them by MALDI-MSI (1, 2). MALDI-MSI is especially interesting for researching for disease markers by analyzing tissue sections in healthy and diseased conditions (3). Here, we analyzed the spatial protein composition of mouse bone tissue.

Methodik

In this study, we used Formalin Fixed Paraffin Embedded (FFPE) bone. At first, tissue sections were mounted onto indium-tin-oxide (ITO)-coated conductive glass slides. Then, deparaffinization and washing with antigen retrieval buffer (pH2) was performed. For tryptic digestion, trypsin solution was sprayed on the tissue slides by a MALDI-matrix spraying device (Image-Prep: Bruker) following by incubation at 37 ºC for three hours. The matrix solution DHB was sprayed on the tissue slices by the MALDI-matrix spraying device and dried. Subsequently, samples were measured by MALDI-MSI (autoflex speed, Bruker Daltonics). Data analysis was performed with flexcontrol 3.3, flexanalysis 3.3, and fleximaging 2.1 Imaging (Bruker Daltonics).

Vorläufige Daten

In our study, we analyzed the spatial distribution of proteins of FFPE bone tissues slices of mice. MALDI-MSI analysis showed many signals representing tryptic peptide ions. The spatial distributions of the signals can be clearly allocated to morphologically distinct areas. E.g. m/z=1275 is present with high signal intensities in cortical bone regions and bone marrow of both tissues. Thus MALDI-MSI gives valuable information about the distribution of distinct proteins. In a future approach we will identify the proteins underlying tryptic peptides.

Neuartige Aspekte

Application of MSI towards the analysis of bone tissue

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Mass Spectrometric Imaging in Malaria Research

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Einleitung

After a first demonstration in 1994 [1], MALDI mass spectrometric imaging (MSI) became a valuable histological tool providing topological information on phospholipids [2], peptides [3], and drug compounds [4] with high reliability, validity, and performance. Lipids are not only essential building blocks of cells but also play an important role in signal transduction, e.g. in malaria parasites, their mosquito vectors, and human hosts. The spatial distribution of lipids was imaged with high mass resolution and mass accuracy from *Plasmodium berghei*-infected and non-infected mouse liver and whole *Anopheles stephensi* mosquito sections

Methodik

Normal and infected mouse liver sections of 8-20 µm thickness were prepared with a cryostat microtome. Mosquito samples were 10-14 µm thick sections, cut after carboxymethyl cellulose (CMC) embedding. An atmospheric pressure scanning microprobe matrix assisted laser desorption (AP-SMALDI) ion source was used for imaging [2]. For positive- and negative-ion measurements 2,5 dihydroxybenzoic acid (DHB) and paranitroaniline (PNA) were homogeneously deposited by means of a dedicated home-built pneumatic sprayer. The AP-SMALDI source was operated with a N_2 laser at 337 nm wavelength with 60 Hz repetition rate. The source was coupled to an Exactive or Q Exactive orbital trapping mass spectrometer (Thermo Fisher Scientific GmbH, Bremen), set to a resolving power between 50,000 and 140,000 at *m/z* = 200.

Vorläufige Daten

The in-house developed software package ‘MIRION’ was used to generate mass images from raw files generated by the mass spectrometer. Matrix cluster peaks were used for internal calibration and ions formed by 30 laser pulses per spot were accumulated prior to detection. Mass spectra from 10-25 µm pixels were obtained with a mass accuracy of ≤ 3 ppm (root mean square). After AP-SMALDI analysis the matrix was removed from sections with 70% ethanol and sections were H&E-stained to correlate the structure with measured m/z images both from mouse liver and *Anopheles* sections.
Anatomical structures and localizations of different classes of phospholipid compounds were identified with high mass resolution and mass accuracy, classified as phosphatidic acid (PA), phosphatidyl ethanolamine (PE), phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylserine (PS), and sphingomyelin (SM), within the mass range of *m/z* 600-900 in positive and negative ion mode. Other lipid classes such as monoglycerides, diglycerides, and triglycerides were also detected and imaged from *Anopheles* sections. Heme was detected in blood vessel regions of mouse liver with high mass accuracy (*m/z* = 616.16) as well as heme fragment ion by MS/MS tissue imaging. Images were generated with a bin width of $\Delta m/z$ = 0.01. The identity of lipid molecules was confirmed by on-tissue MS/MS analysis.
Further MSI studies will be carried out on differential expression of peptides and proteins in the context of malaria infection.

Neuartige Aspekte

High resolution MALDI imaging was employed to investgate infection mechanisms of malaria in mosquitos and mouse liver.

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Full mouse-body imaging by Desorption Electrospray Ionization and statistical analysis of lipid profiles

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Einleitung

Desorption Electrospray Ionization (DESI) is an ambient ionization technique that was first described in 2004. [1] DESI utilizes a pneumatically assisted solvent spray to create primary ions that impact the surface of interest. Upon the impact, analyte molecules are ionized and carried into the mass spectrometer. DESI can be conducted in scanning mode which enables Mass Spectrometry Imaging (MSI). Although DESI has comparatively poor resolution of 100-250 µm, it provides the advantage of minimal sample preparation, especially for tissue samples. It has been shown previously, that the lipid composition of tissue species is characteristic and the phospholipid profile can be utilized to categorize samples. [2]

Methodik

A six days old B6 mouse was sacrificed by inhalational anaesthesia, embedded in 4% aqueous carboxymethyl cellulose (CMC) and was sliced into 30 µm thick full-body sections. Sections were thaw-mounted on glass slides and stored immediately at -85°C until further use. MSI was carried out using a commercially available Prosolia OmniSpray 2D ion source and an Orbitrap Exactive mass spectrometer (Thermo Fisher Scientific GmbH, Bremen, Germany). Spatial resolution was set to 150 µm, greyscale images were created with the DataCube Explorer (FOM Amolf, Amsterdam, Netherlands). Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) were used to analyze lipid profiles. All models and identification maps were created using Medimass and MedImage software (Medimass Ltd, Budapest, Hungary).

Vorläufige Daten

Embedding and slicing of the mouse body yielded plain and intact full-body sections that were suitable for DESI Imaging and haematoxylin and eosin staining. The structure of the mouse body as seen on the H&E stained section was represented in the ion images derived from the MSI experiments. We were able to identify several specific ions, representative for different parts of the mouse body, for example taurochloric acid, which was detected in colon tissue. Not only single ions were found to be specific, but also the full lipid profile was found to be unique for tissue species. The lipid profiles of six different mouse sections were analyzed with multivariate statistical methods to evaluate the reproducibility of the DESI Imaging method and to test the ability to identify unknown murine tissue samples. PCA and LDA models of liver, kidney, lung, colon, brain and muscle spectra were calculated and used as reference data for the identification of further spectra from the same experiments and also for unknown tissue material. The different tissue species were color-coded and combined into a map of different mouse organs. Cross-validation was performed by calculating models from the data of five mouse sections and by identification of the remaining sample. Correct identification of the unknown tissue samples was achieved for 70-98% of spectra, strongly depending on the time between measurements. Identification of spectra was also tested for data from different murine species. The correct identification of organ species was achieved for up to 95% of data.

Neuartige Aspekte

Imaging of full-body mouse sections by DESI
Creation of organ maps using statistical analysis of lipid profiles

Referenzen

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Introduction of a prototype web-based, public domain lipid imaging database – Lipid-MSI

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Einleitung

The interest in mass spectrometry imaging (MSI) applications of biological tissues has been growing exponentially since its introduction in 1995. Among the different target compounds for MSI, lipids have received much attention as they are readily amenable to MS analysis and they are involved in multiple biological processes such as their roles as structural components of cell membranes, their function in the surfactant cycle and their involvement as second messengers in signaling cascades. [1] The number of publications describing lipids in human, animal and plant tissues has increased steadily, especially those on human and rodent samples. As the quality of analysis and the number of identified lipid species depend on numerous experimental MSI parameters (e.g. sample preparation, choice of matrix, matrix deposition technique), the outcome of the experiments can be highly variable and the results confusing. We believe that a database containing lipid species identified in MSI along with detailed sample and MSI acquisition parameters will help scientists greatly, to aid them in their experimental design. We hope that this database will also refine the existing knowledge, so that data mining may reveal yet unidentified relations and correlations in the data.

Methodik

A comprehensive prototype database of the peer-reviewed and published literature on MSI of lipid species was developed as part of a software engineering course given in 2012 at Saarland University (Prof. Dr. Andreas Zeller). A group of seven students developed a database architecture and GUI based on the requirements of the Institute of Bioanalytical Chemistry's mass spectrometry team. Further refinement of the prototype will be performed as part of this year's course, with input from the mass spectrometry community.

Vorläufige Daten

The database in its current state is based on an Apache web-server, PHP and JavaScript as front-end. This dynamic web-based front-end communicates user queries to a computational back-end. The back-end implements scripts for automated retrieval of bibliographic data alongside with standardized PubMed MeSH tags. The NLM Medical Subject Heading provides controlled vocabulary of biomedical terms and thus allows efficient use of search terms. The database connects this bibliographic information with identity of lipids and associated experimental parameters. For MS/MS confirmed lipids, an exemplary entry from LIPID MAPS [2] is imported. In every case, the database refers to the lipid classification system as developed by the LIPID MAPS consortium to provide a generally accepted framework. A fully customizable interpreter handles calculation of adduct ion species without the need for predefined sets of possible adducts, such as currently used by other databases. This implementation allows entering less common adducts without compromising the user friendly interface. For theoretical molecular weight calculations, the computational backend uses data gathered from the LIPD MAPS database directly or, where no entry is available, falls back to freely available LIPID MAPS Perl scripts. In this way, the database stores the lipids' theoretical molecular weights, theoretical *m/z* values of identified adducts and experimentally observed *m/z* values allowing a versatile search. Spatial information is stored as biological nomenclature of compartments (i.e. Organism: "Mus Musculus", Strain: "C57BL/6", Organ: "Brain", Compartment: "Hippocampus", Sub compartment: "Dentate gyrus", Cell type: "pyramidal cells"). Database entries have to be performed manually and are currently entered by one operator, but we envision that researchers may add their own results. We hope that discussions with colleagues will allow us to implement efficient and user-oriented improvements in future versions. We hope that this database will eventually lead to a 'mass spectrometric brain atlas' of frequently examined animals, e.g. rodents.

Neuartige Aspekte

Currently, no database for lipid imaging results is available. Our database is a first prototype containing published, peer reviewed data.

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To Digest or Not to Digest, That's the Question

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Einleitung

MALDI-Imaging Mass Spectrometry (IMS) of proteins in tissue sections combined with Top-Down proteomics represents a powerful approach to biomarker discovery. However, lack of direct identification strategies is preventing its broader use in Proteomics studies.
We have developed a new workflow that combines the spatial information obtained by IMS with the routine identification of proteins from tissue sections by LC-MS. Protein digests were generated by trypsinization on two subsequent tissue sections maintaining the spatial distribution of the peptides. One section was analyzed by IMS. Peptides from the other section were identified by routine LC-MALDI-MS/MS. The new "Spatial Proteomics workflow" allows routine identification of more than 100 proteins from tissue sections as well as their spatial distribution at the 50 µm level.

Methodik

Fresh frozen rat brain, testis samples and human breast cancer biopsies were sectioned to 10 µm slices. Each section was analyzed in parallel with and without tryptic digestion. For the digests, two subsequent slices were placed onto one glass slide and trypsin solution was applied to both of them by supersonic vibration. Tryptic peptides were imaged from one section in a reflector mode MALDI-TOF. The section was coated with CCA matrix. Tryptic peptides were identified by nanoLC-MALDI-TOF/TOF analysis and Mascot searching after elution from the second slice. A software tool was developed that grouped the peptide masses by their associated protein and linked it to the peptide masses in the bottom-up image for visual inspection of their co localization.

Vorläufige Daten

We analyzed rat organs using the new Spatial Proteomics approach as model systems, at the 50 µm spatial resolution level. In both organs, more than 200 peptides present in the images (i.e., 80 %) were identified by the parallel LC-MALDI analysis and approximately 120 proteins localized. As primary validation tools for the assignment of protein distributions in the tissue sections, the intensity and co-localization of 2 or more peptides and the specificity of mapping the peptide molecular weights to 1 or more proteins were used.
As an extension of the established top-down imaging strategy, this bottom-up Spatial Proteomics approach facilitates the identification and simultaneous localization of a much greater number of proteins than was previously possible. Proteins of biological relevance were identified that were previously identified by top-down imaging and top-down sequencing such as [1] thymosin-b4 and LCFA-CoA from rat testis and [2] Cystein-rich intestinal protein 1 (CRIP1) from breast cancer biopsies - a novel marker for metastatic cancer.
HER2+/CRIP1+ specified human breast cancer biopsies were analyzed using the established Spatial Proteomics workflow. CRIP1 was identified as one in 150 proteins. Arg-68 was present predominantly in the methylated form and to a lower extent in the non-modified form. Arginine methylation heterogeneity present in CRIP1 was in agreement with the unexplained top-down imaging peak pattern [2] in breast cancer biopsies.
Globally, the Spatial Proteomics workflow provided protein distributions that are typically invisible in top-down images. These included large (4 MDa titin) and membrane associated proteins such as MARCKS; however, mostly cytosolic, nuclear and cytoskeleton associated proteins were identified. The method may also be useful for protein imaging and identification from FFPE tissue in which top-down analysis is made impossible by protein crosslinks and which is largely accessible from tissue banks.

Neuartige Aspekte

"Spatial Proteomics workflow" allows routine identification of more than 100 proteins from tissue sections.

Rapid assessment of drug and metabolite distribution in whole animal tissues via accurate mass imaging

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Einleitung

Imaging approaches relying on accurate mass measurements typically require long scan speeds that result in lengthy acquisition times and hefty file sizes. Thus, applications toward the assessment of whole body drug distributions have been limited. New methods allowing for ion accumulation prior to FTMS detection, along with online data reduction algorithms, have significantly decreased unnecessary overhead. In this study, rapid imaging of whole body tissues with accurate mass, high resolving power and high dynamic range by MALDI FTMS in broadband mode were successfully obtained in a significantly shorter time frame and allowed for the rapid assessment of drug and metabolite.

Methodik

Male Sprague-Dawley rats (n=3) were administered a 30 mg/kg PO dose of Olanzapine euthanized at 2, 6, and 12 hrs post dose, and flash frozen. Whole-animal carcasses were sectioned at 20 µm thickness and whole-body sections were transferred to MALDI target plates using double-sided tape. CHCA matrix was spray coated onto tissues using the HTX Imaging sprayer. Full MS spectra from m/z 140-1000 were acquired on a 7.0T SolariX (Bruker) equipped with a dual ESI-MALDI source. Data were processed and images were extracted using FlexImaging software (Bruker).

Vorläufige Daten

High resolution (~150,000 @400 m/z) and exact mass (<2 ppm) imaging data were obtained in full scan MS mode over the m/z range of 140 to 1000). Exact mass 2D images of drug showed unique distributions across the whole-body sections. Each whole body section had 24,000 pixels and took 5 hours to acquire. The large sample introduction plate and fast acquisition capabilities of the FTMS made it an ideal platform for rapid whole body mass spectrometric imaging (WB-MSI). This study demonstrated the advantages of effectively enabling a full data set representing a PK time course to be obtained over a course of a few days rather than weeks by traditional approaches.

Neuartige Aspekte

Identification of drug and metabolites in whole body tissue sections by rapid accurate mass full scan imaging.

Software package for MSI instrument control, data acquisition and image processing

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Einleitung

Data acquisition in mass spectrometry imaging (MSI) requires a number of different controllable devices and modules, including the mass spectrometer, a laser, attenuators, x-y-z-stage, cameras and sample illumination. An intuitive and flexible “all in one” software is needed to control these modules properly and to obtain optimized interaction and efficiency of the overall method. Optimization targets include reproducibility, error minimization, user learning curves, automation level and convenience.
MSI data acquisition using high resolution mass spectrometers produces huge amounts of data. Relevant information has to be extracted from these data sets and has to be prepared, processed and reassembled in order to receive meaningful images. Here we describe a complete control and evaluation software package for high resolution MALDI MSI.

Methodik

MCP3 : A software for *AP-SMALDI*$_{10}$ (TransMIT GmbH, Giessen) instrument control and data acquisition. It supports various types of hardware including *LTQ*, *LTQ-FT*, *LTQ Orbitrap*, *Exactive* and *Q Exactive* mass spectrometers (Thermo Fisher Scientific GmbH, Bremen), ionization lasers, attenuators, microprocessor-based controller cards, micrometer x-y-z-stages and sample illumination. MCP3 is written in ObjectPascal. The various device drivers are implemented as ObjectPascal components.
MIRION : The imaging software uses metadata created by MCP3 and supports MCP3 acquisition methods, such as different scan patterns, MS and MS/MS acquisition modes, and ion polarity switching. Furthermore it supports various import and export file formats as well as powerful filters to reduce the input data stream and to provide fast and reliable image processing.

Vorläufige Daten

MCP3 supports the complete set of hardware currently used by us for imaging data acquisition. It supports handshake mode with the mass spectrometer's *Tune* software (Thermo Fisher Scientific GmbH, Bremen), triggers the mass spectrometer, sets illumination of the sample for camera observation, sets laser parameters, triggers the laser, moves the sample in x, y and z direction and synchronizes all activities. It also allows to manually operate the stage and the laser. It enables the user to interact with the modules even during an ongoing imaging measurement, for example for optimizing image acquisition parameters. MCP3 has been developed in close communication and interaction with MSI users in our lab. It includes an easy-to-use, intuitive and widely adaptable user interface which allows to configure MCP3 for individual requirements. The software can be run on three operation levels, for users, experts and service operators. It runs on MS Windows operating systems Windows 7 (32 and 64 bit) and Windows XP.
While earlier versions of MIRION required a full installation of the Thermo XCalibur software, MIRION now can be used with MSFileReader (Thermo Fisher Scientific GmbH, Bremen) and/or imzML data sets. The exchange format imzML allows MIRION to operate on data acquired from a wide range of mass spectrometers. A 64bit version of MIRION is now available which overcomes the former 2 GB data size limitation.

Neuartige Aspekte

All-in-one software package for MSI hardware control, data acquisition and image processing

Adjustment of the laser wavelength to match the absorption properties of matrices increases the ion yield in UV-MALDI mass spectrometry

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Einleitung

Using a laser wavelength that corresponds to a high optical absorption of the utilized matrix is a key element for sensitive UV-MALDI mass spectrometry. Unfortunately, the multitude of possible matrix compounds gets strongly limited by the fixed wavelengths of the common lasers employed for UV-MALDI-MS of 337 nm (N_2-laser) and 355 nm (Nd:YAG-laser). At the example of five cinnamic acid derivatives, we showed recently that a substantial analyte ion yield increase can be achieved if the wavelength is tuned to the individual absorption maxima [1]. Here, we extended this study to approximated conditions of MALDI MS imaging. This was achieved by irradiating fixed positions of homogeneous peptide-matrix samples and by recording the ion yields as a function of laser wavelength and fluence [2]. In addition, we investigated the influence of the wavelength and laser fluence on the intensities of DNA ion signals generated from 3-hydroxypicolinic acid (3-HPA). This standard matrix for the analysis of oligonucleotides is an excellent example for the potential of the described approach because its absorption peaks at about 310 nm, sizeable differing from even the N_2 emission line.

Methodik

A oTOF mass spectrometer was employed [1]. Tunable light was generated with either a dye laser [1] or an optical parametric oscillator (5 ns pulse duration, each). The investigated wavelength range was 290 – 350 nm (5 nm step size) and the laser spot size ~200 x 400 μm^2. Investigated cinnamic acid matrices were: α-cyano-4-hydroxycinnamic acid (HCCA; $\lambda_{max,\ solid\ state}$ = 345 nm), 4-chloro-α-cyanocinnamic (ClCCA; $\lambda_{max,\ solid\ state}$ = 310 nm) and α-cyano-2,4-difluorocinnamic acid (DiFCCA, $\lambda_{max,\ solid\ state}$ = 295 nm). 500 fmol of peptides were added per sample. A mix of DNA reference compounds were investigated with the 3-HPA matrix. In this case, different positions on the sample were irradiated with 600 laser pulses in total.

Vorläufige Daten

Previous studies by Fournier *et al.* showed that after an initial increase during the first few ten exposures peptide ion signals generated from cinnamic acid matrix derivatives like HCCA generally exhibit a steep decline with the number of laser pulses applied on one position of the sample [3].
In our study, this behavior as described by Fournier *et al.* and later by Qiao *et al.* [4] was qualitatively reproduced for the two studied halogenated CCA-derivatives. The most intense analyte ion signals were obtained after a few ten to hundred exposures (at a laser repetition frequency of 10 Hz), which was followed by a gradual decrease of the signal intensities. The time integral over the analyte "ion chromatogram" provides a measure for the overall analyte ion counts that are achievable if a fixed spot is irradiated. The data reveal that a maximum ion yield is found at wavelengths that are close to the highest optical absorption and for which consequently the lowest threshold fluences have been applied [1]. These results are in agreement with the fact that at excitation wavelengths corresponding to a lower optical absorptivity the laser penetration depth increases. Consequently, the analyte ion yield, i.e. the ratio of MALDI-generated analyte ions to the overall laser-desorbed analyte amount and, therefore, the overall analytical sensitivity, decreases.
For the DNA/3-HPA system the highest sensitivity is found around 310 nm, again corresponding to the absorption maximum. In particular, a better signal-to-noise ratio is found at this wavelength as compared to 337 nm, suggesting that the overall ionization efficiency increases. With respect to laser fluence, protonated molecular DNA species dominate the spectra in the optimal fluence range (2-3 times the threshold fluence), while higher abundances of sodium and potassium adducts are obtained at higher laser fluences.

Neuartige Aspekte

MALDI analyte ion yields increase if the excitation wavelength is adjusted to the specific matrix absorptivity

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High Speed AP-MALDI Imaging at high spatial resolution

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Einleitung

AP-MALDI Mass Spectrometry Imaging (MSI) has demonstrated impressing analytical information and specificity. High mass resolution, mass accuracy and high spatial resolution of 2 µm turned it into a sensitive method for molecular histology [1]. The obtained high data quality provides for reliable identification and localization of individual tissue components. The approach has been applied to a number of research areas in pathology, cancer diagnostics, metabolic pathway analysis and plant research, targeting phospholipids [2], peptides [3], proteins [4], drug compounds and metabolites [1]. Similar to other MSI techniques, the method has an analysis speed of about 1 pixel/s. Now, we have developed a data acquisition scheme for Orbitrap^{TM}-based systems that allows for an acquisition speed of up to 8 pixel/s.

Methodik

Data acquisition time in Fourier transform mass spectrometers mostly depends on the targeted value of mass resolution. At the highest mass resolution setting (140,000 at m/z = 200) the Q Exactive^{TM} instrument (Thermo Fisher Scientific GmbH, Bremen, Germany) provides for a full data acquisition time (including MSI operation) of about 1 s per pixel. An asynchronous coupling between the *AP-SMALDI$_{10}$* ion source (TransMIT GmbH, Giessen, Germany) and the Q Exactive mass spectrometer was developed that takes advantage of the actual resolution-dependent data acquisition speed of the instrument. In this mode the xyz-stage moves with a constant speed defined by the current acquisition speed of the mass spectrometer and the anticipated pixel resolution of the MSI analysis.

Vorläufige Daten

Depending on the molecular complexity of the investigated tissue within an imaged m/z range, a certain mass resolution and achieved mass accuracy (and according bin setting) are needed for reliable discrimination of molecular components [2]. After a first survey image, evaluation of data often reveals that mass resolution can be reduced for certain components having no close neighbors in their m/z vicinity, without losing specificity. Reducing mass resolution settings and thus increasing data acquisition speed does not linearly result in a reduction of mass accuracy. While the mass accuracy remains almost constant at a very low ppm level, the overall data acquisition speed is increased almost by the factor of the reduction of mass resolution setting.
At a mass resolution setting of 17,500 at m/z = 200, an acquisition speed of 8 pixels/s was achieved from mouse brain tissue at 10 µm pixel resolution. The total acquisition time of a 315x300 pixel image (3150 µm x 3000 µm area) could be reduced to about 3 hours, still achieving high mass accuracy performance in the low ppm range. Image quality of the high-speed image was found to be similar to that of a conventional-speed high mass resolution image of the same sample.

Neuartige Aspekte

Increased speed of high performance atmospheric pressure MALDI imaging analysis with Orbitrap-based mass spectrometry

Referenzen

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MSI of cuticular lipids on the whole bodies of intact Drosophila melanogaster flies

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Einleitung

Mass spectrometry imaging (MSI) is a dynamically developing area of mass spectrometry with a wide range of applications. MALDI MSI was used for mapping of cuticular lipids on the whole intact *D. melanogaster* flies. Based on this 2-D MSI of 3-D biological objects, the spatial distributions of biologically active compound on the cuticle were studied. MSI experiments of *D. melanogaster* flies were focused specifically on the distribution of male anti-attractants 11-*cis*-vaccenyl acetate (cVA), 3-*O*-acetyl-1,3-dihydroxy-octacosa-11,19-diene (CH5O3) and female pheromone 7Z,11Z-Nonacosadiene (7,11-ND) [1].

Methodik

Because the optimal fixation of the 3-D biological sample on the MALDI target is a crucial condition for a successful MSI experiment, dedicated MALDI targets with integrated complex profiled males and females cells were designed. The MSI experiments of cuticular lipids of virgin and mated 6 days old *D. melanogaster* flies were performed in positive and reflectron mode of LDI-TOF and MALDI-TOF technique. For MALDI-TOF experiments the lithium 2,5-dihydroxybenzoate (LiDHB) [2] matrix was sprayed on the samples by commercial airbrush. All samples were imaged using step size of 100 µm (254 dpi). Acquired raw MSI data were processed with custom-made software into spatially differentiated data and then converted to the 2-D maps of ions intensity maps using Biomap software.

Vorläufige Daten

Dedicated MALDI targets with profiled cells adapted to the dimensions of males and females imagoes of *D. melanogaster* ensured reproducible spatial orientation of flies on the target after spraying and drying. Flies could also be fixed and imaged in both dorsal and lateral orientations. LDI-TOF imaging of fruit flies resulted in spectra rich in TGs signals,but cuticular HCs signal were rather low. For the study of cuticular HCs the MALDI-TOF mode with LiDHB matrix proved to be far more suitable. Homogeneity of the deposit matrix layer was confirmed by SEM images. The visualization of males anti-attractants cVA and CH5O3 distribution was achieved for the first time. The strongest signals of these compounds were located on the tip of the male abdomen. After copulation clear signals of cVA and CH5O3 were observed also on the upper part of female abdomen, which means that the male is marking the female with this mixture of anti-attractants during the copulation. This could be expected based on established behaviour [1]. The female pheromone (7,11-ND) is localized symmetrically in the wings base area. All MSI results were supported by GC-MS analysis of hexane extracts of relevant parts of flies. Based on the our results the MALDI-TOF technique performed on MALDI Micro MX (Waters, UK) appeared to be well suited for 2-D imaging of 3-D biological object.

Neuartige Aspekte

MALDI MSI experiments with *D. melanogaster* enable the visualization of biologically active compounds transferred from male to female during copulation.

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Modellstudien zur Aufnahme von Gd-DTPA durch Kresse und Wasserflöhe mittels µ-Röntgenfluoreszenzanalyse mit Synchrotronstrahlung im Vergleich zur Laserablation ICP-MS

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Einleitung

Das Imaging von Elementverteilungen ist für die biologische und medizinische Forschung von steigendem Interesse. Die Laserablation gekoppelt mit der ICP-MS (LA-ICP-MS) und die Röntgenfluoreszenzanalyse mit Synchrotronstrahlung (SRXRF) sind zwei komplementäre mikroanalytische Techniken für das Elementmapping. Mit beiden Methoden können relativ schnell und einfach Multielementverteilungen in biologischen Proben gemessen werden[1].
Gadolinium wird in der Magnetresonanztomographie kontrastverstärkend eingesetzt. Dabei ist es durch Aminocarboxylate chelatisiert. In seiner freien ionischen Form ist das Gd aber hoch toxisch. Nach der Medikation gelangt das Kontrastmittel über Klärwerke in die Umwelt. In den letzten Jahren entstand dadurch eine positive Gd-Anomalie[2] in Oberflächengewässern. Da bisher nicht bekannt ist, ob das Kontrastmittel dort metabolisiert werden kann, wollen wir mit unserer Forschung den Pfad von Gadolinium-basierten Kontrastmitteln, die aus der Umwelt in biologische Systeme gelangen können, verfolgen[3].

Methodik

Zur Untersuchung der Gd-haltigen Kontrastmittel werden zwei Verfahren angewendet. Zum einen die Röntgenfluoreszenzanalyse mit Synchrotronstrahlung und die Laserablation gekoppelt mit dem ICP-MS. Da die SRXRF annähernd zerstörungsfrei arbeitet, ist es möglich dieselbe Probe nacheinander mit beiden Methoden zu analysieren und die Werte zu validieren.

Vorläufige Daten

Zur Evaluierung wurden die Nachweisgrenzen beider Methoden in den getrockneten Rückständen dotierter Tropfen bestimmt. Die LA-ICP-MS ist dabei mit einer Spotgröße von 50 µm (LOD: 0,78 µm kg^{-1} Gd in dotierten Tropfen) empfindlicher als die SRXRF mit einem Spot von 1,4 × 1 mm^2 (LOD: 15 µm kg^{-1} in dotierten Tropfen).
Modellorganismen (Kressepflanzen und Wasserflöhe) wurden dem Kontrastmittel Gd-DTPA (Magnevist) entweder direkt über das umgebende Medium oder indirekt über die Nahrung ausgesetzt. Im ersten Experiment wurde Gd auf der Haut eines Wasserflohs detektiert, während im Fall der dotierten Nahrung Hotspots im Bereich des Darms gemessen wurden.
Für die Messung von Kresse wurde ein Blatt zuerst mittels µ-SRXRF gescannt und dann mittels LA-ICP-MS untersucht. Die Analyse der Kresse mit beiden Methoden zeigte eine lokale Anreicherung von Gd in den Kapillargefäßen im Vergleich zum umliegenden Gewebe. Durch die zerstörungsfreie Messung mit der µ-SRXRF können die Images mit der LA-ICP-MS validiert werden.

Neuartige Aspekte

Die Bildgebung kann helfen aus elementaren Verteilungen von Gd in biologischen Systemen, Rückschlüsse auf Transportprozesse und die Bioverfügbarkeit zu ziehen.

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Eine kostengünstige computergesteuerte Airbrush-Einheit zur automatisierten Matrixpräparation in der Bildgebenden MALDI-Massenspektrometrie

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Einleitung

Sowohl die analytische Empfindlichkeit als auch die räumliche Auflösung in der Bildgebenden MALDI-Massenspektrometrie (MALDI-MS Imaging) werden maßgeblich durch die Qualität der Matrixapplikation auf die zu untersuchenden Gewebeschnitte bestimmt. Einerseits ist eine Extraktion der Analytmoleküle aus dem Gewebe und Kokristallisation mit der Matrix erforderlich, andererseits sollte es nicht zu einer stärkeren lateralen Diffusion im Präparationsschritt kommen. Auch sollten die erzeugten Kristalle mittlere Durchmesser unterhalb des Laserfokus aufweisen. Eine bewährte Möglichkeit hochwertige homogene Matrixpräparationen zu erzeugen, stellt der Einsatz von Airbrushs aus dem Künstlerbedarf dar. Um die obigen gegenläufigen Anforderungen zu erfüllen, wird die Matrix in der Regel in mehreren Sprüh-Trocknungs-Zyklen manuell aufgebracht. Allerdings bietet diese Herangehensweise nicht nur das Risiko einer hohen Varianz von Präparation zu Präparation, sondern das Verfahren ist für den Anwender auch zeitintensiv. Auf unserem Poster möchten wir einen low-cost-Aufbau vorstellen, mit dem eine Automatisierung erzielt wird. Das System nutzt eine handelsübliche Airbrush, welche mittels zweier Schrittmotoren, einer USB-Motorsteuerung und eines Softwaretools auf einfache Art und Weise mit beliebigen Sprühzyklen betrieben werden kann.

Methodik

Als Airbrush wurde das Modell Infinity (Hacker & Steenbeck) mit einem Düsendurchmesser, von 0.15 mm eingesetzt. Die Airbrush wurde mit einem Hinterdruck von etwa 3 bar N_2 betrieben. Als Matrizes wurden Zimtsäurederivate wie α-Cyano-4-Hydroxyzimtsäure und Benzoesäurederivate wie 2,5-Dihydroxybenzoesäure verwendet. Der Abstand von Ausgangsdüse der Airbrush zur Probe wurde auf etwa 20 cm festgelegt. Zur Bestimmung der Kristallgrößen wurde SEM eingesetzt und Mausschnitte als Realprobenpräparate verwendet. MALDI-MS Imaging-Aufnahmen wurden an einem Synapt G2-S HDMS-Massenspektrometer (Waters) erzeugt.

Vorläufige Daten

Per Hand wird die Infinity Solo über einen flexiblen Druckknopf bedient. Ein vertikaler Druck nach unten öffnet den Gasfluss, während eine leichte Kippbewegung nach hinten eine feine Nadel verschiebt, sodass die Flüssigkeit in den Gasstrom eingesaugt wird. Die beiden Bewegungen lassen sich entkoppeln, indem man den hinteren Gehäuseteil der Airbrush entfernt und die Nadel zur Steuerung des Lösungsmittelstroms mit einem Linear-Schrittmotor verbindet, mit dem die Nadel vor und zurückgeschoben werden kann. Eine zweite identische Linearsteuerung dient dazu, den Gasfluss durch Drücken des Hebels kontrolliert an- und auszustellen. Mittels eines USB-PC-Boards (Stepper Bee, PC Control Ltd.), das mit einem Netbook verbunden ist, lassen sich die Schrittmotoren ansteuern. Die Stepper Bee kommt mit einer Visual Basic-basierten Firmensoftware, die modifiziert wurde, um zum einen eine Null-Punktsabfrage mittels Mikroschalter zu integrieren und zum zweiten Ansteuerroutinen mit variablen Sprüh-/Trockenzyklen zu ermöglichen. In den Experimenten wurden eine variable Zahl von Sprühzyklen von 10 s Dauer verwendet, jeweils gefolgt von 20 s langen Pausen. Zur Unterstützung der Solventverdampfung wurde der Gasfluss in den Pausen nicht unterbrochen. Die Probe wird während des Sprühvorgangs mit einer CCD-Kamera in hoher Auflösung beobachtet. Um die Probenpräparation als Funktion der Zahl der Sprühzyklen und weiterer experimenteller Parameter hinsichtlich ihrer Qualität und Variabilität zu begutachten wurden SEM-Aufnahmen der besprühten Oberflächen angefertigt. Optimierte Präparationsprotokolle wurden angewendet um Maushirnschnitte mit MALDI-Matrix zu applizieren. Von diesen Schnitten wurden MS-Imaging Aufnahmen am MALDI-Synapt-Massenspektrometer angefertigt. Im Vergleich zu manuell betriebenen Airbrush-Systemen erlaubt das vorgestellte System die Möglichkeit, reproduzierbare und automatisierte Probenpräparationen für die Bildgebende Massenspektrometrie zu erzeugen. Die Kosten für das Gesamtsystem liegen um mindestens eine Größenordnung unter den Anschaffungskosten für kommerziell angebotene Matrixapplikatoren für die bildgebende MALDI-Massenspektrometrie.

Neuartige Aspekte

Eine hochwertige, automatisierte Matrixpräparationsstation für die bildgebende Massenspektrometrie lässt sich mit einfachen Mitteln realisieren.

Advances in high resolution mass spectrometry imaging of phospholipids

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Einleitung

Mass spectrometry imaging is a versatile and powerful analytical technique. Our work is focused on further increasing the the biologically relevant information that can be obtained by mass spectrometry imaging. Here we present a number of improvements in instrumentation, sample preparation, measurement parameters and data processing. The discussion will be based on phospholipids in mammalian samples, but the method was also successfully used for other applications including insect and plant specimen.

Methodik

MS imaging experiments were performed with a high resolution atmospheric-pressure imaging source [1] attached to'Exactive Orbitrap' or 'Q Exactive' mass spectrometers (Thermo Scientific GmbH, Bremen). Pixel size was between 2 and 10 µm. Mass accuracy was better than 2 ppm (root mean square) under imaging conditions. Tentative identification based on accurate mass was confirmed by on-tissue MS/MS experiments. MS images were generated with a bin size of $\Delta m/z = 0.01$, which largely eliminates interferences with neighboring peaks in complex samples.

Vorläufige Daten

Phospholipids were investigated in detail in mouse brain and human tumor samples. 'All ion fragmentation' experiments were used to image intact phospholipids simultaneously with their acyl chain and headgroup fragments. This allowed the (tentative) differentiation of isomeric lipids throughout the whole section within one experiment. A complete set of positive and negative ion images was obtained simultaneously by periodically switching the polarity of the ion optics throughout the imaging experiment. This significantly increased the number of lipids that could be identified in one experiment and thus improves the differentiation of tissue types. A coronal mouse brain section was imaged at 2 µm pixel size. This measurement revealed the detailed structure of the lateral ventricle & choroid plexus region.
Statistical analysis tools (including PCA and LDA) were adopted and applied for semiautomatic assignment of tissue types in mouse and human tissue sections. Additional data analysis tools were made accessible by data conversion to the common data format for MS imaging - imzML (www.imzml.org) [2]. This enables a flexible choice of the software best suited for a given application. Measurements from different instruments (data formats) were converted to imzML and displayed in one software tool with identical settings in order to allow for easy comparison.

Neuartige Aspekte

New features of mass spectrometry imaging methodology in phospholipid analysis including 2 µm spatial resolution.

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Inter-Class Separation of Lipids Using UPC2 /MS

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Einleitung

Developing separation based methodology utilizing a single technique is recognized as a common challenge for scientists researching lipidomic and metabolomic applications. Research and development focused on improvements of SFC instrumentation has provided a new technology that utilizes liquid CO_2 as a mobile phase. This new chromatographic instrument is referred as UltraPerformance Convergence Chromatography (UPC^2)
In this application note, UPC^2 is investigated for the analysis of lipids. We explore method variables influencing the peak integrity and chromatographic separation for a mixture of lipid classes. The methodology parameters were explored using lipid class extract standards composed of intra-class components. The method conditions are applied to biological lipids extracts.

Methodik

The ACQUITY UPC^2 system was configured with an ACQUITY SQD (single quadrupole) for the preliminary investigation of chromatographic and MS parameters. The optimal capillary voltage observed for the all the peaks in the lipids mixture was 3.5kV to 4.0kV. The final MS conditions used 3.5kV capillary and 30V cone voltages. Our initial approach was to screen three stationary phases; UPC^2 CSH Flouro-Phenyl, UPC^2 BEH 2-EP, and UPC^2 BEH. Biological extracts were analyzed using a different MS configuration capable of accurate mass determination. The method conditions were applied to biological lipids extracts whereas method adjustments are explored to manipulate the chromatography as per the goal of the analyst.

Vorläufige Daten

Method development screening columns indicated the BEH silica columns provided the best selectivity and peak shape. Injections of the individual lipid mixtures verified current peak assignments. The method used for screening the columns was optimized to focus on lipids with different polar head groups. The initial 12 minute screening method was reduced and performed in 5 minutes.
The degree of saturation affects the retention of the unsaturated analyte to be greater than the saturated analyte. Interestingly, the intra-class separation of LPE and SM standard extracts is observed when using the UPC^2 BEH stationary phase. The other stationary phases which provided broader peaks may be providing a better intra-class separation of the Avanti extract standards. This hypothesis will be explored in future studies.
The variable which resulted in greatest retentivity changes included the addition of a less polar solvent such as acetonitrile to the modifier. In general, using acetonitrile in the modifier improved resolution
The methodology was used to investigate two biological samples, each with distinctly different purposes. The first example examines a mouse heart extract which was investigated using the six minute method for the rapid inter-class *targeted* screening of polar lipids. This proof of concept approach will be used for further studies utilizing MS/MS quantitation assessing increases of phospholipids and sphingolipids during treatment.
The second example investigates neutral lipids present in a cotton seed oil extract. It was observed during the method development process, many neutral lipids elute near the chromatographic void when starting the compositional gradient above 5% modifier. The UPC^2/MS method was modified to retain the neutral lipids by reducing the starting percentage of modifier. The modifier was ramped to elute the polar phosphatidylcholines, which are known to be present as membrane lipid classes in cotton embryos

Neuartige Aspekte

Comprehensive inter-class lipid separation utilizing sub-2µm particle stationary phases and supercritical CO_2 based mobile phases coupled to mass spectrometry.

Identification of reactive lipid peroxidation products by tandem mass spectrometry

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Einleitung

Oxidative stress, defined as imbalance between the production of reactive oxygen species (ROS) and their elimination by antioxidant defense, is most likely related to the pathogenesis of numerous human diseases. ROS can damage virtually all biomolecules including proteins, nucleic acids and lipids. Lipid peroxidation is a major hallmark of oxidative stress due to the high oxidation susceptibility of polyunsaturated fatty acid residues within lipid structures. The analysis of lipid peroxidation products (LPPs) is challenged by the complexity of LPPs generated during oxidation. LPPs can be divided into high molecular weight (HMW), which contain glycerol and the head group, and the low molecular weight LPPs (LMW-LPPs), which are short, saturated or unsaturated aldehydes. Previous studies analyzed LMW- and HMW-LPPs separately using different mass spectrometry (MS) techniques, which often limited the analysis.

Methodik

Recently, we reported a new MS-technique that can simultaneously detect LMW and HMW-LPPs after derivatization with 7-(diethylamino)coumarin-3-carbohydrazide (CHH), which is known for its high carbonyl specificity [1]. Additionally, CHH-derivatives have high proton affinities due to the presence of a tertiary amine and thus can be detected in positive ion mode.

Vorläufige Daten

LPPs, generated by *in vitro* oxidation of different phospholipid classes (PLs), were analyzed before and after CHH-derivatization. The shotgun lipidomics relied on chip-based nanoESI operated in positive ion mode and coupled to an LTQ-Orbitrap-MS. For oxidized phosphatidylethanoamine vesicles, the characteristic fragmentation patterns provided detailed structural information to identify 22 LMW- and 11 HMW-LPP after CHH-derivatization. Oxidation of six different PL classes, i.e. PG, PC, PE, PS, PA, and PI4P, yielded 39, 36, 22, 19, 9, and 6 HMW-LPP-CHH derivatives, respectively. When coupled on-line to RP-HPLC, it was possible to separate LMW- and HMW-LPP and more importantly to separate HMW-LPP by the length of carbon chains. The ionization efficiencies, however, depended strongly on the head group. Thus, only HMW-LPP formed from one PL class can be relatively quantified.

Neuartige Aspekte

Further studies on lipid peroxides in complex biological samples are currently investigate.

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Does male obesity, which results in higher lysophosphatidylcholine levels, have an impact on fertility?

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Einleitung

The human spermatozoa membrane is characterized by a unique fatty acyl composition with significant amounts of highly unsaturated fatty acids, particularly docosahexaenoic acid (22:6), whereby phosphatidylcholine (PC) (16:0/22:6) is the most abundant glycerophospholipid. The large amount of highly unsaturated fatty acyl residues is crucial for the fluidity of the membrane and, therefore, the successful fertilization process, i.e. the fusion between the sperm and the female oocyte. Consequently, however, the spermatozoa are very sensitive to reactive oxygen species (ROS) that are generated under conditions of "oxidative stress" and many pathological conditions. Lipid oxidation of the sperm membrane is accompanied by the loss of oxidatively modified unsaturated residue in *sn*-2 position and the generation of saturated lysophosphatidylcholine (LPC) from common PC. Although other lysolipids are also generated, LPC is the most relevant "marker" lipid due to the high abundance of PC. Oxidative stress is massively involved in the pathology of many diseases; particularly obesity (body mass index (BMI) > 30 kg/m²) negatively affects the male reproductive potential [1]. We will show here that the PC/LPC ratio can be easily determined by MALDI-TOF MS (without the need of major sample workup) and is a reliable measure of sperm quality.

Methodik

The semen parameters of 98 ejaculates of 77 donors were analysed in addition to MS characterization. According to differences in the BMI three groups were defined: normal weight men (BMI < 24.99 kg/m², n=33), over-weight men (BMI 25.00-29.99 kg/m², n=24) and obese men that were subcategorised in obesity I (BMI 30.00-34.99 kg/m², n=10) and obesity II (BMI ≥ 35.00 kg/m², n=8). Prior to MALDI MS lipid extraction was performed according to the protocol of Bligh and Dyer (1959) [2]. All extracts were subsequently mixed with 9-aminoacridine (9-AA) and/or 2,5-dihydroxy-benzoic acid (DHB) matrix. The mass spectra were acquired on a Bruker Autoflex MS device (Bruker Daltonics, Bremen, Germany). Raw data were processed with the software "Flex Analysis" version 2.2.

Vorläufige Daten

Although a maximum of information would be available from the comparison of the positive and the negative ion MALDI spectra, the negative ion spectra of human sperm extracts give always only a single peak. This signal is coming from the presence of the "seminolipid" [3] that represents a strong electrolyte (due to the sulfate residue) and, thus, suppresses all further phospholipids that are normally detectable as negative ions (phosphatidylethanolamine, phosphatidylinositol, etc.). Therefore, exclusively the information available from the positive ion spectra, was included in this study. Under these conditions, the spectra are dominated by phospholipids with quarternary ammonia groups, i.e. PC, LPC and sphingomyelin (SM) are most sensitively. It is an advantage that uncharged lipids such as triacylglycerols and cholesterol are not detectable in the presence of 9-AA [4]. This is a significant difference in comparison to 2,5-dihydroxybenzoic acid (DHB) which is most commonly used for the MALDI analysis of lipids.
The intensities of the peaks of PC 16:0/22:6 (*m/z* = 806.6 and 828.6 corresponding to the H^+ and the Na^+ adduct, respectively) and LPC 16:0 (*m/z* = 496.3 and 518.3) were determined and the ratio of both peaks calculated. Significant differences in the lipid composition and particularly the PC/LPC ratio were observed if the extracts of the sperm from obese donors (Obesity II; BMI ≥ 35.00 kg/m²) were compared with the samples from donors with a lower BMI. There is a considerable reduction of the PC concentration in favour of the concentration of LPC. Thus, the PC/LPC ratio clearly reflects the changes of the lipid composition between normal weight men and extreme obese men. These changes of the lipid composition agree favourably with changes of the clinical parameters [5] that were determined from the same samples: in particular, reduced progressive sperm motility is characteristic of obese donors.

Neuartige Aspekte

MALDI MS is a useful method to correlate changes of the lipid composition with the ferilizing ability of human sperm.

Referenzen

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New MS-based analytical protocol for identification of isobaric oxysterols

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Einleitung

Elevated levels of lipid peroxidation products (LPPs) are associated with numerous human diseases, including neurodegeneration, diabetes and chronic stress related disorders. Some LPPs directly serve as signaling molecules and induce specific cellular responses, such as inflammation and autoimmunity. Others, like reactive carbonyls, show cross-reactivity to nucleophilic biomolecules leading to e.g. loss of function and aggregation of proteins, thus contributing to a disease phenotype. Cholesterol (Chl) is one of the main components of cell membranes and plasma lipidome and can be oxidized *in vivo* via enzymatic and non-enzymatic pathways. Mass spectrometry (MS)-based detection of *in vivo* toxic oxysterols would allow biomarkers identification for different oxidation-related diseases. Non-enzymatic oxidation of Chl results in various compounds with different types, positions and degrees of oxidations, which are often isomeric. Additionally, hydrophobic oxysterols have low ionization efficiencies, often suppressed by intense Chl signals. Additional derivatization is required to specifically distinguish isobaric species and to enhance ionization of oxysterols. Here we present a new consecutive derivatization protocol to identify non-enzymatically derived isobaric oxysterols by tandem mass spectrometry.

Methodik

Cholesterol (1 mmole/L) was oxidized *in vitro* in the presence of $CuSO_4$ (0.5 mmole/L) and ascorbic acid (1 mmole/L) or by a Fenton-like reagent ($CuSO_4$ and H_2O_2, 0.2 mmole/L and 2 mmole/L, respectively) for 70 h at 37 °C in organic (80% aqueous methanol) or aqueous (phosphatidylcholine (PC) vesicles in 3 mmole/L ammonium bicarbonate) solutions. The samples were incubated with 7-(diethylamino)coumarin-3-carbohydrazide (CHH; 10 fold molar excess in 50% aqueous methanol, 1h at 37°C) for derivatization of carbonyl groups and with 4-(dimethylamino)phenyl-isocyanate (DMAPI; 0.2 fold molar excess in triethylamine and dichlormetane, 2h at 65°C) for derivatization of the C3 hydroxy-group. Derivatized samples were analyzed by ESI-LTQ-Orbitrap MS equipped with a robotic nanoflow ion source TriVersa NanoMate.

Vorläufige Daten

Oxidation in organic solvent resulted in eleven oxidation products, but only nine oxysterols were detected in PC vesicles buffer. Oxysterols contained up to three oxygenation sites with combinations of mass shifts of +14, 16, 32 Da representing possible hydroperoxy, hydroxy, keto, and epoxy products. CID spectra of oxysterols showed fragmentation patterns characteristic for a Chl backbone, although identification of oxygenation type was not possible. Additionally, oxysterol characterization was hampered by prominent water loss from the C3 position during ESI, increasing the number of isobaric signals. C3 hydroxyl-group specific derivatization with DMAPI resulted in a characteristic mass shift of 162 Da and a specific reporter ion during CID at m/z 181 allowing to distinguish hydrated and dehydrated oxysterol species. CHH derivatized oxysterols showed a mass shift of 257 Da, a specific reporter ion at m/z 244 and neutral loss ions. Additionally, the tertiary amino-groups of DMAPI and CHH significantly enhanced the ionization efficiency. Consecutive derivatization of oxysterol mixtures allowed to distinguish several isobaric oxysterols including 5,6-secosterol and its aldol condensation product (3,5-dihydroxy-B-norcholestane-6-carboxyaldehyde).

Neuartige Aspekte

The reported new consecutive derivatization of non-enzymatically derived oxysterols allows specific MS identification of isobaric compounds.

MS-based monitoring of linoleic acid nitration mediated by •NO2 radicals.

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Einleitung

(Poly)unsaturated fatty acids (PUFA) are the main building blocks of lipids and important components of lipoproteins and cell membranes. During chronic inflammatory conditions, such as atherosclerosis, oxidation and nitration of PUFA play an important role. *In vivo* nitration of PUFA is induced by nitric oxide ($^{\cdot}NO$) derived species, such as nitrogen dioxide ($^{\cdot}NO_2$) and peroxynitrite anion ($ONOO^-$). Thus a large number of nitroalkenes are generated, which have high electrophilic reactivities towards nucleophilic residues, such as cysteine, histidine and lysine, and can induce protein modifications via Michael addition. Only a few nitration products of PUFA, formed under nitrosative stress conditions, were detected in cell membranes, cardiac tissue, urine and human plasma. The mechanisms of nitroalkenes formation remain to be defined and characterized. Here we present MS approach for the detection of linoleic acid (LA) nitration products and monitoring the kinetics of their formation by multiple reaction monitoring (MRM).

Methodik

LA (0.35 mol/L) was incubated with NO_2BF_4 (0.70 mol/L) (4 h, 20°C). Nitration products were characterized by ESI-LTQ-Orbitrap-MS/MS in negative ion mode. LA nitration products were quantified after 0, 15, 30, 45 and 60 min, 1.5, 2, 2.5, 3 and 4 h. At each time-point the reaction was quenched with water, the organic phase collected, dried and dissolved in chloroform. Samples were analyzed by MRM on an AB SCIEX QTRAP® 4000 system.

Vorläufige Daten

$^{\cdot}NO_2$ mediated nitration of LA under low oxygen tension yielded in total two oxidation and ten nitration products. Specific fragment ions for each product were determined using high resolution MS/MS and the corresponding MRM transitions were optimized. LA nitration was initiated by $^{\cdot}NO_2$ and F^- attacking the double bond in a fast and reversible process, followed by successive losses of H and oxygen produced nitro- and nitroso-LA, while further hydrolysis of nitro-LA yielded hydroxy-nitro-linoleic acid. All products increased over time, reaching the maximal level after 3 h. Di-, tri- and tetra-nitro-LA were formed via $^{\cdot}NO_2$ attack at the double bond of the β-nitroalkyl radical. Their contents increased continuously up to 3 h. After 4 h reaction time, nitro-LA was the main product observed, while the amounts of multiple nitration products significantly decreased. This suggests a reversibility of the nitration reaction, where single or successive losses of nitrous acid lead to accumulation of a nitro-LA.

Neuartige Aspekte

Multiple nitration products of LA, five of them capable to alkylate proteins, were characterized by tandem mass spectrometry.

Overcoming current problems of MALDI-TOF MS analysis of sulphated glycosaminoglycans: improved enzymatic digestibility and reduced sulphate loss

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Einleitung

Glycosaminoglycans (GAG) such as hyaluronan (HA) or chondroitin sulphate (CS) are important polysaccharide constituents of the extracellular matrix (ECM) of tissues such as cartilage, skin or bone [1]. Since the discovery of the "sulphation code", particularly (over)sulphated GAG are experiencing considerable interest because coating of implants with such compounds is known to improve the *in vivo* incorporation of the implants by reducing the inflammatory response of the host organism [2]. Unfortunately, the detailed analysis of the GAG polymer repeating units is a challenging task. There are particularly two problems regarding the MS analysis of (over)sulphated GAG: (a) in contrast to natural GAG, chemically modified GAG are extremely refractive to the digestion with commonly used enzymes (e.g. testicular hyaluronidase or chondroitinase ABC) and (b) using MALDI-TOF MS significant but unwanted sulphate loss occurs. This complicates the analysis of mixtures. We will show here that the inhibition problem can be overcome by using a sufficient excess of the enzymes. We will also show that the positive ion spectra (recorded in the presence of common 2,5-dihydroxybenzoic acid (DHB)) are characterized by a less pronounced sulphate loss in comparison to the negative ion mode.

Methodik

All polysaccharides were kindly provided by Innovent Jena. Digestions were performed by either hyaluronidase or chondroitinase ABC and the yield of oligosaccharides was monitored by using the Morgan-Elson reaction [3]. All used GAG oligosaccharides were commercially available and sucorse octasulphate was purchased from MOLEKULA (Kranzberg, Germany), while the CS tetrasaccharide (CS-4) was obtained by hyaluronidase digestion of the native CS polysaccharide and subsequent purification by TLC [4]. The tetrasaccharide was scraped from the silica gel and re-eluted with water. Its concentration was determined by using a modified carbazol method. The oligosaccharides were analyzed by negative and positive ion MALDI-TOF MS (Bruker-Daltonics, Autoflex^{TM}) using 9-aminoacridine (9-AA) and 2,5-dihydroxybenzoic acid (DHB) as matrices in the negative and positive ion modes, respectively.

Vorläufige Daten

Intact GAG polysaccharides cannot be characterized by MS because the transfer into the gas phase is accompanied by the decomposition of the polymer. Therefore, GAG are normally digested into defined oligosaccharides prior to MS analysis. Unfortunately, this approach is exclusively applicable to natural GAG while the related enzymes are inhibited by chemically (over)sulphated GAG. However, this exclusively holds if an excess of the GAG in comparison to the enzyme is used. As soon as there is an excess (mol/mol) of the enzyme chemically oversulphated CS and HA can be sufficiently digested. Although further investigation of the mechanism are unequivocally required, this is a significant progress since the otherwise applied chemical degradation is accompanied by unwanted side reactions such as the cleavage of the N-acetyl groups or the loss of the sulphate residues. Unfortunately, the sulphate loss does also occur in the gas phase. GAG are normally investigated in the negative ion mode because they are strongly negatively charged. Surprisingly, we found that the positive ion spectra are much more suitable to avoid the sulphate loss. This could be confirmed by using commercially available (either natural of oversulphated) disaccharides of CS, a tetrasaccharide of CS and sucrose octasulphate (SOS) as a readily available model compound: using DHB the proton adduct of the intact molecule was detectable at *m/z* 1158.6 although several sulphate losses were also evident. In contrast, the intact molecule was not detectable as negative ion in the presence of 9-AA as matrix. It is concluded that the positive ion MALDI mass spectra are the method of choice to detect the intact sulphated GAG. This is particularly important if mixtures of compounds with different sulphation patterns are to be characterized. Thus, the use of liquid crystalline matrices (alone or in combination with CsCl [5]) is not an absolute necessity.

Neuartige Aspekte

The results described above will enable an improved MALDI MS-based characterization of chemically modified glycosaminoglycans

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Characterization of new alkanal-phosphatidylethanolamine adducts by tandem mass spectrometry

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Einleitung

The pathophysiology of numerous human disorders, such as arthrosclerosis, diabetes, obesity and Alzheimer´s disease, is accompanied by an increased production of reactive oxygen species (ROS). ROS can oxidatively damage many biomolecules, including lipids, proteins and nucleic acids. In particular, (poly)unsaturated fatty acyl residues within phospholipid (PLs) structures are easily converted by ROS to lipid peroxidation products (LPP) carrying reactive carbonyl groups. Carbonylated LPP can react with nucleophilic groups in proteins (Lys-, Cys-, and His-residues) or phosphatidylethanolamines (PE). Adducts of unsaturated LPP with amino groups in PE were reported before, but little is known about the reactivity of saturated alkanals towards nucleophilic groups of PLs. Multiple compounds can be formed during alkylation of PE head group by reactive alkanals, which require detailed structural elucidation. Here we present a study on new alkanal-PE adducts using consecutive fragmentation (MS^n) and multiple reaction monitoring (MRM) techniques.

Methodik

Dipalmitoylphosphatidylethanolamine (DPPE, 0.1 mmole/L) was incubated with hexanal (400 mmole/L) in aqueous solutions (1 h, 37°C). Lipids were extracted (methanol:chloroform; 1:1, v:v) and analyzed by shotgun lipidomics using ESI-LTQ-Orbitrap-MS. The structure of new DPPE-hexanal adducts was identified by CID-MS^n (n from 2 to 7). Schiff bases were identified by specific reduction with cyanoborohydride. The products were quantified at different time point by MRM on an ESI-QTrap MS.

Vorläufige Daten

Schiff base adducts of single aldehyde and amide adducts were generated in the presence of a low concentration of hexanal (0.5mM). In contrast, at high concentration of aldehyde (25 mM) new PE-hexanal adducts containing dimeric and trimeric hexanal conjugate were detected. Additionally, reduction with cyanoborohydride identified three compounds as Schiff bases. The structures of these new alkyl-adducts were determined by their fragmentation patterns using MS^n experiments. Surprisingly, eight different products were identified, six new compound were founded and two were already known. Studying products at different time point, we could show that Schiff bases and hexanal dimer adducts were formed immediately after hexanal addition to DPPE, whereas amide type products were detected only after longer incubation times. Trimeric hexanal adducts appeared after 15 min and were detected at high levels after 30 min.
The biological relevance of these modifications requires further future investigations but may be relevant in many pathologies.

Neuartige Aspekte

New alkanal-PE adducts were identified using consecutive tandem MS

The muscle (phospho)lipid composition of bulls: an investigation by MALDI MS and 31P NMR

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Einleitung

Meat is increasingly consumed in western societies and provides beside proteins also fatty acids that are essential in human nutrition. Modern techniques of soft ionization MS were so far rarely used to investigate the (phospho)lipid compositions of muscle tissues ("meat") of beef, while classical methods such as gas chromatography / mass spectrometry (GC/MS) are still widely used. Of course, GC/MS provides accurate, quantitative information about the fatty acyl compositions of the individual lipid classes. However, all information about the positions of the fatty acyl residues is lost because hydrolysis of the intact lipids is necessary prior to analysis.
The aim of the present study was to investigate whether a combination between MALDI-TOF MS and high resolution ^{31}P NMR is useful to study the intact (phospho)lipid composition of beef. It is expected that MS is particularly useful to obtain the relative fatty acyl compositions of the individual lipid classes while NMR is the method of choice to obtain quantitative data on the abundance of the different lipid classes. Additionally, it was also planned to investigate whether different nutrition strategies will lead to quantitative and/or qualitative changes of the fatty acyl compositions of the individual lipids.

Methodik

The muscle samples were selected from a feeding experiment with German Simmental bulls. *Longissimus* muscle samples were isolated immediately after slaughter and the lipids were extracted according to Folch [1]. All chemicals, solvents, and the MALDI matrices (9-aminoacridine (9-AA) and 2,5-dihydroxybenzoic acid (DHB)) were obtained in the highest commercially available purity from Sigma-Aldrich. Phospholipid standards were from AVANTI Polar Lipids. DHB was either used as 0.5 M solution in methanol or 100 mg/ml in acetonitrile/water (1:1, *v/v*), while 9-AA was used in a concentration of 10 mg/ml in isopropanol/acetonitrile (60/40, *v/v*)) [2]. MALDI analysis (sometimes combined with TLC) was performed as recently described [3]. The "mixed micelle" approach was used to record the ^{31}P NMR spectra [4].

Vorläufige Daten

MALDI-TOF MS is a fast and sensitive method of lipid analysis that has the additional advantage of tolerating sample impurities such as salts. Therefore, no major purification of the samples is normally required. In dependence on the acidity/basicity of the used matrix, different lipid classes can be differentiated even in complex mixtures: while phosphatidylcholines (PC), sphingomyelins (SM) and triacylglycerols (TAG) as well as the corresponding lysolipids are primarily detectable as positive ions, all further phospholipids such as phosphatidylethanolamines (PE), phosphatidylinositols (PI) and cardiolipins (CL) are much more sensitively detected as negative ions. DHB and 9-AA are the matrices of choice to record the positive and the negative ion mode spectra, respectively. It is also of interest to note that some apolar lipids such as TAG and cholesterol (as well as plasticizers and antioxidants that are common impurities in many plastic materials) are exclusively detectable in the presence of DHB but not 9-AA. Although MALDI MS is basically capable of providing quantitative results if internal standards are used, the MS data were here exclusively used to determine the fatty acyl compositions of the individual lipid classes, while absolute lipid concentrations of the extracts were determined by high resolution ^{31}P nuclear magnetic resonance (NMR) spectroscopy.
According to the obtained data, feeding of the animals (maize versus grass silage) has a significant impact on the lipid composition of the muscles. Feeding with maize silage/grass silage (70/30) results in (a) a higher PL content than feeding with grass silage alone and does also (b) affect the PI/PE ratio. Result (a) is particularly surprising because neither the grass nor the maize silage contain significant amounts of phospholipids but only triacylglycerols [5]. Therefore, further studies are obviously required to clarify the related mechanisms of lipid metabolism.

Neuartige Aspekte

This is the first MALDI MS approach to study the lipid composition of meat.

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Advantages of Ultra-High resolution Time of Flight Mass Spectrometry - Case Studies using Direct Analysis and Separation Techniques

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Einleitung

High resolution mass spectrometry (HRMS) has become a preferred tool in comprehensive differential analysis for metabolomic profiling associated with disease states. In its many forms it is used in the direct analysis of samples (no chromatography) with great results for lipidomics as well as fundamental metabolomics. HRMS has also been used when interfaced to liquid chromatography, gas chromatography, and capillary electrophoresis. Here each of these techniques is applied to the investigation of differential metabolomic profiling associated with different disease states, physiological fluids, and analytical approaches. The complementary nature of the different approaches and the data they provide is emphasized. Novel capabilities including speed of acquisition and fragmention acqusition in CE, high mass accuracy in identification of analytes, and high mass resolving power for the separation of isobaric and isotopic pairings provide for improved information content and selectivity in the analyses.

Methodik

Analyses are performed using a Pegasus GC-HRT for GCMS and Citius LC-HRT for direct analysis, LCMS and CEMS. The CE interface was custom built. Plasma samples are deproteinated using methanol and analyzed using flow injection analysis with ESI ionization to differentially profile lipids from diseased and control rats in the Zucker model. This is compared to CE, GC and UHPLC analysis of the same when interfaced to the similar high resolution mass spectrometer. Urine samples from subjects were analyzed "as is" or desalted using acetonitrile or SPE prior to analysis. GCMS analysis was performed after TMS-derivatization of dried urine or plasma extracts using standard protocols. Acquisition rates varied among the experiments but exceeded 50 s/s in the CE analyses.

Vorläufige Daten

The analysis of diseased rat plasma samples using different analytical platforms provided clear metabolomic differences. Analyses using the different techniques provided correlated and complemetary data on metabolomic differences including amino acids and lipids. The high resolving power provides for superior quanitation of lipids and their various unsaturated forms by reslotuion of isotopic interference. Urine serum samples from normal and diabetic subjects and two forms of cancer are analyzed using CEMS and LCMS and the differences in the levels of metabolites which are up or down regulated with disease are discussed. The ability to leverage mass spectral data having resolving power of up to 100,000 and mass accuracies below 1 ppm is discussed in the context of potential biomarker identification and selective relative quantitation. The enhanced and more facile identification of metabolites is demonstrated using a novel approach to fragment ion creation known as MSc2 in LCMS. The utility of soft ionization in conjunction with EI spectral information is leveraged and CEMS to confidently identify metabolites in GC analyses using high resolution mass spectrometry. Finally, the utility of time of flight as the accurate mass platform is discussed in general, and specifically when interfaced to high efficiency capillary electrophoresis where narrow peaks and the need for fragment ion information is significant. These advances in the technologies and approaches applied to metabolomic analyses create a new path for future investigations and a portfolio of analytical approaches to he metabolomic scientist.

Neuartige Aspekte

Comprehensive MS, CEMS, Metabolomic Complementation, Lipidommics

Lipid changes of lung epithelium cells in a murine model of experimental asthma

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Einleitung

Despite intensive research in the field, many questions regarding the pathogenesis of allergic asthma and the role of lipids in this context remain unclear. In recent years, a central role of the airway epithelium and lipids in asthma became apparent. Among others, ceramides were suggested to be important signaling molecules in asthma. Most studies performed so far focused on detection of lipids by immunohistochemistry and/ or on quantification of selected lipid species by mass spectrometry. To our knowledge, a comprehensive study of all major lipid classes in airway epithelium cells by mass spectrometry has not been performed.
In order to investigate lipid alterations in the pathogenesis of asthma, we aimed to determine lipid composition of murine tracheal airway epithelium from healthy mice and mice that were sensitized and challenged with the relevant antigen house dust mite extract.

Methodik

Epithelium cells were obtained by gently skimming the mice epithelium using a sterile polyethylene swab. Lipids on the swab were extracted using the Bligh & Dyer method and separated by head groups using normal phase (NP)-HPLC. Online detection was performed using a high resolution hybrid Apex-Qe FT-MS system (Bruker Daltonics, Bremen, Germany) equipped with a 7 Tesla actively shielded superconducting magnet and an Apollo Dual ESI/ MALDI ion source. Mass spectra were summed up according to the respective Rf values of the lipid groups of interest and analyzed using LipID[1] allowing the verification of the head groups and the overall fatty acid composition including the degree of saturation.

Vorläufige Daten

The developed method can be used for simultaneous determination of individual lipid species of fourteen major lipid classes (Cer, TAG, PG, CL, PE, LPG, LPE, PI, LPI, PS, LPS, SM, PC, FA). Isobaric PC and PE species were baseline-separated using the LC system. The assay linearity was typically greater than two orders of magnitude and correlation coefficients were > 0.99. Typical limits of detection (LOD, defined as S/N=3) for most lipid classes were below 0.1 µ*M*. The limits of quantitation (LOQ, defined as S/N=10) were in the range from 0.02 to 0.5 µ*M*.
By using this non-targeted approach, we were able to identify a variety of lipid species belonging to 8 different lipid classes (PG, PC, PS, Cer, SM, PE, PI, LPC) in murine tracheal airway epithelium cells. 14 lipid species belonging to six different lipid classes (SM, PG, PC, PS, PI and Cer) were significantly different within the two groups. All measured ceramide (5/8 significant, $p < 0.05$, Mann-Whitney Test) and sphingomyelin (2/15 significant, $p < 0.05$, Mann-Whitney Test) species were increased in treated animals compared to controls.
These results indicate that ceramide accumulation in murine lung epithelium after house dust mite challenge is of importance in the pathogenesis of asthma. Furthermore, increased sphingomyelin levels suggest *de novo* formation of ceramides after challenge in treated animals.

Neuartige Aspekte

First detailed investigation of lipid changes after sensitization and challenge of mice with house dust mite extract compared to controls.

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New methods to determine free fatty acids in complex mixtures

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Einleitung

The liver is an extremely important organ and involved in a large variety of metabolic pathways. Since the liver is particularly involved in the metabolism of lipids, there is a considerable amount of free fatty acids (FFA) that may be converted into triacylglycerols or oxidized as fuel. Since FFA contribute also to important liver disease such as the “non-alcoholic fatty liver disease”, their detailed qualitative and quantitative analysis is highly important. The so far established approach of FFA analysis is based on gas chromatography / mass spectrometry (GC/MS) that is rather time-consuming. Therefore, we have addressed the question whether more convenient methods can be used as alternate techniques. These methods comprise a commercially available test kit, the matrix-assisted laser desorption and ionization (MALDI) mass spectrometry (MS) and the high resolution nuclear magnetic resonance (NMR) spectroscopy.

Methodik

A commercially available kit was used to determine the absolute amounts of FFA. This test was performed according to the manual and the obtained data were compared with the NMR results: FFA were in situ derivatized with 2-chloro-4,4,5,5-tetramethyl-dioxaphospholane and the integral intensity of the corresponding ^{31}P resonance compared with the intensity of an internal standard. The NMR spectra were recorded on a Bruker AVANCE 300 MHz NMR device. Finally, the relative ratios of the individual FFA were determined by negative ion MALDI MS in the presence of 1,8-bis-(dimethylamino)-naphthalene that was recently suggested as an excellent matrix for FFA analysis. UV (337 nm) MALDI mass spectra were obtained on a Autoflex I device (Bruker Daltonics, Bremen) in the reflector mode.

Vorläufige Daten

Based on the obtained results it can be stated that there is a very suitable alternative method compared to the relatively expensive GS/MS method for the analysis of free fatty acids. As in the analysis of phospholipids this method comprises a combination of ^{31}P NMR and MALDI MS. By ^{31}P NMR, we obtain exact absolute concentrations if the time-dependence of the spectra is taken into account. The relative contribution of the individual fatty acids we obtained by MALDI MS. Again, it is important to maintain a certain time frame. For a detailed analysis, it is essential to use the results which are recorded immediatly after loading the target into the MALDI mass spectrometer. Otherwise, when exceeding the time frame, no accurate results can be obtained. As long as time the window is observed with the combination of both methods, an accurate FFA analysis is possible. Regarding complex mixtures, this approach is more reliable than the colorimteric approach. This commercially available kit is not suitable for the determination of the overall FFA concentration in a given mixture because highly unsaturated FFA such as arachidonic (20:4) or docosahexaenoic (22:6) acid are determined much less sensitively than saturated ones

Neuartige Aspekte

^{31}P NMR in combination with MALDI MS is a useful methodological couple to determine FFA concentrations in a crude mixture.

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Umfassende Lipidstruktur-Kandidaten Generierung aus MALDI-CFR-PSD- und MALDI-QIT-TOF-MS/MS-Fragmentionen-Spektren

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Einleitung

Die Matrix-unterstützte Laser-Desorptions/Ionisations-Massenspektrometrie (MALDI-MS) hat sich mittlerweile als Methode zur Analyse von Lipiden etabliert [1]. Dies erweitert den Anwendungsbereich von MALDI-MS aus der Proteomik hin zur Lipidomik mit dem Ziel einer möglichst umfassenden Identifizierung der Lipidzusammensetzung von biologischen Proben (z.B. Zellen, Gewebe, Blut, usw.). Dabei eignen sich besonders die Fragmentionen-Spektren der Lipid-Quasimolekülionen (z.B. H-, Na- oder Li-Addukte) zur Charakterisierung der Lipidstrukturen [2]. Derartige Daten können mittels ein- oder mehrstufiger Tandem-MS (MS/MS bzw. MS^n) entweder durch "Post-Source-Decay" (PSD) in einem "Curved-Field Reflectron" (CFR) Flugzeitanalysator (TOF) oder durch "Collisional Induced Dissociation" (CID) in einer 3D-Quadrupol-Ionenfalle (QIT) erzeugt werden.
Die Produktionen aus Niedrigenergie-Fragmentierungen (wie sie durch PSD bzw. in einer 3D-Ionenfalle generiert werden) wurden für verschiedene Lipidklassen bereits umfassend beschrieben [2], sodass eine automatische Erzeugung möglicher Lipidstrukturen für die Messung unbekannter Lipide erfolgversprechend erscheint.

Methodik

Für die Messung der PSD-Spektren wurde ein MALDI-CFR-TOF-MS (Axima-CFRplus, Shimadzu) und für die CID-Spektren ein Hybrid MALDI-QIT-TOF-MS/MS (AXIMA-Resonance, Shimadzu) Instrument, das über eine MS^n-fähige Quadrupol-Ionenfalle gekoppelt an einen Flugzeitanalysator verfügt, eingesetzt. Zur MALDI-Präparation wurden in der Lipidanalytik übliche Matrix-Substanzen (z.B THAP, ATT, DHB, 9-AA) für den positiven und negativen Ionenmodus verwendet [3].
Der Lipidstruktur-Generator arbeitet zweistufig: (1) Für jeden einzelnen Peak im Fragmentionen-Spektrum des unbekannten Lipids werden alle möglichen Erklärungen durch bekannte Fragmente oder Neutralverluste gesucht. (2) Durch Kombination von Lipidbausteinen wie Glycerin oder Sphingosin, Phosphat, Phospholipid-Kopfgruppen, Fettsäuren, Zuckern und Kationisierung werden alle möglichen Precursor-Ionenstrukturen erzeugt, aber nur diejenigen als Kandidaten akzeptiert, die für alle Fragment-Ionen eine Erklärung besitzen.

Vorläufige Daten

Unsere Erfahrungen zeigen, dass unter den gewählten experimentellen Bedingungen üblicherweise alle MS/MS Signale erklärt werden können, wobei die Identifizierung eines unbekannten Lipids erreicht werden kann, wenn alle essentiellen Strukturinformationen im MS/MS-Spektrum vorhanden sind. Fehlende Information (z.B. keine MS/MS-Peaks, welche die Fettsäure-Zusammensetzung charakterisieren) führt zu größeren Lösungsmengen, die aber in jedem Fall die korrekte Lösung beinhalten. In manchen Fällen genügt sogar ein einzelnes Fragmention zur Identifikation eines Lipids.
Der Algorithmus wurde zuerst für Triacylglyzeride und Phospholipide im positiven Ionenmodus entwickelt und später für Glykolipide und den negativen Ionenmodus erweitert. Viele Erklärungsregeln für Fragmente und Neutralverluste konnten vom positiven auf den negativen Modus übernommen werden. Auch die komplexen Lipidstrukturen der Cardiolipine wurden untersucht und in die Methode aufgenommen.
Falls die Strukturgenerierung zu mehreren Lipid-Kandidaten für ein MS/MS-Spektrum eines unbekannten Lipids führt, so können mit Hilfe einer kleinen Datenbank gemessener Fragmentionen-Spektren, deren Peaks vollständig annotiert wurden, die theoretischen Massenspektren aller Strukturkandidaten simuliert werden und mit der Messung des Unbekannten verglichen werden.

Neuartige Aspekte

Etablierung eines Algorithmus zur umfassenden Strukturgenerierung aller Lipid-Kandidaten für MALDI-basierende MS/MS-Spektren auf Basis der Erklärung aller Fragmentionen.

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Calibration of Traveling Wave Ion Mobility Mass Spectrometer (TW IM-MS) to Estimate Collision Cross Sections (CCSs) of Glycans

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Einleitung

Glycosylation is one of the most common post-translational protein modifications. The bound glycans play important roles in their function and influence characteristics such as cellular transport or resistance to enzymatic degradation. The development of methods for the analysis of glycans proved to be challenging, due to their immense structural diversity and complexity.[1] The constituting monosaccharide building blocks can exhibit the same mass, but different stereochemistry, and possess multiple functional groups at which new glycosidic bonds can be formed. Accordingly, glycans often exist as multiple isomers with identical atomic composition, but different structure. This limits most of the currently available methods including mass spectrometry.

Methodik

A promising technique to provide an additional separation step for carbohydrate analysis is ion mobility mass spectrometry (IM-MS). After ionization of the samples with a nano-ESI-source the ions travel through a gas-filled cell aided by an electric field and are separated according to their shape and size, allowing the differentiation of isomers with the same mass. While the drift time of an ion depends on the instrument parameters, the collision cross section (CCS) is a molecular property that can be compared and calculated theoretically. Due to the inhomogenic electric field in traveling wave (TW) instruments it is not possible to directly measure absolute CCSs. Therefore, an external calibration is necessary to estimate them.

Vorläufige Daten

Analogous to a protocol that is already established for the CCS estimation of proteins we here present a calibration strategy for glycans.[2] Glycan calibrants were released from a series of well-characterized and commercially available glycoproteins (e.g. fetuin, ovalbumin, ribonuclease B). Subsequently, their underlying absolute CCSs were measured in He and N_2 using a modified drift tube Synapt IM-MS instrument.[3]
Using this calibration method it is possible to estimate CCSs of glycans with commercially available hybrid TW IM-MS instruments (Synapt G2-S, Waters). Our data demonstrate that the glycans of one glycoprotein and their resulting fragments are in principle sufficient to yield a calibration of the required quality. Additionally, our experiments show that carbohydrate isomers with the same mass can be separated and identified by IM-MS based on their CCSs. The easy to follow protocol allows to include the estimation of CCSs in routine measurements. In the future this could therefore be used to implement the CCS as an additional structural criterion in carbohydrate databases.

Neuartige Aspekte

Estimation of collision cross sections (CCSs) of glycans using traveling wave ion mobility mass spectrometry (TW IM-MS).

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Rab5 knockdown by RNA interference: functional study by shotgun lipidomics

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Einleitung

How do cells control the number and size of membrane organelle? Recently, Zeigerer et al. showed that the knock down of the small GTPase Rab5 - a master regulator of endosome biogenesis, causes a marked reduction in the number of early and late endosomes and of lysosomes in the mouse liver. To study the effect of liver endosome loss on lipid metabolism we performed a shotgun lipidomic analysis of mouse liver and serum samples of wt and Rab5 knockdown animals. The systematic analysis encompassing a large fraction of liver and serum lipidome shed light on the mechanistic relationship between the endosome biogenesis and lipid homeostasis.

Methodik

3, 4, 5, 10, and 15 days after delivery of siRNA to mouse hepatocytes *in vivo* the liver and sera of wt and Rab5 knockdown animals were taken for a shotgun lipidomic analysis. Internal standards for lipid quantification were added to the samples and lipids were extracted with a modified Folch protocol [2]. Mass spectrometric analysis was performed on a Q Exactive (Thermo Fisher Scientific) mass spectrometer equipped with a robotic nanoflow ion source TriVersa (Advion Biosciences). Lipid species were identified using LipidXplorer software [3].

Vorläufige Daten

In this study, a top-down shotgun lipidomic approach was employed to characterize the serum and liver lipid profile of wt and Rab5 knockdown mice 3, 4, 5, 10, and 15 days post siRNA injection. For quantification of all identified lipid species internal standards were spiked into the samples prior to lipid extraction by a modified Folch protocol. Total lipid extracts were directly infused into a Q Exactive mass spectrometer and species identified and quantified using LipidXplorer software. The analysis encompassed > 200 individual lipid species from 15 major classes within less than 7 min acquisition time [4] with no carry-over of lipid material between the samples. Shotgun profiling of sera from Rab5 knockdown animals indicated a strong increase of Cholesterol, Triacylglycerol, Diacylglycerol, Sphingomyelin, and Cholesterolesters in the serum of the animals depleted of Rab5 when compared to respective controls. The peak accumulation occurred 5 days post injection – which correlates with the down-regulation of Rab5 in the liver. Lipid analysis of liver samples revealed an accumulation of intracellular Cholesterol and Cholesterolesters while no changes of membrane lipids were observed. These results suggest a link between endocytosis and metabolism.

Neuartige Aspekte

Endosome loss promotes lipid accumulation in both liver and serum.

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Fucose migration in gaseous protonated N-glycopeptides

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Einleitung

Fucosylation of *N*-glycans is frequently found in nature and is involved in many processes such as recognition events, cell adhesion or cancer development [1]. Recently, it has been shown that fucose residues which are α1,3-linked to the *N*-acetylglucosamines (GlcNAc) located in the antennae can rearrange by migration between the terminal branches of protonated *N*-glycan ions. Evaluation of the resulting fragment ion spectra might lead to misinterpretation of the analyte structure. However, it has been demonstrated that fucoses α1,6-linked to the proximal HexNAc of the chitobiose unit do not migrate prior to fragmentation [2]. In this contribution, we demonstrate that fucoses attached α1,3 to the Asn-linked HexNAc of glycopeptides obtained from plant lectins also exhibit rearrangement processes preceeding fragmentation.

Methodik

Glycopeptides were obtained by enzymatic proteolysis of galactose-specific lectins from *Trichosanthus anguina* and *Dolichos lablab* as well as from bovine IgG preparations. For *N*-glycopeptide enrichment ZIC-HILIC SPE based separation of the resulting glycosylated analyte species was employed [3]. Structure elucidation of *N*-glycosylated peptides was performed by low-energy CID experiments using a nanoESI Q-Tof MS instrument.

Vorläufige Daten

Glycopeptides obtained by enzymatic digestion of galactose-specific plant lectins were submitted to low-energy CID experiments. Evaluation of the resulting MS/MS spectra revealed the presence of paucimannosidic type *N*-glycans, i.e., the mannosylchitobiosyl core region (Manα1–6(Manα1–3)Manβ1–4GlcNAcβ1–4GlcNAcβ1) is modified by addition of xylose to the β-Man residue and an α1,3 linked fucose to the Asn-linked HexNAc. Fragment ions attributed to losses of either a pentose (xylose) or a desoxyhexose (fucose) or both starting from the intact precursor ions as well as from fragment ions resulting from subsequent cleavage of two hexoses (mannoses) were observed. In contrast, prior to the liberation of the third hexose the pentose had to be cleaved off since no loss with an increment of 162 u starting from $Y_{3\alpha}/Y_{3\beta}$ could be detected indicating the linkage of the xylose to the innermost mannose. Similarly, loss of the proximal *N*-acetylhexosamine (*N*-acetylglucosamine, GlcNAc) leading to Y_0 arises exclusively subsequent to cleavage of the fucose thus proving the expected core fucosylation. However, careful inspection of the MS/MS data revealed the presence of additional fragment ion peaks which are not consistent with the structure described above. Fragment ions of B-type formed by cleavage of the glycosidic bond between the proximal and the distal HexNAc building blocks of the chitobiose unit exhibit a mass shift corresponding to an additional desoxyhexose which can only be explained by a migration of the fucose residue prior to fragmentation. Double cleavages give rise to further fragment ions that point to the same rearrangement process. In contrast, collisional activation of glycopeptide ions of mammalian origin comprising a fucose linked α1,6 to the proximal HexNAc do not show any evidence for migration of the fucose residues. These distinct fragmentation patterns might be used for the discrimination between α1,6- and α1,3-linked fucoses in *N*-glycopeptide ions.

Neuartige Aspekte

Unexpected rearrangement processes of core α1,3-linked fucose in gaseous protonated glycopeptides

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Lipid identification through MS and MS/MS analyses combined with new database search software

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Einleitung

Lipids are a large and diverse group of molecules that include fats, sterols, phospholipids, glycerides and many others. The main biological functions of lipids include energy storage, as structural components of cell membranes, and as important signaling molecules. These findings have led to an increasing interest into lipid analysis at large scale during the past years, also coined ”Lipidomics”. The huge structural variety makes their analysis quite demanding. Today mass spectrometry and liquid chromatography mass spectrometry represent versatile tools in the field of lipid analysis, also offering the possibility for molecular structural identification. One of the most important aspects of mass spectrometric lipid data analysis is the structural identification of lipids using precursor and MS/MS data.

Methodik

Glycerophospholipids standards (Avanti Polar Lipids) from different lipid classes were analyzed. For each lipid mass spectra in positive and negative mode were acquired on FTICR, ion trap, Q-TOF and MALDI-TOF/TOF instruments and raw data exported to the SimLipid software (Premier Biosoft). Individual lipids were identified by a structure database search using the SimLipid software.

Vorläufige Daten

In this study we analyzed a number of standard lipids using MALDI-TOF/TOF, MALDI-FTMS and ESI-QTOF and ESI-iontrap (infusion) techniques. For most analyses positive ionization mode was used, negative mode was successfully applied to the analysis of lipids with an acidic characteristics such as PA, PG and PE.
The lipid identification software used MS and MS/MS spectra containing peak list data for lipid profiling and structural elucidation. The program accepts experimental MS and MS/MS data obtained by mass spectrometry in text formats (e. g. MS Excel, mzData and mzXML) and native file formats from all used mass spectrometers. Lipids were identified by searching precursor molecular weights and MS/MS fragments against known lipid structures available in a database provided by SimLipid and fragment masses calculated based on the lipid structures.
All lipids were unequivocally identified by database searching (typically rank 1 to 3 in the result list) independent of the mass spectrometer used and the ionization technique involved. The lipids were identified within MS/MS error tolerance settings that are adequate for the various mass analyzers (0.005-0.2 Da). The various tested lipid classes and their covalent gross structures, such as the head group type and the fatty acyl chain composition were correctly attributed in the search results in all cases. However, the localization of double bonds by this approach was not possible due to lack of specific fragments; here chemical derivatization remains to be required.
An instrument independent platform for lipid identification was established in this work, which can now be used in lipid characterization or lipidomics by LC-MS/MS, infusion-ESI or TLC-MALDI (thin layer chromatography) workflows.

Neuartige Aspekte

An automated lipid identification platform was validated with various mass spectrometry platforms.

Impact of ESI-MS combined with immunodetection for research on zoonotic pathogens like influenza viruses for discrimination of isomeric receptor gangliosides

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Einleitung

Gangliosides are a variable class of glycosphingolipids (GSLs) composed of a ceramide portion (*N*-acylsphingosine) as lipid anchor, and an oligosaccharide moiety containing one or more sialic acid residues [1]. Gangliosides with terminal Neu5Acα2-3Galβ1-4GlcNAc- or Neu5Acα2-6Galβ1-4GlcNAc-sequences are potential receptors for influenza A viruses [2]. The hemagglutinins of human and avian influenza viruses are supposed to discriminate between these two isomeric gangliosides in the initial binding and infection process. This discriminatory potential is believed to determine at least to some degree the host-specificity of zoonotic influenza viruses. Change in binding specificity may be one reason for crossing the species barrier of zoonotic viruses. The thin-layer chromatography (TLC) overlay technique is an appropriate method to distinguish between these differently sialylated gangliosides using specific antibodies. Mass spectrometry (MS) allows for elucidating the monosaccharide sequence of the glycan core and, in particular, the linkage position of the sialic acid as well as the structure of the ceramide moiety of individual ganglioside species. In the present study we matched three complementary methods comprising (1) TLC separation of ganglioside mixtures, (2) ganglioside detection with sialyloligosaccharide-specific antibodies, and (3) electrospray ionization (ESI) MS analysis of gangliosides after extraction from the silica gel of immunopositive bands.

Methodik

The ganglioside mixture employed was isolated from human granulocytes and purified as described elsewhere [3]. Gangliosides were separated on silica gel precoated high-performance thin-layer chromatography (TLC)-plates. For use in the TLC overlay assays, the silica gel was fixed with polyisobutylmethacrylate (plexigum) and after blocking with bovine serum albumin the primary antibody was allowed to bind. After washing the second antibody conjugated to alkaline phosphatase was applied. Subsequent incubation with 5-bromo-4-chloro-3-indolyl phosphate (BCIP) yielded a blue precipitate. After removal of the plexigum, the silica gel of immunostained bands was scraped off, gangliosides were extracted, and analyzed by use of nanoESI-QTof MS in negative ion mode. More detailed structure information was obtained from low-energy collision induced dissociation (CID) experiments.

Vorläufige Daten

The ganglioside mixture of human granulocytes contains five major GSLs, GM3 as well as α2-3- and α2-6-sialylated gangliosides with nLc4- and nLc6-core structures, which can be easily separated by TLC. The separation is primarily based on the number of sugar units. Due to the substitution of the sphingosine moiety with different fatty acids, mainly C16 and C24, gangliosides containing the same sugar moiety occur as double bands. Furthermore, double bands of two isomeric structures only differing in type of sialylation are completely separated by TLC as well, i.e., IV^3nLc4Cer (Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1Cer) and IV^6nLc4Cer (Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1Cer). TLC overlay assays of the ganglioside mixture were performed with anti-Neu5Acα2-3Galβ1-4GlcNAc-R and anti-Neu5Acα2-6Galβ1-4GlcNAc-R antibodies. Gangliosides with nLc4- and nLc6-core structure exhibiting Neu5Ac in either α2-3- or α2-6-linkage were distinguished unambiguously. For detailed characterization and verification of the proposed structures, the silica gel of immunostained bands was scraped off and GSLs were extracted from the silica gel prior to nanoESI-QTof MS analysis. In the negative ion mode, all molecular species were detected as deprotonated singly charged ions and structures of the major components were assigned to monosialylated nLc4Cer. In CID-experiments of the precursor ions with *m/z* 1626.99 and 1627.04, full sets of Y- and C-ions and some B- and Z-ions were detected confirming the proposed structures of terminally sialylated gangliosides with nLc4-cores. Moreover, $^{0,2}X_4$-ions of *m/z* 1405.95 and $^{0,4}A_2$- and $^{2,4}A_3$-ions eliminating CO_2 of *m/z* 306.15 and 468.16, respectively, were detected. These ions are diagnostic for α2-6-linked sialic acid [1, 4] and allow for discrimination between gangliosides with terminal Neu5Acα2-3Galβ1-4GlcNAc- or Neu5Acα2-6Galβ1-4GlcNAc-structures, which each may act as specific receptors for certain types of zoonotic avian and/or human influenza A viruses.

Neuartige Aspekte

The combined method offers a promising tool for screening of specific ganglioside receptors of zoonotic influenza viruses.

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Herstellung eines C13 markierten internen Standard für quantitative Glykomik Messungen

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Einleitung

Im Bereich der Glykananalytik sind aufwendige Probenvorbereitungen notwendig um die gewünschten Zuckerstrukturen aus komplexen Proben zu extrahieren. Die Rückführbarkeit der Daten ist daher besonders wichtig. Im Gegenteil zu Proteinanalysen im Bereich Proteomik, gibt es in der Glykomik wenige verlässliche Quantifizierungsstrategien und Standards, da dieser Forschungsbereich noch relativ jung ist [1]. Wir haben deshalb versucht einen C13 markierten internen Standard aus Hefezellwand zu extrahieren um zu untersuchen, ob sich dieser als isotopenmarkierter Referenzstandard für Glykomik Anwendungen eignet. Dieser Ansatz ist besonders interessant, da die isotopenmarkierte Hefezellwand aus *Pichia pastoris* Fermentationen, basierend auf C13 markierter Glukose, stammt und daher eine billige Alternative zu anderen Standardprodukten darstellt [2].

Methodik

Es wurde versucht ein homogenisiertes C13 markiertes Hefezellwandmaterial aus *Pichia pastoris* zu gewinnen und dieses mit verschiedenen massenspektrometrischen Techniken auf dessen Anwendungsmöglichkeiten zu untersuchen. Aufgrund der hohen Stabilität der Hefezellwand, ist eine geeignete Extraktionsmethoden unumgänglich. Nach dem Homogenisationsschritt der Hefezellwand und Herstellung eines Hefezellwandpulvers, wurden die Lipide entfernt und die Proteine mittels Acetonpräzipitation ausgefällt. Danach folgte ein enzymatischer PNGase F Verdau für die N-Glykane, während die O-Glykcane mittels Beta-Elimination, einer chemischer Hydrolyse, extrahiert wurden. Aufgrund der Probenkomplexität wurde Permethylierung als Derivatisierungs-und Aufreinigungsschritt der massenspektrometrischen Detektion vorgeschaltet. Die isotopenmarkierten Hefezellwandproben wurden mittels MALDI-TOF-MS und direct infusion Orbitrap-FTMS untersucht. Der Markierungsgrad wurde bestimmt und zur weiteren Materialcharakterisierung wurden Maltosestandards in verschiedenen Größen (Malt4-Malt7) und Konzentrationen zur Hefezellwand zugegeben.

Vorläufige Daten

In dieser Arbeit geht es um die Untersuchung der Möglichkeit einen internen Standard aus C13 isotopenmarkierter *Pichia pastoris* Hefezellwand herzustellen. Es wurde hierzu versucht N- und O-Glykane aus der Hefezellwand zu extrahieren. Das Probenscreening mit der Probenmatrix DHB und Positiv-Ionen Reflektron MALDI-TOF-MS zeigte, dass C13 markierte N- und O-Glykane in der Probe vorhanden sind. Experimente mit MALDI- und Orbitrap-MS zeigten einen Markierungsgrad von 99.3% und lieferten somit den Beweis, dass die Glykane unter die angewendeten Fermentationsbedingungen vollständig markiert wurden. N-Glykane konnten nicht reproduzierbar aus den Hefezellwänden extrahiert werden, daher ist eine weitere Extraktionsoptimierung für diese Strukturen notwendig. Da die Probenvorbereitung von O-Glykanen ein reproduzierbares Größenmuster von Hex_2-Hex_9 lieferte, kann dieses Muster für die Quantifizierung von Glykanen in verschiedenen Massenbereichen eingesetzt werden. Gleichzeitig konnte mit der Reproduzierbarkeit dieser Daten die generelle Homogenität des Materials nachgewiesen werden. Zur weiteren Charakterisierung des isotopenmarkierten Hefezellwandmaterials wurden verschiedene Konzentrationen von kommerziell erhältlichen Maltosestandards (Malt4-Malt7) zugegeben und Direktanalyse mit Orbitrap-FTMS Detektion durchgeführt. Die Wiederfindungsraten zeigten, dass eine präzise Quantifizierung von 10-100 nmol absolut unter diesen Bedingungen möglich ist, aber ein interner Standard unbedingt notwendig ist um die Probenvorbereitungsverluste und die instrumentellen Unsicherheiten auszugleichen. Zusammenfassend zeigten diese Quantifizierungsexperimente die potentielle Anwendbarkeit des Hefezellwandmaterials und die Vorteile der Einsetzung von internen Standards für die Quantifizierung von biologischen Proben.

Neuartige Aspekte

Erstmals wurde versucht einen C13-isotopenmarkierten internen Standard aus Hefezellwand zu extrahieren und ihn auf seine Anwendbarkeit für Glykomik zu prüfen

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Glycosphingolipid receptors of Shiga toxin 2e: structures and membrane microdomain association in porcine brain endothelial cells elucidated by TLC/ESI MS

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Einleitung

Shiga toxin (Stx)-producing *Escherichia coli* (STEC) elicit vascular damage diseases in humans and animals [1]. Pigs are known to develop edema disease through STEC producing Stx2e, which represents the most frequent Stx2 subtype in porcine feces. Although Stx2e-mediated brain vascular injury is the key event in development of neurologic signs, the glycosphingolipid (GSL) receptors of Stx2e of pig brain endothelial cells (PBCECs) and their molecular organization in the plasma membrane have not been investigated so far. Here we report on the structural characterization of Stx receptors from PBCECs and their localization in *lipid rafts* isolated as detergent-resistant membranes (DRMs) using the thin-layer chromatography (TLC) overlay assay combined with ESI MS.

Methodik

PBCECs from the brains of ~6-month-old pigs were grown until confluence and DRMs were prepared by sucrose gradient centrifugation [2]. Lipids were extracted from total cells and DRMs, respectively, with chloroform/methanol; co-extracted phospholipids and triglycerides were saponified by mild alkaline treatment. Stx2e receptors of PBCECs were identified by TLC overlay assays using Stx2e-containing bacterial culture supernatants and polyclonal antibodies against Stx2e-receptors globotriaosylceramide (Gb3Cer) and globotetraosylceramide (Gb4Cer) [3]. Structural characterization of Stx2e receptors by mass spectrometry was performed with a Q-TOF instrument equipped with a nanoelectrospray manipulator. GSL samples were dissolved in MeOH, 2% formic acid and analyzed in the positive ion mode. Collision-induced dissociation (CID) was performed with argon as collision gas [4, 5].

Vorläufige Daten

The orcinol stain of the TLC-separated neutral GSLs of PBCECs along with well characterized GSL reference mixtures suggested the presence of Gb3Cer and Gb4Cer. TLC overlay assays performed with specific anti-Gb3Cer and anti-Gb4Cer antibodies revealed the expression of Gb3Cer and Gb4Cer in total lipid extracts as well as in DRM fractions prepared from PBCECs, respectively. In order to unravel preferential binding of Stx2e toward PBCEC-derived Gb3Cer or Gb4Cer species, TLC overlay assays were performed with Stx2e derived from a human (Stx2e (h)) and a pig (Stx2e (p)) STEC isolate. In comparison to the orcinol stain, the TLC overlay assays using Stx2e (h) and Stx2e (p) clearly indicated binding of both Stx2e-variants to Gb3Cer and Gb4Cer, whereby a remarkably stronger interaction to Gb4Cer than to Gb3Cer could be observed for both Stx2e-variants.

For mass spectrometric identification of Gb3Cer and Gb4Cer lipoforms the silica gel of immunopositive bands derived from total lipid extracts and DRM fractions was scraped off the plate and the GSLs eluted from silica gel were submitted to MS^1 analysis followed by CID of selected precursor ions. The overview MS^1 spectra of Gb3Cer- and Gb4Cer-immunopositive bands obtained from total cells as well as DRM fractions revealed a huge diversity of Gb3Cer and Gb4Cer species and suggest considerable ceramide variability. This holds true especially for Gb3Cer where variants with an exceptional high degree of hydroxylation of ceramide were observed as ions of highest abundance. The tentatively assigned structures were further verified by low-energy CID.

Neuartige Aspekte

Identification and structural characterization of *lipid raft*-associated Stx2e receptors in porcine brain capillary endothelial cells

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A simple MALDI MS-based Method to detect Plasmalogens in complex Lipid Mixtures - The Use of 2,4-Dinitrophenylhydrazine as a reactive Matrix

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Einleitung

There is increasing evidence that MALDI MS is a useful method of lipid research [1]: MALDI MS is fast, sensitive, tolerates sample impurities to a considerable extent and provides simple mass spectra because singly charged ions are nearly exclusively detectable. In particular, the considerable robustness of the MALDI ionization process paves the way for applying selected auxiliary agents, for instance, salts such as CsCl that help to overcome assignment problems of different lipid species (with varying fatty acyl residues) in complex lipid mixtures [2]. 2,4-dinitrophenylhydrazine (DNPH) is a classical derivatization agent and a "reactive" matrix that helps to convert aldehydes (and with a lower efficiency also ketones) into derivatization products that are stable under high vacuum conditions. We will demonstrate that it is a very useful and simple approach to monitor the presence of plasmalogens (alkenyl-ether phosholipids) in cellular extracts without the need of using highly sophisticated MS equipment. It will also be shown that DNPH is a useful agent to derivatize selected oxidation products of lipids that are generated by the cleavage of the olefinic residues.

Methodik

All chemicals, solvents, the applied MALDI matrices (9-aminoacridine (9-AA) and 2,5-dihydroxybenzoic acid (DHB)) and a 0.2 M DNPH solution (in phosphoric acid) were obtained from Sigma-Aldrich. Phospholipid standards were from AVANTI Polar Lipids and used as supplied. TLC/MALDI analysis was performed as recently described [3, 4].
DHB was either used as 0.5 M solution in methanol or as a solution of 100 mg/ml in acetonitrile/water (1:1, v/v), while 9-AA was used in a concentration of 10 mg/ml in isopropanol/acetonitrile (60/40, v/v)) [5]. Lipid extracts from spermatozoa were obtained according to the Bligh & Dyer method and the spectra recorded as previously described [1]. Derivatization with DNPH was performed either in solution or directly on a TLC plate.

Vorläufige Daten

Some cells (e.g. ruminantia spermatozoa) and tissues (e.g. brain and heart) contain in addition to common diacyl (phospho)lipids also significant amounts of ether lipids. Alkenyl-ether lipids (plasmalogens) are of particular interest because they are considered as natural antioxidants because the alkenyl-ether linkage has a much higher reactivity with reactive oxygen species (ROS) than the olefinic residues within the fatty acyl chains. In combination with a complex fatty acyl pattern, the unequivocal identification of plasmalogens is a challenging task - in particular if only a simple TOF device without MS/MS capacity is available. Fortunately, plasmalogens can be easily identified by simple chemical derivatization and without the need of sophisticated MS equipment: plasmalogens are extremely sensitive against traces of acids that convert the plasmalogen into a lyso lipid (lacking the ether residue) and the corresponding aldehyde. The lyso lipid can be easily identified while the simultaneously generated aldehyde is not detectable under conditions of high vacuum. We will show that the derivatization of the generated aldehydes by DNPH is a convenient method to overcome this problem. The derivatization products are best detected as negative ions and this helps to minimize interferences with bulk phospholipids such as PC. Samples of boar spermatozoa will be used to illustrate the power of this derivatization. Of course, the DNPH derivatization may be also performed directly on a TLC plate and gives - subsequent to MALDI MS - direct information about the contribution of plasmalogens to the individual lipid classes. Finally, it will be shown that the applied highly acidic conditions are very helpful to overcome overlap problems between different adducts and lipids with different fatty acyl compositions. It is concluded that this is a very simple and convenient approach that can be used on all MALDI mass spectrometers.

Neuartige Aspekte

A simple method of plasmalogen analysis was developed and applied to a physiologically-relevant sample.

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Quantification of Free Fatty Acids by Ultraviolet Laser Desorption Ionization Mass Spectrometry Utilizing Drosophila Wings as Sample Substrates

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Einleitung

GC-MS is the common method for identification and quantification of free fatty acids (FA). However, to increase their volatility, esterification is required. Underivatized FAs can be analyzed using ESI and MALDI mass spectrometry, but not without pitfalls. For example, solvent systems have to be compatible and quantification is not straightforwardly obtained over a wide concentration range. Abundant matrix-derived background ion signals can furthermore hamper the MALDI-MS analysis.
Recently, we demonstrated that *Drosophila* wings can serve as an alternative sample substrate for UV-LDI-MS of a variety of lipids, fatty acids, fatty acid methyl esters, and triglycerides [1]. Mass spectra obtained this way are characterized by a low background with only a few signals resulting from endogenous compounds and by the preferential detection of lipid compounds if crude mixtures are applied to the wings. Here, we show that the method also provides excellent quantitative results for both saturated and unsaturated fatty acids over 3 orders of magnitude. Second, we introduce a protocol that allows easy switching from one adduct ion system (e.g., complexation with potassium) to another (e.g., sodium). Finally, optimized protocols were applied to the analysis of crude extracts of liver tissue containing a variety of different FAs - from 16:0 to 22:6.

Methodik

Wings were dissected from dead *D. melanogaster* flies and fixed to a MALDI sample plate using adhesive tabs. Saturated and unsaturated FA reference compounds were from Sigma-Aldrich and dissolved in chloroform or chloroform/methanol mixtures. 0.2 µl of sample solution were applied per wing. Spectra were acquired in the positive ion mode on an orthogonal-TOF mass spectrometer (QSTAR pulsar i, AB Sciex) with the oMaldi2 ion source operated at 2 mbar of N_2.The fine vacuum ion source increases the vacuum stability and produces particular cold ions by collisional cooling. Samples were irradiated with the uniform beam (Φ_{focal}=200 µm) of a nitrogen laser (λ=337 nm, τ=3 ns), operated at 30 Hz. 1800 laser pulses were applied to acquire one mass spectrum.

Vorläufige Daten

All quantitative experiments were conducted in the positive-ion mode because this provided the highest sensitivity in the low ten pmol range of spotted FAs. If untreated wings were used as sample substrates, FAs are predominantly detected as $[FA+K]^+$ species, most probably because of the high concentration of potassium in the wing. Switching to other alkali adduct ions can almost quantitatively be achieved by washing the wings with sodium- or lithium-bases followed by washing with organic solvent. Because of the higher stability of the sodium adducts this feature will be most helpful in tandem MS analyses. Saturated FAs were diluted in chloroform and mixed with an internal standard (C15:0) before preparation on an untreated wing. Using the signal of the internal standard as reference, quantification of saturated FAs is possible with an excellent regression coefficient R^2>0.99 over three decades and moreover produces the same ion response / slope also for FA species of different chain lengths (C16-C20). Moreover, the presence of various saturated/unsaturated FAs in the mixture (up to: 6 were tested) does not seem to deteriorate the quantification (i.e., there is no obvious ion suppression effect). Quantification of unsaturated fatty acids necessitated a change of solvent system to a mixture of methanol and chloroform. With such solvent systems, similarly high regression coefficients of R^2>0.99 were obtained for these species over three decades. Clean mass spectra dominated by FA ion signals are also produced when crude sample extracts are applied, e.g., obtained from liver tissue. Protocols allowing accurate quantification of highly unsaturated FAs are currently developed. However, it still remains to be determined if FAs of different polarities, i.e., with strongly different degrees of unsaturation, can simultaneously be detected quantitatively. If successful, this could be regarded as a real milestone in lipid analysis because GC/MS is much more time-consuming and laborious.

Neuartige Aspekte

A new UV-LDI-MS-based method for rapid and accurate quantification of free fatty acids is presented.

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Fully automated chip-nanoelectrospray combined with collision induced and electron transfer dissociation for rapid diagnosis of Fabry disease

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Einleitung

Fabry disease, a lysosomal storage disorder (LSD), is characterized by the absence or reduction of α-galactosidase A activity with accumulation of globotrioasylceramide especially in blood vessels. Early diagnostic is crucial to initiate the treatment formula and to prevent the major deterioration of the tissues. In recent years, mass spectrometry (MS) based on either electrospray ionization (ESI) or matrix-assisted laser desorption/ionization (MALDI) was demonstrated as one of the most proficient analytical tools for studies targeting LSDs. For the detection of α-galactosidase activity from dried blood spots (DBS) in Fabry patients *vs.* healthy controls, novel diagnostic methods based on a chemical substrate and quantification of the reaction mixture by MS were developed [1,2]. Although capable to provide early detection of the disease, occasionally these approaches reported false-negative results because of the limited reproducibility, reduced sensitivity and throughput of the MS experiments as well as the carry-over from sample to sample, which might occur during successive ESI infusions. To eliminate these drawbacks of DBS-MS methods, we propose here a novel platform for rapid and reliable diagnostic of Fabry disease, based on enzyme assay and fully automated chip-nanoESI MS, collision induced dissociation (CID) and electron transfer dissociation (ETD) MS/MS.

Methodik

The general workflow encompassed: 1) α-galactosidase extraction from DBS of the Fabry patient and the healthy donor; 2) enzymatic cleavage of a substrate GLA-S from a cocktail containing GLA-S and an internal standard, GLA-IS; 3) high throughput MS identification of GLA-S, GLA-IS, GLA-P, the product of GLA-S cleavage, and α-galactosidase corresponding ions; 4) GLA-P confirmation by CID and ETD MS^2. Fully automated chip-based (+)nanoESI MS was performed on a high capacity ion trap (HCT) ultra PTM (Bruker Daltonik, Bremen) coupled to a NanoMate 400 robot (Advion BioSciences, Ithaca). CID was carried out at variable RF amplitude using He as the collision gas. ETD was performed using fluoranthene as the anionic reagent and methane as the supporting gas for anion production.

Vorläufige Daten

The procedure described above was applied to 11 healthy donors and 11 Fabry patients, screened in high throughput mode. Since the results were similar we have chosen to present one example. In the case of the healthy donor, the signal corresponding to GLA-P ions, exhibited an absolute intensity of 1.3×10^6 counts/s, while the signal afferent to the GLA-IS had an absolute intensity of 0.5×10^5 counts/s. As a result, GLA-P/GLA-IS ratio was 26. Contrastingly, MS^1 of DBS reaction products from Fabry patient, measured under the same conditions, indicated a low intensity signal of 0.4×10^6 counts/sec for GLA-P and a GLA-P/GLA-IS ratio of 2. This significant difference found in GLA-P/GLA-IS demonstrated that the alteration of the activity and/or expression of α-galactosidase in Fabry subjects may be reliably monitored by chip-based nanoESI MS. Moreover, in the case of the healthy subject, a series of signals typical for a protein was observed. 46205.5 Da calculated molecular weight (MW) was found in agreement with the reported MW (46 kDa) of mature lysosomal form of α-galactosidase. However, no signals corresponding to α-galactosidase were detected in the case of the patient, an indication that the enzyme is either absent or exists in a quantity below even the detection limit of NanoMate HCT MS. In the last stage of analysis GLA-P structure was confirmed by CID and ETD used complementarily. This first employment of ETD in rapid diagnosis of a LSD was possible due to the ability of chip-nanoESI to form multiply charged cations. Exhibiting three -NH-CO- bonds, GLA-P compound was an ideal candidate for ETD fragmentation to substantiate -NH-CO- linkages. Observed c_1, z_1 and c_2 ions corroborated for the structure of GLA-P and, together with the information obtained by CID, contributed to the complete characterization of the enzymatic assay product.

Neuartige Aspekte

A novel method based on NanoMate-HCT MS, CID and ETD MS/MS was introduced for rapid diagnosis of Fabry disease.

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Introducing Orbitrap mass spectrometry for ganglioside biomarker discovery in human brain cancer: application to astrocytoma

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Einleitung

Astrocytomas (AcTs), a group of central nervous system neoplasms are characterized by a predominant cell type derived from an immortalized astrocyte [1]. As a group of severe diseases, AcTs are usually detected by imaging methods, frequently at a late stage wherefrom their poor prognosis. The only practical alternative is the early detection, at a resectable stage, which should be based on biomarker discovery before clinical symptoms arise. Therefore, currently, the research is focused on the development of efficient analytical methods able to discover AcT molecular fingerprints.
So far, the modern advances in proteomics and mass spectrometry (MS) have leaded to the detection of protein markers of high usefulness in AcT early diagnosis [2]. Gangliosides, another class of brain molecular markers, are known to play an important role in brain tumor development, progression and treatment. Therefore, in the past decade MS was introduced in brain ganglioside research and emerged as a reliable, versatile and sensitive method for the discovery of species exhibiting a biomarker role [3].
In this study, Orbitrap MS is for the first time introduced in human brain ganglioside research in general and for the investigation of ganglioside expression and structure in a human AcT specimen in particular.

Methodik

Crude ganglioside mixtures from AcT, its surrounding tissue (ST) and a normal brain tissue (NT) used as a control were extracted using the protocol described previously [3]. MS was conducted on an Orbitrap Velos Pro instrument (Thermo Scientific, Bremen, Germany) tuned in negative ion mode. The instrument was equipped with a nano source for electrospray and two detectors, an independent linear ion trap and an Orbitrap detector, producing a high signal-to-noise ratio.
Collision-induced dissociation (CID) was employed for multistage MS (MS^n) experiments using helium as the collision gas. For MS^n sequencing, the precursor ions were selected within an isolation width of 1u. Fragmentation involved normalized collision energy of 50 and an activation q value of 0.225.

Vorläufige Daten

A total number of 37 different ganglioside species in AcT, 40 in ST and 56 in NT were attributable to the signals detected by MS screening. While AcT and ST were found to contain no less than 18 identical species, NT exhibited only 1 common structure with ST and 2 with AcT. Regarding the sialylation status, while in AcT only 5 monosialotetraoses (GM) species were identified, a number of 18 GM structures were found in NT. Unlike NT, AcT is basically dominated by species with high sialylation content: 14 disialylated (GD), 11 trisialylated (GT), 6 tetrasialylated (GQ) and one pentasialylated (GP) glycoforms were identified. On the other hand, as compared with ST, both AcT and NT showed a lower overall sialylation profile. According to previous studies, in the case of astrocytoma, the hypersialylation might confer a growth advantage to AcT cells. From this perspective, our findings corroborate for AcT cell invasion in the investigated surrounding tissue.
As the MS mapping indicated that AcT, ST and NT share one doubly deprotonated molecule at *m/z* 1063.31, attributable to GT1(d18:1/18:0), this particular ion detected in AcT ganglioside extract was chosen for detailed carbohydrate sequence analysis. Optimized CID MS^2-MS^4 provided straightforward data supporting for the first time the presence of GT1c isomer in an astrocytoma tissue.
These first results obtained by Orbitrap MS and multistage CID MS lead to biological and clinical implications that are able to open new perspectives in the field of brain tumor gangliosides. In view of the technical performances in terms of resolution and sensitivity as well as the generation of highly accurate data with biological impact, we believe that Orbitrap MS has a remarkable potential to be introduced in brain cancer research as a routine method for discovery of tumor-associated markers or specific therapeutic targets.

Neuartige Aspekte

High resolution mass spectrometry on Orbitrap instrument is for the first time introduced in human brain ganglioside research.

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Chip-nanoelectrospray quadrupole time-of-flight tandem mass spectrometry of human meningioma gangliosides

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Einleitung

Meningiomas are a group of tumors of the meninges, the membranes surrounding the brain and spinal cord. Meningiomas occur from the arachnoid cap cells, which enclose and adhere to the dura mater. In the investigation and treatment of human brain tumors, nowadays a massive effort is oriented towards the discovery of molecular markers, which allow early tumor detection and development of novel and more efficient therapeutic schemes. A group of such bioindicators and potential tumor specific targets is represented by gangliosides, a class of complex glycosphingolipids containing sialic acid, highly expressed in the cells of the nervous system. In the last years, our group was intensively involved in development of efficient mass spectrometric (MS)-based approaches for ganglioside biomarker discovery in defined regions of healthy human brain and brain tumors such as gliosarcoma, hemangioma and brain metastasis of lung adenocarcinoma [1-3]. In this paper we report on the first application of an advanced MS system encompassing a high resolution instrument coupled with fully automated chip-based nanoelectrospray for compositional and structural analysis of gangliosides expressed in human brain meningeoma. High performance thin layer chromatography (HPTLC) and laser densitometry were complementarily used for quantification.

Methodik

Meningeoma gangliosides were extracted and purified following the procedure described by us previously [1-3]. HPTLC was performed on glass backed HPTLC-plates (Merck, Germany). HPTLC separated fractions were subjected to laser densitometric scanning (LKB 2202 Laser Ultrascan, LKB, Bromma, Sweden) at 580 nm.
MS and MS/MS experiments were conducted on an orthogonal quadrupole time-of-flight instrument (QTOF Micro, Waters, Manchester, U.K.) operating in the negative ion mode. QTOF MS was coupled to a NanoMate 400 robot incorporating nanoelectrospray Chip technology (Advion BioSciences, Ithaca, USA). MS/MS was performed by collision-induced dissociation (CID) at low energies using argon as the collision gas. The mass deviation did not exceed 40 ppm, which is in the normal range for this type of instrument.

Vorläufige Daten

In this work, a detailed investigation of ganglioside composition followed by fragmentation analysis of individual species in a human adult meningioma specimen was carried out using an advanced mass spectrometric method based on nanoESIchip QTOF MS and CID MS/MS developed in our laboratory.
HPTLC and densitometric analyses were complementarily used for ganglioside fraction profiling and quantitative evaluation of their proportions in meningioma. HPTLC and densitometry indicated that the amount of gangliosides in this tumor is approximately 20 times lower than in the normal adult cerebellar tissue.
MS of ganglioside mixture extracted from meningioma evidenced a rather rich molecular ion pattern, demonstrating not only the presence of a high number of glycoforms but also an unexpected diversity and several less common ceramides for certain components. 34 distinct glycosphingolipid components of which five asialo, one GM4, nine GM3, two GM2, two GD3, nine GM1 and six GD1 differing in their ceramide compositions were identified. According to *m/z* values, all ganglioside species present long-chain bases (LCBs) with 18 carbon atoms, while the lengths of the fatty acid vary from C11 to C25. In agreement with HPTLC assessment, several signals such as those at *m/z* 1149.99, 1233.93 and 1261.91, which are among the most intense in the spectrum, belong to GM3 group. Nine distinct ions were attributable to GM3 variants; six of these glycoforms bear dihydroxy and three trihydroxy saturated sphingoid bases and C16, C18, C22 and C24 fatty acid chains in their Cer residues.
Simultaneous fragmentation of meningioma-associated GM1 (d18:1/24:1) and GM1 (d18:1/24:0) by tandem MS using CID confirmed the postulated structures of the ceramide moieties and provided data on the glycan core, which document that for each of the GM1 (d18:1/24:1) and GM1 (d18:1/24:0) forms both GM1a and GM1b isomers are expressed in the investigated meningioma tissue.

Neuartige Aspekte

Chip-nanoESI QTOF MS and CID MS/MS method was for the first time applied for structural characterization of human meningioma gangliosides.

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Strategies of immobilization of Metal-based Nucleic Acid and quantification by ICP-MS

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Einleitung

Our ambition is the development of different strategies for detecting DNA in a very low concentration using a metal-coded affinity tag technique (MeCAT). The method is based on a chemical labeling, but instead of isotopes, different lanthanide ions in macrocyclic complexes are used [1]. For the quantitative detection of target DNA, inductively coupled plasma MS (ICP-MS), a technique that allows detection of metals with high precision and low detection limits, is used.

Methodik

After labeling of thiol-functionalized ssDNA probes with lanthanide-chelate-complex, the labeled probes are purified by HPLC. The labeling reactions are followed by HPLC-FT-ICR-MS and the amounts of labeled ssDNA probes are determined by ICP-MS. After labeling, target DNA is captured via hybridization with biotinylated ssDNA probes and MeCAT-labeled-ssDNA probes onto solid support. After enrichment the dsDNA is eluted by an enzymatic reaction from the solid support [2, 3]. Therefore, a number of different enzymes were tested to determine their effect on elution efficiency. The concentration of the target DNA is determined via measurement of the metal amount and internal standard calibration by ICP - MS.

Vorläufige Daten

The capture of target DNA by different modified ssDNA probes and enrichment of hybrid-DNA-MeCAT-complex offers a sensitive and absolute quantification via inductively coupled plasma MS. The use of different types of enzymes to elute the DNA complex from the support allows a wide application range.

Neuartige Aspekte

We show here a new sensitive method to detect target DNA using MeCAT-tag

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Peptide-Based Multi-Metal Tag for Biomolecule Labeling

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Einleitung

The labeling of biomolecules with lanthanide ions by means of metal-chelating ligands allows their quantification via inductively coupled plasma mass spectrometry (ICP-MS). The sensitivity of the method can be significantly improved with tags containing more than one metal atom, as previously shown, e.g. with polymeric tags [1].
Here we report first attempts to utilize solid phase peptide synthesis (SPPS) to produce short peptide-based multi-metal tags. The performance of these multi-metal tags was initially evaluated by labeling of thiol-modified oligonucleotide probes and their application in ICP-MS-based DNA-quantification assay.

Methodik

The chelator 1,4,7,10-tetraazacyclododecane N,N´,N´´,N´´´-tetra acetic acid (DOTA) was purchased as DOTA-NHS ester and attached to the free side chain amino group of Fmoc-Lysin-OH. Subsequently, the Fmoc-Lysin(DOTA)-OH was metalated with terbium.
Fmoc-Lysin-(DOTATb)-OH was used as building block in SPPS. Following standard Fmoc-protocol several short peptides were synthesized containing Glycin as spacer between the Lysin-(DOTATb) residues. Maleimide hexanoic acid was coupled to the N-terminus, thus providing a reactive moiety to attach the multi-metal-tag to thiol-bearing biomolecules. Finally, peptides were cleaved from the resin with 90% TFA.
The multi-metal peptide tags were first applied to label thiol-modified oligonucleotides. The peptide-oligonucleotide conjugates were put to test as reporter probes in ICP-MS based sequence specific nucleic acid quantification assay (for details see accompanying poster).

Vorläufige Daten

Different strategies for solid phase synthesis of DOTA-peptide conjugates were reviewed by Z. Kovacs and co-workers [2]. In contrast to the protocols mentioned there, we used an already metalated DOTA-amino acid derivative as building block. We synthesized short peptides harboring 2,3 and 4 lanthanide atoms. The metal chelate seems to be sufficiently stable under SPPS conditions, even during the final cleavage step with 90% TFA. Loss of metal was observed to a small extent of about 1% per incorporated Lysin-(DOTATb). However, this might become one of the limiting factors for the synthesis of peptides with higher metal content.
The multi-metal tags were successfully applied to label thiol-modified oligonucleotides in solution. First trials with two-fold labeled reporter probes in nucleic acid quantification assay showed the expected two-fold signal increase in ICP-MS.
The most interesting result is the stability of the DOTA-lanthanide complex under (harsh) organic synthesis conditions. At least when working with stable isotopes, the strategy to first metalate the DOTA and to employ the metal coordination to protect the carboxyl groups might offer an alternative to the established method, that is to use tert-butyl-protected DOTA-derivatives and to metalate as final step. Furthermore, our approach provides the possibility to incorporate different metals site-specific into one peptide sequence.

Neuartige Aspekte

metalated amino acid as building block in solid phase peptide synthesis
short peptide-based multi-metal tag for signal amplification in ICP-MS

Referenzen

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Sequence Specific DNA-Quantification via ICP-MS using Lanthanide-Labeled Probes and Ligase-Based Signal Amplification

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Einleitung

Here we describe an approach for sequence specific quantification of nucleic acids based on metal-labeled probes and analysis via ICP-MS. The sequence specificity is obtained by hybridization of the target DNA with complementary artificial oligonucleotide probes. Two types of probes are used: lanthanide-labeled reporter probes and biotinylated capture probes. To enhance the assays sensitivity we employed signal amplification with ligase detection reaction.

Methodik

Derivatives of the chelator 1,4,7,10-tetraazacyclododecane N,N´,N´´,N´´´-tetra acetic acid (DOTA) were used to label the thiol-endmodified reporter probes with lanthanide atoms.
Hybrids of target DNA, reporter probe and capture probe were formed in solution and subsequently immobilized on streptavidin coated well-plates. After removal of unbound reporter probes the captured hybrids were eluted. Lanthanide contents were analyzed via ICP-MS (Element XR, Thermo scientific) equipped with a concentric nebulizer and a cyclonic spray chamber.
In addition to this basic assay, we applied linear ligase reaction as an preceding amplification step [1]. The 9°N-DNA-Ligase catalyzes the formation of a phosphodiester bond between biotinylated probe and metalated probe when adjacent on the target. Repeated thermal cycling results in an linear increase of ligation product.

Vorläufige Daten

The nucleic acid quantification with metal-labeled probes works for our simplified model systems. In basic assay, formation, capture and release of the target-probe-hybrids is quantitative with single stranded target, thus, the measured amount of lanthanide is equal to the target-DNA input. The basic assays range is 10 - 1000 fmol single stranded target.
Zhang et al., simultaneously working on the same field, showed with their most recently published proof-of-concept study the multiplexing strength of a similar approach [2]. They utilized capture probe functionalized magnetic microparticles and achieved detection limits of 500 - 2000 fmol single stranded target DNA.
Our method is inferior in terms of multiplexing, but one step ahead in terms of sensitivity, especially with the enzyme-based signal amplification. Preceded linear ligase reaction resulted in an 18-fold signal increase after 60 cycles. And in contrast to the basic assay it works equally well with single and double stranded target. Disadvantageous is the narrow range: 0.5 - 10 fmol. The upper limit is always given by the streptavidin wells biotin capacity and could be overcome by using another solid support for capture.
In our further experiments we will try to employ several means of signal and target amplification, because an marked increase of sensitivity is needed to meet the requirements of real DNA-quantification tasks.

Neuartige Aspekte

heterogeneous assay for sequence specific nucleic acid quantification using lanthanide-labeled probes, streptavidin-biotin affinity, ligase-based signal amplification and ICP-MS detection

Referenzen

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Unravelling the Interaction between Gadolinium and Transferrin by Mass Spectrometric Analysis

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Einleitung

Gadolinium (Gd) is used as contrast agent during magnetic resonance imaging (MRI) since 1988. Because free Gd^{3+} is highly toxic, it is chelated by polyaminocarboxylic acids. The coordination avoids the release of ionic Gd into the blood during MRI. Nevertheless, release induced by transmetallation, especially by free iron (Fe^{3+}), is well possible. In this case, coordination and distribution of Gd^{3+} by Transferrin (Tf) might occur.
Transferrin is the iron transporting protein in the human organism. It contains two metal ion binding sites, whereas each site generally coordinates tightly, but reversibly one Fe^{3+}. Under physiological conditions, iron only occupies ~ 25% of the binding sites, thus allowing the coordination of other metal ions present in the blood.

Methodik

Monitoring the interaction of human serum transferrin with Gd^{3+} can be performed by means of molecular and atomic mass spectrometry (MS) methods. In the first part of the work, the interaction was monitored by means of reversed-phase liquid chromatography (LC) coupled to electrospray ionization-time of flight-mass spectrometry (ESI-ToF-MS). Validation of the obtained data was performed by polyacrylamide gel electrophoresis (PAGE) in the second part. The use of laser ablation (LA) hyphenated to inductively coupled plasma-mass spectrometry (ICP-MS) represents a sensitive and selective method for the detection of protein-bound metals inside the focused protein bands in the dried gel.

Vorläufige Daten

Using LC hyphenated to ESI-MS, identification of different Tf isoforms was carried out. Considering the critical pH dependence of the protein-metal-complex a sensitive LC method was developed for the metalloprotein. Kind and content of the coordinated metal was readily determined based on the shift in molecular mass. This way, the uptake of one Gd^{3+} by Tf was detected. Protein species containing Fe^{3+} and Gd^{3+} simultaneously were not identified. Validation of the data was achieved by PAGE. After focusing the protein bands, the gels were dried without any staining process. Afterwards, the gels were ablated by LA and transferred to ICP-MS achieving time-resolved line spectra. Data processing was done by home-made software to detect the elemental distribution of Gd in the ablated area of the gel. Based on the intensity distribution of Gd in the generated image, the protein band was visualized and the coordination of Gd by Tf was demonstrated.

Neuartige Aspekte

Interaction of Transferrin and Gadolinium monitored by means of PAGE and LA-ICP-MS

Studying the intracellular chemistry of cisplatin by elemental speciation analysis

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Einleitung

Since the discovery of its anticancer properties more than three decades ago, cisplatin is still in the first line to fight cancer.[1],[2],[3],[4] The majority of the knowledge on the intracellular chemistry of the drug is based on assumptions, deduced from in vitro solution chemistry. It is safe to assume that cisplatin being an electrophile will form adducts with thiol- containing biomolecules once inside the cell. In this work, the use of liquid chromatography in combination with inductively coupled plasma mass spectrometry (LC-ICP-MS) enabled accurate intact cisplatin quantification in cell model experiments.

Methodik

Cisplatin was spiked using sub-µM concentration levels of the drug on cell extracts. Moreover, cells were exposed to 5 µM drug, lysed at different time points and fractionated. The cytosolic fraction was further separated into protein and small molecule fraction by the use of centrifugal filtration (10 kDa cut-off). ICP-MS addressed the quantification of drug uptake, intracellular distribution and quantification of protein bound drug versus low molar mass fraction. Chromatographic separation of cisplatin species was based on pentafluorophenylpropyl siloxane bonded- and on porous graphitic carbon stationary phases. Glutathione in reduced and oxidized form were studied via HILIC-ESI-MS-MS.

Vorläufige Daten

The *in vitro* experiments revealed novel insights into kinetic aspects of cell associated cisplatin reaction. A half-life of 2 hours and pseudo first order decay kinetics was found for cisplatin in cell extracts. These model experiments showed that a significant fraction of intact cisplatin was forming adducts inside cell, before hydrolysis was occurring. Therefore thiol binding is highly likely. Pseudo first order kinetics resulted from the large excess of potential binding partners offered in the cell extract. Pronounced protein adduct compared to low molecular weight biomolecule adduct formation was readily observed within the first hour of incubation. Drug accumulation experiments showed that rapid diffusion was the predominant cisplatin uptake mechanism; since the cell associated cisplatin concentration resembled the cisplatin concentration in the medium already after 1 hours of exposure and remained constant during the monitoring. Moreover, the Pt was readily distributed over all cell compartments including nucleus. Active uptake of cisplatin would imply that other low molecular weight Pt species would be present in the cytosol. However, at any point of investigation intact cisplatin was the predominant species in the cytosol. Model experiments addressing the drug efflux showed that intracellular Pt concentration was significantly decreased once the drug exposure stopped. The observed removal was biphasic, while intact cisplatin was removed rapidly by nearly 100% within one hour, Pt –associated to high molecular weight biomolecules decreased only to 30-40% of their initial concentration within 24 hours. Presumably, the rapid and pronounced protein binding upon drug uptake together with the fact that these protein adducts are only slowly removed from the cells, is in accordance to the known nephrotoxicity of cisplatin, which can be described as a heavy metal toxicity characterized by inhibition of crucial enzymes, binding to thiol compounds and damage cell membrane.

Neuartige Aspekte

Application of quantitative mass spectrometric methods enabled the investigation of the intracellular chemistry of cisplatin in clinically relevant concentrations.

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High throughput analysis of differentially induced metabolites using metaXMCS and automated fragmentation tree alignment.

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Einleitung

The qualitative and quantitative analysis of all metabolites represents the metabolite profile of a plant at a given instant. In addition to factors like stage and state of a plant, biotic stresses e.g. leaf herbivory or bacterial infection could significantly alter the metabolite profile of a plant. Differentially regulated metabolites in such stress condition comprise interesting classes of compounds which are either the outcome of a perturbed metabolism or which help to restore the normal metabolism. In metabolomics, the characterization of such metabolites induced due to particular biotic stress is still a challenging task.
High-throughput analysis of metabolites using modern LCMS techniques can be combined with automated fragmentation trees alignment for rapid characterization of metabolites. This combination, although in its infancy, promises to help not only in automated identification of the known metabolites but also in categorizing unidentified metabolites on the basis of their fragmentation patterns which strongly correlate with the chemical nature.
In current work, we analyze differentially induced masses in plants infested with *S. littoralis* or infected with *P. syringae* in comparison with the control. We will also comment on the correlation of chemical classes of differentially induced metabolites with that of applied biotic stresses.

Methodik

Arabidopsis thaliana col-0 plants were grown under standard growth conditions. Plants at early flowering stage (25 days old) were used for all experiments. Wild type plants serve as control. First plant set is subjected to *S. littoralis* leaf herbivory while the second set is subjected to *P. syringe* infection. Plants were cut into above and below ground parts and extracted separately using methanol:water (80:20, v:v). Extracts were analyzed using UHPLC (C-18 RP column) coupled to Orbitrap MS. Mass spectra were acquired at various fragmentation energies in CID or HCD mode.
Analysis of differentially up/down regulated masses was performed using metaXCMS. Extracted masses will be categorized into related chemical classes using the automated fragmentation tree alignment approach.

Vorläufige Daten

metaXCMS analysis showed total of 449 masses that are 5 fold up or down regulated ($P < 0.05$) in particular biotic stress treatment. Compared to control plants, 114 and 199 masses are exclusively induced in *P. syringae* infected plants or *S. littoralis* infested plants respectively. There are 191 overlapping masses in the two biotic stress treatment plant sets hinting induction of some common metabolic pathways.
The detailed XCMS analysis and characterization of differentially induced masses into chemical classes will be reported shortly.

Neuartige Aspekte

High throughput and automated identification of differentially induced metabolites by combining metaXCMS analysis with alignment of fragmentation trees.

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Storage and retrieval of information in xenobiotic metabolism studies to improve decision making and knowledge sharing between scientists, facilities, locations

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Einleitung

Current informatics approaches for xenobiotic studies limit the progress of scientists due to the restrictions of predefined software workflows and the difficulty on passing on one's knowledge to peers. The ability to share discoveries and knowledge on the biotransformations of NCE's/classes for universal use in an organization and to feed previously discovered information into today's experiments holds the potential to unlock the potential of historical data. The approach defined in this paper uses the latest generation hardware and informatics to accurately screen samples, confidently interrogate datasets and efficiently disseminate data in a highly flexible format. This system allows users to elucidate metabolic pathways, store, access and interrogate each other's data and benefit from previous experience, bringing an encyclopedia of knowledge from the organization's best scientists to bear on the pertinent challenges.

Methodik

The samples were analyzed on accurate mass MS platforms equipped with sub 2µ LC systems. Metabolite analysis of several compounds was performed using a prototype version of a scientific information system software. The software contains a scientific library function which is used to enter and retrieve information about the compounds being analyzed and is used as an interface to disseminate information. The samples were automatically processed to provide a comprehensive list of metabolites which can then be filtered or interrogated to suit both the analytical and reporting needs of the user.

Vorläufige Daten

Metabolite data was analyzed using novel peak picking algorithms with advanced multicore processing capabilities. Processing takes advantage of built in scientific library functionality which manages both compound and metabolite identification specific criteria and allows users to define and generate metabolite relationships within a single LCMS software package. Users are able to both build and retrieve metabolite relationship information across datasets and samples that were historically difficult to manage as a single report. Integration of in silico tools, such as; metabolite prediction, isotopic pattern analysis and intelligent mass defect filtering will be discussed. We will also demonstrate how drug metabolism departments will have the ability to manage instrument and server assets remotely across the network. This network based infrastructure also facilities scientifically sharing and disseminating data and knowledge across labs and across networks.
DMPK datasets are processed for both generating qualitative structural and relationship pathways as well as for quantitative analysis.

Neuartige Aspekte

Putting the datasets into meaningful context within a single, integrated processing and reporting environment.

Gold(I) phosphine coordination to de novo designed peptides

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Einleitung

The study of metals in medicine can have a significant impact in the fight against many diseases particularly cancer and HIV which claim millions of lives each year. Aurothiomalate for example has been shown to have anti-arthritic properties, auranofin has been proposed for the treatment of psoriasis. We have used ESI and MALDI combined ion mobility-mass spectrometry to examine the reaction of the gold phosphine complex $AuPEt_3Cl$, with a number of amino acids and synthetic peptides. It is our intention to investigate the coordination of peptide sequences, which posses well defined secondary structures, to gold(I) phosphine compounds based on auranofin. Ultimately, with the view of developing targeting derivatives of auranofin with improved biodistribution and bioactivity.

Methodik

ETD was implemented on a Synapt G2 mass spectrometer, which incorporates three Travelling-Wave SRIG's. For ETD fragmentation, a glow discharge source was used to fill the Trap T-Wave with quadrupole isolated reagent anions. During acquisition, the source polarity and quadrupole set mass are switched to allow multiply charged cations to interact with reagent anions in the Trap T-Wave. ETD/CID product ions were separated according to their ion mobilities in the IMS cell. Upon exiting the IMS cell the ions enter the Transfer T-Wave which can be used to provide supplemental activation prior to the ToF. MALDI acquisitions used a solid state Nd:YAG laser (λ = 355nm), operated at a pulse rate of 1000Hz with 180sec/acquisition with CHCA as the matrix.

Vorläufige Daten

The precise site of binding of $AuPEt_3$ to TRI23C can be determined by both ETD and CID fragmentation methods. Following CID of the $[M+\{AuPEt_3\}+4H]^{5+}$ and $[M+\{AuPEt_3\}+6H]^{7+}$ ions generated by ESI no sequence-specific ions containing the gold based complex were observed. The reverse was found following ETD-generated mass spectra of the $[M+\{AuPEt_3\}+4H]^{5+}$ and $[M+\{AuPEt_3\}+6H]^{7+}$ multiply charged precursor ions. Limited sequence coverage was obtained for the triply and quadruply charged peptide-Au complex species. These ions produced abundant charge-reduced species which did not allow the site of the Au binding to be precisely determined. However, the sequence coverage obtained by ETD fragmentation of the 5+ and 7+ peptide-Au complex allowed the site of Au binding to be located upon the cysteine residue, as expected. The percentage of sequence coverage was 97% and 100% for the 5+ and 7+ respectively. Following CID of the $[M+\{AuPEt_3\}+1H]^{1+}$ ion generated by MALDI a percentage sequence coverage of 100% was achieved, which also allowed for the determination of the Au binding site. We provide methods for the determination of Gold(I) binding to a number of peptides/proteins. The structural information inferred from the amount of peptide sequence coverage obtained using CID/ETD for ESI is shown to be charge-state dependent.

Neuartige Aspekte

Investigation of the coordination of novel peptide sequences, which posses well defined secondary structures, to gold(I) phosphine compounds.

Investigating the applicability of direct analysis ion mobility for the analysis of synthetic polymers.

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Einleitung

Nowadays, polymeric materials are inescapable and cover a broad range of applications in areas as diverse as automobiles, textiles, packaging, and medicine. For more demanding and specific applications, higher performing materials are required and the preparation of highly complex polymers is a major challenge. As a direct consequence, sophisticated techniques are required for the in-depth characterization of macromolecules. The usual characterization tools such as Nuclear Magnetic Resonance (NMR) and Gel Permeation Chromatography (GPC) are extensively used. Nevertheless, both techniques are averaging methodologies since they only provide pieces of information about the polymer mixture instead of affording data on individual macromolecules. For several years, mass spectrometry (MS) has emerged as a characterization method as ubiquitous as the SEC or NMR

Methodik

MS can be used to generate detailed structural information from individual oligomers. It is possible to gain detailed molecular structural information when MS is combined with soft ionization methods, such as electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI). In the study undertaken here High Definition Mass Spectrometry (HDMS) has been utilised to analyse synthetic polymeric products. It is a combination of high resolution mass spectrometry and high efficiency ion mobility (IM). IM mass spectrometry is a rapid orthogonal gas phase separation technique that allows another dimension of separation to be obtained.

Vorläufige Daten

Two polylactide samples were used to investigate the ability of ion mobility separation in combination with direct analysis ESI (direct ES+ infusion experiment) to provide polymer characterisation data. The samples were cationised with sodium, lithium and caesium. Using travelling wave IM the molecular structure of the polylactides were characterised through the determination of the relative size of the cationised species, which can be used to infer changes in molecular conformation and shape. In addition the separation of the singly charged and doubly charged polymer distributions was easily achieved. The size and shape generated for the singly charged polylactide samples are linear, with a correlation coefficient r=0.99. From the observed drift time values for +2 species of polylactide it can be seen that the doubly charged species were not linear. This is seen clearly in the drift time vs molecular weight plot generated. The study undertaken warrants further investigation of the enhanced peak capacity that Direct Analysis-IMS-MS can enable in polymer characterisation applications. Mass measurement errors of <2 ppm have been illustrated. The unique separation power of ion mobility has enabled data to be generated which infers a change in the shape of the polymer structure and provides a new routine route to polymer characterisation.

Neuartige Aspekte

Ion mobility delivers an extra dimension of separation for direct analysis of polymers allowing better structural characterization.

Towards understanding Combustion – Flame chemistry analysis with advanced molecular-beam mass spectrometry

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Einleitung

Combustion today provides the dominant share of global energy consumption and will remain a significant contribution to the energy portfolio in the coming decade and potentially beyond. Considering combustion emissions and climatic effects, it is mandatory to investigate and design efficient and clean combustion technologies. Also, with respect to limited fossil fuel resources, alternative fuels and combustion processes are being discussed and developed. It is well-known that biofuels, especially oxygenates, exhibit different combustion behavior and may form different pollutants emissions than their conventional fossil counterparts [1]. Information on the detailed combustion chemistry is thus necessary, which is provided with dedicated experiments using advanced diagnostic techniques.

Methodik

For such detailed insight into the combustion chemistry of different fuels, laminar premixed flames are commonly used as model flames because of their simple fluid dynamics. Here, a non-commercial time-of-flight molecular-beam mass spectrometric (TOF-MBMS) system with a high mass resolution is used for the simultaneous detection of almost all species occurring in these flames, including in-situ detection of radicals. Different ionization methods are used to minimize fragmentation of the detected species, and special calibration strategies are necessary to obtain quantitative data. We have recently coupled in-situ gas chromatography (GC) from the same volume to the MBMS analysis to identify different isomers of stable species by analyzing pre-sampled gas.

Vorläufige Daten

Selected results are shown to highlight the advantages of this combination of techniques for the analysis of combustion chemistry. High-resolution in-situ time-of-flight MBMS with electron ionization (EI) was used to detect stable and reactive species profiles of reactants, intermediates and products. With the novel in-situ combination of EI-MBMS with GC from the same volume, separation of isomers for stable species is possible, enhancing the information gained about the combustion process. Identical sampling for both methods significantly reduces potential interpretation ambiguities and measurement errors. This is particularly useful when complex fuel reaction sequences are studied.
To illustrate the potential of this method combination, exemplary results of three different studies will be presented. Starting from an overview of the analysis of combustion reaction mechanisms [2], applications to the evaluation of biofuel combustion will be presented with a third focus on the development of low-temperature combustion strategies [3].
Acknowledgements: Partial funding by SFB 686 TP B3 is gratefully acknowledged.

Neuartige Aspekte

Molecular-beam mass spectrometry and gas chromatography are coupled to get a more detailed isight into combustion processes.

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Optimierung eines GC-APCI-MS-Systems für bioanalytische Anwendungen

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Einleitung

Die *atmospheric pressure chemical ionisation* (APCI) ist eine attraktive weiche Ionisierungstechnik für GC-MS-Untersuchungen. In den letzten Jahren haben verschiedene Hersteller APCI-Quellen für GC-MS-Kopplungen eingeführt. Die Ausschöpfung des Potentials der GC-APCI-MS hängt jedoch stark von Details der Konstruktion der APCI-Quellen sowie von der Optimierung verschiedener experimenteller Parameter ab. Die nachfolgend beschriebenen Untersuchungen wurden mit einem GC-APCI-Q-TOF-MS-System der Firma Agilent durchgeführt.

Methodik

Als Modellanalyte für die Optimierung dienten Benzophenon, Methylpalmitinat, Methylstearat und Cocainhydrochlorid mit einer Konzentration von 100µg/mL. Gemessen wurde im positiven Ionenmodus. Die temperaturprogrammierte GC-Trennung erfolgte in einer relativ kurzen Zeit von fünf Minuten. Die APCI-Quelle wurde durch Implementierung eines Feuchtigkeitssensors modifiziert. Zudem erfolgte eine kontrollierte Einspeisung von Stickstoff mit variabler Feuchtigkeit. Es wurden Parameter, wie die Position der GC-Kapillare, der Fluss des Trockengases, die Stromstärke an der Corona - Nadel, die Feuchtigkeit in der Ionenquelle, das Makeup - Gas und der Fluss des Makeup - Gases variiert. Außerdem wurde bei offener und geschlossener Ionenquelle gemessen und es wurden verschiedene Gase in die Quelle eingeführt.

Vorläufige Daten

Alle vier Substanzen des Gemisches konnten mit hoher Empfindlichkeit nachgewiesen werden, in den meisten Fällen als $[M+H]^+$ - Ion. Bei offener Quelle und geringem Feuchtigkeitsgehalt in der Ionenquelle konnten Radikalkationen nachgewiesen werden. Es wurden auch andere Gase wie zum Beispiel Ammoniak in die Ionenquelle eingespeist. Dies hatte zur Folge, dass nur noch Benzophenon und Cocain, die eine höhere Gasphasenbasizität als Ammoniak besitzen, protoniert wurden. Bei Molekülen wie Methylstearat und Methylpalmitinat mit geringerer Gasphasenbasizität konnte unter typischen Messbedingungen kein Signal mehr erhalten werden. Die unterschiedlichen Optimierungsschritte zeigten, dass die Ionisierung und die Empfindlichkeit von verschiedensten Faktoren abhängig sind. Ziel ist es, die optimierten Bedingungen des GC-APCI-MS-Systems auf Bestimmungen von Oxysterolen zu übertragen, was derzeit erprobt wird.

Neuartige Aspekte

Implementierung eines Feuchtigkeitssensors; Kontrollierte Einspeisung von Stickstoff mit variabler Feuchtigkeit

HILIC-ICP-MS Speciation of Gadolinium containing contrast agents in surface water samples and plants

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Einleitung

For more than 20 years Gadolinium-based contrast agents are widely used in medical diagnostics to enhance the contrast of images in magnetic resonance imaging (MRI). These gadolinium (Gd) complexes are very stable and thus exhibit much less toxicity compared to the free Gd^{3+} ion. However, up to now little is known about their behaviour in the environment. While contrast agents are found in urban surface water, the fate of these Gd complexes in the flora and fauna is from interest.

Methodik

Because of the very low concentrations of Gd species in environmental samples a hydrophilic interaction chromatography (HILIC) coupled with inductively coupled plasma mass spectrometry (ICP-MS) were used to detect Gadolinium-based contrast agents.

Vorläufige Daten

Surface water samples from the Teltow channel, near Berlin, were investigated over a distance of 5 km downstream from the influx of a wastewater treatment plant. The total concentration of gadolinium increased significantly from 50 to 990 ngL^{-1} due to the influx of the contrast agents in the waste water. After complete mixing with the river water, the concentration remained constant over a distance of at least 4 km. Two main substances [Dotarem® (Gd-DOTA) and Gadovist® (Gd-BTDO3A)] have been identified in the river water using standards. A gadolinium-based contrast agent, possibly Gd-DOTA (Dotarem®), was also detected in water plant samples taken from the Teltow channel. Therefore, uptake of contrast agents [Gadovist® (Gd-BTDO3A), Magnevist®(Gd-DTPA), Omniscan® (Gd-DTPA-BMA), Dotarem®(Gd-DOTA), and Multihance® (Gd-BOPTA)] by plants was investigated in a model experiment using Lepidium sativum (cress plants). HILIC–ICP-MS was used for identification of different contrast agents, and a first approach for quantification using aqueous standard solutions was tested. For speciation analysis, all investigated contrast agents could be extracted from the plant tissues with a recovery of about 54 % for Multihance® (Gd-BOPTA) up to 106 % for Gadovist® (Gd-BT-DO3A). These experiments demonstrate that all contrast agents investigated are transported from the roots to the leaves of the cress plants where the highest content was measured.

Neuartige Aspekte

Quantitative speciation studies are urgently needed to understand the fate of the contrast agents species in the environment.

Experimental investigation of the low-temperature oxidation of dimethyl ether using molecular-beam mass spectrometry

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Einleitung

Molecular-beam mass spectrometry (MBMS) is a powerful tool to study complex reaction systems like the fuel destruction and oxidation sequences in combustion. This method allows the detection of reactive species such as radicals in flames, and hydroperoxides and ketohydroperoxides in the low-temperature oxidation process [1]. A fundamental understanding of the low-temperature combustion mechanism is beneficial especially for enhancing the fuel efficiency of engines as well as the design and control of innovative engine concepts such as homogenous charge compression ignition (HCCI) engines. Such detailed understanding of the low-temperature combustion mechanism is promoted by experimental studies on the laboratory scale using well-designed flames and other simplified configurations such as jet-stirred reactors, flow reactors, and rapid compression machine.
In the present work, an apparatus for the analysis of the low-temperature oxidation of different fuels was designed, which includes a flow reactor and an in-situ MBMS system. Dimethyl ether (DME) was chosen as a first target because of its simple molecular structure and its potential as a clean alternative to diesel fuel [2]. Considering its importance as a future renewable fuel, more reliable data on its low-temperature oxidation are desired including detailed quantitative species concentrations.

Methodik

The in-situ MBMS system consists of the source chamber connected to the flow reactor, a differentially pumped intermediate chamber and the ionization chamber connected to an electron-ionization time-of-flight (TOF) mass spectrometer. The oxidation products were sampled at the outlet of the reactor by a quartz nozzle. The molecular beam formed behind a copper skimmer was detected by the TOF-MS, and the signal was amplified and fed to the computer system.
The flow reactor made of fused silica has an inner diameter of 8 mm and a length of 1500 mm. Argon as the diluent, oxygen and DME were controlled by mass flow controllers, mixed in an inlet flow tube (inner diameter 4 mm), and finally flowed into the reactor.

Vorläufige Daten

To test the performance of the apparatus, the oxidation of DME was studied at stoichiometric condition at near atmospheric pressure and in the temperature range from 400 to 1100 K. The preliminary data with this new set-up are similar to our previous study on DME oxidation [3]. With the combination of the flow reactor with the MBMS system, however, unstable species could now be detected. This includes for example low-temperature intermediates with m/z 64 which could not be observed in the previous study where only stable products and intermediates were accessible. In the temperature region used in this study, the oxidation of DME occurs in two stages. At lower temperatures below around 750 K, the negative temperature coefficient (NTC) of DME was observed. In this region, formaldehyde was detected as the main intermediate; small concentrations of final oxidation products such as CO, CO_2 and H_2O were also observed. Above the NTC region at slightly higher temperatures, no reaction was seen and the concentration of DME kept nearly constant. In the second stage of the oxidation at temperatures above around 850 K, DME was consumed rapidly, and large amounts of CO, CO_2 and H_2O were formed. Furthermore, some hydrocarbon intermediates such as CH_4, C_2H_2, C_2H_4 and C_2H_6 were also detected.

Neuartige Aspekte

Molecular-beam mass spectrometry combined with a flow reactor for the investigation of different fuels in the low-temperature regime.

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Antibody Characterization by Orbitrap Mass Spectrometry

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Einleitung

In the development and production phases of monoclonal antibodies the evaluation of 3D structure, amino acid sequence and glycosylation pattern are essential steps in the analysis. Important prerequisites for intact protein characterization and top-down analysis by mass spectrometry are the resolving power and mass accuracy as provided by the Orbitrap mass analyzer. However, resolving power of large intact protein mass measurements requires a very low pressure in the Orbitrap mass analyzer to prevent fast transient decay caused by collisions with residual gas.

Methodik

We have modified a Thermo Fisher Scientific Orbitrap instrument for improved high molecular weight ion characterization. The instrument modifications relate to the implementation of a switching valve in the C-trap collision gas supply used to control respectively reduce the pressure in the Orbitrap mass analyzer as well as changes in the instrument control software. Reduction of the gas load in the C-trap/HCD collision cell allows for the reduction of the pressure in the Orbitrap mass analyzer and hence reduces the decay of transients of intact proteins and allows for the detection of more discrete beats in the transient of high molecular weight ions, thus increasing the upper mass limit of proteins isotopically resolved in online analysis up to ~50 kDa.

Vorläufige Daten

To demonstrate the improved instrument performance resulting from the implemented modifications we have analyzed an intact monoclonal antibody in non-reduced and reduced condition by LC-MS. The intact antibody respectively the separated light and heavy chains were analyzed in Full MS experiments as intact protein species as well as with top-down experiments using the different and complementary fragmentation techniques in-source CID (SID), CID, HCD and ETD. The obtained data demonstrate the wealth of information that can be achieved from an antibody sample in a limited amount of time and with a rather simple experimental setup.

Neuartige Aspekte

Analysis of intact antibody to achieve isotopically reduced molecular ions as well as sequence information from an intact, non-reduced antibody.

Resonance-Enhanced Multiphoton Ionization with Circularly Polarized Light: Chiral Carbonyls

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Einleitung

The combination of circular dichroism and mass spectrometry is a young developing research field which promises to become a new analytical tool for mass selective probing of chirality. Such a chiral sensor opens new possible application e.g. for studies of chiral catalysis or for analysis of chiral biomolecules by laser desorption techniques such as matrix-assisted laser desorption ionization (MALDI) or laser desorption photoionization (LD-PI). This poster introduces to the basics of the corresponding method called CD-REMPI (circular dichroism resonance enhanced multiphoton ionization) [1-4]. This will be performed at the example of 3-MCP (3-methylcyclopentanone) and a review of our results on other cyclic carbonyls.

Methodik

REMPI, on the one-hand, combines optical spectroscopy with mass spectrometry and thus allows two-dimensional selectivity (with the two parameters: UV-wavelength and molecular mass). CD, on the other hand, is a frequently used effect to discriminate between the two enantiomers of chiral substances. Since CD is a method of optical spectroscopy it is well incorporated in the REMPI process which is based on UV-spectroscopy. The combination of REMPI and CD is achieved by using circularly instead of linearly polarized laser light.

Vorläufige Daten

This poster will introduce to the basics of REMPI and CD-spectroscopy by focusing on the two main pillars of REMPI, namely spectroscopy and mass spectrometry, for the example of the chiral molecule 3MCP. It will present results on some other cyclic ketones as representatives of carbonyls. Comparison of the CD-REMPI results and conventionally measured CD values demonstrate the reliability of the new method, for the chemical class of carbonyls, but also for aromatic substances such as phenylethanol. As a first demanding practical application it is planned to use this method as analytical tool for research on nanocatalysis of size-selected metal clusters [5] with the goal of enantio-selectivity. This research is performed in the ultrahigh vacuum. CD-REMPI-MS is ideally adapted to monitor chiral product molecules, or their enantiomeric purity under these experimental conditions. In addition, this poster will address some new interesting features of chiral spectroscopy performed via resonance enhanced multiphoton ionization, such as cumulative circular dichroism and circular dichroism of molecular ions. For further details see [Boesl U, Bornschlegl A, Logé Ch, Titze K, (2013) Anal Bioanal Chem accepted for publ.].

Neuartige Aspekte

This method allows enantio-sensitive, mass-selective probing of chiral molecules. It is based on resonance-enhanced multiphoton-ionization and special time-of-flight mass spectrometry.

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Entwicklung eines IMS TOF-MS mit einer Laser basierten Ionisation unter Normaldruck

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Einleitung

Das im Arbeitskreis vorhandene Ionen-Mobilitätsspektrometer (IMS) von der *Drägerwerk AG* erzeugt Ionen bei Normaldruck mit einer ^{3}H-Quelle über einen Chemischen Ionisations (CI) Prozess.
Um die Gruppe der Analyten auf schwer protonierbare Spezies zu erweitern, soll die vorhandene ^{3}H-Quelle durch eine Laser gestützte Ionisation ersetzt werden, wobei der vorhandene Aufbau (vgl. Methode) weitestgehend erhalten bleiben soll. Durch die geänderte Ionisationsmethode werden zusätzlich andere Ionenspezies erzeugt (z.B. Radikalkationen anstelle von protonierten Ionen). Des Weiteren kann durch die Wahl der Wellenlänge eine selektive Ionisation von Analyten erlangt werden.

Methodik

Der experimentelle Aufbau wurde bereits früher ausführlich beschrieben [1]. An dieser Stelle soll lediglich ein kurzer Überblick gegeben werden. Ein selbstgebautes und ein von der *Drägerwerk AG* erworbenes IMS sind mit einem selbstgebauten Flugzeit-Massenspektrometer (TOF-MS) verbunden. Zur Ionisation wurde jeweils die vorhandene ^{3}H-Quelle verwendet. Um die Ionen vom Normaldruckbereich in den Hochvakuumteil des TOF-MS zu leiten, werden diverse differentielle Pumpstufen verwendet.
Um die Ionenausbeuten der verwendeten Ionisationsmethoden (Multi Photonen Ionisations [MPI] vs. CI) zu vergleichen wurde ein weiteres selbstgebautes IMS verwendet, besteht aus einem Glasrohr, auf welches mit 16 Elektroden gezogen wurden. Die elektronischen Komponenten zur Ansteuerung und Datenerfassung wurden aus dem vorherigen Experiment entnommen.

Vorläufige Daten

In früheren Experimenten [1] konnte gezeigt werden, dass bei dem Transfer der Ionen von dem Normaldruckbereich in den Hochvakuumteil des TOF-MS ein hoher Verlust der Ionen stattfindet. Daher sollte zunächst der Einfluss der Ionisationsmethode auf die Ionenausbeute untersucht werden.
Dazu wurden erste Messungen mit Aceton und Toluol durchgeführt. Die entstehenden und transferierten Ionen wurden mit einem Faraday Cup detektiert. Im Anschluss wurde die ^{3}H-Quelle durch einen Laser ausgetauscht. Es wurde ein Nd:YAG-Laser verwendet, dessen 4. Harmonische ausgekoppelt und zwischen einem angebrachten Repeller und dem IMS-Eingang gespiegelt wurde.
Da Toluol bei 266 nm über einen MPI Prozess gut ionisiert werden kann, wurden die Messungen für Toluol wiederholt. Vergleichsmessungen mit Aceton zeigten keine auswertbaren Signale.
Im Falle von Toluol können im Vergleich der beiden Ionisationsarten mehrere Effekte beobachtet werden. Das erhaltene Signal wächst um das 10-fache mit einer starken Peakverbreiterung und ausgeprägtem Tailing. Des Weiteren verschiebt sich das Maximum des Signals um ca. 2,5 ms zu späteren Driftzeiten.

Neuartige Aspekte

Vergleich der Ionenausbeuten von einer MPI und einer CI durch eine ^{3}H-Quelle bei Normaldruck.

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Analysis of Local Dynamics of Human Insulin and a Rapid-acting Insulin analog by Hydrogen Deuterium Exchange Mass Spectrometry

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Einleitung

Human insulin, used by diabetics to regulate blood sugar, was first introduced as a recombinant therapeutic drug nearly 30 years ago. It is, however, very challenging to adequately characterize the higher-order structures of biotherapeutic products and to monitor the changes in oligomeric stability. Human insulin and insulin lispro have identical primary structure, except for the transposition of a pair of amino acids. Lispro is one of the rapid-acting insulin analogs, which has higher tendency to dissociate than human insulin. In this study, we present an analytical workflow to allow us to detect the difference in the oligomeric dynamics using a Hydrogen Deuterium Exchange Mass Spectrometry (HDX MS).

Methodik

Drug products, human insulin (Humulin R) and lispro (Humalog), were reduced and digested online by pepsin. Deuterium labeling, quenching, and injection to on-line pepsin digestion were prepared using a robotic sample manager. Labeling experiments in 0, 0.5, 5, 10, 60, and 180 min interval were duplicated for both samples. The peptic digests were separated on a UPLC system dedicated for HDX experiments at 0°C. Q-TOF mass spectrometer was used to measure the deuterium incorporation of identified peptides. The amount of deuterium was determined by automated HDX data processing software, DynamX 2.0. The HDX results were compared in uptake curves, heat maps, and 3D-structures, which were enhanced features of the software.

Vorläufige Daten

We obtained 98% of sequence coverage for both human insulin and lispro. The coverage map of the peptic digests demonstrated that the efficient digestion was achieved with a high redundancy score resulted by multiple overlapping peptides. From peptide HDX determination, two regions were revealed distinctive different values in deuterium uptakes between human insulin and lispro; the N terminus of chain A, and a region adjacent to the C terminus of chain B. We attributed this localized behavior to the relation of hexamerization and dimerization, respectively. Furthermore, characteristic profiles that showed different deuteration margins between two insulins were determined, which was also consistent with their involvement in hexamer and dimer formation.

Neuartige Aspekte

Automated HDX analysis on Insulin and Lispro peptides for characterization of higher-order structures and monitoring changes in oligomeric stability

GC/APLI-FTMS – a new sensitive method to detect aromatic compounds in complex mixtures

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Einleitung

Many analytical tools are available to characterize compounds in complex mixtures. Suitable methods for the characterization of complex mixtures by mass spectrometry are hyphenated techniques such as LC/MS or GC/MS. The new method GC/APLI-FTMS has been developed for the detection of aromatic compounds in complex mixtures. This technique combines GC as a high performance separation method, APLI as a very sensitive ionization method for aromatic compounds, and FTMS which is known as a method for very accurate mass measurements. Furthermore, APLI is a non-destructive, soft ionization method in contrast to common GC/MS systems with EI. Therefore, this hyphenation method is well suited for the characterization of volatile aromatic compounds in complex mixtures like waste water or oil samples.

Methodik

GC/APLI has been coupled to a solarix 12 T FT-ICR FT-ICR instrument to detect polycyclic aromatic hydrocarbons (PAHs) and polycyclic aromatic sulfur heterocycles (PASHs) in fuel oil and gas oil. Waste water samples were analyzed by concentration of specific PAHs. Typical GC temperature gradients (5°C/min and 10°C/min) were used. 1 mL of sample dilutions (between $1{:}10^3$ and $1{:}10^6$ of oil sample) have been injected on the VF-5ms (0.25um film thickness, 30m, 0.25 mm ID) GC column. Mass spectra were acquired at 1Hz resulting in a resolving power of 250,000 at *m/z* 200. A transfer temperature of 290°C between GC and ion source was used.

Vorläufige Daten

The elemental compositions of PAHs and PASHs in gas oil and fuel oil as well as PAHs in waste water have been analyzed by exact mass and retention time in positive ion mode. Samples with different amount of sulfur between 10 ppm to more tan 1000 ppm have been tested. APLI as an extremely sensitive method for the selective ionization of aromatic compounds combined with GC is a perfect method to detect PAHs and PASHs without fragmentation of the molecular ions. Therefore, compounds can be easily identified just by retention time and accurate mass combined with the isotopic pattern of the molecular ion. Extracted ion chromatograms (EICs) with 1 mDa mass tolerance have been used to identify isomers of PAHs and PASHs. Species with a small mass difference with similar retention times can also be separated due to high resolving power of the FT-ICR MS technique. The sensitivity of GC/APLI has been compared with GC/APCI for the detection of dibenzothiophenes to evaluate this new analytical method. All results have shown that GC/APLI-FTMS allows the detection and characterization of low abundant PAHs and PASHs in complex mixtures. Therefore, this new analytical technique may be a new method to detect and quantify very low amounts of PAHs in waste water or even drinking water.

Neuartige Aspekte

GC/APLI combined with FT-ICR for detecting low amounts of PAHs of complex mixtures.

Ion mobility in Matrix-Assisted Laser Desorption/Ionization – Mass Spectrometry Imaging (MALDI-MSI)

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Einleitung

Over the years, Mass Spectrometry Imaging (MSI) has become a widely used analytical technique to visualize the spatial distribution of a large variety of substances of biological interest, such as lipids, peptides and proteins. The state-of-the-art imaging technique developed by our group provides for a spatial pixel resolution of less than 10µm and a mass resolving power of the employed orbital trapping mass spectrometers of R > 100,000 (m/z = 200), generating ion images with a bin size of $\Delta m/z = 0.01$ [1]. One limitation of this technique is the inability to separate and identify isomers, isobars and conformers which are commonly encountered when analysing complex biological substances.

Methodik

Ion mobility based separation employing the traditional drift tube technique (IMS) at low electric fields (< 500 V/cm) [2] has proven to be an excellent techniques to separate and detect isomers and isobars. Separation of compounds in these techniques is based on differential velocities the ionised substances attain in an electric field at atmospheric pressure, based on their mass, shape and size. A combination of IMS and MSI will obviously increase specificity in imaging analyses of complex biological samples. Ion mobility effects observed when using various setups in front of the atmospheric pressure inlet of the mass spectrometer were therefore investigated.

Vorläufige Daten

We present initial results obtained from ion mobility studies where differences in drift times were observed depending on mass and shape of the ions and instrumental parameters. Standard samples for MALDI-MSI analysis were scanned by the laser and ion drift times were recorded depending on instrumental parameters. By varying the opening times of the C-trap in the mass spectrometer, substance-specific differences in the resultant spectra were observed, providing data for the development of controlled ion mobility separation in MALDI-MSI analyses.

Neuartige Aspekte

Ion mobility was investigated in atmospheric pressure MALDI ion sources depending on various instrumental parameters.

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Selected Mobility Accumulation (SMA) in a Trapped Ion Mobility – Mass Spectrometer (TIMS-MS)

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Einleitung

Recently, a new type of ion mobility analyzer – the "Trapped Ion Mobility Spectrometry" (TIMS) analyzer was introduced, which produces results similar to conventional drift cell analyzers in a physically smaller design (~10X) and at much lower operating potentials. However, an analysis via TIMS as described thus far proceeds too rapidly (~2 ms/peak) for any mass analyzer except time-of-flight (TOF) to follow. In the present a new technique - selected mobility accumulation (SMA) – is introduced, which matches the timescale of the mobility analysis with that of "low duty cycle" mass analyzers (e.g. FTMS) and techniques (e.g. ETD). SMA is achieved by efficiently accumulating ions of the desired mobility for long (300 ms) periods of time

Methodik

In the present work, a TIMS analyzer is incorporated as a section of an ion funnel in the ion source of a mass spectrometer. As described previously, in "broadband" mode, ions are initially trapped in the TIMS analyzer wherein gas flow pushing ions downstream is counterbalanced by an electric field holding the ions back. After trapping, the electric field is slowly decreased so that ions are eluted according to mobility. Eluting ions are mass analyzed in the orthogonal time-of-flight analyzer to produce a TIMS – MS spectrum. To perform selected mobility accumulation (SMA) experiments, the electric field is modified to form a "plateau" within the TIMS trap on which ions of a selected mobility or mobility range can be accumulated.

Vorläufige Daten

The mobility analysis in a TIMS device may be viewed as roughly the same as that in a conventional IMS drift cell except that, in the conventional cell an electric field pushes ions through a stationary gas whereas in the TIMS analyzer a retarding electric field holds ion in place against a moving gas. IIons, initially trapped in the TIMS analyzer, elute according to mobility – lowest mobility first – as the strength of the retarding electric field is reduced. The "shape" of the retarding electric field, potential vs. axial position, has a profound effect on the number and types of ions that are initially trapped and the timing of their elution.
The effects of various retarding field gradient profiles were explored, and a plateau in the gradient profile at about 4% below the highest retarding gradient resulted in the optimal SMA results, i.e. mobility selection resolution of greater than 50 and duty cycle > 50%. In one series of tests *m/z* 1222 ions from Agilent tunemix (PN G1969-85000) , which have a a Ko of about 0.72, were able to be selectively accumulated for 300 ms without signs of saturation. Ejection of these ions required less than 100 ms, thus the fundamental limit on the duty cycle is much better than 50%. In further tests, higher amplitude RF confining fields resulted in longer accumulation times before the onset of saturation. Also, higher pressures at the entrance to the TIMS analyzer leads to higher resolution SMA but lower accumulated ion abundances.

Neuartige Aspekte

A new method of selected mobility accumulation (SMA) of ions in a trapped ion mobility spectrometry (TIMS) analyzer.

Systematic evaluation of ultrahigh resolution MS instrument parameter to optimize topdown analysis

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Einleitung

The combination of ultrahigh resolution together with the complementary dissociation techniques collision induced dissociation and electron transfer dissociation enables thorough topdown protein characterization.
Compared to peptides, intact proteins have a much higher number of fragmentation channels and therefore fragment ion intensities are usually lower compared to peptide fragment ions. This requires averaging of MS/MS spectra to increase the data quality but also makes it more difficult to do topdown analysis on LC timescale.
With the introduction of the compact highfield Orbitrap mass analyzer topdown experiments can be done at LC time scale if the experimental parameters are chosen appropriately. This study evaluates the different instrument parameters to increase both spectral quality and speed of analysis.

Methodik

A hybrid ion trap – Orbitrap mass spectrometer equipped with a compact high-field orbitrap and electron transfer dissociation capabilities has been used throughout all experiments (Thermo Fisher Scientific, Bremen, Germany). Standard proteins were purchased from Sigma-Aldrich (St. Lois, MO, USA).

Vorläufige Daten

Proteins in the range of 8 kDa to 30 kDa have been used to study the effect of various experimental parameters on the speed and spectral quality for topdown MS/MS. Amongst those were precursor ion charge state, AGC target value for precursor cations and ETD anions, resolution, ETD reaction time, Orbitrap vacuum quality, number of averaged spectra or transients etc.
Consistently across the studied proteins, the precursor ion charges states with the highest number of charges gave best fragmentation efficiencies. They all had their maximum fragmentation efficiency at around 5 ms for ETD reaction time. AGC target value, resolution and number of averaged spectra have a direct influence on the speed of analysis. Here, recommendations can be deduced from the systematic evaluation of those parameters for the best compromise between spectral quality and speed of analysis.

Neuartige Aspekte

Systematic evaluation of different instrument parameters for highest spectral quality at LC timescale.

Einzelpartikelanalytik mittels ICP-MS: Möglichkeiten und Herausforderungen pneumatischer Probeneintragssysteme zur Charakterisierung von Goldnanopartikeln.

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Einleitung

Nanopartikel besitzen häufig außergewöhnliche Eigenschaften und haben damit unzählige neue Anwendungen in Industrie, Medizin und Forschung hervorgebracht. Eingesetzt in Farben und Lacken, Industrie- und Haushaltsprodukten und sogar als Lebensmittelzusatzstoffe werden sie kontinuierlich in die Umwelt freigesetzt.
Sowohl zur Charakterisierung dieser Nanopartikel, als auch zur Bewertung ihres Gefährdungspotentials für Mensch und Umwelt sind deshalb robuste und empfindliche Analysemethoden zur Bestimmung von Partikelgröße, Größenverteilung und chemischer Zusammensetzung nötig.
Eine attraktive Möglichkeit zur Partikelcharakterisierung stellt dabei die Massenspektrometrie mit induktiv-gekoppeltem Plasma (ICP-MS) dar. Neben bereits bekannten Kopplungen dieser Analytik mit den unterschiedlichsten Trenntechniken erlaubt diese Methode auch, zur Untersuchung von Nanopartikeln vollständig auf Kopplungstechniken zu verzichten.

Methodik

Eine vollständige Ionisierung der Partikel im Plasma und die anschließende elementspezifische Detektion ermöglichen die direkte Charakterisierung von Partikeldispersionen mittels ICP-MS im Einzelpartikelmodus (engl. single particle mode, SP-ICP-MS) [1].
In dieser Arbeit wurden dazu wässrige Dispersionen von Goldnanopartikeln mit schmaler Größenverteilung und mittleren Partikelgrößen zwischen 20 nm und 100 nm untersucht. Zum Einsatz kamen zwei unterschiedliche konventionelle Probeneintragssysteme (PC^3, gekühlte Zyklonsprühkammer bzw. APEX Q, beheizte Zyklonsprühkammer und Desolvatisierungseinheit) mit kontinuierlichem Probeneintrag. Die mit dem Einzelpartikelmodus einhergehende Forderung, trotz des polydispersen Zerstäuberaerosols nicht mehr als einen Partikel je Zeitintervall ins Plasma einzutragen, wurde durch eine entsprechende Verdünnung der Proben sichergestellt.

Vorläufige Daten

Mit Hilfe beider Probeneintragssysteme konnten einzelne Goldnanopartikel mit Größen bis zu 20 nm (PC^3) bzw. 30 nm (APEX Q) erfolgreich detektiert werden. Die Detektionseffizienzen lagen, abhängig von Größe und verwendetem Probeneintragssystem, zwischen $5.9*10^{-5}$ cpa und $1.1*10^{-4}$ cpa (engl. counts per atom). Auch Größenverteilungen in polydispersen Proben, die Verteilungen von zwei oder mehr Partikel-größen beinhalten, konnten mit Hilfe der Einzelpartikelanalytik entsprechend wiedergegeben werden.

Neuartige Aspekte

Charakterisierung von einzelnen Goldnanopartikeln mittels ICP-MS

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Ionische Flüssigkeiten als Matrices für die AP-MALDI MS

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Einleitung

Seit Entdeckung der Matrix-unterstützten Laser-Desorption/Ionisierung (MALDI) durch Karas und Hillenkamp hat sich die Massenspektrometrie als eine der meistgenutzten Methoden für die Detektion, Identifizierung und Charakterisierung biologischer Proben etabliert. [1] Diese Entwicklung führte zu einem Aufschwung in der Entwicklung und Verbesserung weiterer Ionisierungstechniken.
Im Jahr 2000 konnte durch Laiko *et al.* erstmals der Vorteil bei Atmosphärendruck zu arbeiten mit den Vorteilen der MALDI kombiniert werden. [2] Die Probenbehandlung und Untersuchung wird hierbei unter Atmosphärendruck durchgeführt. Dadurch wird die Probeneinfuhr dramatisch beschleunigt und es können darüber hinaus auch Analyten untersucht werden, die nicht vakuumkompatibel sind. Die Entwicklung der AP-MALDI MS eröffnet somit den Zugang zur Untersuchung zahlreicher neuer biologischer Proben, welche unter herkömmlichen MALDI-MS-Bedingungen nicht analysierbar wären. [3]
Eine der einfachsten und am häufigsten verwendeten Präparationsarten ist die sogenannte “dried-droplet“-Methode. Die eingeschränkte Reproduzierbarkeit der Spektren, sowie schwankende Signalintensitäten in der MALDI MS führen jedoch zu Problemen in der quantitativen Analytik. Um reproduzierbare Ergebnisse zu erhalten, müssen gezielt sogenannte “hot spots“ lokalisiert und ablatiert werden, an denen Analyt und Matrix aufkonzentriert vorliegen. Zur Umgehung dieses Problems können ionische Flüssigkeiten als eine neue Klasse an Matrices verwendet werden. Diese ermöglichen aufgrund einer Durchmischung eine homogenere Verteilung des Analyten in der flüssigen Matrix. [4]

Methodik

Eine selbstgebaute AP-MALDI-Ionisierungsquelle, welche die Vorteile einer Ionisierung bei Atmosphärendruck (API) und der Matrix-unterstützten Laser Desorption/Ionisierung (MALDI) miteinander vereint, wurde konstruiert. Der Ionisierungsprozess findet hierbei außerhalb des Massenspektrometers in einer separaten Probenkammer statt.
Innerhalb der Kammer wird ein Teil der Probe durch die dritte Harmonische eines Nd:YAG-Lasers (355 nm, 15 Hz, 3.8 mJ) desorbiert/ionisiert. Die erzeugten Ionen werden über einen geheizten Stickstoffträgergasstrom, durch eine Kapillare, in das Massenspektrometer eingetragen. Als Massenanalysator dient ein Flugzeitmassenspektrometer (ToF).
Es wurden diverse ionische Flüssigkeiten hergestellt und auf ihre Tauglichkeit als Matrix in der AP-MALDI MS zum Nachweis verschiedener Stoffklassen untersucht.

Vorläufige Daten

Es wurden Strategien gewählt, verschiedene ionische Flüssigkeiten durch eine möglichst einfach gehaltene Synthese herzustellen. Als Grundlage dienten die bekannten MALDI-Matrices 2,5-Dihydroxybenzoesäure (DHB), α-Cyano-4-Hydroxyzimtsäure (α-CHCA) und Sinapinsäure (SA).
Aus diesen konnten die Matrices DHBB und SATEA erfolgreich hergestellt werden. DHBB besteht aus DHB als Anion und Butylamin als Kation. SATEA setzt sich aus Sinapinsäure als Anion und Triethylamin als Kation zusammen. Die Charakterisierung der erhaltenen ionischen Flüssigkeiten erfolgte mittels ^{1}H-Kernspinresonanzspektroskopie.
Mit Hilfe dieser ionischen Flüssigkeiten konnten unterschiedliche Analyten unter Atmosphärendruck desorbiert/ionisiert und anschließend massenspektrometrisch detektiert werden. Die selbstgebaute Probenkammer erlaubte eine umfangreiche Optimierung unterschiedlicher experimenteller Parameter um einen empfindlichen und zugleich robusten Nachweis zu gewährleisten.

Neuartige Aspekte

Optimierung des Laserablationsprozesses in der AP-MALDI MS.
Verwendung ionischer Flüssigkeiten als Matrices in AP-MALDI MS.

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IR-MALDI-Ionenmobilitätsspektrometrie für den Nachweis von Neuroleptika und Pestiziden in Flüssigkeiten

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Einleitung

ESI und MALDI sind zwei Ionisationsmethoden, die erstmalig die fragmentfreie Ionisation großer biologisch relevanter Moleküle erlaubten. MALDI mittels gepulster UV-Laser wurde durch die Bowers-Gruppe in die Ionenmobilitäts (IM)-Spektrometrie eingeführt [1]. Mit der UV-MALDI gehen zwei wesent-liche Nachteile einher: eine aufwendige Probenvorbereitung und die fehlende Echtzeit-fähigkeit. Die Anwendung gepulster IR-Laser löst das erste Problem, die Probenvorbereitung, da die Biomoleküle in ihrer natürlichen Umgebung, im Wasser, untersucht werden können [2]. Die Anwen-dung der IR-Flüssig-strahl (IR-FL)-MALDI-Quelle löst das zweite Problem, die Echtzeituntersuchung der Proben, in Verbindung mit Fließinjektionssystemen [3,4]. In dieser Arbeit wird IR-MALDI erstmals zur direkten Unter-suchung wässriger Lösungen in die IM-Spektrometrie eingeführt. IR-MALDI beruht auf der Frei-setzung von Ionen nach Anregung der O-H-Streckschwingung der Wassermoleküle durch IR-Laser-pulse.

Methodik

In der Arbeit wurden zwei in der eigenen Werkstatt hergestellte IM-Spektrometer eingesetzt. Das erste IM-Spektrometer besteht aus der Ionisationsregion und einer Driftröhre, während das zweite IM-Spektrometer zusätzlich noch eine zweite Driftröhre zur Desolvatisierung der Ionen und ein Bradbury-Nielsen-Ionentor besitzt. Beide IM-Spektrometer können wahlweise mit zwei Ionisationsquellen ausgestattet werden: einer Tropfenquelle und einer Mikrostrahlquelle. Die Ionisationsregion kann zudem gepulst betrieben werden, was eine verzögerte Extraktion der Ionen in die Driftröhre erlaubt. Als IR-Laser wurde ein IR-OPO (IR-Opolette HE 2731, 20 Hz, Opotek Inc., Carlsbad, CA, USA) eingesetzt.

Vorläufige Daten

Schwerpunkt dieser Arbeit ist die Charakteri-sierung der IR-MALDI-Quelle mit dem Ziel der analytischen Anwendung sowie eines tieferen Verständ-nisses der Freisetzung der Ionen aus der Flüssigkeit und der resultierenden IM-Spektren. Der Einfluss physika-lischer Größen wie der Temperatur und der Leistungsdichte der IR-Strahlung auf Intensität und Form der Peaks in den IM-Spektren wird untersucht. Die leicht asym-metrischen IM-Peaks werden durch eine exponentiell modifizierte Gauß-Funktion quantitativ beschrie-ben. Mit zunehmen-der Temperatur in der Ionisationsregion werden die IM-Peaks schmaler und sym-me-trischer. Als opti-male Leistungsdichte wird ein Wert von $5 \cdot 10^7$ W cm^{-2} bestimmt. Weiterhin werden zwei Möglich-keiten untersucht, die vollständige Desolvati-sie-rung der Ionen über die Erhöhung der Desolvatations-zeit zu erreichen. Während die erste Methode auf der verzögerten Extraktion der Ionen aus der Ionisationsregion beruht, wird im zweiten Fall eine zusätzliche Driftstrecke zur Desolvatisierung vor der eigentlichen Driftröhre zur Trennung der Ionen imple-men-tiert. Analytische Kenngrößen der IR-MALDI-IM-Spek-tro-metrie, wie die Nachweisgrenze und der Linearitätsbereich des Nachweises verschiedener Neuro-leptika und Pestizide, werden mit der ESI-IM-Spektrometrie sowie der ESI-MS und IR-FL-MALDI-MS verglichen.

Neuartige Aspekte

Implementierung der IR-Flüssigstrahl-MALDI-Quelle in ein Ionenmobilitätsspektrometer und Charak-terisierung der Freisetzung der Ionen sowie analytischer Kenngrößen.

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Spray-Ionisation mit einer handelsüblichen Airbrushpistole

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Einleitung

Die Analyse von großen Biomolekülen bedarf einer schonenden Ionisationstechnik. Dazu kommen unter den Atmosphärendruck-(AP) Ionisationsmethoden bevorzugt MALDI und ESI zum Einsatz.[1,2]
Für ESI wird zwischen einer Kapillare und dem Einlass in das Massenspektrometer eine Spannung angelegt und durch die Kapillare geleitete Analytlösung fein zerstäubt. Durch Verdampfungs- und Ladungsabstoßungsprozesse werden Quasimolekülionen erzeugt. Vorteile sind die geringe Probenvorbereitung und die Möglichkeit einer direkten Kopplung zwischen Chromatographie und Massenspektrometrie. Erhaltene Spektren sind von Ladungsserien geprägt und können gut ausgewertet werden.
In einer kürzlich veröffentlichten Arbeit konnte gezeigt werden, dass bei IR-MALDI an Flüssigkeiten die Ionisation durch bloße Evaporation des Analyt-Lösungsmittelgemischs erfolgt.[3]
Um ein ähnliches Verhalten ohne Verwendung eines Lasers zu untersuchen, wurden Experimente zur Sprayionisation MS durchgeführt. Dabei wird ähnlich der ESI ein Aerosol gebildet, jedoch ohne dass eine Spannung anliegt. Der Aufbau ist somit einfach und kostengünstig. Eine Miniaturisierung sowie eine Kopplung der Spray-Ionisation an einen akustisch levitierten Tropfen spielen in der weiteren Forschung eine zentrale Rolle.

Methodik

Mit einer kommerziell erhältlichen Airbrushpistole (AB-200, Sogolee) wurden verschiedene Analytlösungen fein zerstäubt und die so entstandenen Aerosole ohne nachgeschaltete Ionisation in einem Atmosphärendruck-Flugzeitmassenspektrometer (HTOF, Tofwerk) untersucht. Es wurden Trägergasfluss, Analytzugabe, Abstand und Versatz unabhängig voneinander angepasst. Begleitend wurden mit bildgebenden Methoden die Größe und Verteilung der Tröpfchen in der Aerosolfahne analysiert. Um die zugrundeliegenden Prozesse mit ESI zu vergleichen, ist es möglich ein Potential (0-5kV) zwischen Aerosolquelle und Einlass des MS anzulegen.

Vorläufige Daten

Die mit diesem Aufbau erhaltenen Spektren zeigen Signale protonierter Analyten sowie die Bildung von Aggregaten. Die Signalintensitäten sowie das Aggregationsverhalten hängen dabei stark von den experimentellen Parametern wie dem angelegten Potential und dem pH-Wert der zerstäubten Lösung ab.
Die Methode ermöglicht es, Analyten aus unterschiedlichen Stoffklassen effektiv und mit guten Nachweisgrenzen zu ionisieren. So konnten beispielsweise Monosaccharide neben Peptiden unterschiedlicher Größe wie Aspartam (294 Da) und Lysozym (14.3 kDa) nachgewiesen werden. Bei großen Molekülen treten, ähnlich der ESI, bevorzugt Mehrfachladungen auf, kleine Moleküle werden bevorzugt aggregiert detektiert.
Die vorgestellten Ergebnisse demonstrieren eine vielseitige Anwendbarkeit der Sprayionisation für unterschiedliche analytische Fragestellungen. Zudem zeichnet sich die Methode durch die geringe Probevorbereitung und einen einfachen, robusten Aufbau aus.

Neuartige Aspekte

Massenspektrometrische Signale werden, ohne anlegen einer Spannung, allein aufgrund von Verdampfungsprozessen in einem Aerosol erhalten.

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Neuartiges Verfahren zur weichen Elektronenstoßionisation in einem Flugzeitmassenspektrometer

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Einleitung

Für die meisten Moleküle erreicht die Ionisierungswahrscheinlichkeit bei Elektronenstoßionisation bei Elektronenenergien im Bereich von 30-100 eV ein Maximum. Unterhalb von 30 eV fällt die Ionisierungswahrscheinlichkeit dagegen sehr rasch ab. Dies ist mit ein Grund, weshalb EI bislang nicht bei niedrigen Ionisierungsenergien eingesetzt wurde. Der zweite, schwerwiegendere Grund liegt in der geringen Elektronenausbeute, die durch die Potentialverteilung zwischen Filament und Ionenquelle verursacht wird und durch die freigesetzten Elektronen stark negativ beeinflusst wird. Durch die freigesetzten Elektronen bildet sich eine negative Ladungswolke um das Filament, die das ursprüngliche Potentialfeld zwischen Filament und Ionenquelle abschirmt [1].
Durch Optimierung der Potentialverteilung in einer Elektronenkanone ist es gelungen, die Elektronen-ausbeute bei Ionisierungsenergien bis nahe 10eV auf einem vergleichbar hohen Niveau wie bei harter Ionisation (70eV) zu halten. Diese Elektronenkanone wurde in ein TOF-Massenspektrometer integriert.

Methodik

Die neuartige Elektronenquelle wurde in ein Flugzeitmassenspektrometer (BenchTOF-dx, ALMSCO International [2]) integriert. Damit können Elektronen erzeugt werden, deren Energie über einen weiten Bereich abstimmbar ist. Diese Elektronen werden fokussiert und in eine Ionisierungskammer geleitet, die zugleich als Extraktionskammer dient. Die Erzeugung von Ionen direkt in der Extraktionskammer ohne die Notwendigkeit eines verlustbehafteten Ionentransfers ermöglicht maximale Transmissionsraten. Damit werden bei der Aufnahme des gesamten Massenspektrums Empfindlichkeiten erreicht, die im Bereich von SIM-Signalen liegen.Durch die geringe Fragmentierung bei Ionisierung mit niederenergetischen Elektronen werden Matrixinterferenzen unterdrückt. Durch die qualitativ veränderte Fragmentierung bei weicher und harter Ioniserung werden neue Unterscheidungsmerkmale zur Identifizierung von Substanzmustern erzeugt. Dies reduziert Matrixeinflüsse, verbessert das Signal/Rausch-Verhältnis und die Detektionsgrenzen in der Spurenanalytik.

Vorläufige Daten

Mit der o.g. Anordnung wurden sowohl GC/MS als auch GCxGC/MS-Analysen bei harter und weicher Ionsierung durchgeführt. Damit wurden verschiedene Stoffgruppen (Alkane, Aromaten, PCBs etc.) charakterisiert. In allen Fällen konnte bei weicher Ionisierung eine deutliche Verringerung der Fragmentierung und eine starke Erhöhung des Molekülions gegenüber der 70eV Ionisierung erreicht werden. Für manche Substanzen kann die Fragmentierung ganz unterdrückt werden, so dass lediglich das Molekülion und der Isotopenpeak generiert werden. Gleichzeitig auftretende isobarische Substanzen, die aus der Chromatographiesäule koeluieren (z.B. PCB Kongenere in Kombination mit Dioxinen), können durch die Unterdrückung der Fragmentierung besser identifiziert werden. Quantitative Vergleiche des Molekülions bei harter und weicher Ionisierung ergeben absolute Intensitäten in der gleichen Größenordnung. Damit besteht die Möglichkeit durch schnelles Umschalten der Ionisierungsenergien quasi-kontinuierliche harte und weiche Chromatogramme zu erzeugen. Die komplementären Informationen aus diesen Chromatogrammen können für die Auswertung komplexer Matrices genutzt werden. Durch die Verwendung einer universellen Ionisierungsmethode, die sich gleichzeitig für harte und weiche Ionisierung eignet und über die Steuerung von elektrischen Feldern schnell umschaltbar ist, eröffnen sich vielfältige Möglichkeiten für GC/MS- und GCxGC/MS-Anwendungen.

Neuartige Aspekte

Eine neuartige Elektronenoptik ermöglicht den Einsatz einer weichen Elektronenstoßionisation für Ionisierungsenergien unterhalb 20eV bei hohen Elektronenausbeuten für GC/MS Anwendungen.

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Ultra-high resolution mass spectrometry without super-conducting magnet: Studying the derivatization reaction in crude oil with a research-type Orbitrap MS

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Einleitung

The development of societies throughout the world is driven by the need for affordable energy. Until now fossil-based materials remain the major source of energy because of its convenience and comparatively low cost.[1] This includes more extensive use of heavier fractions of crude oil that have not been valued as highly in the past. Due to a high amount of heteroatoms such as sulfur and nitrogen in the high-boiling fractions, these resources need more chemical upgrading. Major efforts are directed toward analyzing and characterizing the polyaromatic heterocycles in crude oil to facilitate chemical upgrading processes. [2]
Owing to the incredible complexity of crude oil mixtures, ultra-high-resolution mass spectrometry is the method-of-choice for crude oil analysis. Now, instead of the standard instrument for ultra-high resolution – FT-ICR MS – an additional instrument is available that is capable of reaching the necessary resolutions and accuracies. A high-field Orbitrap analyzer that is capable of longer transients provides more comprehensive and indispensable data. Meanwhile selective derivatization methods are developed to combine analytical methods with a selective chemical component.

Methodik

10 mg bitumen and 8 eq. C_2H_5I were dissolved in 250 µL dry 1, 2-dichloroethane (DCE). A solution of 2 eq. silver tetrafluoroborate in 120 µL DCE was added and yellow AgI precipitated immediately. After 24 h, the precipitate was removed by centrifugation and washed with 800 µL DCE.
Mass spectra were recorded using LTQ-Orbitrap Elite mass spectrometry (Thermo Fisher Scientific, Bremen) that is capable to run transients up to 3s, allowing a resolution 800 000 at *m/z* 400 and equipped with an ESI source. The solution of 25 µL sample with 1 mL dichloromethane was injected by syringe with a flow rate 5 µL/min at a voltage 5 kV. External mass calibration was performed using a tune mix solution.

Vorläufige Daten

The derivatization of crude oil samples allows ionizing non-polar components that are otherwise not ionizable by electrospray. Thiophenium salts are being formed during the derivatization reaction that dissociate in solution and therefore, show pre-formed ions that allow the ionization of such non-polar components. Different reactands were investigated to study these derivatization reactions to see if the selectivity towards a class of components in a very complex crude oil mixture is given. For this, different alkylating reactands were studied, including methyl iodide, D3-methyl iodide, ethyl iodide, *n*-propyl iodide and *i*-propyl iodide. All reactands were studied using both a number of selected standard compounds and different crude oil samples.
The yield of alkylation reached >99% when using ethyl iodide as alkylating agent. Additional studies with crude oil samples show that sulfur compounds show up a high selectivity for the derivatization reaction. Additionally, nitrogen components could be ionized when sulfur species were present.
With this procedure it is possible to characterize the types and classes of compounds present in crude oil fractions with a high selectivity for sulfur-containing and nitrogen-containing polyaromatic heterocycles and the high field Orbitrap MS allows to follow the details of this reaction without the use of a superconducting magnet.

Neuartige Aspekte

Orbitrap Elite mass spectrometry coupled with Electrospray ionization aided by derivatization was used to characterize the crude oil.

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Impact of source geometry on signal intensities of ions generated by low temperature plasma ionization for mass spectrometry

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Einleitung

Recent introduction of ambient ionization techniques, i.e. ionization techniques working at atmospheric pressure and open to the ambience, revolutionized applications in mass spectrometry. Minimizing the overall analysis time, biological material theoretically can now be introduced *as is* and then rapidly analyzed, so that unwanted metabolic alterations before analysis are kept to a minimum. Ionization such as by low temperature dielectric barrier discharge plasma (LTPI) [1] belongs to these ambient techniques; its mild, non-invasive conditions and the easy set up are very promising for future applications with the potential to ionize compounds even from living tissue [2]. In a dielectric barrier discharge (DBD) plasma, at least one of the electrodes is separated by an insulator from the plasma gas [3]. The gas is hardly heated by the discharge, it can spread over a larger area, and the current is limited by rapid collapse of the arc discharges. Consequently, high gas flows are not required for cooling the electrode like in the “plasma jet”. In order to design an optimal set up, we investigated the dependence of signal intensities in the mass spectrum on different configurations of this cold plasma ion source.

Methodik

A homemade DBD plasma source was used employing a borosilicate capillary, supplied with an AC voltage of 7.5 kV at a frequency of 25 kHz and helium 5.0 as plasma gas (monitored by flow meter Digital Flow Check, Alltech). The device was coupled to an Esquire 3000^+ ion trap MS from Bruker Daltonics (software Data Analysis 3.3, Esquire Control 5.3). Different parameters of the plasma source were investigated to impact signal intensity of ionization products of air and a reference substance, respectively: flow rate of the plasma gas, distance between the electrodes, electrode width, outlet distance, strength of the dielectric, distance of the sample to the MS inlet, distance of the plasma source to the sample, and different target materials.

Vorläufige Daten

Increasing gas flow rates up to 20 mL/min caused a hyperbolic increase in signal intensity of ions originating from plasma ionization of air; above this flow rate there was no further increase in signal intensity. No effect on the signal intensity was observed increasing the distance of the electrodes to each other until plasma ignition failed. Signal intensity was slightly decreased with wider electrodes; however, with wider electrodes the discharge zone between the electrodes moves further away from the outlet of the plasma source. With decreasing distance of the electrodes to the outlet of the plasma source, signal intensities were increased; however, shorter distances of the electrodes to the outlet require better insulation of the electrodes in order to prevent flashover. In comparison, signal intensity showed an exponential decay with increasing distance of the sample to the MS inlet and the distance of the plasma source to the sample, eventually due to reactive collisions of the generated ions with air molecules on their way to the MS inlet or the sample, respectively.
A thinner dielectric lead to higher signal intensities on one hand, but on the other hand to higher temperatures presumably due to the increased charge transfer and impaired heat dissipation causing instability of the plasma source capillary.
Teflon, glass, and paper were tested as target materials for LTPI. Whilst paper showed the most uniform distribution upon drying of the reference substance, it also had the highest background noise compared to Teflon and glass. Teflon and glass as target materials provided good signal-to-noise ratios with very low background signal intensities; but on the other hand, the reference substance on the target formed a crystalline ring upon drying, similar to MALDI.

Neuartige Aspekte

We built a DBD-LTP ion source for ambient mass spectrometry and identified critical parameters for optimization of its geometry.

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HDMS Compare Software Tool for Complex Materials Comparisons

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Einleitung

Many industries routinely handle complex samples on a daily basis. Examples of such set of analytes include crude oil, bio-oil, crude oil fractions and derivatives, advanced polymer blends, and complex chemical formulations. Typically, numerous analytical tools are used to investigate and characterize these samples – including various mass spectrometric techniques. The data acquired reflect the complexity of the materials being analyzed and contain myriad components. Consequently, data analysis is a significant challenge, requiring powerful and intuitive tools that are functional and easy to use on a routine basis. Frequently, it is necessary to make comparisons between complex samples; for example when a particular compound fails to perform correctly, or the same sample has been treated or processed in different ways. In these cases, a visual comparison of data from the samples is extremely difficult, and identifying key differences is particularly problematic. Here we present a powerful software tool in combination with ion mobility (IM) to resolve the sample complexity.

Methodik

The analysis of different asphaltene samples was accomplished using a SYNAPT G2 HDMS QTOF mass spectrometer equipped with ion mobility cell (Waters) and an inlet system consisting of a so-called ASAP probe (Waters) for sample introduction. The asphaltenes were mixed into a thick slurry using toluene as solvent. The ASAP probe was dipped into the slurry and inserted to the source of the mass spectrometer. Ionization of the samples was achieved via traditional Atmospheric Pressure Chemical Ionization (APCI) mechanisms. A manual, stepwise temperature ramp was implemented over the range 150 °C to 650 °C, with 100° increases after every minute elapsed and a total acquisition time of 10 minutes. The ion mobility data were then processed using HDMS Compare software.

Vorläufige Daten

A novel software approach with HDMS Compare was successfully applied to make comparisons between complex samples using hybrid technologies as ion mobility and time of flight mass spectroscopy data. ASAP-IM-MS(/MS) is a fast analytical tool for identification or control of specific compounds in oil samples. In the usual approach the TIC and full spectra for the two acquisitions looked broadly similar. With HDMS Compare the key areas of significant differences between the two samples were clearly visualized and identified with two different, contrasting colours. This visualization is called Fusion Map and showed the ions with more significance in each sample. Regions of interest were easily selected in “slices”, or expanded in the Zoomed Map view – this allowed further detailed interrogation of important sample differences. Selected ions or regions of interest can be exported to MassLynx, e.g. for structure elucidation. HDMS Compare software offers analysts, who are frequently required to draw important analytical conclusions based on comparisons between complex samples, a powerful and versatile interactive tool to facilitate their decision making workflow.

Neuartige Aspekte

New software for comparison and characterization of complex samples analyzed by direct sample introduction and ion mobility MS is described.

Food packaging: Strategies for rapid phthalate screening in real time by ambient ionization tandem mass spectrometry

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Einleitung

Phthalate monitoring in food stuffs is of great interest to monitor for regulated phthalates that could leach into food commodities from packaging and food processing. The inherent abundance of phthalates in the environment presents analytical challenges due to the high risk for sample contamination ranging from the equipment used for sample preparation to high risk for carryover. Ambient ionization permits ionization of samples with little to no sample preparation, which presents the means to directly ionize samples including packaging, as well as the food commodity itself. The Direct Analysis in Real Time (DART) ambient ionization technique coupled with tandem mass spectrometry offers the ability to rapidly fingerprint phthalates in food packaging and screening for phthalates in food matrices. Characteristic MS/MS fragment patterns in combination with the rapid DART ionization technique coupled with the high scan speed of the latest ion trap mass spectrometer enables a very high throughput analytical method.

Methodik

A fourth generation DART ionization source (DART-SVP) was fitted with a motorized sampling set-up and interfaced to a Velos Pro ion trap mass spectrometer for a rapid MS/MS phthalate screening method. Ten phthalate standards were directly analyzed in liquid form from glass melting point capillary tubes in order to optimize the MS/MS instrument parameters. Various collected food packaging ranging from lid gasket seals, plastics wrapping, styrofoam cups, plastic utensils and exercise equipment were directly scrutinized for the presence of regulated phthalates without any sample treatment. Analysis time per sample was less than 10 seconds per sampled area on the objects.

Vorläufige Daten

Several of the regulated phthalates, which are isomeric, needed to be clearly distinguished from one another, especially if there could be a mixture of phthalates in a single sample. Simply screening for the presence of these compounds in full MS mode, even with high resolution instruments is not enough without any separation methods in the case of the DART direct ionization technique, and in order to differentiate between the compounds there is a huge advantage in generating clean MS/MS spectra. Of the nearly 25 consumer products screened using the DART-MS/MS method the most commonly observed phthalates were DnOP, DEHP. DiDP and DiNP, all of which are regulated in the US and European Union countries [1] in food contact material at the low ppm detection level.

Neuartige Aspekte

Direct screening and confirmation of the presence of regulated phthalates in food packaging and consumer products requiring no sample preparation.

Referenzen

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Untersuchungen zu Matrixeffekten bei der LA-ICP-MS bei der Analyse von Kunststoffen

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Einleitung

Die Laserablation gekoppelt mit der Massenspektrometrie mit induktiv gekoppeltem Plasma (LA-ICP-MS) ist eine vielversprechende Technik, die die Probenvorbereitungszeit in der Elementspurenanalyse von Feststoffen signifikant reduzieren kann. Die Anwendungsgebiete reichen von der Geochemie, über die biomedizinische Forschung bis hin zu den Materialwissenschaften. Trotz allem hat sich die Laserablation bei der Analyse von Kunststoffen wegen des Mangels an matrixangepassten zertifizierten Referenzmaterialien noch nicht durchsetzen können. Umfangreiche Studien der FH Münster zeigten, dass verschiedene Kunststoffe sehr unterschiedlich mit der Laserstrahlung wechselwirken [1]. Das oft als interner Standard verwendete Kohlenstoffisotop ^{13}C reicht dabei nicht aus, um diese Unterschiede zu korrigieren [2]. Als ein Teil des MaxLaP-Projektes (Matrixeffekte bei der Laserablation von Polymeren) wurden matrixangepasste Standards auf Polyethylen- und Acrylnitril-Butadien-Styrolbasis entwickelt und diese für die Kalibrierung der LA-ICP-MS genutzt. Anhand dieser Materialien werden die Matrixeffekte bei der Laserablation näher untersucht.

Methodik

Mittels Laserablation können Materialmengen im ng - Bereich von Kunststoffmaterial abgetragen und der Analyse mittels ICP-MS zugeführt werden. Die zur Kalibrierung des Verfahrens notwendigen Standards wurden mittels Knetkammer und Schneckenextruder hergestellt. In die Standards wurden Br, Cd, Cu, Cr, Fe, Sb in rein organischer, rein anorganischer und gemischter Form ihrer Verbindungen eingearbeitet. Die quantitative Zusammensetzung der Materialien wurde mittels ETV-ICP-OES, DC-arc-OES, RFA und ICP-MS nach Mikrowellendruckaufschluss überprüft. Die Homogenität der Einarbeitung wurde mittels µ-SyRFA und LA-ICP-MS untersucht.

Vorläufige Daten

Die Analyse der Standardmaterialien zeigte eine quantitative Einarbeitung der Analyten in die Polymermatrix. Dabei ließen sich die organischen Verbindungen homogener als ihre anorganischen Vertreter einarbeiten. Die Kalibrierung der LA-ICP-MS ist mit diesen Standardmaterialien möglich.
Zur Untersuchung der Matrixeffekte während der LA-ICP-MS und der matrixunabhängigen Kalibrierung für Kunststoffe wurden der Einfluss der chemischen Verbindung der Additive, die Größe der bei der Laserablation gebildeten Partikel und die Art des Kunststoffes auf die Laserablation analysiert. Es konnte gezeigt werden, dass die chemische Form der Additivzusätze einen Einfluss auf die Sensitivität der Analyse hat, da sich die Additive in unterschiedlich großen Partikeln des Laseraerosols anreichern.

Neuartige Aspekte

Produktion von dotierten Kunststoffstandardmaterialien zur Kalibrierung der LA-ICP-MS und Untersuchung von Matrixeffekten dieser Technik

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First field application of an on-line TD-REMPI-SP-TOFMS setup for detection of particle bound polycyclic aromatic hydrocarbons

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Einleitung

Laser based mass spectrometry became an important tool for the on-line detection of individual single aerosol particles. The detection of health relevant organic molecules is often complicated due to a high degree of fragmentation in single particle laser mass spectrometry. Therefore, an analytical tool that uses soft ionisation techniques would be desirable. To accomplish this, mainly used one-step laser desorption/ionisation can be replaced by a two-step approach. Therefore, particle bound compounds can be volatilised by thermal desorption by using a heated metal surface. A subsequently triggered laser pulse can be then used for a soft laser ionisation reducing the degree of fragmentation. The chosen wavelength and intensity of the laser pulse can influence the selectivity of the ionisation process.

Methodik

A custom designed aerosol mass spectrometer was used. It consists of a differentially-pumped inlet system that removes the gas phase of an aerosol and forms a particle beam that crosses two cw-laser beams for laser velocimetry. Hereby, the light scattered by single particles (SP) is detected with photo-multipliers. With a known distance of the laser beams the velocity of individual particles and their aerodynamic diameter are calculated. Afterwards, particles impinge on a heated metal surface (500°C) for thermal desorption (TD). According to the individual particle velocity, a KrF-excimer laser (λ=248nm) is triggered a few microseconds delayed for resonance enhanced multi-photon ionisation (REMPI) selective for polycyclic aromatic hydrocarbons (PAH). Ions are analysed and detected with a reflectron time-of-flight mass spectrometer (TOFMS).

Vorläufige Daten

The TD-REMPI-SP-TOFMS setup was successfully tested for the first time in the field during a measurement campaign in Augsburg, Germany in winter 2010 with the aim to analyse the impact of domestic wood combustion on ambient aerosol with an urban background and to compare the results with other instruments like the Aerodyne aerosol mass spectrometer (AMS) and off-line filter samples, that were later analysed with GC-MS techniques. In difference to other methods, there was a special focus on aerosol particles with aerodynamic diameters larger than 1 µm with the TD-REMPI-SP-TOFMS setup. About 360.000 particle events were recorded with this method during the campaign. Data analysis of the acquired mass spectra showed, that PAH could be found on 15.7% of the observed particles. 2.1% of the spectra showed besides peaks typical for PAH a peak at m/z=234, that is dedicated to retene, a C_4-alkylated derivate of phenanthrene. Retene is a marker for combustion of coniferous wood. The comparison to results of other instruments that were used during the campaign, showed that there are differences in the time series, diurnal variation of occurrence and size distributions of PAH-containing and PAH-free particles. AMS data and off-line filter samples detected wood combustion related aerosols during the evening ours, while TD-REMPI-SP-TOFMS data detected them during rush hours. That indicates that mainly agglomerates and particles re-suspended by traffic were detected with this method.

Neuartige Aspekte

The TD-REMPI-SP-TOFMS setup was successfully applied in a field campaign for on-line detection of particle bound PAH with source apportionment.

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Chemische Gasphasenionisation auf Basis eines nicht-radioaktiven pulsbaren Elektronenstrahlers

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Einleitung

In vielen Anwendungsgebieten der Gasanalyse oder Gasdetektion sind Messsysteme erforderlich, welche sowohl eine hohe Sensitivität, als auch eine ausreichende Selektivität und kurze Antwortzeiten aufweisen. Anwendungsbeispiele hierfür sind die Analyse von Atem [1], die Qualitätskontrolle und die Sicherheitstechnik. Ein Ansatz hierfür, ist die Kombination der chemischen Gasphasenionisation, welche zu extrem geringen Nachweisgrenzen für viele Substanzen [2] führt, mit der Flugzeit-Massenspektrometrie, welche eine geeignete Trennung der meisten Analyte leisten kann. Allerdings führen konkurrierende Ionisationsprozesse zu einer Diskriminierung von Substanzen mit einer – je nach betrachteter Polarität - im Verhältnis geringeren Protonen- oder Elektronenaffinität, weshalb bei einer Ionisation bei Atmosphärendruck häufig eine Vortrennung des Stoffgemisches durch einen Gaschromatographen erforderlich ist. Um eine simultane Detektion der Analyte durchführen zu können, muss die Anzahl der für einen Ladungsaustausch zur Verfügung stehenden Reaktantionen daher deutlich über der Anzahl der Analytionen liegen [3]. Mögliche Ansätze hierfür sind PTR-MS [3] und SIFT-MS [4], welche jeweils auf einer Hohlkathoden- oder einer Mikrowellen-Entladung basieren. In dieser Arbeit stellen wir eine Methode vor, welche auf der Ionisation durch einen Elektronenstrahler basiert. Dabei ist es möglich, sowohl Anionen als auch Kationen zu untersuchen. Weiterhin besteht durch ein Pulsen des Elektronenstrahlers die Möglichkeit, die Reaktionskinetik der Substanzen zu untersuchen [5] und diese ggf. als weiteres Trennungsverfahren zu verwenden.

Methodik

Für die Ionisation der Reaktantionen wird ein pulsbarer Elektronenstrahler (Optimare Analytik) verwendet. Die mittlere kinetische Energie der Elektronen beträgt dabei um 10keV. Das Analytgas sowie Wasserdampf werden durch einen Massenflussregler in den Ionisationsraum geleitet. Dieser hat eine Länge von ungefähr 100 mm und einen Durchmesser von 16,2 mm und besteht aus 15 Elektroden. Diese dienen dazu, ein elektrisches Feld aufzubauen, welches die sich bildenden Ionen in Richtung des Transferbereichs zum Massenspektrometer transportiert und gleichzeitig einer Bildung von Wasserclustern entgegenwirkt. Der Feldverlauf kann jeweils an eine Detektion der Anionen oder Kationen angepasst werden. Der Druck im Ionisationsraum kann eingestellt werden. Am Ende des Ionisationsraumes gelangen die Ionen durch ein Pinhole in einen Transferbereich und von dort in ein lineares Flugzeit-Massenspektrometer.

Vorläufige Daten

Erste Ergebnisse zeigen, dass eine Ankopplung des Ionisationsraumes an das Massenspektrometer erfolgreich durchgeführt werden konnte. Der Transferbereich und das Massenspektrometer sind dabei so ausgelegt worden, dass Massen von unter 19 u (H_3O^+) bis 117 u ($((CH_3H_6O)_2H^+)$) detektiert werden konnten. Prinzipiell ist auch eine Detektion größerer Massen möglich, bisher aber noch nicht vorgenommen worden. Untersuchungen der sich im Wasserdampf ohne die Zugabe eines Analytgases bildenden Kationen zeigen, dass sich je nach Druck im Ionisationsraum und je nach dort anliegendem elektrischen Feld, unterschiedliche Wassercluster im Massenspektrometer nachweisen lassen. So wird bei 2 mbar und 120 V/cm hauptsächlich das Masse-zu-Ladungs-Verhältnis m/z = 19 aufgezeichnet, welches dem protonierten Wassermolekül (H_3O^+) zugeordnet werden konnte. Bei einer Verringerung der Feldstärke treten vermehrt auch größere Wassercluster auf. Aufgrund der vergleichsweise geringen Protonenaffinität von H_3O^+ stellt dieses Reaktantion die Grundlage für die später gewünschte Gasphasenionisation durch Protonierung dar. Eine gezielte Erzeugung großer Mengen von H_3O^+ durch die ständige Fragmentierung größerer Wassercluster soll daher die gleichzeitige Ionisation mehrerer Analyte ermöglichen. In diesen Versuchen konnten ebenfalls geringe Spuren einer Substanz m/z = 32 nachgewiesen werden. Diese werden auf Restgas im Wassertank des Aufbaus zurückgeführt. Wahrscheinlich handelt es sich daher dabei um O_2^+. Durch die Zufuhr von mit Aceton angereicherter Luft konnte bei 2 mbar und 120 V/cm im Spektrum zusätzlich m/z = 59 nachgewiesen werden. Dabei handelt es sich wahrscheinlich um das protonierte Aceton ($((CH_3H_6O)H^+)$), welches in dieser Form typischerweise bei einer chemischen Gasphasenionisation auftritt.
Durch die Steigerung der elektrischen Feldstärke vor dem Einlass der Ionen in den Transfer konnte weiterhin eine Fragmentierung des Acetons durchgeführt werden. Dabei traten m/z = 15, m/z = 31 und m/z = 43 zusätzlich im Spektrum auf. Eine gezielte Fragmentierung der jeweiligen Ionen könnte bei der späteren Identifikation einer unbekannten Substanz verwendet werden.

Neuartige Aspekte

Die Verwendung eines nicht-radioaktiven pulsbaren Elektronenstrahlers für die chemische Gasphasenionisation bei 2 mbar zur Bildung von Anionen und Kationen

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Determination of trace-level odorous components from a polluted air sample using a hyphenated TD–GC/TOF MS technique and data analysis software

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Einleitung

The release of odorous compounds into the environment can cause great nuisance to communities and have a negative impact on human health.
These substances often have very low (ppb) odor thresholds and can be difficult to identify within the complex matrix of a polluted air sample. Furthermore, some of these species, particularly sulfur compounds, can be labile during analysis in non-inert systems.Traditionally, detection of odorous species has involved the use of olfactometry. However, this method is subjective to the human nose, and therefore a robust chromatographic method is desirable.
In this poster, we describe the use of thermal desorption (TD)–gas chromatography to analyse an air sample collected from the waste outlet of a biofuel plant.

Methodik

A 5 L sample of polluted air was mixed with 15 L of clean nitrogen contained within a nalophane bag. From this, a sample volume of 1000 mL was pumped onto an inert-coated three-bed sorbent tube packed with quartz wool, a porous polymer (Tenax® TA) and a molecular sieve (SulfiCarb™). Combined, these inert sorbents are capable of retaining labile analytes over a wide volatility range (C_3 to n-C_{30}). For thermal desorption a TD 100 instrument from Markes was used, analytes were detected using a BenchTOF-dx GC-TOF MS from Almsco International coupled to a Thermo Trace Ultra GC.

Vorläufige Daten

1000 mL polluted air was re-collected and re-analysed under low-split conditions. The sample was interrogated for sulfur compounds using TargetView™ compound identification software. TargetView uses advanced algorithms to eliminate unwanted background, identify individual peaks in complex chromatograms, and match them to customised target libraries or large commercial libraries. Eight sulfur compounds were identified within the sample from a 22-compound library. Several odorous compounds were identified, some at trace levels, including methanethiol, dimethyl disulfide and dimethyl trisulfide. Each identified compound showed a strong spectral agreement with the library spectra. Other species (*e.g.* solvents) were observed at higher levels.
The combination of an inert-coated TD flow path, inert tubes and re-collection capabilities of Markes' TD instrumentation has preserved trace-level sulfur species following sample pre-concentration. The inherent sensitivity of BenchTOF-dx and the production of reference-quality spectra has allowed TargetView software to identify odorous species at low levels and with high confidence in a complex polluted air sample.

Comprehensive 2D-GC combined with the latest developments in time-of-flight MS analysis for improved speciation of compound classes in petrochemical analyses

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Einleitung

Precise characterisation of petrochemical samples is crucial for quality control, and also to understand the reactions that take place during refining processes.Comprehensive two-dimensional gas chromatography (GC×GC) offers significant advantages over conventional chromatography for such analyses, with its vastly expanded separation space and the added benefit of highly structured groupings of compound classes.
However, classifying compounds solely according to their presence in simple graphical regions may not always be reliable. For example, the well-structured alkylbenzene classes show significant crossover with indanes and cycloalkanes, making construction of 'templates' for automated compound identification challenging.
This study shows how chemical qualifiers can ease the construction of the sophisticated 'templates' needed for reliable automated compound classification in GC×GC/TOF MS analyses.

Methodik

Experiments were conducted on a BenchTOF-dx GC-TOF MS from Almsco International coupled to an Agilent 7890A GC with a Zoex ZX1 thermal loop modulator. The primary oven was set at 60°C for 2 min, then 1.5°C/min to 153°C for 0 min, then 2°C/min to 280°C for 17.5 min. Colums used were HP PONA (50 m × 200 µm × 0.5 µm) and SGE SolGelWax (2.5 m × 100 µm × 0.1 µm), as required by UOP method 990-11 for the analysis of distillates. The mass spectrometer was operated at a data rate of 50 Hz with a mass range 40 – 400 amu.

Vorläufige Daten

A UK diesel sample was analysed by GC×GC coupled to a TOF MS (BenchTOF-dx, ALMSCO International), resulting in a complex chromatogram containing thousands of peaks .
Computer Language for Identifying Chemicals (CLIC) expressions were used to identify key chemical classes, such as the alkylbenzenes and alkylnaphthalenes
CLIC incorporates a number of chemical identifiers (using both chromatographic and mass spectral characteristics) to allow fast and accurate classification of analytes of interest.
Incorrect classifications were minimal, despite close elution with other chemical classes.
The combination of sub-unit mass resolution, accurate relative intensities and powerful software allows chemical class templates to be prepared automatically using mass spectrometric chemical identifiers. This significantly minimises the time spent on data processing, and allows easy construction of templates for screening subsequent samples
The 'classical' EI spectra produced by BenchTOF-dx allow confident identification of target analytes even within highly complex matrices. In particular, accurate relative intensities minimise the likelihood of incorrect identification

Neuartige Aspekte

BenchTOF-dx generates 'classical' EI spectra, enabling comparison with libraries while delivering maximum sensitivity at high data rates demanded by GCxGC.

Developing the Universal Microarray: Analysis of Immobilized Peptides with Nano-assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry

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Einleitung

Our vision is to develop a universal microarray enabling the analysis of various kinds of biomolecules. This universal microarray would allow the investigation of many different biological reactions on one single slide. To our mind, the only way to realize this vision is the development of a microarray that can be analyzed by mass spectrometry.
We present here a promising approach that is based on the use of a nanostructured silicon surface as solid support for the covalent immobilization of a peptide-photolinker construct. This kind of nanostructured surface is known to mediate the desorption and ionization of biomolecules in laser desorption/ionization mass spectrometry [1].

Methodik

A suitable NALDI support was produced by subjecting (111)Si wafer to a metal-assisted chemical etching process, yielding a porous Si surface (pSi) with Si nanowires [2]. The NALDI support was silanized in order to implement epoxy groups on the surface. Using the epoxy groups, a peptide-photolinker construct was covalently attached. The enzymatic deacetylation of the peptide by a histone deacetylase was selected as a model reaction, carried out directly on the nanostructured Si surface. After all, NALDI-TOF mass spectra were acquired with a MALDI-TOF/TOF mass spectrometer (UltraflexII, Bruker Daltonics).

Vorläufige Daten

We were able to show that the produced nanostructured Si surface can be used in NALDI-TOF mass spectrometry. On the NALDI support the peptide-photolinker constructs were successfully immobilized. The analysis by NALDI-TOF mass spectrometry showed that the cleavage of the photolinker can be induced by the laser of the mass spectrometer and that a high-quality mass spectrum can be obtained for the released peptide. In addition, we were able to show that the enzymatic deacetylation of the immobilized peptide by a histone deacetylase is working on the NALDI support.
One of the great advantages of the approach presented herein lies in the fact that the mass spectrometry measurements are performed in absence of any matrix compound. This enables tandem experiments (every spot is analyzed for several times) to simulate multistep enzyme reactions and biological pathways.

Neuartige Aspekte

Analysis of peptides covalent immobilized on a nanostructured Si surface with NALDI-TOF mass spectrometry.

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