



48. Jahrestagung der Deutschen Gesellschaft für Massenspektrometrie

01. bis 04. März 2015
Wuppertal



Datum: Sonntag, 01.03.2015

13:45	Workshop Ionisation unter Atmosphärendruck Teil 1	Workshop Lipidomics und Lipidanalytik Teil 1
-	Ort: I28	Ort: I27
15:15	Chair: Hendrik Kersten	Chair: Robert Ahrends, Dominik Schwudke
15:15	Kaffeepause	
-	Ort: Foyer	
15:45		
15:45	Workshop Ionisation unter Atmosphärendruck Teil 2	Workshop Lipidomics und Lipidanalytik Teil 2
-	Ort: I28	Ort: I27
17:15	Chair: Hendrik Kersten	Chair: Robert Ahrends, Dominik Schwudke
17:15	Tagungseröffnung	
-	Ort: K33	
18:00		
18:00	Wolfgang-Paul-Vorlesung	
-	Ort: K33	
19:00		
19:00	Welcome Reception	
-	Ort: Foyer	
22:30		

Präsentationen

Wolfgang-Paul-Vorlesung

Zeit: Sonntag, 01.03.2015: 18:00 - 19:00 · Ort: K33

Midwinter Reflections on the Progress of Biological Mass Spectrometry

Catherine E. Costello

Boston University School of Medicine, USA; cecmsms@bu.edu

Datum: Montag, 02.03.2015

8:30	Plenarvortrag I: Ionization at atmospheric pressure: why and how		
-	Ort: K33		
9:15			
9:15	Ola: Bildgebende Massenspektrometrie I	Olb: Umwelt- & Lebensmittelanalytik I	Olc: Grundlagen der Massenspektrometrie
-	Ort: K33	Ort: K32	Ort: I26
10:15			
10:15	Agilent Research Summer		
-	Ort: K33		
10:45			
10:45	Kaffeepause		
-	Ort: Foyer		
11:10			
11:10	Olla: Proteomics I	Ollb: Metabolomics	Ollc: Instrumentelle Entwicklungen
-	Ort: K33	Ort: K32	Ort: I26
12:50			
12:50	Mittagspause / Lunchseminare		
-			
14:20			
14:20	Plenarvortrag II: How to speed up high-performance mass spectrometry		
-	Ort: K33		
15:05			
15:05	Wolfgang-Paul-Studienpreis		
-	Ort: K33		
15:50			
15:50	Kaffeepause		
-	Ort: Foyer		
16:05			
16:05	Ollla: Ionisationsmethoden I	Olllb: Lipide & Phospholipide I	Olllc: Gasphasenreaktionen, Fragmentierungen & Strukturaufklärung
-	Ort: K33	Ort: K32	Ort: I26
17:25			
17:25	Kaffeepause		
-	Ort: Foyer		
17:40			
17:40	Gastvortrag: Der Weg wissenschaftlicher Erkenntnisse in die Politik - oder wie wird Wissenschaft wirksam?		
-	Ort: K33		
18:40			
18:40	PI: Postersession I		
-	Ort: Foyer		
20:25			

Präsentationen

Plenarvortrag I

Zeit: Montag, 02.03.2015: 8:30 - 9:15 · Ort: K33

Ionization at atmospheric pressure: why and how

Andries Bruins

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Ola: Bildgebende Massenspektrometrie I

Zeit: Montag, 02.03.2015: 9:15 - 10:15 · Ort: K33

Ola1: MALDI-2: Sensitive MS Imaging with Laser-Induced Postionization at 5 Micrometer Pixel Size

Jens Soltwisch¹, Hans Kettling^{1,2}, Simeon Vens-Cappel^{1,2}, Marcel Wiegelmann¹, Johannes Müthing^{1,2}, Klaus Dreisewerd^{1,2}

¹Institute for Hygiene, University of Münster, Germany; ²Interdisciplinary Center for Clinical Research (IZKF), University of Münster, Germany; dreisew@uni-muenster.de

Ola2: Spatially resolved detection of contact and systemic pesticides in plants using DESI-MSI

Stefanie Gerbig¹, Hubertus Brunn², Bernhard Spengler¹, Sabine Schulz¹

¹Justus Liebig Universität Gießen, Deutschland; ²Landesbetrieb Hessisches Landeslabor, Gießen, Deutschland; stefanie.gerbig@anorg.chemie.uni-giessen.de

Ola3: Mass spectrometry imaging for characterizing parasite host interactions in malaria.

Saleh Mahmud Khalil¹, Andreas Römpf¹, Jette Pretzel², Katja Becker², Bernhard Spengler¹

¹Institute of Inorganic and Analytical Chemistry, Justus Liebig University Giessen, Germany; ²Biochemistry and Molecular Biology, Interdisciplinary Research Center, Justus Liebig University Giessen, Germany; saleh0207@gmail.com

Olb: Umwelt- & Lebensmittelanalytik I

Zeit: Montag, 02.03.2015: 9:15 - 10:15 · Ort: K32

Olb1: Analyse von Fruchtsäften mittels HPLC/CZE-IMS-qTOF-MS

Oliver Schmitz, Susanne Stephan

Universität Duisburg-Essen, Angewandte Analytische Chemie; oliver.schmitz@uni-due.de

Olb2: A non-targeted LC-HRMS multi-method for the detection of food allergen trace contaminations

Robin Korte, Jens Brockmeyer

Westfälische Wilhelms-Universität Münster, Deutschland; r.korte@posteo.de

Olb3: DART-FT-ICR-MS-Studie zur Abgabe von Polydimethylsiloxanen in Lebensmittel beim Gebrauch von Silicongummi-Haushaltsartikeln

Jürgen Gross

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Olc: Grundlagen der Massenspektrometrie

Zeit: Montag, 02.03.2015: 9:15 - 10:15 · Ort: I26

Olc1: Studying viral assemblies with mass spectrometry and XFELs

Charlotte Uetrecht

Heinrich Pette Institut, Deutschland; lottiu@yahoo.de

Olc2: UV-MALDI-MS Analysis of Non-Covalent Streptavidin-Biotin Complexes with a 6-Aza-2-thiothymine Matrix: Effect of Wavelength and Fluence on their Detection

Andreas Schnapp¹, Marcel Wiegelmann¹, Jens Soltwisch¹, Klaus Dreisewerd^{1,2}

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Olc3: Fragmentierung von Ethylaminosubstituierten Farbstoffen mittels CID und PD

Claus Gernert, Jürgen Grotemeyer

Christian Albrechts Universität zu Kiel, Deutschland; gernert@phc.uni-kiel.de

Agilent Research Summer

Zeit: Montag, 02.03.2015: 10:15 - 10:45 · Ort: K33

N-Maleoyl Amino Acids as Novel Alkylating Agents for Mass-Spectrometric Detection of Cysteine-Containing Peptides – a Complex Comparison for Complex Proteomics

Wiebke Nadler^{1,2,3}, **Regina Berg**⁴, **Philipp Walch**^{2,3}, **Mathis Baalman**³, **Christoph Rösl**^{1,2}

¹Deutsches Krebsforschungszentrum, Deutschland; ²Heidelberg Institute for Stem Cell Technology (HI-STEM); ³Ruprecht-Karls-University Heidelberg; ⁴Department of Organic Chemistry, University of Basel; wiebke.nadler@hi-stem.de

Olla: Proteomics I

Zeit: Montag, 02.03.2015: 11:10 - 12:50 · Ort: K33

Olla1: Towards a mass spectrometric Western Blot: Multiplexed Targeted Protein Quantification by Gel-LC-MS/MS

Marc Gentzel, **Mukesh Kumar**, **David Drechsel**, **Andrej Shevchenko**

MPI of Molecular Cell Biology and Genetics, Dresden, Deutschland; gentzel@mpi-cbg.de

Olla2: A universal workflow for efficient enrichment of proteins in mass-spectrometry based proteomics

Florian Bonn, **Jürgen Bartel**, **Knut Büttner**, **Michael Hecker**, **Andreas Otto**, **Dörte Becher**

Institut für Mikrobiologie, Universität Greifswald, Deutschland; fbonn@uni-greifswald.de

Olla3: Characterization of an Improved Ultra-High Resolution Quadrupole Time of Flight (UHR-QTOF) Instrument for Proteomics Applications

Stephanie Kaspar, **Markus Lubeck**, **Annette Michalski**, **Oliver Raether**, **Christoph Gebhardt**, **Stuart Pengelley**

Bruker Daltonik GmbH, Bremen, Deutschland; Stuart.Pengelley@bruker.com

Olla4: Thermal Proteome Profiling

Mikhail Savitski, **Friedrich Reinhard**, **Gerard Drewes**, **Marcus Bantscheff**

Cellzome GmbH, Deutschland; marcus.x.bantscheff@gsk.com

Olla5: Uncovering molecular details of protein "misfolding - aggregation" using affinity- and ion mobility- mass spectrometry: Physiological and Parkinson- Synucleins

Michael Przybylski¹, **Kathrin Lindner**¹, **Nicolas Pierson**², **Ying Zhang**³, **Brindusa-Alina Petre**^{1,3}, **Stefan Schildknecht**¹, **Michael Gross**³, **David Clemmer**²

¹Steinbeis Centre for Biopolymer Analysis & Biomedical Mass Spectrometry, University of Konstanz, and Rüsselsheim, Germany; ²Department of Chemistry, Indiana University, Bloomington, USA; ³Department of Chemistry, Washington University St.Louis, USA; michael.przybylski@stw.de

Ollb: Metabolomics

Zeit: Montag, 02.03.2015: 11:10 - 12:50 · Ort: K32

Ollb1: Negative Ion Electrospray Tandem Mass Spectrometry of Polyketides

Jürgen Schmidt

Leibniz Institute of Plant Biochemistry Halle (Saale), Germany; jschmidt@ipb-halle.de

Ollb2: Combination of proteomic and metabolomic approaches in plant stress research

Andrei Frolov^{1,2}, **Elena Lukasheva**³, **Tatiana Bilova**^{1,2}, **Gagan Paudel**^{1,2}, **Uta Greifenhagen**^{1,2}, **Dominic Brauch**^{1,2}, **Elena Tarakhovskaya**³, **Juliane Mittasch**⁴, **Natalia Osmolovskaja**³, **Gerd Ulrich Balcke**⁵, **Carsten Milkowski**⁴, **Claudia Birkemeyer**⁶, **Ludger Wessjohann**⁷, **Ralf Hoffmann**^{1,2}

¹Universität Leipzig, Faculty of Chemistry and Mineralogy, Institute of Bioanalytical Chemistry; ²Center for Biotechnology and Biomedicine (BBZ), Leipzig; ³Saint-Petersburg State University, Faculty of Biology, Department of Plant Biochemistry and Physiology; ⁴Martin Luther University Halle-Wittenberg, Interdisciplinary Center for Crop Plant Research (IZN); ⁵Institute of Plant Biochemistry, Department of Cell and Metabolic Biology; ⁶Universität Leipzig, Faculty of Chemistry and Mineralogy, Institute of Analytical Chemistry; ⁷Institute of Plant Biochemistry, Department of Bioorganic Chemistry; andrei.frolov@bbz.uni-leipzig.de

Ollb3: Online EC/(LC)/ESI-MS for investigating the oxidative metabolism of roxarsone

Lisa Frensemeier, **Lars Büter**, **Uwe Karst**

University of Münster, Institute of Inorganic and Analytical Chemistry, Corrensstr. 28/30, 48149 Münster, Deutschland; lisa.frensemeier@uni-muenster.de

Ollb4: Metabolic pathway driven targeted metabolomics – a “quickstep” from mass spectrometric raw data to biologically relevant conclusions

Anrdea Kiehne, **Aiko Barsch**, **Verena Tellström**, **Heiko Neuweiger**

Bruker Daltonik GmbH, Bremen, Deutschland; Verena.Tellstroem@bruker.com

Ollc: Instrumentelle Entwicklungen

Zeit: Montag, 02.03.2015: 11:10 - 12:50 · Ort: I26

Ollc1: Improving resolving power in complex mixtures by combining tunable synchrotron radiation with advanced mass spectrometric techniques

Arnas Lucassen

Physikalisch-Technische Bundesanstalt, Deutschland; arnas.lucassen@ptb.de

Ollc2: Hochempfindliches Fourier-Transform-Massenspektrometer mit nicht-destruktiver Ionendetektion

Michael Schmidt¹, Albrecht Brockhaus¹, Stefan Butzmann¹, Thorsten Benter², Alexander Laue³, Michel Aliman³

¹Institut für Sensorik und messtechnische Systeme, Bergische Universität Wuppertal; ²Institut für Physikalische und Theoretische Chemie, Bergische Universität Wuppertal; ³Carl Zeiss SMT GmbH; mschmidt@uni-wuppertal.de

Ollc3: Coupling thin layer chromatography with mass spectrometry - a practical approach for matrix-loaded samples

Hans Griesinger¹, Katerina Matheis¹, Jürgen Schiller³, Beate Fuchs³, Michael Schulz²

¹Merck KGaA, Department of Bioanalytical Chemistry, Deutschland; ²Merck KGaA, Instrumental Analytics R&D, Deutschland; ³Universität Leipzig, Institut für Medizinische Physik und Biophysik, Deutschland; hans.griesinger@merckgroup.com

Ollc4: Proton-Transfer-Reaction Mass Spectrometry Coupled with FastGC: High Selectivity in Near Real-Time

Philipp Sulzer¹, Rene Gutmann¹, Lukas Fischer^{1,2}, Jens Herbig¹, Simone Jürschik¹, Alfons Jordan¹, Eugen Hartungen¹, Gernot Hanel¹, Lukas Märk¹, Matteo Lanza^{1,2}, Kostiantyn Breiev^{1,2}, Tilmann D. Märk^{1,2}

¹IONICON Analytik GmbH., Eduard-Bodem-Gasse 3, 6020 Innsbruck, AUSTRIA; ²Institut für Ionenphysik und Angewandte Physik, Leopold-Franzens Universität Innsbruck, Technikerstr. 25, 6020 Innsbruck, AUSTRIA; philipp.sulzer@ionicon.com

Ollc5: RECENT ADVANCES IN TIME-OF-FLIGHT MASS SPECTROMETRY: WHAT CAN MULTIPLEXING DO FOR YOU?

Gerhard Horner², Laura McGregor³, Nick BUKOWSKI³, Leonhard Pollack¹, Stefan Koschinski¹

¹Markes International GmbH, Deutschland; ²five technologies GmbH, Deutschland; ³Markes International Ltd., UK; ghorner@markes.com

Plenarvortrag II

Zeit: Montag, 02.03.2015: 14:20 - 15:05 · Ort: K33

How to speed up high-performance mass spectrometry

Yury O. Tsybin^{1,2}, Konstantin O. Nagornov¹, Kristina Srzentic¹, Anton N. Kozhinov¹

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Wolfgang-Paul-Studienpreis

Zeit: Montag, 02.03.2015: 15:05 - 15:50 · Ort: K33

Charakterisierung und Optimierung von Corona - Mikroplasma initiierten Ionisationsprozessen zur Anwendung in der Atmosphärendruckionisations - Massenspektrometrie

Sonia Klee

Tofwerk AG, Schweiz; klee@tofwerk.com

Mechanistische Studien zur Mizoroki-Heck- und zur Kobalt(I)-katalysierten Diels-Alder-Reaktion mithilfe massenspektrometrischer Methoden

Lukas Fiebig

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OIIa: Ionisationsmethoden I

Zeit: Montag, 02.03.2015: 16:05 - 17:25 · Ort: K33

OIIa1: Highly time-resolved two-dimensional mass spectrometric imaging of molecular combustion and pyrolysis product concentrations in a burning cigarette

Ralf Zimmermann¹, Thorsten Streibel¹, Romy Hertz-Schünemann¹, Kevin McAdam², Chuan Liu², Sven Ehlert¹

¹Uni Rostock, Deutschland; ²BAT R&D, UK; ralf.zimmermann@gsf.de

OIIa2: Laserspektroskopische Untersuchung an heterosubstituierten Halogenbenzolen mittels REMPI- und MATI-Spektroskopie

Sascha Krüger, Jürgen Grotemeyer

Christian-Albrechts-Universität zu Kiel, Deutschland; krueger@phc.uni-kiel.de

OIIa3: Matrixeffekte bei der LC/MS-Analyse von Vitamin D-Metaboliten mittels verschiedener Ionisierungstechniken

Sebastian Hagenhoff, Heiko Hayen

Westfälische Wilhelms-Universität Münster, Institut für Anorganische und Analytische Chemie, Deutschland; sebastian.hagenhoff@uni-muenster.de

OIIa4: Three-layer set-up for standardized quantification of salicylic acid in dried blood spots by desorption electrospray ionization (DESI) mass spectrometry

Markus Siebenhaar^{1,2,3}, Kai Küllmer³, Nuno Miguel de Barros Fernandes³, Volker Hüllen³, Carsten Hopt^{1,2}

¹Instrumental Analysis and Bioanalysis, Department of Biotechnology, Mannheim University of Applied Sciences; ²Institute of Medical Technology, University of Heidelberg and Mannheim University of Applied Sciences, Paul-Wittsack-Str. 10, 68163 Mannheim, Germany; ³Roche Diagnostics GmbH, Sandhofer Straße 116, 68305 Mannheim; Markus.Siebenhaar@roche.com

OIIIb: Lipide & Phospholipide I

Zeit: Montag, 02.03.2015: 16:05 - 17:25 · Ort: K32

OIIIb1: LMP-EX a multimolecular omics approach for systems biology

Robert Ahrends, Cristina Coman

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OIIIb2: Analysis of the lipid composition of adipose tissues by spectroscopic methods

Yulia Popkova¹, Andrej Meusel¹, Jana Breittfeld², Dorit Schleinitz², Johannes Hirrlinger^{3,4}, Dirk Dannenberger⁵, Peter Kovacs², Jürgen Schiller¹

¹University of Leipzig, Medical Faculty, Institute of Medical Physics and Biophysics, Härtelstrasse 16-18, 04107 Leipzig, Germany; ²University of Leipzig, IFB Adiposity Diseases, Liebigstrasse 21, 04103 Leipzig, Germany; ³University of Leipzig, Medical Faculty, Carl-Ludwig-Institute for Physiology, Liebigstrasse 27, 04103 Leipzig, Germany; ⁴Dept. of Neurogenetics, Max-Planck-Institut für Experimentelle Medizin, Hermann-Rein-Str. 3, D-37075 Göttingen, Germany; ⁵Leibniz Institute for Farm Animal Biology, Institute of Muscle Biology and Growth, Wilhelm-Stahl-Allee 2, D-18196 Dummerstorf, Germany; yulia.popkova@medizin.uni-leipzig.de

OIIIb3: Gender-specific Differences in Blood Plasma Lipidomes of Healthy Females and Males

Susanne Sales¹, Juergen Graessler², Marc Bickle¹, Carlo Cannistraci³, Kim Ekroos⁴, Andrej Shevchenko¹

¹Max Planck Institute of Molecular Cell Biology and Genetics, Germany; ²Department of Medicine III, University Hospital Carl Gustav Carus, TU Dresden, Germany; ³Biotechnology Center TU Dresden, Germany; ⁴Zora Biosciences Oy, Espoo, Finland; sales@mpi-cbg.de

OIIIb4: Lipidomics screens of human lung tissue together with histological characterization allow studying lipid metabolic perturbation in cancer and COPD

Lars F. Eggert¹, Julia Müller², Verena Scholz¹, Torsten Goldmann^{2,3}, Dominik Schwudke^{1,3}

¹Division of Bioanalytical Chemistry - Research Center Borstel, Parkallee 1-40, 23845 Borstel, Germany; ²Division of Clinical and Experimental Pathology - Research Center Borstel, Parkallee 1-40, 23845 Borstel, Germany; ³Airway Research Center North – German Center for Lung Research, Wöhrendamm 80, 22927 Grosshansdorf, Germany; leggers@fz-borstel.de

OIIIc: Gasphasenreaktionen, Fragmentierungen & Strukturaufklärung

Zeit: Montag, 02.03.2015: 16:05 - 17:25 · Ort: I26

OIIIc1: A new TEMPO-Active Ester Reagent for Peptide Structure Analysis by Free Radical Initiated Peptide Sequencing (FRIPS) Mass Spectrometry

Mathias Schäfer¹, Christian Ihling², Francesco Falvo¹, Isabel Kratochvil², Andrea Sinz²

¹Department of Chemistry, University Cologne, Greinstrasse 4, 50939 Köln, Germany; ²Institute of Pharmacy, Martin-Luther University Halle-Wittenberg, Wolfgang-Langenbeck-Str. 4, D-06120 Halle (Saale), Germany.; mathias.schaefer@uni-koeln.de

OIIIc2: First Principles Calculation of Electron Ionization Mass Spectra

Christoph Bauer, Stefan Grimme

Universität Bonn, Deutschland; christoph.bauer@thch.uni-bonn.de

OIIIc3: Structure of Full-Length p53 Tumor Suppressor Probed by Chemical Cross-Linking and Mass Spectrometry

Christian Arlt, Christian Ihling, Andrea Sinz

Institute of Pharmaceutical Chemistry & Bioanalytics, Martin-Luther University Halle-Wittenberg; christian.arlt@pharmazie.uni-halle.de

OIIIc4: Identification, recognition structures and affinities of nitrated tyrosine sites by affinity mass spectrometry

Claudia Andries^{1,2}, Stefan Slamnoiu¹, Laura Ion^{1,2}, Michael Przybylski¹, Brindusa-Alina Petre^{1,2}

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Gastvortrag

Zeit: Montag, 02.03.2015: 17:40 - 18:40 · Ort: K33

Der Weg wissenschaftlicher Erkenntnisse in die Politik - oder wie wird Wissenschaft wirksam?

Uwe Schneidewind

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PI: Postersession I

Zeit: Montag, 02.03.2015: 18:40 - 20:25 · Ort: Foyer

Analytische Anwendungen

PI01: Combining thin-layer chromatography, antibody-overlay detection and DESI-FT-ICR-MS for the analysis of Shiga toxin glycosphingolipid receptors

Katja Steckhan¹, Christoph Große Kracht¹, Lothar Veith², Carsten Engelhard³, Daniel Steil¹, Iris Meisen¹, Johannes Müthing¹, Michael Mormann¹

¹Institute for Hygiene, University of Münster, Robert-Koch-Straße 41, 48149 Münster, Germany; ²Tascon GmbH, Mendelstraße 17, 48149 Münster, Germany; ³Institute for Analytical Chemistry, University of Siegen, Adolf-Reichwein-Straße 2, 57068 Siegen, Germany; k_stec01@uni-muenster.de

PI02: Massenspektrometrische Analyse von Biotensiden am Beispiel der Rhamnolipide

Beate Behrens¹, Jeannine Engelen², Heiko Hayen¹

¹Westfälische Wilhelms-Universität Münster, Deutschland; ²Bergische Universität Wuppertal, Deutschland; behrensb@uni-muenster.de

PI03: Bestimmung von polaren Metaboliten in Algenextrakten mittels HILIC-ESI-MS

Alexander Schriewer, Heiko Hayen

Westfälische Wilhelms-Universität Münster, Deutschland; alexander.schriewer@wwu.de

PI04: Bringing Triple Quadrupole Detection Limits to a New Standard –in Theory and in Practice

Ralf Falter, Thomas Glauner, Laszlo Toelgyesi

Agilent Technologie, Deutschland; ralf_falter@agilent.com

PI05: Identification of urinary peptides triggering social behavior of Danio Rerio by mass spectrometry

Astrid Markgraf¹, Jens Christoffers², Gabriele Gerlach¹

¹Institute of Biology and Environmental Sciences, Carl von Ossietzky University, D- 26111 Oldenburg, Germany; ²Institute of Chemistry and Center of Interface Science, Carl von Ossietzky University, D-26111 Oldenburg, Germany; astrid.markgraf@uni-oldenburg.de

PI06: Advancements in (MA)LDI based mass spectrometry imaging techniques in the field of chemical ecology

Filip Kaftan¹, Jerrit Weißflog¹, Samay Pande², Purva Kulkarni³, Mayuri Napagoda⁴, Tino Jaschinski⁵, Aleš Svatoš¹

¹Max Planck Institute for Chemical Ecology, Deutschland; ²Institute of Integrative Biology, Department of Environmental Systems, Zürich, Switzerland; ³Chair for Bioinformatics, Friedrich Schiller University Jena, Jena, Germany; ⁴Faculty of Medicine, University of Ruhuna, Department of Biochemistry, Sri Lanka; ⁵Institute for Inorganic and Analytical Chemistry, Friedrich Schiller University Jena, Germany; fkraftan@ice.mpg.de

Bildgebende Massenspektrometrie

PI07: Determination of the platinum distribution in Caenorhabditis elegans after Cisplatin incubation by LA-ICP-MS

Barbara Crone¹, Julia Bornhorst², Michael Aschner³, Tanja Schwerdtle², Uwe Karst¹

¹Institute of Inorganic and Analytical Chemistry, University of Münster, Münster, Germany; ²Institute of Nutritional Science, University of Potsdam, Nuthetal, Germany; ³Department of Molecular Pharmacology, Neuroscience, and Pediatrics, Albert Einstein College of Medicine, Bronx NY, USA; barbaracrone@wwu.de

PI08: Exploring the head and neck cancer by MALDI FT-ICR mass spectrometry imaging

Lukas Krasny¹, Franziska Hoffmann², Günther Ernst², Jan Hendrik Kobarg³, Dennis Trede^{3,4}, Theodore Alexandrov^{4,5,6}, Vladimír Havlicek¹, Orlando Guntinas-Lichius⁷, Ferdinand von Eggeling^{2,7,8}, Anna C. Crecelius^{9,10}

¹Institute of Microbiology, v.v.i., Prague, Czech Republic; ²Institute of Physical Chemistry, Friedrich Schiller University Jena, Jena, Germany; ³Steinbeis Innovation Center SCiLS Research, Bremen, Germany; ⁴SCiLS GmbH, Bremen, Germany; ⁵Center for Industrial Mathematics, University of Bremen, Bremen, Germany; ⁶European Molecular Biology Laboratory, Heidelberg, Germany; ⁷Department of Otorhinolaryngology, Jena University Hospital, Jena, Germany; ⁸Leibniz Institute of Photonic Technology (IPHT), Jena, Germany; ⁹Jena Center for Soft Matter (JCSM), Friedrich Schiller University Jena, Jena, Germany; ¹⁰Laboratory of Organic and Macromolecular Chemistry (IOMC), Friedrich Schiller University Jena, Jena, Germany; kobarg@scils.de

PI09: Laser ablation dielectric barrier discharge imaging MS for direct molecular analysis of pharmaceuticals in tablets

Tim Elseberg¹, Christina Herdering¹, Michael Sperling², Uwe Karst¹

¹University of Münster, Institute of Inorganic and Analytical Chemistry; ²European Virtual Institute for Speciation Analysis (EVISA); tim.elseberg@uni-muenster.de

PI10: Improved spatial resolution in the analysis of FFPE tissue after tryptic digestion

Janine Beckmann, Detlev Suckau, Michael Becker, Arndt Asperger

Bruker Daltonik GmbH, Bremen, Deutschland; Arndt.Asperger@bruker.com

PI11: Untersuchung der Verteilung von Silbernanopartikeln nach oraler Gabe im Gastrointestinaltrakt von Ratten mit LA-ICP-MS

Dörthe Dietrich¹, Dr. Antje Vennemann², Prof. Dr. Martin Wiemann², Prof. Dr. Uwe Karst¹

¹Westfälische Wilhelms-Universität Münster, Deutschland; ²IBE R&D gGmbH; d_diet03@uni-muenster.de

PI12: Quantitative Bioimaging of Pd-tagged Photosensitizers in Tumor Spheroids by LA-ICP-MS

Ann-Christin Niehoff^{1,2}, Rebecca Niehaus², Michael Sperling², Uwe Karst²

¹Graduate School of Chemistry, University of Muenster; ²Institute of Inorganic and Analytical Chemistry, University of Muenster; a.niehoff@uni-muenster.de

PI13: AP-SMALDI mass spectrometry imaging of metabolites in insects using high resolution in mass and space

Dhaka Bhandari, Matthias Schott, Andreas Römpf, Andreas Vilcinskas, Bernhard Spengler
Justus Liebig University Giessen, Deutschland; Dhaka.R.Bhandari@anorg.Chemie.uni-giessen.de

PI14: Differentiating Macrophages in atherosclerotic plaques using matrix-assisted laser desorption/ionization mass spectrometry imaging

Pegah Khomehghir-Silz¹, Andreas H. Wagner², Andreas Römpf¹, Markus Hecker², Bernhard Spengler¹

¹Institute of Inorganic and Analytical Chemistry, Justus Liebig University, Germany; ²Institute of Physiology and Pathophysiology, University of Heidelberg, Germany; Pegah.Khomehghir@anorg.chemie.uni-giessen.de

Dateneanalyse

PI15: Customized data processing pipeline based on command line tools

Henrik Thomas, Andrej Shevchenko

MPI-CBG, Deutschland; thomas@mpi-cbg.de

Element- & Speziationsanalytik

PI16: LC-ICP-MS and LC-ESI-MS for the Speciation Analysis of Iodine

Maria Viehoff¹, Chun Kong Mak¹, Michael Sperling², Uwe Karst¹

¹University of Muenster, Institute of Inorganic and Analytical Chemistry, Germany; ²European Virtual Institute for Speciation Analysis (EVISA), Muenster, Germany; mariaviehoff@uni-muenster.de

Gasphasenreaktionen, Fragmentierungen & Strukturaufklärung

PI17: Resonanzverstärkte Multiphotonenionisation von Triethylbenzolen

Heinke Thurn, Jürgen Grotemeyer

Christian Albrechts Universität zu Kiel, Deutschland; thurn@phc.uni-kiel.de

Grundlagen der Massenspektrometrie

PI18: Photofragmentierung von Azofarbstoffen im sichtbaren Wellenlängenbereich

Martin Clemen, Jürgen Grotemeyer

Christian-Albrechts-Universität zu Kiel, Deutschland; clemen@phc.uni-kiel.de

PI19: Revisiting Classical MALDI Matrices: The Effect of Laser Wavelength

Annika Koch¹, Andreas Schnapp¹, Marcel Wiegelmann¹, Jens Soltwisch¹, Klaus Dreisewerd^{1,2}

¹University of Münster, Institute of Hygiene, Biomedical Mass Spectrometry, Robert-Koch-Str. 41, 48149 Münster, Germany; ²Interdisciplinary Center for Clinical Research (IZKF) Münster; Annika.Koch@uni-muenster.de

PI20: Influence of transfer capillary temperature on adduct formation in AP-MALDI MS

Anna Schultheis, Bernhard Spengler

Justus Liebig University Giessen, Germany; anna.schultheis@anorg.chemie.uni-giessen.de

Hyphenated Techniques

PI21: Sensitivity of detection of 12 aminoglycoside antibiotics by positive ESI-MS - comparison between HILIC and reverse phase methods

Jens Boertz¹, Olga Shimelis², Emily Barrey², Dave Bell², Craig Aurand²

¹Sigma-Aldrich, Fluka, Buchs SG, Switzerland; ²Sigma-Aldrich, Supelco, Bellefonte, USA; jens.boertz@sial.com

PI22: Rapid LC-MS/MS determination of dioxin and digitoxin in biological fluids with minimal matrix effects

Jens Boertz¹, Xiaoning Lu², Hillel Brandes², Stacy Squillario², David Bell², Wayne Way²

¹Sigma-Aldrich, Fluka, Buchs SG, Switzerland; ²Sigma-Aldrich, Supelco, Bellefonte, USA; jens.boertz@sial.com

PI23: Determination of thyroid hormones in biological fluids by LC-MS with online solid phase extraction

Jens Boertz¹, Xiaoning Lu², Dave Bell²

¹Sigma-Aldrich, Fluka, Buchs SG, Switzerland; ²Sigma-Aldrich, Supelco, Bellefonte, USA; jens.boertz@sial.com

PI24: Thermogravimetry hyphenated to ultra-high resolution FT-ICR-mass spectrometry using atmospheric pressure chemical ionization (APCI) as novel approach for evolved gas analysis

Toni Miersch¹, Christopher P. Rüger¹, Martin Sklorz¹, Ralf Zimmermann^{1,2}

¹Joint Mass Spectrometry Centre / Chair of Analytical Chemistry, University of Rostock, Rostock, Germany; ²Cooperation Group Comprehensive Molecular Analysis, Helmholtz Zentrum München, Neuherberg, Germany; toni.miersch@uni-rostock.de

Instrumentelle Entwicklungen

PI25: Near-IR laser induced desorption sampling of acoustically levitated liquids

Carsten Warschat¹, Arne Stindt¹, Andreas Bierstedt¹, Ulrich Panne^{1,2}, Jens Riedel¹

¹BAM Federal Institute for Materials Research and Testing, Berlin, Germany; ²HUB Humboldt University of Berlin, Germany; Carsten.Warschat@bam.de

PI26: Ein Orbitrap-Prototyp zur hochauflösenden Untersuchung von Erdöl

Alessandro Vetere, Wolfgang Schrader

Max-Planck-Inst. für Kohlenforschung, Deutschland; vetere@kofo.mpg.de

PI27: Assessing the Peptide Quantitation Performance of a Newly Developed Triple Quadrupole Instrument

Ralf Falter, Volker Gnau, Christine Miller

Agilent Technologie, Deutschland; ralf.falter@agilent.com

PI28: Eine DBD zur in-situ Erzeugung von Reaktantionen für negative chemische Ionisation

Sascha Albrecht¹, Fred Stroh¹, Thorsten Benter²

¹Forschungszentrum Jülich GmbH, Deutschland; ²Bergische Universität Wuppertal, Deutschland; s.albrecht@fz-juelich.de

Life Sciences & Forensik

PI29: Increasing Depth of Coverage in Data Independent Acquisition

Joerg Dojahn¹, Christie Hunter², Ben Collins³, Ludovic Gillet³, Rudi Aebersold³

¹AB SCIEX, Deutschland; ²AB SCIEX, USA; ³ETH Zurich, Zurich, SWITZERLAND; joerg.dojahn@absciex.com

PI30: A targeted lipidomics approach for ceramide analysis in platelet

Bing Peng, Cristina Coman, Robert Ahrends

Leibniz-Institut für Analytische Wissenschaften - ISAS - e.V., Deutschland; bing.peng@isas.de

PI31: Metabolic profiling of the battle field of competing white-rot fungi

Riya C Menezes¹, Marco Kai², Aleš Svatoš¹

¹Max Planck Institute for Chemical Ecology, Jena, Deutschland; ²Universität Rostock, Rostock, Deutschland; rmenezes@ice.mpg.de

PI32: Online Photostability Study of Pharmaceutical Substances

Diego Zulkiewicz Gomes^{1,2}, Jaber Assaf¹, Thomas Schulze¹, Maria Kristina Parr¹

¹Freie Universität Berlin, Deutschland; ²IPT – Institute for Technological Research, Brazil; diegozq@zedat.fu-berlin.de

Lipide & Phospholipide

PI33: To which extent are oxidized phospholipids digestible by the enzyme phospholipase A2? - A mass spectrometric and NMR spectroscopic study

Jenny Schröter, Zschörnig Kristin, Fuchs Beate, Süß Rosemarie, Schiller Jürgen

University of Leipzig, Faculty of Medicine, Institute of Medical Physics and Biophysics; Jenny.Schroeter@medizin.uni-leipzig.de

PI34: Lipidomics of Alzheimer's Disease using an Integrated Microfluidic-Ion Mobility-MS Device

Gunnar Weibchen¹, Steven Lai², David Heywood², Angela Doneanu², Jim Murphy², James Langridge³, Giuseppe Astarita²

¹Waters GmbH, Deutschland; ²Waters Corporation, Milford, MA, USA; ³Waters Corporation, Manchester, UK; gunnar.weibchen@waters.com

Metabolomics

PI35: The Use of Fragment Ion and Collision Cross Section for Confident Identification from LC-Ion Mobility-MS Metabolomics Data

Gunnar Weibchen¹, Giorgis Isaac², Giuseppe Astarita², Steven Lai², Adam Ladak², James Langridge⁴, John Shockcor², Andy Borthwick³

¹Waters GmbH, Deutschland; ²Waters Corporation, Milford, MA; ³Nonlinear Dynamics, Newcastle, UK; ⁴Waters Corporation, Manchester, UK; gunnar.weibchen@waters.com

PI36: Unravelling 2-deoxy-2-fluoro-D-glucose metabolism in plant tissue using mass spectrometry and NMR

Amol Fatangare¹, Christian Paetz², Hans Peter Saluz³, Ales Svatos¹

¹Mass spectrometry and proteomics research group, Max Planck institute for chemical ecology, Jena, Germany; ²Biosynthesis / NMR research group, Max Planck institute for chemical ecology, Jena, Germany; ³Cell and molecular biology department, Hans Knoll institute, Jena, Germany.; afatangare@ice.mpg.de

PI37: Synthese und massenspektrometrische Charakterisierung von Aib-haltigen antibiotischen Peptiden

Annegret Laub

Leibniz-Institut für Pflanzenbiochemie, Deutschland; Annegret.Laub@ipb-halle.de

Native MS & non-covalent Interactions

PI38: Advances in Native Mass Spectrometry-based methods for the analysis of Non-Covalent Protein complexes

Jonathan P. Williams¹, Marc Kipping¹, Malcolm Anderson², Lidia Jackson², Kevin Giles², Jeff Brown²

¹Waters GmbH, Deutschland; ²Waters Corporation, Manchester, UNITED KINGDOM; marc.kipping@waters.com

PI39: Bacteriophage endolysin activity is modulated by quaternary state.

Boris Krichel¹, Matt Dunne², Rob Meijers², Charlotte Uetrecht¹

¹Heinrich Pette Institut, Deutschland; ²European Molecular Biology Laboratory (EMBL), Meyerhofstrasse 1, DE-69117 Heidelberg, Germany.; boris.krichel@hpi.uni-hamburg.de

Petrolomics, Energy & Combustion

PI40: Thermal/optical Carbon Analysis coupled with Photoionization Time-of-Flight Mass Spectrometry: Fine Particulate Matter from a Marine Engine

Hendryk Czech¹, Olli Sippula^{2,3}, Martin Sklorz¹, Thorsten Streibel^{1,3}, Ralf Zimmermann^{1,3}

¹Joint Mass Spectrometry Centre University of Rostock, Chair of Analytical Chemistry, Germany and Helmholtz-Zentrum München, CMA (Comprehensive Molecular Analytics), Germany; ²University of Eastern Finland, Department of Environmental Science, Fine Particle and Aerosol Technology; ³Helmholtz Virtual Institute of Complex Molecular Systems in Environmental Health (HICE) - www.hice-vi.eu; hendryk.czech@uni-rostock.de

Cross-Linking

PI41: Characterization of the PKD2-ARF1 Complex by Chemical Cross-Linking and Mass Spectrometry

Björn Häupl, Dirk Tänzler, Christian Ihling, Andrea Sinz

Martin-Luther-Universität Halle-Wittenberg, Deutschland; bjorn.haeupl@pharmazie.uni-halle.de

PI44: Characterization of the Interaction between Intrinsically Disordered Gab1 and Grb2 by Cross-Linking/Mass Spectrometry

Nadia Mallock¹, Dirk Tänzler¹, Katharina Mandel², Marc Lewitzky², Stephan Feller², Andrea Sinz¹

¹Department of Pharmaceutical Chemistry and Bioanalytics, Institute of Pharmacy, Martin-Luther-University Halle-Wittenberg, Germany; ²Tumor Biology Section, Institute of Molecular Medicine, Martin-Luther-University Halle-Wittenberg, Germany; nadia.mallock@pharmazie.uni-halle.de

PI51: Automated Assignment of MS/MS Cleavable Cross-Links in Protein 3D-Structure Analysis

Michael Götze¹, Jens Pettelkau², Romy Fritzsche², Christian Ihling², Mathias Schäfer³, Andrea Sinz²

¹Institut für Biochemie und Biotechnologie, MLU Halle, Deutschland; ²Institut für Pharmazie, MLU Halle, Deutschland; ³Institut für Organische Chemie, Universität Köln, Deutschland; christian.ihling@pharmazie.uni-halle.de

Proteomics

PI42: SpheriCal® - Monodisperse Polyester Dendrimers as Universal Mass Calibrants in Mass Spectrometry

Jens Boertz¹, Scott M. Grayson², Michael Malkoch³

¹Sigma-Aldrich, Fluka, Buchs SG, Switzerland; ²Tulane University, Department of Chemistry, New Orleans, USA; ³Polymer Factory Sweden AB, Stockholm, Sweden; jens.boertz@sial.com

PI43: SILAC-Based Secretome Analysis of Non-Small Cell Lung Cancer Cell Lines

Konstanze Bosse¹, Silvia Haneder², Christian Ihling¹, Thomas Seufferlein³, Andrea Sinz¹

¹Martin-Luther-Universität Halle, Deutschland; ²Spherotec GmbH Martinsried, Deutschland; ³Universitätsklinikum Ulm, Deutschland; konstanze.bosse@pharmazie.uni-halle.de

PI45: Human bone marrow stromal cell-derived osteoblast matrix vesicle proteome and functions are regulated by sulfated glycosaminoglycan derivatives

Johannes Schmidt¹, Stefanie Kliemt^{1,2}, Carolin Preissler³, Stephanie Möller⁴, Martin von Bergen^{1,5}, Ute Hempel³, Stefan Kalkhof¹

¹Helmholtz-Centre for Environmental Research - UFZ, Leipzig, Germany; ²B CUBE Center for Molecular Bioengineering, TU Dresden, Germany; ³Institute of Physiological Chemistry, TU Dresden, Germany; ⁴Biomaterials Department, INNOVENT e.V., Jena, Germany; ⁵Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University, Denmark; johannes.schmidt@ufz.de

PI46: Monitoring PPAR γ induced changes in glycolysis by selected reaction monitoring mass spectrometry

Andreas Hentschel, Cristina Coman, Robert Ahrends

Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V.; andreas.hentschel@isas.de

PI47: Proteomic study of the impact of Magnesium implants on osteoblasts

Maryam Omid¹, Anna Burmester², Parnian Kiani¹, Marcel Kwiatkowski¹, Berengere Luthringer², Regine Willumeit², Hartmut Schlüter¹

¹Department of Clinical Chemistry, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; ²Helmholtz-Zentrum Geesthacht; Institute of Materials Research; Structural Research on Macromolecules; Geesthacht, Germany; momidi@uke.de

PI48: Uncontrolled modification of redox-active cysteines in proteins and peptides

Malte Bayer, Simone König

Core Unit Proteomics, Interdisciplinary Center for Clinical Research, University of Münster; maltebayer@web.de

PI49: Low abundant N-linked glycosylation in wild-type hen egg white lysozyme is localized at non-consensus sequons

Arndt Asperger¹, Kristina Marx¹, Christian Albers¹, Laura Molin², Odra Pinato²

¹Bruker Daltonik GmbH, Bremen, Deutschland; ²Chelab Silliker, Resana, Italy; arndt.asperger@bruker.com

PI50: A closer look at the quality of microwave digests

Vera Jüngst, Marion Bäumlisberger, Ute Bahr, Michael Karas

Goethe Universität Frankfurt, Deutschland; juengst@pharmchem.uni-frankfurt.de

PI52: New Workflows for Identification and Profiling of Disulfide Bonds in Biopharmaceuticals

Jan Wiesner, Antje Kozicki, Anja Resemann, Rainer Paape, Lars Vorweg, Kristina Marx, Andrea Kiehne, Ralf Hartmer, Carsten Baessmann, Detlev Suckau, Wolfgang Jabs, Zoltan Centnar

Bruker Daltonik GmbH, Deutschland; Zoltan.Centnar@bruker.com

PI53: Exploring the potential of the last generation UHR-Q-TOF for rapid generation of accurate information on proteoforms distribution and relative abundance

Wolfgang Jabs, Stuart Pengelley, Christian Albers, P.O Schmit P.O Schmit, Verena Tellström

Bruker Daltonik GmbH, Deutschland; Verena.Tellstroem@bruker.com

PI54: High quantification efficiency for discovery and validation approaches on a Q-TOF platform

Stephanie Kaspar¹, Pierre-Olivier Schmit², Ulrike Schweiger-Hufnagel¹, Aiko Barsch¹, Stuart Pengelley¹

¹Bruker Daltonik GmbH, Bremen, Deutschland; ²Bruker Daltonique S.A., Wissembourg, France; Stuart.Pengelley@bruker.com

PI55: MeCAT - Using light for cleavability

David Benda, Sabine Ufer, Sebastian Beck, Michael W. Linscheid

Humboldt-Universität zu Berlin, Deutschland; david.benda.1@chemie.hu-berlin.de

PI56: Displacement chromatography as enrichment step in phosphoproteomics

Parnian Kiani, Marcel Kwiatkowski, Hartmut Prof. Dr. Schlüter

Mass Spectrometric Proteomics, Institute of Clinical Chemistry, University Medical Center Hamburg-Eppendorf (UKE); p.kiani@uke.de

PI57: Mass spectrometric based approaches for studying oligomerization of β -amyloid peptide at amino acids level

Andries Claudia¹, Ion Laura¹, Michael Gross², Petre Brindusa Alina^{1,2}

¹Alexandru Ioan Cuza University, Iasi, Romania, Rumänien; ²Washington University, St. Louis, Missouri, USA; andries.claudia17@gmail.com

PI58: A top to bottom approach for in-depth characterization of therapeutic monoclonal antibodies

Martin Samonig^{1,2}, Kai Scheffler^{1,3}, Christian Huber^{1,4}

¹Christian Doppler Laboratory for Innovative Tools for Biosimilar Characterization; ²Thermo Fisher Scientific, Dornierstraße 4, 82110 Germering, Germany; ³Thermo Fisher Scientific GmbH, Im Steingrund 4-6, 63303 Dreieich, Germany; ⁴University of Salzburg, Department of Molecular Biology, Division of Chemistry and Bioanalytics, Hellbrunnerstrasse 34, 5020 Salzburg, Austria; kai.scheffler@thermofisher.com

PI59: Targeted quantification of myofibrillar myopathy aggregate related proteins via PRM

Katalin Barkovits¹, Alexandra Maerkens^{1,2}, Rudi A. Kley², Matthias Vorgerd², Katrin Marcus³

¹Department of Medical Bioanalytics, Medizinisches Proteom-Center, Ruhr-University Bochum; ²Department of Neurology, Neuromuscular Centre Ruhrgebiet, University Hospital Bergmannsheil, Ruhr-University Bochum; ³Department of Functional Proteomics, Medizinisches Proteom-Center, Ruhr-University Bochum; katalin.barkovits@rub.de

PI65: Identifizierung von intrazellulären Cisplatin-Protein Addukten mithilfe des fluoreszierenden Cisplatin-Analogen CFDA-Cisplatin

Sandra Kotz¹, Maximilian Kullmann², Anya Kalayda², Ulrich Jaehde², Sabine Metzger¹

¹Universität zu Köln, Deutschland; ²Universität Bonn, Deutschland; skotz@uni-koeln.de

Umwelt- & Lebensmittelanalytik

PI60: UPLC Ion Mobility Mass Spectrometry: A New Approach to Authentication and Routine Screening of Ginsenocide Isomers in Functional Food Products.

Michael McCullagh¹, Davor Turkovic², Rob Lewis¹, David Douce¹

¹Waters Corporation, UK; ²Waters GmbH, Deutschland; davor_turkovic@waters.com

PI61: The Combining of an Integrated Microfluidic Device with CCS Ion Mobility Screening for the Analysis of Pesticide Residues in Food.

Michael McCullagh¹, Davor Turkovic², Christoph Thomas², David Douce¹

¹Waters Corp., UK; ²Waters GmbH, Deutschland; christoph_thomas@waters.com

PI62: Screening and Quantitation of About 350 Pesticides in Fruit Juices with Positive/Negative Switching LC/MS/MS

Zicheng Yang¹, Louis Maljers¹, Arnd Ingendoh²

¹Bruker Daltonics Inc., USA; ²Bruker Daltonik GmbH, Deutschland; Arnd.Ingendoh@bruker.com

PI63: Screening und Quantifizierung abwasserbürtiger Spurenstoffe in einer Uferfiltrationstransekte mittels UHPLC-HRMS

Patricia van Baar, Florian Wode, Uwe Dünnbier

Berliner Wasserbetriebe, Deutschland; patricia.vanbaar@bwb.de

PI64: A DESI MS based screening method for phthalates in consumer goods

Sabine Schulz¹, Sebastian Wagner¹, Stefanie Gerbig¹, Dieter Bohn², Detlef Sialff³, Herbert Wächter⁴, Bernhard Spengler¹

¹Justus-Liebig-Universität, Deutschland; ²Landesbetrieb Hessisches Landeslabor, Deutschland; ³Landesuntersuchungsamt Rheinland-Pfalz, Deutschland; ⁴Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, Deutschland; sabine.schulz@anorg.chemie.uni-giessen.de

Datum: Dienstag, 03.03.2015

8:30	Plenarvortrag III: Merging mass spectrometry and IR spectroscopy		
-	Ort: K33		
9:15			
9:15	OIVa: Proteomics II	OIVb: Bildgebende Massenspektrometrie II	OIVc: Hochauflösende Massenspektrometrie
-	Ort: K33	Ort: K32	Ort: I26
10:15			
10:15	Mattauch-Herzog-Förderpreis		
-	Ort: K33		
10:45			
10:45	Kaffeepause		
-	Ort: Foyer		
11:10			
11:10	OVa: Cross-Linking	OVb: Lipide & Phospholipide II	OVc: Ionenmobilität
-	Ort: K33	Ort: K32	Ort: I26
12:50			
12:50	Mittagspause / Lunchseminare		
-			
14:20			
14:20	Plenarvortrag IV: Human Proteomes: From basic science to understanding drug action		
-	Ort: K33		
15:05			
15:05	Preis Massenspektrometrie in den Biowissenschaften		
-	Ort: K33		
15:50			
15:50	PII: Postersession II		
-	Ort: Foyer		
17:35			
17:35	Mitgliederversammlung		
-	Ort: K33		
18:35			
19:30	Konferenzdinner		
-	Ort: Brauhaus Wuppertal		
23:00			

Präsentationen

Plenarvortrag III

Zeit: Dienstag, 03.03.2015: 8:30 - 9:15 · Ort: K33

Merging mass spectrometry and IR spectroscopy

Jos Oomens^{1,2}

¹Radboud University Nijmegen, Institute for Molecules and Materials, Niederlande; ²Van 't Hoff Institute for Molecular Sciences, University of Amsterdam, Niederlande; j.oomens@science.ru.nl

OIVa: Proteomics II

Zeit: Dienstag, 03.03.2015: 9:15 - 10:15 · Ort: K33

OIVa1: Age-related changes of advanced glycation patterns in Arabidopsis thaliana

Tatiana Bilova^{1,2,3}, **Dominic Brauch**^{1,2}, **Uta Greifenhagen**^{1,2}, **Elena Tarakhovskaya**³, **Claudia Birkemeyer**⁴, **Ludger Wessjohann**⁵, **Andrej Frolov**^{1,2}

¹Institut fuer Bioanalytische Chemie, Fakultät fuer Chemie und Mineralogie, Universität Leipzig; ²Biomedizinisch-Biotechnologisches Zentrum (BBZ), Universität Leipzig; ³Biological Faculty of Saint-Petersburg State University, Russia; ⁴Institut fuer Analytische Chemie, Fakultät fuer Chemie und Mineralogie, Universität Leipzig; ⁵Natur- und Wirkstoffchemie, Leibniz-Institut fuer Pflanzenbiochemie; bilova.tatiana@gmail.com

OIVa1: Probing pH-Dependent Protein G α Surface Topology Alterations by Fast Photochemical Oxidation of Proteins and Mass Spectrometry

Yelena Yefremova¹, **Mahmoud Al-Majdoub**¹, **Kwabena F.M. Opuni**¹, **Cornelia Koy**¹, **Yuetian Yan**², **Michael Gross**², **Michael O. Glocker**¹

¹Proteome Center Rostock, University Medicine Rostock, Rostock, Germany; ²Washington University in St. Louis, St. Louis, Missouri, USA; yelena.yefremova@uni-rostock.de

OIVa3: Oxidative degradation of N ϵ -fructosylamine-substituted peptides in heated aqueous systems

Uta Greifenhagen, **Viktor Sekovski**, **Marko Damjanovic**, **Andrej Frolov**, **Ralf Hoffmann**

Institut für Bioanalytische Chemie, Fakultät für Chemie und Mineralogie; Biotechnologisch-Biomedizinisches Zentrum, Universität Leipzig, Deutschland; uta.greifenhagen@bbz.uni-leipzig.de

OIVb: Bildgebende Massenspektrometrie II

Zeit: Dienstag, 03.03.2015: 9:15 - 10:15 · Ort: K32

OIVb1: Mass Spectrometry Imaging of Biological Tissue: An Approach for Multicenter Studies

Andreas Römpf¹, **Jean-Pierre Both**², **Alain Brunelle**³, **Ron M. A. Heeren**⁴, **Olivier Laprevote**⁵, **Brendan Prideaux**⁶, **Alexandre Seyer**³, **Markus Stoekli**⁷, **Donald F. Smith**⁸, **Bernhard Spengler**¹

¹Justus-Liebig-Universität Giessen, Deutschland; ²French Atomic Energy Commission (CEA-LIST), Saclay, France; ³Centre de Recherche de Gif, CNRS, Gif-sur-Yvette, France; ⁴The Maastricht Multimodal Molecular Imaging institute (M4I), Maastricht University, Maastricht, The Netherlands; ⁵Chimie Toxicologie Analytique et Cellulaire, Université Paris Descartes, Paris, France; ⁶Public Health Research Institute, UMDNJ, Newark, NJ, United States; ⁷Novartis Institutes for BioMedical Research, Basel, Switzerland; ⁸National High Magnetic Field Laboratory, Florida State University, Tallahassee Florida, United States; Andreas.Roempf@anorg.Chemie.uni-giessen.de

OIVb2: Genaueste Einblicke in die Klimageschichte anhand der Bestimmung von Lipidbiomarkern in marinen Sedimenten mittels LDI FTICR Imaging

Lars Wörmer¹, **Susanne Alfken**¹, **Marcus Elvert**¹, **Jens Fuchser**², **Julius S. Lipp**¹, **Matthias Zabel**³, **Kai-Uwe Hinrichs**¹

¹Organic Geochemistry Group, MARUM Center for Marine Environmental Sciences and Department of Geosciences, University of Bremen, Germany; ²Bruker Daltonik GmbH, Deutschland; ³Inorganic Geochemistry Group, MARUM Center for Marine Environmental Sciences and Department of Geosciences, University of Bremen, Germany; Jens.Fuchser@bruker.com

OIVb3: Combining label-free whole-cell MALDI MS biotyping and imaging mass spectrometry for in-situ monitoring of histone deacetylase drug target activation

Bogdan Munteanu^{1,2}, **Björn Meyer**^{1,2}, **Elke Burgermeister**³, **Carolina v. Reitzenstein**^{1,2}, **Matthias P. Ebert**³, **Carsten Hopf**^{1,2}

¹Center for Applied Research in Biomedical Mass Spectrometry (ABIMAS); ²Instrumentelle Analytik und Bioanalytik Hochschule Mannheim; ³Universitätsklinikum Medizinische Fakultät Mannheim der Universität Heidelberg; munteanu@hs-mannheim.de

OIVc: Hochauflösende Massenspektrometrie

Zeit: Dienstag, 03.03.2015: 9:15 - 10:15 · Ort: I26

OIVc1: High precision mass measurement and separation of nuclear isomers with a multiple-reflection time-of-flight mass spectrometer

Christine Hornung¹, Samuel Ayet^{1,2}, Timo Dickel^{1,2}, Jens Ebert¹, Hans Geissel^{1,2}, Emma Haettner², Ronja Knöbel², Ivan Miskun², Stephane Pietri², Wolfgang R. Plaß^{1,2}, Sivaji Purushothaman², Moritz Pascal Reiter¹, Ann-Kathrin Rink¹, Helmut Weick², Peter Dendooven³, Marcel Diwisch¹, Florian Greiner¹, Fabian Heiße², Christian Jesch¹, Nasser Kalanta-Nayestanaki², Johannes Lang¹, Wayne Lippert¹, Iain Moore⁴, Alexandre Pikhitelev⁵, Ilkka Pohjalaine⁴, Andrej Prochazka², Manisha Ranjan³, Christoph Scheidenberger^{1,2}, Maya Takechi², John S. Winfeld², Xiaodong Xu^{1,2}, Mikhail I. Yavor⁶

¹JLU Gießen, Germany; ²GSI Helmholtzzentrum für Schwerionenforschung GmbH, Darmstadt, Germany; ³KVI, University of Groningen, 9747 AA Groningen, The Netherlands; ⁴University of Jyväskylä, FI-40014 Jyväskylä, Finland; ⁵Institute for Energy Problems of Chemical Physics, Russian Academy of Sciences, Chernogolovka, Russia; ⁶Institute for Analytical Instrumentation, Russian Academy of Sciences, 190103 St. Petersburg, Russia; christine.hornung@physik.uni-giessen.de

OIVc2: Analysis of polar trace components in middle distillate fuel by ultra-high resolution mass spectrometry using ESI and GC-APCI

Christopher Paul Rüger¹, Elize Smit², Martin Sklorz¹, Stefan De Goede³, Ralf Zimmermann^{1,4}, Egmont Rohwer²

¹Joint Mass Spectrometry Centre / Chair of Analytical Chemistry, University of Rostock, Rostock, Germany; ²Department of Chemistry, University of Pretoria, Pretoria South Africa; ³Sasol Technology, Fuels Research, Sasol, Sasolburg, South Africa; ⁴Cooperation Group Comprehensive Molecular Analysis, Helmholtz Zentrum München, Neuherberg, Germany; christopher.rueger@uni-rostock.de

OIVc3: Adding a chemical dimension to the analysis of heavy crude oils with ultra-high resolution mass spectrometry

Xuxiao Wang, Wolfgang Schrader

Max-Planck-Institut für Kohlenforschung; wuxiao@kofo.mpg.de

Mattauch-Herzog-Förderpreis

Zeit: Dienstag, 03.03.2015: 10:15 - 10:45 · Ort: K33

ESI Mass Spectrometry for Tracking Down Elusive Organometallics

Konrad Koszinowski

Georg-August-Universität Göttingen, Deutschland; kkoszin@gwdg.de

OVa: Cross-Linking

Zeit: Dienstag, 03.03.2015: 11:10 - 12:50 · Ort: K33

OVa1: Improving cross-linked peptide identification in large protein complexes across different high-resolution LC/MS/MS platforms

Christof Lenz^{1,2}, Chung-Tien Lee^{1,2}, Olexandr Dybkov¹, Reinhard Lührmann¹, Henning Urlaub^{1,2}

¹Max-Planck-Institut für Biophysikalische Chemie, Göttingen, Deutschland; ²Universitätsmedizin Göttingen (UMG), Deutschland; christof.lenz@mpibpc.mpg.de

OVa2: Monitoring Conformational Changes in PPAR β/δ by Chemical Cross-Linking, Photo-Affinity Labeling, and Mass Spectrometry

Rico Schwarz, Dirk Tänzler, Knut Kölbl, Christian Ihling, Andrea Sinz

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OVa3: Chemical crosslinking of TSHR-ECD

Marcus M. B. Nagel^{1,2}, Jörg Schaarschmidt¹, Jens Meiler³, Stefan Kalkhof², Ralf Paschke¹

¹University of Leipzig, Division of Endocrinology and Nephrology; ²Helmholtz Centre for Environmental Research – UFZ, Department of Proteomics, Leipzig, Germany; ³Vanderbilt University, Center for Structural Biology, Nashville, USA; marcus.nagel@ufz.de

OVa4: Incorporation of Photo-Methionine into Calmodulin for Photo-Cross-Linking/MS Studies

Christine Piotrowski, Christian Ihling, Andrea Sinz

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OVa5: Mass spectrometric analysis of post-translational modifications in T cells

Christian Freund

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OVb: Lipide & Phospholipide II

Zeit: Dienstag, 03.03.2015: 11:10 - 12:50 · Ort: K32

OVb1: Lipids originating from *Mycobacterium tuberculosis* (Mtb) are detected in plasma of Tuberculosis Patients

Nicole Zehethofer^{1,2}, Jan Heyckendorf³, Ulrich E. Schaible¹, Christoph Lange³, Dominik Schwudke²

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OVb2: A simple MALDI MS-based method to identify ether lipids in complex lipid mixtures of spermatozoa

Beate Fuchs¹, Karin Müller², Jürgen Schiller¹

¹University of Leipzig, Faculty of Medicine, Institute of Medical Physics and Biophysics, Härtelstr. 16-18, D-04107 Leipzig; ²Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Str. 17, D-10315 Berlin; beate.fuchs@medizin.uni-leipzig.de

OVb3: LPPdb: a new MS-centered database of modified lipids

Zhixu Ni^{1,2}, Ivana Milic^{1,2}, Dieter Weber^{1,2}, Andrea Annibal^{1,2}, Ralf Hoffmann^{1,2}, Maria Fedorova^{1,2}

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OVb4: Shotgun Lipidomics for Dissecting Lipid-protein Assemblies

Sophie Aycirix¹, Hermeto Gerber², Patrick Fraering², Andrej Shevchenko¹

¹Max Planck Institute of Molecular Cell Biology and Genetics, Deutschland; ²Ecole Polytechnique Fédérale de Lausanne (EPFL), Brain Mind Institute and School of Life Sciences, Switzerland; aycirix@mpi-cbg.de

OVb5: LC-MS2 based Lipid Mediator Profiling reveals insights into signaling cascades during *Mycobacterium tuberculosis* infection

Matthias Krajewski¹, Bhesh Paudyal², Ulrich Schaible², Dominik Schwudke¹

¹Forschungszentrum Borstel, Deutschland, Bioanalytische Chemie; ²Forschungszentrum Borstel, Deutschland, Zelluläre Mikrobiologie; mkrajewski@fz-borstel.de

OVc: Ionenmobilität

Zeit: Dienstag, 03.03.2015: 11:10 - 12:50 · Ort: I26

OVc1: Eliminating systematic errors in the LC-MS/MS analysis of vitamin D in human serum

Dietrich Volmer, Yulin Qi

Universität des Saarlandes, Deutschland; Dietrich.Volmer@mx.uni-saarland.de

OVc2: The new Agilent IMS QTOF, how it works and where it does add advantages

Thiemann Joachim, Ralf Falter

Agilent Technologie, Deutschland; joachim.thiemann@agilent.com

OVc3: Weiterführende Untersuchungen zum Vergleich von APLI und APCI bei der Anwendung in der Ionenmobilitätsspektrometrie

Marvin Ihlenborg, Björn Raupers, Jürgen Grotemeyer

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OVc4: Gas-Phase Microsolvation of Ubiquitin: Identification of Crown Ether Binding Sites

Melanie Göth¹, Xiao Jakob Schmitt², Stephan Warnke², Gert von Helden², Kevin Pagel^{1,2}

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OVc5: Analysis of isomeric lipids by high resolution ion mobility-mass spectrometry

Michael Gröbl¹, Stephan Graf¹, Miroslav Lisa², Michal Holcapek², Julio Sampaio³, Bernhard Dick⁴, Bruno Vogt⁴, Richard Knochenmuss¹

¹Tofwerk, Schweiz; ²University of Pardubice, Czech Republic; ³Lipotype, Germany; ⁴Bern University Hospital, Switzerland; groessler@tofwerk.com

Plenarvortrag IV

Zeit: Dienstag, 03.03.2015: 14:20 - 15:05 · Ort: K33

Human Proteomes: From basic science to understanding drug action

Mathias Wilhelm^{1,2}, Judith Schlegl², Hannes Hahne¹, Mikhail Savitski³, Amin Moghaddas Gholami¹, Susan Kläger¹, Daniel Martinez Molina⁴, Pär Nordlund⁴, Marcus Bantscheff³, Gerard Drewes³, Bernhard Küster¹

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Preis Massenspektrometrie in den Biowissenschaften

Zeit: Dienstag, 03.03.2015: 15:05 - 15:50 · Ort: K33

Stable isotope labelling and mass spectrometric analysis of peptides allows tracking of isotopic flux in microbial communities

Martin von Bergen¹, Jana Seifert²

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PII: Postersession II

Zeit: Dienstag, 03.03.2015: 15:50 - 17:35 · Ort: Foyer

Analytische Anwendungen

PII01: Veränderungen des organischen Bodenkohlenstoffs bei Waldbränden

Diana Hofmann¹, Bernhard Steffen¹, Kai-Uwe Eckhardt², Peter Leinweber²

¹Forschungszentrum Jülich, Deutschland; ²Bodenkunde der Universität Rostock; d.hofmann@fz-juelich.de

PII02: Detection of interfering substances of enzyme-based glucose measurement in complex matrices by Flowprobe™ micro extraction mass spectrometry

Tanja Gaissmaier¹, Makus Siebenhaar^{1,2,3}, Vanya Todorova², Carsten Hopf^{1,3}, Volker Hüllen²

¹Instrumental Analysis and Bioanalysis, Department of Biotechnology, Mannheim University of Applied Sciences; ²Roche Diagnostics GmbH, Sandhofer Straße 116, 68305 Mannheim; ³Institute of Medical Technology, University of Heidelberg and Mannheim University of Applied Sciences; tanja.gaissmaier@gmail.com

PII03: Improved Determination of Allergenic Fragrances in Detergents and Personal Care Products in Multiple Reaction Monitoring GC-MS/MS

Gordon van't Slot, Verena Tellström

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PII04: Direct Analysis of Degradation Products in Lithium-ion Batteries Using Low-temperature Plasma Ambient Desorption/Ionization High-Resolution Mass Spectrometry

Christopher Kuhlmann¹, Inessa Pastushkina¹, Sascha Nowak², Carsten Engelhard¹

¹Universität Siegen, Deutschland; ²MEET - Münster Electrochemical Energy Technology, Deutschland; christopher.kuhlmann@uni-muenster.de

PII05: Automated top-down mass spectrometry of hemoglobin for a clinical application

Didia Coelho Graça¹, Adelina E. Acosta-Martin^{1,2}, Wolfgang Jabs³, Ralf Hartmer³, Lorella Clerici², Markus Meyer³, Kaveh Samii⁴, Yury O. Tsybin⁵, Denis Hochstrasser^{1,2}, Pierre Lescuyer^{1,2}, Alexander Scheri^{1,2}, Zoltan Centnar³

¹Department of Human Protein Sciences, Faculty of Medicine, Geneva University, Geneva, Switzerland; ²Department of Genetic and Laboratory Medicine, Geneva University Hospitals, Geneva, Switzerland; ³Bruker Daltonik GmbH, Bremen, Germany; ⁴Division of Hematology, Geneva University Hospital, Geneva, Switzerland; ⁵Biomolecular Mass Spectrometry Laboratory, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland; Zoltan.Centnar@bruker.com

PII06: Electrospray ionization of aromatic amino compounds

Andreas Kiontke¹, Andrej Frolov², Ariana Oliveira¹, Carolin Oswald¹, Claudia Birkemeyer¹

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Bildgebende Massenspektrometrie

PII07: Multiple mass spectrometric imaging analyses from single tissue section with Desorption Electrospray Ionization (DESI) on a oa-TOF mass spectrometer

Gunnar Weibchen¹, Emrys Jones², Emmanuelle Claude²

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PII08: Enhancement of weak analyte signals in human disease-related MALDI MS images by applying fast randomized denoising and compression algorithms

Matthias Schwartz, Björn Meyer, Bernhard Wirnitzer, Carsten Hopf

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PII09: Imaging mass spectrometry (IMS) to discriminate breast from pancreatic cancer metastasis in FFPE tissues

Rita Casadonte¹, Mark Kriegsmann², Friederike Zweyner³, Katrin Friedrich⁴, Gustavo Barreton⁴, Mike Otto^{1,3,5}, Sören Deininger⁶, Rainer Paape⁶, Eckhard Belau⁶, Detlev Suckau⁶, Daniela Aust⁴, Christian Pilarsky⁴, Jörg Kriegsmann^{1,3,5}, Arndt Asperger⁶

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PII10: 3D MALDI Imaging of Mouse Heart after Myocardial Infarction

Michael Becker¹, Lena Hauberg-Lotte², Judith Lotz³, Janina Oetjen², Dennis Trede⁹, Michaela Aichler⁶, Wolfgang Dreher⁷, Moritz Wildgruber⁸, Klaus Steinhorst⁵, Jan Hendrik Kobarg⁵, Stefan Schiffler⁹, Stefan Heldmann³, Herbert Thiele³, Peter Maass⁴, Axel Walch⁶, Theodore Alexandrov⁴, Arndt Asperger¹

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PII11: LA-ICP-MS to Study the Distribution of Copper in Wilson's Disease Liver Samples

Oliver Hachmöller¹, Michaela Aichler², Ann-Christin Niehoff¹, Michael Sperling¹, Axel Walch², Uwe Karst¹

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PII12: Improving ion-signal reproducibility in MALDI MSI using an internal standard

Sarah Aboulmagd¹, Diego Esteban-Fernández¹, Boris Neumann^{2,4}, Alberto Lázaro³, Alberto Tejedor³, Michael Linscheid¹

¹Institut für Chemie, Humboldt-Universität zu Berlin, Deutschland; ²Proteome Factory, Magnusstraße 11, 12489 Berlin, Germany; ³Renal Physiopathology Laboratory, Department of Nephrology, IISGM-Hospital General Universitario Gregorio Marañón, Universidad Complutense de Madrid, Madrid, Spain; ⁴Charité-Universitätmedizin Berlin, Institute of Pharmacology, Hessische Straße 3-4, 10115 Berlin, Germany; sarah.aboulmagd80@gmail.com

PII13: Pre-separation of ions in an atmospheric-pressure MALDI ion source based on differential mobility in a frequency-asymmetric alternating electrical field

Christof Barth, Bernhard Spengler

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Datenanalyse

PII14: Chromatographic resolved mass defect plots: A new analytical tool for the interpretation of high-resolution MS data.

Thomas Gröger^{1,2}, Benedikt Weggler^{1,2}, Ralf Zimmermann^{2,1}

Helmholtz Zentrum München – German Research Center for Environmental Health GmbH, Deutschland; ²University of Rostock; thomas.groeger@helmholtz-muenchen.de

Element- & Speziationsanalytik

PII15: Metal labelling of antibodies

Oleksandra Kuzmich, Michael Linscheid

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Gasphasenreaktionen, Fragmentierungen & Strukturaufklärung

PII16: Reactivity of Hydrated Monovalent First Row Transition Metal Ions $M+(H_2O)_n$, $M = Cu$ and Zn toward C_6H_5Cl , C_6H_5Br , C_6H_5I and C_3H_7I

Ina Herber, Martin Beyer

Institut für Ionenphysik und Angewandte Physik, Universität Innsbruck, Österreich; Ina.Herber@uibk.ac.at

PII17: Untersuchung der Fragmentierungen von auf Cyclen basierenden Ligandensystemen mittels ESI-MS/MS und MS³

Markus Plaumann^{1,2}, Dorit Kemken¹, Thomas Dülcks¹, Dieter Leibfritz¹

¹Universität Bremen, Institut für Organische und Analytische Chemie, Deutschland; ²Otto-von-Guericke Universität Magdeburg, Institut für Biometrie und Medizinische Informatik, Deutschland; markus.plaumann@med.ovgu.de

Grundlagen der Massenspektrometrie

PII18: Kinetic Energy Release und Fragmentierungswege substituierter Aniline

Sarah Seulen, Jürgen Grotemeyer

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PII19: The Extraction of Maximum Information from Individual Ion Arrivals And Its Application to Extending the Dynamic Range of IMS-oaTOF-MS Data

Martin Green¹, Darrell Williams¹, Garry Scott¹, Tony Gilbert¹, Martin Palmer¹, Nick Tomczyk¹, Keith Richardson¹, Mark Wrona², Alexander Muck³, Mathias Hofmann³

¹Waters Corporation, Wilmslow, UK; ²Waters Corporation, Milford, MA, USA; ³Waters GmbH, Eschborn, GE; alex_muck@waters.com

Hochauflösende Massenspektrometrie

PII20: Expediting Peptide Mapping with High Resolution LC-MS

Kai Scheffler¹, Tabiwang Arrey², Eugen Damoc², Maciej Bromirski²

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Hyphenated Techniques

PII21: Analysis of multiple toxins by LC-MS/MS: In-depth analysis of column selectivity

Jens Boertz¹, Emily Barrey², Olga Shimelis²

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PII22: Sensitive and fast analysis of aflatoxin M1 in milk at picogram levels using LC-MS/MS

Jens Boertz¹, Ken Espenscheid², Emily R. Barrey², Olga I. Shimelis², Michael Ye², Jim Brown², Ed Mauney²

¹Sigma-Aldrich, Fluka, Buchs SG, Switzerland; ²Sigma-Aldrich, Supelco, Bellefonte, USA; jens.boertz@sial.com

PII23: TLC-MALDI-MS investigation of a multi-compound flu medication

Michael Schulz², Hans Griesinger¹, Michaela Oberle², Knut Behrend³, Katerina Matheis¹

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PII24: Influence of the silica gel layer thickness on the quality of TLC-MALDI mass spectra of lipids

Hans Griesinger¹, Katerina Matheis¹, Beate Fuchs³, Jürgen Schiller³, Michael Schulz²

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Instrumentelle Entwicklungen

PII25: Exploring Impact of Dynamic Accumulation for Improving MS/MS Quality of QqTOF Data

Joerg Dojahn¹, Nic Bloomfield², Christie Hunter², Sean Seymour²

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PII26: Design and Development of a New, High Performance 20kV HED Detector with Improved Efficiency and Low Noise Characteristics

Ralf Falter, Layne Howard, Micahel Ugarov

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PII27: Coupling of Acoustically Levitated Droplets with Ion Mobility Spectrometry

Aleksandra Michalik-Onichimowska^{1,2}, Toralf Beitz², Jens Riedel¹, Ulrich Panne^{1,3}, Hans-Gerd Löhmannsröben²

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Life Sciences & Forensik

PII28: Identification of human milk derived proteolytic glycopeptides by use of ion mobility separation (IMS) and subsequent low energy collision-induced dissociation

Gottfried Pohlentz, Michael Mormann, Julia Krägenbring, Klaus Dreisewerd, Johannes Müthing

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PII29: Enrichment of Glycoproteins from Human Breast Milk and Structural Characterization of N-Glycosylation

Julia Krägenbring, Gottfried Pohlentz, Michael Mormann

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PII30: Enhanced Confirmation Criteria for Reducing False Positive Rates (FPR) in Toxicology Screening using High Resolution QToF Accurate Mass Analysis

Tony Drury, Stuart Pengelley

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PII31: Introducing ion mobility mass spectrometry in glycomics of Schindler's disease

Mirela Sarbu¹, Jasna Peter-Katalinic², David Clemmer³, Michael Przybylski⁴, Alina Zamfir¹

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Lipide & Phospholipide

PII32: The maturation of sperm is accompanied by changes of the (phospho-)lipid composition – A MALDI-TOF MS Investigation of murine epididymal spermatozoa

Ariane Nimptsch¹, Susanne Pyttel², Jan Böttger³, Ulrike Jakob⁴, Jürgen Schiller¹

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PII33: Phospholipid-Profiling mittels HILIC-ESI-MS/MS

Christian Vosse, Heiko Hayen

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Metabolomics

PII34: Metabolomics Profiling using Atmospheric Pressure Gas Chromatography-MS

Gunnar Weibchen¹, Vladimir Shulaev³, Ghaste Manoj^{3,4}, Steven Lai², Carolina Salazar³, Nobuhiro Suzuki³, Janna Crossley³, Ron Mittler³, James Langridge², Giuseppe Astarita², Fulvio Mattivi⁴

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PII35: A novel high resolution MS/MS Human Metabolite Spectral Library enabling rapid and accurate metabolite identification in human metabolomics studies

Zhendong Li², Mingguo Xu², Yiman Wu², Chiao-Li Tseng², Tao Huan², Wei Han², Jaspaul Tatlay², Tran Tran², Aiko Barsch¹, Carsten Baessmann¹, Liang Li², Stuart Pongelley¹

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PII36: Quantifizierung von TMAO und verwandter Metabolite im Plasma mit HILIC-MS/MS im Kontext ernährungsphysiologischer Studien

Ralf Krüger¹, Maria Pfeuffer¹, Michael Emp², Pablo Steinberg², Alexander Roth¹, Achim Bub¹

¹Max Rubner-Institut, Karlsruhe; ²Tierärztliche Hochschule Hannover; ralf.krueger@mri.bund.de

PII37: An extraction strategy for systems biology

Cristina Coman, Fiorella Solari, Andreas Hentschel, René Zahedi, Robert Ahrends

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PII65: Lysosomal Storage Diseases: Development of ESI- ion trap diagnostic assay for MPS II and MPS VI. Multiplexing possibilities

Laura Ion^{1,2}, Claudia Andries^{1,2}, Michael Przybylski¹, Brindusa-Alina Petre^{1,2}

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Native MS & non-covalent Interactions

PII38: Characterization of a Cisplatin-DNA-Antibody and its Corresponding Antibody-Antigen-Complexes by high-mass Q-ToF MS and MS/MS

Lena Ruhe¹, Yves Hachenberger¹, Ulrike Hochkirch¹, Johanna Hofmann², Jürgen Thomale³, Michael W. Linscheid¹

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PII39: Mass-to-charge-dependent suppression of trapped ions

Mario Kompauer, Bernhard Spengler

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Petrolomics, Energy & Combustion

PII40: Analysis of crude oil mixtures by Atmospheric pressure photoionization Fourier Transform Mass Spectrometry (APPI-FTMS)

Matthias Witt, Arnd Ingendoh

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Cross-Linking

PII42: Evaluation of native cross-links in elastin

Christoph U. Schräder, Andrea Heinz, Christian E.H. Schmelzer

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PII45: Protein structure prediction guided by crosslinking constraints – a systematic evaluation of the impact of the crosslinking spacer length

Tommy Hofmann¹, Axel Fischer², Jens Meiler², Stefan Kalkhof¹

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Proteomics

PII41: Characterisation of SIL universal antibody and SIL human proteins for quantitative mass spectrometry

Jens Boertz¹, Pegah R. Jalili², James J. Walters², Gordon Nicol², Kevin Ray²

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PII43: Influence of glycoforms on the tryptic digestion efficiency of immunoglobulin G based biopharmaceuticals

David Falck¹, Rosina Plomp¹, Bas J. Jansen¹, Dietmar Reusch², Markus Habeger², Manfred Wuhrer^{1,3}

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PII44: Seeking marker peptides for tracking age-related changes in elastic tissues

Angela Cristina Mora-Huertas, Christian E.H. Schmelzer, Christoph U. Schröder, Reinhard H.H. Neubert, Andrea Heinz

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PII46: A data independent strategy for a multi-omic approach to investigate obesity treatment within a mouse model

Gertjan Kramer¹, Nicholas Dekker¹, Marc Kipping², Lee A Gethings³, Victoria Lee⁴, Robert J Beynon⁴, James Langridge³, Johannes P.C. Vissers³, Johannes M.F.G. Aerts¹

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PII47: MS optimization for identification and quantification of TMT labeled peptides

Florent Jouy, Stefan Kalkhof, Sina Riemschneider

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PII48: Complex Regional Pain Syndrome: Targeted Resolvin and Neuropeptide Analysis

Anna Schildt¹, Tanja Schlereth², Frank Birklein², Simone König¹

¹Core Unit Proteomics, Interdisciplinary Center for Clinical Research, University of Münster; ²Department of Neurology, University Medical Center Mainz; anna.schildt@uni-muenster.de

PII49: CoFGE mapping of flour proteins for protein aeroallergen database

Marina Hanneken, Björn Teichert, Simone König

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PII50: Metal labelling for quantification of post translational sugar modifications of proteins

Stefanie Ickert, Sebastian Beck, Michael W. Linscheid

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PII51: Improved glycopeptide analysis using acetonitrile enriched sheath gas and oxonium ion dependent ETD

Kristina Marx, Andrea Kiehne, Markus Meyer, Zoltan Centnar

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PII52: Comparison of peptide separation techniques using strong cation exchange materials

Vahid Golghalyani, Marion Bäumlisberger, Ute Bahr, Michael Karas

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PII53: Investigation of the composition of protein mixtures extracted from muscle tissue with a picoseconds infrared laser

Refat Nimer¹, Marcel Kwiatkowski¹, Sebastian Kruber², R. J. Dwayne Miller², Hartmut Schlüter¹

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PII54: Proteome analysis of beewolf-associated symbiotic 'Streptomyces philanthi' revealed bacterial factors essential for survival under in vivo stress conditions.

Taras Nechitaylo¹, Tobias Engl¹, Yvonne Hupfer², Martin Kaltenpoth¹, Aleš Svatoš², Natalie Wielsch²

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PII55: Application of Metal Coded Affinity Tagging to Bdellovibrio bacteriovorus

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PII56: Comparison of Thermolysin and Neutrophil Elastase for Peptide Mapping (LC-MS/MS) to fill the Gaps in the Tryptic Digest

Björn Mautz, Hans Rainer Völger, Hans Koll, Michael Mølhøj

Pharma Research and Early Development (pRED), Large Molecule Research, Dept. of Mass Spectrometry, Roche Innovation Center Penzberg, Roche Diagnostics GmbH, Germany; bjoern.mautz@roche.com

PII57: An assessment of label-free quantification approaches for protein quantification in complex samples

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PII58: Investigation of amino acid mutations in the plasma protein C1-Esterase-Inhibitor in a human population

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PII64: Influence of Different Buffer Cations on Charge-State Distribution and Collisional Cross Sections of Native-Like Protein Ions in Native Mass Spectrometry

Christoph Hage^{1,2}, Annika Butterer¹, Debbie Dewaele¹, Frank Sobott^{1,3}

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Umwelt - & Lebensmittelanalytik

PII59: Discovery of Pesticide Protomers Using Routine Ion Mobility Screening

Michael McCullagh¹, **Davor Turkovic**², Kieran Neeson¹, Jeff Goshawk¹, David Eatough¹, Chris Carver¹

¹Waters Corporation, UK; ²Waters GmbH, Deutschland; davor_turkovic@waters.com

PII60: Using the Routine Separation Dimension and Identification Criteria of UPLC Ion Mobility to Enhance Specificity in Profiling Complex Samples.

Michael McCullagh¹, Davor Turkovic², **Christoph Thomas**², Kieran Neeson¹, Jeff Goshawk¹, Christopher Carver¹, David Douce¹

¹Waters Corp., UK; ²Waters GmbH, Deutschland; christoph_thomas@waters.com

PII61: Enhanced reduction of matrix effects using LC-MS/MS with online extraction for the rapid quantitation of antibiotics in milk

Louis Maljers¹, **Arnd Ingendoh**²

¹Bruker Daltonics Inc., USA; ²Bruker Daltonik GmbH, Bremen, Deutschland; Arnd.ingendoh@bruker.com

PII62: Verfolgung der photochemisch induzierten Bildung gebundener Rückstände von Benzotriazol mit gelöstem organischem Material mittels ultra-hochauflösender Massenspektrometrie

Julia Raeke, Bettina Seiwert, Caroline Davis, Cindy Weidauer, Thorsten Reemtsma

Helmholtz-Zentrum für Umweltforschung GmbH - UFZ, Deutschland; julia.raeke@ufz.de

PII63: Eliminating Matrix Effects During Multi-residue Pesticide Analysis by Extensive Dilution Using A New Triple Quadrupole MS With Enhanced Sensitivity

Ralf Falter, Andreas Reimann, Thomas Glauner

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Datum: Mittwoch, 04.03.2015

8:30 Plenarvortrag V: Analysis of Protein Binding to Anticancer Metallodrugs
- Ort: K33

9:15

9:15 **OV1a: Life Sciences & Forensik**
- Ort: K33

OV1b: Hyphenated Techniques
Ort: K32

OV1c: Ionisationsmethoden II
Ort: I26

10:35

10:35 **Kaffeepause**
- Ort: Foyer

11:00

11:00 **OV1a: Proteomics III**
- Ort: K33

OV1b: Umwelt- & Lebensmittelanalytik II
Ort: K32

OV1c: Kohlenhydrate
Ort: I26

12:00

12:00 **Abschlussvortrag: Plasma in Hollywood**
- Ort: K33

12:45

12:45 **Tagungsabschluss**
- Ort: K33

13:00

Präsentationen

Plenarvortrag V

Zeit: Mittwoch, 04.03.2015: 8:30 - 9:15 · Ort: K33

Analysis of Protein Binding to Anticancer Metallodrugs

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OVla: Life Sciences & Forensik

Zeit: Mittwoch, 04.03.2015: 9:15 - 10:35 · Ort: K33

OVla1: Altersbestimmung von Kugelschreibereinträgen durch Lösungsmittel, Harze und Binder.

Andrea Hahn¹, Peter Seiler¹, Fritz Köhler¹, Dieter Kirsch²

¹BKA, Kriminaltechnisches Institut, KT 41 Physikalisch-chemische Urkundenprüfung; ²BKA, Kriminaltechnisches Institut, KT 12 Zentrale Analytik II; Andrea.Hahn@bka.bund.de

OVla2: Structural investigation of co-assembled clathrin adaptor protein complexes

Johannes Heidemann¹, Maria Marta Garcia-Alai², Anna Gieras², Rob Meijers², Charlotte Uetrecht¹

¹Heinrich-Pette-Institut, Deutschland; ²EMBL Hamburg Unit, European Molecular Biology Laboratory (EMBL), Hamburg, Deutschland; Johannes.Heidemann@hpi.uni-hamburg.de

OVla3: At the edge of lipidOMICS and proteOMICS: mobility mass spectrometry of lipid-protein adducts

Maria Fedorova^{1,2}, Ivana Milic^{1,2}, Zhixu Ni^{1,2}, Eva Griesser^{1,2}, Ralf Hoffmann^{1,2}

¹Institute of Bioanalytical Chemistry, Faculty of Chemistry and Mineralogy, Universität Leipzig; ²Center for Biotechnology and Biomedicine, Universität Leipzig; maria.fedorova@bbz.uni-leipzig.de

OVla4: LUX Score: Cheminformatics Approach to Compute Lipidome Homology

Chakravarthy Marella¹, Andrew Torda², Dominik Schwudke^{1,2,3}

¹Research Center Borstel, Deutschland; ²Centre for Bioinformatics, University of Hamburg; ³Airway Research Center North, German Center for Lung Research; cmarella@fz-borstel.de

OVlb: Hyphenated Techniques

Zeit: Mittwoch, 04.03.2015: 9:15 - 10:35 · Ort: K32

OVlb1: Investigation of UV transformation products of xenobiotics in the aquatic environment by means of liquid chromatography and mass spectrometry

Jörg Roscher, Uwe Karst

Westfälische Wilhelms-Universität Münster, Deutschland; joerg.roscher@uni-muenster.de

OVlb2: Electrochemistry coupled to LC/MS for the identification and characterization of reactive xenobiotic metabolites and their protein adducts

Tina Wigger^{1,2}, Lars Büter^{1,2}, Uwe Karst¹

¹University of Münster, Institute of Inorganic and Analytical Chemistry, Corrensstr. 28/30, 48149 Münster, Germany; ²NRW Graduate School of Chemistry, Wilhelm-Klemm-Str. 10, 48149 Münster, Germany; tina.wigger@uni-muenster.de

OVlb3: Differential Protein Labeling with Electrochemically Generated Reactive Intermediates

Lars Büter^{1,2}, Helene Faber¹, Tina Wigger^{1,2}, Uwe Karst^{1,2}

¹Institut für Anorganische und Analytische Chemie, Universität Münster; ²NRW Graduate School of Chemistry, Universität Münster; lars.bueter@uni-muenster.de

OVlb4: Study on highly aromatic complex mixtures such as crude oil asphaltenes with online and offline SEC-MS

Lilla Molnárné Guricza, Wolfgang Schrader

Max-Planck-Institut für Kohlenforschung; guricza@kofo.mpg.de

OV1c: Ionisationsmethoden II

Zeit: Mittwoch, 04.03.2015: 9:15 - 10:35 · Ort: I26

OV1c1: Characterization of a versatile low temperature plasma torch by optical emission spectroscopy and time-of-flight mass spectrometry

Andreas Bierstedt, Jens Riedel

BAM, Deutschland; andreas.bierstedt@bam.de

OV1b2: Mass spectrometry of oligopeptides in the presence of alkali halides using desorption/ionization induced by neutral cluster impact

Andre Portz¹, Markus Baur², Julian Heep¹, Christoph Gebhardt³

¹Justus-Liebig-Universität Giessen, Deutschland; ²Fakultät Angewandte Naturwissenschaften, Hochschule Esslingen, Deutschland; ³Bruker Daltonik GmbH, Bremen, Deutschland; andre.portz@ap.physik.uni-giessen.de

OV1b3: Fundamentals and Applications of Plasma-based Ambient Desorption Ionization Mass Spectrometry in Bioanalysis and Lithium-Ion Battery Research

Carsten Engelhard¹, Anastasia Albert², Christopher Kuhlmann¹, Jacob T. Shelley³, Britta Vortmann^{2,4}, Sascha Nowak^{2,4}

¹University of Siegen, Germany; ²University of Muenster, Germany; ³Kent State University, Kent, USA; ⁴MEET Battery Research Center, Muenster, Germany; engelhard@chemie.uni-siegen.de

OV1b4: LILBID-MS reveals structural insights into a unique hybrid FoVo rotor and its integration into the ATPase

Nina Morgner

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OV1a: Proteomics III

Zeit: Mittwoch, 04.03.2015: 11:00 - 12:00 · Ort: K33

OV1a1: Identification and relative label-free quantification of lactosylated peptides in bovine raw milk and processed milk products

Sanja Milkovska^{1,2}, Ralf Hoffmann^{1,2}

¹Institute of Bioanalytical Chemistry, Faculty of Chemistry and Mineralogy, Universität Leipzig; ²Center for Biotechnology and Biomedicine, Universität Leipzig; sanja.milkovska@bbz.uni-leipzig.de

OV1a2: Multiplexed 2D MRM-based protein biomarker quantitation of plasma proteins from free flaps – a pilot study

Jing-zhi Yang¹, Andrew Percy², Jun-Cong Yang², Christoph Borchers², Uwe von Fritschen³, Juliane C Finke³, Michael O Glocker¹

¹Proteome Center Rostock, University Medicine and Natural Science Faculty, University of Rostock, Rostock, Germany; ²University of Victoria - Genome British Columbia Proteomics Center, Vancouver Island Technology Park, Victoria, BC, Canada; ³Division of Plastic Surgery and Hand Surgery, HELIOS Clinic Emil von Behring, Berlin, Germany; jingzhi.yang@uni-rostock.de

OV1a3: Rapid extraction of intact proteins from tissues via desorption by impulsive excitation

Marcel Kwiatkowski¹, Marcus Wurlitzer¹, Maryam Omid¹, Refat Nimer¹, Sebastian Kruber², R.J. Dwayne Miller², Hartmut Schlüter¹

¹Mass Spectrometric Proteomics, Institute of Clinical Chemistry, University Medical Center Hamburg-Eppendorf (UKE), Germany; ²Atomically Resolved Dynamics Department, Max Planck Institute for the Structure and Dynamics of Matter, Hamburg, Germany; m.kwiatkowski@uke.de

OV1b: Umwelt- & Lebensmittelanalytik II

Zeit: Mittwoch, 04.03.2015: 11:00 - 12:00 · Ort: K32

OV1b1: Flowsystem on-line gekoppelt mit einem LC/qTOF-MS zur systematischen Untersuchung der Photolyse unter umweltrelevanten Bedingungen

Bettina Seiwert, Cindy Weidauer, Thorsten Reemtsma

Helmholtz-Zentrum für Umweltforschung, Deutschland; bettina.seiwert@ufz.de

OV1b2: On-line Analysis of Organic Emissions from Residential Wood Combustion with Single-Photon Ionization Time-of-Flight Mass Spectrometry (SPI-TOFMS)

Hendryk Czech¹, Olli Sippula^{2,3}, Miika Kortelainen², Jarkko Tissari², Thorsten Streibel^{1,3}, Ralf Zimmermann^{1,3}

¹Joint Mass Spectrometry Centre University of Rostock, Chair of Analytical Chemistry, Germany and Helmholtz-Zentrum München, CMA (Comprehensive Molecular Analytics), Germany; ²University of Eastern Finland, Department of Environmental Science, Fine Particle and Aerosol Technology; ³Helmholtz Virtual Institute of Complex Molecular Systems in Environmental Health (HICE) - www.hice-vi.eu; hendryk.czech@uni-rostock.de

OV1b3: Automated fast screening of TOF-MS data

Benedikt Weggler^{1,2,4}, Maximilian Jennerwein^{2,3}, Thomas Gröger^{1,2}, Ralf Zimmermann^{1,2,4}

¹Joint Mass Spectrometry Centre, Cooperation Group "Comprehensive Molecular Analytics" Helmholtz Zentrum Muenchen; ²GermanybJoint Mass Spectrometry Centre, Institute of Chemistry, Chair of Analytical Chemistry University of Rostock; ³ASG Analytik Service GmbH, Trentiner Ring 30, 86356 Neusäss; ⁴Helmholtz Virtual Institute of Complex Molecular Systems in Environmental Health – Aerosol and Health (HICE); benedikt.weggler@helmholtz-muenchen.de

OVIIc: Kohlenhydrate

Zeit: Mittwoch, 04.03.2015: 11:00 - 12:00 · Ort: I26

OVIIc1: Separation of Carbohydrate and Glycoprotein Isomers using Ion Mobility-Mass Spectrometry

Johanna Hofmann^{1,2}, Hannes Hinneburg^{2,3}, Heung S. Hahn^{2,3}, Daniel Kolarich^{2,3}, Peter H. Seeberger^{2,3}, Kevin Pagel^{1,2}

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OVIIc2: Influence of glycoforms on the tryptic digestion efficiency of immunoglobulin G based biopharmaceuticals

David Falck¹, Rosina Plomp¹, Bas J. Jansen¹, Dietmar Reusch², Markus Habberger², Manfred Wuhrer^{1,3}

¹Center for Proteomics and Metabolomics, Leiden University Medical Center, Leiden, The Netherlands; ²Pharma Biotech Development Penzberg, Roche Diagnostics GmbH, Penzberg, Germany; ³Division of BioAnalytical Chemistry, VU University Amsterdam, Amsterdam, The Netherlands; d.falck@lumc.nl

OVIIc3: MALDI TOF and ESI IT MS Characterization of highly sulfated Glycosaminoglycan-Oligosaccharides

Katharina Lemmnitzer¹, Joanna Blaskiewicz², Jürgen Schiller¹, Jörg Rademann²

¹Institut für Medizinische Physik und Biophysik, Medizinische Fakultät, Universität Leipzig; ²Institut für Pharmazie, Freie Universität Berlin; katharina.lemmnitzer@medizin.uni-leipzig.de

Abschlussvortrag

Zeit: Mittwoch, 04.03.2015: 12:00 - 12:45 · Ort: K33

Plasma in Hollywood

Achim von Keudell

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Midwinter Reflections on the Progress of Biological Mass Spectrometry

Costello, Catherine E.

Boston University School of Medicine, USA

A long and snowy winter provides a rare opportunity to stop and take stock of where we've been and where we're going. In the twenty years since moving across the river from MIT to Boston University School of Medicine, our laboratory members (and the community at large) have witnessed remarkable changes in our research field, and these have made mass spectrometry much more amenable to addressing the pressing needs of unique bioforms and personalized medicine. Increased sensitivity allows determinations of bioactive peptides in individual patients, top-down sequencing allows assignment of multiple post-translational modifications to individual protein molecules, ion mobility allows structural studies of individual glycoforms and molecular complexes, ultra high resolution allows definition of individual compositions in closely-spaced peaks, MALDI imaging allows monitoring of the spatial distributions of individual components within tissue slices, and new dissociation modes increase the possibility for fully elucidating individual molecular structures. Each of these expanded capabilities has role to play in meeting important, and previously unapproachable, challenges in the detection and treatment of disease. Illustrations from ongoing studies will provide examples of state-of-the-art mass spectrometry in action, and suggest future possibilities.

Ionization at atmospheric pressure: why and how

Bruins, Andries

University of Groningen, Niederlande

Stichworte: ionization, atmospheric pressure, LC/MS, fragmentation, sensitivity

The proliferation of atmospheric pressure ionization mass spectrometers was triggered by the invention of electrospray ionization by Fenn and coworkers in 1984. Its development into reliable instruments for everyday use took place at the end of the 1980s and was driven by the need for the coupling of liquid chromatography with MS. All LC/MS systems developed since 1975 were designed for ionization in an electron ionization source or chemical ionization source inside a vacuum envelope. Such designs had to deal with the incompatibility between high liquid flow rates from the LC and the pumping capacity of the vacuum system of the MS. Introduced in 1980, thermospray came a long way to meet the demands of the user, but it was not suitable for continuous use over a long period of time. Horning et al. were the first to build an APCI system for LC/MS in 1974, but although very promising, their design did not lead to a breakthrough.

Ionization at atmospheric pressure requires the transfer of ions from atmospheric pressure into the vacuum envelope of the mass spectrometer. Due to strong cooling during expansion into vacuum, a mixture of ions and evaporated solvents will lead to condensation of water and other vapors on sample ions and creation of ion-solvent clusters of unknown size. Examples of designs to avoid this problem include a curtain gas (Sciex) and a heated transfer capillary (Thermo).

Inside the vacuum one needs to transfer ions into the mass analyzer, and remove neutrals via vacuum pumps. Separation of ions from neutrals is done by means of RF-only quadrupoles, step-wave lenses, S-lenses and other devices from different manufacturers. The common goal of all such ion guides is to increase the ions-to-gas ratio, and thereby increase sensitivity of the mass spectrometer. Transportation inside the vacuum may be assisted by forward electric fields that accelerate ions. Sensitivity may be increased by higher voltage drops, but the acceleration of ions and collisions with background gas often leads to fragmentation and partial loss of molecular ions. The ion transport discussed so far is independent from the way ions were generated. On the atmospheric side there is a wide choice of ionization methods. The majority of users choose electrospray, either at high flow for general use and high-throughput quantitation, or at low liquid flow rate for proteomics applications. Results obtained by electrospray are very good; most users and several manufacturers ignore the importance of techniques such as APCI and APPI that good alternatives for thermally stable samples. APCI is often sold as an option, rather than as a standard part of an LC/MS system. APCI and APPI are much less susceptible to suppression of sample ionization due to co-eluting matrix components.

Examples of ion generation from solid samples are DART, DESI, and a range of related "ambient sampling methods". When we look at the basics of mechanisms of ionization it is clear that electrospray makes use of ions in the liquid phase, while APCI and APPI ionize samples in the gas phase. The "magic" of DART is a combination of rapid heating and evaporation of a sample followed by chemical ionization in the gas phase. Whatever the initial step of ion formation of a sample, what we observe in the mass spectrometer is the final result of interaction of sample ions with background gas composed of air, nitrogen, and solvent vapor in the atmospheric source, and the following passage from atmosphere into the vacuum and into the mass analyzer. Do not be surprised if the spectrum observed does not fit in our simplified ideas of ion stability and reactivity.

How to speed up high-performance mass spectrometry

Tsybin, Yury O. (1,2); Nagornov, Konstantin O. (1); Srzentic, Kristina (1); Kozhinov, Anton N. (1)

1: École Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland; 2: Spectroswiss Sàrl, 1015 Lausanne, Switzerland

Ion detection time in FTMS is a substantial cost to pay on the experimental time balance sheet. Extreme complexity of biological samples requires as high throughput as possible, and even then the dynamic range of protein concentration in proteomic samples would not be addressed completely. The situation gets more demanding when ultra-high resolution (>500k in a 200-2000 m/z range) is needed, e.g., as in neutron encoded multiplexed protein quantitation via SILAC approach. Other multiplexed quantitation approaches, e.g., 10-plex tandem mass tag (TMT), may require a moderate resolution of 60k at 100 m/z . However, reaching even this level of performance is time-consuming and throughput-limiting (costs about 100 ms of ion detection on a modern Orbitrap FTMS with absorption mode FT – which is a four-fold increase compared to the widely employed 6-plex TMT approach). New sources of molecular information, e.g., isotopic fine structure of peptides, have shown to be of a value for proteomics, but required resolution performance is not readily achievable on the timescale of a typical proteomics experiment. Similar situation is in the metabolomics in general and in lipidomics in particular applications of high-resolution MS. Finally, biological diversity of diagnostic proteoforms requires mass spectrometry to be performed not on short, <3 kDa, peptides as in bottom-up proteomics, but on longer ones, 3-15 kDa, as in middle-down proteomics, or even on intact proteoforms in a top-down approach. Therefore, increasing the rate of ion detection in FTMS is highly anticipated. The direct approaches to address this challenge is to increase the electromagnetic fields strength. However, already today both ultra-high-field Orbitrap and ICR (21 T) are running close to the technological limits. What are other, complementary, approaches to speed up ion detection in FTMS? Recent experiments demonstrate that coherence of ion motion in FT mass analyzers, both ICR and Orbitrap, is significantly better than the FT uncertainty principle requires. That opens two avenues for speeding up FTMS: instrumentation for a better measurement of very coherent ion motion (frequency multiples, high-order harmonics, and multi-transient measurements) and advanced signal processing (non-FT or improved FT) that employs less strict uncertainty principles. Here we will discuss our recent advances in both these directions and present our vision for future developments.

Merging mass spectrometry and IR spectroscopy

Oomens, Jos (1,2)

1: Radboud University Nijmegen, Institute for Molecules and Materials, Niederlande; 2: Van 't Hoff Institute for Molecular Sciences, University of Amsterdam, Niederlande

Over the past decade, the application of IR spectroscopy to mass selected ions in a tandem mass spectrometer has revolutionized structure determination in ion chemistry. While the density of ions in a mass spectrometer is many orders of magnitude too low for recording an absorption spectrum, application of powerful tunable laser sources allows one to obtain a photodissociation spectrum. On account of their wide tunability over the fingerprint range of the IR spectrum, infrared free-electron lasers have been instrumental in enabling this development.

Areas of active research at the FELIX facility include the binding geometries of metal ions to biological molecules, the structures of dissociation products of protonated (and deprotonated) peptides and the IR spectra of ionized polyaromatic species of astrochemical interest. I will give an update on recent results of these studies, focusing on peptide dissociation studies.

Human Proteomes: From basic science to understanding drug action

Wilhelm, Mathias (1,2); Schlegl, Judith (2); Hahne, Hannes (1); Savitski, Mikhail (3); Gholami, Amin Moghaddas (1); Kläger, Susan (1); Martinez Molina, Daniel (4); Nordlund, Pär (4); Bantscheff, Marcus (3); Drewes, Gerard (3); Küster, Bernhard (1)

1: Technische Universität München, Germany; 2: SAP, Germany; 3: Cellzome, Germany; 4: Karolinska Institute, Sweden

Proteomes are characterized by large protein-abundance differences, cell-type- and time-dependent expression patterns and post-translational modifications, all of which carry biological information that is not accessible by genomics or transcriptomics. Here we present a mass-spectrometry-based draft of the human proteome and a public, high-performance, in-memory database for real-time analysis of terabytes of big data, called ProteomicsDB. The information assembled from human tissues, cell lines and body fluids enabled estimation of the size of the protein-coding genome, the analysis of messenger RNA and protein-expression profiles of human tissues revealed conserved control of protein abundance and integration of drug-sensitivity data enabled the identification of proteins predicting resistance or sensitivity. The same overall technology also allows tracking drug actions in cells and lysates on a target-class or proteome-wide scale with many implications for drug discovery. We present examples for new uses of existing drugs, the identification of toxicity targets and the elucidation of the mechanism of action of drugs in cancer cells.

Wilhelm *et al.* Mass-spectrometry-based draft of the human proteome. *Nature* 2014 May 29;509(7502):582-7.

Savitski *et al.* Tracking cancer drugs in living cells by thermal profiling of the proteome. *Science* 2014 Oct 3;346(6205):1255784

<https://www.proteomicsdb.org/>

Analysis of Protein Binding to Anticancer Metallo drugs

O'Connor, Peter; Wootton, Chris; Sadler, Peter

University of Warwick, Vereinigtes Königreich

In cancer patients up to 99% of some metal based chemotherapy agents injected into the body do not actually reach the intended DNA target.

Most of the compound reacts along the way with peptides, proteins, and various other biomolecules, thought to contribute the dangerous side effects patients can commonly suffer, e.g. nausea & hair loss.

A new approach is to use localised activation of inert metallo pro-drug to ensure these toxic compounds are only active where they are needed – near cancerous tissue. Meaning less of the drug is wasted reacting in other parts of the body, causing fewer side effects in these areas, this can also translate into reduced dosages, especially since some of these drugs have been shown to be an order of magnitude more potent than Cisplatin under comparable conditions.

Not only have these new photoactivatable complexes already been shown to have potent cytotoxicity, it has been found they bind to DNA effectively with a different mechanism of action to more traditional drugs such as Cisplatin. Meaning these compounds could be effective against Cisplatin-resistant strains of cancer, which increase in frequency year upon year.

FT-ICR MS offers ultra-high resolving power and unparalleled mass accuracy providing the highest possible confidence during analysis of these unique and previously unexplored post translational modifications. We show tandem FT-ICR mass spectra of novel proteins/peptides and their metallo drug-modified counterparts in order to deduce not only the binding sites of said drugs to biopolymers, but also to investigate the varying identity of the modifications post-photoactivation and present examples of their unique behaviour under electron capture dissociation (ECD). ECD in particular was found to be critically important for retaining these modifications during MS/MS analysis.

Der Weg wissenschaftlicher Erkenntnisse in die Politik - oder wie wird Wissenschaft wirksam?

Schneidewind, Uwe

Wuppertal Institut für Klima, Umwelt, Energie, Deutschland

Wie entsteht "wirksame" Wissenschaft, die nicht nur in der Scientific Community wahrgenommen wird, sondern Impulse für die gesellschaftliche Entwicklung auslöst?

Welche Anforderungen an Wissenschaftler/innen und den wissenschaftlichen Prozess sind zu stellen, um den gesellschaftlichen Impact zu erhöhen? Warum gewinnt die Forderung nach "wirksamer" Wissenschaft aktuell so an Bedeutung? Auf diese Fragen geht der Vortrag ein und erläutert sie an aktuellen Beispielen aus der Forschung des Wuppertal Institutes für Klima, Umwelt und Energie.

Plasma in Hollywood

von Keudell, Achim

Ruhr-Universität Bochum, Deutschland

Plasmen sind alltäglich. Jeder Stern am Himmel ist ein Plasmaball. Auf der Erde werden Plasmen sichtbar als Blitze oder Polarlichter. Plasmen finden Anwendung als Lichtquellen in Neonröhren, Autoscheinwerfern und Plasmafernsehern, oder als Energiequelle der Zukunft wie bei der Kernfusion. Plasmen leuchten, üben Kräfte aus, werden instabil und können ganze Kinofilme motivieren. Selbst der ehemalige Gouverneur von Kalifornien wird von einem Plasma angetrieben!

In diesem Vortrag soll an Hand von vielen Beispielen aus Hollywood die Wirklichkeit und die Fiktion der Plasmaforschung gegenüber gestellt werden. Lehnen sie sich zurück und untersuchen wir gemeinsam die eigenwillige Interpretation der Physik durch amerikanische Filmemacher.

Charakterisierung und Optimierung von Corona - Mikroplasma initiierten Ionisationsprozessen zur Anwendung in der Atmosphärendruckionisations - Massenspektrometrie

Klee, Sonja

Tofwerk AG, Schweiz

The aim of this work was the development of a corona-microplasma based APCI source that efficiently generates protonated molecules in a controllable manner.

From the fundamental characterization of the complex ion-molecule chemistry prevailing in API sources a generally applicable ionization mechanism regarding the formation of protonated molecules using proton bound water clusters as reagent ions was inferred and validated. This in-depth analysis was used for the development of a clean, stable and controllable APCI source, which incorporates an ion activation stage, operating with electrical radio frequency fields in the first differential pumping stage of the mass spectrometer. The combination of both significantly increased the ionization efficiency towards typical APCI amenable analytes and also considerably widens the analyte range.

Mechanistische Studien zur Mizoroki-Heck- und zur Kobalt(I)-katalysierten Diels-Alder-Reaktion mithilfe massenspektrometrischer Methoden

Fiebig, Lukas

Boehringer Ingelheim Pharma GmbH & Co. KG, Deutschland

Ionische Palladium- und Kobalt-Komplexe, deren Untersuchung als reaktive und transiente Intermediate in Mizoroki-Heck- und Diels-Alder-Reaktionen in Lösung schwierig ist, wurden unter definierten Bedingungen in der Gasphase gebildet. Mit der Kombination aus Tandem-MS, mehrstufigen Ion/Molekül-Reaktionen und der Bestimmung exakter Ionenmassen wurden intrinsische Eigenschaften der Komplex-Ionen und insbesondere deren Gasphasen-Reaktivitäten gegenüber Modell-Substraten untersucht. Zur geregelten Überführung der Neutralreagenzien in die Gasphase wurde die Stoßgas-Zuleitung der linearen Ionenfalle eines LTQ Orbitrap Massenspektrometers modifiziert.

N-Maleoyl Amino Acids as Novel Alkylating Agents for Mass-Spectrometric Detection of Cysteine-Containing Peptides – a Complex Comparison for Complex Proteomics

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Cysteine is unique among the proteinogenic amino acids due to its ability to form disulfide bonds. Though this property is of vital importance for establishing protein structures and effecting biological processes, it causes difficulties for the mass spectrometric identification of cysteine-containing peptides. In the course of a bottom-up proteomics experiment, proteins are fragmented into peptides using a proteinase (e.g. trypsin). Disulfide bridges can mask the tryptic recognition site, thus preventing proper cleavage. On the peptide level, two distinct peptides can be linked via their thiol groups, or intramolecular disulfide bonds can be formed. When subjected to mass spectrometric analysis with subsequent comparison to a database of linear peptide fragments, these branched fragments cannot be identified or even lead to false identifications. A common approach to overcome these problems is the reduction and covalent modification of the sulfhydryl groups prior to tryptic digest.

In this study, we have analyzed the suitability of several N-maleoyl compounds for the modification of cysteine-containing peptides in complex proteomics experiments. All novel compounds contain a maleoyl moiety, but differ in the N-substituent. The rationale behind this design is the modular combination of a group enabling an operationally simple Click chemistry approach for cysteine alkylation with residues of varying hydrophilicity and size in order to control their chromatographic and mass spectrometric properties. Compounds not commercially available were synthesized from inexpensive precursors according to the generic two-step approach proposed by Rich *et al.* (Journal of Medicinal Chemistry, 1975). These N-maleoyl amino acids were fully characterized and their hydrolytic stability in different aqueous buffer solutions was determined photospectrometrically. As for their reactivity and ionization characteristics in mass spectrometry applications, the substances were compared with five established alkylating agents, i.e. iodoacetamide (IAA), 4-vinylpyridine, (3-acrylamidopropyl)-trimethylammonium chloride, 2-bromoethylamine and N-ethyl maleimide (NEM). In a reactivity assay performed with bovine serum albumin as model substrate, the novel N-maleoyl amino acids and NEM showed the highest reactivity with a 100-fold molar excess of these compounds with respect to the number of cysteine residues present in the protein. However, the ionization of a synthetic peptide was considerably more efficient after its modification with 4-vinylpyridine. Modification with N-maleoyl isoleucine could likewise improve the ionization in comparison to NEM or IAA, though to a lower extent. The ionization data were substantiated by density functional theory calculations (DFT) on the gas phase basicities of these compounds with methyl thiol groups added as a model for the cysteine residues.

An established workflow for large scale proteomics is SDS-page based prefractionation coupled with liquid chromatography-tandem mass spectrometry and subsequent MS/MS analysis (GeLC-MS). As proof-of-principle experiment, *E. coli* lysates were electrophoretically separated and the region between 43 and 54 kDa was excised from eleven parallel lanes. Each of the parallel slices was treated with a different compound and the peptides were tryptically digested and desalted. Following separation on two serially installed C18 columns, peptides were analyzed with an Agilent 6550 QTOF LC/MS instrument (equipped with an iFunnel Dual AJS ESI source). The resulting data were searched with SpectrumMill against an *E. coli* SwissProt database. Several thousand different peptides were identified per sample, whereof several hundred contained cysteines, thus proving the compatibility of all compounds with this general mass spectrometric workflow. Analysis of peptide identifications as a function of retention time showed that the modification with N-maleoyl isoleucine led to the separation of the modified from the unmodified peptide populations along the LC run. While peptides without modified cysteines elute rather early, modified peptides show increased retention times.

To analyze if unspecific alkylations observed with synthetic peptides translate into lower relative identification rates for more complex analyses, a shotgun experiment with *E. coli* lysates was performed. The relative occurrence of each amino acid in the identified peptides was calculated for each of three replicates per compound and results were normalized to the composition of the search database. By modification of the sulfhydryl groups the recognition of cysteine could be increased by a factor of at least 2.5. Major differences between the reagents can be observed with regard to the relative identification of cysteine- and methionine-containing peptides. The highest relative identification

rate of cysteine-containing peptides is reached with a 10 000-fold excess of iodoacetamide. However, this is at the expense of a reduced relative identification of methionine-containing peptides due to unspecific reactions. It can be concluded that, depending on the specific experimental requirements, N-maleoyl alanine, N-maleoyl beta alanine, N-maleoyl valine, N-maleoyl isoleucine as well as the well-established alkylating agents N-ethyl maleimide, 4-vinylpyridine, and iodoacetamide are adequate choices for cysteine modification for large-scale proteomics experiments, while (3-acrylamidopropyl)-trimethylammonium chloride, bromo-ethyl-amine and N-2-aminoethylmaleimide are less suited.

Acknowledgment

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ESI Mass Spectrometry for Tracking Down Elusive Organometallics

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Organometallics are of *pivotal importance* to modern organic chemistry, either as stoichiometric reagents (Grignard and organozinc compounds, organocuprates etc) or as catalytic intermediates (organopalladium complexes, organoiron species etc). For optimizing the reactions involving these components, their *mechanism* must be understood. In particular, the exact nature of the organometallic species formed in situ needs to be known. To this end, various analytical methods are employed, among which *ESI mass spectrometry* appears particularly promising. This technique not only affords detailed stoichiometric information, but also provides valuable further insight if complemented by *gas-phase reactivity studies*.

My group and I have developed routines for directly probing highly reactive and elusive organometallics by ESI mass spectrometry. To avoid the decomposition of water- and oxygen-sensitive organometallic species, the *careful exclusion of air* is of key importance. Moreover, we employ *mild ESI conditions* to suppress unwanted decomposition reactions of thermolabile complexes. The resulting intact ions are identified on the basis of their *m/z* ratio, their isotope pattern, and their fragmentation behavior.

Under optimized conditions, we are able to *detect highly reactive and sensitive* organomagnesium, -iron, -cobalt, -palladium, -copper, -zinc, -aluminum, and -indium species in intact form.^[1-7] Besides mononuclear complexes, we often also observe the corresponding polynuclear homologues. The *aggregation equilibria* inter-relating these species strongly depend on the solvent and the counter-ion. Apparently, the ESI process itself results in a shift of the equilibria toward higher aggregation states because of the analyte enrichment in the ESI nanodroplets. As MSⁿ experiments reveal, the different aggregates strongly differ in their *microscopic reactivity*; for organocuprate ions, e.g., the absence or presence of a Li⁺ counter-ion has a dramatic effect. In several cases, the reactions observed in the gas phase match the *elementary steps* postulated in catalytic cycles and corroborate the latter. Analyzing elusive organometallics by ESI mass spectrometry, thus, can give unprecedented insight into these fascinating species and, in the long run, will assist in the rational development of new and better reagents and catalysts.

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Stable isotope labelling and mass spectrometric analysis of peptides allows tracking of isotopic flux in microbial communities

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Microbial communities are key for geobiochemical cycles like sources and sinks of methane but are like the microbiome also crucial for maintaining human health. For understanding the functionalities of interaction and interdependency within the consortia the metaproteomics approach was developed. Metaproteomics is defined as the assessment of present proteomes of at least two—and in the best case of all—species in an environmental or organismal sample at a certain condition. Although quantitative proteomes do provide functional insights into the blueprint of physiology the mere abundance of a protein is still not necessarily conclusive for its activity. For assessing the actual active part of the physiological blueprint of the different members of a consortium we developed protein based stable isotope probing (Protein-SIP) approach. Herefore a consortium is fed with an isotopically labelled substrate and the incorporation of the isotopes (can be ^{13}C , ^{15}N , or ^{34}S) into the proteins is detected by mass spectrometry analysis of the peptides. The combination of metaproteomics with Protein-based stable isotope probing (protein-SIP) enables the detection of functional active species and allows an exact quantification of the heavy labeled isotope incorporation. In combination with metagenomics this functional metaproteomics approach can be used to reconstruct carbon and nitrogen fluxes in complex microbial communities. Features of protein-SIP like the high dynamic range of incorporation, the labeling ratio and the distribution of the isotopologue pattern were characterized in the previous years and are nowadays used to describe the microbial activities in soils, aquifers and the gastrointestinal tract of animals and humans.

MALDI-2: Sensitive MS Imaging with Laser-Induced Postionization at 5 Micrometer Pixel Size

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Stichworte: MALDI, MS Imaging, Postionization, Ion yield, Lipids

Einleitung

In MALDI, only a fraction of the desorbed molecules is also ionized [1]. This problem is aggravated in highly-resolved MALDI imaging where only small amounts of material are available and because of ion suppression effects. We used a wavelength-tunable postionization (PI) laser to initiate secondary MALDI-like ionization processes in the gas phase. This way, the ion yields for numerous lipid classes (e.g., cholesterol, certain phospho- and glycolipids) and liposoluble vitamins increased by up to two orders of magnitude. Critical parameters for initiation of the secondary ionization processes are pressure of the cooling gas in the ion source, laser wavelength, pulse energy and delay between the laser pulses. The technology could enable sensitive MALDI-MS imaging with lateral resolution below 10 micrometers.

Experimenteller Teil

A Synapt G2-S HDMS mass spectrometer (Waters) was used. The MALDI ion source was modified for (i) operation at elevated N₂ buffer gas pressure (up to 4 mbar) [2], (ii) for achieving an effective focal laser spot size of 5 μm diameter, and (iii) for adopting the beam of a wavelength-tunable PI laser (OPO, versaScan, GWU Lasertechnik, λ=213–2550 nm, τ~6 ns). The beam of the PI laser was intersecting the initial MALDI plume at a distance of ~500 μm above the sample surface and with adjustable delay. Positive and negative ion mode mass spectra were recorded with 2,5-dihydroxybenzoic acid (DHB) and norharmane matrices, respectively. To achieve a uniform microcrystalline coating of tissue slices, a sublimation/recrystallization protocol was used.

Ergebnisse

In the positive ion mode, in particular lipid species that form [M+H]⁺ ions benefitted strongly from the postionization step [e.g., cholesterol, phosphatidylethanolamines (PE), -serines (PS) and galactosylceramides (GalCer)]. Partially, these compounds (e.g., cholesterol, PE) were detected with MALDI-2 signal intensities that were even surpassing those of the PCs, which are typically dominating the lipid profiles as recorded by conventional MALDI-MSI. About 2-3 more membrane lipid species could tentatively be assigned from mouse brain tissue and high image contrasts were obtained at 5 μm-pixel size from only 20 laser shots. In addition, liposoluble vitamins A, D₃ and E, all detected as [M+H]⁺ ions, could be imaged.

In the negative ion mode, strongly elevated [M-H]⁻ ion signals of numerous membrane lipids (including PE and PS) were generated from the mouse brain tissue. The MALDI-2-MSI analysis of plant (apple) tissue moreover showed that also oligosaccharides (detected as [M-H]⁻ species) benefit from the postionization in a similar manner.

Key parameters for a high MALDI-2 ion yield are (i) a sufficient buffer gas pressure in the ion source (2-3 mbar), which differentiates the method from conventional single and multi-photon ionization performed under high vacuum [1, 3, 4], (ii) a photon energy exceeding the two-photon ionization threshold of the matrix (~310 nm for DHB), and (iii) a suitable pulse delay and laser pulse energy range. Together with the observation of strongly elevated abundances of protonated and deprotonated matrix molecules, that can potentially serve as proton donors and acceptors, these findings suggest that the MALDI-2 ionization processes could be similar to those discussed for conventional MALDI-MS [5].

Given the high signal intensities which we obtained by MALDI-2-MSI from only 5 μm-wide pixels we hypothesize that the technology could enable sensitive MS imaging with even higher lateral resolution in the 1-2 μm range.

Neuer Aspekte

The ion yields in MALDI-MS imaging are boosted by initiation of secondary MALDI-like ionization processes

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Spatially resolved detection of contact and systemic pesticides in plants using DESI-MSI

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Stichworte: Desorption Electrospray Ionization, Pesticides, Mass Spectrometry Imaging

Einleitung

Desorption Electrospray Ionization (DESI) was introduced in 2004 [1] and has gained high interest as a versatile tool in ambient mass spectrometry (MS). DESI is operated by producing a nebulized spray of charged micro-droplets, directed towards the sample. This enables in-situ analysis of molecules on surfaces of solid samples. DESI can be operated in MS Imaging (MSI) mode for spatially resolved analysis by scanning of the entire sample area using computer controlled moving stages. A lateral resolution of 35 to 200 μm can be achieved allowing insights into the location of analytes across the sample. Low sample preparation requirements provide for fast analysis as it is demonstrated here for the analysis of pesticides in and on plant material.

Experimenteller Teil

For the investigation of contact pesticides, commercially available pesticide formulations were applied to *Cotoneaster horizontalis*. One spray (COMPO Schädlingfrei plus, COMPO GmbH und Co. KG) contained rapeseed oil and pyrethrins which act as natural insecticides. The other spray (Zierpflanzenspray Lizetan® plus, Bayer CropScience Deutschland GmbH) contained synthetic insecticides methiocarb and imidacloprid. Both sprays were applied to a leaf, dried and analyzed by DESI-MSI. Dimethoate as a systemically acting pesticide was spiked to potting soil of *Kalanchoe blossfeldiana* using plant protection tablets (Combistäbchen Lizetan, Bayer CropScience Deutschland GmbH). Uptake of pesticides into the plant was monitored. DESI-MSI was carried out using a home-built ion source coupled to an orbital trapping mass spectrometer (Exactive, Thermo Fisher Scientific Inc., Bremen, Germany).

Ergebnisse

The distribution of contact pesticides on plant surfaces is a highly interesting topic for agricultural industry, since a homogenous coverage with pest repellents is required for optimal plant protection. DESI-MSI was used to analyze contact insecticides on leaves using *Cotoneaster horizontalis* as a model system. Contact pesticide sprays were applied until complete moistening of the leaves to ensure distribution of active ingredients all over the sampling area. Comparison of natural water-based COMPO spray and synthetic butane-based Lizetan spray, using a lateral resolution of MSI of 50-100 μm , revealed differences in pesticide distribution that could be related to droplet size and speed of drying. Natural compounds of the COMPO spray showed partial and inhomogeneous coverage of the investigated leaf while synthetic insecticides depicted more homogeneous spreading on the leaf.

Systemic pesticides are incorporated into the plant and form an internal protection against pests. The homogenous distribution throughout the plant is hence an important criterion for efficiency. Analysis of systemic pesticides in plants was carried out by spiking the potting soil of *Kalanchoe blossfeldiana* with 33 mg/kg dimethoate. Progression of the pesticide into the plant was monitored by DESI-MS analysis of the sap, emerging from the petiole after removal of a leaf. After 25 days, a stem from the lower part of the plant was removed and sectioned into 20 μm thick slices. MS imaging of dimethoate revealed distribution in different compartments of the section. Highest signal intensities were observed in the area of phloem and xylem which form the transport system of the plant. Furthermore, primary plant metabolites like sugars and amino acids were detected. 60 days after application, a leaf was removed and cross-sections were prepared. At this time, dimethoate showed a homogenous distribution. These experiments show the first application of DESI-MSI for laterally resolved analysis of pesticides in plants.

Neuer Aspekte

Rapid and simple mapping of contact and systemic pesticides in plants using DESI-MSI

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Mass spectrometry imaging for characterizing parasite host interactions in malaria.

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Stichworte: Anopheles mosquitos, Insect, High-resolution mass spectrometry imaging, MALDI Imaging

Einleitung

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. Malaria still represents a severe threat to human health and welfare. The causative agent *Plasmodium*, undergoes a complex life cycle in its mosquito vector and its mammalian host. To understand the anatomical structure, differential localization, and abundance of (patho)physiologically relevant molecules in malaria vectors and host cells, we developed a high-resolution matrix assisted laser desorption/ionization mass spectrometry imaging method for malaria research. The focus of this study was to reveal the spatial differences in lipid biochemistry within the pathogen-infected and healthy-control samples and to identify differentially expressed phospholipid markers.

Experimenteller Teil

Mouse liver sections of 10-20 μm thickness were prepared with a microcryotome. Mosquito samples were cut in 20 μm thick longitudinal sections with a microcryotome, after carboxymethyl cellulose embedding. A high-resolution atmospheric-pressure MALDI imaging ion source (AP-SMALDI10, TransMIT GmbH, Giessen) was used. The source was coupled to an orbital trapping mass spectrometer (Q Exactive, Thermo Scientific GmbH, Bremen), set to a mass resolving power between 50,000 and 140,000 at $m/z = 200$.

Ergebnisse

AP-MALDI MSI analysis with a mass accuracy of better than 2 ppm allowed for substance identification with high confidence. *Plasmodium berghei*-infected *Anopheles* mosquitos revealed lipid-based differences of PE and ether PI around dissected midguts and whole mosquito sections (20 μm thickness) from different biological replicates. Furthermore, altered expression of these lipid markers has been observed in *P. berghei*-infected mouse liver sections. We believe that this novel approach has a great potential to support future studies on the pathophysiology of malaria and parasite host cell interactions as well as drug discovery approaches.

Neuer Aspekte

High resolution in mass and space for understanding the pathogenesis of malaria infection.

Analyse von Fruchtsäften mittels HPLC/CZE-IMS-qTOF-MS

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Stichworte: IMS; Fruchtsaft, IMS-qTOF-MS, Collision cross sector

Einleitung

The coupling of classical separation methods like capillary zone electrophoresis (CZE) or high performance liquid chromatography (HPLC) to ion mobility mass spectrometry (IM-MS) provides an interesting technique for the analysis of complex samples due to an additional separation dimension compared to usual CZE-MS or HPLC-MS systems.

Analytes like structural isomers which coelute in HPLC and have the same m/z can be further separated in the IM-dimension by their size-to-charge ratio. In CZE, all uncharged compounds elute at the same time with the electroosmotic flow which makes a separation in the IM-dimension interesting. Here, we present the application of HPLC- and CZE-IMS-qTOF for the analysis of complex samples like citric fruit juices, which contain isomeric substances like flavonoid glycosides.

Experimenteller Teil

Coupling of HPLC as well as CZE to the Agilent IM- qTOF 6560 was realized and used for non-targeted analysis of citric fruit juices. Reversed Phase HPLC measurements were carried out with a linear gradient and coupled to the MS using electrospray ionization (ESI). For CZE experiments an ESI-MS interface with a sheath liquid flow of 5 $\mu\text{L}/\text{min}$ was used. All samples were measured in qTOF only mode, where ions just pass the IMS drift tube, and in IM mode, where ions are trapped and released into the drift tube in certain packages. Nitrogen with a pressure of 4 torr was used as drift gas.

Ergebnisse

HPLC and CZE were successfully coupled to IMS-qTOF. Comparing data acquired in IM mode to data obtained in a normal qTOF only mode shows the advantage of IMS, giving a higher peak capacity because analytes are separated by retention or migration time and by the drift time of their ions. This leads to a more detailed fingerprint of complex samples. HPLC measurements of orange juice show examples for isomeric compounds like flavonoid glycosides having the same retention time and the same m/z and would therefore appear as one substance in usual LC-qTOF runs. Here it can be shown that coeluting substances are further separated by their drift time in IMS. The same can be found in CZE-IMS-qTOF applications. Additionally, neutral substances like sugars which elute with the electroosmotic flow (EOF) and can therefore not be separated in CZE show several peaks in the IM drift spectrum.

Neuer Aspekte

Vorstellung des Agilent 6560 in Kombination mit CZE und HPLC

A non-targeted LC-HRMS multi-method for the detection of food allergen trace contaminations

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Stichworte: Nut allergens, Multi-Method, LC-HRMS, Peptide markers, Protein isoforms

Einleitung

Mass spectrometry based proteomics methods have been increasingly raising in interest in molecular allergology over the last decade [1]. Up to 17% of the European population are estimated to suffer from food allergy, an IgE-mediated overreaction against specific food proteins that may have life-threatening consequences[2]. As reliable and cost-effective analytical methods are needed to guarantee for a safe and satisfying product variety for allergic patients mass spectrometry is progressively gaining importance for the development of novel approaches to the detection of allergens in foodstuffs[3].

We here present the development of to the best of our knowledge the first comprehensive LC-HRMS multi-allergen-screening method for six different allergic nuts in processed food.

Experimenteller Teil

Marker peptides were identified by LC-MS/MS analysis of tryptic digests from six relevant nut species on a LTQ Orbitrap XL mass spectrometer (Thermo Fisher). Peptide sequences were verified by their fragment spectra using the software PEAKS 7. For each peptide the analytical specificity was checked both *in silico* via a database search (BLAST, UniProtKB-Plants) and experimentally through analysis of several blank matrices with relevant contamination potential. Only peptides that proved species-specific and well detectable in representative food samples were considered appropriate markers.

Specificity and sensitivity of the developed method was assessed by spiking experiments of nut proteins in ice cream matrix and statistical validation according to DIN 32645.

Ergebnisse

Using bottom-up proteomics 44 marker peptides were identified for the detection of almond, cashew, hazelnut, peanut, pistachio and walnut. All marker peptides are specific against other nut species and relevant matrices and were well detectable in a protein rich model food matrix. A deeper survey of the marker peptides showed that some originate only from certain isoforms of a major allergen that might not be present in all nut cultivars. We therefore strongly advise to include at least 3-4 marker peptides in MS based methods to ensure the reliable detection of allergens.

Based on these findings an analytical LC-MS method for contaminations of nuts in processed foods was developed using a LTQ Orbitrap XL mass spectrometer. Analysis of spiked ice cream samples and validation based on linear regression analysis gave limits of detection (LOD) below 12 mg/kg for at least three marker peptides from almond, cashew, hazelnut, peanut and pistachio and below 36 mg/kg for walnut respectively. This demonstrates that MS based analysis of allergens can compete with the so far more common methods ELISA and PCR in terms of analytical sensitivity. In contrast to ELISA assays our MS method also provides exact information about the detected protein sequences and allows for multiplex allergen analysis. By using the allergenic proteins as analytical targets it furthermore avoids the risk of false negative results that indirect detection methods like PCR face due to a potential discrimination of DNA and proteins.

Neuer Aspekte

First multi-screening method for food allergens using LC-HRMS.

Survey to the effect of allergen isoforms onto peptide based allergen detection.

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DART-FT-ICR-MS-Studie zur Abgabe von Polydimethylsiloxanen in Lebensmittel beim Gebrauch von Silicongummi-Haushaltsartikeln

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Stichworte: DART-MS, Haushaltsartikel, Silicongummi, Lebensmittel, FT-ICR-MS

Einleitung

Direct Analysis in Real Time (DART) [1] ist eine effiziente Methode zur schnellen Analyse von Polydimethylsiloxanen (PDMS, Silicongummi); dies wurde bereits zur Massenkalisierung im positiv-Ionen DART-Modus genutzt [2]. Ebenso ließen sich Produkte aus Silicongummi bezüglich ihrer Tendenz analysieren, PDMS beim Gebrauch abzugeben [3]. Beispielsweise wurden Silicon-Backformen, Teigschaber, Schnuller oder Trinksauger untersucht. Es zeigten sich bei allen Artikeln intensive DART-Spektren von PDMS und demzufolge vergleichbare Freisetzung von PDMS. Auch im Bereich von Lebensmittelqualität und -sicherheit spielt DART-MS eine Rolle [4]. Hier werden nun Ergebnisse zur Freisetzung von PDMS beim Gebrauch von flexiblen Silicongummi-Backformen präsentiert. Mittels DART-MS ist ein direkter Nachweis von PDMS auf der Oberfläche der Backwaren möglich.

Experimenteller Teil

Die positiv-Ionen DART-Spektren wurden an einem Bruker ApexQe FT-ICR-Massenspektrometer (Bruker Daltonik, Bremen) mit ESI-MALDI-Kombiquelle aufgenommen. Daran wurde eine DART-SVP-Quelle (IonSense, Saugus, MA) verwendet, die über eine zusätzliche Pumpstufe (VapurInterface) am ESI-Interface montiert wurde. Die Proben, kleine Stücke von Muffins, wurden manuell unter die im 45°-Winkel montierte Quelle positioniert. Helium wurde als DART-Gas bei 300°C eingesetzt. Die Ionen wurden im RF-only Hexapol für 1,0 s akkumuliert und dann in der ICR-Zelle analysiert [3]. Es wurden 8–16 Transienten für ein Breitband-Spektrum mit 1 MB Datensatz akkumuliert. Die externe Massenkalisierung wurde mit einer ionischen Flüssigkeit [5] oder mit Siliconöl [2] erstellt. Die Gerätesteuerung erfolgte mit Bruker ApexControl 3.0.0, die Auswertung der Spektren mit Data Analysis 4.0.

Ergebnisse

Eine grobe Quantifizierung der bei einer DART-Messung von Silicongummi-Artikeln freigesetzten Menge an PDMS ließ sich dadurch erreichen, das seine Eichreihe mit Siliconöl auf einem Objektträger aus Glas erstellt wurde. Darauf basierend wurden PDMS-Abgaben von >20 µg bis > 100 µg während der Messdauer von 16 s für eine Messung ermittelt. Dann wurden Extraktionsexperimente durchgeführt, bei denen PDMS von Backformen für eine Stunde in Rapsöl bei 180 °C einen Backvorgang simulierte. Dabei wurden Werte von 1 µg PDMS pro mg Öl gefunden.

Schließlich wurden reale Backexperimente vorgenommen und Muffins (Mehl, Zucker, Butter, Eier, Backpulver, teils auch Kakao) aus Blechformen mit denen aus Silicon-Backformen verglichen. Von der Oberfläche der Muffins aus Silicon-Backformen war PDMS direkt und deutlich nachweisbar. Die Identität von PDMS wurde anhand der exakten Massen sowie den Isotopenmustern der PDMS abgesichert. Die Ergebnisse deuten stark auf eine Anreicherung von PDMS an bestimmten Stellen der Backwaren. Bei allen Muffins traten zudem meist intensive Signale auf, die $[M+NH_4]^+$ -Ionen und zugehörigen Cluster-Ionen von Butterfetten zugeordnet werden konnten. Auch die zugehörigen Muffin-Backpapiere wurden überprüft. Sie enthielten große Mengen von Tributylacetylcitrat, einem Weichmacher, der allerdings mit der verwendeten Methode nicht in den Backwaren nachgewiesen wurde.

Die Auswahl der geringen Anzahl von Produkten erfolgte zufällig und die hier aufgeführten Werte sollten nicht verwendet werden, um die Produkte exakt dieser Anbieter zu diskreditieren. Vielmehr legen bisherige Ergebnisse nahe, dass alle Artikel aus Silicongummi dieses Verhalten zeigen. Es wird daher angeregt, detaillierte Untersuchungen zur Freisetzung von PDMS aus Silicongummi in lebensmittelanalytischen oder materialprüfenden Laboren anzustellen.

Neuer Aspekte

Haushaltsartikel aus Silicongummi können beim Gebrauch PDMS-Oligomere freisetzen, die hier mittels DART-FT-ICR-MS direkt auf der Oberfläche von Backwaren nachgewiesen wurden.

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Studying viral assemblies with mass spectrometry and XFELs

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Stichworte: native mass spectrometry, structural biology, sample delivery, single particle diffraction

Einleitung

Recent successes in femtosecond X-ray protein nano-crystallography and imaging of single particles demonstrate the prospects of X-ray free-electron lasers (XFELs) for biophysics. Due to the intense and short femtosecond pulses, diffraction patterns of a single particle can be recorded before damaging occurs. Thereby, the major bottleneck in structural biology to obtain large high quality crystals may be overcome. Since the sample is destroyed by the X-ray pulse, a full dataset is acquired in many shots of identical particles. The technique can be used to study structural changes in *e.g.* protein complexes in a time-resolved manner.

Experimenteller Teil

However, current sample delivery techniques suffer from an increased background lowering the resolution or are inefficient wasting precious samples. Moreover, the reconstruction of a single structure requires extensive computational sorting.

Using native mass spectrometry (MS) for sample delivery, sample consumption would be reduced. Local increased concentrations of a sample can be achieved in ion traps to optimise the interaction rate with the FEL beam. MS is especially attractive for reaction monitoring as minor or transient species can be selected for imaging.

Ergebnisse

With such an online purification, low populated species can be studied, which would otherwise pass unnoticed. Additional components, like ion mobility or dipole alignment, enable data pre-sorting according to shape and orientation. This could tremendously lower the computational costs to sort diffraction patterns and lead to orders of magnitude shorter calculation times.

An introduction to XFELs, sample delivery systems and design considerations for the mass spectrometer are presented.

Additionally, most recent native MS data on processing and assembly of non-structural coronavirus proteins will be presented, which form the viral replication complex, and other viral systems my group is currently working on.

Neuer Aspekte

New method in structural biology

UV-MALDI-MS Analysis of Non-Covalent Streptavidin-Biotin Complexes with a 6-Aza-2-thiothymine Matrix: Effect of Wavelength and Fluence on their Detection

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Stichworte: MALDI-MS, ATT matrix, Non-covalent complexes, Streptavidin-biotin, Laser wavelength

Einleitung

The analysis of non-covalent complexes by MALDI-MS poses a challenge. Factors that all can result in their unintended dissociation are the acidic character of most MALDI matrices, the requirement of analyte-matrix co-crystallization, and the thermal load imparted during the MALDI process. Best results are frequently obtained with the near pH-neutral 6-aza-2-thiothymine (ATT) matrix [1,2]. Interestingly, this compound exhibits a peak absorption close to 265 nm, far off the standard MALDI wavelengths of 337 and 355 nm. Here, we investigated the influence of the laser wavelength and that of the laser fluence on the ion generation of streptavidin-biotinyl glucagon complexes desorbed from the ATT matrix.

Experimenteller Teil

Samples were prepared from aqueous solutions using the dried-droplet method. MS experiments were performed with an oTOF-mass spectrometer that was equipped with an optical parametric oscillator laser (OPO; versaScan, GWU-Lasertechnik) and provided tunable laser light with 5 ns pulse duration. Ion signals were recorded for excitation wavelengths between 213-360 nm (step size: 10 nm). The elliptical laser spot size on the target was ~200x400 μm^2 .

Ergebnisse

Intact non-covalent streptavidin-biotin complexes were detected with optimal ion yields in a wavelength-fluence band between 300 and 340 nm, which is covering the 337 nm emission line of the N_2 -laser but not that the 355 nm output of the frequency-tripled Nd:YAG laser. With regard to the laser fluence, maximum yields were obtained for fluences exceeding the ion detection threshold by a factor of 3 to 5.

In line with our previous results on the detection of gastrin I-peptide-complexes [2], an increased laser penetration depth at these wavelengths of reduced optical absorption on the falling slope of the matrix absorption band appears to support the detection of the complex, while a too low absorption at even higher wavelengths prevents an efficient ionization. The ejection of larger volume elements could lead to an overall process that is similar to IR-MALDI, which is generally regarded as providing “softer” MALDI conditions.

Because streptavidin is highly absorbing at lower UV-wavelengths, the second maximum at 240 nm observed for the detection of the non-absorbing gastrin I-peptide complexes is not available. This is presumably caused by direct photodissociation of the streptavidin monomer. For wavelengths between 290 and 300 nm, only the monomers were detected with high signal intensities. Despite of its high affinity (dissociation constant $\sim 10^{-15}$ M [3]) and the effective collisional cooling in the oMALDI ion source, the thermal load acquired within the MALDI process under these conditions appears here as too high to allow for an intact desorption of the complex.

The results demonstrate that careful adjustment of all critical sample and irradiation parameters including the excitation wavelength is generally necessary to enable the intact MALDI-MS analysis of weakly non-covalent bound receptor-ligand complexes.

Neuer Aspekte

Detection of intact streptavidin-biotin complexes by MALDI-MS.

Referenzen

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Fragmentierung von Ethylaminosubstituierten Farbstoffen mittels CID und PD

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Stichworte: Laserfarbstoffe, Fragmentierung, CID, Fragmentierungsmechanismus

Einleitung

Coumarine, Oxazine und Rhodamine sind sowohl als Laserfarbstoffe als auch als Label für Photofragmentierungen bekannt. Im Rahmen dieser Arbeit wurde die Fragmentierung unterschiedlicher Farbstofflabel mit Ethylaminoseitengruppen mittels CID und PD untersucht. Für die PD Messungen wurde ein neues Experiment aufgebaut, sodass es möglich ist mittels Nanosekundenpulsen unterschiedlicher Wellenlängen Untersuchungen in der ICR Zelle durchzuführen. Hierbei zeigte sich ein ungewöhnliches Fragmentierungsverhalten der Ethylaminoseitengruppe unter Bildung eines Azarinringes abhängig von der direkten Umgebung der Seitengruppe und der verwendeten Wellenlänge.

Experimenteller Teil

Alle Messungen wurden mit einem 7.05 T FT-ICR Massenspektrometer der Firma *Bruker Daltonik* (Bremen) durchgeführt. Die unterschiedlichen Proben wurden mittels Elektrospray Ionisation in die Gasphase überführt, die entsprechenden Ionen in der ICR-Zelle isoliert und anschließend durch CID (*Collision Induced Dissociation*) mit Argon als Stoßgas sowie PD (*Photodissoziation*) mit unterschiedlichen Wellenlängen fragmentiert. Hierzu wurde ein Nd:YAG Laser von *Continuum* zur Fragmentierung mit Nanosekundenpulsen bei einer Wellenlänge von 532 nm, 355 nm sowie 266 nm verwendet. Alle verwendeten Substanzen wurden aus Grundchemikalien von *Sigma Aldrich* synthetisiert.

Ergebnisse

Bei den durchgeführten Fragmentierungsreaktionen mittels CID und PD an den substituierten Farbstofflabeln wurden Fragmente sowohl aus der Ethylaminoseitengruppe als auch aus den Farbstofflabeln selbst beobachtet. Neben den erwarteten Fragmenten der Ethylaminogruppe wurden weitere Fragmente beobachtet, die nur unter Beteiligung der benachbarten Alkylgruppen möglich sind. Anhand unterschiedlicher Farbstoffe konnte der Einfluss der Größe sowie der unterschiedlichen Substitutionen untersucht werden, sodass ein zur Bildung der Verluste zugrunde liegender Fragmentierungsmechanismus entwickelt werden konnte. Neben der Abhängigkeit der Abspaltungen von der direkten Umgebung der Seitengruppe konnte auch der Einfluss der Wellenlänge untersucht werden. Insgesamt konnte gezeigt werden, dass die unterschiedlichen Farbstofflabel bei Fragmentierungsuntersuchungen mittels CID und PD viele Abspaltungen bilden und dass Photofragmentierung bei unterschiedlichen Wellenlängen einen großen Einfluss auf die Bildung und Intensität der unterschiedlichen Fragmente hat. Ebenso wie bei den Xanthenfarbstoffen [1] zeigt sich auch bei den substituierten Coumarinen und Oxazinen eine Verletzung der *Even Electron Rule*, da bei diesen Fällen ebenfalls unterschiedliche Radikale beobachtet werden konnten.

Neuer Aspekte

Fragmentierung von Farbstofflabeln mittels CID und PD

Referenzen

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Towards a mass spectrometric Western Blot: Multiplexed Targeted Protein Quantification by Gel-LC-MS/MS

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Stichworte: Quantitative Proteomics, Targeted Protein Quantification, Gel-LC-MS/MS

Einleitung

Introduction

Quantification of protein abundance changes in biological research is commonly performed by Western Blots or ELISA assays. However, these methods are often impeded by the availability and specificity of antibodies, dynamic range and multiplexing capabilities. Synthesis of isotopic labeled peptides for protein quantification by mass spectrometry is laborious, expensive and inflexible for small-scale discovery studies.

We have developed a fast, inexpensive and flexible pipeline for targeted multiplexed protein quantification that relies on the recombinant expression of artificial proteins comprising isotopic labeled reporter peptides for several proteins and Gel-LC-MS/MS. Absolute quantification is achieved by calibration with a quantified protein standard and this method may substitute Western Blots and/or ELISA assays in selected biological and clinical applications.

Experimenteller Teil

Methods

Based on observation of previous label-free LC-MS/MS analyses for each targeted protein the most abundant 4 to 8 tryptic peptides were selected. The peptides were translated into a single artificial gene that was chemically synthesized (Life Technologies). The gene was subcloned into a bacterial expression vector and expressed in a Lys, Arg dual-auxotroph *E.coli* strain in media complemented with $^{13}\text{C}^{15}\text{N}$ -Arg and ^{13}C -Lys (Silantes). The artificial protein was submitted to SDS gel electrophoresis and the protein band was combined with the gel slice containing the protein(s) of interest, co-digested with trypsin in-gel and analyzed by LC-MS/MS on a Orbitrap Velos (Thermo Fisher Scientific) employing the programs Progenesis LC-MS (Nonlinear Dynamics) and/or Xcalibur Qualbrowser (Thermo Fisher Scientific).

Ergebnisse

Preliminary Results & Discussion

Different methods for protein quantification by isotopic labeled peptides (AQUA, [1]), recombinant expression of concatenated peptides [2] or single protein fragments in *E. coli* [3,4] or wheat germ extracts [5] have been reported previously but have not gained wide-spread application.

In our methodical approach each protein is represented by several most abundant tryptic peptides, which improves reliability of quantification. Reporter peptides for multiple proteins are combined into a single artificial gene that is chemically synthesized and available "on-demand". With high efficiency we have expressed artificial genes comprising more than 30 peptides representing up to 8 proteins and a molecular weight up to 77kD. SDS gel electrophoresis removes the majority of bacterial background and omits the necessity of purification steps and native solubility of the artificial protein. The gel slice containing the proteins of interest and the gel band containing the quantitative standard are combined and co-digested in a single reaction.

We have evaluated the capabilities of this method in proof-of-principle experiments. A chimeric protein of 41kD comprising 31 peptides representing 5 proteins was expressed. Full-length expression was confirmed and incorporation of the heavy isotope label was higher than 98%. Five proteins were spiked as an equimolar mixture at different amounts from 20 fmol to 25 pmol per lane into a dominant background of 50 μg *E. coli* lysate. The lysate was separated by SDS-gel electrophoresis, gel slices were excised, combined with gel bands with the chimeric protein, digested with trypsin and respective proteins were then quantified by Gel-LC-MS/MS.

Based on the abundance ratio of “light” and “heavy” peptide counterparts relative quantification of spiked proteins was accurate ($R^2=0.96$) over a dynamic range from 2 fmol to 2.7 pmol of the injected target proteins which enabled their quantification at 0.17-1.94 ng amounts despite dominating ($>10^4$ -fold) excess of background *E.coli* proteins.

Neuer Aspekte

Flexible multiplexed targeted protein quantification by Gel - LC MS/MS.

Referenzen

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A universal workflow for efficient enrichment of proteins in mass-spectrometry based proteomics

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Stichworte: Protein Enrichment, Proteomics, Solid Phase Extraction, Highly Diluted Samples

Einleitung

In modern proteomics protein enrichment from diluted samples like body fluids or culture supernatants is a common task. Enrichment is achieved for example by protein precipitation, ultra-filtration or solid phase enrichment (SPE). But all established workflows have certain drawbacks. We have developed an optimized protocol for protein SPE with Strata-Clean beads, leading to bias-free, reproducible results in protein enrichment in very dilute samples. Our protocol [1] is a user-friendly, one-pot method and compatible with GeLC-MS and gelfree workflows. We further have enhanced the scope of StrataClean beads for protein storage on the beads at ambient temperature.

Experimenteller Teil

We have introduced a priming step for removal of contaminants by acidic hydrolysis. Two workflows were established: a GeLC-MS workflow with optimized protein elution and a gel-free workflow comprising on-bead digest with trypsin. The optimized GeLC-MS workflow was compared with classic techniques. We have demonstrated enrichment from extremely diluted samples (0.1 µg/ml) and unbiased protein purification by LC-MS. Further, we suggest protein storage on the desiccated beads at ambient temperature as a novel application allowing for shipping of samples in a very cost effective way.

Ergebnisse

Due to quantitative removal of contaminants by bead-priming before protein purification, we assured reproducible and highly efficient protein binding of the StrataClean beads. Several steps of the gel-based workflow were optimized to improve protein elution from the beads. We compared StrataClean enrichment from extremely diluted samples with detergent assisted TCA precipitation and demonstrated highly efficient protein enrichment by a factor of 10,000 with StrataClean in a GeLC-MS experiment. By our new workflow we were able to identify 50 % more proteins reproducible in three technical replicates as with the classic precipitation workflow.

With the gel-free workflow we were able to prove bias free protein enrichment after on-bead protein digest. This is a highly interesting option for label-free quantitative proteome studies, including absolute protein quantification.

Finally, we established easy protein shipping at ambient temperature. We were able to show that after desiccation of the loaded beads proteins are stable without cooling for more than 10 days. This allows for long range shipping of protein samples at low cost without necessity of elaborated cooling logistics.

The combination of highly efficient, bias free protein enrichment, protein purification from extremely diluted samples, GeLC-MS and gel-free analyses and convenient protein shipping at full advantage of the one-pot/ fast protocol of SPE by StrataClean beads makes our new protocol the first universal method for protein enrichment in mass-spectrometry based proteomics.

Neuer Aspekte

A protocol for superior protein enrichment from highly diluted samples and protein storage at ambient temperature.

Referenzen

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Characterization of an Improved Ultra-High Resolution Quadrupole Time of Flight (UHR-QTOF) Instrument for Proteomics Applications

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Stichworte: bottom-up, proteomics, QTOF, cell lysate

Einleitung

In shotgun proteomics it is desirable to identify and quantify a large number of individual peptides from complex samples, such as tryptic digests of human plasma samples or whole cell lysates in the shortest possible time. Complexity and concentration range, however, pose a great challenge to the MS instrumentation in terms of sensitivity, resolution and dynamic range. Several hardware modifications of a bench-top UHR-TOF instrument were carried out and evaluated addressing these particular performance aspects.

Experimenteller Teil

To test the impact of these modifications on proteomics performance, different complex tryptic digests were analyzed with nano-flow UHPLC and a CaptiveSpray ion source connected to the impact II instrument (Bruker Daltonik). For peptide identification the MaxQuant software package was used (*Nature Biotechnology* **26**, 1367 - 1372 (2008)).

Ergebnisse

For higher sensitivity at fast acquisition speed, ion extraction from the collision cell into the orthogonal acceleration of the TOF-analyzer was improved by using a novel collision cell design. Increased resolution without changing the effective flight path could be achieved with a modified reflectron. In addition, a faster detector (reduced width of individual ion signals) led to further improvements in resolving power. Comparison with previous design clearly shows an increased resolving power resulting in a full sensitivity resolution (FSR) of 50,000.

Using an optimized detector digitizer combination, a threefold higher dynamic range was observed. However in complex samples, the dynamic range is also limited by the capability of the instrument to resolve nearly isobaric compounds. The dynamic range detectable in proteomics samples has been investigated. For one of the most difficult proteomics samples - undepleted plasma - we could cover a dynamic range of 4 orders of magnitude. We will show further results evaluating the impact of the novel hardware features on performance improvements for proteomics applications.

Neuer Aspekte

Improvements to several hardware components allow identification and quantification of complex proteomics samples with very high dynamic range

Thermal Proteome Profiling

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Stichworte: Quantitative Massenspektrometrie, Targetidentifizierung, Proteomik

Einleitung

Changes in the thermal stability of proteins are frequently used to study ligand binding and, with the recent development of the cellular thermal shift assay (CETSA), can now be observed in living cells (1), enabling the monitoring of target engagement, which is a key parameter in drug discovery. By combining the CETSA method with multiplexed quantitative mass spectrometry, we established the proteome-wide determination of protein thermal stability in intact cells as an independent and complementary strategy for the characterization of cellular proteotypes. We further demonstrate that monitoring thermal stability across cellular proteomes in different states, such as under drug treatment, enables the identification of direct physical interaction partners and downstream effectors as markers of target engagement and drug efficacy.

Experimenteller Teil

Cells were cultured under differential conditions, such as drug treatment. In an alternative method, the cells were extracted first, and the extracts were treated with drug. For each condition, the cell or cell-extract sample was divided into 10 aliquots. Aliquots were subjected to heating at the indicated temperatures. Samples of intact cells were subsequently subjected to extraction with PBS. After digestion with trypsin, each sample was labeled with a different TMT10 isotope tag. Subsequently, all samples from each condition were mixed and analyzed by means of LC-MS/MS. The obtained reporter ion intensities were used to fit a melting curve and calculate the melting temperature T_m of each protein separately for the two conditions.

Ergebnisse

The thermal stability of proteins can be used to assess ligand binding in living cells. We have generalized this concept by determining the thermal profiles of more than 7000 proteins in human cells by means of mass spectrometry. Monitoring the effects of small-molecule ligands on the profiles delineated more than 50 targets for the kinase inhibitor staurosporine. We identified the heme biosynthesis enzyme ferrochelatase as a target of kinase inhibitors and suggest that its inhibition causes the phototoxicity observed with vemurafenib and alectinib. Thermal shifts were also observed for downstream effectors of drug treatment. In live cells, dasatinib induced shifts in BCR-ABL pathway proteins, including CRK/CRKL. Thermal proteome profiling provides an unbiased measure of drug-target engagement and facilitates identification of markers for drug efficacy and toxicity.

Neuer Aspekte

Neue Massenspektrometrie basierte Methode um die Protein-Bindung bioaktiver Substanzen in Zellen zu messen.

Referenzen

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Uncovering molecular details of protein "misfolding - aggregation" using affinity- and ion mobility- mass spectrometry: Physiological and Parkinson- Synucleins

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Stichworte: Alpha-Synuclein, Physiological and pathophysiological synucleins, oligomerisation-aggregation, ion mobility-MS, affinity-MS

Einleitung

A large variety of cellular processes are based on the formation and dynamics of multi- and supramolecular protein assemblies, and several diseases, previously thought to be unrelated, such as cancer and neurodegenerative diseases, are characterised by "misfolded" protein aggregates. Chemical structures and reaction pathways of pathophysiological aggregates are only poorly characterised at present. "Soft-ionisation" mass spectrometry (MS), such as HPLC-electrospray-MS, is often unsuitable to direct analysis of reaction pathways and intermediates in aggregation. Recently, ion mobility- MS (IM-MS) has been emerging as a new tool for analysis of protein aggregation due to its *concentration-independent* gas phase separation capability.

Experimenteller Teil

Although α Syn and β Syn are closely related with a sequence homology of 62%, β Syn has a different triplet sequence (70-72), VFS, lacks the 11- amino acid stretch Gly73-Glu83 and is devoid of any fragmentation and aggregation. The fragmentation specificity of α Syn motivated us to examine mutants with modified fragmentation and aggregation propensity. Focusing on the substitution of β -sheet- with α -helix- favoring residues, we designed several α Syn mutants with substitutions of three to six aa residues within the hydrophobic aggregation-promoting domain (70-76). All mutant synucleins were prepared by expression in *E.Coli* and purified by semipreparative HPLC.

Ergebnisse

First applications of IM-MS to the *in vitro* oligomerization of α -synuclein (α Syn), a key protein for Parkinson's disease, enabled the identification of hitherto unknown degradation and aggregation products. Time- dependent studies of the *in vitro* oligomerization- aggregation of α Syn provided the first identification of a specific autoproteolytic fragmentation, particularly a highly aggregation-prone fragment by cleavage at V71/T72 in the β -breaking triplett VVT(70-72) in the central aggregation domain [1]. The corresponding recombinant α Syn(72-140) fragment showed substantially faster aggregation and high neurotoxicity compared to the intact protein. The recent development of combined, online bioaffinity-MS methods [2] enabled first direct ("top-down") structural studies *in vivo*, such as from brain homogenate. Applications of affinity-MS will be discussed using epitope-specific α Syn-antibodies [3] for the characterization of oligomers and interactions *in vivo*. Most recently, specific mutations of the central (70-72) triplett in synucleins, and affinity-MS provided breakthrough results, such as mutation of the VFS(70-72) triplett from physiological β Syn into α Syn completely abolished neurotoxic aggregation [4].

Neuer Aspekte

Ion mobility- and affinity-MS are powerful tools for the molecular elucidation of structures and intermediates of α synuclein aggregation.

Referenzen

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Negative Ion Electrospray Tandem Mass Spectrometry of Polyketides

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Stichworte: negative ion electrospray tandem mass spectrometry, polyketides, stilbenes, flavonoids, anthraquinones

Einleitung

Polyketides are widely distributed in plant organisms [1]. During the last twenty years mass spectrometry, especially soft ionization methods as electrospray in combination with tandem mass spectral methods represent an useful tool for structural investigations of such compounds [2-5]. The present lecture deals with the mass spectral behavior of representative model compounds from different important polyketide classes (stilbenes, flavones, isoflavones, flavonols, flavanones, flavanols, phloroglucinols, anthraquinones and bisanthraquinones) under negative ionization conditions. The aim of this study is to compare representative compounds possessing a substituted 1,3-dihydroxy benzene moiety as a common structural feature like resveratrol, apigenin, genistein, kaempferol, naringenin, catechin, emodin and hypericin for a classification of the observed fragmentations in a more general way.

Experimenteller Teil

The negative ion electrospray high-resolution MSⁿ and HCD data of the selected representative model compounds were obtained from an Orbitrap Elite instrument (Thermo Scientific GmbH Bremen, Germany) by direct infusion.

Ergebnisse

The stilbene resveratrol shows an unusual fragmentation displaying not only an intermediate methyl loss, but also a rearrangement to a naphthol-like fragment ion as indicated by a comparison of the HCD spectra of naphth-1-ol and naphth-2-ol with that of the key ion at m/z 143 obtained by MS² measurements. In case of resveratrol, apigenin and genistein the loss of carbon suboxide (C₃O₂) under negative ionization can be assigned as an important fragmentation step indicating the presence of a substituted 1,3-dihydroxy benzene moiety as a common structural feature [2, 4]. On the other hand, in all compounds investigated the prominent unusual loss of CO₂ from the [M-H]⁻-ion is of special interest. Other fragmentations being typical for the polyketides discussed are successive losses of ketene (CH₂CO) and CO units as well as specific structure-dependent key fragments. Furthermore, it is demonstrated that high-resolution MS² and MS³ measurements also allow a differentiation of the origin of isobaric ions. It can be stated that in addition to positive ion electrospray MS/MS data negative ion high-resolution tandem mass spectrometry represents a powerful analytical tool for the structural characterization of such compounds.

Neuer Aspekte

Comparison of the mass spectral behavior of different polyketide classes under negative ionization to generalize the observed fragmentations

Referenzen

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Combination of proteomic and metabolomic approaches in plant stress research

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Stichworte: AGEs, glycation, post-translational modifications, tandem mass spectrometry, plant environmental stress

Einleitung

Glycation is a non-enzymatic post-translational modification formed by reaction of reducing sugars (aldoses and ketoses) with amino groups of proteins forming so-called Amadori and Heyns compounds, respectively. Their further oxidation and formation of α -dicarbonyls (glycoxidation) yield advanced glycation end-products (AGEs) known for their pro-inflammatory effects in humans. Though AGEs readily form during thermal processing of foods, it may occur also during the life time of crop plants. In this context, it is important to know the patterns of AGEs in crop plants and effect of environmental stress on their dynamics.

Experimenteller Teil

The models of high light, drought and metal stress were established with *Arabidopsis thaliana*, *Nicotiana tabacum* and *Brassica napus*. The leaves were harvested before stress application and in multiple points throughout the stress period. The analytical strategy (applied to *A. thaliana* and *B. napus*) relied on the combination of LC-based bottom-up proteomics (LC x LC-ESI-Orbitrap-LIT-MS/MS data dependent acquisition experiments), untargeted and targeted metabolomics (GC-EI-Q-MS) and model glycation experiments with synthetic peptides (LC-QqTOF-MS and MS/MS).

Ergebnisse

Even unstressed *A. thaliana* and *B. napus* plants displayed rich patterns of glycated and glycoxidated proteins (up to 386 and 900 modified peptides, respectively), representing mostly regulatory pathways, protein and nucleic acid metabolism. The product structures were comprehensively characterized with ESI-MS/MS. Light and metal stress resulted in increased Amadori/Heyns product formation (mostly triose- and pentose-derived). The number of AGE-modified sites (dominating with arginine residues) was essentially increased only under drought stress conditions (44 unique sequences), while all stresses resulted in significant increase in intensities of corresponding signals. However, the majority of AGE-modified sites did not resemble glycated ones. In contrast, all stresses caused significant increase of tissue carbohydrate contents without elevation of free α -dicarbonyl levels, indicating autoxidation of sugars with immediate binding of α -dicarbonyls to proteins as the most probable scenario. Reactivity towards peptides, glycation and AGE-formation potentials of individual sugars were characterized *by in vitro* experiments with synthetic peptides. Thereby, *D*-glucoso-6-phosphate showed the highest reactivity, while dihydroxyacetone phosphate, *D*-glyceraldehyde and *D*-ribose demonstrated the highest potential for AGE formation.

Neuer Aspekte

The stress-related patterns of plant glycation and glycoxidation are characterized for the first time. The mechanisms and precursors proposed.

Online EC/(LC)/ESI-MS for investigating the oxidative metabolism of roxarsone

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Stichworte: Electrochemistry, Metabolism, Roxarsone, Arsenic

Einleitung

Electrochemistry (EC) coupled to electrospray ionization mass spectrometry (ESI-MS) has been proven to be a valuable tool for the simulation of the oxidative drug metabolism. Application of EC/ESI-MS enables the generation and identification of potential phase I metabolites of xenobiotics as well as the investigation of the phase II metabolism and the reactivity of the oxidation products towards biomolecules.

In the present study, the metabolism of the arsenic containing drug roxarsone (ROX) is simulated. ROX is an animal feed additive that is frequently used since 1944 and is presumed to be excreted unmetabolized from the animal organism. However, the detailed metabolic pathway is not entirely known and for this reason, toxic effects to humans cannot be excluded.

Experimenteller Teil

The oxidative metabolism of ROX has been simulated by hyphenation of an EC cell with ESI-MS. Application of a potential ramp from 0-2500 mV and the presentation of the data in form of a three-dimensional mass voltammogram (MV) enabled the identification of the oxidation products and provided the potential of the highest conversion rate.

Addition of a peptide solution (e.g., glutathione (GSH)) directly after the EC cell facilitated the simulation of the phase II metabolism of ROX. By extending the setup with a high performance liquid chromatography (HPLC) system between EC and MS, the reactivity of the oxidation products towards proteins has been investigated. The HPLC system was necessary to remove salts and excessive metabolites from the protein fraction.

Ergebnisse

The oxidative transformation of the arsenic containing drug ROX has been investigated to simulate the phase I metabolism by means of EC coupled online to ESI-MS. The generation of a mass voltammogram, where mass spectra are plotted against the applied potential, enabled a broad overview on the generated oxidation products. Oxidizing ROX, many different products were detected. Next to mono-, di- and tri-hydroxylated ROX, also aminated compounds as well as the corresponding dehydrogenated species were found. In addition to hydroxylation and amination, a carbon-arsenic-bond cleavage took place and thus, the toxic inorganic species arsenate (As(V)) was formed.

Besides the studies of the phase I metabolism of ROX, investigations on the reactivity of the products towards biomolecules were carried out as well. In first experiments, the phase II metabolism was simulated by adding a solution of the tripeptide GSH to the effluent of the EC cell via a T-piece. In this case, two different adducts with transformation products of ROX could be detected. When the experimental setup was extended by an HPLC system, protein adduct formation was found. For adduct formation experiments, two model proteins were selected: The relatively small, homogeneous whey protein b-LGA and the more complex protein HSA. In both cases, adduct formation with the same oxidation products that showed reactivity towards GSH was observed. Hence, using EC coupled to (LC)/ESI-MS, not only the identification of potential phase I metabolites is possible, but also the estimation of the phase II metabolism as well as the toxicity of the compounds.

Neuer Aspekte

The oxidative metabolism of ROX was simulated successfully resulting in toxic arsenic species and intermediates that show reactivity towards biomolecules.

Metabolic pathway driven targeted metabolomics – a “quickstep” from mass spectrometric raw data to biologically relevant conclusions

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Stichworte: QTOF, metabolomics, coffee, metabolic pathway

Einleitung

Biologists often think in terms of biological pathways and try to interpret results derived from Transcriptomics, Proteomics or Metabolomics experiments by making use of these. In addition to the interpretation of known target compounds, scientists in Metabolomics research often also intend to observe significant changes in abundances of as yet unidentified metabolites. Modern full scan high resolution QTOF instruments provide the advantage that both researcher's requests can be answered using the same data set. In this study a profiling for compounds contributing to the differentiation of coffee according to the assigned strength is performed.

Experimenteller Teil

LC-MS data was acquired on a compact QTOF (Bruker) and evaluated using a non-targeted workflow. This non-targeted approach enabled differentiation of coffee types based on their assigned flavor intensity and identified trigonelline (N-methyl-nicotinic acid) as one characteristic compound for weak coffee. We postulated that other compounds contained in metabolic pathways involved in generating trigonelline or derived from trigonelline might have higher abundance in weak coffee as well. Using a novel software tool we queried the elemental composition for trigonelline in the KEGG database (<http://www.kegg.jp/>). This returned several metabolic pathways involved in trigonelline metabolism.

Ergebnisse

The nicotinate metabolic pathway was selected for automatically creating a targeted profiling list containing all metabolites of this pathway map. The initial screening list was extended by several compounds known to be characteristic for coffee.

The software tool also enabled to quickly screen for the presence of the compounds by generating high resolution Extracted Ion Chromatograms (hrEIC) with narrow mass tolerance window for the target compounds. Interactive views allowed for a quick evaluation of the compounds detected within the samples. The tentative identity of the compounds screened for was substantiated by taking into account accurate mass and isotopic pattern information. For further statistical evaluation all data was exported for PCA calculation. This revealed a clustering according to the coffee intensity as expected based on the previous non-targeted profiling results. The targeted profiling revealed chlorogenic acid and quinic acid as further compounds responsible for differentiating weak and strong coffee, respectively. This statistical targeted evaluation guided the purchase of reference standards for the final confirmation of the identity of chlorogenic and quinic acid.

In summary a novel workflow for combined non targeted and pathway driven targeted metabolomics base on the same data high resolution QTOF data files will be presented.

Neuer Aspekte

Deriving biomarker panels by using knowledge about metabolic pathways after the initial identification of a single marker.

Improving resolving power in complex mixtures by combining tunable synchrotron radiation with advanced mass spectrometric techniques

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Stichworte: MBMS, TOF, Isomer Selectivity, Reactiv Mixtures, Multiplexing

Einleitung

Reactiv flow systems, for instance laminar premixed flames, jet stirred reactors, photolysis reactors, pyrolysis and oxidation flow reactors or counter flow flames pose a very strong challenge to the mass spectrometric analysis. The Mixture sampled by sample, drawn from this systems by for instance a molecular beam to preserve both the composition at the sampling position and reactive components as for instance radicals, does contain a large set of very similar components. If Fragmentation is not avoided it is virtually impossible to distinguish between contributions from Fragmentation and components with the same mass actually present in the mixture.

Experimenteller Teil

About a decade ago this was overcome by combining molecular beam time of flight mass spectrometers with tunable Vacuum ultra violet synchrotron radiation. With this technique it is not only possible to avoid fragmentation by ionizing only slightly above the ionization threshold but also to identify isomers by their specific ionization characteristics. Identification and quantification relies on reference ionization data (cross sections and thresholds). This works very well for small molecules even at the low mass resolution caused by the continuous ionization source. For larger molecules, however, this fails because of the high number of possible isomers which are than also overlapped by unresolved elemental compositions with the same nominal mass.

Ergebnisse

With a state of the art orthogonal reflectron time of flight mass spectrometers introduced recently at Sandia National Laboratories, it is now possible to separate species based on their elemental compositions at continuous ionization, which was previously only possible in pulsed techniques. This allows to identify and quantify a much larger set of species unambiguously. In this presentation some specific examples from a variety of systems are shown where new and interesting intermediate molecules in reactions could be identified because of this capability. In larger systems the point where it is impossible to separate different isomers is still reached very quickly. This is especially true for radical species which often have very similar ionization energies and cross sections. Furthermore the basis of reference data is very limited. Many of this limitations can be overcome by Photo electron photo ion coincidence spectrometry. Some results are shown where this technique was recently employed for flame analysis for the first time.

Neuer Aspekte

Novel combination of high mass resolution with isomer selectivity in one instrument.

Hochempfindliches Fourier-Transform-Massenspektrometer mit nicht-destruktiver Ionendetektion

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Stichworte: breitbandige Ionenfalle, hochauflösend, Ionenspeicherung

Einleitung

In der modernen Massenspektrometrie werden Quadrupole hauptsächlich für Transferstrecken und als Massenfilter eingesetzt. Quadrupol-Ionenfallen erlauben zudem eine Akkumulation und Beobachtung der Ionen, was diese Instrumente für viele Anwendungen attraktiv macht. Gleichwohl konnten sich Massenanalytoren, die auf Quadrupol-Ionenfallen basieren, in der Praxis bisher nicht durchsetzen. Die Gründe hierfür liegen in dem begrenzten Auflösungsvermögen sowie niedrigen Messgeschwindigkeiten, verglichen mit FFT-basierten Instrumenten. Diese Nachteile lassen sich jedoch umgehen, indem man die Ionenbewegung – ohne die Ionen zu extrahieren – durch Messung von Influenzströmen detektiert und das Massenspektrum mittels Fourier-Transformation berechnet [1, 2]. Im Folgenden wird ein FFT-basierter Quadrupol-Ionenfallen-Massenanalysator vorgestellt und dessen Leistungsfähigkeit diskutiert.

Experimenteller Teil

Kernstück des vorgestellten Spektrometers ist eine typische, kompakte Paul'sche Teilchenfalle. Über eine Bohrung in der Ringelektrode lassen sich beschleunigte Elektronen injizieren, mit denen ein Analytgas direkt in der Quadrupol-Ionenfalle ionisiert werden kann. Ein hochfrequentes Speicherfeld sorgt für eine stabile Speicherung der erzeugten Ionen innerhalb der Zelle. Durch eine geeignete Anregung, beispielsweise einem Rechteckpuls, werden die Ionen aus der Mitte der Ionenfalle heraus beschleunigt. Die dadurch angeregte Ionenschwingungen erzeugen auf den beiden Deckelektroden Influenzladungen, die über empfindliche und rauscharme Verstärker breitbandig detektiert werden. Mit Hilfe der Fourier-Transformation lässt sich aus dem aufgenommenen Influenzladungssignal das Massenspektrum berechnen. Dabei ist die Influenzladungsmessung grundsätzlich nicht-destruktiv, so dass die Ionen auch nach einer Messung noch stabil gespeichert sind und für weitere Analysen zur Verfügung stehen.

Ergebnisse

Zur Charakterisierung des Systems werden verschiedene Analyten, darunter unter anderem flüchtige organische Verbindungen (BTX) sowie Toluol in Stickstoff, in niedrigen Konzentrationen in die Teilchenfalle eingelassen und mittels Elektronenstrahl ionisiert. Es lassen sich Konzentrationen im unteren ppb-Bereich nachweisen. Bei Massen unter 200 amu wurde eine Massenauflösung von $m/\Delta m > 18000$ erzielt. Da interessante Gaskomponenten häufig nur in Spuren eines dominanten Hintergrundgases vorkommen, z.B. BTX in Luft, sind die Fragen nach Dynamik und Empfindlichkeit von besonderer Relevanz. Hier beweist die Messmethode ihre besonderen Fähigkeiten, denn durch ein vorher berechnetes Anregungssignal können gezielt bestimmte Störkomponenten über eine resonante Anregung aus der Zelle entfernt werden. Da die Ionenpopulationen auch nach Abklingen ihrer Anregung weiter gespeichert bleiben, kann – falls gewünscht – auch mehrfach angeregt werden, um eine insgesamt längere Schwingungsdauer und damit eine noch höhere Massenauflösung zu erreichen. Damit vereint das hier vorgestellte System die generellen Vorteile von Ionenfallen mit den Vorteilen gängiger Fourier-Transform-Spektrometer, wie z.B. Ionenzyklotronresonanz-Massenspektrometer oder der Orbitrap.

Neuer Aspekte

Hochempfindliches und präzises Fourier-Transform-Massenspektrometer auf Basis der Paul-Falle mit Influenzstrommessung.

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Coupling thin layer chromatography with mass spectrometry - a practical approach for matrix-loaded samples

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Stichworte: thin layer chromatography, mass spectrometry, TLC-MS interface, instrumentation, sample preparation

Einleitung

A straightforward way to couple thin layer chromatography (TLC) with mass spectrometry (MS) is the elution-based TLC-MS interface [1]. It is a semi-automatic system to extract analytes from the TLC plate and transfer them online into the mass spectrometer. It is suitable for all thin layer materials and every eluent that is LC-MS compatible. The interface can be easily connected to any kind of LC-coupled mass spectrometer. Therefore the fields of application are quite broad. In this lecture we will present applications with matrix-rich samples.

Experimenteller Teil

All experiments were performed on newly developed HPTLC plates with a reduced separation layer thickness. After chromatographic separation the analytes were extracted with acetonitrile/water (95:5, v/v) + 0.1 % formic acid and transferred online into the MS with a flow rate of 0.2 mL/min. Electrospray ionization (ESI) was used in positive and negative mode.

Ergebnisse

Thin layer chromatography allows sample preparation and chromatographic separation in one step. This is possible because of the high sample matrix tolerance of TLC. Through the coupling of thin layer chromatography with mass spectrometry (TLC-MS) unequivocal substance identification is possible [2].

We show how the elution-based TLC-MS interface can be used for the development of TLC-MS methods for matrix-loaded samples in the areas of pharmaceutical ingredients, cosmetic actives and consumer products. Application data is presented for the analysis of methylisothiazolinone (MIT) in wall paint and skin cream.

It is shown that the use of thinner TLC plates leads to lower detection limits, increased sensitivity and improved S/N ratios [3]. The sample matrix can be clearly separated from the target analytes. This results in highly unaffected mass spectra due to a very low level of ion suppression.

Neuer Aspekte

Direct coupling of thin layer chromatography with mass spectrometry via an elution-based TLC-MS interface using newly developed thinner TLC plates.

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Proton-Transfer-Reaction Mass Spectrometry Coupled with FastGC: High Selectivity in Near Real-Time

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Stichworte: FastGC, PTR-MS, TOF-MS, Trace Gas Analysis, Selectivity

Einleitung

Gas Chromatography (GC) and Proton-Transfer-Reaction Mass Spectrometry (PTR-MS) are two complementary analytical methods. GC on the one side is the gold standard in terms of selectivity and substance identification. However, one drawback of GC is that it is time consuming, which makes real-time analysis impossible. PTR-MS on the other hand has a minimum response time of about 100 ms and thus can be considered as an online and real-time method, but with a somewhat limited selectivity (e.g. no separation of isomers). Here, we present a novel instrumental setup where we combine the advantages of both methods, i.e. we integrate a (fast)GC column into a PTR-MS instrument. This tremendously increases the selectivity but does not considerably limit the PTR-MS' real-time capabilities.

Experimenteller Teil

PTR-MS is a well established technology that utilizes H_3O^+ , NO^+ , O_2^+ or Kr^+ , respectively, as reagent ions for chemical ionization of trace compounds [1]. The major benefits of this technology are real-time quantification capabilities, high sensitivity (up to 4700 cps/ppbv [2]) and low limits of detection (ppqv range [2]). For this study we modified a PTR-MS instrument by equipping it with a resistively heated fastGC column and a switching mechanism that allows the user to choose between direct injection and fastGC PTR-MS mode. When the instrument is switched to fastGC mode, a sample loop gets filled with the gas entering the instrument's inlet line. Subsequently, the content of the sample loop is injected into the fastGC column.

Ergebnisse

We exemplify the fastGC process while Manuka tea and spruce resin are analyzed, respectively. In H_3O^+ direct injection mode for both samples a peak in the mass spectra appears at m/z 137.13, which can correspond to a multitude of protonated monoterpenes that are not further distinguishable. After switching to fastGC mode the different compounds contributing to m/z 137.13 are separated according to their retention times in the column. The whole process is completed in less than 1 min, which is when the instrument can be switched back to direct injection mode. Thus, by performing an automated fastGC run every 10 to 60 mins (depending on the expected dynamics within the isomers) the two main advantages of SRI-MS and GC-MS are combined: real-time quantification and extremely high selectivity.

The fastGC mode offers an additional advantage for PTR-MS measurements, namely the separation of compounds with sample concentrations so high, that the direct inlet PTR-MS analysis would be derogated. Ethanol is such a compound of high abundance in wine head space samples, which would normally require a dilution of the sample. In FastGC mode, the ethanol elutes the column early and the analysis of other compounds at later times thus remains unaffected [3].

We compare the advantages of fastGC PTR-MS in terms of selectivity to those of switching the reagent ions (e.g. from H_3O^+ to NO^+). The latter has already proven to be a valuable tool for substance identification [4] and isomer separation [5] as changing the ion chemistry in the drift tube can lead to the formation of unambiguous product ions. Here we present unpublished data of methyltryptamine isomers.

We acknowledge funding through the PIMMS ITN which is supported by the European Commission's 7th Framework Programme under Grant Agreement Number 287382.

Neuer Aspekte

We integrate a fastGC column into a PTR-MS instrument, which considerably improves selectivity but preserves near real-time capability.

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RECENT ADVANCES IN TIME-OF-FLIGHT MASS SPECTROMETRY: WHAT CAN MULTIPLEXING DO FOR YOU?

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Stichworte: Time-of-flight mass spectrometry, multiplexing

Einleitung

Time-of-flight mass spectrometry (TOF MS) has made considerable advances in recent years, with the elimination of mass discrimination (crucial for GC couplings), increased sensitivity and compact instrument designs making the technique more amenable. In this presentation, we will discuss advances in TOF MS technology which will further increase its applicability.

Experimenteller Teil

In reaction kinetics, the simultaneous quantitation of hydrogen with other organics is desirable and can be accommodated by the mass range of modern TOFs. However, the bulk medium (e.g. inert carrier gas) will be present at concentrations many orders higher than species of interest. The term 'multiplexing' is used to describe the combination of multiple data streams into a single, comprehensive stream. Multiplexing can be applied to mass spectrometry in a number of ways but, for the purpose of this presentation, we will focus on the use of alternate 'high-pass, low-pass' filtering of mass and extending the dynamic range of an instrument by alternating the ionisation period between consecutive scans.

Ergebnisse

The fast switching (up to 100 Hz) between experiments allows extremely low mass range (e.g. m/z 0.5-3.5) to be recorded alternately with a more typical organic mass range (e.g. m/z 10-800), solely eliminating the otherwise detrimental ions - in this case helium - while retaining high time resolution and maximum sensitivity.

Switching the ionisation period between consecutive scans can enhance the dynamic range of an instrument by up to 2 orders of magnitude.

Fast multiplexing of TOF parameters during acquisition can greatly improve the performance of a TOF MS. Exemplary applications for extending the mass range and the dynamic range of a given instrument will be presented.

Neuer Aspekte

Fast multiplexing of TOF parameters extends mass range (low mass range and typical organic mass range) and the dynamic range.

Highly time-resolved two-dimensional mass spectrometric imaging of molecular combustion and pyrolysis product concentrations in a burning cigarette

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Stichworte: photo ionisation, imaging, combustion processes, TOFMS

Einleitung

Photoionisation mass spectrometry (PIMS) is an established fast on-line analysis technique for organic compounds highly-dynamic combustion processes. A new sampling and data analysis approach allow the mapping/imaging of trace products of combustion e.g. in the tip of a burning cigarette.

Experimenteller Teil

Photoionisation mass spectrometry (PIMS) is an established fast on-line analysis technique for highly-dynamic combustion processes. A capillary-microprobe sampling system (μ -probe) with PIMS can be used for direct, localized examination of organic vapours from burning or pyrolyzing objects (μ -probe PIMS). This approach now was further developed to a spatial/temporal resolved, in situ imaging method for combustion/pyrolysis products in e.g. a burning cigarette tip. Repetitive experiments were performed at different sampling positions.

Ergebnisse

The time resolved PIMS-sequences were combined to spatially resolved, time-dependent "concentration-images" for different compounds. Quantitative images of e.g. nitrogen monoxide-, benzene- and oxygen-concntrations in the burning tip of a cigarette were measured during a 2-second lasting puff with 100ms time resolution. Different formation and destruction zones in the reaction region were observed and time/space-resolved combustion-kinetic data was obtained.

The classical formation/destruction mechanisms of e.g. NO, oxygen and benzene during a cigarette-puff (fuel-NO formation, re-burn, gas phase re-formation via HCN) were space- and time-resolved observed. The data sequence will also be shown as a video sequence, depicting the changes in the spatial combustion product distribution during the 2 sec. lasting, highly dynamic combustion and pyrolysis processes during a single cigarette puff.

Neuer Aspekte

neuen Methode (sampling, Datenauswertung) auf Basis der Photoionisationsmassenspektrometrie zum zeit- und ortsaufgelösten mapping/imaging von organischen Verbindungen in Vverbrennungsprozessen, insb.der Feststoffverbrennung

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

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Stichworte: Ionenspektroskopie, Ionisierungsenergie, Anregungsenergie, Schwingungsstruktur

Einleitung

Ionenspektroskopie ist eine essentielle Methode zur Untersuchung der vibronischen Struktur von molekularen ionischen Spezies und angeregten Neutralen. Es wurden Halogenbenzole bezüglich ihrer vibronischen Struktur des ersten angeregten Zustands (S1) mittels ResonanceEnhancedMultiPhotonIonization (REMPI) Spektroskopie untersucht, der ionische Grundzustand (D0) mit Hilfe der MassAnalyzedThresholdIonization (MATI) Spektroskopie. Ein besonderes Augenmerk widmet sich der Frage, ob aromatische Spezies ihre Planarität bei elektronischer Anregung oder Verlust eines Elektron beibehalten. Die Aufnahme der MATI-Spektren erfolgt in einem Mehrphotonenprozess über resonante Zwischenzustände. Es wird für jedes untersuchte Molekül ein komplementärer Satz an MATI-Spektren erhalten. Diese Spektren erlauben Rückschlüsse auf die während der Ionisation aktiven Schwingungsmoden. Besondere Aktivität von Schwingungsmoden, z.B. in Form von Progressionen, geben wichtige Hinweise auf Geometrieverzerrungen.

Experimenteller Teil

Das Experiment besteht aus einem mit einstufiger Ionenquelle und Reflektoren ausgerüstetem Time of Flight (ToF)-Massenspektrometer [1] und zwei separat (355 nm, Nd:YAG) gepumpten, durchstimmbaren Farbstofflasern. Die Dye-Wellenlänge beider Laser wird frequenzverdoppelt. Der Aufbau lässt somit Zwei-Farben-Experimente zu. Ein Überschall 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.

Ergebnisse

REMPI und MATI Untersuchungen an einer systematischen Reihe von Halogenbenzolderivaten ergaben ein detailliertes Bild der vibronischen Struktur im ersten elektronisch angeregten Zustand (S1) und kationischen Grundzustand (D0) von 1,2-Dichlor-3-fluorbenzol (1,2,3-DCFB), 1,2-Dichlor-4-fluorbenzol (1,2,4-DCFB), 1,3-Dichlor-2-fluorbenzol (1,3,2-DCFB), 1,3-Dichlor-5-fluorbenzol (1,3,5-DCFB) und 1,4-Dichlor-2-fluorbenzol (1,4,2-DCFB). Für das 1,2,4-DCFB, 1,3,2-DCFB, und 1,4,2-DCFB konnte gezeigt werden, dass es während der elektronischen Anregung zu einer Abweichung der Planarität entlang der Koordinate einer out-of-plane Mode kommt. Außerdem konnte gezeigt werden, dass es bei diesen Molekülen während der Ionisation aus dem ersten elektronisch angeregten Zustand heraus zu einer Replanarisierung der aromatischen Struktur kommt.

Das REMPI-Spektrum von 1,3-Dichlor-2-fluorbenzol zeigt eine ungewöhnlich hohe Aktivität einer niederfrequenten out-of-plane-mode (17b²). MATI-Spektren über verschiedene Moden zeigen eine Verletzung der $\Delta v=0$ Vorzugsregel. Außerdem lässt sich im MATI-Spektrum über die 17b-Mode eine kurze, dreigliedrige Progression der Mode 17b mit einem Shift des Frank-Condon-Maximums zu niedrigeren Energien beobachten [2]. Das MATI Spektrum über die out-of-plane Mode (17b²) im 1,2,4-DCFB zeigt sich eine, bisher in der Literatur unbekannte, sechsgliedrige Progression der 17b-Schwingungsmode. Jedes Mitglied der Progression ist zusätzlich in ein Triplet aufgespalten. Desweiteren lieferten oben genannte Untersuchungen sehr genaue Werte für die elektronische Anregungsenergie (AE) und Ionisierungsenergie (IE). Die durchgeführten quantenchemischen Rechnungen zeigten gute Übereinstimmung zwischen Theorie und Experiment. Ebenfalls war mit Hilfe der quantenchemischen Rechnungen möglich REMPI und MATI Spektren zu simulieren.

Neuer Aspekte

Geometrieänderung bei Anregung und Ionisation

Referenzen

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Matrixeffekte bei der LC/MS-Analyse von Vitamin D-Metaboliten mittels verschiedener Ionisierungstechniken

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Stichworte: DBDI, LC/MS, SPE, Vitamin D, Metabolite

Einleitung

Vitamin D und seine Metabolite spielen eine essentielle Rolle in der Calcium-Homöostase und sind daher ein wichtiger Faktor für die Knochengesundheit. Die Serumkonzentrationen von 25-Hydroxyvitamin D₃ und anderer Metabolite werden dabei als Biomarker für den Vitamin D-Status herangezogen. Zur Analyse von Vitamin D-Metaboliten in humanem Serum wurde die Hochleistungsflüssigchromatographie in Kopplung mit der hochauflösenden Massenspektrometrie (LC/HRMS) eingesetzt. Aufgrund des weiten Polaritätsbereichs der untersuchten Metabolite wurde die dielektrisch behinderte Entladungsisolation (DBDI) [1], welche auf einem Niedrigtemperaturheliumplasma basiert, verwendet und mit etablierten Verfahren zur Ionisation bei Atmosphärendruck (API) verglichen.

Experimenteller Teil

Zur Anreicherung der Metabolite und Abtrennung von Matrixbestandteilen wurde eine Festphasenextraktion (SPE) der humanen Serumproben durchgeführt. Hierzu wurden die Materialien Waters Oasis[®] HLB und Supelco HybridSPE[®]-Phospholipid Ultra verwendet und miteinander verglichen. Die Ionisations- und Fragmentierungseigenschaften sowie die auftretenden Matrixeffekte bei Einsatz der DBDI, der chemischen Ionisation bei Atmosphärendruck (APCI) und der Elektrospray-Ionisation (ESI) wurden untersucht und verglichen.

Ergebnisse

Bei der Analyse der extrahierten Serumproben mittels APCI, DBDI und ESI wurden positive Matrixeffekte beobachtet, die vermutlich durch Phospholipide verursacht werden. Diese Matrixeffekte konnten durch die Optimierung der SPE unter Verwendung einer Zirkon-beschichteten Silica-Phase (Supelco HybridSPE[®]-Phospholipid Ultra), welche Phospholipide entfernt, drastisch reduziert werden. Die DBDI zeigt im Vergleich zur APCI ähnliche Eigenschaften bezüglich der Ionisation und Fragmentierung sowie der analytischen Leistungsfähigkeit. Beide Ionisationsverfahren weisen für höher hydroxylierte Metabolite teilweise intensive Wasserverluste auf, welche zu multiplen Fragmentationen pro Analyt führen.

Neuer Aspekte

Anwendung der DBDI zur Analyse von Vitamin D-Metaboliten mittels LC/HRMS und Untersuchung der Matrixeffekte.

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Three-layer set-up for standardized quantification of salicylic acid in dried blood spots by desorption electrospray ionization (DESI) mass spectrometry

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Stichworte: Bioanalytical methods, drug monitoring, desorption electrospray ionization, mass spectrometry, pharmaceuticals

Einleitung

Desorption electrospray ionization (DESI) mass spectrometry has recently been introduced as an emerging technology for direct therapeutic drug monitoring in dried blood spots (DBS) [1,2]. For absolute quantification of various drugs, however, available DBS methods typically require manual application of small molecule internal standards other than the analyte [3]. Aiming to avoid manual handling steps and with industrial standardization in mind, we developed a three layer setup for robust quantification of salicylic acid directly from DBS.

Experimenteller Teil

A dioctyl sodium sulfosuccinate (DONS) weave facilitating sample spreading was combined with a cellulose layer containing isotope-labeled salicylic acid as internal standard. The third layer is a filter paper that was eventually analyzed by DESI-MS. Using this setup, we developed a quantification method for salicylic acid (SA) from whole blood that covers the entire therapeutic range (30-300 mg/L). Validation of the method was achieved by comparison with LC-MS/MS quantification and results of a cobas[®] 6000 analyzer.

Ergebnisse

Compared to previous quantification approaches by DESI MS [2,4], the three-layer setup provided increased sensitivity with a lower limit of quantification (LLOQ) of 20 mg/L SA in whole blood. As a measure of variance, the relative standard deviation (RSD) was used over six sample spots. Quantification experiments showed good reproducibility with $RSD \leq 14\%$ and a correlation coefficient of 0.99. We also showed the applicability to dried plasma spots with a LLOQ of 8 mg/L SA in human plasma.

Evaluation of suitability and robustness was achieved by single blind testing. We obtained recovery rates of 95% up to 113% for four samples with $RSD \leq 14\%$. The RSD of the calibration points was given with $< 10\%$ and a correlation coefficient of 0.988.

We successfully tested a three layer setup in DESI-MS applications for introduction of an internal standard into blood samples. This approach obviates the need for any manual sample handling steps.

Rapid quantification of drugs or metabolites such as salicylic acid by DESI-MS analysis of DBS on pre-manufactured three-layer cartridges may be a promising approach for future therapeutic drug monitoring, product control or novel MS-based Point of Care devices.

Neuer Aspekte

novel method for internal standard introduction in ambient mass spectrometry (3 layer method)

Salicylic acid quantification on DBS by DESI-MS

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LMP-EX a multimolecular omics approach for systems biology

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Stichworte: lipidomics, proteomics, multi molecular analysis, PPAR signaling

Einleitung

In order to understand molecular behavior or trends at the systems levels a deep coverage of many molecular species is required. This is especially true for the lipidome, metabolome and proteome, since they are deeply intertwined at different functional layers including signaling, metabolism and cellular integrity. To address this challenge, we introduce here **LMP-EX** a strategy that allows the quantitative analysis of **L**ipids, **M**etabolites and **P**roteins from one sample with an excellent reproducibility, in an unbiased fashion and with equal efficiency compared to uni-molecular workflows. In summary, the results of this work place LMP-EX as a superior strategy that enables exciting new directions in multiple areas of systems biology ranging from differentiation processes to clinical applications.

Experimenteller Teil

To achieve this goal we setup a biphasic organic extraction protocol LMP-EX (Lipid, Metabolite, Proteins-Extraction), applied the protocol to lipidomics, metabolomics and proteomics workflows and investigated the applicability of the established protocol to analyses complex systems such as the PPAR signaling system during the onset of adipogenesis. To do so an adaption of a methyl-tert-butyl ether (MTBE)/ methanol lipid extraction protocol [1] was carried out and lipids, metabolites and proteins were subjected to an individual MS based workflow including selected reaction monitoring and high resolution MS and MS/MS experiments.

Ergebnisse

Here, we established a protocol which can deliver a more comprehensive picture of an entire biological system by extending the detection capabilities to more than one molecular class. With this strategy we were able to access 360 lipids, 70 metabolites and 3327 proteins making this method to the most comprehensive approach for a multi-molecular analysis. To illustrate the benefits, of the developed strategy, LMP-EX was applied to the analysis of PPARG signaling in stromal stem cells [2]. Here major molecular remodeling processes for all 3 molecular classes were revealed, which are needed for terminal differentiation into adipocytes. The LMP-EX workflow introduces a novel concept to systems biology allowing for the first time the simultaneous analysis of three different molecular classes from one sample, making this strategy to an excellent and simple approach for systems biology. Therefore this method will supplement common knowledge and identify novel central hubs important for metabolic control. The outcomes of this work have important consequences toward advancing the development of mass spectrometry driven omics workflows as a supportive technique for understanding complex biological systems, in particular for integrative studies combining mass spectrometry data with that from multiple streams, such as genomics, transcriptomics or high content screening approaches. As mass spectrometry instrumentation continuously advances and gains in sensitivity and speed are made, it can be expected that with this established strategy even more molecular species will quantitatively accessible in near future.

Neuer Aspekte

LMP-EX a multimolecular extraction procedure for quantitative biology.

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Analysis of the lipid composition of adipose tissues by spectroscopic methods

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Stichworte: Adipose tissue, double bond content of lipids, MALDI MS, NMR spectroscopy, gas chromatography.

Einleitung

During the last decades many studies focused on the investigation of obesity-related (bio)markers such as adipocyte-derived peptides [1], but minor interest has been paid to the investigation of the lipids, especially triacylglycerols (TAGs), and fatty acyl compositions of adipose tissues [2].

In this study we combined the strengths of NMR and MS to investigate the impact of the diet on the lipid composition of different adipose fat depots of mice. Beside the localization of the fat (brown, visceral and subcutaneous fat) diet (high fat (HF) vs. standard (SD)) induced differences of lipid composition were of particular interest. All obtained data were also compared with established methods of lipid analysis, particularly thin-layer chromatography (TLC) and gas chromatography (GC) [3].

Experimenteller Teil

Animal experiments were performed according to the German guidelines of laboratory animal care. Male mice (n = 6, 3 × standard diet (SD, 11% fat content) and 3 × high fat high sugar diet (HF, 54% fat content); age of 8-12 weeks) were set on diets for 12 weeks. Tissue lipids were extracted using methyl-tert-butyl ether (MTBE) [4]. Adipose tissue lipid compositions were first analyzed by means of MALDI MS (Bruker Autoflex). Qualitative analysis was additionally approved by HPTLC. Quantitative analysis was performed by means of GC (Autosys XL (Perkin Elmer) with a flame ionization detector) and NMR spectroscopy. (¹H and ³¹P HR NMR (Bruker AVANCE-600 at 600.13 MHz for ¹H and Bruker DRX-600 at 242.88 MHz for ³¹P respectively)

Ergebnisse

Both diets consist almost exclusively of triacylglycerols (TAG), beside minor amounts of phosphatidylcholines (in particular PC 16:0/18:2 (*m/z* 758.6 and 780.6) and PC 16:0/20:4 (*m/z* 782.6 and 804.6)) in the "standard" food. However, the overall fatty acyl compositions of the TAGs in both diets differ significantly: the SD is characterized by the presence of TAGs with longer (particularly C18), unsaturated fatty acyl residues (e.g. *m/z* 901.7 -TAG 54:6), whereas the high fat diet contains nearly exclusively shorter, saturated fatty acyl residues (e.g. *m/z* 661.5 - TAG 36:0).

The lipid composition of the diet supplied to the mice has a strong impact on the lipid composition of the adipose tissues. There are considerable differences depending on the diet and only moderate differences between the types of adipose tissue. All spectra are dominated by the presence of TAGs while there are also some PC species, particularly in brown fat. The observed TAG fatty acyl patterns resemble closely the composition of the SD. In contrast, the adipose tissue extracts at conditions of HF diet are dominated by shorter, saturated fatty acyl residues which are also predominantly present in the HF diet.

The relative fatty acyl compositions of both diets and adipose tissues of interest have also been determined by gas chromatography. Longer-chain unsaturated fatty acids (particularly linoleic acid) are nearly exclusively present in the SD, while shorter and saturated ones (particularly lauric and myristic acid) represent the majority of the fatty acids in tissues of mice fed with HF diet. Absolute quantitative data regarding the double bond content were finally obtained by high-resolution NMR spectroscopy, which further supported the above mentioned findings.

In conclusion, the diet composition should be carefully checked prior to performing animal experiments. It is extremely important to use identical diet compositions in order to avoid misinterpretations of experimental results.

Neuer Aspekte

The incorporation of nutritional fatty acids into tissues was studied by a couple of different spectroscopic methods.

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Gender-specific Differences in Blood Plasma Lipidomes of Healthy Females and Males

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Stichworte: human plasma, gender differences, lipidomics

Einleitung

Lipidomics of human tissues and fluids continuously gains importance for the identification of new biomarkers as risk indicators for a wide variety of diseases. However, due to the differences in analytical methods and biased selection of study populations there are no generally accepted reference values of lipid concentrations. We aimed to define sex-specific reference values and their variance for all major lipid classes and species of human blood plasma. To this end we analyzed a cohort comprising 36 males and 35 females, aged 19 to 33 years, whose clinical blood test and BMI were within clinically accepted ranges.

Experimenteller Teil

For shotgun lipidomics lipids were extracted by MTBE [1]. Gangliosides were extracted according to Fong et al. [2] with minor modifications. Eicosanoids were recovered by solid-phase extraction as described in [3]. Shotgun analyses were performed on a QExactive mass spectrometer (Thermo Fisher Scientific) equipped with a robotic ion source TriVersa (Advion BioSciences) [4]. LC-MSMS analyses were performed on a QTRAP 5500 (AB Sciex). Reference lipid standards spiked in each sample prior extraction were validated by comparison with “quantitative LIPID MAPS standards” supplied by Avanti. Lipids were quantified using LipidXplorer software [5]. Statistical differences between the groups were analyzed using Mann-Whitney *U* test.

Ergebnisse

280 lipid species from 27 lipid classes were quantified by shotgun lipidomics and LC-MSMS analyses. Using non parametric tests we established statistically significant ($p < 0.01$) sex-specific differences for 77 out of 280 lipid species and 17 out of 27 lipid classes. Generally, we found that women have a significantly higher amount of phospho- and glycerophospholipids, while men have more lyso- and ether-lipids. An unsupervised explorative analysis using principal component analysis showed that the lipidomic profile not only revealed sex-specific differences, but also the use of contraceptive drugs inside the female cohort. In addition, we observed strong impact of sex-specific hormones and of the sex-hormone binding protein in females, however free testosterone did not affect the plasma lipidome in males. Furthermore, although all study subjects were young, healthy, and had no metabolic disorders, their lipidomes fall into several compositionally distinct clusters. We demonstrated that hormonal drugs including common contraceptives is a major factor affecting the lipidome in females and their impact has to be carefully considered when compiling study cohorts and interpreting the outcome of lipidomics screens.

Neuer Aspekte

In-depth analysis of sex-specific differences in the plasma lipidome of healthy young individuals.

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Lipidomics screens of human lung tissue together with histological characterization allow studying lipid metabolic perturbation in cancer and COPD

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Stichworte: Lipidomics, FT-ICR-MS, lung tissue, COPD, lung cancer

Einleitung

Lipids are very important for respiratory functions in the lung. Gas exchange is located in the alveoli, which contain specialized cells to produce firstly the pulmonary surfactant and maintain secondly a barrier to the environment. The surfactant covers the alveoli and contains mostly saturated phospholipids as well as proteins. To date there is relatively little known about the lung lipidome and how the lipid metabolism changes in diseases like cancer or inflammation. Chronic obstructive pulmonary disease (COPD) is an inflammation in the lung caused by smoking and one of the major causes of death worldwide. The correlation between lipidome data and histological characterizations for COPD and cancer are explored in this study.

Experimenteller Teil

Human lung tissue samples were acquired from lung biopsies of cancer patients. All tissue sections were divided to (1) enable histological characterisation using HOPE^[1] methodology and (2) to perform lipid extraction and shotgun lipidomics. For lipidome analysis the tissue samples were homogenized in KCl buffer using Ultra-Turrax (IKA, Staufen, Germany). Lipids were extracted using a methyl-*tert*-butyl-ether/methanol/water solvent system^[2]. Shotgun lipidomics were applied on a Apex Qe FT-ICR mass spectrometer (Bruker Daltonics, Bremen, Germany) using a chip based nano electrospray ion source (TriVersa NanoMate, Advion, Ithaca, USA). For lipid identification LipidXplorer^[3] was used. Free cholesterol (FC) was derivatized with acetyl chloride^[4] and quantified by quadrupole time-of-flight mass spectrometry (Q-ToF-MS). All lipid quantification were based on addition of internal standards prior extraction.

Ergebnisse

Electrospray ionization was optimized using several salt additives. We found that 2.9 mM ammonium acetate in the sample matrix for positive ionization and 0.05 mM ammonium chloride for negative ionization yielded the highest signal intensities (~10⁶) and best dynamic range. The use of ammonium chloride in the negative ion mode is very advantageous because signals for phosphatidylcholines (PC), lysophosphatidylcholines (LPC) and sphingomyelins (SM) could be detected exclusively as chloride adduct. Addition to the spray solvent led to at least two ion populations of acetate and formiate adducts as well as chloride adducts, whose abundance could not be controlled because of the sample matrix. Spectra complexity was reduced and we were able to distinguish phosphatidylserines (PS) and PC solely by their exact mass. As a result we could unequivocally identify lipids containing odd numbered fatty acid chains. Furthermore, the sensitivity for the detection of phosphatidylinositol (PI), PS and phosphatidylglycerols (PG) molecules was improved when compared to commonly used ammonium acetate as additive. For the subsequent shotgun lipidomics analyses a reproducibility of approximately 10 % was found. Quantification of FC using automated flow injection analyses enabled a good reproducibility of better than 5 %. With our approach we were able to identify and quantify approximately 300 lipids from 11 lipid classes. In our current dataset we predominantly identified phospholipids like PC, PG, PI, PS, phosphatidylethanolamine and SM. Neutral lipids like triacylglycerols (TAG) or cholesterol esters were also detected. Saturated fatty acid containing PCs, PGs and PIs were identified as characteristic lipids of the surfactant. The quantity of these lipids potentially indicates the health status of alveolar lung tissue. Our data show that surfactant lipids are decreased in abundances in most cancer tissues. We are at the beginning to understand how increased inflammation, necrosis and cancer types are correlated to specific lipid signatures.

Neuer Aspekte

We apply lipidomics screens to study human lung tissue and correlate our data to histological features.

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A new TEMPO-Active Ester Reagent for Peptide Structure Analysis by Free Radical Initiated Peptide Sequencing (FRIPS) Mass Spectrometry

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Stichworte: TEMPO; Chemical Cross-Linking, Tandem-MS, Free Radical Initiated Peptide Sequencing

Einleitung

To enable an effective analysis of cross-linked peptides by electrospray ionization (ESI) and/or matrix assisted laser desorption ionization (MALDI) tandem MS, we and others have developed novel cross-linking (XL) reagents, which decompose more easily than typical peptide bonds and additionally exhibit a characteristic fragmentation behavior upon collision activation, thereby allowing selective detection and sequence analysis by MS³ [1,2; and references therein]. To satisfy these requirements potential XL reagents have to contain a labile covalent bond located within the linker region, which performs a selective and preferred cleavage or facilitates fragmentation by loss of small neutrals upon collision activation in the gas phase.

Experimenteller Teil

Cross-linking

XL reactions of the TEMPO-Bz-linker with L-amino acids were conducted at room temperature (30min) before quenching with ammonium bicarbonate. XL reactions with peptides (50 µM) were conducted in 20mMHEPES buffer, pH 8.0 or 8.5 respectively. After the XL, cross-linked test peptide 1 and M13 peptide were digested with trypsin (Promega, cleaving C-terminally of Lys and Arg) at 37 °C for 3 h.

MS

Nano-HPLC/Nano-ESI-Orbitrap-MS, Offline Nano-ESI-Orbitrap-MS and ESI-LTQ-Orbitrap-MS were performed as described in detail elsewhere.[3] Exact ion masses of all precursor and fragment ions were determined in the orbitrap analyzer (R= 30 000 at m/z 400; external calibration: $\Delta m \leq 3$ ppm; internal standardization with substance P: $\Delta m \leq 1$ ppm).

Ergebnisse

For that special application and purpose we synthesized a homobifunctional active ester XL-reagent containing a TEMPO (2,2,6,6-tetramethylpiperidine-1-oxy) moiety connected to a benzyl group (Bz), termed TEMPO-Bz-linker [3]. The aim for designing this novel cross-linker was to facilitate MS analysis of cross-linked products by free radical initiated peptide sequencing (FRIPS) [4,5]. The TEMPO-Bz-linker was reacted with all 20 proteinogenic amino acids as well as with model peptides to gain detailed insights into its fragmentation mechanism upon collision activation. The ultimate goal of this *proof-of-principle* study was to evaluate the potential of the TEMPO-Bz-linker for chemical cross-linking studies to derive 3D-structure information of proteins. Our studies were motivated by the well documented instability of the central NO-C bond of TEMPO-Bz reagents upon collision activation [5]. The fragmentation of this specific bond was investigated in respect to charge states and amino acid composition of a large set of precursor ions resulting in the identification of two distinct fragmentation pathways. Molecular ions with highly basic residues are able to keep the charge carriers located, *i.e.*, protons or sodium cations, and consequently decompose via a homolytic cleavage of the NO-C bond of the TEMPO-Bz-linker. This leads to the formation of complementary open-shell peptide radical cations, while precursor ions that are protonated at the TEMPO-Bz-linker itself exhibit a charge-driven formation of even-electron product ions upon collision activation. MS³ product ion experiments provided amino acid sequence information and allowed determining the cross-linking site. Our study fully characterizes the CID behavior of the TEMPO-Bz-linker and demonstrates its potential, but also its limitations for chemical cross-linking applications utilizing the special features of open-shell peptide ions on the basis of selective tandem MS analysis [3].

Neuer Aspekte

Introduction of a new Bifunctional TEMPO-Active Ester Reagent

Chemical Cross-Linking by Free Radical Initiated Peptide Sequencing (FRIPS) Mass Spectrometry

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First Principles Calculation of Electron Ionization Mass Spectra

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Stichworte: Quantum Chemistry, Molecular Dynamics, Molecular Modeling, Fragmentation Pathways, Reaction Kinetics

Einleitung

Recently, we have presented the novel Quantum Chemistry Electron Ionization Mass Spectra (QCEIMS) method, based on a mixed quantum-classical molecular dynamics (QC-MD) protocol.[1] In essence, we simulate an ensemble of molecular ions assumed to have undergone an (e,2e) process with a certain impact excess energy distribution by propagating the nuclei classically on a quantum chemical potential energy surface (PES). By this fully parallelized stochastic/dynamic algorithm, one obtains in most cases a reasonable computed mass spectrum in a sensible amount of computation time.

Experimenteller Teil

We have used the QCEIMS program in conjunction with various semi-empirical and density functional theory (DFT) PES. No further modification to the protocol was made, except the introduction of uniform instead of localized velocity scaling regarding the internal conversion heating process for large molecules. The ensemble sizes, that is the number of individual QC-MD trajectories varied from 300 to 1000, yielding theoretical base peaks with ≈ 100 counts. We have also implemented into our approach a matching score[2] (0 = no overlap computed/experimental 1000 = complete overlap) for evaluation of our calculated mass spectra.)

Ergebnisse

We present two QCEIMS applications that we have recently published.[3, 4]

Nucleobase Fragmentations

We subjected the five canonical nucleobases adenine, cytosine, thymine, uracil and guanine (from here on abbreviated as A, C, T, U, and G) to our simulation protocol. For A, we found that all major fragmentations are open-chained, as the central bond in the purine ring system is substantially weakened upon electronionization. The matching score was also expectedly increased when going from semi-empirical PES to a DFT PES, and all major peaks were found in the computed mass spectrum.

For C, T, U, and G, we could also reproduce experimental EI mass spectra quite well (scores up to 700, manuscript presently under revision). We took into account low-energy tautomer populations of C and G, thereby significantly improving our predictions. From a mechanistic point of view, our simulations yield a complex fragmentation network of an ensemble of molecular ions, including such well-known fragmentation pathways as the retro-Diels-Alder reaction, i.e., in the case of C the expulsion of HNCO.

Organic Drug Molecules

Using QCEIMS together with semi-empirical quantum chemical PES, we were able to simulate fragmentation processes of large organic molecules (nominal mass ≈ 800 atomic mass units) to a satisfactory degree.

For the well-known natural product and anticancer drug taxol, we correctly calculated the base peak at m/z 105 (the benzoyl cation), as well as several other important peaks. While there are still some systematic deficiencies, related mostly to the shortcomings of semi-empirical PES, we submit that with the advent of QCEIMS a significant step towards routine computational mass spectra prediction of sizeable organic molecules has been taken.

Neuer Aspekte

Brute-force fragmentation-simulations help elucidate reactionmechanisms and may bring about first-principles-type, routine mass spectra prediction without a priori assumption of fragmentationmechanisms.

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Structure of Full-Length p53 Tumor Suppressor Probed by Chemical Cross-Linking and Mass Spectrometry

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Stichworte: p53, tumor suppressor, chemical cross-linking, Orbitrap-MS, stable isotope labeling, 3D-structure

Einleitung

The tumor suppressor p53 acts as a DNA sequence-specific transcription factor, which induces or represses a wide range of target genes that are involved in cell cycle control, senescence, and apoptosis in response to genotoxic stress. Intracellular levels of p53 and its function are regulated by factors, such as post-translational modifications, degradation, and interactions with a broad palette of other proteins [1]. Briefly, p53 exerts its function as the “guardian of the genome” via a complex interplay of independently folded and intrinsically disordered (natively unfolded) domains. The transactivation domain (*N*-terminus) and regulatory domain (*C*-terminus) are such disordered regions. Here, we describe our efforts to obtain 3D-structural information of p53’s *C*-terminal region by chemical cross-linking and mass spectrometry.

Experimenteller Teil

¹⁵N-labeled- and non-labeled human p53 were overexpressed as HLT fusion protein [2] in *E. coli* BL21 (DE3) following a published protocol [3]. Isotope-labeled and non-labeled p53 were mixed at a molar ratio of 1:1 (10 μM) and pre-incubated for 16 hours at 4°C to form mixed p53 tetramers. Cross-linking reactions were carried out using a 50-fold molar excess of the homobifunctional amine-reactive cross-linker BS²G. Samples were proteolyzed with Asp-N and trypsin and the resulting peptide mixtures were analyzed by nano-HPLC/nano-ESI-MS/MS using an Orbitrap Fusion Tribrid mass spectrometer (Thermo Fisher Scientific). Cross-linked peptides were identified with the in-house software StavroX Vers. 3.4.5 [4].

Ergebnisse

The tumor suppressor protein p53 contains a high percentage of intrinsically disordered regions, making it inherently difficult to be studied by conventional methods for protein 3D-structure analysis. Here, we present a chemical cross-linking/MS approach for deriving structural information of full-length wild-type p53 in solution. To discriminate between intra- (within one p53 monomer) and intermolecular (between two p53 monomers) cross-linked products an isotope labeling approach was established that relies on a mixture of non-labeled (¹⁴N) and fully ¹⁵N labeled protein. Chemical cross-linking of full-length p53 was performed in the absence of DNA. Formation of cross-linked products was monitored by SDS-PAGE, revealing a decrease in the signal of monomeric p53 and the appearance of signals corresponding to tetrameric p53. Both the dimer and the tetramer p53 bands were excised from the gel, enzymatically digested, and subjected to LC/MS/MS analysis. In conclusion, seven intermolecular cross-links and four intramolecular cross-links were found. For example, the cross-link between Lys-357 in the tetramerization domain of p53 with Lys-357 from another p53 molecule was identified, resulting in the appearance of characteristic patterns in MS-analysis composed of signals for the ¹⁴N, mixed ¹⁴N/¹⁵N, and ¹⁵N species. Tandem MS analysis of these species confirmed the cross-linked products based on the characteristic mass shifts caused by isotope labeling. The identified cross-links were mapped into a previously published SAXS model of the p53 tetramer in a DNA-free state [5]. All intramolecular cross-links are in good agreement with this model, but the majority of intermolecular cross-links were much longer than the distance BS²G is able to bridge (Cα-Cα distance 26Å), except the cross-link between Lys-357 and Lys-357. Therefore our cross-linking data between different p53 molecules indicate a high flexibility in the regulatory domain of full-length p53 in the absence of DNA and a more compact arrangement of p53 monomers within the tetramer.

Neuer Aspekte

3D-structural analysis of the intrinsically disordered *C*-terminal region of p53

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Identification, recognition structures and affinities of nitrated tyrosine sites by affinity mass spectrometry

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Stichworte: Protein oxidation, tyrosine nitration, 3-nitrotyrosine antibodies, affinity-mass spectrometry

Einleitung

Protein tyrosine nitration is known to occur in various acute and chronic inflammatory diseases and is thought to be involved in their pathology. Most identification of nitrations had been derived from the use of anti-3-nitrotyrosine (3-NT) antibodies; however, only scarce information of properties and molecular specificities of 3-NT- antibodies has been hitherto reported [1]. Immunoanalytical methods such as Western blot, ELISA and immuno-electron microscopy, employing different 3-NT- antibodies [2, 3], have been used for overall detection of nitrations, but these method do not provide identification of nitration sites and structures as only in combination with mass spectrometry can be obtained [4].

Experimenteller Teil

For the identification of nitrated sites in Eosinophil cationic protein (ECP), mixtures of proteolytic peptides were bound for 2 hrs at RT to a microaffinity column with immobilized 3NT antibodies. After washing, bound peptides were eluted by mild acidic treatment (0.1 % TFA) and the resulting nitrated peptides were identified by nano-ESI-FTICR-MS. A direct online combination of bioaffinity and MS, was employed for the simultaneous characterization and quantification of the interaction of synthetic model nitrated peptides with anti-3NT-antibody. The online affinity-MS approach utilized a surface-acoustic wave biosensor [5], with the anti-3NT antibody immobilized on a chip surface. Following association of peptides, dissociation was performed using an acidic buffer into the interface for ESI-MS analysis.

Ergebnisse

To enable the unequivocal identification of specific 3-NT residues in two eosinophil proteins (ECP and EDN) purified from eosinophil granules of patients with eosinophilia a proteolytic affinity-MS extraction ("PROFINEX"- MS) approach was developed. EDN was cysteine- carbamidomethylated and then digested in solution with thermolysin, used instead of trypsin due to the low number of Arg and Lys residues in the N-terminal part of the protein. The mixture of proteolytic peptides produced was submitted to the affinity column, incubated for 2 h, and the supernatant non-binding peptides removed by washing and analyzed by MALDI-TOF-MS as a control. The remaining antibody-peptide complex was then dissociated at slightly acidic conditions, and the elution fraction analyzed by high resolution FTICR- mass spectrometry. The ESI-FTICR-MS revealed a single Tyr-peptide, EDN (²⁹VINNY(NO₂)QRRCKNQNTF⁴³) with a monoisotopic mass corresponding to a nitration at Tyr³³ with a mass increment of 103 Da (45 Da for the Tyr³³-nitro group, 58 Da for Cys³⁷-carbamidomethyl). Accessible surface areas were calculated using the Surface Racer program. The spatial orientation was compared for all tyrosine residues in EDN and showed that the Tyr³³ residue has the highest surface accessibility (100.8 Å²), compared with other tyrosines. These results showed that the affinity differences between the nitrated ECP peptides correlate with a specific sequence environment of the tyrosine residues. The SAW-MS biosensor results showed that the affinity of the nitrated ECP peptide was completely abolished upon replacing the positively charged residues Arg²⁸, Arg³⁴, Arg³⁶, and Lys³⁸ by alanine, thus confirming the importance of cationic amino acids in the vicinity to the nitration site for affinity binding. Affinity determinations of the nitrated peptide in comparison to the intact ECP protein using the SAW biosensor provided dissociation constants of approximately 6 nM for the nitrated peptide ECP (24–41), and 28 nM for the intact ECP.

Neuer Aspekte

Bioaffinity- MS combines the specificity of affinity determination with the molecular selectivity, speed, and sensitivity of MS.

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Age-related changes of advanced glycation patterns in *Arabidopsis thaliana*

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Stichworte: tandem mass spectrometry, glycation, AGEs, Amadori products, carbonyls, plant ageing, A. thaliana

Einleitung

The contents of soluble carbohydrates and production of reactive oxygen species (ROS) gradually increase throughout plant ontogenesis. The combination of these factors favors protein glycation, sugar autoxidation and lipid peroxidation, enhancing thereby formation of α -dicarbonyls and further - advanced glycation end products (AGEs). The latter are known for their pro-inflammatory effect in animal organism. Moreover, in mammals, AGEs are the well-known markers of ageing accumulating over animal's life span. In contrast, no information about age-related changes of plant AGE patterns is available. Here we provide this information and characterize possible mechanisms of AGE formation.

Experimenteller Teil

A. thaliana plants were harvested at 6, 8 and 11 weeks after seed germination and ROS production was characterized by biochemical approaches. The analytical strategy (applied to *A. thaliana* and *B. napus*) relied on the combination of LC-based bottom-up proteomics (LC x LC-ESI-Orbitrap-LIT-MS/MS data dependent acquisition experiments), untargeted and targeted metabolomics (GC-EI-Q-MS) and model glycation experiments with synthetic peptides (LC-QqTOF-MS and MS/MS).

Ergebnisse

During the five weeks of the experiment, the plants showed gradual increase of the tissue levels of sugars and ROS. LC-MS/MS analysis with subsequent database search revealed 588 AGE-modified peptides representing 771 advanced glycated sites. The number of corresponding specifically modified residues decreased in the order: N^ε-carboxymethyllysine (CML) > N^ω-carboxymethylarginine (CMA) > methylglyoxal-derived hydroimidazolone (MGH) > glyoxal-derived hydroimidazolone (Glarg) > N^ω-carboxymethylarginine (CEA) > argpyrimidine > pyrroline > dihydroargpyrimidine. Though only few new modification sites appeared with time, essential age-related changes in abundances of glycosylated proteins were observed: 97 proteins changed their abundance over time (78 of which increased and 19 decreased their abundance). Most of these proteins were involved in signaling and protein metabolism pathways. Surprisingly, glycation patterns were less abundant and comprised arginine-derived Amadori/Heyns products, that was proved by tandem mass spectrometry, to the best of our knowledge, for the first time. As only minimal overlap between early and advanced glycated sites was observed, we propose "oxidative glycosylation" i.e. advanced glycation via monosaccharide autoxidation and carbonyl formation to be the main pathway of AGE formation.

Neuer Aspekte

Monosaccharide autoxidation was the main AGE formation source in ageing plants. Arginine-derived Amadori/Heyns products were characterized for the first time.

Probing pH-Dependent Protein G'e Surface Topology Alterations by Fast Photochemical Oxidation of Proteins and Mass Spectrometry

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Stichworte: Protein G'e, Fast Photochemical Oxidation of Proteins, ESI-MS, CD-spectroscopy, protein structure changes

Einleitung

Protein G'e (consisting of three nearly identical IgG-binding domains with linker regions in between and an N-terminal His-tag) is widely used for immobilization and purification of antibodies from body fluids [1]. Antibodies can be released from protein G by dropping the pH from neutral to acidic ranges. In this study we analyzed whether the pH-shift caused structure changes of protein G'e which might be responsible for the antibody release [2]. This was done by using ESI-MS [3] and by Fast Photochemical Oxidation of Proteins (FPOP). In FPOP, hydroxyl radicals rapidly oxidize solvent-exposed amino acid residues. Tryptic digestions and LC-MS/MS analyses of chemically modified peptides pinpoint structural differences with changes in accessibilities of amino acid residues on the peptide level [4].

Experimenteller Teil

Protein G'e (10 mM) was dissolved in 50 mM NH₄OAc (pH 7) and 2% AcOH (pH 3), respectively [1]. Then 200 mM Gln and 150 mM H₂O₂ (5 µl, each) were added. FPOP was conducted in a capillary tube at KrF excimer laser power 24.3 mJ/pulse (flow rate: 24.6 µl/min). Reactions were quenched with 10 µl of 200 mM Met and 1 µg catalase. The control sample was handled in the same manner, except it was not laser-irradiated. Oxidation rates were checked on a Bruker MaXis Q-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany). Proteins were digested with trypsin/LysC at enzyme to substrate ratios of 1:2, overnight. Peptides were analyzed on an LTQ-Orbitrap mass spectrometer (Thermo Fisher, Waltham, MA) [5].

Ergebnisse

A global shift in the charge structure of protein G'e from a native-like (maximum intensity at the [M+10H]¹⁰⁺ ion) to a denatured conformation (maximum intensity at the [M+20H]²⁰⁺ ion) was observed by ESI-MS when sprayed from solutions with neutral and acidic pH, respectively. These results agree with CD-spectroscopy of protein G'e. At neutral pH protein G'e shows a predominant α -helical structure, whereas in acidic buffer the CD spectrum of protein G'e indicates lesser secondary structure features. Surface topology alterations at the domain level were mapped by FPOP. Under neutral pH conditions, Met20, Met23, and Met29 (located in the N-terminal flanking region which is not part of the IgG-binding domains of protein G'e) were instantly chemically oxidized. By contrast, oxidations of Trp88, Trp158, Trp228 residues (these are located in the middle of their respective IgG-binding domains and are part of the respective hydrophobic cores) were found in high extent upon FPOP treatment. Similarly, oxidations of Pro113 and Pro183 residues (located in the linker sequences in between the globularly folded IgG-binding domains) were observed as major oxidation events. When switching to acidic conditions, oxidation yields of Met and Pro residues remained unchanged. However, FPOP-induced Trp oxidation decreased as compared to that under neutral conditions. Finally, Tyr48 was found to be oxidized by FPOP exclusively upon acid denaturation. As Tyr48 is not accessible in the native protein structure, oxidation of this residue is explained by unfolding of the protein G'e domain assembly. This opening up of the globular assembly of the three IgG-binding domains with respect to each other could be associated with a simultaneous tightening of the individual domain folds by which hydrophobic areas should be covered and diminish Trp oxidation. In sum, the suggested protein G'e structural changes match with the observed changes in IgG-binding and antibody release under acidic conditions.

Neuer Aspekte

Structural changes of protein G'e associated with pH-shift are probed on the peptide level, resulting in a dynamic structure model.

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Oxidative degradation of N ϵ -fructosylamine-substituted peptides in heated aqueous systems

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Stichworte: AGEs, carbohydrates, glycation, synthetic peptides, tandem mass spectrometry

Einleitung

Protein glycation is a non-enzymatic post-translational modification formed by reaction of reducing sugars with N-terminal and lysyl amino groups. Resulting Amadori compounds readily oxidize ('glycooxidation')^[1] yielding advanced glycation end-products (AGEs), a heterogeneous group of compounds demonstrating a clear pro-inflammatory effect in mammals. Alternatively, carbohydrates can oxidatively degrade to α -dicarbonyls, which are highly-reactive towards lysyl and arginyl residues also yielding AGEs ('oxidative glycosylation')^[2]. While the latter processes have been studied on protein-modeling peptidesystems^[3], AGE formation via Amadori peptide degradation has not been studied. This is, however, important for food quality, as many foods containing Amadori products are heat-treated.

Experimenteller Teil

The glycated peptides Ac-AK_{Amadori}ASAXFL-NH₂ (X = S, H, R, K, D, E) and their unmodified analogues (serving as control) were incubated at 95 °C for up to 4 h. The incubation solution contained phosphate buffer (0.1 mol/L, pH 7.4) without further additives or with FeSO₄ (18 or 590 μ mol/L) alone or complemented by ascorbic acid (60 μ mol/L or 1.2 mmol/L), respectively. RP-HPLC-ESI-QqTOF-MS was used for relative quantification of the analytes by integration of extracted ion chromatograms ($m/z \pm 0.1$). Reaction products were identified by their fragmentation patterns and exact mass (ESI-Orbitrap-MS) and partially confirmed by synthetic standards (identical retention times and fragmentation pattern).

Ergebnisse

The Amadori compound degraded to less than 10% within 15 min of incubation and was quantitatively lost after 30 min regardless of the incubation condition. The two major products, the unmodified and carboxymethyl (CML)-containing peptide, were equally fast formed (70 and 51% of maximum amount at 5 min), suggesting a direct formation from the Amadori peptide, i.e. 'glycooxidation' route to CML. Further evidence was found by (i) the identification of an N^ε-(ethanallyl)lysine-containing peptide, an intermediate for CML formation by retroaldol-reaction of the Amadori peptide, which appeared with intermediate kinetics, and (ii) identification of CML only in position 2 (original glycation site) in peptides containing an additional lysyl or arginyl residue in position 6 equally susceptible to the 'oxidative glycosylation' pathway to CML/CMA.

Six additional peptides were identified with lysine modifications derived from carbohydrates, and three from oxidation. Oxidation- and carbohydrate-derived products could be distinguished by their formation under different oxidative conditions: whereas carbohydrate-derived products showed increased amounts with higher Fe^{II} concentration, oxidation-derived products behaved opposite. Phenylalanine was oxidized to tyrosine independently from the lysine modification. Tyrosine and allysine formation is radical-dependent^[4,5] and occurred only to minor or non-detectable amounts in control incubations. Contrary, peptide fragments, observed equally in all incubations, might rather be formed by hydrolysis at labile peptide bonds.

Neighboring residues affected both Amadori degradation and product formation. Faster degradation of the Amadori residue by neighboring basic residues did not increase the formation of the two major products.

Neuer Aspekte

Amadori degradation was studied in a protein-modeling system at 95 °C. Product formation pathways were revealed.

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Mass Spectrometry Imaging of Biological Tissue: An Approach for Multicenter Studies

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Stichworte: mass spectrometry imaging, multicenter studies, multimodal imaging, data format imzML, data handling and processing

Einleitung

Mass spectrometry imaging has become a popular tool for probing the chemical complexity of biological surfaces. This led to the development of a wide range of instrumentation and preparation protocols. It is thus desirable to evaluate and compare the data output from different methodologies and mass spectrometers.

Experimenteller Teil

Here we present an approach for the comparison of mass spectrometry imaging data from different laboratories (often referred to as multicenter studies). This is exemplified by the analysis of mouse brain sections in five laboratories in Europe and the United States. The instrumentation includes MALDI-TOF, MALDI-QTOF, MALDI-FTICR, AP-MALDI-Orbitrap and Cluster TOF-SIMS.

Ergebnisse

Experimental parameters such as measurement speed, imaging bin width and mass spectrometric parameters are discussed. All datasets were converted to the standard data format imzML and displayed in a common open-source software with identical parameters for visualization, which facilitates direct comparison of MS images. The imzML conversion also allowed exchange of fully functional MS imaging datasets between the different laboratories. The experiments ranged from overview measurements of the full mouse brain to detailed analysis of smaller features (depending on spatial resolution settings), but common histological features such as the corpus callosum were visible in all measurements. High spatial resolution measurements of AP-MALDI-Orbitrap and TOF-SIMS showed comparable structures in the low micrometer range.

We discuss general considerations for planning and performing multicenter studies in mass spectrometry imaging. This includes details on the selection, distribution and preparation of tissue samples as well as on data handling. Such multicenter studies in combination with ongoing activities for reporting guidelines, a common data format (imzML) and a public data repository can contribute to more reliability and transparency of MS imaging studies.

Neuer Aspekte

Flexible approach to compare and combine MS imaging platforms across different laboratories.

Genaueste Einblicke in die Klimageschichte anhand der Bestimmung von Lipidbiomarkern in marinen Sedimenten mittels LDI FTICR Imaging

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Stichworte: Imaging, MALDI, Geochemie, Bohrkern, FTMS

Einleitung

Marine Mikroorganismen passen je nach Habitat und Umgebungstemperatur den Aufbau ihrer Membranen durch Modifikation der Lipidzusammensetzung an. Diese strukturelle Information bleibt auch nach der Sedimentierung erhalten, da die Verbindungen auch über geologische Zeiträume erhalten werden. Daher sind sedimentierte Lipidbiomarker eine wichtige Informationsquelle zur Rekonstruktion der Klimageschichte.

Experimenteller Teil

Konventionelle Biomarkeranalyse (Extraktion gefolgt von LCMS-Analysen) ist laborintensiv und erfordert Sedimentstücke in cm-Größen, wodurch die zugängliche zeitliche Auflösung häufig niedrig ist. Hier präsentieren wir eine neue Methode, die auf Laser Desorptions Fourier Transformations Massenspektrometrie (FTICR MS) beruht. Diese Methode wurde für die Detektion von Glycerol Dialkyl Glycerol Tetraethern (GDGTs) in Sapropelen aus marinen Sedimenten des östlichen Mittelmeers angewandt. Diese Zellbausteine stammen von einzelligen Meeresorganismen, Archaeen, die sowohl heute als auch damals den weltweiten Ozean bevölkern. In den Sedimenten konserviert, können diese Lipide als sogenannte Proxies genutzt werden. Sie liefern, stellvertretend für direkte Messungen, Informationen über vorzeitliche Meeresbedingungen und werden in den meisten Fällen repräsentativ als Proxy für Meerestemperaturen eingesetzt^[1].

Ergebnisse

Durch das Abrastern eines Sedimentkerns mittels LDI FTICR MS werden die relativen Intensitäten der GDGTs direkt aus Sedimentkernen gewonnen und können mit der zum Zeitpunkt der Sedimentierung herrschenden Meeresoberflächentemperatur korreliert werden^[2]. Die hohe räumliche Auflösung des Lasers (hier: 250 µm) in Kombination mit dem extremen MS-Auflösungsvermögen des FTICRs ist die Voraussetzung zur Gewinnung relevanter Daten. Da die resultierenden Massenspektren sehr komplex sind, wird zur Beschränkung des Massenbereichs ein Quadrupol zum Vorfiltern benutzt. Diese neuartige Methode ermöglicht somit die Untersuchung vorzeitlicher Klima- und Umweltveränderungen in bislang unvorstellbarer zeitlicher Auflösung.

Neuer Aspekte

Hochauflösendes MALDI imaging von geologischen Bohrkernen zur Erhöhung der zeitlichen Auflösung von Daten für die Klimageschichte

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Combining label-free whole-cell MALDI MS biotyping and imaging mass spectrometry for in-situ monitoring of histone deacetylase drug target activation

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Stichworte: Whole-Cell MALDI MS, Target engagement, Histones, Tumor models, Imaging

Einleitung

Epigenetics describes the heritable changes in gene expression that do not **require** changes in nucleotide sequence. Research revealed that cancer is not only a genetic disease but can also result from a deregulation in epigenetic enzymes e.g. histone deacetylases (HDAC)(1). HDAC inhibitors are focus of research. Drug binding and target activation are prerequisites of efficacy and need to be monitored during drug development. State of the art techniques require reagents and multiple preprocessing steps and reagent-free in-situ analysis of engaged drug targets or target-proximal pharmacodynamic signatures in solid tumors remains challenging. MALDI biotyping (2) demonstrated that HDACi potencies can be label-free quantified in whole-cells and MALDI-IMS revealed the distribution of acetylated histones and thus confirming the tumor-selective pharmacodynamic responses (2).

Experimenteller Teil

Application of Whole-Cell MALDI MS biotyping to detect histone core proteins under whole cell conditions without performing acid extraction or LC-purification. Monitoring the pharmacodynamic fingerprint of HDAC inhibitor (HDACi) drug target activation and evaluation of HDACi drug potencies on pharmacodynamic histone fingerprints.

Development of a proteins MALDI- mass spectrometry imaging technique to visualize the histone acetylation state in solid tumors and to monitor the proximal biomarkers of HDACi drug target activation in a gastric cancer mouse models.

Ergebnisse

Histone Core proteins can be directly detected from whole cells by Whole-Cell MALDI MS without prior acid extraction and purification, by increasing the MALDI matrix acidity. Whole-Cell MALDI MS is suitable to study the target engagement of pharmaceutical drugs (HDACi) under whole-cell conditions and the label-free evaluation of their IC50 values relying on histone acetylation-specific mass shifts.

By applying MALDI mass spectrometry imaging on the histone mass shifts the spatiotemporal distribution of acetylated histones and thus the tumor-selective pharmacodynamic responses in a mouse model of gastrointestinal cancer could be confirmed. Taken together, the combined power of Whole-Cell MALDI biotyping and MALDI mass spectrometry imaging suggests that the monitoring of drug-induced mass shifts could provide a suitable platform in pharmacology, clinical oncology and drug discovery.

Neuer Aspekte

Quantification of HDACi on their pharmacodynamic fingerprint. In-situ analysis of drug target activation of HDACi in solid tumor models.

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High precision mass measurement and separation of nuclear isomers with a multiple-reflection time-of-flight mass spectrometer

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Stichworte: Time-of-flight mass spectrometer, Multiple-reflection, Nuclear isomer separator, Exotic nuclei

Einleitung

Nuclear isomers are excited metastable states of nuclei. Their long lifetimes originate from their shape, spin or spin projection. The properties of nuclear isomers can differ from the ground state properties besides their half-life e.g. their decay properties. Applications of nuclear isomers affect a broad field of research including new possibilities for clean energy storage to impact on nuclear astrophysics and the production of the elements in the universe. Particularly in the region of exotic nuclei, with an extreme neutron-to-proton ratio, these excited states can only be produced, measured and separated under challenging conditions. The multiple-reflection time-of-flight mass spectrometer (MR-TOF-MS) [1,2] is an ideal tool to provide new information and opportunities.

Experimenteller Teil

The MR-TOF-MS has been developed as part of the FRS Ion Catcher [3] at GSI in Darmstadt. Here exotic nuclei are produced at relativistic energies and are separated in-flight in the fragment separator (FRS). They are slowed down and thermalized in a cryogenic stopping cell, before they are transported in a radio frequency (RF) quadrupole beam line towards the MR-TOF-MS where mass measurements and ion separation are performed. In the MR-TOF-MS [4] the ions are accumulated, cooled and injected in bunches by a linear RF trap. In the analyzer they are reflected multiple times to enlarge their flight path by orders of magnitude to obtain high resolving powers in a very short measurement duration.

Ergebnisse

The performance of the MR-TOF-MS was improved. The kinetic energy of the ions in the time-of-flight section of the MR-TOF-MS has been increased to 1300eV. Mass resolving powers (FWHM) for ^{133}Cs of 120,000, 220,000 and 420,000 have been obtained in 2.3 ms, 4.6 ms and 18.3 ms, respectively. A novel RF quadrupole-based switchyard was recently developed and commissioned. It provides the opportunity to additionally inject calibration ions into the RF beam line by merging beams from up to 5 beam lines.

Projectile and fission fragments of Uranium at the FRS produced at 1000 MeV/u were measured for the first time directly with a MR-TOF-MS at low yields (few ions per hour), among them ^{213}Rn with a half-life of only 19.5 ms. Due to the single ion sensitivity of the device, it can perform these mass measurements.

In the region of the chart of nuclei of the doubly magic nucleus ^{132}Sn several nuclei have long-lived nuclear isomers. Ground and excited state of ^{133}I were measured simultaneously with the MR-TOF-MS. This allows the direct determination of directly the mass of both states and therefore the excitation energy of the isomeric state. Furthermore the isomeric ratio can be measured at the same time. This will lead to a better understanding of the production mechanism of exotic nuclei at accelerator facilities.

The MR-TOF-MS is not only suited for mass spectrometry. It is also used, equipped with a Bradbury-Nielsen gate, as an isobar and nuclear isomer separator. In the MR-TOF-MS the ground and excited state of an exotic ion can be separated spatially. This was demonstrated in an experiment in October 2014. With the opportunity to produce a pure ion beam which consists of one excitation state only, new possibilities arise in the novel field of mass resolved decay spectroscopy in nuclear physics.

Neuer Aspekte

High resolution MR-TOF-MS with single-ion sensitivity enable measurement and separation of nuclear isomeric states of rare short-lived nuclei.

Referenzen

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Analysis of polar trace components in middle distillate fuel by ultra-high resolution mass spectrometry using ESI and GC-APCI

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Stichworte: Diesel, ESI, GC-APCI, FT-ICR-MS

Einleitung

Fossil fuel analysis is still a challenging analytical task, even for light and middle distillate fuels. Polar compounds, such as oxygenated or heterocyclic species, are especially interesting due to their influence on physical properties, e.g. corrosivity or viscosity [1,2]. State-of-the-art techniques are chromatographic techniques, such as gas-chromatography (GC) and comprehensive 2-D GC (GCxGC) [3]. Typically these instruments are equipped with time-of-flight mass analyzers, supplying excellent acquisition speed, but usually lacking in resolving power. In the presented work we applied high-resolution mass spectrometry with classical direct infusion electrospray ionization (ESI) in positive and negative mode to cover basic and acidic species. Furthermore a hyphenation of GC to atmospheric pressure chemical ionization (APCI) was used for the determination of medium polar compounds.

Experimenteller Teil

Four reference (Europe-EN590, California, Swedish-MK1, USA-US2D) and nine commercial diesel samples were investigated. The polar fraction of each sample was extracted by mixing fuel with methanol. For analyzes analyses with ESI the extract was further diluted with methanol. For GC-APCI-analyses the neat fuel diluted in a methanol/toluene-mixture was injected. Mass spectrometric analyses were carried out using a Bruker-solariX-FT-MS and Bruker-apex-FT-MS (both equipped with a 7T magnet) for ESI and GC-APCI respectively. For (+/-)-ESI-analyses 200 scans were accumulated, a resolving power of roughly 150000 @ m/z 200 was obtained and each sample was analyzed in triplicate. For the GC-APCI-analyses a BPX5-column was used and a resolving power of roughly 340000 @ m/z 200 was obtained with a scan frequency of 0.8Hz.

Ergebnisse

Using ESI, the reference diesel samples showed a completely different pattern in -class distribution when compared to that from the commercial diesel samples, e.g. in positive polarity mode the CHN₁-class was significantly more abundant in the commercial diesel samples. This behaviour can be partially explained by considering the origin of the samples, i.e. blending and distillation. Very low intensities for the standard diesel indicate a low content of polar species, leading to a higher percentage variation between the replicates. In negative polarity mode mainly compounds with oxygen and/or sulphur were found, whereas in positive mode mainly pure hydrocarbons and nitrogen containing species were observed. Nevertheless some compound classes, such as CHN₁ and CHO_x, were found in both data sets, but a different pattern for the double bond equivalent distribution was obtained. Therefore it can be assumed that different functional groups were detected for species with the same elemental composition.

The GC-APCI-hyphenation enables isomeric/isobaric differentiation and minimises matrix effects. Even at high acquisition rates the chromatographic features cannot be distinguished, but some information can be gathered based on the retention time index (RI). Sophisticated data analyses by self-written MATLAB routines compared measured RI values and data from the NIST database for limiting proposed elemental compositions. As a result the +1O₁ subclass showed 13 peaks when summing up all mass spectra for the GC run to mimic direct infusion, whereas 42 species were detected using the retention time resolved data processing. This multidimensional information was visualized by using a variation of the traditional Kendrick plot, where GC retention times are used instead of nominal mass values on the x-axis. By applying this visualization-technique the standard diesel samples can be well separated from the commercial diesel samples.

Neuer Aspekte

- investigation of polar compounds in diesel fuels using (+/-)-ESI and (+)-APCI
- use of GC-APCI hyphenation to FT-ICR-MS for diesel analysis

Referenzen

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Adding a chemical dimension to the analysis of heavy crude oils with ultra-high resolution mass spectrometry

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Stichworte: Ultra-high resolution, mass spectrometry, chemical method, electrospray ionization, crude oil.

Einleitung

Fossil-based materials including heavy crude oil are still the main supply of energy. The presence of even only a small percentage of non-hydrocarbon elements (such as N-, S-, and O-) in crude oil is deleterious and harmful for refining catalysts and environments. Crude oil is considered the most complex mixture in nature. Chemical methods in combination with ultrahigh resolution mass spectrometry (the high-field Orbitrap FTMS and FT-ICR MS) coupled with ESI provide an effective and feasible approach to selectively analyze the nonpolar sulfur species in whole crude oils without any fractionation and chromatographic separation.

Experimenteller Teil

DBT was dissolved in dry 1, 2-dichloroethane (DCE) and was reacted with a certain amount of deuterated derivatization reagents (C_2H_5I) in the presence of silver tetrafluoroborate ($AgBF_4$) for 4 h. The precipitate was removed by centrifugation and washed with dichloromethane. The same procedure was further applied to a mixture of standard aromatic CH-, N-, O- and S-containing compounds and a heavy bitumen. C_2H_5I was replaced by CD_3I and C_2D_5I in heavy bitumen reaction.

Mass spectra were recorded using a 7 T FT-ICR MS (Thermo Fisher Scientific, Bremen) and a high-field Orbitrap FTMS equipped with an ESI source. External mass calibration was performed using a tune mix solution.

Ergebnisse

As known, electrospray ionization (ESI) coupled with ultrahigh resolution mass spectrometry is an excellent method to selectively analyzing polar compounds; nevertheless, most of components of crude oil are non- or less-polar. Herein, we introduce the chemical methods to crude oil to selectively analyze the S-containing species under positive ESI conditions.

DBT was selected as an example to optimize the reaction condition while the reaction was monitored by 1H NMR analysis. These optimized conditions were then employed to a mixture of standard aromatic CH-, N-, O- and S-containing compounds, DBT, anthracene (ANTH), dibenzofuran (DBF) and acridine (ACR), and after reaction, only DBT was selectively tagged. The same conditions were finally introduced into the real heavy bitumen, and normal C_2H_5I was replaced by the deuterated alkylation reagents (CD_3I and C_2D_5I), by which, the specific information of D atoms which are not present at high abundance in crude oil allows unambiguously discriminating the nonpolar sulfur species by CD_3 or CD_5 group from original crude oil in positive ESI. As a reference, the original heavy bitumen was prepared with the solvent DCE containing 0.2 % AcOH, and polar N- and NS-species were observed by protonation.

The results show that the S1- and S2-species were selectively tagged showing a DBE range (double bond equivalent) from 2- 25. Coordination chemistry, nucleophilic substitution together with relevant methodology play role in selective analysis of sulfur-containing compounds.

Chemical methods in combination with the ultrahigh resolution mass spectrometry coupled with ESI provide an effective approach to selectively analyze the nonpolar sulfur species in whole crude oils without any fractionation and chromatographic separation.

Neuer Aspekte

Combining chemical reactions with ultra-high mass spectrometry is used to selectively analyzing crude oil.

Improving cross-linked peptide identification in large protein complexes across different high-resolution LC/MS/MS platforms

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Stichworte: cross-linking, protein complex, high-resolution MS

Einleitung

Most cellular proteins do not function in isolation, but assemble into higher order complexes to perform their physiological roles. Conventional high-resolution structural methods such as NMR or X-ray crystallography show intrinsic limitations in the analysis of large or dynamic protein complexes. To overcome these, chemical cross-linking combined with mass spectrometry (XL-MS) is applied more and more to identify spatially proximal protein components, thereby generating distance constraints that can be used to generate or refine structural models. We have optimized workflows to apply XL-MS to Megadalton protein complexes, and will discuss improvements and limitations regarding (i) sample preparation and separation, (ii) mass spectrometric analysis on different high-resolution MS platforms, (iii) data processing and (iv) implementation into structural modeling.

Experimenteller Teil

Protein complexes of different sizes were chemically cross-linked with bisulfosuccinimidyl suberate (BS3), reduced, alkylated and tryptically digested into (cross-linked) peptides. Additional separations were performed either on the protein (SDS-PAGE) or peptide (SEC, RP-HPLC) level to enrich cross-linked species. The final samples were analysed by nanoLC/MS/MS using ion-trap and beam-type CID on several instrument platforms, including different Orbitrap (Thermo Q Exactive, Q Exactive HF, Orbitrap Fusion) and Quadrupole-Time of Flight mass spectrometers (AB SCIEX TripleTOF 5600+). Data analysis was performed using pLink 1.18 and 2.0 beta software (Chinese Academy of Sciences) [1], and searching against custom-made protein sequence databases. Cross-links were tested for plausibility by matching against existing structural models where possible.

Ergebnisse

The success of XL-MS studies of large protein complexes depends on careful optimization of experimental parameters. Whereas cross-linked complexes consisting of few components can be separated from non-cross-linked proteins by SDS-PAGE, larger complexes have to be trypsinized in solution. Here, peptide/peptide cross-links can be enriched from the large excess of non-cross-linked or “dead-end” peptides e.g. by size exclusion chromatography (SEC).

Optimization of nanoLC/MS/MS conditions revealed that both general instrument properties and parameter optimization contribute significantly to the confident identification of larger numbers of cross-links. MS/MS acquisition speeds and MS/MS spectral quality are of major importance, as even after enrichment peptide/peptide cross-links are a minority in the complex mixtures analysed. With regard to CID activation, beam-type, high-resolution CID (HCD) significantly outperformed ion-trap CID. In addition, optimization of the CID collision energy provided significant improvements irrespective of the instrument generation used.

Another hurdle towards the routine use of XL-MS experiments for structural biology lies in the database search algorithms used for the identification of cross-linked species. While significant improvements have been implemented, there is still a lack of reliable statistical means for results evaluation such as an unbiased, global False Discovery Rate (FDR) analysis, resulting in a significant need for manual evaluation of large datasets.

We tested the plausibility of database search results by matching identified cross-links including their scores, numbers of spectral counts and the resulting distance constraints against available structural models generated by high-resolution X-ray crystallography for smaller, well-characterized protein complexes. We found that a surprisingly high percentage of the identified crosslinks matched within plausible tolerance, demonstrating the validity of the approach. Application of the improved overall methodology on Megadalton-sized protein/protein complexes resulted in the

identification of >1000 crosslinks for individual complexes, highlighting the need for statistical tools to validate the results in a more automated fashion.

Neuer Aspekte

Chemical cross-linking and mass spectrometric analysis (XL-MS) for the structural analysis of large protein complexes

Referenzen

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Monitoring Conformational Changes in PPAR β/δ by Chemical Cross-Linking, Photo-Affinity Labeling, and Mass Spectrometry

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Stichworte: Bpa, photo-affinity labeling, BS²G-D₀/D₄

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 [1]. Peroxisome proliferator-activated receptors (PPARs) belong to the subfamily of nuclear receptors that are involved in metabolic processes. One subtype, PPAR β/δ , is thought to be connected with the development of several chronic diseases and presents an important drug target [2].

Experimenteller Teil

The ligand-binding domain (LBD) of PPAR β/δ (amino acids 166-441) was cross-linked with the amine-reactive homobifunctional cross-linker bis(sulfosuccinimidyl)glutarate (BS²G)-D₀/D₄. For photo-affinity labeling (PAL) studies, several variants of the LBD of PPAR β/δ , in which specific amino acids were exchanged against p-benzoyl-L-phenylalanine (Bpa) [3], were cross-linked using a home-built UV-irradiation chamber. After *in-gel* or *in-solution* digestion, cross-linked samples were analyzed by nanoHPLC/nano-ESI-MS/MS with an UltiMate 3000 RSLC system (Thermo Fisher Scientific) coupled to an Orbitrap Fusion Tribrid mass spectrometer (Thermo Fisher Scientific) equipped with a Nanospray Flex ion source (Thermo Fisher Scientific). Cross-links were evaluated with the software StavroX [4]. The distance constraints imposed by the cross-links served to monitor conformational changes in PPAR β/δ upon binding of the agonists GW1516 and GW0742.

Ergebnisse

Cross-linking experiments with BS²G indicate a high flexibility of the LBD of PPAR β/δ . Both in the absence and in the presence of ligands, the majority of cross-links involved amino acids 185-210. In the presence of the agonists GW1516 and GW0742, additional cross-links were found from the *N*-terminus of the LBD to lysines K322 and K422/K423 suggesting a large conformational change. To confirm this, additional cross-linking experiments with full-length PPAR β/δ are currently performed. To obtain additional information about conformational changes induced by ligand binding, PAL studies are in progress. For these experiments, the phenylalanine residues F177, F189, F210, and F245 as well as the *C*-terminal tyrosine Y441 were exchanged against Bpa. All site-specific mutations were validated by MS/MS experiments. One cross-link was found in all experiments connecting Bpa177 to isoleucine I378, as both residues are located on opposite helices. Surprisingly, Bpa cross-links were often identified as two distinct species even before MS/MS experiments were conducted: One species is the intact cross-linked product, one species shows the loss of one water molecule. This finding indicates that after Bpa cross-linking the hydroxyl group is eliminated already in solution resulting in the formation of an alkene. CID, HCD, and ETD fragmentation experiments of Bpa cross-linking products are currently performed.

Neuer Aspekte

Chemical cross-linking, photo-affinity labeling, conformational changes in PPAR β/δ upon ligand binding

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Chemical crosslinking of TSHR-ECD

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Stichworte: Chemical Crosslinking, Orbitrap-MS, TSHR, computational modelling, protein structure

Einleitung

Chemical crosslinking is a powerful tool for the elucidation of protein structures especially those that cannot be solved by classical approaches like X-ray or NMR. The major advantages of this method are its high sensitivity and the possibility to study low purity proteins. Furthermore, experiments can be executed under native-like conditions [1].

The Thyroid Stimulating Hormone-Receptor (TSHR) is a Glycoprotein Hormone-Receptors (GPHR), which is of high interest for the fundamental understanding of membrane proteins and potential medical applications. A particular characteristic of GPHRs is the large Extracellular Domain (ECD) that includes the glycohormone binding site which is of special interest [2].

The aim of this study is to create the first experimental based model of the TSHR-ECD.

Experimenteller Teil

Cross-linking reactions were performed using DST (disuccinimidyl tartrate), BS₃ (bis(sulfosuccinimidyl) suberate), sulfo-EGS (ethylene glycol bis(sulfosuccinimidylsuccinate)), BS(PEG)₅ (bis(succinimidyl) penta(ethylene glycol)). All cross-linking reactions were conducted in 1X PBS-buffer, pH 7.2 at a protein concentration of 3.3 or 2.5 μM. The protein to cross-linker ratio was 1:100 or 1:200 (n:n) respectively and the incubation was carried out at room temperature for 60 or 120 min before the reaction was quenched with an equimolar amount of ammonium bicarbonate buffer. Afterwards, the proteins were in-gel digested with trypsin. MS-measurements were performed with an LTQ-Orbitrap XL (Thermo Fisher Scientific Inc.) online coupled to an NanoAcquity-UPLC (Waters Corporation) or an Orbitrap Fusion Tribrid-MS (Thermo Fisher Scientific Inc.) online coupled to an UltiMate 300 HPLC (Dionex Corporation).

Ergebnisse

For decades the structure of TSHR and especially the TSHR-ECD have been studied by scientists, but still no atomistic model of this protein is available. Much of the insights into the structure of the GPHR are based on the crystal structure of the Follicle stimulating Hormone-Receptor (FSHR)-ECD [3], which has a moderate sequence identity of ~40% to the TSHR-ECD [4]. This enabled our working group to create homology models of the TSHR-ECD which could be filtered and probed using our crosslinking data.

In total, we identified 41 inter- and intramolecular crosslinks (XL). Six intramolecular crosslinks (XL) were detected in the unbound state of the TSHR-ECD, of which, four represented the C-terminal part, which was not covered by any structural model so far.

Furthermore, we found 22 XLs for the TSHR when bound to TSH. Two were intramolecular XLs for the TSHR-ECD and 10 were intermolecular XL between the bound TSH and its receptor. These XLs delivered valuable information about the relative orientation, position and interaction between the two proteins and their respective structures.

While 18 XLs of the TSHR-ECD in complex with TSH matched perfectly the best-scored predicted TSHR structure, four XL did not fulfill the criterion stating that distance between the Cβ-atoms should be smaller than the sum of the spacer length plus the side chains of the two connected amino acids. However, three of these four divergent XL were located on the protein terminus, which was predicted to be highly flexible. Interestingly, the remaining XL, obtained with DST fits better in a second modelling cluster (22.4 Å in maximum) and also depends strongly on the chosen template for the modelling process. By applying chemical crosslinking in combined with MS and molecular modeling our working group was able to deliver an experimental based model of the TSHR-ECD.

Neuer Aspekte

First experimental based structure model for the GPCR TSHR-ECD are obtained using chemical crosslinking and computational modelling.

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Incorporation of Photo-Methionine into Calmodulin for Photo-Cross-Linking/MS Studies

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Stichworte: Photo-Cross-Linking, Photo-Methionine, E. coli

Einleitung

Photo-cross-linking is an auspicious tool to obtain 3D-structural information of a protein and to gain insights into protein-protein interactions. The advantage of photo-cross-linking is that different amino acid side chains can be targeted, while the mainly used chemical cross-linkers prefer to react with primary amine or hydroxy groups [1]. If photoactivatable amino acids are incorporated into the proteins of interest, no external cross-linker has to be added [2]. Here, we present the successful incorporation of the photo-reactive diazirine analogon of methionine into the 17-kDa protein calmodulin (CaM), which is a highly conserved Ca^{2+} -binding signaling protein that plays an important role in a variety of cellular processes. CaM contains nine methionines that can potentially be replaced by photo-methionine (pMet) [3].

Experimenteller Teil

For the incorporation of pMet into CaM, *E. coli* was grown in mineral salts medium supplemented with pMet during overexpression of the recombinant protein. His₆-tagged CaM was enriched from cell extracts using immobilized metal ion affinity chromatography and size exclusion chromatography. Cross-linking experiments were performed in 20 mM HEPES buffer (pH 7.4) with a free Ca^{2+} concentration of 30 nM. The photo-cross-linking reaction was induced by UV-A irradiation (360 nm, 8000 J/cm²). Mass spectrometric analysis of cross-links was done after in-solution digestion with trypsin and GluC by nano-HPLC/nano-ESI-MS/MS on an Ultimate 3000 RSLC nano system coupled to an Orbitrap Fusion Tribrid mass spectrometer. Data were analyzed with the StavroX software 3.4.9 [4].

Ergebnisse

The incorporation of pMet into CaM is straight-forward as the translation machinery accepts pMet instead of methionine. Therefore, no additional components, e.g. tRNAs, were required for the expression of pMet-labeled CaM. After purification of pMet-labeled CaM, the successful incorporation of pMet was validated by peptide fragment fingerprint MS analysis. In all samples, all nine methionines of CaM were found to be replaced by pMet. Calculation of the pMet incorporation rate was performed for CaM samples obtained at different timepoints after induction. The highest pMet incorporation rate (>30%) was obtained at 3h after induction. Obtained pMet-labeled CaM was used for photo-cross-linking experiments resulting in the identification of several intramolecular cross-links within CaM, in which different pMet residues (positions 36, 76, 144 and 145) are involved. The identified cross-links are in agreement with the proposed structure of CaM at low Ca^{2+} concentration and demonstrate that short valuable distance information can be obtained by the incorporated photoactivatable amino acids and subsequent photo-cross-linking.

Neuer Aspekte

Incorporation of pMet is easily performed in *E. coli* using a mineral salts medium without incubation on complex media.

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Mass spectrometric analysis of post-translational modifications in T cells

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Stichworte: tyrosine phosphorylation, sortase-mediated ligation

Einleitung

Post-translational modifications of proteins are ubiquitous and can dramatically alter their function. They often act as molecular switches that reflect the "on" or "off" state of a protein within the living cell. Therefore, trapping the state of these modifications under certain cellular conditions represents an important task in modern proteomics research.

Experimenteller Teil

- SILAC

-18O/16O labeling [1]

- Affinity purification [2]

- Quantitative mass spectrometry [3,4]

- Sortase mediated ligation [5]

Ergebnisse

Here, we follow two key modifications of biological systems, namely palmitoylation and phosphorylation, by mass spectrometry. For palmitoylation, we use established acyl-biotin exchange chemistry to map the subset of proteins that is reversibly palmitoylated in primary T cells from healthy human donors. Similarly, phospho-protein interactions can be mapped by the combination of affinity purification, chemical labeling and quantitative mass spectrometry. Therefore, our approach offers a powerful mean to infer protein function from biologically complex samples

Neuer Aspekte

Phosphorylation-dependent interactome mapping of "structured" phosphosites

Sortase-mediated ligation for affinity-MS

Palmitome and phosphoproteome analysis of human primary T cells

Referenzen

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Lipids originating from *Mycobacterium tuberculosis* (Mtb) are detected in plasma of Tuberculosis Patients

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Stichworte: LC-MS, tuberculosis, lipidomics, high-resolution mass spectrometry

Einleitung

The human plasma lipidome is highly complex. While some “shotgun lipidomics” approaches rely solely on high resolution mass spectrometry for lipid identification¹, tandem mass spectrometry is required to specifically identify fatty acid residues bound to particular lipid species². Odd-numbered fatty acid residues are of low abundance in human plasma. However, *Mycobacterium tuberculosis* (Mtb)³, the causative agent of tuberculosis (Tb), contains odd-numbered fatty acids in high abundance. To address the question whether a tuberculosis infection can be detected based on the analysis of phospholipids containing odd-numbered fatty acid residues in human plasma samples, we developed a method to specifically identify these species in plasma. This will allow for identification of potential mycobacterial-derived lipid species in plasma samples of infected individuals.

Experimenteller Teil

Plasma samples were obtained from Tb patients before and during anti-Tb treatment and from healthy controls. 50µl plasma was extracted using methyl-tert-butyl ether and normal phase-HPLC was used for separation of lipid classes. Online detection was performed using a high resolution hybrid Apex-Qe FT-MS system (Bruker Daltonics, Bremen). Mass spectra were summed up according to the respective retention time ranges of the lipid classes of interest, smoothed, baseline subtracted and peak lists were exported using customized VBA scripts (DataAnalysis 4.0). Lipids were assigned using LipidXplorer if the mass error was better than 3ppm⁴. Tandem mass spectrometry experiments were performed on a Q Exactive Plus (Thermo Fisher Scientific, Bremen) for identification of the fatty acid composition of the major phospholipid classes.

Ergebnisse

Proof-of-principle experiments were performed using a small subset of patient samples (n=3) since study enrolment is still ongoing because anti-Tb treatment requires six months for completion.

By using the described LC-MS approach, we were able to quantify more than 300 lipid species in Tb patients' plasma samples. Identified lipid species were analysed using targeted tandem mass spectrometry experiments for identification of phospholipid species containing odd-numbered fatty acids. We were able to quantify more than 50 lipid species containing odd-numbered fatty acids in plasma samples obtained from Tb patients and from healthy controls, accounting for ~ 2 mol% of total identified plasma lipids. In this subset of lipid species identified, we were particularly interested in phospholipid species containing the mycobacterial specific tuberculostearic acid (TSA). In mycobacterial lipid extracts, TSA is primarily found in its esterified form, where it is incorporated into phosphatidylinositol species containing saturated fatty acids as the second fatty acid residue, for example PI(19:0/16:0).

In plasma lipid extracts of Tb patients, we found that TSA is mainly incorporated into phosphatidylinositol and phosphatidylethanolamine species containing poly unsaturated fatty acids as second fatty acid residue.

Neuer Aspekte

Detection of mycobacterial cells wall lipids released into hosts' plasma during infection.

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A simple MALDI MS-based method to identify ether lipids in complex lipid mixtures of spermatozoa

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Stichworte: MALDI-TOF MS, ether phospholipids, phospholipase A₂, 2, 4-dinitrophenylhydrazine, plasmalogens

Einleitung

There is increasing evidence that MALDI MS is a useful method of lipid analysis [1]: MALDI MS is fast, sensitive, and tolerates sample impurities to a considerable extent [2]. As phospholipase A₂ (PLA₂) cleaves selectively the *sn*-2 acyl group of phospholipids, PLA₂ is very helpful to differentiate the residues in *sn*-1 and *sn*-2 positions. Furthermore, the classical reagent 2,4-dinitrophenylhydrazine (DNPH) is a "reactive" matrix that helps to convert aldehydes into stable products. We will demonstrate a simple approach to monitor the presence of plasmalogens (alkenyl-ether phospholipids) in spermatozoa extracts. 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.

Experimenteller Teil

All chemicals, solvents, PLA₂ and 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.

Ergebnisse

Spermatozoa 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: 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 combining enzymatic digestion/chemical derivatization and without the need of sophisticated MS equipment: (1) PLA₂ digestion converts the plasmalogen into a lyso-plasmalogen lacking the *sn*-2 acyl moiety and (2) already traces of acids convert the (acid sensitive) plasmalogen into a lysolipid (lacking the ether residue) and the corresponding aldehyde. The lysolipid 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 strategy. 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 spermatozoa 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.

Neuer Aspekte

A simple method to identify plasmalogens in spermatozoa lipids was developed. This approach is also applicable to other physiologically-relevant samples.

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LPPdb: a new MS-centered database of modified lipids

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Stichworte: lipidomics, lipid peroxidation, database, data management, lipid profiling

Einleitung

Lipid peroxidation products (LPP) generated during oxidative stress are recognized as important biomarkers of numerous human disorders. LPP are extremely diverse in their structures and thus require sophisticated detection protocols. In recent years, we established several strategies combining TLC and HPLC with MALDI- and ESI-MS to analyze LPP, such as carbonylated, hydroxylated, and head group modified phospholipids, oxysterols, eicosanoids, oxidized and nitrated fatty acids. These strategies allowed us to identify many new LPP that have not been reported before. As the currently available lipid databases, such as LIPIDMAPS, LipidHome, and HMDB, do not specifically collect information on modified lipids, we initiated a knowledge MS-centric database of oxidized lipids termed LPPdb.

Experimenteller Teil

LPPdb consists of two main components: LPPdb client and LPPcloud website (www.uni-leipzig.de/~oxlpp). The web-client structure was designed using python language [1] with Pandas data analysis library [2] for advanced data processing and PySide [3] for client interface. The LPP entries are parsed and stored externally in MariaDB database server [4] by LPPdb client: chemical formula, exact mass, m/z , taxonomy, and IUPAC name, structure, MS/MS information (fragment ions, neutral losses and MRM transitions), identification/quantification protocols (LC and MS specific conditions), known pathways, and reference DOI. Derivatization reagent specific information is also supported. Unique LPPid are generated automatically based on the key properties of each entry.

Ergebnisse

LPPdb is maintained as a crowd-sourcing database, which allows all users to generate their private LPP databases and to exchange information with others. The LPPdb client is a fundamental client software, which was designed to generate and manage all LPP database entries, whereas LPPcloud provides an online web service with a query function of a public LPP database contributed by the LPPdb community. The LPPdb client contains five modules, which cover the whole workflow including entry generation, method management, database management, database query, and entry viewer. The architecture of LPPdb allows a flexible use of private and LPPcloud databases. A preliminary dataset obtained from MS data can be used for advanced LPPdb searches to generate a list of identified LPP supported by the corresponding tandem mass spectra from the private database, collaborators database and LPPcloud to accelerate data analysis of complex biological samples. Confirmed entries can be exported in "LPP.txt" and further used as supplementary files for publications. Thus a new strategy to manage and analyze mass spectra of LPP was established.

The current version of LPPdb contains general, MS related and published information to assist MS/MS interpretations. So far more than 400 entries have been generated including oxysterols, nitrated and oxidized fatty acids, eicosanoids, hydroxylated, truncated, glycated, and head group modified phospholipids, carbonylated low and high molecular weight LPP from different phospholipid classes. Researches working on oxidized lipids can get access to LPPdb and use it for the identification of oxidized lipids and for the enrichment with newly identified LPP.

Neuer Aspekte

The LPPdb provides a web-client based database to organize and share MS-based lipidomics data for oxidized lipids.

Referenzen

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Shotgun Lipidomics for Dissecting Lipid-protein Assemblies

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Stichworte: γ -secretase, shotgun lipidomics, lipid-protein assemblies

Einleitung

We combined immunoaffinity purification and shotgun lipidomics to determine the lipidome associated with gamma-secretase, a multi-subunit membrane protein complex. γ -Secretase complex is a protease that catalyzes the final intramembrane cleavage of the β -amyloid precursor protein during the neuronal production of the amyloid β -peptides, the causative agents of Alzheimer's disease (AD). It has become an important therapeutic target for the development of drugs to slow down or prevent the pathogenesis of AD.

Experimenteller Teil

Whole membrane lysates were prepared from CHO cells stably overexpressing the human γ -secretase complex according to [1]. After ultracentrifugation step, the membrane protein lysate was incubated overnight at 4°C with anti-Flag M2 affinity beads. The γ -secretase complex was purified by gel filtration chromatography. Lipids were extracted using a modified Folch protocol from each collected fraction. Cholesterol was derivatized with acetylchloride prior to the analysis [2]. Lipid extracts spiked with internal standards were infused into a Q Exactive mass spectrometer (Thermo Fisher Scientific) via a robotic nanoflow electrospray ion source (Triversa NanoMate, Advion BioSciences). Lipids were identified by LipidXplorer software [3].

Ergebnisse

We applied top-down shotgun lipidomics approach to quantify 53 lipid species from 11 major classes associated with affinity-purified γ -secretase complex with a ratio of lipids to proteins of 393. PC, PE, PS, PI and cholesterol were the major lipid classes associated with the complex, while SM, ether lipids, lysolipids and PG were detected in lesser amounts, consistently with previous studies [4]. Glycerophospholipidome was dominated by dioleoyl PC 18:1/18:1, PE 18:1/18:1, PI 18:1/18:1 and monooleoyl PS 18:0/18:1 species. Glycerophospholipids were enriched with medium chain length moieties: 30% of C16 and 60% of C18 with mainly one unsaturation (74%), two unsaturations (3%) and no unsaturation (23%). Cholesterol is a major component of lipid bilayer membrane. Some studies described cholesterol as a strong interactor of the γ -secretase complex. In our study, cholesterol represented 7% of the total lipid class associated with the γ -secretase (ratio cholesterol/protein= 30). We also observed a strong affinity between cholesterol and the complex after a second size exclusion step of the fraction enriched in affinity-purified enzyme.

In this study, we described the direct lipid environment of the gamma-secretase complex by a top-down shotgun lipidomics approach. We identified the main lipid classes which could stabilize the structure and have a direct influence on the function of the membrane enzyme.

Neuer Aspekte

Endogenous lipids associated with the active γ -secretase complex quantified by top-down shotgun lipidomics.

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LC-MS² based Lipid Mediator Profiling reveals insights into signaling cascades during *Mycobacterium tuberculosis* infection

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Stichworte: lipid mediators, pathogen-induced inflammation, plasma, lung homogenate, LC-MS²

Einleitung

Lipid mediators are bioactive derivatives of ω -3 and ω -6 polyunsaturated fatty acids (PUFAs). They play crucial roles in the signalling network during microbial infections and mark the inflammation status of the host [1]. Lipid mediators such as prostaglandins and leukotrienes act in a pro-inflammatory manner, whereas resolvins and maresins have anti-inflammatory and pro-resolving properties, respectively [2]. Both mediator types exert their regulatory effects by binding to G protein-coupled receptors that mediate neutrophil influx and macrophage stimulation [3]. Only the quantitation of these constitutional isomers using LC-MS² approaches [4] enables a comprehensive insight into the regulation of immune cell activities.

Experimenteller Teil

Wild type (WT) mouse strain (C57BL/6) was used to perform a time course experiment applying aerosol infection with *Mycobacterium tuberculosis* (M.tb.). During 100 days of infection WT plasma (150 μ l) and lung homogenate (1.0 ml) were analysed for lipid mediators. After Bligh&Dyer extraction [5] total lipids were loaded solid phase extraction (SPE)-free on a PhenomenexTM Luna C18(2) column for LC separation (Agilent 1100). Acidified mobile phase (flow rate: 10 μ l/min) comprised water, acetonitrile, ammonium acetate, methanol, methyl tert-butyl ether, isopropanol. Full MS² spectra were acquired in the negative ion mode using a Q-TOF UltimaTM equipped with an electrospray ion source.

Ergebnisse

LC-MS² analysis enabled the assignment of 28 lipid mediators of our pool of standards. In this manner, the developed LC gradient allowed SPE-free loading of total lipid extracts on the column. Remaining phospholipids on column could be removed largely during a chromatographic run. The 5 deuterium-labeled internal standards for quantitation, PGE2-d9, RvD2-d5, LTB4-d4, 5-HETE-d8 and AA-d11, co-eluted with their respective endogenous species. Selected reaction monitoring of the 23 reference compounds showed a linear response in a range of 50-2500 fmol on column ($r^2 > 0.99$). The limit of detections was determined to be 50 fmol for PGE2-d9, 62.5 fmol for RvD2-d5 and 156 fmol for 5-HETE-d8 on column when 250 μ l growth medium (DMEM, containing 10% FCS) was processed as matrix. Extraction efficiencies were 104 ± 15 % (PGE2-d9), 105 ± 13 % (RvD2-d5) and 110 ± 14 % (5-HETE-d8). We quantified 28 molecular species in plasma and 36 in lung homogenate during M.tb. infection. M.tb. seemed to perturbate the host's oxidized PUFA metabolism. When M.tb. infection were studied in a time course extensive changes in the lipid mediator profiles were observed. The M.tb. infection changed the pool of di-hydroxylated PUFAs and prostaglandins. Several metabolites are present only upon M.tb. infection. 9 lipid mediators were changed significantly (5: increased; 4: decreased) in plasma compared to the uninfected WT control, among them the pro-inflammatory PGE2, the bioactive 12-HETE and the pathway marker 14-HDoHE. The pro-inflammatory LTB4 was detected only at day 30 after M.tb. infection. Our pilot experiments might help to understand the specificity of lipid signalling during M.tb. infection. Therefore, in our future work we will apply the LC-MS² platform for a systematic analysis of infection-induced inflammation specific to selected pathogens.

Neuer Aspekte

A SPE-free LC-MS² method to read out on lipid mediator level the inflammation status of the host upon infection.

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Eliminating systematic errors in the LC-MS/MS analysis of vitamin D in human serum

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Stichworte: Vitamin D, LC-MS/MS, Ion mobility, Systematic errors, Accuracy

Einleitung

LC-MS/MS determination of concentration levels of 25-hydroxyvitamin D in human serum is difficult for a number of reasons, including the low ionization efficiency and problems with selectivity. The latter problems are only partially addressed through stable isotope internal and reference standards. Many issues of method selectivity remain unresolved, because isobaric ions have the potential to interfere in, even in MS/MS mode. This study was concerned with systematic errors and accuracy of LC-MS/MS assays for vitamin D in human serum and suggests an alternative solution for avoiding/minimizing these interferences. For this, we compared different sample preparation techniques and utilized a combination of low-resolution triple quadrupole, ultra-high resolution Fourier transform ion cyclotron MS and differential ion mobility spectrometry.

Experimenteller Teil

After initial protein precipitation, human serum samples were extracted using liquid/liquid, solid-phase or plate micro-extraction. Separation of vitamin D metabolites was achieved using a PFP column and compounds ionized by electrospray ionization. Low resolution tandem MS was conducted on a quadrupole-quadrupole linear ion trap instrument (QqLIT) in multiple reaction monitoring (MRM) mode; ultra-high resolution characterization experiments on a 7 Tesla quadrupole-Fourier-transform ion cyclotron resonance (qFTICR) MS. Overlap of peaks was simulated using MatLab; the Gaussian function was used to reconstruct the peak shape and peak areas. Differential ion mobility (DMS) separations were performed between ESI source and entrance quadrupole q_0 of the QqLIT instrument. No chemical modifier was added to enhance DMS separations, to improve ruggedness of the method.

Ergebnisse

In this study, we have systematically investigated the range of isobaric compounds present in human serum for the main vitamin D metabolite, 25-hydroxyvitamin D ($[M+H]^+$, m/z 401), which is commonly measured to determine vitamin D status of individuals. We were specifically interested in those compounds that have the potential for causing interferences in MS/MS as a result of common product ions. During the experiments, we varied the sample preparation technique for extraction of 25-hydroxyvitamin D from serum, to compare their impact on the range of co-extracted isobaric interferences during electrospray ionization. We have shown by detailed high resolution FTICR mass spectrometry experiments that multiple isobaric compounds were present in the sample extracts from human serum, several of which influenced peak areas measured for 25-hydroxyvitamin D using the LC-MS/MS assay in multiple reaction monitoring (MRM) mode. At least one of these isobars was an exogenous compound, the structure of which was fully elucidated, as will be shown in the presentation. We believe that the interference was probably introduced by our analytical methodology; other interferences were endogenous molecules from the serum matrix. We have also evaluated differential ion mobility spectrometry as a filtering step, to successfully remove or reduce the level of the isobaric interferences. Our experiments demonstrated that under the specific experimental conditions chosen in our assay, up to 20% of the vitamin D signal originated from interfering components in the sample extracts, which were indistinguishable from vitamin D using the regular assay, even under MS/MS conditions. We therefore believe that differential ion mobility has the potential to significantly decrease systematic errors, and thus improve the accuracy of vitamin D assays.

Neuer Aspekte

Detailed experimental investigation of the 'isobaric space' of interferences of vitamin D from endogenous and exogenous compounds in human serum

The new Agilent IMS QTOF, how it works and where it does add advantages

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Stichworte: Ion Mobility, Native MS, Metabolomics, CCS

Einleitung

Ion mobility spectrometry (IMS) is a gas phase ion separation technique that can be coupled to mass spectrometry. The separation is carried out in a drift tube filled with an inert buffer gas and the ions are transmitted across the drift tube using an electric field gradient. Larger ions traverse the drift tube at a slower pace than the smaller ions due to their larger collision cross section, thus providing the basis for ion separation.

The drift time information obtained using this technique allows the user to directly calculate the collision cross section (CCS) for ions of interest.

The CCS information is related to the structure of ions and it can be used in biological, structural, kinetic and thermodynamic studies of molecules.

Experimenteller Teil

In this presentation we will present a new uniform-field ion mobility-quadrupole time-of-flight mass spectrometer (IMS-QTOF-MS).

The uniform low field drift cell is independent in its resolution from RF voltages and therefore effects from ion heating can be separately controlled and is overall very low. The design of the ion optics with the drift cell after the entrance funnels and before the quadrupole enables us to have constant drop in pressure downstream the ion optics, thus guaranteeing the structural integrity of all kinds of analytes.

Ergebnisse

The application examples for Metabolites, Carbohydrates, Peptides and native Protein (complexes) will illustrate, that the system adds great advantages to dedicated applications. On top of the superior sensitivity of the systems, it does provide drift separation and CCS information which can be used for identification or confirmation of compounds without any intensive drift tube calibration.

We will demonstrate the sensitivity and separation of isobaric compounds with an urine sample. Proving the concept of the system, we can show that more compounds can be found with the IMS system, than with a non IMS system of same performance class. We can also demonstrate that a non IMS system can lead to wrong relative quantification information.

Carbohydrates do show a great complexity by linkage. Ion mobility is very often the only possible TOF coupled technology to separate these types of molecules which we will show on one example.

We have also evaluated the Agilent low field drift tube ion mobility device for IM native MS applications, using Nano electrospray ionization.

CCS values for Concanavalin A and Cytochrome C are shown. The data will illustrate that for Con A with a charge state of 20, several conformers with CCS values lower than the literature values can be found.

Neuer Aspekte

Particularly easy and accurate determination of the CCS of compounds.

High IMS resolution for separation of isobaric compounds.

Weiterführende Untersuchungen zum Vergleich von APLI und APCI bei der Anwendung in der Ionenmobilitätsspektrometrie

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Stichworte: APCI, APLI, Atmosphärendruck IMS

Einleitung

Nach ersten erfolgreichen Vergleichsmessungen von APCI und APLI Quellen an einem selbstgebauten IMS bei Atmosphärendruck, sollen weiterführende Vergleichsmessungen an einem kommerziell verfügbaren IMS der Firma Dräger durchgeführt werden.

Durch die Verwendung eines kommerziellen Aufbaus soll eine größere Vergleichbarkeit der erhaltenen Driftzeitspektren mit der Literatur erhalten werden. Es wird zum jetzigen Zeitpunkt davon ausgegangen, dass eine starke Signalverbreiterung bezogen auf die Dauer der Ionisation erhalten wird.

Experimenteller Teil

Ein kommerziell erworbenes IMS der Firma Dräger (Dräger Safety, Dräger IMS 5000) wurde für den Betrieb unter APCI Bedingungen und einer abwechselnden Messung unter APLI Bedingungen umgebaut.

Zur Ionisation unter APCI Bedingungen steht eine ^3H -Quelle zur Verfügung. Für APLI Untersuchungen wird ein Nd:YAG Laser (Surelite I, Continuum, 4. Harmonische, 266 nm) verwendet.

Ergebnisse

Durch die Änderung der Ionisationsmethode bei ansonsten nahezu vergleichbaren Bedingungen können mehrere Effekte in den erhaltenen Driftzeitspektren beobachtet werden.

Neben der möglichen Ionisation von kleinen aromatischen Verbindungen mit einer Polarität kleiner als Wasser, werden bei den APLI Untersuchungen für alle hier untersuchten Substanzen bereits bei geringen Laserleistungen höhere Ionenausbeuten im Vergleich zu APCI erreicht.

Auffällig bleibt jedoch eine annähernd vergleichbare Halbwertsbreite der Signale. Durch sehr kurze Ionisationsdauern durch einen Laserpuls (~ 10 ns) im Vergleich zum Extraktionspuls bei APCI (~ 110 μs) wäre eine entsprechende Verringerung der Halbwertsbreite zu erwarten. Mehrere der folgenden Effekte sind vorstellbar.

Es werden Fragmente mit unterschiedlichen Driftzeiten auf Grund eines Leiter-Wechsel-Mechanismus und durch Ionen-Molekülreaktionen mit dem Probegas und Driftgas erhalten. Auch Cluster-Ionen unterschiedlicher Zusammensetzung wären so denkbar.

Als Folge von zeitlicher, energetischer und räumlicher Verteilung der Moleküle vor dem Repeller werden zusätzlich langsamere Ionen erzeugt.

Der Energieübertrag der Photonen auf die Moleküle ist so groß, dass eine explosionsartige Verteilung der Ionen in alle Raumrichtungen mit großer kinetischer Energie stattfindet.

Neuer Aspekte

Qualitative und quantitative Vergleichbarkeit von Ionenausbeuten und Driftzeitspektren erhalten durch APCI und APLI mit 266 nm.

Gas-Phase Microsolvation of Ubiquitin: Identification of Crown Ether Binding Sites

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Stichworte: ion mobility-mass spectrometry, microsolvation, ubiquitin, crown ether, gas-phase structure

Einleitung

There is ongoing debate about the extent to which protein structure is retained after electrospray ionization and transfer into the gas phase. It is widely accepted that the repulsion between equal charges is one of the major determinants: low charge states typically adopt compact, native-like conformations, while high charge states tend to unfold. Intermediate charge states can show a multitude of coexisting conformations.[1] Recently, we showed that not only Coulomb repulsions but also the much more subtle coordination of charged side chains onto the protein backbone can severely influence the conformation of gas-phase proteins.[2] Here, we are investigating these effects further by comparing the impact of solvent-exposed charged residues with those involved in intramolecular salt bridges.

Experimenteller Teil

In order to study the influence of side chain-backbone coordination on the structure of a gas-phase protein, we non-covalently attached different amounts of crown ether (CE, 18-crown-6) to the charged lysine side chains of a series of ubiquitin mutants (Sigma Aldrich). The resulting protein-CE complexes were analyzed *via* tandem MS and ion mobility-mass spectrometry (IM-MS) using a commercially available traveling wave IM-MS instrument (Waters, Synapt G2-S).

Ergebnisse

18-crown-6 is known to strongly bind to protonated lysine and, with considerably lower affinity, arginine side chains in gas-phase proteins. In order to study the impact of such CE microsolvation on the protein structure, we here investigated ubiquitin mutants, in which either all lysine residues (*noK*), or all but one specific lysine (*K6*, *K11*, *K27*) are replaced by arginine. Independent of the charge state, complexes with up to five CEs were observed for *wt* ubiquitin as well as all mutants at gentle conditions. CID experiments revealed, however, that there are significant differences in the relative dissociation energy between lysine and arginine-bound CE.

IM-MS experiments showed that ubiquitin ions of high (unfolded) and low charge states (compact) essentially retain their shape upon CE complexation. At intermediate charge states on the other hand, *wt* ubiquitin undergoes an unusually distinct compaction with increasing number of attached CEs. This is in agreement with previous observations [2] and can be explained by the fact that the CE solvates the protonated lysine side chains and prevents them from a structure-breaking coordination to the protein backbone. In contrast to *wt* ubiquitin, no significant structural compaction upon CE binding was observed for the *noK* mutant, which likely results from the weak binding of CE to arginine. A similar trend was also observed for the *K11* and *K27* mutants, in which the only remaining lysine is involved in a strong intramolecular salt bridge. Surprisingly, however, a clear compaction was found for *K6* CE complexes, in which the lysine side chain is solvent-exposed and easily accessible for complexation.

Taken together, our results indicate that the unfolding induced by coordination of charged side chains to the protein backbone strongly depends on the involved residues. Solvent-exposed lysines exhibit a much stronger impact on the protein structure than those buried in salt bridges.

Neuer Aspekte

Identifying crown ether binding sites with ion mobility-mass spectrometry using different ubiquitin mutants.

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Analysis of isomeric lipids by high resolution ion mobility-mass spectrometry

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Stichworte: Isomer separation, ion mobility, lipids, steroids, sterols

Einleitung

Ion mobility spectrometry coupled to mass spectrometry (IMS-MS) is becoming increasingly popular, but most commercial systems have insufficient ion mobility resolution to separate isomeric compounds. We report on a bench-top IMS-MS that delivers resolution beyond 200, under true low field conditions, with 50% duty cycle. The benefits of this system coupled to TOF-MS are demonstrated for the analysis of isomeric biomolecules.

Experimenteller Teil

All measurements were carried out on a ToFwerk IMS-TOF. The system comprises an ESI source, a 10 cm desolvation tube, a 20 cm drift tube (both made from resistive glass) and a ToFwerk HTOF TOF-MS. Measurements were carried out in both positive and negative ion modes (applied ESI potential approximately 2kV) with the desolvation and drift tube kept at pressures between 1 and 1.4 bar and 150°C with nitrogen as the buffer gas. The reduced field strengths were near 2 Td.

Ergebnisse

IMS separates molecules based on their collision cross sections. Since this depends on the geometry of molecules, IMS is an attractive method for isomer separation. In most cases resolving power above 100 is required to distinguish isomers, a range which lies beyond most instruments. Here we show separation of isomeric biomolecules in a gas-tight multiplexed IMS drift cell at pressures of up to 1.4 bar. Combined with multiplexing postprocessing techniques, ion mobility resolving power above 200 was obtained. Multiplexing also increases ion transmission over 200 times and S/N ratios 10 times compared to conventional pulsed mode.

For phospholipids and triacylglycerols, we show the separation of regioisomers (different positions of acyl chains on the glycerol skeleton), double bond positional isomers and stereoisomers (double bond geometry). We also show that, based on accurate reduced mobility measurements, identification of lipid species is feasible even in complex samples such as total lipid extracts.

Additionally, high resolution IMS-MS was investigated for the analysis of closely related isomeric steroids and their metabolites which are commonly included in clinical urine steroid profiles. We demonstrate that the technique is able to separate isomers of hydroxyprogesterones or cortisone metabolites. Finally, we also present data that highlights separation of isomeric sterols (cholesterol derivatives).

Neuer Aspekte

Analysis of isomeric lipids, steroids and sterols by ion mobility-mass spectrometry.

Altersbestimmung von Kugelschreibereinträgen durch Lösungsmittel, Harze und Binder.

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Stichworte: Altersbestimmung, Kugelschreiber, Polymeranalytik

Einleitung

In Kugelschreibereintragungen finden mit der Zeit chemische Veränderungen der verschiedenen Komponenten statt.[1] Diese chemischen Veränderungen können zur Altersbestimmung der Eintragung und somit des Vertrags oder Dokuments verwendet werden. Zu den Komponenten in den Kugelschreiberpasten gehören Lösungsmittel, Farbstoffe, Harze, Binder und weitere Additive. In den letzten Jahren wurden verschiedene analytische Methoden zur Altersbestimmung von Kugelschreibereintragungen entwickelt, die auf den Lösungsmitteln in den Pasten basieren. Das KTI entwickelte hierzu eine HPLC-UV Methode, mit der eine Alterungsbestimmung über das Lösungsmittel Phenoxyethanol durchgeführt wird.[2] Diese Methode wird bereits in der Fallarbeit eingesetzt und ermöglicht die Altersbestimmung einer Kugelschreibereintragung bis zu einem Zeitraum von 6 Monaten.

Experimenteller Teil

Zur Erweiterung des Datierungszeitraums wird auch das Alterungsverhalten von Harzen, Bindern und Additiven in Kugelschreibereintragungen untersucht. In den letzten Monaten wurde im Zuge des Monopoly Projektes 2011 eine massenspektrometrische Methode entwickelt, mit der Harze, Bindemittel und Additive sensitiv detektiert werden. Dazu gehören Probenaufbereitung, HPLC und hochauflösende Massenspektrometrie. Verschiedene Ionisationsmethoden, wie die Electrospray Ionization (ESI), die Atmospheric Pressure Chemical Ionization (APCI) und die Direct Analysis in Real Time (DART) wurden verwendet. Für einen großen Anteil der Analyte ist die positive ESI die geeignetste Ionisierungsmethode.

Ergebnisse

Verschiedene chemische Komponenten von Kugelschreibertinten wurden massenspektrometrisch analysiert, charakterisiert und die Massenspektren und Fragmentspektren werden in einer Datenbank zusammengefasst. Eine qualitative Unterscheidung zwischen verschiedenen Pasten und Eintragungen ist bereits möglich. Analysen von gealterten Proben zeigen, dass Harze, Binder und Additive sehr langsam altern. Sowohl die Ausgangsverbindungen wie auch die Abbauprodukte sind über einen langen Zeitraum von mehreren Jahren bis Jahrzehnten in Kugelschreibereintragungen detektierbar.

Neuer Aspekte

Analytik von Harzen, Bindemitteln und Additiven in Schreibmitteln und Altersbestimmung von Kugelschreibereinträgen über mehrere Jahre.

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Structural investigation of co-assembled clathrin adaptor protein complexes

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Stichworte: Native Mass Spectrometry, Endocytosis, Protein complexes, Lipid-binding

Einleitung

Clathrin-mediated endocytosis is the major trafficking route from the plasma membrane to the cytoplasm and thus indispensable for many cellular processes. The remodeling of the membrane during endocytosis requires a mechanical force to be transmitted to the membrane. In this context, clathrin adaptors were found to have two important functions. They link clathrin to the plasma membrane and also couple the membrane to the polymerizing actin cytoskeleton.

Experimenteller Teil

Lipid binding of single membrane-binding domains of clathrin adaptors from yeast and fungi was analyzed. Furthermore, complex stoichiometry and clues on the complex topology were obtained.

Ergebnisse

Here, we show that the membrane-binding domains of two clathrin adaptors co-assemble in a lipid-dependent manner and obtain structural information of the protein complex by native mass spectrometry.

Neuer Aspekte

Structural information on co-assembled clathrin adaptor protein complexes

At the edge of lipidOMICS and proteOMICS: mobility mass spectrometry of lipid-protein adducts

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Stichworte: Lipidomics, lipid peroxidation, lipid-protein adducts, LC-MS, ion mobility, proteomics

Einleitung

Many human diseases are accompanied by chronic inflammation and closely connected to oxidative stress (OS). OS can oxidize virtually all biomolecules including lipid peroxidation generating multiple electrophilic products capable to modify nucleophilic sites in proteins. The heterogeneous nature of lipid peroxidation products (LPP) and the large variety of LPP-protein adducts demands sophisticated strategies to identify such protein modifications by mass spectrometry, which starts with a detailed profiling of reactive LPP produced under OS. The combination of the lipidomics data provide the basis for a proteome-wide analysis of LPP-peptide adducts. Here we present lipidomics of OS-derived LPP combined with proteomics of modified proteins for high-throughput identification of lipid-protein adducts both in vitro and in vivo.

Experimenteller Teil

The analysis of the LPP relied on their specific derivatisation followed by LC-MS^{1,2} to construct a database (LPPdb) of reactive LPP³, which was used for search for LPP-adducts in synthetic model peptides separated by RPC or HILIC and analyzed using high resolution ESI-Orbitrap- and ESI-IMS-MS (Synapt G2 Si). The CID spectra revealed certain rules to distinguish different types of LPP-peptide adducts. Additionally, IMS-MS allowed the separation and characterization of conformational isomers formed between peptides and phospholipid-derived LPP. This information allowed finally the in-depth profiling of complex LPP-protein adduct mixtures in cell models of oxidative stress by combining LC-MS relying on extended exclusion lists with different fragmentation techniques (CID and ETD).

Ergebnisse

The newly developed LC-MS protocol relying on the specific derivatisation of carbonylated LPP identified more than 150 low- and high-molecular weight reactive lipids generated by in vitro oxidation of phospholipid (PL) mixtures and complex cellular extracts. This information was used to establish an open database (termed LPPdb³) consisting of MS-centered information on oxidized lipids. Reactive LPP entries allowed the characterization of LPP-peptide adducts using different LC, MS, and IM-MS techniques. The acquired CID tandem mass spectra allowed distinguishing Schiff bases, Michael adducts, and dehydrated cyclic hemiacetals formed by LPP on Lys-, Cys-, and His-residues. IMS-MS allowed the separation of different classes of compounds (peptide vs lipid vs peptide-lipid) and various structural conformers within a single peptide-lipid adduct, driven most probably by the orientation of the hydrophobic lipid along the peptide backbone.

The information obtained on the in vitro systems was the applied to a cardiomyocyte model of OS. Lipid extracts were derivatized (reactive LPP), identified, and relatively quantified by nanoRPC-ESI-MS. The 25 dominating LPP species (e.g. alkanals, alkenals, alkadienals, alkatrienals, and oxo-carboxylic acids) were dynamically generated and degraded with different kinetics. Several PL-bound aldehydes were identified for the first time after OS. Furthermore, the protein extracts were digested, analyzed by LC-MS, and the library of reactive LPP was used for identification of LPP-protein adducts. All protein modifications significantly increased during OS. The combination of lipidomics and proteomics allowed the identification of more than 200 proteins modified by LPP species of which many are involved in calcium signaling pathways, regulation of actin cytoskeleton, focal adhesion, and phosphatidylinositol signaling system. Biochemical and microscopy studies confirmed LPP-derived impairment of Ca-signaling and cytoskeletal protein distribution.

Neuer Aspekte

The combined lipidomics/proteomics-strategy provides powerful approach to analyze lipid-modified proteins in OS-related diseases and cellular physiology.

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LUX Score: Cheminformatics Approach to Compute Lipidome Homology

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Stichworte: LUX Score, Cheminformatics, Lipidome

Einleitung

Lipids play major role in human health and disease. To understand their function, model organisms like *D. melanogaster*, *S. cerevisiae*, *C. elegans* etc. are commonly employed. However, it is known that lipid structures in these model organisms are different from humans. To extrapolate the functional implications of model organism's lipidome to humans, a criterion is necessary. We hypothesized that lipid structures and their structural relation to each constituent of a given lipidome might be the best link to function. Lipidome Juxtaposition Score (LUX Score) describes the-overall-structural-differences between lipids of any given pair of cells/tissues or organisms. We develop the LUX Score with the aim to provide a tool that facilitates systems biology research.

Experimenteller Teil

The workflow to determine LUX Score starts by combining all lipids from a set of lipidomes into a unique list. It is followed by conversion of lipid structures to SMILES. Next, we determine similarity between all pairs of SMILES from the unique list. Thereafter, we applied Principal Component Analysis (PCA) to convert pairwise structural similarity values of SMILES into three dimensional chemical space coordinates. Next, we determine the extent of overlap between a pair of lipidomes by comparing the spatial distribution their respective lipids in the chemical space. As a proof of principle we tested the accuracy of LUX Score(s) by comparing lipidomes of yeast strains that were grown at 24°C and 37°C [1].

Ergebnisse

SMILES (Simplified Molecular Input Line Entry Specification) is a linear representation of three-dimensional structure of a molecule, routinely used for storing and retrieving chemical structures from databases [2]. We tested common algorithms for retrieving structures from chemical libraries. Canonical SMILES were initially developed for this purpose [3]. Canonical SMILES provide one unique representation of a given chemical structure even though, theoretically, one could write many SMILES for one structure. However, we found out that isomerism of lipids could not be represented with canonical SMILES. As result we developed template-based SMILES based on LIPIDMAPS Structure Drawing Tools [4] that keep relationships between lipid classes based on a chemical nomenclature. This approach also resulted in one unique SMILES but enabled us to utilize sequence analysis algorithms such as Bioisosteric similarity, Smith-Waterman, SMILIGN and Levenshtein distance. Among the six methods tested, Levenshtein distance provided most consistent similarity scores. We show that PCA produces unique coordinates for all 30150 lipids of the LIPIDMAPS structure database and cluster lipids according to the defined nomenclature. Plotting lipids in orthogonal principal component planes provided an intuitive visualization system for navigating within the yeast lipidome comprising 248 unique lipids. We measure the extent of overlap between a pair of lipidomes by calculating the average distance between their unique lipids in the PCA chemical space. Thus, LUX Score is a numerical measure of the combined structural differences between a pair of lipidomes. LUX Score based clustering of yeast lipidomes brought the wild-type BY4741 and mutant-Elo1 strains together, indicating that the metabolic alteration caused by the Elongase mutation was smaller than the temperature effect. For the mutant strains Elo2 and Elo3 we noticed stronger influence of the Elongase mutation, separating them from the wild-type BY4741 and mutant-Elo1 strains.

Neuer Aspekte

For the first time, a homology measure is described for lipidomes.

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Investigation of UV transformation products of xenobiotics in the aquatic environment by means of liquid chromatography and mass spectrometry

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Stichworte: Emerging Contaminants, UV-degradation, HPLC-MS, MS(n)

Einleitung

The wide use of medicines, pesticides, and personal care products leads to increasing concentrations of xenobiotics in the aquatic environment [1,2]. Since conventional waste water treatment plants degrade these contaminants insufficiently, additional processes (advanced oxidation processes, AOP) are investigated and already tested in pilot projects [3]. Several AOPs have been investigated, including ozonation, UV-radiation, Fenton's reaction, and combinations thereof. They all aim at the production of hydroxyl radicals, which undergo further reactions with the contaminants. In literature, many impressive degradation efficiencies for a wide range of common contaminants have been presented [3-5], but often only the decreasing concentration of the investigated xenobiotic is presented, without any information regarding reaction products.

Experimenteller Teil

Two common xenobiotics occurring in the aquatic environment are Diclofenac and Sulfamethoxazole, which are known to be easily degraded with AOPs. In this project, they were degraded by means of pure UV radiation (220-500 nm). After different radiation times, the samples were analyzed by liquid chromatography-mass spectrometry (LC-MS). This way, a kinetic profile for both, the degradation process of each xenobiotic and the occurring degradation products, could be recorded. The use of a time of flight mass spectrometer (TOF-MS) allows determination of mass to charge ratios with high accuracy, so that sum formulas can be proposed. Additional fragmentation with a 3D-ion trap provides information about the fragmentation behavior and allows the prediction of tentative chemical structures.

Ergebnisse

Both investigated xenobiotics show a high number of products after UV radiation. For each substance, at least one occurring product is unstable against further UV radiation. Its intensity first increases to a maximum followed by a decrease of this product, which indicates its subsequent degradation. Other products appear after different radiation times and show increasing intensities until they reach a stable maximum intensity. In case of Diclofenac, the isotopic patterns of the products show that the metastable product still carries one chlorine atom, whereas for all other products, no chlorine can be found anymore.

Detection by means TOF-MS reveals sum formulae for the degradation products with low mass deviations. Together with obtained fragmentation spectra, tentative structures of some of the products can be presented.

Some of the detected degradation products have already been described in the literature, but the presented work includes a range of previously undescribed products, which are of particular interest for further experiments regarding their reactivity and structural characterization. Furthermore, a comparison between pure UV radiation and UV/H₂O₂ as another AOP is carried out to see, if the addition of hydrogen peroxide accelerates the degradation process and if it leads to the same products.

Neuer Aspekte

Photochemistry/LC-MS of xenobiotics allows the identification of their UV degradation pathways.

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Electrochemistry coupled to LC/MS for the identification and characterization of reactive xenobiotic metabolites and their protein adducts

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Stichworte: Metabolism, Protein adducts, Electrochemistry

Einleitung

Adduct formation of reactive metabolites with proteins is considered as a process contributing to the toxicity of xenobiotics. Therefore, the identification and characterization of reactive metabolites and their protein adducts is of great importance in toxicological research. Electrochemistry coupled online to liquid chromatography and mass spectrometry (EC/LC/MS) is a purely instrumental method that has proven to be a valuable tool not only for simulating the metabolic transformation reactions of xenobiotics but also for studying the generation of their protein adducts. In this presentation, we introduce the general principle and setup of this method and its application for analyzing the metabolic processes of polycyclic aromatic hydrocarbons (PAHs), a group of ubiquitous environmental contaminants.

Experimenteller Teil

The solution containing the analyte is pumped through a commercially available electrochemical cell, in which the molecules are oxidized on the surface of the working electrode. Generated oxidation products are either detected directly by means of mass spectrometry or after an additional separation step using an integrated HPLC system. For studying adduct formation, the setup is extended by a second syringe pump and a T-piece, which allows the addition of biomolecules into the effluent of the EC cell. Identification and characterization of generated protein adducts is achieved by LC/MS analysis of the intact proteins as well as their peptides obtained by tryptic digestion.

Ergebnisse

Pyrene and benzo[a]pyrene were selected as PAH model compounds and oxidized within an electrochemical thin-layer cell, equipped with a boron-doped diamond (BDD) working electrode. The generated oxidation products were analyzed using high resolution mass spectrometry (HRMS) in order to obtain their exact masses. By applying a potential ramp, mass voltammograms were recorded, which show the obtained mass spectra in dependency on the applied voltage in a three dimensional plot. These plots give a fast overview of the oxidation behavior of the analytes and may provide first information on the formation of reactive species. For both investigated model compounds, quinones were identified as the major oxidation products. Further characterization of these oxidation products was achieved by means of HPLC separation as well as MS/MS analysis.

Since quinones represent a class of metabolites that generally show a high potential to form adducts, the next step was to study the reactivity of the electrochemically generated PAH metabolites towards biomolecules. For this purpose, the tripeptide glutathione as well proteins such as β -lactoglobulin and human serum albumin were added to the effluent of the electrochemical cell and allowed to react with the oxidation products. Generated adducts were detected and identified by means of (LC)/HRMS. In case of protein adducts, additional experiments were performed with the aim to obtain further information about the nature and the site of the protein modification. Therefore, the proteins were digested with trypsin and the resulting peptides were analyzed by means of LC/HRMS. Using this approach, the binding of PAH metabolites to free thiol groups in proteins was demonstrated.

Neuer Aspekte

Reactive PAH metabolites and their protein adducts are generated and characterized by means of EC/LC/MS.

Differential Protein Labeling with Electrochemically Generated Reactive Intermediates

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Stichworte: Protein labeling, Mass spectrometry, Electrochemistry, Reactive intermediates, Hyphenated techniques

Einleitung

In the field of proteomics, the identification as well as the quantification of proteins in complex mixtures is required. The identification of proteins can be performed via the identification of a specific enzymatic peptide. The determination of the number of a certain amino acid in a protein provides additional information, which allows a simplified identification. Therefore, within this study, cysteine residues in proteins were labeled with electrochemically generated reactive intermediates. The formation of reactive quinoid intermediates via dehydrogenation leads to a selective Michael-type addition of these electrophilic structures to the nucleophilic thiol group of cysteine residues. Electrochemical generation of reactive intermediates of compounds in their native and isotope labeled form provides a defined mass difference of the differentially labeled biomolecules.

Experimenteller Teil

The hyphenation of an electrochemical cell, equipped with a boron-doped diamond working electrode, to electrospray ionization MS (ESI-MS) allows the immediate detection of generated peptide adducts. Applying a potential ramp from 0 - 2500 mV and presenting the data in form of three dimensional mass voltammograms enables a broad overview of the generated products.

An extension of the instrumental set-up by means of liquid chromatography (LC) allows 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 can be performed online prior to MS detection.

Ergebnisse

Electrochemical oxidation of the two phenolic species phenol and $^{13}\text{C}_6$ -phenol or acetaminophen and D_4 -acetaminophen, respectively, resulted in the generation of reactive intermediates. The obtained quinoid structures benzoquinone or *N*-acetyl-*p*-benzoquinone imine (NAPQI) were subsequently allowed to react with the tripeptide glutathione (GSH). The formed adducts were identified by means of ESI-MS. With the help of mass voltammograms, the optimum oxidation potential for the generation of reactive intermediates was determined. Furthermore, it could be shown that the native and the isotopic enriched form of the reactive intermediates formed GSH adducts to the same extent. Thus, a defined mass difference for the differentially labeled peptide could be detected in both cases.

By adding a protein to the effluent of the EC cell, the online modification of proteins was obtained. In order to separate and identify the protein adducts, EC was coupled to LC/ESI-MS. The protein carbonic anhydrase I (CAI), which is highly abundant in red blood cells, was incubated with the reactive intermediates. For phenol and $^{13}\text{C}_6$ -phenol, quantitative conversion of the free thiol groups was achieved. Moreover, only one adduct was formed, which could be traced back to adduct formation of CAI with benzoquinone. In contrast, labeling after oxidation of acetaminophen and D_4 -acetaminophen was not quantitative and next to an adduct with NAPQI, a further adduct with a dehydrogenated dimer of acetaminophen was detected. Thus, it could be shown that the system phenol/ $^{13}\text{C}_6$ -phenol is better suited for protein labeling than acetaminophen/ D_4 -acetaminophen.

After enzymatic digestion, a mass difference of differentially labeled, cysteine containing peptide was observed. This can be traced back to the heavier, labeled species of phenol. Cysteine was identified to be the only binding site in the protein. Based on the obtained mass difference, a simplified identification of cysteine containing peptides in complex mixtures is possible.

Neuer Aspekte

Differential labeling and tryptic digestion of proteins allows the detection of a defined mass difference for cysteine containing peptides.

Study on highly aromatic complex mixtures such as crude oil asphaltenes with online and offline SEC-MS

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Stichworte: Asphaltenes, hyphenated techniques, size exclusion chromatography, high resolution mass spectrometry

Einleitung

Asphaltenes are the heavy components present in crude oil and exemplify a very diverse fraction. They have a wider range of polarity than other fractions, can be highly aromatic and show different solubilities. Furthermore, asphaltene compounds fairly differ in their molecular sizes.¹

Chromatographic separation is an excellent tool to simplify extreme complex mixtures. The sample simplification and reduced matrix effect make possible to get deeper information about samples containing large number of different compounds using mass spectrometry (MS) for detection.²

Size exclusion chromatography (SEC) is the method where the separation is taking place according to hydrodynamic ratio. Therefore, the coupling of SEC and MS offers a good opportunity to effectively analyze asphaltenes on a molecular level.³

Experimenteller Teil

Size exclusion chromatography was performed using an Agilent 1100 HPLC system. As optimized separation conditions, a combination of two PSS SDV (styrene-divinyl benzene copolymer) analytical columns (300 x 8.0 mm ID, 5 µm) were used with pore sizes of 1000 Å and 100 Å, respectively as stationary phase and tetrahydrofuran as mobile phase.

Mass spectrometric analysis was performed on a research-type LTQ-Orbitrap Elite MS equipped with a combination of APPI and APCI source. In addition to MS, UV detector at 300 nm was used, as well.

Ergebnisse

For the investigation of highly aromatic complex structures such as asphaltenes, a coupling system of SEC and MS was developed. Compounds present only in small amounts can be detected more sensitively due to the separation. SEC presents the method of choice for separation of asphaltenes because solvents can be used that dissolve the compounds and here the results of online and offline coupling are compared.

The separation results only in one but fairly broad chromatographic peak with a time width of 6 min where all components were separated according to their size. The detection of this separation can be done both online - where each second two scans could be recorded - and offline where fractions within a time range of 1 min was collected for detailed analysis. The results show that there still is a remarkable similarity between the online and offline coupling results. The main advantage of offline coupling is that higher mass resolution can be applied, so even deeper information can be obtained, although some separation might be lost due to the fraction collection.

The mass range of asphaltene molecules detected by MS is 200-800 Da. The results from the individual fractions cover this whole mass range. Compounds having larger masses are eluting first and the smaller ones are detected later due to the different exclusion from the pores of the packing material. The properties of the compounds from the different fractions during separation are discussed in this presentation.

Neuer Aspekte

SEC coupling with high resolution MS (online and offline) is applied to analyze highly aromatic complex mixtures such as asphaltenes.

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Characterization of a versatile low temperature plasma torch by optical emission spectroscopy and time-of-flight mass spectrometry

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Stichworte: Ambient ionization, Low temperature plasma, Dielectric Barrier Discharge (DBD), Emission spectroscopy, ToF-MS

Einleitung

In recent years, several designs of stable atmospheric pressure plasma sources have been developed and characterized. [1,2] One of the most prevalent devices is the dielectric barrier discharge (DBD), where high voltages in the kHz frequency range are used to ignite a bright plasma between two separated electrodes. The DBD is inexpensive and robust running at low plasma temperatures, resulting in little thermal stress for the sample. The DBD can additionally be used for ambient, solvent-free analysis of untreated samples. Regarding these advantages the DBD is a suitable candidate as a powerful ionization source in mass spectrometry. Our goal is a further understanding of the participating mechanisms and reactions in the plasma by using mass spectrometry and emission spectroscopy.

Experimenteller Teil

The DBD source consists of a simple toolbox, resulting in the possibility of a rapid change of different working modes. The individual parts are all commercially available mass products and entirely disposable. It involves a borosilicate glass tube, different types of copper mesh as external powered electrode, a stainless steel acupuncture needle as inner grounded electrode and a Swagelok Tee-piece. High voltage is provided by an adjustable input power source and a low cost flyback high voltage transformer. Helium serves as discharge gas at an adjustable flow, controlled by a mass flow meter. All experiments were conducted using an orthogonal time-of-flight mass spectrometer with an atmospheric pressure inlet capillary. Spectral imaging was conducted on an in-house 3D-optical emission spectroscopy tomograph.

Ergebnisse

The ultimate goal is the use of a plasma source as an effective post-ionization source for mass spectrometry, avoiding at the same time fragmentation and competitive chemistry. For DBDs, several competitive modes of operation resulting in different ionization pathways have been discussed. Our used toolbox allows for a wide variety of simple and easy to change set-ups of the DBD source. In all set-ups, formation of reactive species was found to be highly effective, but further understanding of the participating mechanisms and reactions in the plasma is desired for maximum ionization efficiency with a minimum of subsequent fragmentation.

Blank spectra of ambient air show intensive formation of water cluster series, up to higher masses of 1 kDa, as well as smaller contributions of reactive oxygen and nitrogen species. The DBD source was additionally used to desorb and ionize different analyte compounds, *e.g.* drugs, polymers and organometals, deposited between the exit of the glass tube and the mass spectrometer. Depending on the used setup either the plasma itself or the plasma-activated gas stream exits the glass tube and interacts with the sample directly where it desorbs and ionizes surface molecules in the ambient environment.

To yield further insight into the discharge region, a 3D spectral imaging characterization was performed. Spectra were acquired at ~ 70.000 different positions of the plasma, mapping the electronic temperature, electron density and reactive species number densities. A preliminary analysis of the obtained results indicates that the postulated ionization mechanisms need revision.

The obtained knowledge of the plasma processes allows us to selectively switch between the formation of only radical cations and only protonated ions. Besides the geometrical arrangement of electrodes, the flow rate seems to have the biggest influence on the formation of the particular species.

Neuer Aspekte

Tunable DBD source for the formation of either radical or protonated ions.

Spatial and Spectral imaging of DBD source.

Referenzen

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Mass spectrometry of oligopeptides in the presence of alkali halides using desorption/ionization induced by neutral cluster impact

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Stichworte: Soft desorption/ionization, alkali-peptide complexes

Einleitung

Oligopeptides in natural environment are often exposed to relatively high concentrations of alkali halides, hence the investigation of biomolecules in this particular environment is of high importance. Furthermore, peptides can build non-covalently bonded complexes with metal ions; a promising field of research in this context is the study of metal complexes acting as target-selective proteases catalysing the hydrolytic cleavage of disease-related oligopeptides or proteins [1,2]. In this work we show the applicability of desorption/ionization induced by neutral SO₂ clusters (DINeC) for the study of biomolecules in their natural environment as well as for the understanding of their interaction with metal ions.

Experimenteller Teil

Bare oligopeptides and metal complexes were desorbed and ionized from a SiO₂-substrate by neutral SO₂ clusters with a mean size of 10³ to 10⁴ molecules seeded in a pulsed He beam. The impacting polar clusters both provide the energy for desorption as well as they serve as a transient matrix in which the analyte molecule is dissolved during cluster surface impact. The latter effect lowers the energy barrier for desorption which thus takes place very softly and without fragmentation of the analyte [3,4]. The samples were prepared by drop casting the respective aqueous solution with alkali halide concentrations up to an excess of a factor of 1000 and absolute concentrations up to 1 mol/l.

Ergebnisse

Using DINeC as desorption/ionization method, clear spectra of the intact biomolecule [M+H]⁺ as well as of the intact complex of a biomolecule with an alkali ion X, [M+X]⁺, were detected even in the presence of a large excess of salt in the original solution. The ratio between metal complexes [M+X]⁺ and bare biomolecules [M+H]⁺ can be controlled by using different preparation procedures. The effect was traced back to phase separation of alkali halides and biomolecules during the drying cycle of the preparation; the ratio of metal complexes and bare biomolecules was found to decrease within the first main group. Furthermore, DINeC was efficiently combined with ion trap mass spectrometry also allowing for structural analysis of the non-fragmented metal complexes due to its MSⁿ capabilities. The intact metal complexes [M+X]⁺ and [M+X+H]²⁺ were isolated and fragmented in the ion trap, the fragments were attributed to respective sequences of Angiotensin II. Both, fragments carrying an alkali ion as well as bare fragments could be identified. Based on the distribution of these fragments, a localization of the metal ion in Angiotensin II molecules was possible. According to our results, alkali ions are mainly bound to the carboxyl groups of aspartic acid and phenylalanine located at the terminal groups of the molecule. The localization and binding strength seems to be stronger for Li and Na compared to K.

Neuer Aspekte

clear spectra from biomolecule/salt mixtures, desorption of intact complexes of peptides with alkali ions, localization of alkali ion in peptides

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Fundamentals and Applications of Plasma-based Ambient Desorption Ionization Mass Spectrometry in Bioanalysis and Lithium-Ion Battery Research

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Stichworte: LTP, Orbitrap, ambient desorption/ionization

Einleitung

Ambient desorption/ionization mass spectrometry (ADI-MS) aims to enable direct analysis of gaseous, liquid, and/or solid samples in the ambient environment. In ADI-MS, different types of desorption/ionization sources are classified according to their basic method of operation, namely spray-based, laser-based, and plasma-based sources.

In this presentation, the applicability of plasma-based sources for both direct qualitative and quantitative analysis will be discussed. One benefit of using a desorption/ionization source is the possibility to skip a preceding chromatography run if high mass resolution is available at the detector. This, in turn, significantly reduces the amount of solvents required per analysis and sample throughput improves.

Experimenteller Teil

A home-built low-temperature plasma probe (LTP, based on the original work by Harper *et al.* [1]) was coupled to a high-resolution mass spectrometer (HRMS, model Exactive HCD, Thermo Fisher). After careful optimization of source parameters and sample-to-probe geometry [2], desorption/ionization of dried analyte spots is facilitated using the afterglow of the dielectric-barrier discharge that extends out of the LTP probe. Depending on the compounds of interest, additional heating may be applied to aid thermal desorption from the sample target.

Ergebnisse

Several examples will be discussed, in which LTP-HRMS was found useful for screening applications. In contrast to qualitative analysis, quantitative analysis with plasma-based ADI-MS is still challenging but recent reports indicate that it is very well feasible if methods are developed carefully. Here, a method for rapid and quantitative analysis of pesticides in fruits by QuEChERS pretreatment and LTP-HRMS will be discussed [3]. Lastly, LTP-MS was applied for direct analysis of lithium-ion battery (LIB) compounds. After controlled aging of model electrolytes, degradation products were successfully identified and helped to further understand the reaction mechanisms in LIB [4]. Based on our results, LTP-HRMS is considered a useful and complementary technique for the mass spectrometric toolbox.

Neuer Aspekte

Quantitative analyses with low-temperature plasma ambient desorption/ionization high resolution mass spectrometry (LTP-HRMS)

Referenzen

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LILBID-MS reveals structural insights into a unique hybrid FoVo rotor and its integration into the ATPase

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Stichworte: non-covalent MS, ATPase, C-ring, LILBID

Einleitung

In recent years mass spectrometry (MS) evolved into a powerful method to analyse the stoichiometry and connectivity of multiprotein complexes.

While soluble complexes have long since been targeted successfully by MS, the hydrophobic membrane complexes remained challenging. Especially suited to membrane proteins is the LILBID-MS technique (Laser Induced Liquid bead Ion Desorption-MS).

Experimenteller Teil

It employs an on-demand droplet generator which introduces 50µm droplets of the analyte into the vacuum where they are irradiated by an IR-laser pulse. This leads to the explosive expansion of the droplet setting the solvated ions free. Then they can be analyzed by time of flight MS.

Ergebnisse

LILBID allows the measurement even of membrane protein complexes lacking any soluble parts, such as isolated ATPase c-rings. Here I will present results from the Na⁺ F₁F₀ ATP synthase of *A. woodii*, which has a unique F₀V₀ hybrid rotor, for which three genes (*atpE₁*, *atpE₂*, *atpE₃*) encode different c-subunits (the V-type like *c₁* (four trans membrane helices (TMH)) and the identical F-type *c₂/c₃* (two TMH)). Earlier results had shown, that these always result in a c-ring of a fixed stoichiometry of 9:1 (*c₁* : *c_{2/3}*).

With the help of deletion mutants we could show that subunits *c₁* and *c₂* are essential for a functional ATP synthase. MS analysis of the c rings isolated from these ATPase mutants revealed that the c subunit stoichiometry remains unaltered for those mutants that still contain the V-type *c₁* subunit, while in the case of Δc_1 the loss of *c₁* is compensated for by two additional copies of *c_{2/3}*.

Deletion of an essential c subunit leads to the assembly of incomplete ATPases, which allowed us to investigate the steps involved in the integration of the c ring into the ATPase.

Neuer Aspekte

analysis of hybrid F₀V₀ rotor, assembly of c-ring into ATPase

Identification and relative label-free quantification of lactosylated peptides in bovine raw milk and processed milk products

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Stichworte: Amadori product, boronate affinity chromatography, lactosylation, milk processing, relative label-free quantification

Einleitung

The microbiological safety of milk products is guaranteed by different types of thermal treatment providing also longer shelf life times. These elevated temperatures and the high content of lactose, however, favor Maillard reactions yielding in the early stage Amadori products at lysine residues (lactulosyllysyl). Extensive lactosylation of milk proteins modifies lysine residues and thereby decreases the protein nutritional value and reduces their degradation by enzymes after consumption with heavily glycosylated sequences being resistant to proteolysis. Furthermore, lactosylated milk proteins may trigger an immune response and thereby trigger food allergies. Although such severe side effects have been discussed, compelling data supporting these concerns are missing demanding comprehensive studies determining the modification sites and degrees of proteins in processed milk products.

Experimenteller Teil

Lactosylation induced in milk proteins during thermal processing including the modification sites were determined in bovine raw milk (RM), raw colostrum (RC), pasteurized milk (P), ultra high temperature (UHT) milk, and infant formulas (IFs). Proteins were extracted, digested with trypsin, lactosylated peptides were enriched with boronate affinity chromatography, and analyzed by nLC-ESI-MS/MS (CID and ETD mode). A retention time-based inclusion list of all potentially lactosylated peptides was used in a subsequent nLC-ESI-MS/MS (ETD mode) to retrieve the peptide sequences and the modification sites in all milk samples. Lactosylated peptides identified with high confidence scores and additionally manually confirmed were quantified by integration of the corresponding extracted ion current chromatograms (XICs).

Ergebnisse

In total, we could identify 260 unique lactosylated peptides corresponding 123 lactosylation sites (LS) in 28 proteins. Most peptides were singly lactosylated, but a few di-lactosylated peptides were also identified mostly in UHT and IF. All five analyzed IF samples contained even triply lactosylated peptides indicating higher glycation levels. Correspondingly, the highest number of lactosylated peptides and unique lactosylation sites (106) were identified in IF. Several lactosylation sites were already present in un-processed raw milk. The whey proteins β -lactoglobulin and α -lactoglobulin as well as kappa-casein appeared to contain the most lactosylation sites, although this might be linked to their high concentrations allowing a better analysis.

Additionally, all 260 identified lactosylation sites were quantified using the signal intensities of the precursor ions acquired for all milk samples. The quantitative data provided the basis to evaluate the impact of different thermal processes on the glycation degree relative to untreated raw milk. The highest lactosylation degree was determined in infant formulas. UHT contained most lactosylation sites at lower quantities, although the relative content depended very much on the protein. Pasteurized milk contained much lower levels that were similar to RC and RM. Thus, both RC and RM contained already a relatively high basic lactosylation degree that increased only for harsher thermal treatment conditions (UHT) and additional drying (IF). Interestingly, the lactosylation levels varied very much among the three UHT and five IF samples obtained from different brands indicating that the complete technical process and not only the sterilization step determines the overall lactosylation degree in both milk products.

Neuer Aspekte

Twenty-five novel lactosylation sites were identified in bovine milk proteins including six proteins that were not known to be lactosylated.

Multiplexed 2D MRM-based protein biomarker quantitation of plasma proteins from free flaps – a pilot study
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Stichworte: Plasma, Protein Quantitation, Multiple reaction monitoring (MRM), Multiplexed, Ischemia and Reperfusion

Einleitung

Free flap transplantation has become the gold standard to cover complex wounds of the human body. However, so-called “ischemia and reperfusion injuries” of the transplants are unavoidable during surgery; potentially leading to complications and even loss of the transplant. Monitoring tissue-related processes on the molecular level is desired to assess individual transplant quality when clinical signs are not visible [1]. Since blood is circulating all tissues it contains proteins secreted from them. We collected human plasma samples from the feeding artery and the effluent vein of free flaps during surgery to study their respective protein profiles by LC-MRM-MS [2]. In this pilot study we investigated abundances of two proteins (MCSF and calgranulin A) in human plasma samples from eight patients.

Experimenteller Teil

Proteins in 20 µl plasma were denatured, reduced and alkylated. After digesting with trypsin, 5 stable-isotope-labelled standard (SIS) peptides were spiked-in. Peptide mixtures were desalted by solid phase extraction, then eluted with 600 µl 55% ACN in 0.1% FA and lyophilized. For injection onto the high-pH RPLC system, peptides were rehydrated with 1600 µl 10mM ammonium hydroxide. After LC fractionation of the peptide mixture into 47 wells of a 96 well plate, eluates were lyophilized. Before analysis at low-pH RPLC/MRM-MS, neighboring fractions were combined into 13 fractions for each sample in 100 µl of 0.1% FA. Fractions were analyzed on a tripe quadrupole mass spectrometer (6490; Agilent technologies), MRM data were processed with Mass Hunter Quantitative Analysis software (version B. 07.00).

Ergebnisse

Five proteotypic peptides were chosen to represent MCSF and calgranulin A. Chromatographic peaks were manually inspected for peak-related information (retention time, peak width and area) to ensure correct selection of MRM fragments for integration. For quantitation the peak area filter was set to 300 counts. Peaks areas above 300 were determined to calculate protein concentrations.

For MCSF, the peptide FNSVPLTDTGHER with $[M+3H]^{3+}$ ion signal (m/z 491.58) and its $y7^+$ fragment ion (m/z 815.37), was chosen. The corresponding SIS peptide ($[M+3H]^{3+}$ at m/z 494.91) and its $y7^+$ fragment (m/z 825.37) was used as reference. Retention time of these peptides is 5.7 min at low pH, and the optimal collision energy is 17 V. For calgranulin A, the selected peptide was GADVWFK ($[M+2H]^{2+}$ at m/z 411.71, SIS peptide: m/z 415.72) and its $y3^+$ ion was recorded at m/z 480.26 (SIS fragment: 488.27). Retention time of these peptides is 8.6 min, and the optimal collision energy is 9 V. Using these two transitions it was possible to determine precise quantities of both proteins in all 16 plasma samples.

The averaged MCSF concentration in artery plasma was 21.2 ng/mL (\pm 6.4), whereas that in venous plasma was 32.0 ng/mL (\pm 9.3); a 1.5-fold upregulation ($p < 0.05$). Calgranulin A concentration in artery plasma was 3.4 ng/mL (\pm 1.3), whereas that in venous plasma was 12.8 ng/mL (\pm 10.9); a 3.75-fold upregulation ($p < 0.05$). Standard deviations reflect biological variances.

MCSF has been reported to increase during tissue inflammation, indicating macrophage activation in the flaps [3]. Calgranulin A, as part of calprotectin, has been discovered to control macrophage activity during

ischemia/reperfusion, causing fibrosis and tissue damage [4]. Our study proves that the risk of tissue damage during transplantation can be monitored by LC-MRM-MS and has the potential for early assessment of free flap quality.

Neuer Aspekte

MRM-MS determines differential expression of MSCF and calgranulin A from free-flaps during transplantation for early tissue quality assessment.

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Rapid extraction of intact proteins from tissues via desorption by impulsive excitation

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Stichworte: protein extraction, tissue samples, PIRL-DIVE, proteomic, mass spectrometry

Einleitung

Protein extraction from tissue is a critical and time-consuming step in every protein analysis. Proteins must be released from their native environments using homogenization and further isolation methods, which vary depending on the nature of the tissue and proteins of interest. An appropriate protein extraction procedure shouldn't change their chemical compositions and functions like enzymatic activities. Proteolytical and other degradations should be avoided during the extraction process. In this work we examined the potential of a picosecond-IR laser (PIRL) [1] to extract proteins from tissue samples through desorption by impulsive excitation (DIVE) of intramolecular vibrational states of water molecules [2].

Experimenteller Teil

To test the suitability of DIVE for protein extraction, several experiments were performed. First, mouse muscle and liver tissue samples were irradiated by PIRL. The condensates of the ablation plumes were analyzed by SDS-PAGE to investigate whether a broad range of proteins can be extracted by PIRL-DIVE and if these are suitable for immunoblotting. Furthermore, the integrity and enzymatic activity of ablated proteins were investigated by mass spectrometry. For this purpose PIRL-desorbed and non-desorbed RNase A were analyzed on peptide and protein level to investigate the chemical compositions of the ablated molecules. The enzymatic activities of PIRL desorbed trypsin and human blood plasma were investigated by mass spectrometry after incubation with α -casein respectively angiotensin I (Ang 1-10).

Ergebnisse

With DIVE almost 1 mg of proteins were extracted from 37.5 mm³ mouse muscle tissue in less than five minutes, whereas the conventional extraction procedure lasted more than three days with a total protein yield of 175 μ g. The SDS-PAGE of the PIRL plume condensate showed a huge number of bands covering proteins from a few kDa up to several hundred kDa. LC-MS/MS analysis of selected bands revealed that even large proteins like titin (approx. 4 MDa) were extracted in a huge quantity. In addition, PIRL protein extracts were generated from mouse liver tissue for immunoblotting. Detection of highly glycosylated CEACAM1 protein showed that even labile PTMs survived the DIVE process. A detailed investigation of the chemical composition of RNase A in the PIRL plume condensate by MALDI-MS and nanoUPLC-ESI-QTOF-MS/MS on peptide and protein level revealed that the chemical composition wasn't altered by DIVE. Furthermore, enzymatic activities of DIVE treated trypsin as well as DIVE treated human blood plasma proteins were retained. LC-MS analysis of the incubate of trypsin with α -casein showed a number of signals for tryptic α -casein peptides, whereas no signals for the intact α -casein protein were observed. Detection of Ang 1-8, Ang 6-9, Ang 4-10 and Ang 1-7 in the incubate of the condensate of the DIVE plume of human blood plasma with Ang 1-10 showed that Ang 1-10 metabolizing enzymes were still active after PIRL irradiation. Since MALDI spectra of the incubate of ablated blood plasma and non-ablated blood plasma with Ang 1-10 showed very similar patterns, it can be assumed that almost all of the enzyme activities were maintained after DIVE extraction. In summary, DIVE facilitates an ultrafast protein extraction from tissues in a high quantity and quality. DIVE is compatible with common protein analytical techniques, minimizes time and increases the yield of extracted proteins.

Neuer Aspekte

PIRL-DIVE is a new, ultrafast and innovative method for extracting proteins from tissues in a high quantity and quality.

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Flowsystem on-line gekoppelt mit einem LC/qTOF-MS zur systematischen Untersuchung der Photolyse unter umweltrelevanten Bedingungen

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Stichworte: Transformationsprodukte, Pathway, Photolyse, Flowsystem, Spektren-Datenbank

Einleitung

Transformationsprodukte (TP) in komplexen Umweltproben zu detektieren und einzelnen Chemikalien zuzuordnen ist aufgrund der Vielzahl möglicher Reaktionen und Produkte nicht einfach. Noch schwieriger wird es, wenn man auch Aussagen über Pathways machen möchte. Daher ist es sinnvoll, einzelne Transformationsprozesse im Labor nachzustellen und schrittweise deren Komplexität zu erhöhen. Für den photolytischen Abbau von Carbamazepin gibt es in der Literatur unterschiedliche Pathways [1, 2, 3]. Diese wurden vor allem basierend auf den teilweise nicht vollständig bestätigten Strukturvorschlägen der Abbauprodukte aufgestellt. Das Fließsystem ist in der Lage schnell eine systematische Untersuchung von Ausgangsstoffen und seinen Transformationsprodukten vorzunehmen. Eine gemeinsame Analyse der erhaltenen LC/MS-Spektren erleichtert dann das Aufstellen von Pathways und kann über Plausibilitätsprüfungen und gemeinsamer Analyse der MS/MS-Spektren auch zu einer verbesserten Strukturaufklärung beitragen.

Experimenteller Teil

Das Flowsystem besteht aus einer Anordnung von Spritzenpumpen und Ventilen, die über eine gemeinsame Software gesteuert werden. Grundlage ist ein kontinuierlicher Fluss, in den die Analyte (eventuell mit bestimmten Zusätzen) als Plugs injiziert werden. Diese Plugs werden dann in einer Schnecke aus FEP-Schlauch eingeleitet und von einer Xenonlampe, die sich in einer Kammer befindet, die thermostatiert werden kann, gleichzeitig bestrahlt. Über die Flussrate lässt sich die Bestrahlungsdauer verändern. Des Weiteren kann auch die Bestrahlungsstärke der Xenonlampe variiert werden. Über einen Loop erfolgt die Injektion der einzelnen Plugs in das LC/MS-System, wo diese mit einer schnellen, kurzen Gradienten mittels UPLC/HRMS vermessen werden. Die Auswertung der LC/MS-Chromatogramme der Analyte und deren Analoga findet dann gemeinsam mit Unifi (Waters) statt.

Ergebnisse

Die online-Kopplung des Photolyse-Systems zu einem LC-qTOF ermöglicht ein systematisches schnelles Screening von anthropogenen Spurenstoffen und deren Transformationsprodukten, wodurch man auch für vermeintlich gut untersuchte Substanzen wichtige Zusatzinformationen erhält. Dies soll nun im Folgenden am Beispiel von Carbamazepin (CBZ) und dessen Analoga verdeutlicht werden. Durch Vergleich der LC/MS-Chromatogramme mit und ohne Bestrahlung kann man eine Aussage zur generellen Abbaubarkeit der Ausgangssubstanzen unter umweltrelevanten Bedingungen vornehmen. CBZ-epoxid und auch das dihydro-diOH-CBZ werden durch direkte Photolyse innerhalb von 3h nicht abgebaut, während Oxcarbazepin zu 100% abgebaut wird. Dies beweist das CBZ-epoxid nicht ein zentrales TP im Pathway darstellen kann und unter umweltrelevanten Bedingungen nicht zur Bildung von Acridin und BQD führt, wie teilweise in der Literatur [1] vermutet wurde. Die Auswertung der LC/MS-Chromatogramme findet für CBZ und dessen Analoga (Ox-CBZ, 3-OH-CBZ, diOH-CBZ, Epoxid-CBZ, Acridin) gleichzeitig statt, wodurch die Identifizierung bekannter und neuer unbekannter TPs wesentlich erleichtert wird. Diese Strategie verdeutlicht auch, dass gleiche exakte Massen (m/z 224.0706) und damit gleiche Summenformeln ($C_{14}H_9NO_2$) aus den Analoga entstehen, die aufgrund ihrer Retentionszeit aber unterschieden werden können. Eine Zuordnung eines Strukturvorschlages allein aufgrund der Fragmente ist teilweise nur mit dem Wissen über die Ausgangsstoffe möglich. Ein Ziel des Flowsystems ist es dabei auch eine Datenbank von Transformationsprodukten zu erstellen, in der Informationen wie Summenformeln, Retentionszeit, Isotopenmuster und MS/MS-Spektren gespeichert sind. Diese Informationen können dann für ein umfassendes Umweltmonitoring genutzt werden. Die zusätzliche Information über den oder die Ausgangsstoffe, die zum jeweiligen TP führen, kann dabei wertvolle Hinweise geben.

Neuer Aspekte

Flowsystem zur on-line-Generierung von TPs analoger Substanzen, die zusammen ausgewertet werden erleichtert die Strukturaufklärung und ermöglicht ein Verifizieren aufgestellter Pathways.

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On-line Analysis of Organic Emissions from Residential Wood Combustion with Single-Photon Ionization Time-of-Flight Mass Spectrometry (SPI-TOFMS)

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Stichworte: single-photon ionization (SPI), non-negative matrix factorization (NMF), principal component analysis (PCA), wood combustion, burning phase

Einleitung

According to Directive 2009/28/EC, the Commission of the European Union fixed targets for its member states to reach a mean percentage of 20% renewable energy of the final energy consumption in the whole European Union until 2020, for example by the combustion of wood. Therefore wood combustion, in particular during winter, contributes significantly to indoor and outdoor air pollution. Wood smoke and wood combustion-derived particles are known for their toxicological effects. Thus, it is necessary to characterise the emission of different wood combustion appliances, whose characteristics change qualitatively as well as quantitatively with ongoing advances on wood stoves. However, time-resolved measurements for volatile and intermediate-volatile organic compounds (VOC and IVOC) are limited.

Experimenteller Teil

Six consecutive batches of three common European types of firewood (beech, birch and spruce; batch weight: 2.5 kg; total duration: 4 h) were burned in a modern wood log fired masonry heater. The temporal variation of emissions of single organic compounds was studied by single-photon ionization time-of-flight mass spectrometry (SPI-TOFMS) with 118 nm VUV-photons. SPI refers to a soft ionization technique, thus leading to molecular ions, low fragmentation and no adducts [1]. Every compound with ionization energy below the photon energy becomes ionized whereby the ionization efficiency strongly depends on the compound class [2]. VUV-photons of 118 nm were generated by multiple frequency increasing of the fundamental radiation of 1,064 nm of a Nd:YAG-laser (energy of 40mJ @355nm).

Ergebnisse

Emissions of the whole combustion cycle and temporal variations were discussed. Short-chain carbonyls, alkenes and dienes, furan/isoprene and benzene as nonspecific decomposition products revealed highest abundances, but m/z of phenolic species and anhydrous sugars as building blocks of the wood polymers lignin, cellulose, and hemicellulose were also observed. Regarding the different batches, more than 50% of the overall intensity for all considered m/z occurred during the combustion of the first two batches. Mainly m/z of components with not less than one double bond and low O/C ratio were significantly enhanced in the first or first two batches, which was ascertained by single and double Grubb's Outlier Test. Furthermore, the high temporal resolution down to 1s facilitates to investigate the combustion process more in detail. Within one batch, two different temporal trends were perceived: The first group of m/z diminished steadily, whereas the second group had its maximum at the beginning as well, but passed through a minimum and increased again towards the end of the batch. Mainly m/z of benzene, naphthalene, butadiene, vinylacetylene, and propene belong to the second group, remaining m/z to the first group. In one spruce experiment, generally increased intensities were observed, but not defrayed in equal proportions of all m/z . Compared to another spruce experiment, primary decomposition products of wood polymers are more enhanced than secondary decomposition products, such as benzene or small alkenes. Finally, the molecular signatures of burning phases ('ignition', 'stable combustion' and 'ember') were examined by using non-negative matrix factorization (NMF) [3,4] and principal component analysis (PCA) in sequence. Marker substances for wood or biomass combustion, such as phenolic species or anhydrous sugars, exhibited highest relative abundance during 'stable combustion', whereas 'ember' was distinctly characterized by unsaturated hydrocarbons, such as benzene or naphthalene, through pyrosynthesis; in 'ignition', secondary decomposition products dominated.

Neuer Aspekte

Burning phases of wood were determined by NMF from VOC and IVOC and subsequently characterized on a molecular level.

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Automated fast screening of TOF-MS data

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Stichworte: Automated Compound Classification, Fast Screening, Quantification

Einleitung

Sophisticated separation techniques like GCxGC-TOFMS are well established for the comprehensive characterization of complex sample compositions that contain a large number of compounds. Due to the complex amount of information the data evaluation is difficult and time consuming. To cope with this, advanced scripting algorithms based on knowledge based rules (KBR) were in house developed and applied to GCxGC TOFMS data. Thereby, thousand different compounds can reliably be classified in a short time period based on their fragmentation pattern. The quantification of these classes is challenging. Based on the response factors and group specific response curves of a set of clearly separable standards and carbon numbers the ultra-fast screening method could be accomplished by a comprehensive quantification of these compounds.

Experimenteller Teil

For the analysis by GCxGC-TOFMS, an Agilent 6890 gas chromatograph equipped with a LECO Pegasus 4D detector, including a Pegasus IV TOF-MS (LECO, USA) using helium as the carrier. An Optic 3 inlet system (ATAS GL, Netherlands) was used. Data collection was performed using LECO ChromaTOF® software v. 4.50.8. Chromatographic separation was performed on a 60 m × 0.25-mm i.d. × 0.25-µm df BPX5 (SGE, Australia) column and then a 1.9 m × 0.1-mm i.d. × 0.10-µm df BPX50 (SGE, Australia) column

Ergebnisse

If implemented properly, this automated screening approach allows samples with over 4000 peaks to be screened in less than 20 min without a deep knowledge of the sample composition. Compared to previously published approaches the accuracy of the classification algorithms could be increased significantly by adding fragmentation patterns, molecular ion information and specific molecular fragments. Combining this approach with an interpolation of response factors or an average response, covering the main compounds and carbon numbers is possible and can be used for a nearly complete quantification of e.g. middle distillates.

Neuer Aspekte

Automated Compound classification based on fragmentation pattern

Group-type quantification

Separation of Carbohydrate and Glycoprotein Isomers using Ion Mobility-Mass Spectrometry

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Stichworte: Ion mobility-mass spectrometry, glycans, protein glycosylation, collision cross section, travelling wave

Einleitung

Complex carbohydrates are of great importance for a variety of biological functions. They can be linked to proteins as post-translational modifications or exist as free oligosaccharides, for example in milk. Currently, the vast majority of complex carbohydrates are characterized using mass spectrometry-based techniques (MS) that are directly descending from those established in proteomics. Measuring the molecular weight of a sugar, however, immediately poses a fundamental problem: entire classes of the constituting monosaccharide building blocks exhibit an identical atomic composition and, consequently, also an identical mass.[1] Therefore, carbohydrate MS data can be highly ambiguous and often it is simply not possible to clearly assign a particular molecular structure.

Experimenteller Teil

A promising approach to overcome the above-mentioned limitation is to implement an additional gas phase separation step using ion mobility-mass spectrometry (IM-MS). Here, ions travel through a gas-filled cell aided by an electric field and are separated according to their mass, charge, and shape. While the drift time of an ion depends on the underlying instrument conditions, the collision cross section (CCS) is a molecular property that can be compared and calculated theoretically. Due to the inhomogeneous travelling wave field utilized in most of the commercially available instruments, however, CCS estimation requires careful calibration.[2,3]

Ergebnisse

Here, we demonstrate the potential of IM-MS to be used as a tool for the separation and identification of isomeric carbohydrates and glycopeptides. First, six synthetic carbohydrate isomers that differ with respect to their composition, connectivity, or configuration were analyzed individually and as mixtures. Our data reveal that carbohydrate linkage- and stereoisomers, which are difficult to distinguish using established techniques such as liquid chromatography or NMR, can be separated and unambiguously identified on basis of their CCS. When mixed, even minor isomeric components with relative concentrations as low as 0.1% were still clearly detectable.

Second, we recently extended our investigations to glycopeptides. First preliminary data show that the position of the carbohydrate within the peptide sequence can be clearly identified using IM-MS when multiple glycosylation sites are present. More importantly, however, our data reveal that also glycopeptides, which merely differ in the regiochemistry of the attached glycan can be distinguished using gas-phase fragmentation and subsequent IM-MS analysis.

These results indicate that IM-MS is an exceptionally effective tool for the structural analysis of complex carbohydrates. The full benefit of this method will become apparent once CCS data of carbohydrates and oligosaccharide fragments will be deposited in databases.

Neuer Aspekte

Analysis of carbohydrate isomers using ion mobility-mass spectrometry.

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Influence of glycoforms on the tryptic digestion efficiency of immunoglobulin G based biopharmaceuticals

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Stichworte: Bottom-up proteomics, Glycoform profiling, Biopharmaceuticals, nanoLC-MS, Tryptic digestion biases

Einleitung

Bottom-up proteomics is employed frequently in biological and clinical research. Nowadays, it is becoming increasingly popular for the analysis of biopharmaceuticals in discovery, development and batch control. The digestion of the target protein by proteases, generally by trypsin, into more easy to analyse peptides is a key step in bottom-up proteomics. Thus, a robust analysis needs to incorporate an efficient and unbiased tryptic digestion step. It has been observed previously that incomplete tryptic digestion can introduce biases into glycoform profiles[1].

Therefore, we investigated the preferential tryptic digestion of glycoforms of two biopharmaceutical formats: 1. an immunoglobulin G1(IgG1) monoclonal antibody(mAb) and 2. Flebogamma, an intravenous IgG(IVIG) from healthy donor pools. Via denaturation experiments, we collected evidence for the 3D-structure dependence of the biases.

Experimenteller Teil

The tryptic digestion of the target protein into glycopeptides was followed over time. The digestion of protein with an intact 3D-structure was compared to that of protein denatured by various methods. These included denaturation, reduction and alkylation as a gold-standard as well as a novel, save and efficient protocol for IgG denaturation. Glycopeptides from the conserved glycosylation site in the F_C-part of IgG were detected by a nanoLC-ESI-q-TOF-MS method[2]. In addition, the glycoproteins and glycopeptides of partially digested samples were separated by gel electrophoresis and the residual glycoproteins were reanalysed after complete in-gel tryptic digestion. By relative quantitation of the glycopeptides and estimation of their absolute abundances, we could visualize a preference of trypsin for the digestion of certain glycoforms.

Ergebnisse

In general, we proved that the glycoforms of the IgG1 mAb containing high mannose and hybrid type glycans in the Fc portion were preferentially digested into the glycopeptides compared to those glycoforms containing complex type glycans. The biases observed in IVIG were smaller, because this formulation did not contain high mannose or hybrid glycans. The main bias in IVIG was created by preferential digestion of bisected species. Interestingly, the α 2-3 linked sialic acid containing species in the IgG1 mAb were amongst the first to be digested while the α 2-6 linked sialic acid containing species in IVIG were not preferred. Comparing the tryptic digestions of non-denatured and denatured protein revealed that the biases are much less pronounced in denatured samples. Additional experiments proved that this effect is independent of the increased digestion efficiency. A significant part of the digestion biases must therefore be caused by the differences in antibody 3D structure induced by the different glycoforms.

Our simple, novel pre-treatment method — acid incubation and evaporation — exhibited a denaturation efficiency comparable to the gold standard (see *Methods*) for IgG while significantly reducing the hands-on time and the toxicity of the reagents involved. It additionally offers the advantage of performing the tryptic digestion under optimal buffer conditions.

In conclusion, proper denaturation and digestion completeness are key to avoiding biases in bottom-up proteomics based glycoform profiling. However, the digestion biases have implications beyond glycoproteomics. For example, relative protein quantitation (even with isotopically labelled standards) in two samples could be biased by differences in glycoform profiles, if complete tryptic digestion is not achieved.

Neuer Aspekte

Trypsin preferentially digests certain glycoforms of IgG. This preference is extensively induced by the specific 3D structure of the glycoform.

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MALDI TOF and ESI IT MS Characterization of highly sulfated Glycosaminoglycan-Oligosaccharides

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Stichworte: sulfated glycosaminoglycans, oligosaccharides, loss of sulfation

Einleitung

Glycosaminoglycans (GAG) are natural, acidic polysaccharides which occur in the extracellular matrix of many tissues [1]. Certain growth factors and cytokines are known to bind to GAG. This process is assumed to be of significant relevance for wound healing and tissue regeneration [2]. However, the role of the sulfation pattern regarding GAG-protein interactions is unknown. Thus, well characterized GAG (oligosaccharides) with defined sulfation patterns are urgently required for further studies. The mass spectrometric characterization of these highly sulfated compounds is challenging due to the lability of the sulfate residues.

Here, the characteristics of a persulfated (ps) tetrasaccharide of hyaluronan (HA-4) will be investigated by ESI IT and MALDI TOF MS and compared with the model compound Arixtra.

Experimenteller Teil

Arixtra (as 12 mg/mL solution in physiological saline) was purchased from Glaxo Smith Kline and either directly used as supplied or after exhaustive dialysis to reduce the salt content.

The persulfated HA-tetrasaccharide (psHA-4) was obtained from HA-4 (obtained by digestion of the HA polysaccharide by hyaluronidase) by exhaustive sulfation with sulfur trioxide pyridine complex.

MALDI TOF mass spectra were acquired on a Bruker Autoflex MS and ESI IT spectra on a Bruker Amazon SL. 9-Aminoacridine (9-AA) is the matrix of choice to record negative ion spectra due to its pK of about 10. Selected positive ion spectra were also recorded in the presence of DHB. For ESI MS a mixture of methanol and distilled water (2:1, v/v) was used as solvent system.

Ergebnisse

ESI and MALDI are "soft" ionization techniques and can be widely used in structural studies. (Highly) sulfated carbohydrates are very refractive and normally result in poor ion yields and/or loss of sulfate residues [3].

To verify the capabilities and limitations of both MS methods to characterize newly synthesized sulfated carbohydrates, a reliable reference compound is unequivocally needed. "Fondaparinux" (trade name "Arixtra"), a synthetic, commercially available pentasaccharide with 8 sulfate residues, is a very useful compound for that purpose.

Arixtra could be detected by MALDI MS in negative and positive ion mode without the need of desalting, i.e. in the presence of physiological salt concentration. We could show that the negative ion mode provides generally higher sensitivity whereas the intact ion is detectable as positive ion with higher intensity. Additionally, the number of sodium counter ions determines the number of detectable sulfate residues, i.e. the MALDI spectral quality decreases with a decreasing salt concentration.

The inevitable [4], significant loss of sulfate during the MALDI process complicates the evaluation of newly synthesized compounds. Since ESI MS is a "softer" method it is more suitable to determine the extent of sulfation [5] although the presence of salt suppresses the ionization process. After exhaustive dialysis, Arixtra could be detected as intact ion with 8 (53%) sulfate residues although loss of one (33%) and more than one (14%) sulfate residue does also occur. Similar data can be obtained when the persulfated HA-4 is investigated. However, the intensity of the intact persulfated Hyaluronan-Tetrasaccharide ion accounts only for 11%.

We are currently working on the establishment of an improved desalting method to exclude partial sulfate loss during the dialysis process.

Neuer Aspekte

This is the first (MALDI and ESI) mass spectrometric study of such artificially highly sulfated oligosaccharides.

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Combining thin-layer chromatography, antibody-overlay detection and DESI-FT-ICR-MS for the analysis of Shiga toxin glycosphingolipid receptors

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Stichworte: DESI, FT-ICR, glycosphingolipids

Einleitung

Glycosphingolipids (GSLs) are integral part of the cell membrane of vertebrates and play important roles in many biological processes [1]. They participate in cell recognition and serve as receptors for numerous pathogens [2] or virulence factors, such as Shiga toxins (Stxs) released by enterohemorrhagic *E.coli* (EHEC) [3]. GSLs are comprised of a polar oligosaccharide head group as well as an unpolar ceramide part made of a fatty acid and sphingosine. Structural characterisation of the molecular heterogeneity demands a combination of separation techniques such as thin-layer chromatography (TLC) and mass spectrometry. Here, we demonstrate the use of desorption electrospray ionisation [4] Fourier-transform ion cyclotron resonance mass spectrometry (DESI-FT-ICR-MS) for the analysis of GSLs separated by TLC and detected by immunostaining techniques.

Experimenteller Teil

GSLs from human erythrocytes were applied bandwise onto normal phase silica TLC plates, separated, and detected by antibody-overlay assays. To that end plates were incubated with either a specific anti-GSL antibody or a Stx, its respective toxin-specific antibody followed by incubation with a secondary antibody and staining with 5-bromo-4-chloro-3-indolyl phosphate (BCIP) giving rise to bright blue bands. For DESI measurements, the plate was placed onto a movable sample stage and analytes were desorbed and ionised directly from the plate by use of methanol as a solvent spray. Mass analysis was done with a Fourier-transform ion cyclotron resonance mass spectrometer equipped with a 7 T magnet. A CO₂ laser was used for infrared multiphoton dissociation (IRMPD) MS² experiments.

Ergebnisse

GSLs could be separated according to their oligosaccharide chain lengths, e.g. globotriaosylceramide (Gb3Cer) and globotetraosylceramide (Gb4Cer). Furthermore, separation depending on the length and saturation of the fatty acid was possible to some extent. GSLs were detected using anti-GSL antibodies, alkaline phosphatase (AP) conjugated secondary antibodies and the staining reagent BCIP. Alternatively, GSL receptors could be detected specifically by Stxs and the latter were recognised by AP conjugated secondary antibodies. Here we show the use of Stx2a which binds preferentially to Gb3Cer. Stained bands were examined by DESI-FT-ICR-MS. Analytes could be desorbed and ionised directly from the TLC plate and were detected even in the presence of other biomolecules such as antibodies and staining reagent. Scanning the plate in the direction of chromatographic development furnished 2D spectra which showed the distribution of the different GSL species on the plate. Scanning of just a single band gave rise to mass spectra of unseparated GSLs of similar polarity. Due to the high mass accuracy of the FT-ICR mass spectrometer all ionic species could be assigned to GSL structures. Gb4Cer detected by antibody-overlay assays could be detected with good signal-to-noise-ratios at amounts down to 20 ng per band. For further structure elucidation, precursor ions were isolated in the ICR cell and fragmented by IRMPD. MS/MS experiments yielded B- and Y-type ions from cleavages of the glycosidic bonds, as well as typical fragment ions allowing characterisation of the sphingosine portion.

The combination of TLC and DESI-FT-ICR-MS offers a suitable alternative to conventional methods for GSL analysis such as ESI-MS or MALDI-MS. DESI provides desorption and ionisation of analytes without preceding sample preparation, thus, analyte loss is largely reduced. Ions can be probed very accurately with the FT-ICR mass spectrometer and MS/MS experiments enable structure assignments.

Neuer Aspekte

TLC and antibody-overlay detection in combination with DESI-FT-ICR-MS is a new, alternative method for the analysis of glycosphingolipids.

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Massenspektrometrische Analyse von Biotensiden am Beispiel der Rhamnolipide

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Stichworte: Biotenside, Rhamnolipide, LC-MS/MS, GC-MS, SPE

Einleitung

Rhamnolipide (RL) gehören zu den Tensiden mikrobiellen Ursprungs. Sie zeichnen sich durch eine geringe Toxizität und eine gute Bioabbaubarkeit aus und eignen sich daher z.B. für die Anwendung als Emulgatoren in Kosmetika und Lebensmitteln. RL bestehen aus ein bis zwei Rhamnoseeinheiten (mono-/di-RL), welche mit bis zu drei β -Hydroxyfettsäuren mit einer Kettenlänge von C8-C14 verbunden sind. Die mikrobielle Produktion der RL in Gemischen sowie ihre strukturelle Vielfalt erfordern effiziente analytische Methoden, um qualitative und quantitative Informationen über die Zusammensetzung der RL-Probe zu erhalten. Chromatographische Methoden gekoppelt mit einer massenspektrometrischen Detektion haben sich für die Identifizierung von RL als geeignet erwiesen [1,2].

Experimenteller Teil

Für die Analyse der Rhamnolipid-Proben wurde die HPLC gekoppelt mit der Tandem-Massenspektrometrie (LC-MS/MS) angewendet. Die freie Carboxylgruppe ermöglicht die Ionisierung mittels Electrospray (ESI) im negativen Modus. Für die Charakterisierung der RL-Proben wurden verschiedene Scan-Experimente (Full Scan, Produktionen-Scan, Vorläuferionen-Scan und MRM) herangezogen. Zusätzlich wurde die Gaschromatographie-Massenspektrometrie (GC-MS) mit Elektronenionisation zur Bestätigung der identifizierten Rhamnolipide anhand der Zusammensetzung ihrer β -Hydroxyfettsäuren eingesetzt.

Ergebnisse

Die analysierten Rhamnolipide wurden durch den Mikroorganismus *Pseudomonas putida* KT2440 in einem Fermentationsprozess hergestellt [3]. Das zellfreie Probenmaterial wurde durch die Arbeitsgruppe von Prof. Blank (RWTH Aachen) zur Verfügung gestellt. Als Alternative zur bisher üblichen Flüssig-Flüssig-Extraktion wurde die Festphasenextraktion (SPE) als Probenvorbereitung angewendet. Die SPE wurde hinsichtlich der Durchführung der Wasch- und Elutionsschritte optimiert. Die quantitative Elution aller Analyten ist von der Konzentration der Rhamnolipide im Probenmaterial abhängig und wird durch die Mischung Acetonitril/Ameisensäure ($\geq 2\%$) erreicht. Nach der Aufarbeitung erfolgte die Analyse mittels HPLC-(ESI)-MS und HPLC-(ESI)-MS/MS. Mono-RL verschiedener Kettenlängen wurden anhand ihrer deprotonierten Molekülionen $[M-H]^-$ und ihrer spezifischen Fragmente in MS/MS-Experimenten identifiziert und in ihrer relativen Verteilung bestimmt. Zusätzlich wurde die komplementäre Analyse der Fettsäuren mittels GC-MS nach Derivatisierung zu den korrespondierenden Fettsäuremethylestern durchgeführt. Die Identifizierung erfolgte über die Retentionszeiten von Standards und spezifischen Fragmenten aus der Elektronenionisation. Mit den genannten Methoden wurde ein Rhamnolipid mit der seltenen Zusammensetzung von Rhamnose (Rha) verbunden mit β -Hydroxydecansäure (C10) und β -Hydroxyundecansäure (C11), Rha-C10-C11 und das Isomer Rha-C11-C10, identifiziert. Die Existenz eines Rhamnolipids mit dieser Zusammensetzung wurde bisher in der Literatur nur einmal erwähnt und konnte nicht mit anderen Methoden als der HPLC-MS bestätigt werden [4].

Neuer Aspekte

Anwendung der Festphasenextraktion als Alternative zur bisher üblichen Probenaufarbeitung und Identifizierung eines seltenen Rhamnolipids mittels komplementärer LC-MS/MS- und GC-MS-Experimente

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Bestimmung von polaren Metaboliten in Algenextrakten mittels HILIC-ESI-MS

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Stichworte: HILIC-ESI-MS, Redox-Cofaktoren, Flussanalyse, Probenvorbereitung von Algenzellen

Einleitung

Cofaktoren wie NAD^+/NADH , $\text{NADP}^+/\text{NADPH}$ oder FAD/FADH_2 sind essentiell für die Redox-Homöostase. Des Weiteren spielen Metabolite wie Glutathion, ATP, ADP und AMP eine wichtige Rolle im Zellgleichgewicht. Glutathion kann freie Radikale abfangen und so die Redox-Fähigkeit der Zellzyklen zu regulieren,[1] während ATP der universelle und essentielle Energieträger in jeder Zelle ist.

Zur Bestimmung der Redoxzustände und der Regenerationsrate von Cofaktoren in Zellen und Organismen ist es notwendig, deren Flussverteilung in Stoffwechselwegen zu bestimmen. Das Ziel der Flussanalyse ist die Quantifizierung von metabolischen Flüssen im Zentralstoffwechsel von Mikroorganismen.[2] Die Ermittlung der Flussverteilung kann durch ^{13}C - und/oder ^{15}N -Markierungsexperimente im Zellwachstum und anschließender massenspektrometrischer Analyse der Verhältnisse unterschiedlich markierter Analyten erfolgen.

Experimenteller Teil

Bei der Methodenentwicklung wurde der Fokus auf die Metabolite FAD , NAD^+ , NADH , NADP^+ , NADPH , AMP , ADP , ATP sowie Glutathion in oxidiert und reduzierter Form gelegt. Die Hydrophile Interaktionsflüssigkeitschromatographie (HILIC) bietet eine Möglichkeit polare bis sehr polare Analyten chromatographisch zu separieren. In Verbindung mit einem hochauflösenden Massenspektrometer können zudem verschieden isotopenmarkierte Analyten unterschieden werden. Die Entwicklung einer Trennung wurde mit Standards auf einer HILIC-Säule durchgeführt, während die Detektion im negativen ESI-Modus erfolgte.

Die Anwendbarkeit der entwickelten Methode wurde anhand von Algenextrakten getestet, die aus *Chlamydomonas reinhardtii*-Kulturen nach Zellaufschluss und anschließender Flüssig-Flüssig-Extraktion gewonnen wurden. Zum Aufschluss der Zellen wurden verschiedene Verfahren evaluiert, die die Zellwände mechanisch zerstören, um die intrazellulären Metabolite für die Extraktion zugänglich zu machen.

Ergebnisse

Mithilfe der HILIC konnte eine Trennung der zumeist sehr polaren Cofaktoren erfolgreich entwickelt werden. Bei der Entwicklung der Trennung wurden neben verschiedenen HILIC-Säulen auch Mixed-Mode-Säulen getestet. Dazu wurden sowohl die Zusammensetzung der mobilen Phase als auch die Pufferkonzentration und der pH-Wert des Puffers verändert.

Zur optimalen Trennung wurde der Anteil des organischen Lösungsmittels sowie die Pufferkonzentration in der Probenlösung variiert. Bei dieser Untersuchung konnten die besten Ergebnisse mit einem Acetonitril-Anteil von 75% und einem 50 mM Ammoniumacetat-Puffer (pH 5,76) erreicht werden.

Die Modellkultur *Chlamydomonas reinhardtii*, bereitgestellt von der Arbeitsgruppe von Prof. Michael Hippler (Westfälische Wilhelms-Universität Münster, Institut für Biologie und Biotechnologie der Pflanzen), wurde dazu verwendet verschiedene Aufarbeitungsmethoden miteinander zu vergleichen. Zum einen wurde ein Zellaufschluss nach Wase *et al.* mit Methanol, Chloroform und Wasser durchgeführt.[3] Zum anderen wurde ein Aufschluss mittels Kugelmühle und anschließender Flüssig-Flüssig-Extraktion mit Wasser und Chloroform angewendet.[4] Weiterhin wurde der Aufschluss mit einem Nebulizer sowie durch Ultraschall durchgeführt.

Das Potential der vorgestellten Methode zur ^{13}C - bzw. ^{15}N -basierten Flussanalyse wird am Beispiel der Bestimmung der Markierungsgrade von Metaboliten in Algenextrakten demonstriert.

Neuer Aspekte

HILIC-ESI-MS Trennung von zehn polaren Redox-Cofaktoren in einem Lauf zur Flussanalyse mittels LC-ESI-MS.

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Bringing Triple Quadrupole Detection Limits to a New Standard –in Theory and in Practice

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Stichworte: S/N, IDL, detection limits, instrument detection limits

Einleitung

We report on the use of an instrument detection limit (IDL) based on random standard deviation (RSD) of a series of replicates as a universal measure of “ion efficiency” and analytical utility. The IDL methodology for LC/MS is illustrated for the analysis of samples from food safety and environmental applications and data show a significant improvement in limits of quantitation. The obtained IDL levels are therefore independent of the noise measurement as long as the noise comes as a small fraction of the measured signal.

Experimenteller Teil

Sample preparation

Extracts of black tea obtained using QuEChERS and dispersive SPE were diluted 1:20 with acetonitrile and then spiked with several pesticides presenting high risk relevance in the concentration range of 0.2ppt to 100ppb. Steroids spiked in neat solvents were directly injected into the mass spectrometer using an injection volume of 20 μ L. Chromatography was performed under gradient conditions using 2.1x100mm columns-Eclipse-Plus C18 (1.8 μ m particle size) and Poroshell120 (2.7 μ m particle size) for pesticide and steroid analysis, respectively.

Ergebnisse

More specifically, due to the random nature of the ion flow to the detector the standard deviation (SD) of the number of arrivals in a unit of time follows that of the Poisson distribution, and depends on the average number of ions N as

Neuer Aspekte

S/N definition compared to Instrument Detection Limit (IDL) methode

Identification of urinary peptides triggering social behavior of *Danio Rerio* by mass spectrometry

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Stichworte: Peptides, zebrafish, MHC, olfactory signals, chemical signals

Einleitung

Imprinting in zebrafish is a learning process that is limited to a short time period during early development and leads to an irreversible change in behavior [1]. In a narrow time window (day 6 post fertilization) zebrafish larvae imprint on olfactory cues of siblings, which results in a preference of kin over non kin [2]. The biochemical mechanisms underlying this complex process are poorly understood. Particularly, the triggers which are necessary to impress a lifelong memory remain elusive. However, accumulating data suggests that major histocompatibility complex (MHC) dependent peptides, released by the urine, are implicated in olfactory imprinting.

Experimenteller Teil

We kept different group of siblings (30 zebrafishes) in 1.5 liters freshwater for 24 hours. Subsequently we performed a solid-phase extraction to enrich the peptide concentration and to separate them from electrolytes and low-molecular matrices. This extract was probed with an ESI-Q-TOF mass spectrometer to get distinct primary peptide sequences out of the diverse mixture of molecules. Afterwards the zebrafish groups were genotyped for their MHC class II alleles by SSCP gel to find secreted peptide specific for the respective MHC background.

Ergebnisse

Proteins of the major histocompatibility complex (MHC) play a fundamental role in discriminating ‘self’ and ‘non-self’ in the immune system by presenting pathogen-derived short peptides to lymphocytes. In vivo calcium imaging shows, that some olfactory bulb neurons are highly tuned to MHC peptides with a detection threshold at 1 pM or lower, demonstrating that fish can smell MHC peptides at physiological concentration [3]. The aim of this project is to identify putative signal peptides in the urine of zebrafish involved in olfactory imprinting. In comparison with several zebrafish families with different MHC background, we want to limit the identified set of urinary peptide to single peptides specific for the respective MHC type.

Neuer Aspekte

For the first time we were able to identify peptides in the urine of zebrafish presumably involved in olfactory imprinting.

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Advancements in (MA)LDI based mass spectrometry imaging techniques in the field of chemical ecology

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Stichworte: MALDI MSI, AP-SMALDI MSI, Drosophila melanogaster, surface lipids, cVA, CH5O3, 1, 8-Di(piperidiny)-naphthalene, 14-Diaza[5]helicene

Einleitung

The field of chemical ecology investigates the various ways in which organisms interact chemically. For example symbioses, parasitism, chemical defense agents, attractions of pollinators etc. Imaging techniques are important tools providing information about the exact location of production, storage and release of these chemicals. Especially techniques based on matrix-assisted laser desorption ionization mass spectrometry (MALDI MS) are suited for the analysis of wide spectrum of biological samples (e.g. plants, insects, microorganisms) giving high spatial resolution and detailed information about the chemical compounds present in the tissues, cells or other biological structures.

Experimenteller Teil

Despite of the advancement of these MALDI based techniques the low amount of present compound and the 3-D character of the sample require an improvement in sensitivity and prevention of mass shift¹ because of the non-planar character of the sample. Additionally we are focused on small molecules, some of which are not easily ionizable. Therefore we were designing and screening for novel matrix systems to improve sensitivity and expand the scope of possible analytes. Furthermore we are employing state of the art of hardware e.g. AP-SMALDI Orbitrap based MS to push the limits in terms of spatial and mass resolution, giving new insights in several ecologically relevant issues.

Ergebnisse

1) We found that the simply pre-purified commercially available diatomaceous earth² (Celite) applied by spraying, mediates the laser desorption ionization (LDI) of surface lipids on *Drosophila melanogaster* flies. Using this approach, very clean mass spectra were obtained without any interfering signals which are related to Celite.

2) Two novel matrices, 1,8-Di(piperidiny)-naphthalene and 1,14-Diaza[5]helicene³, showed improved sensitivity especially towards lipid based analytes. Furthermore these matrices showed high vacuum stability making them useful for prolonged MALDI MSI experiments in a negative mode. Owing to their superbasic nature they are able to deprotonate weak acidic analytes in solution already during sample preparation. Thus the ionization takes place prior to the laser desorption. The matrices themselves are not deprotonated, which prevents the formation of interfering signals.

3) MALDI MSI technique was used to investigate cross-feeding interactions in bacteria biofilms via spatial distribution of amino acids (histidine and tryptophan) which were formed into crystals on the surface of the biofilm. A synthetic system was constructed between *Acinetobacter baylyi* and *Escherichia coli*, in which both strains reciprocally exchange amino acids. The MALDI MSI results were supported by HR MS analysis of surface crystals. The spatial distribution of cocultured cross-feeding cooperators was investigated by fluorescence microscopy.

Neuer Aspekte

Commercially available diatomaceous earth Celite was successfully used for ionization of embedded molecules in LDI MSI experiments of *D. melanogaster*.

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Determination of the platinum distribution in *Caenorhabditis elegans* after Cisplatin incubation by LA-ICP-MS

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Stichworte: Bioimaging, LA-ICP-MS, C. elegans, Cisplatin

Einleitung

Cisplatin is one of the most important and frequently used cytostatic drugs within the treatment of cancer. The cytostatic effect is based on binding to the DNA in tumor cells by forming intra- and interstrand crosslinks. The associated DNA deformations and the interference with DNA repair mechanisms lead to apoptosis in cancer cells. Poly(ADP-ribose)polymerase-1 (PARP-1) is a protein that is involved in several biological pathways including DNA repair mechanisms. The nematode *Caenorhabditis elegans* (*C. elegans*) is occupying the poly(ADP-ribose)metabolism enzyme 1 (pme-1), which is an ortholog of human PARP-1. Loss of this enzyme is resulting in a disturbed DNA damage response. The bioavailability of Cisplatin in *C. elegans* was analyzed by laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS).

Experimenteller Teil

Experiments were performed using a laser ablation system based on a Nd:YAG laser with a wavelength of 213 nm, coupled to a quadrupole-based inductively coupled plasma mass spectrometer. Besides the dry aerosol, a ruthenium solution as the internal standard was simultaneously introduced, to improve the plasma stability. Possible interferences of e.g. [⁴⁰Ar¹⁵⁵Gd] were minimized in the kinetic energy discrimination mode (KED) with a He/H₂ gas mixture as cell gas. The ablation of the nematodes was performed in a multiline scan. Laser parameters were optimized to obtain optimal spatial resolution and elemental information. Furthermore, to validate these data a total reflection x-ray fluorescence (TXRF) method was applied.

Ergebnisse

The distribution of platinum in *C. elegans* after Cisplatin incubation was determined. Therefore, L4 stage wildtype worm and pme-1 deletion mutants were treated with different concentrations of Cisplatin. Characteristics of this animal, such as its genetic manipulability and the well-characterized genome makes it a suitable model system. The uptake of Cisplatin was investigated by visualization of the distribution of platinum. Thus, images with a lateral resolution of 5 µm were generated. The elemental mapping indicated that Cisplatin is located in the head of the worms. Furthermore, it was shown that higher Cisplatin concentrations and longer incubation times led to higher platinum concentrations within the worm.

Neuer Aspekte

The uptake and accumulation of Cisplatin in *C. elegans* were analyzed successfully by LA-ICP-MS.

Exploring the head and neck cancer by MALDI FT-ICR mass spectrometry imaging

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Stichworte: MALDI, MSI, FT-ICR, Head and neck, Cancer

Einleitung

Head and neck cancer comprises of malignant tumors located in the oral cavity, the throat, and the upper aerodigestive tract. To improve individualized therapy, the spatial molecular characterization is crucial. The aim of this study is to use MALDI mass spectrometry imaging (MALDI imaging) to discover the spatial lipidome of head and neck cancer.

Experimenteller Teil

MALDI imaging enables the simultaneous analysis of hundreds of molecular compounds in a single measurement. In this study, head and neck cancer samples have been analyzed with a Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometer in the mass range of m/z 200 to 1,500 and a spatial resolution of 50 μm . Consecutive sections were stained by H&E and co-registered with the MALDI imaging datasets. For computational evaluation the spatial segmentation approach was used which provides spatial features by statistically determine similarities between spectra. The resulted segmentation map was overlaid with the histological image and validated by an experienced pathologist. A subsequent comparative analysis yielded discriminating m/z values.

Ergebnisse

We performed the MALDI FT-ICR imaging workflow together with the computational evaluation on four different cancer samples. The high mass resolution of the FT-ICR imaging datasets yielded large data sets, each of a size of about 100 GB. The clusters of the spatial segmentation coincide with morphological structures apparent in the microscopy images. Subsequently, co-localized masses have been found, which discriminate prominent morphological structures.

The results show that the morphological changes observed in the investigated head and neck tumor samples are predominantly reflected in the MALDI FT-ICR imaging data. We found m/z values that discriminate different morphological areas. The high mass accuracy and high mass resolving power of FT-ICR mass spectrometer enables to match the discovered peaks against lipidomics databases.

Neuer Aspekte

The spatial segmentation approach applied for lipidomic analysis reveals new insights into the metabolic organization of carcinoma tissue.

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Laser ablation dielectric barrier discharge imaging MS for direct molecular analysis of pharmaceuticals in tablets

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Stichworte: Laser ablation, molecular imaging, Pharmaceuticals, Dielectric barrier discharge, small molecules

Einleitung

Worldwide 10% of all used drugs are counterfeits.[1] To identify counterfeits, several parameters, e.g., the total amount and lateral distribution of the active pharmaceutical ingredient (API), appearance, packing or excipients can be compared with the original product. While most of these parameters can be analyzed by several analytical methods, the analysis of the surface distribution of the API is generally limited to spectroscopic methods and MALDI-MS.[2]

Laser ablation (LA) coupled to a dielectric barrier discharge interface (DBDI) and orbitrap MS is used to obtain high resolution molecular images. The information of these imaging experiments can be used to identify counterfeits via their API distribution and gather further information of the ingredients homogeneity.

Experimenteller Teil

Two commercially available pain killers with the API caffeine (50 mg), acetylsalicylic acid (250 mg) and acetaminophen (200 mg) were used as test substances. These tablets were placed inside the ablation cell without any sample pretreatment. A 213 nm Nd:YAG laser with a spot size of 25 μm is used to linewise ablate material from a surface. This material is transported with a nitrogen gas stream to a dielectric barrier discharge where it is ionized. The ions are detected by a high resolution orbitrap mass spectrometer. Extracted ion chromatograms of the API are aligned with a housemade software to generate high resolved molecular images in mass and space.

Ergebnisse

The commonly used methods to analyze the lateral API distribution comprise some disadvantages. LA-DBDI-MS is able to overcome these limitations. The high resolution mass spectrometer offers a new dimension of sample information in contrast to the spectroscopic methods. The exact m/z value allows the identification of an API by its accurate mass. To gather this information, contrary to MALDI experiments, no sample pretreatment, such as matrix application, is needed.

Neuralgin® and Thomapyrin® are analyzed as test substances by LA-DBDI-MS. The two respective active pharmaceutical ingredients are caffeine, acetylsalicylic acid and acetaminophen with identical concentration, although different excipients are used. It is investigated whether this is reflected in the surface distribution of the API. Therefore, molecular images of caffeine, acetylsalicylic acid and acetaminophen are recorded for both, Neuralgin® and Thomapyrin® and compared regarding their surface homogeneity.

It was shown that LA-DBDI-MS is a suitable tool for molecular imaging and that this method is promising to identify counterfeit drugs by deviations in the lateral distribution of the API compared to the original product. Furthermore, this method could help to optimize manufacturing routes to increase the homogeneity of the active components and thereby improve the intake into the human body and the stability of the tablet.

Neuer Aspekte

High resolved molecular MS imaging of small molecules in pharmaceutical preparations by LA-DBDI-MS without sample pretreatment.

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Improved spatial resolution in the analysis of FFPE tissue after tryptic digestion

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Stichworte: imaging, FFPE, MALDI, brain tissue

Einleitung

In recent years, MALDI imaging has proven to be a powerful method for clinical research. Although imaging of protein distributions has had substantial impact in this area, three challenges does still have to be addressed: the detection of high molecular weight (>30kDa), the identification of m/z signals and the accessibility of formalin-fixed, paraffin embedded (FFPE) tissues. Trypsin digestion is seen as a promising approach for improving the above points and we present here an update on our efforts to develop a robust protocol for on-tissue digestion.

Experimenteller Teil

Mouse and rat brains tissue sections were mounted on conductive glass slides. Digestion was preceded by paraffin removal and antigen-retrieval treatment. Enzyme and matrix application was performed by a piezoelectric sprayer. Incubation at elevated temperatures (37 – 50 °C) was conducted in a custom-made humid chamber. MS data was acquired on a MALDI-TOF instrument operating in reflector mode. Results were visualized and evaluated using commercially available software.

Ergebnisse

On-tissue digestion adds more complexity to sample preparation for MALDI Imaging, which makes development of a robust protocol challenging. Spatial resolution, number of peptides generated and identification rate are considered important for a successful experiment, but optimizing these can be diametrically opposed. We found that high humidity and extended digestion time increased the effectiveness of the digestion, but also on-tissue delocalization of peptides. Short (<2h) incubation at above-physiological temperature (50 °C) was determined as a good compromise between digestion efficiency and peptide delocalization. Low NH_4HCO_3 concentration helped to preserve tissue integrity, while elevated TFA concentration facilitated peptide ionization. We also demonstrate how detection of spatially co-localized peptides by Pearson correlation can lend an additional level of confidence to identification results.

Neuer Aspekte

An optimized protocol for on-tissue digestion, considering several parameters to achieve efficient digestion at good spatial resolution for FFPE samples.

Untersuchung der Verteilung von Silbernanopartikeln nach oraler Gabe im Gastrointestinaltrakt von Ratten mit LA-ICP-MS

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Stichworte: Element-Bioimaging, Einzelpartikelanalyse, Nanopartikel, Silber, LA-ICP-MS

Einleitung

Silbernanopartikel (AgNP) finden wachsende Anwendung sowohl im Gesundheitssektor, als auch im täglichen Leben. Beispiele sind Wundverbände, Operationsbesteck und Prothesen, die AgNP enthalten oder mit ihnen beschichtet werden,^[1] sowie den Einsatz in Raumsprays, Waschmitteln und als Überzug oder Imprägnierung in der Kleidung. Dabei soll die antibakterielle Wirkung gegen geruchsverursachende Bakterien in Unterwäsche und Socken helfen.^[2] Den häufigsten Expositionsweg der AgNP stellt die orale Aufnahme dar. Um mehr über die Verteilung im Körper zu erfahren, werden Ratten oral AgNP verabreicht und die Verteilung der AgNP in den Organen des Gastrointestinaltraktes wird bildlich dargestellt. Die am weitesten verbreitete Methode zur ortsaufgelösten Bestimmung von Spurenelementen ist die Laserablation mit induktiv gekoppeltem Plasma (LA-ICP-MS).^[3]

Experimenteller Teil

Dünnschnitte des Magens und der drei Darmabschnitte Duodenum, Jejunum und Ileum von Ratten wurden mittels LA-ICP-MS auf die Verteilung der Silbernanopartikel untersucht. Dabei handelt es sich um Organe von Ratten, denen 1000 mg AgNP pro Kilogramm Körpergewicht verabreicht wurde. Die Schnitte wurden linienweise mit einer Messfleckgröße von 5µm und einem Vorschub von 10µm/s ablatiert. Die Messungen wurden im Einzelpartikelmodus des Massenspektrometers mit einer Messzeit von 10 ms durchgeführt. Es wurde eine Kalibrationsreihe mit Silbernanopartikeln verschiedenen Durchmessers in Leber und Gelatine erstellt. Zusätzlich wurden Schnitte der entsprechenden Magen- und Darmabschnitte von Kontrolltieren untersucht, denen keine AgNP verabreicht wurden.

Ergebnisse

Die erhaltenen Intensitäten zu jedem Messpunkt wurden zu einem dreidimensionalen Bild zusammengesetzt. In allen untersuchten Abschnitten des Gastrointestinaltraktes der behandelten Ratten konnte Silber in unterschiedlichen Konzentrationen nachgewiesen werden. Die erhaltene Intensität eines Silbernanopartikel hängt direkt mit dem Volumen und somit dem Partikeldurchmesser zusammen. Durch die externe Kalibration mit Leber- und Gelatinestandards konnte so der Durchmesser der detektierten Partikel im Gewebe äquivalent zur Intensität berechnet werden.

Die Silberverteilung korreliert in allen Abschnitten gut mit der Struktur des Gewebes. In Magen, Duodenum und Jejunum ist Silber vornehmlich in der Mukosa zu finden. Dies zeigt, dass Partikel ins Gewebe aufgenommen werden. Im Duodenum sind in einem Bereich der Mukosa Signale mit einer sehr hohen Intensität zu finden. Das Spektrum dieses Bereichs zeigt keine Hinweise auf einzelne Nanopartikel. Daher könnte die hohe Intensität in diesem Fall auf die gleichzeitige Detektion mehrerer Partikel zurückzuführen sein. Alle anderen Bereiche zeigen mit der verwendeten Methode eine gute Auflösung der einzelnen Partikel mit einer durchschnittlichen berechneten Partikelgröße von 40-60 nm. Im Bereich der Muskelschicht konnte in keinem der Gewebeschnitte Silber nachgewiesen werden. Hingegen sind in allen Gewebeabschnitten Signale mit geringerer Intensität im äußeren Bereich des Gewebes nachweisbar. Das Spektrum zeigt hier keine Hinweise auf Nanopartikel. Diese Signale sind auch im Ileum zu finden, in dem in den anderen Bereichen kein Silber nachzuweisen ist. In den Gewebeschnitten der Kontrollratte konnte kein Silber nachgewiesen werden. Daher kann davon ausgegangen werden, dass detektiertes Silber in den Proben der behandelten Ratte ausschließlich auf die Fütterung mit den AgNP zurückzuführen ist.

Neuer Aspekte

Anwendung der Einzelpartikelanalyse im Imaging.

Auflösung einzelner Silbernanopartikel in Gewebeschnitten.

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Quantitative Bioimaging of Pd-tagged Photosensitizers in Tumor Spheroids by LA-ICP-MS

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Stichworte: Imaging, LA-ICP-MS, Tumor Spheroid, Photosensitizer

Einleitung

Photosensitizers are frequently used as drugs in photodynamic therapy. Due to accumulation of the photosensitizer within malignant tissue and subsequent illumination with light of a specific wavelength, highly reactive oxygen species arise, which are able to induce apoptosis of the tumor cells. The distribution of the photosensitizer in tissues can be monitored by fluorescence microscopy. However, the major restrictions of this technique are its high limits of detection. Laser ablation coupled to inductively coupled plasma mass spectrometry offers much higher sensitivity enabling the investigation of the fate of the photosensitizers. Tagging of the drugs with Pd enabled the detection by means of ICP-MS.

Experimenteller Teil

Experiments were performed with a 213 nm Nd:YAG laser using a spot diameter of 10 μm , which was coupled to the ICP-MS system. For examination and stabilization of the plasma conditions, a ruthenium solution was introduced as internal standard into the stream of the dry aerosol coming from the laser ablation unit. Possible interferences of e.g. $[\text{}^{40}\text{Ar}+\text{}^{64}\text{Cu}]^+$ were minimized by utilization of a collision/reaction cell in kinetic energy discrimination (KED) mode with He/H₂ as gas mixture. The visualization was achieved by a line by line scan and subsequent merging. Laser parameters, including pulse energy, scan rate, and repetition frequency, were tuned such that optimal signal and spatial resolution were obtained.

Ergebnisse

The quantitative distribution of Pd in biological matrices was determined. Spheroidal cell cultures were used as a model system for malignant tissues and were incubated with different concentrations of Pd-tagged photosensitizers. Additionally, effects of the dosage form of the photosensitizer were elucidated by incubation of the tumor spheroids with the pure substance and poly(lactic-co-glycolic acid) (PLGA) nanoparticles. The incubated cells were embedded and sliced into 5 μm thin cryosections. The cellular uptake and intracellular accumulation of the drugs was investigated by visualization of the distribution of Pd. Thus, images with a lateral resolution of 10 μm were generated. For quantification, matrix-matched standards based on gelatin were prepared. The enrichment of the drug occurs within the first cell layers of the spheroid. In case of incubation with the pure substance an accumulation of the drug in specific areas can be shown, while the nanoparticles are distributed more homogenously.

Neuer Aspekte

The examination of cellular uptake and intracellular accumulation of Pd-tagged drug delivery systems using LA-ICP-MS is presented.

AP-SMALDI mass spectrometry imaging of metabolites in insects using high resolution in mass and space

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Stichworte: Paederus riparius, Harmonia axyridis, insects, high-resolution mass spectrometry imaging, MALDI Imaging

Einleitung

Insects have tremendous taxonomic and molecular diversity and are among the most successful organisms on earth. Due to this high diversity, insects are highly attractive as biological resources. The synthesis, transportation, and storage of metabolites in insects are still unclear. Chemical imaging by mass spectrometry provides non-targeted, label-free imaging, capable of visualizing hundreds of metabolites in a single scan. Here, we present atmospheric pressure high-resolution scanning microprobe matrix assisted laser desorption/ionization mass spectrometry imaging (AP-SMALDI MSI) as a technique for whole-insect tissue imaging for visualizing the distribution of metabolites in tissue sections and organs of *Paederus riparius* and *Harmonia axyridis*.

Experimenteller Teil

Tissue sections were obtained using a cryotome after optimizing various preparation techniques, such as using carboxymethyl cellulose (CMC) or tragacanth gum as embedding medium. Matrix was applied using a high-resolution matrix-preparation robot (SMALDIPrep, TransMIT GmbH, Giessen, Germany). An atmospheric-pressure high-resolution MALDI imaging source (AP-SMALDI10, TransMIT GmbH, Giessen, Germany) was used for ion imaging. The source was coupled to an orbital trapping mass spectrometer (Exactive or Q Exactive Orbitrap, Thermo Fisher Scientific GmbH, Bremen). Mass resolution of the orbital trapping mass spectrometer was set to 140,000 at m/z 200.

Ergebnisse

High-quality m/z images with a bin width of ± 5 ppm, based on high lateral resolution and high mass accuracy, were generated using the imaging software package 'Mirion'. All measurements were performed with a mass accuracy of better than 2 ppm (root mean square of mass error). Organ specific compounds were identified for brain, nerve cord, eggs, gut, ovaries and malpighian tubules of *Paederus riparius*. Three ganglia of the nerve cord, which feature dimensions of 250x500 μm , were measured with 10 μm spatial resolution. In every single experiment, mapping of different compound classes was possible. This approach improved the understanding of metabolite distributions in the insects. Concentration differences and distributions of defensive compounds and its analogues could be visualized in whole-insect transverse sections. Without labeling, different key lipids, specific for individual organs, were assigned with a resulting morphological-structure specificity higher than with staining or immune-histological methods.

Neuer Aspekte

Sample preparation for insect tissue sections. Understanding metabolite distributions in insects by using high resolution in mass and space.

Differentiating Macrophages in atherosclerotic plaques using matrix-assisted laser desorption/ionization mass spectrometry imaging

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Stichworte: MALDI Imaging, Macrophages, Atherosclerosis

Einleitung

Macrophages are large phagocytes playing myriads of roles in innate and adaptive immunity. Multiple properties show that macrophages are by far more than just immune cells. They are present during the entire process of tissue repair and/or remodeling. To better understand their roles and functions, macrophages are classified into several subpopulations, according to their polarization state *in vitro*. These phenotypes are likely not to correspond to what occurs *in vivo*, but can be taken as an approach to describing differentiation and classification. Distinguishing macrophages in tissue samples, such as atherosclerotic plaques, by using classical histological methods, is a difficult though vital task for understanding mechanisms of plaque formation. Mass spectrometry imaging (MSI) is an alternative approach and an important complement.

Experimenteller Teil

Three types of macrophages, M0 (non-polarized macrophages), M1 (pro-inflammatory) and M2 (anti-inflammatory) macrophage cells were applied to a glass slide by pipetting a cell solution and then allowing to dry. The dry samples were covered with a matrix (2,5-dihydroxybenzoic acid) using a pneumatic sprayer (SMALDIprep, TransMIT GmbH, Giessen, Germany) prior to analysis. The cells were measured with a high-lateral-resolution AP MALDI source (AP-SMALDI10, TransMIT GmbH, Giessen, Germany), coupled to a high-mass-resolution orbital trapping mass spectrometer (Q Exactive, Thermo Fisher Scientific GmbH, Bremen, Germany). A lateral resolution of 50 to 100 μm in a mass range of m/z 300-1600 was chosen for the described experiments.

Ergebnisse

In this work several macrophage phenotypes, which were activated by different stimulants *in vitro*, could be distinguished based on their lipid profile, obtained from atmospheric-pressure matrix-assisted laser desorption/ionization mass spectrometry imaging (AP-MALDI-MSI) experiments [1]. Selected ion images were generated with the imaging software packages Mirion (V24_64_63) and MSiReader. Ion images with a mass error below 3 ppm were subjected to principal compound analysis (PCA) in order to find lipid markers for each macrophage type. The results show a sufficient discrimination of the three macrophage phenotypes, and potential markers were found.

Neuer Aspekte

Differentiation of macrophages by using MALDI mass spectrometry imaging

Referenzen

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LC-ICP-MS and LC-ESI-MS for the Speciation Analysis of Iodine

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Stichworte: Speciation Analysis, Quantification, Thyroid Hormones

Einleitung

The thyroid gland controls many metabolic reactions in the human organism through the production of hormones influencing the formation of proteins in different cells. Two active forms of thyroid hormones, thyroxine and 3,5,3'-triiodothyronine, are formed by a reaction of the glycoprotein thyroglobulin with iodine. Those and the inactive form 3,3'-diiodothyronine occur in selected tissue, where the ratio among the three compounds provides information about the activity of thyroid hormones. Their analysis in selected tissues can thus help to understand the iodine balance in humans. The required amount of iodine uptake is 180-200 µg/day, while an excess of iodine, e.g. resulting from the ingestion of iodine-based pharmaceuticals, may cause serious problems for the thyroid gland, resulting in hyperthyroidism.

Experimenteller Teil

Liquid chromatography coupled to an electrospray ionization-mass spectrometer (LC-ESI-MS) is used for the identification of unknown iodine species. Due to the substance-specific ionization efficiency of the species, quantification with this method is difficult, while liquid chromatography-inductively coupled plasma-mass spectrometry (LC-ICP-MS) allows the precise quantification even of unknown species. As plasma stability is affected by a varying composition of the mobile phase, a gradient compensation system is introduced to ensure a constant composition of the mobile phase.

Ergebnisse

Using liquid chromatography with a C18 reversed phase column, a sufficient separation of thyroxine, 3,5,3'-triiodothyronine and 3,3'-diiodothyronine within ten minutes was achieved. An analytical gradient elution program was optimized and a mixture of the three hormones was resolved and then identified by the on-line coupled electrospray ionization-high resolution-mass spectrometry. For a quantification a counter gradient after separation was applied. Thus, a constant ratio of the mobile phases was introduced into the ICP-MS. Since a constant response of the ICP-MS respecting to different iodine species, 2-amino-3-iodophenol was selected to be the internal standard.

Neuer Aspekte

A method for the identification and quantification of iodine-containing species using LC-ESI-MS and LC-ICP-MS was developed.

Resonanzverstärkte Multiphotonenionisation von Triethylbenzolen

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Stichworte: REMPI, 1, 3, 5-Triethylbenzol, 2, 4-Triethylbenzol

Einleitung

1,3,5-Triethylbenzol wird häufig als supramolekulares Modellsystem verwendet, da es eine Reihe von schwachen Wechselwirkungen ausüben kann, die in der Summe dazu führen ein Gast-Moleküle stark und spezifisch zu binden [1]. 1,3,5-Triethylbenzol kann über Präorganisation enthalpische Effekte ausbilden, die energetisch ungünstige Bindungskonformationen in Komplexen ausgleichen. Obwohl es häufig verwendet wird, ist über die elektronischen Eigenschaften des Moleküls wenig bekannt. Die Ionisierungsenergie wurde mit Cyclo-Voltammetrie bestimmt [2].

Im Vergleich zu dem 1,3,5-Triethylbenzol hat das 1,2,4-Triethylbenzol eine stark unterschiedliche räumliche Struktur und kann damit weniger gerichtete Interaktionen auf Komplexpartner ausüben. Um diese Effekte zu untersuchen wurden 1,3,5- und 1,2,4-Triethylbenzol mittels resonanzverstärkter Multiphotonen-ionisation (REMPI) vermessen und somit konnten Aussagen über ihre vibronische Struktur getroffen werden.

Experimenteller Teil

Es wurde ein Molekularstrahlexperiment an einem modifizierten ReTOF (*Bruker*) durchgeführt. Die verwendeten Substanzen wurden ohne weitere Aufreinigung verwendet (*Sigma Aldrich*). Der Molekularstrahl wurde mittels eines gepulsten Ventils und Argon (Hintergrunddruck 3-4 bar) erzeugt. Der Probenbehälter kann geheizt werden. Zur Anregung und (1+1)-Ionisierung der Triethylbenzole wurde ein von 265 nm bis 280 nm durchstimmbarer Farbstofflaser (Coumarin 153, Scanmate, *Lambda Physics*) verwendet, der von einem frequenz-verdoppelten Nd:YAG-Laser mit 355 nm (*SpitLight, InnoLas*) gepumpt wird. Die Messungen wurden von einer im Arbeitskreis programmierten Software über ein Oszilloskop (LSA1000Series, *LeCroy*) und eine MCP aufgenommen [3].

Ergebnisse

Die Triethylbenzole konnten erstmals wellenlängenabhängig untersucht werden. Durch die vom 1,3,5-Triethylbenzol verschiedene räumliche Struktur des 1,2,4-Triethylbenzols sind die möglichen Interaktionen mit Gast-Molekülen andere, was zu einer anderen Komplex-Ausbildung führt. Im Massenspektrum treten bei beiden Isomeren neben dem Molekülion unerwartete Methyl- und Ethyl-Fragmente in hoher Intensität auf. Diese wurden bezüglich ihrer vibronischen Struktur untersucht und mit den vibronischen Strukturen der Triethylbenzole und mit der von Mesitylen verglichen. Außerdem wurden die Abhängigkeiten der Fragmentbildung von der Temperatur und der eingestrahlten Laserleistung untersucht.

Neuer Aspekte

REMPI-Spektren von 1,3,5-Triethylbenzol, 1,2,4-Triethylbenzol und ihren Fragmenten.

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Photofragmentierung von Azofarbstoffen im sichtbaren Wellenlängenbereich

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Stichworte: FT ICR-MS, ESI, Photofragmentierung, Azofarbstoffe, Kinetik

Einleitung

Photonen des sichtbaren Bereichs des elektromagnetischen Spektrums führen in der Regel zu Schwingungsanregungen im Molekül. Sobald die eingestrahlte Energie nicht mehr im Molekül umverteilt werden kann, kommt es zu Fragmentierungen [1]. Besonders gut wird sichtbares Licht von farbigen Molekülen, z. B. Azofarbstoffen, absorbiert.

Das Interesse gilt hierbei dem Mechanismus, nach dem eine Fragmentierung abläuft [2]. Vor allem dann, wenn es sich um Abspaltungen handelt, die nicht ohne weiteres durch die Molekülstruktur erklärt werden können, wie es bei den untersuchten Azofarbstoffen der Fall ist. Hierbei helfen auch die Aufnahmen kinetischer Messreihen, um die Anzahl absorbierter Photonen und kinetische Konstanten bestimmen zu können.

Experimenteller Teil

Alle Massenspektren wurden mit einem Bruker APEX III FT-ICR Massenspektrometer mit einem 7.05 T Magneten (*Bruker Daltonik*, Bremen) aufgenommen. Die Proben wurden in EtOH gelöst, in MeOH/H₂O verdünnt und mittels ESI-Quelle in die Gasphase gebracht.

Die Fragmentierung erfolgte durch Laseraktivierung (VisPD) in der ICR-Zelle. Hierfür wurden die entsprechenden Ionen in der Zelle gespeichert und isoliert. Ein Argon Ionen cw-Laser (*Innova 70, Coherent*, Santa Clara, CA, USA) im singleline Modus bei 488 nm wurde zur Fragmentierung verwendet. Über einen selbstgebauten Shutter konnte die Einstrahldauer des Lasers reguliert werden. Zur Aufnahme der Kinetiken wurde ein Bereich von 0.01 bis 3.0 s abgedeckt.

Die Farbstoffe wurden durch Azokupplung der Anilinderivate oder durch Alkylierung von 2-Aminoazotoluol synthetisiert.

Ergebnisse

Das mit Abstand größte Fragment bei carbonsäurehaltigen Azofarbstoffen ist der Verlust von Wasser aus dem protonierten Molekülion. Hierfür muss der Farbstoff an der Carbonsäuregruppe protoniert werden und die C-O-Bindung brechen. Untersuchungen mit Deuteriumoxid bestätigen diese Vorgänge in den unterschiedlichen Molekülen.

Auffällig ist, dass die weiteren Fragmente nur durch kombinierte Verluste erklärbar sind. So tritt beim Ethylrot ein Fragment auf, das in Summe einer Abspaltung von C₃H₁₀O entspricht. Der kombinierte Verlust von C₃H₈ und H₂O ist die wahrscheinlichste Erklärung dieses Fragments. Der Verlust von C₃H₈ aus dem protonierten Molekülion kann nicht beobachtet werden. Des Weiteren zeigen MS³-Untersuchungen keinen Verlust von C₃H₈, wenn Wasser schon abgespalten wurde. Dies wird weder nach erneuter Laserbestrahlung noch durch Stoßaktivierung erreicht. Demnach spricht an dieser Stelle alles für einen konzertierten Fragmentierungsmechanismus. Hinweise für einen konsekutiven Mechanismus gibt es keine. Es ist jedoch denkbar, dass nach dem Wasserverlust zwei isomere Produkte entstehen. Zum einen das thermodynamisch stabile, welches beobachtet wird, zum anderen ein labiles, welches so schnell weiter reagiert, dass eine Beobachtung im FT-ICR-MS nicht möglich ist.

Die starke Favorisierung des Wasserverlustes bei Protonierung der Carbonsäure lässt nicht auf einen weiteren Protonierungsort schließen, was konsistent mit dem Fehlen von Fragmenten ohne Kombination mit dem Wasserverlust ist. Eine Umverteilung der Energie über die Schwingungsniveaus des Moleküls ist eine mögliche Erklärung weiterer Fragmentierungswege. Im Mittel sind alle Ionen durch die schnelle Zyklotronbewegung dem gleichen Photonenfluss ausgesetzt.

Durch unterschiedlich lange Einstrahlzeiten des Argon Ionen Lasers werden kinetische Profile der unterschiedlichen Fragmente erhalten. Durch Bestimmung von Ratenkonstanten einzelner Reaktionskanäle ist ein besseres Verständnis des gesamten Reaktionsweges und damit auch des Fragmentierungsverhaltens von Azofarbstoffen möglich. Dies zeigt, dass für den Verlust von Wasser aus diesen Farbstoffen die Absorption eines Photons ausreichend ist.

Neuer Aspekte

Aufnahme von kinetischen Profilen der Fragmente aus diversen Azofarbstoffen und Bestimmung der entsprechenden Ratenkonstanten.

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Revisiting Classical MALDI Matrices: The Effect of Laser Wavelength

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Stichworte: MALDI, Matrix, Laser wavelength, Absorption, Ion yield

Einleitung

Either N₂ or frequency-tripled Nd:YAG-lasers are typically used for UV-MALDI mass spectrometry. However, their emission wavelengths of 337 and 355 nm are not necessarily matching the peak absorption of some particular powerful MALDI matrices. Numerous studies have shown that an optimal UV-MALDI performance is only obtained when the laser wavelength coincides with a high optical absorption [1,2]. Here we determined the absorption profiles of selected matrices in solution and in solid state. By use of a wavelength-tunable UV-laser we compared the performance characteristics of selected compounds at standard and optimized wavelengths. Adjustment of the excitation wavelength could improve the limits of detection and therefore offer new possibilities for sensitive MALDI mass spectrometry.

Experimenteller Teil

Solid state absorption spectra were obtained in diffusive reflection geometry using an integrating sphere (SR-260, Shimadzu) and a dual beam spectrophotometer (UV-2102PC, Shimadzu). MS experiments were conducted with a prototype orthogonal-extracting time-of-flight mass spectrometer [1]. A wavelength-tunable (λ = 213 to 2550 nm) optical-parametric oscillator (OPO) laser with 5 ns pulse duration (versaScan, GWU Lasertechnik) served for desorption/ionization. To obtain precisely controlled irradiation conditions, the focal spot size of the laser beam (about ~150 x 300 μm²) was controlled with a diode array and the pulse energy monitored online. Samples were prepared from aqueous solutions using the dried droplet method. Analyte-to-matrix ratios were optimized for each system.

Ergebnisse

The spectrophotometric data show that numerous matrices for which excellent MALDI performances have been reported actually exhibit absorption profiles for which the absorption maxima are substantially shifted towards shorter wavelengths. In some cases, also a second maximum at higher wavelengths is found. For example, 9-aminoacridine, a matrix widely used for the analysis of lipids in the negative ion mode, has peak absorptions at 265 nm and 405 nm, and 2-mercaptobenzothiazole, a powerful matrix for glycolipids, exhibits a peak absorption at 320 nm. For both compounds the absorption is substantially lower at standard MALDI wavelengths. We present first data on the comparison of the MS performance of selected matrices and analyte systems at the two standard wavelengths of 355 nm, 337 nm and the individual optimal wavelength according to the absorption profile.

Neuer Aspekte

Solid-state absorption profiles of powerful UV-MALDI matrices and first data on the MALDI performance at the standard and optimized wavelengths.

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Influence of transfer capillary temperature on adduct formation in AP-MALDI MS

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Stichworte: adduct formation, ionization mechanism, atmospheric pressure MALDI

Einleitung

Adduct formation of analyte molecules with charge carriers other than protons is often observed with various ionization methods including atmospheric-pressure matrix-assisted laser desorption/ionization (AP-MALDI). Using laser desorption/ionization (LDI) it was found rather early that increasing the laser irradiance influences adduct formation, in a way that sodiated molecular ions overcome their protonated analogues in signal intensity. It was derived that a cluster decay mechanism might be responsible for the attachment.^[1] Parts of this mechanism later became part of the lucky-survivor model.^[2]

We observed similar effects in AP-MALDI experiments at high laser fluences with increasing temperature of the transfer capillary of an orbital trapping mass spectrometer. We assume a rather similar mechanism of cluster decay for these experiments.

Experimenteller Teil

Various matrices and analytes were used in combination with potential adduct ions in different concentrations. Solutions were mixed and sprayed onto glass slides for analysis.

Sample preparation was performed with a commercial matrix sprayer (SMALDIprep, TransMIT GmbH, Giessen, Germany) as used for MALDI mass spectrometry imaging, optimized for forming small matrix crystals in uniform and homogeneous layers. This leads to a high shot-to-shot reproducibility, allowing for a statistical data analysis.

A high-resolution MALDI imaging ion source (AP-SMALDI10, TransMIT GmbH, Giessen) was used in non-imaging mode on an orbital trapping mass spectrometer (Q Exactive, Thermo Fisher Scientific GmbH, Bremen). The temperature of the transfer capillary was altered while laser fluences were held as high as possible, for the described experiments.

Ergebnisse

It was found that with elevated laser fluences and increasing capillary temperature, sodium adduct signals of peptides tend to become stronger, compared to their protonated analogues. This eventually resulted in sodium adduct signals becoming more intense than signals of the protonated molecules. We also observed individual cases where sodium adduct signals increased without reaching the signal intensities of the protonated species and cases where both did not change in signal intensities.

The observations can be explained with a model of cluster formation upon desorption under laser irradiation. Here, molecular clusters, formed of analytes enclosed in a shell of matrix molecules, protons and other ions, undergo evaporation of the neutral, more volatile components, while sodium ions remain in the cluster because of their higher enthalpy of evaporation. With higher temperature of the surrounding atmosphere, larger clusters would eventually evaporate down to individual analyte molecules, which are then attached and ionized by the remaining sodium ions of the evaporated cluster and detected as molecular ion signals. At lower temperatures, these clusters (containing substantial amounts of sodium ions) would not be recognized as molecular signals, due to incomplete cluster evaporation.

Since sodium is an omnipresent ion in typical samples, we switched to other potential adducts, such as potassium ions and also varied analytes and matrix compounds, to verify the above observations. First results show that matrix identity does not alter the observations significantly. Adduct ion concentrations were found to be less influencing, but a certain concentration level is needed to allow for overcoming the intensity of protonated molecules. Chemical identity of the analyte, on the other hand, seems to be a significant factor, as for example found for L-tryptophan,

showing a rapid drop of sodium adduct signal intensity with elevated temperature. Here, analyte-structural effects of alkali ion coordination may play a concurrent role.

Neuer Aspekte

Transfer capillary temperature influences abundance of protonated and alkali-attached molecular ions, which can be explained with a cluster decay mechanism.

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Sensitivity of detection of 12 aminoglycoside antibiotics by positive ESI-MS - comparison between HILIC and reverse phase methods

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Stichworte: Reverse phase, ion pairing reagents, ionization efficiency, ESI-MS

Einleitung

The sensitivity of LC-MS detection depends on efficient formation of ions during the ionization and presence of sharp peaks during chromatographic separation. A study was undertaken to examine the influence of both parameters on the sensitivity of detection of aminoglycosides. The sensitive analysis of this antibiotic class is important in veterinary testing and for regulatory compliances. Ion pairing agents are used as mobile phase modifiers in the reverse phase to ensure compounds retention. HILIC chromatography can be used with polar compounds that are harder to retain in the reverse phase. Both modes were thoroughly studied for application towards aminoglycoside analysis. The heavier aminoglycoside compounds were found to have better detection sensitivity in the presence of ion pair agent.

Experimenteller Teil

HILIC separation of aminoglycosides under gradient conditions was undertaken using formic acid as the mobile phase modifier and the superficially porous HPLC column. Post-column infusion of ion pair agents was done to measure their effects on the compounds ionization. The Reverse phase separation was done using HFBA as an ion pair agent, a C18 HPLC column and gradient conditions. Integrated peak areas from all methods formed the basis for sensitivity comparisons. The studies used AB Sciex QTRAP 3200 instrument and Agilent's HPLC system.

Ergebnisse

Data showed significant differences between aminoglycoside peak areas from HILIC and RP methods. The study found that mobile phase composition such as percent organic and the presence of ion pair reagent as well as differences in the chromatographic peak shapes are all important contributors to these effects. The presence of ion pair agent alone contributed to less efficient ionization of spectinomycin, streptomycin and geneticin and to more efficient ionization for gentamicin, neomycin and tobramycin. These effects loosely correlated with the number of ionizable amino-groups present in the structure of the antibiotics. Both MRM transitions and the formation of the parent ion showed very similar effect in both magnitude and trend.

At analytes' elution time, HILIC method had less percent of organic solvent in the mobile phase than the reverse phase method. Sharper peaks were generally produced under HILIC separation conditions except apramycin, gentamicin and neomycin had sharper peaks in the reverse phase mode. The combined effects of all of the above phenomena contribute to better sensitivity for neomycin (+700%), tobramycin (+140%), gentamicin (+380%) and hygromycin (+40%) when reverse phase mode with ion pair agent is used. Puromycin(+86%), spectinomycin(+74%), and streptomycin (+12%) gave higher detection sensitivity under HILIC mode.

Neuer Aspekte

Reverse phase is the preferred mode of neomycin detection since ion pairing reagents contribute to better ionization efficiency in ESI-MS.

Rapid LC-MS/MS determination of dioxin and digitoxin in biological fluids with minimal matrix effects

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Stichworte: digoxin, digitoxin, LC-MS/MS, matrix effects, biological fluids

Einleitung

The cardiac glycosides digitoxin and digoxin are widely prescribed for treating congestive heart failure. Digitoxin differs from digoxin only in that digoxin possesses an additional OH group. In fact, digoxin is one of the OH-metabolites of digitoxin. Therefore, there is a need for simultaneous determination of digitoxin and digoxin in biological fluids. Unfortunately, the current immunoassay-based methods are not able to effectively differentiate these two compounds due to their similar chemical structures. In addition, the immunoassays typically involve labor-intensive and time consuming steps.

The present work describes a rapid, selective and sensitive LC-MS/MS method for determination of digitoxin and digoxin in plasma with a simple sample clean-up technique for simultaneous removal of proteins and phospholipids.

Experimenteller Teil

Sample Preparation by HybridSPE-PLus

A 100 µL of spiked rat plasma/serum sample was added to the HybridSPE[®]-PLus plate well, followed by the addition of a 300 µL of protein precipitation solvent, acetonitrile with 1% formic acid.

The plate was sealed on top with a sealing film and secured on a vibrator, and agitated by vibration at 1000 rpm for 2 min. The plate was transferred to a vacuum manifold, and the seal was peeled off. Vacuum was applied at 10 in Hg for 4 min to pull the sample through the plate. The flow-through was collected and transferred to autosampler vials for direct injection for LC-MS analysis.

Ergebnisse

Digitoxin and digoxin are ionized primarily as sodium adducts under the conditions with 50% acetonitrile containing 0.1% formic acid as the mobile phase. Further experiments show such sodium adduct ions are hardly fragmented in MS/MS regardless of collision energy and gas pressure, thus not amenable to be monitored in MS/MS mode.

Digitoxin and digoxin generate primarily ammonium adducts under the conditions with mobile phase 50% methanol containing 10 mM ammonium formate and low desolvation temperature (100 °C).

The ammonium adduct ions of digitoxin and digoxin are readily fragmented. The MRM transitions 782.5/635.5 and 798.5/651.5 were chosen for LC-MS/MS quantification of digitoxin and digoxin, respectively.

The limit of detection (LOD) is about 0.01 ng/mL, 20x lower than that of the LC/SIM mode.

Matrix Effects and Sample Preparation

The ESI-MS spectra of the rat plasma are dominated with highly intense ions of multiple phospholipid species. In contrast, these phospholipids are not observed after the samples were prepared with HybridSPE-PLus. This indicates the phospholipids were effectively eliminated employing HybridSPE-PLus.

Matrix Effects on the LC-MS/MS Signal Responses and Reproducibility

The ion signals of both analytes from the samples prepared by standard protein precipitation are only 1/3-1/4 as intense compared to the samples prepared by HybridSPE-PLus, resulting in low recoveries of the analytes. Furthermore, the ion signals intensities of both analytes from standard protein precipitation tend to decrease with repeated injections, leading to irreproducible quantitative results. In contrast, the sample prepared employing the

HybridSPE-Plus provides high recoveries (>90%) and reproducible (<6%) results. These results indicate the matrix effects were significantly reduced by the HybridSPE-Plus.

Neuer Aspekte

A rapid, selective and sensitive LC/MS/MS method for determination of digitoxin and digoxin in plasma/serum was developed using HybridSPE-Plus.

Determination of thyroid hormones in biological fluids by LC-MS with online solid phase extraction

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Stichworte: thyroid hormones, LC-MS, SPE, biological fluids

Einleitung

Thyroid hormones play critical roles in the regulation of biological processes such as growth, metabolism, protein synthesis, and brain development. Specifically, thyroid hormones, thyroxine or T₄ and T₃, are essential for development and maintenance of normal physiological functions. For a clinical laboratory, measurements of total T₄ and total T₃, along with estimates of free T₄ (FT₄) and free T₃ (FT₃), are important for the diagnosis and monitoring of thyroid diseases. Most clinical laboratories measure thyroid hormones using immunoassays.

The present work exploited online SPE with LC/MS for rapid determination of T₄, T₃ and 3,3',5'-triiodo-L-thyronine (rT₃) from biological matrices.

Experimenteller Teil

Sample Processing Procedure

A 100 µL of rabbit plasma spiked with a desired amount of T₄, T₃ and rT₃, were protein precipitated by addition of 25 µL zinc chloride and 200 µL methanol, agitated by vortex for 1 min, and then centrifuged at 9000 g x 3 min. The resulting supernatant was collected and directly injected for LC/MS analysis.

Ergebnisse

An online SPE-LC/MS method was developed for the determination of thyroid hormones in biological fluids. The method exploits RP-Amide or C8 as the trapping column and Phenyl phase as the separation column, respectively.

The preliminary experiments demonstrated that, under the optimized conditions, both RP-Amide and C8 effectively trapped the thyroid hormones extracted from spiked rabbit plasma sample which had been protein precipitated. In both traps, sharp peak shapes were observed. However, the RP-Amide traps are advantageous over the commonly used C8 traps with higher signals and recoveries. Additionally, RP-Amide traps are more flexible with washing solvent as it is compatible with 100% aqueous mobile phases and can be used with up to 20% methanol with minimal sample loss.

Neuer Aspekte

An online SPE-LC/MS method was developed for the determination of thyroid hormones in biological fluids.

Thermogravimetry hyphenated to ultra-high resolution FT-ICR-mass spectrometry using atmospheric pressure chemical ionization (APCI) as novel approach for evolved gas analysis

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Stichworte: evolved gas analysis (EGA), ultra-high resolution mass spectrometry, thermal analysis, pyrolysis, lignocellulosic biomass

Einleitung

In this study, the hyphenation of a commercial thermobalance to ultra-high resolution Fourier transform ion cyclotron resonance mass spectrometry (UHR-FTICR-MS) is presented by means of evolved gas analysis (EGA). Thermo induced processes, such as thermodesorption and/or pyrolysis of complex mixtures, can be characterized at the molecular level due to the high mass resolving power and high mass accuracy of FT-ICR-MS [1]. In the following, we focus on the most crucial part: the hyphenation between atmospheric pressure thermobalance and FT-ICR-MS with atmospheric pressure chemical ionization (APCI) [2].

Experimenteller Teil

This difficulty could be solved by applying an over-pressure of about 50 mbar in the thermobalance (Netzsch STA 409PG) by blocking the relief valve and creating a driving force for the analyte to move into the ion source. By using an ultra-high resolution mass spectrometer (Bruker apex-Qe generation II), evolving isobaric compounds, even from complex mixtures like crude oil or biomass pyrolysis, could be resolved at the m/z scale with a mass resolution of 280.000 @ $m/z=400$. It turned out that mass spectra were reproducible by means of pattern and base signal, although the absolute intensities varied. As proof of principle, the thermal behavior of different grounded log woods and pellets was investigated.

Ergebnisse

Exact mass measurements allow the time-resolved calculation of elemental composition of the detected ions. Our results confirmed that the developed approach is suitable to distinguish between different kinds of wood by their specific molecular pattern of evolved compounds, their thermal behavior in the thermobalance and their compound class distribution. Up to 1.000 sum formulas could be assigned in the average spectra. The obtained mass spectra were dominated by different derivatives and pyrolysis products of a few precursor structures, such as the lignin monomers coniferyl and sinapyl alcohol, and high abundances in the range of $m/z=200$ and $m/z=350$. Oxygenated species (CHO_n , $1 \leq n \leq 10$) were the prevailing compound classes with a maximum at CHO_3 and CHO_4 for softwoods and hardwoods, respectively, which is caused by the selectivity of the ion source towards polar compounds, such as the thermal decomposition products of the three main wood components: lignin, cellulose and hemicellulose. Except in resin-containing pine wood, pyrolysis was the dominant thermo-induced process leading to the formation of a variety of volatiles and intermediate-volatiles. Moreover, literature-known wood pyrolysis markers were confirmed according to their elemental composition, such as coniferyl aldehyde ($\text{C}_{10}\text{H}_{10}\text{O}_3$), sinapyl aldehyde ($\text{C}_{11}\text{H}_{12}\text{O}_4$) and several decomposition products from abietic acid ($\text{C}_{20}\text{H}_{30}\text{O}_2$) [3]. The spectra of birch-bark pellets showed a high abundance at higher m/z ratios in range of about $m/z=430$, originating from decomposition products of betulin ($\text{C}_{30}\text{H}_{50}\text{O}_2$) and betulinic acid ($\text{C}_{30}\text{H}_{48}\text{O}_3$), which represent up to 34% of dry mass of the birch bark [4]. However, the system reveals its limitations through the missing m/z -scale below $m/z=100$, carryover effects in the heated transfer line between thermobalance and atmospheric pressure ion source, and an error containing mass signal in the thermobalance due to low weighed portions and pressure conditions during the temperature program.

Neuer Aspekte

Deeper look into pyrolysis of lignocellulosic biomass by high resolution mass spectrometry as novel approach for evolved gas analysis.

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Near-IR laser induced desorption sampling of acoustically levitated liquids

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Stichworte: Laser Desorption Ionization; Levitated Droplets; Ambient Mass Spectrometry

Einleitung

Acoustic levitation of droplets has matured into a powerful tool for containerless handling of microliter samples. In microfluidic systems the absence of confining walls is greatly beneficial because it can effectively suppress agglomeration and contamination of the sample originating at the liquid/solid interfaces. In a previous contribution we have introduced a contact free laser spray droplet-MS interrogation scheme using glycerol as a chromophore for the $\lambda = 10.6 \mu\text{m}$ output of a CO₂ desorption laser. [1] Its low vapour pressure, however, makes glycerol an unfavourable solvent. Here we present a new design, utilizing the $\lambda = 2.94 \mu\text{m}$ output of a diode pumped Er:YAG laser for excitation, opening the laser spray to any OH-group containing solvents.

Experimenteller Teil

Levitation of the droplets is performed by an acoustic levitator consisting of a transducer (40 kHz, MTH, Henstedt-Ulzburg, Germany) coupled to a titanium sonotrode opposing a spherical reflector at a resonant distance of $n/2 \lambda_{\text{US}}$ (λ_{US} being the acoustic wavelength of the ultrasonic field). The levitated droplets are placed in sound pressure minima positioned coaxially to the inlet of a time-of-flight mass spectrometer (API HTOF-MS, Tofwerk, Thun, Switzerland). Laser induced desorption is carried out by the $\lambda = 2.94 \mu\text{m}$ output of a diode pumped Er:YAG laser (Pantec Engineering AG, Rugell, Liechtenstein). For an effective detection of nonpolar analytes a home-built dielectric barrier discharge plasma torch can be added as a post-ionisation source.

Ergebnisse

The previously used CO₂-laser (10.4 μm) limited the solvent to glycerol and was consequently substituted by an Er:YAG laser (2.94 μm). This wavelength is efficiently absorbed by a large variety of hydroxy containing solvents like water or alcohols. In the previous experiments, a spray based ionization mechanism was postulated. The higher vapour pressure of an aqueous or methanolic solution should thus lead to a more efficient ionization since evaporation results in a finer nebula. Compared to glycerolic solutions, once formed, the smaller droplets undergo an even faster evaporation. Consistently, a greatly enhanced ion yield can be observed at the newly used wavelength.

The main features are the analyte signals in and signals of solvent clusters. According to the analyte solubility, the spectra are dominated by rather methanol clusters in case of methanol/water mixtures accompanied by only weak water cluster signals. These findings corroborate the earlier made postulate that the observed ionization occurs mainly by charge separation during rapid spray nebulization. Accordingly, the observed ions are protonated molecules $[x M+z H]^{z+}$ or analyte/solvent clusters incorporating one or more protons $[x M+y H_2O+z H]^{z+}$. Nonpolar analytes that cannot get protonated cannot be detected.

However, non-proton affine analytes can be detected by incorporating an additional dielectric barrier discharge ion source. This post-ionization step leads to spectra with protonated as well as radical ion species. For instance, Ibuprofen that was not detectable without post-ionisation can be post-ionized to detectable $[\text{Ibu}+\text{H}]^+$ and $[\text{Ibu}]^{+\bullet}$ species. This opens up a multitude of possibilities for laser spray ionization of levitated droplets in a vast number of applications including for instance microfluidic systems.

Neuer Aspekte

An improved laser-induced spray ionization of liquid samples is presented. Depending on the analytical demands, additional post-ionization can be applied.

Referenzen

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Ein Orbitrap-Prototyp zur hochauflösenden Untersuchung von Erdöl

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Stichworte: FTMS, Orbitrap, Erdöl, Spectral Stitching

Einleitung

Einer eingehenden Analytik von Erdölen stehen diverse Probleme entgegen. Insbesondere die Komplexität erlaubt häufig nur Untersuchungen auf Bulkigenschaften. Massenspektrometrische Methoden hingegen bieten eine gute Möglichkeit zur Charakterisierung auf molekularer Ebene. Angesichts der enormen Anzahl von mehreren 10.000 zu untersuchenden Verbindungen und der damit zu erwartenden Anzahl isobarer Ionen ist ein hohes Massenaufklärungsvermögen ein unerlässliches Kriterium. Gold-Standard in der Erdölanalytik ist dementsprechend die Untersuchung mittels FT-ICR-Massenspektrometrie. Mit Einführung von Hochfeld-Orbitrap-Zellen im Jahre 2010 konnte das Auflösungsvermögen der Geräte verbessert und die Lücke zu marktüblichen FT-ICR-Geräten weiter geschlossen werden. Wir präsentieren hier die Nutzung eines Orbitrap-Prototypen mit einer Auflösung $> 800.000 @ 400 \text{ Th}$ zur massenspektrometrischen Analyse von Erdölen unter Nutzung der "Spectral Stitching Methode".[1]

Experimenteller Teil

Zur Durchführung der Studie wurde ein schweres Erdöl zunächst in Toluol auf 250 ppm verdünnt.

Die erhaltene Lösung wurde anschließend per Direktinjektion nach Photoionisation bei 10,0/10,6 eV an einem Orbitrap Elite Prototypen jeweils wie folgt vermessen:

- Full Scan im Massenbereich 200 - 1200 Th
- Spectral Stitching im Bereich 200 - 1200 Th; Segmentbreite 30 Th; Überlapp 5 Th

Für drei verschiedene Transienten/Auflösungen (768 ms; 1,5 s; 3 s) wurden dabei jeweils insgesamt 250 Einzelscans aufgenommen und aufsummiert. Die Interpretation der Daten erfolgte mit Hilfe von Composer (Sierra Analytics, Version 1.0.6).

Ergebnisse

Für die massenspektrometrische Analyse von Erdölen sind auf instrumenteller Seite zwei Faktoren von entscheidender Bedeutung: Empfindlichkeit bzw. Ionentransmission und eine hinreichend hohe Auflösung über einen weiten Massenbereich. Der klassische Ansatz mittels FT-ICR-Massenspektrometrie verspricht, eine entsprechende Magnetfeldstärke vorausgesetzt, hier ausgezeichnete Resultate. Nachteilig wirken sich jedoch die verminderte Ionentransmission, insbesondere in niedrigen Massenbereichen, und die mit steigendem m/z reziprok abfallende Massenaufklärung aus.

Orbitrap Elite Massenspektrometer erlauben ab Werk eine maximale Transientendauer von 768 ms, was einer Auflösung von 240k @ 400 Th (FWHM) entspricht. Im von uns verwendeten, darauf basierenden, Prototypen kann die Transientendauer darüberhinaus verdoppelt, bzw. auf ca. 3 s vervierfacht werden. Dies erlaubt unter realen Bedingungen eine Auflösung von $>800k @ 400 \text{ Th}$ (nominell 960k). Diese Auflösung liegt nur noch geringfügig unter der von FT-ICR-Geräten, die bei einer Feldstärke von 7T die Marke von 1.000.000 erreichen.

Durch den gegenüber der FT-ICR-Technik schwächer ausgeprägten Auflösungsverlust bei steigendem Massenbereich wird bei 1.000 Th noch immer eine Auflösung von ca. 500k erreicht. Von besonderem Interesse im Bereich der Erdölanalytik ist die massenspektrometrische Unterscheidung zwischen C_3 und SH_4 , was einer Massendifferenz von 3,4 mDa entspricht. Eine Auflösung von 500k im Bereich von 1.000 Th erlaubt es, diese Unterscheidung noch bis zu einem Peakintensitätsverhältnis von etwa 1:10 sicher zu treffen.

Durch die Anwendung des "Spectral Stitching" kann, bei gleichbleibender Gesamtmessdauer, die Anzahl beobachteter und zugeordneter Signale um den Faktor 3-4 erhöht werden. Der im Vergleich zu einer analogen Studie mit einem FT-

ICR-Massenspektrometer geringe Zugewinn kann der grundsätzlich besseren Ionentransmission zugeschrieben werden.[1] Der Informationsgewinn ist bei allen untersuchten Auflösungen ähnlich. Bei längeren Transienten steigt die Gesamtzahl der zugeordneten Signale erwartungsgemäß hauptsächlich im Bereich über 600 Th aufgrund der zugewonnen Massenauflösung.

Neuer Aspekte

FT-Orbitrap-MS kann durch erhöhte Auflösungen als günstige und schnelle Alternative zur FT-ICR-MS für Untersuchungen komplexer Proben verwendet werden.

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Assessing the Peptide Quantitation Performance of a Newly Developed Triple Quadrupole Instrument

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Stichworte: QQQ, Peptide Quantification, Proteine Digests

Einleitung

Sample complexity and low concentration of some biomarkers are the main challenges in biomarker verification methodology based on multiple reaction monitoring (MRM). Consequently, the development of MRM-based methods with stable isotope-labeled standards peptides in biological fluids has focused on improving method sensitivity and increasing the dynamic range by lowering detection limits. Furthermore robustness and reproducibility are essential for using these assays in high-throughput translational research environments.

This work demonstrates the sensitive and robust determination of human plasma peptides using a newly designed triple quadrupole mass spectrometer. Improvements include a new optimized Q1 ion transfer optics and a novel ion detector that uses a high voltage conversion dynode with low noise characteristics.

Experimenteller Teil

Improved Signal with Higher HED Voltage

- The impact of the high voltage dynode on response was measured using HED voltages of -10, -15, -18 and -20 kV
- Results (average of n=3, shown as % gain over the -10 kV) to the right demonstrate a clear benefit to the higher HED voltage (30-80% increase in response at -20 kV) **Anti-HER2/neu mAb Digest** Following the same protocol as for the enolase experiment, a mAb digest was analyzed in order to test higher *m/z* product ions and glycopeptide behavior.
- Results (average of n=3, shown as % gain over the -10 kV) to the right demonstrate a clear benefit to higher HED voltage (60-120% increase in response at -20 kV) for higher *m/z* ions

Ergebnisse

- The 6495 QQQ with Agilent JetStream Technology provides outstanding sensitivity at standard flow rates with an LLOQ of 5 amol on-column
- Ultimate sensitivity is achieved with nanoflow LC/MS on the HPLC-Chip/6495 where we achieved a 500 zmol LLOQ
- With a complex plasma digest matrix, the 6495 QQQ showed excellent response and retention time reproducibility over an extended period (0.5 mL of plasma digest injected over 3.5 weeks!)
- Improved performance in peptide quantitation can be achieved by the increased precursor ion transmission, enhanced detection efficiency (HED voltage up to -20kV) and extended *m/z* range of the 6495 QQQ

Neuer Aspekte

Peptide Quantification with high accuracy

Eine DBD zur in-situ Erzeugung von Reaktantionen für negative chemische Ionisation

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Stichworte: Dielektrisch behindertes Plasma, Ionenquelle, Chemische Ionisation

Einleitung

Die chemische Ionisation (CI) bei Atmosphärendruck wird zumeist noch mit radioaktiven Ionenquellen betrieben, da diese einen hohen und konstanten Ionenstrom liefern. CI-Massenspektrometer werden unter anderem für die Messung atmosphärischer Spurengase verwendet. Da diese Geräte mobil sein müssen sind radioaktive Ionenquellen aufgrund der gesetzlichen Bestimmungen nur bedingt einsetzbar. Die präsentierte Dielektrisch-Behinderte-Entladungs-Ionenquelle (DBD) kann genutzt werden, um Reaktantionen in-situ zu erzeugen, so können zum Beispiel negative Nitrationen mit einer Mischung aus Stickstoff und Sauerstoff in der DBD erzeugt werden. Die DBD Ionenquelle wird mit einer radioaktiven Ionenquelle verglichen um Aussagen über ihre Brillanz treffen zu können.

Experimenteller Teil

Die DBD wird mit 20 kHz und ca. 10 kV betrieben. Als Dielektrikum dient 1 mm Keramik und der Abstand zwischen der Elektrode und dem Dielektrikum beträgt 1 mm, hier brennt das Plasma. Das Plasma wird mit Stickstoff oder Sauerstoff betrieben, abhängig vom Reaktantion das erzeugt werden soll. Die Hochspannungselektrode ist in der Keramik die auch als Dielektrikum dient schlüssig befestigt. Als Gegenelektrode dient eine Edelstahlkapillare, die das Plasma von der Reaktionszone, in der die chemische Ionisation betrieben wird, trennt. Durch diese Kapillare werden die Reaktantionen in die Reaktionszone transportiert. Die Reaktionszone befindet sich in einem Ionentrichter, der bei 50 hPa betrieben wird. Nach der chemischen Ionisation werden die gebildeten Analytionen mit einem Time-of-Flight Massenspektrometer gemessen.

Ergebnisse

Die gute Steuerbarkeit der Chemie im Plasma, welche vom Analytgasstrom getrennt brennt und eine Chemie, die prominente Reaktantionen für die negative chemische Ionsisation hervorbringt, macht die DBD zu einer vielseitigen Ionenquelle für die chemische Ionsisation. Es wird aufgezeigt, dass thermodynamisch stabile Reaktantionen wie negativ geladene Nitrat-, Carbonat-, Iodid- und Schwefelfluorid-Ionen im Plasma der DBD erzeugt werden können. Nitrationen können schon aus Spuren von Stickstoff in Sauerstoff erzeugt werden aber auch aus Spuren von Sauerstoff in Stickstoff, um die Produktion von Ozon gering zu halten. Ebenso können Carbonationen aus Spuren von Kohlenstoffdioxid in Sauerstoff erzeugt werden. Geringe Beimengungen von Iod im ppb-Bereich sorgt für die Produktion von Jodidionen. Auch Schwefelhexafluorid lässt sich im Plasma ionisieren. Zudem kann auf klebrige und korrosive Vorgängermoleküle, wie z.B. Salpetersäure zur Erzeugung von Nitrationen verzichtet werden. Dadurch kann die Chemie in der Ionenquelle relativ schnell umgestellt werden. Auch die Stabilität der Ionenquelle ist hervorragend, da die Chemie in der Ionenquelle nicht durch den Analyten gestört wird. Stärkeren Einfluss auf die erzeugte Ionenmenge hat der Gasfluss durch die DBD, dieser kann aber mit einem Flussregler stabil gehalten werden. Nach dem Einschalten der Entladung braucht es etwa 15 min bis die produzierte Ionenmenge auf einem stabilen Niveau bleibt, dieser Effekt geht vermutlich mit der Aufheizphase der Ionenquelle und der Konditionierung der Wände einher. Bisherige Vergleiche mit veröffentlichten Nachweisgrenzen (NWG) von amosphärischen Spurengasen geben Anlass zur Annahme, dass die Kombination aus der DBD Ionenquelle und der verwendeten Transferstufe, überdurchschnittlich gute NWG erzielen. Daher soll der Vergleich einer radioaktiven Ionenquelle mit der DBD zeigen wie brillant die DBD Ionenquelle ist.

Neuer Aspekte

Gesundheitlich und logistisch problematische radioaktive Elemente in der Ionenquelle für die negative CI können durch eine DBD-Ionenquelle ersetzt werden.

Increasing Depth of Coverage in Data Independent Acquisition

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Stichworte: variable windows, SWATH acquisition, ADC, TDC, detector, DIA

Einleitung

Data independent acquisition (DIA) strategies have been used to increase the comprehensiveness of data collection while maintaining high quantitative reproducibility. In DIA, larger fixed-width Q1 windows are stepped across the mass range in an LC timescale, transmitting populations of peptides for fragmentation, and high resolution MS and MS/MS spectra are acquired. Previous work has shown that using more narrow Q1 windows can improve peptide detection and increase sample coverage. Here both method and instrumentation advancements will be explored to continue to increase depth of sample coverage.

Experimenteller Teil

The MS analysis was performed on a modified quadrupole time of flight instrument equipped with an ADC detection system. DIA data collection was done using SWATH™ acquisition with prototype acquisition software to explore a variety of acquisition strategies. The DIA data was interrogated using a comprehensive yeast spectral library created from many data dependent experiments. Results assessment was performed using Excel tools.

Ergebnisse

Original work exploring variable window size and more narrow windows demonstrated that increasing the number of total Q1 windows from 24 to 60 windows provided an increase in confident peptide detections with good quantitative reproducibility of ~15%. To enable higher sample loads, the dynamic range of the detection system was extended by switching from a TDC based detection system to an ADC based system on a modified TripleTOF system. At the higher sample loads, we next applied increasingly narrow Q1 windows during SWATH acquisition to continue to improve the S/N in MS/MS. The number of windows was extended from 60 to 100 windows across the 400-1250 precursor m/z range while maintaining a cycle time of 3.2 secs. This provided a 20% increase in confident peptide detections with 20% or better CVs across replicates [1]. Further optimization of longer chromatography and investigations of impact on other proteomes will be discussed.

Neuer Aspekte

Using variable windows for SWATH acquisition

Referenzen

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A targeted lipidomics approach for ceramide analysis in platelet

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Stichworte: lipidomics, ceramide, LC/ESI SRM, platelet, absolute quantification

Einleitung

Platelets are the central building block of coagulation and hemostasis and directly linked to or affected by several metabolic diseases including diabetes, dyslipidemia and metabolic syndrome. Due to the fact that these metabolic dysfunctions might directly impact the platelet lipid composition and therefore the platelet function, it is necessary to develop a comprehensive picture of how lipids are altered and regulated in platelets. We initially focus on ceramides which are participating in the regulation of cell response such as senescence, apoptosis and autophagy [1-2]. To obtain a deep view into the ceramide lipidome we developed a selected reaction monitoring (SRM) approach to detect and quantify ceramides with high sensitivity and accuracy.

Experimenteller Teil

To obtain a comprehensive understanding how ceramides are regulated, it is essential to develop reliable quantification approaches. Initially, we performed lipid extraction using methyl-tert-butyl ether (MTBE) [3] to isolate lipids from human and mouse platelet pellets. Then lipid suspension was analyzed with a SRM based LC/MS lipidomics approach, which allows us to perform sensitive screening as well as absolute quantification of ceramides (spiked with internal standards) over a wide dynamic range.

Ergebnisse

By applying the established method, we were able to identify 160 ceramide species at fatty acid scan species level in human platelets. In mice platelets we identified around 60 ceramides species and quantified them with an excellent reproducibility over a dynamic range of 5 orders of magnitude. In these mice platelets, we achieved a coefficient of variation (CV) of 10% for technical reproducibility and a CV of 17% for the biological replicates, reflecting the good reproducibility of the ceramide analysis. When we compared the ceramide lipidome between human and mice platelets, the relative long chain base (LCB) concentration of d18:1 was the most abundant in both samples. LCB d17:0, d17:1, t17:0 and t18:0 were only found in human platelets. In general, most ceramide classes were found with similar peak area in both samples, only lactosylceramide was found less abundant in mice platelets. To test our developed approach for human platelet lipidome analysis, we compared our targeted analysis approach against a shotgun lipidomics approach. In comparison with shotgun lipidomics, more lipid species were identified in the targeted analysis LC/ESI SRM approach. In contrast, classes such as plasmalogen phosphatidylethanolamine (pPE), plasmalogen phosphatidylcholine (PCO), phosphatidic acid (PA) were only detected by shotgun analysis. Therefore, shotgun and LC based targeted lipidomics are complementing each other and should be applied both for a comprehensive lipidomics study.

Neuer Aspekte

A quantitative LC/ESI SRM approach for ceramides analysis at fatty acid scan species level.

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Metabolic profiling of the battle field of competing white-rot fungi

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Stichworte: wood-degrading fungi, pigments, secondary metabolites

Einleitung

Schizophyllum commune, the renowned white-rot fungus produces secondary metabolites like pigments when accosted by various white-rot fungi in nature. It can be assumed that different mycelial reactions like these can activate different genes thereby eliciting different metabolic pathways in the organism. Many microbial gene clusters may be silent under standard laboratory growth conditions. One promising approach to trigger the activation of cryptic biosynthetic pathways is the co-cultivation of two or more microorganisms in the same confined environment, leading to the production of new metabolites. Such compounds, produced dynamically, are of potential interest as new leads for drug discovery. Thus, this study focuses on metabolites produced by *S. commune* during interspecies crosstalk with wood-decay fungi - *Hypholoma fasciculare*, and *Pleurotus ostreatus*.

Experimenteller Teil

To investigate the fungal cocultures, agar plate based confrontation assays were performed between *S. commune* and, *H. fasciculare* and *P. ostreatus*. From the co-cultured plates, mycelial strips were cut out from the interaction zone and from the periphery of fungi, respectively. Each sample was subjected to a methanol:ethyl acetate extraction and finally diluted in methanol. UHPLC–ESI–MS/MS was performed with the diluted extract. Full scan mass spectra were generated using 30,000 m/Δm resolving power. PCA was performed on the full-scan data of the samples using Metaboanalyst 3.0, a web-based tool for metabolomic data processing, statistical analysis, and functional interpretation.

Ergebnisse

In self-paired cultures the fungi did not exhibit induction of pigmentation, while coloured substances and discolouration of the medium was observed when *S. commune* interacted with *H. fasciculare* and *P. ostreatus*. A greenish blue/sometimes black pigment developed at the bottom of the plate within the domain occupied by *S. commune* at 12 h postcontact. The intensity of this pigmentation increased with the duration of contact and was visible in the interaction zones of the fungi. LESA-HRMS was performed on the confrontation assay plates directly from the surface of the agar. Analyzing the interaction zone, a mass signal corresponding to the pigments indigo, indirubin and isatin were observed. Statistical analyses of the different zones of the fungal cocultures showed a distinction between metabolites produced by *S. commune* interacting with *H. fasciculare* and *P. ostreatus*. There was also a difference noted in the mycelial metabolites from those that were secreted. These patterns suggest that in *S. commune* different effectors are elicited in response to different competitors.

In order to allow scientists observations of natural phenomena in the most direct and non-invasive ways, new tools have constantly been developed. Because of the complexity of microbial extracts, advanced analytical methods (e.g., mass spectrometry based metabolomics) are the key for the successful detection and identification of co-culture-induced metabolites. Thus, we showed that a direct technique like LESA and an indirect one like LC-MS are efficient tools to identify compounds which have a relatively low solubility in water and organic solvents, necessitate only low invasion and are label-free applications requiring minimal sample preparation. Therefore, they are well suited for biological applications.

Neuer Aspekte

The techniques open up avenues for investigations of fungal interactions with co-occurring organisms to obtain information on their mechanisms

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Online Photostability Study of Pharmaceutical Substances

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Stichworte: photostability, product identification, reduction of animal studies, kinetics

Einleitung

Study of the photostability of pharmaceutical substances is becoming increasingly important in drug development. Thus pharmaceutical industries are also requested by the regulations agencies to provide data for new or renewed licences of medicines.

The aim of this work is to evaluate the photostability of drug substances by using a modified HPLC, which consists of a tailored online photoreactor with back-flush and two column system. Coupling to a mass selective detector allows to identify the newly formed photoproducts.

By this method, it is possible to obtain a straightforward answer on the degradation via photolysis of different substances *in-vitro* and to predict the photostability in early stage of drugs development. This may also help to reduce animal experiments in the future.

Experimenteller Teil

Ketoprofen aqueous solution (pH 7.0) was irradiated with UVA during different periods of time. The peaks were collected and analysed by LC-MS single quadrupole and also by ESI-TOF.

The photoreactor consist of a UVA lamp surrounded by a braided and twisted capillary inside an Aluminium tube. The temperature is also controled and monitored during all the experiment. After the substances run throught the photoreactor and the degradation products are formed, they are first trapped in a Aqua C18 column and then eluted in reverse flow in a second column (Onyx monolythis C18).

Ergebnisse

Irradiation of the nonsteroidal anti-inflammatory agent ketoprofen with UVA resulted in decreasing amounts of the target compound. First order kinetics was found as best model. Several photoproducts were detected after irradiation in aqueous solution (pH 7.0), when maintaining the temperature in the reactor at 25-30°C. Mass spectrometric characterization supported the structures reported from literature.

These data showed the potential for the use of the new device for fast and easy photostability studies, that may help to reduce time consuming *in-vitro* experiments and animal trials. Targeted MS/MS-based methods may be generated using the results obtained by this online-irradiations for use *in-vitro* and *in-vivo*. Scale-up may also be realized for the generation of reference material for quantification as well as for toxicity testing.

Neuer Aspekte

A new online-hv-HPLC device is presented that allows easy study of photodegradation and direct coupling to MS(/MS) for product identification.

To which extent are oxidized phospholipids digestible by the enzyme phospholipase A₂? - A mass spectrometric and NMR spectroscopic study

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Stichworte: MALDI-TOF MS, ESI MS, Lipid Oxidation, Phospholipase A₂.

Einleitung

Free fatty acids are released through phospholipase A₂ (PLA₂), which cleaves the fatty acyl residue in *sn*-2 position of the corresponding phospholipid (PL) [1]. During inflammatory diseases, reactive oxygen species (such as HOCl) lead to the formation of oxidatively modified PLs (e.g. chlorohydrin generation). Oxidation of PLs may significantly influence their digestibility through PLA₂. Furthermore, the position of the unsaturated fatty acyl residue (*sn*-1- or *sn*-2-position) and the modification of the headgroup (phosphatidylcholine (PC) versus phosphatidylethanolamine (PE)) may lead to major differences with respect to the PLA₂ activity. Therefore, the aim of this study is to investigate these aspects using a combination of MALDI-TOF and ESI-IT MS as well as high resolution NMR and HPTLC.

Experimenteller Teil

All chemicals, solvents, PLA₂ and the applied MALDI matrices (9-aminoacridine and 2,5-dihydroxybenzoic acid) were obtained from Sigma-Aldrich. Stock solutions of PLs were purchased from AVANTI Polar Lipids and used as supplied. The PL oxidation was performed as recently described [2,3] using either HOCl or the Fenton system (Fe²⁺/H₂O₂). The oxidized PLs were enriched by extraction with organic solvents and chromatographically purified. MALDI-TOF and ESI-IT mass spectra were recorded on a Bruker Autoflex and Bruker Amazon, respectively. HPTLC experiments were performed applying unmodified silica gel (Merck) and chloroform, ethanol, water, and triethylamine (35:35:7:35, v/v/v/v) as mobile phase. High resolution NMR spectra were recorded either on a Bruker AMX 300 or AVANCE 600 MHz spectrometer.

Ergebnisse

Isolated standards of PC (PC 16:0/18:2 (PLPC)) as well as two PEs (PE 16:0/16:0 (DPPE) and 16:0/18:2 (PLPE)) with defined fatty acyl compositions were oxidized with HOCl under identical conditions to enable the direct comparison of the oxidative effect of HOCl regarding the unsaturated fatty acyl residues and - in the case of the PEs - the PL headgroup, too. All investigated, unsaturated PLs react readily with HOCl. Using a sufficient excess of HOCl, PLPC could be quantitatively converted into the corresponding dichlorohydrin if the reaction was performed in absence of chloride. There were only minor amounts of the corresponding lysophosphatidylcholine (LPC 16:0), i.e. a hydrolytic cleavage does not occur. Whereas the unmodified PLPC can be easily digested by PLA₂, all used analytical methods clearly indicate (with the aid of the PC/LPC ratio) that the corresponding chlorohydrin product is digested less efficiently. However, digestion products of the chlorohydrin modified PLPC are detectable using a higher enzyme concentration. Thus, inactivation of PLA₂ is mediated by the chlorohydrin product of PLPC. Similar data were obtained if chain-shortened oxidation products of PLPC (such as 1-palmitoyl-2-(9-oxo-nonaic acid)-*sn*-phosphatidylcholine) or hydroperoxides of PLPC were investigated. Compared to the studied PC, the investigations of PEs regarding their oxidation capability are much more complex. PEs can be modified at both the (unsaturated) fatty acyl residues and the (amine) headgroup. For instance, the presence of HOCl leads to the formation of mono- and dichloramines, which are rapidly converted into other products such as the corresponding aldehydes, nitriles or imines. Hence, the examinations of these products are significantly aggravated by the cross reactivity of the amino group. According to our current data, however, modifications of the headgroup are less important in comparison to the fatty acyl residues that agree with the specificity of the enzyme PLA₂.

Neuer Aspekte

The reduced digestibility of oxidized phospholipids by PLA₂ was proven by different MS and spectroscopic methods.

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Lipidomics of Alzheimer's Disease using an Integrated Microfluidic-Ion Mobility-MS Device

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Stichworte: Metabolomics, Lipidomics, Lipid, Ion Mobility, Alzheimer's disease

Einleitung

Alzheimer's disease (AD) is the most common cause of adult dementia, but the cause of this inexorable neurodegenerative disease remains still elusive. Alterations in lipid pathways have been associated with AD. Here we used a novel MS device to conduct an untargeted lipidomic investigation of AD human tissues.

Experimenteller Teil

A microfluidic-ion mobility-MS device was optimized for MS analysis of lipids in complex biological extracts. The integrated microfluidic device was fabricated from resistant ceramic materials that permit operation at high pressure with sub 2 μm particles, leading to highly efficient LC separations of lipid molecules. Lipids were separated using 150 μm ID x 100 mm devices packed with reversed phase C18, 1.7 μm particles at flow rates of 3 $\mu\text{l}/\text{min}$. By integrating microscale LC components into a single platform design, the devices avoided problems associated with capillary connections and the need to keep the system free of leaks, blockages, and excessive dead volume. Such an integrated microfluidic-ion mobility-MS device had a performance comparable to analytical scale LC-MS analysis.

Ergebnisse

We used an integrated microfluidic-ion mobility-MS device to survey frozen tissue samples from clinically characterized AD patients and age-matched controls. Mobile phases and analysis times were similar to regular LC methods using analytical-scale columns. Data was collected using both negative and positive mode in the data-independent acquisition mode with an alternate low and elevated collision energy method to acquire both precursor and product ion information in a single analytical run. Lipids profiles were processed using multivariate and pattern-recognition tools to group the observed changes in lipids, which were identified using online database search. Travelling-Wave ion mobility was integrated into the novel microfluidic-MS device to give improved peak capacity and CID fragmentation specificity. Ion mobility-derived collision cross sections provided orthogonal physicochemical data that were used with retention time, accurate mass and MS/MS data to increase confidence of lipid identification. The information obtained was integrated with clinical data to generate testable hypotheses on the functional significance of the lipid abnormalities observed in AD.

Neuer Aspekte

Microfluidic-ion mobility-MS-based lipidomics reveals novel molecular alterations in Alzheimer's disease.

The Use of Fragment Ion and Collision Cross Section for Confident Identification from LC-Ion Mobility-MS Metabolomics Data

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Stichworte: Metabolomics, Lipidomics, ion mobility, collisional cross-section (CCS)

Einleitung

Mass spectrometry based technologies often couple with UPLC-Ion Mobility-MS to measure the level and variation of metabolites in biofluids. Data generated through metabolomics studies can yield important metabolic insights into disease onset and progression. The identification of unknown compounds is often a bottleneck in large scale metabolomics profiling. UPLC-Ion Mobility-MS based metabolomics generate large and complex datasets and data analysis, interpretation and identification is the slowest step in any metabolomics workflow. In this study, we introduce a simple and novel bioinformatics tool that has been specifically developed for the large scale analysis of UPLC-Ion Mobility-MS data for confident identification of small molecules based on fragment ion and collisional cross-section.

Experimenteller Teil

Urine samples were collected from a single healthy individual. The urine was centrifuged and 600 μ L of the urine supernatant was taken and diluted with 1800 μ L of water. The urine was divided into three groups: control, low dosed (LD) and high dosed (HD). To create a working differential urine sample 11 different drugs were spiked to the LD and HD urine. A simple gradient elution at a flow rate of 500 μ L/min was applied with a mobile phase consisting of water and acetonitrile both in 0.1% formic acid on a reversed phase AQUITY column (HSS T3, 2.1x100 mm, 1.8 μ m). The data were acquired on SYNAPT G2-S HDMSTM system operated in positive mode using the MSE or HDMSE data acquisition technique.

Ergebnisse

The software adopts an intuitive workflow approach to performing comparative UPLC-Ion Mobility-MS metabolomics data analysis. The workflow starts with UPLC-Ion Mobility-MS raw data file loading then retention time alignment and deconvolution, followed by analysis that creates a list of features. The features are then identified based on exact mass, MS/MS fragment, isotope distribution, retention time and collisional cross-sectional area (CCS). A simple dataset consisting three sample mixtures was created to illustrate the processing and identification of features. A control urine (no spiking), LD urine (spiked with 4 LD drugs) and HD urine (spiked with 4 HD drugs). According to the experimental design the software should: Identify all the spiked standards, classify three distinct groups and show high level of relative abundance of the spiked standards for the LD and HD groups compared to control. To improve the confidence in the compound identification theoretical fragmentation of a candidate list of compounds and their calibrated CCS was used. The resulting 'in silico' fragmentation was matched against the measured/observed fragments and CCS of the compound database. For the in silico fragmentation, the candidate molecules are selected from a compound database based on the exact mass. Using this list of candidates the fragmentation algorithm generates all possible fragments for a candidate compound in order to match the fragment mass with the measured peaks. The identification data can be stored and used to perform fragment and CCS database searches. This allows building custom databases of fragment information, CCS and retention time which can then be used to perform future identifications when using the same compound database. The results showed that the spiked standards were identified as top rank based on MS/MS fragment and CCS compared to identification based on retention time and exact mass. The functionality of the software will be demonstrated using biological samples.

Neuer Aspekte

A novel and confident identification based on fragment ion and CCS of UPLC-Ion Mobility-MS data from metabolomics and lipidomics datasets.

Unravelling 2-deoxy-2-fluoro-D-glucose metabolism in plant tissue using mass spectrometry and NMR

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Stichworte: Arabidopsis thaliana, FDG (2-deoxy-2-fluoro-D-glucose) metabolism, Mass spectrometry, NMR, F-maltose.

Einleitung

2-Deoxy-2-Fluoro-D-glucose (FDG) is a structural glucose analogue commonly used as a radioactive glucose surrogate in clinical diagnostics and animal studies. It mimics the glucose distribution and it was assumed that after uptake, it is metabolized via glycolysis pathway to FDG-6-phosphate but not further^[1]. However, numerous papers describe the fate of FDG to FDG-6-P and further metabolites in the animal cells^[2]. FDG has also been employed in plant radiotracer studies but its metabolism in plant cells is not yet characterized. Here, we applied FDG to *Arabidopsis* leaf and analyzed leaf extract for fluorine (¹⁹F) metabolites using MS and NMR. We demonstrate that FDG metabolism in plant cells is considerably different than animal cells and goes beyond FDG-6-P.

Experimenteller Teil

Mature leaves of *A. thaliana* (short day plants, 6-7 week, early flowering stage) were gently pricked on the abaxial surface. Five microliter of FDG (20 mg.mL⁻¹) was immediately applied in the pricked spots. Four hours later leaves were extracted using Chloroform:Methanol:Water (1:2:1). Aqueous fraction was analyzed by LC-MS/MS and NMR for the presence of ¹⁹F-containing compounds.

Ergebnisse

Elucidating FDG metabolism in plants is a crucial aspect for establishing its application as a radiotracer in plant imaging. Here, we describe the metabolic fate of FDG in model plant species, *Arabidopsis thaliana*. LCMS and direct infusion MS results confirmed the presence of 5 different ¹⁹F containing metabolites in the extract. In total, we putatively identified above ¹⁹F containing metabolites as FDG (*m/z* 181.0513), F-gluconic acid (*m/z* 197.0464), FDG-6-P (*m/z* 261.0180), F-maltose (*m/z* 343.1051), and UDP-FDG (*m/z* 567.0434) on the basis of known literature information, their exact mono-isotopic mass (± 5 ppm mass error) and MS/MS fragmentation analysis. Characterization of purified compounds using NMR confirmed identification of ¹⁹FDG-6-P (*m/z* 261.0180), and ¹⁹F-maltose (*m/z* 343.1051) as major end products of ¹⁹FDG metabolism in *A. thaliana* leaf cells.

Neuer Aspekte

Elucidation of FDG metabolic fate in plant cells.

¹⁹F-maltose biosynthesis

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Synthese und massenspektrometrische Charakterisierung von Aib-haltigen antibiotischen Peptiden

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Stichworte: Festphasenpeptidsynthese, UHPLC-ESI-HR-Tandem-Massenspektrometrie von Peptaibolen

Einleitung

Peptaibole sind Sekundärmetaboliten von parasitären Pilzen, die sich durch ein interessantes biologisches Wirkspektrum auszeichnen [1]. Dabei handelt es sich um Peptide, die durch hohe Gehalte nicht-proteinogener Aminosäuren charakterisiert sind. Die dadurch verursachte Bildung helikaler Sekundärstrukturen ist für die membranmodifizierenden Eigenschaften der Peptaibole verantwortlich [2]. Durch die Entwicklung von Synthesestrategien zur Totalsynthese von Naturstoffen können die so dargestellten Peptaibole als Referenzsubstanzen für die Strukturaufklärung von zuvor isolierten Verbindungen sowie zu umfangreichen biologischen Untersuchungen genutzt werden [3, 4].

Experimenteller Teil

Die Festphasenpeptidsynthese der Peptaibole wurde mittels Fmoc-Strategie automatisiert in einem Peptidsynthesizer ResPep SL (intavis Bioanalytical instruments) durchgeführt. Die abschließende *N*-Acetylierung wurde an dem festphasengebundenen Reaktionsprodukt manuell vorgenommen. Die Aufreinigung erfolgte mittels Säulenchromatographie sowie präparativer HPLC. Die Charakterisierung der dargestellten Verbindungen wurde mittels ESI-HR-MSⁿ unter Verwendung von stoßinduzierten Dissoziationsmassenspektren (CID, *collision induced dissociation*) im positiven und negativen Ionenmodus mit einem Orbitrap Elite Massenspektrometer (ThermoFisher Scientific) durchgeführt. Die chromatographische Trennung der zu untersuchenden Probenlösung erfolgte mittels UHPLC (Dionex UltiMate 3000, Thermo Scientific) mit einer RP-C18-Säule (Hypersil GOLD; Thermo Scientific). Als mobile Phase wurden ein Gemisch aus Wasser und Acetonitril (jeweils mit 0.2% Ameisensäure) unter Verwendung eines Gradientensystems verwendet.

Ergebnisse

Im Rahmen dieser Arbeit war es möglich, die Primärstruktur der bislang unbekanntenen Chilenopeptine A und B und der Albupeptine A und C mit Hilfe von UHPLC-ESI-HR-MSⁿ-Messungen im negativen sowie positiven Modus aufzuklären und somit die vorgeschlagenen Sequenzen der nativen Strukturen zu bestätigen. Durch den Einsatz des enantiomerenreinen *D*-Isovalins (*D*-Iva) bei der Synthese der Albupeptine A und C konnte die Zuordnung der absoluten Konfiguration des Isovalins (*D*-Iva) in diesen Verbindungen mittels NMR bestimmt werden. Am Beispiel des Chilenopeptins A wird das unterschiedliche Fragmentierungsverhalten der $[M+H]^+$ - und $[M+2H]^{2+}$ -Ionen im MS²-Modus diskutiert. Bei den $[M+H]^+$ -Ionen bilden sich im MS² Fragmente der *b*-Reihe. Im Gegensatz dazu weist das $[M+2H]^{2+}$ -Ion im MS²-Spektrum zwei charakteristische Fragmente auf, die aus dem Bruch der labilen Bindung zwischen Aib-Pro resultieren. In diesem Fall werden insbesondere ein *N*-terminales *b*-Fragment- und ein *C*-terminales *y*-Ion beobachtet.

Neuer Aspekte

Strukturaufklärung und Totalsynthese neuer Peptaibole aus chilenischen Pilzen

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Advances in Native Mass Spectrometry-based methods for the analysis of Non-Covalent Protein complexes

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Stichworte: Intact, Protein, Complexes, Non-covalent, ETD

Einleitung

Native Mass Spectrometry involves the generation, transmission and detection of intact non-covalently assembled protein-protein and protein-ligand complexes which often yields a narrow charge-distribution at high m/z predominantly using nano-ESI. The high m/z capability of time-of-flight mass analysers has made them the instrument of choice for the study of such complexes. Here, we investigate the high m/z characteristics of a quadrupole/ion mobility/time-of-flight instrument (Q-IM-TOF) incorporating a dual stage conjoined source ion guide for the transmission and subsequent fragmentation using CID and/or ETD of typical assemblies. We will present examples such as human haemoglobin (64 kDa), ADH (147 kDa), GDH (335 kDa) and GroEL (802 kDa). Size exclusion UPLC-MS was also investigated using non-denaturing conditions in order to preserve the native complexes.

Experimenteller Teil

A Q-IM-TOF mass spectrometer (Synapt G2-S) was operated in both CID and ETD modes. ETD was performed in the trap travelling wave ion guide (containing He) and CID was effected by raising the ions energy as they entered the trap or transfer ion guide (containing Ar). Direct infusion was conducted by nanoESI using pre-cut PicoTip emitters. SE-UPLC made use of an Acquity UPLC I-Class Bio System with a BEH200 SEC 1.7 μm , 4.6 x 150 mm column. The complexes were injected into an aqueous mobile phase containing 10mM Ammonium Formate at a flow rate of 150 $\mu\text{L}/\text{min}$.

Ergebnisse

We will present data detailing the extent to which ETD is a valuable additional and complementary fragmentation technique to CID for such species, facilitating extensive cleavage of the protein backbone of the sub-units from within the homo- and hetero-complexes. For the nano-ESI infusion studies which combined CID and ETD, the protein complexes were infused separately. The ETD of human haemoglobin will be shown to have the potential for phenotyping human hemoglobinopathies. Moreover, the applied on-line SE-UPLC MS separation technique has significant potential for future top-down MS studies of proteins in their denatured or native states whilst offering a de-salting step prior to MS.

Neuer Aspekte

Novel use of ETD using Synapt G2-S for potential surface mapping and phenotyping of protein complexes in their native state.

Bacteriophage endolysin activity is modulated by quaternary state.

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Stichworte: Virology, Native Mass Spectrometry, Biotechnology

Einleitung

Endolysins are produced in the final stage of bacteriophage infections to lyse bacterial peptidoglycan causing the cell wall to rupture killing the bacterial host cell. The timing of lysis is strongly regulated and is thought to involve a molecular switch.

Experimenteller Teil

Here three bacteriophage endolysins were studied with focus on quaternary structure influencing lytic activity. We found that free C-terminal domain (CTD) plays an essential role for endolysin higher order assemblies.

Native mass spectrometry (MS) was used to analyze subunit stoichiometry of intact endolysins *in vacuo* and to determine ratios of different species in solution. By this means coexisting oligomeric states of wild type endolysins were identified and confirmed after CID (collision induced dissociation). The distribution of species was then compared to lytic activity.

Ergebnisse

The most abundant oligomer, a 2:2 hetero-tetramer consisting of the CTD and full length protein, is likely the most active oligomeric species. Mutants affecting the CTD production relative to the full length protein influence the oligomeric state of the endolysin and reduce lytic activity. In order to restore activity His-tagged CTD was titrated to monomeric full length protein and analyzed via native MS and in a lysis activity assay. Up to a threefold molar excess of CTD the lysis activity increases carried mainly by the 2:2 and 3:1 hetero-tetramer. However, full wild type activity was never recovered. These results demonstrate how enzymatic activity is regulated by switching the relative abundance of differently active protein complexes.

Neuer Aspekte

Secondary translation of endolysin subunit via ribosomal binding site

Relation between oligomeric state and lytic activity

Thermal/optical Carbon Analysis coupled with Photoionization Time-of-Flight Mass Spectrometry: Fine Particulate Matter from a Marine Engine

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Stichworte: single-photon ionization (SPI), resonance-enhanced multiphoton ionization (REMPI), hyphenation, fine particulate matter (PM), marine engine

Einleitung

Worldwide 90% of goods are transported by ships, mainly operating on heavy fuel oil (HFO) with an average fuel-sulfur content of 2.7%. Thereby, several million tons of fine particulate matter (PM) are emitted per year, composed of soot, metal oxides, sulfates and nitrates, but also organics adsorbed on particle surface [1]. Previous studies identified ship-related PM as a perpetrator of cardiovascular diseases and lung cancer [2]. Furthermore, it affects the radiative forcing of the Earth depending on its composition [3]. In 2020, the legal fuel-sulfur content for ships will be statutorily restricted to 0.5% in coastal areas or even to 0.1% in sulfur emission control areas (SECA). Therefore, the qualitative and quantitative changes in emission have to be evaluated.

Experimenteller Teil

PM of HFO and diesel fuel (DF) from a marine engine was collected on quartz fiber filters and analyzed by a thermo/optical carbon analyzer (TOCA) which was coupled to a time-of-flight mass spectrometer (TOFMS) using single photon ionization (SPI) at 118 nm and resonance-enhanced multi photon ionization (REMPI) at 266 nm [4]. Among the quantification of organic (OC) and elemental carbon (EC) by “*ImproveA*” temperature protocol, approximately 20% of the evaporating compounds are alienated to the TOFMS, which allows the detection of molecular ions of single compounds in thermodesorption-like (25°C – 280°C) and pyrolysis-like fraction (280°C – 580°C). REMPI refers to a selective ionization technique for aromatics, whereas SPI is regarded as a more universal technique of soft ionization.

Ergebnisse

HFO-derived particles contained the triple quantity of OC (38.6 mg/m³) compared to DF-derived particles (14.3 mg/m³), but approximately the same quantity of EC (HFO: 8.0 mg/m³; DF: 8.6 mg/m³), which finds also expression in the different ratios of OC/EC (LFO: 4.8; HFO: 1.8). In the thermodesorption-like OC-fraction of DF, the mass spectrum is dominated by methyl esters of fatty acids, such as oleic acid, linolic acid and palmitic acid, which are known as biodiesel components. The thermodesorption-like OC-fraction of HFO showed mainly alkylated dibenzothiophenes and phenanthrenes along with other low-weight polycyclic aromatic hydrocarbons (PAH). In the pyrolysis-like OC-fractions some similar thermal fragments occurred for both fuels. Sulfur-containing thermal fragments as well as large PAH seems to be characteristic for HFO. This observation was examined more in detailed by running a modified Student’s t-test [5] for the mass spectra of both fuels and the respective thermal fraction. It turned out that alkanes, (low-alkylated) 2- to 4-ring PAH and corresponding oxy-PAH were significantly enhanced on the DF-particles, whereas HFO-particles contained significantly more n-ring-PAH with $n \geq 5$ and PAH with a high degree of alkylation. Moreover, the combustion was investigated by comparing ratios of parent and alkylated PAH in fuel and particulate emission. Parent PAH are mainly formed by flame synthesis while alkylated PAH in the emission indicate unburned fuel. Compared to the fuel, diesel fuel particles are enriched in parent PAH, but for HFO the distribution of alkylation remains constant. Hence, two different fuel-dependent combustion processes can dominate in the same engine leading to different type of PAH with different toxicity equivalent factor (TEF). Thus, toxicological studies must follow to assess whether the potential change in effect of ship-related PM on human health.

Neuer Aspekte

PM from a marine engine was analyzed by thermal/optical carbon analysis coupled to time-of-flight mass spectrometry with photoionization.

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SpheriCal® - Monodisperse Polyester Dendrimers as Universal Mass Calibrants in Mass Spectrometry

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Stichworte: SpheriCal, monodisperse, mass calibration

Einleitung

Proteins or peptides were an obvious choice to meet the need for calibrants during early mass spectrometry studies of high mass ranges ($m/z > 1000$) because these large biomolecules were widely available and monodisperse. However, they typically exhibited poor stability, and therefore required care during storage and sample preparation. While traditional linear polymers within a similar mass range can be easily prepared with enhanced stability, they exhibit polydispersity which can complicate accurate mass identification. Dendrimers, however, are a class of synthetic macromolecules that are prepared using an iterative step-wise synthesis, and therefore offer high molecular weights and monodispersity in addition to chemical robustness. A family of polyester dendrimers have been prepared and evaluated for use as calibrants.

Experimenteller Teil

A library of polyester dendrimers were prepared according to previously reported techniques with molecular weights ranging from 700 to 30000 u. MALDI-TOF-MS was used to characterize crude reaction mixtures to verify that each step in the synthesis was driven to completion, and to confirm the monodispersity of the product after workup. MALDI-TOF-MS sample preparation utilized 9-nitroanthracene as the matrix, sodium trifluoroacetate as the counter ion and tetrahydrofuran as the solvent. All chemicals and solvents were purchased from Sigma-Aldrich and used without further purification. Mass spectrometric analysis was performed using a Bruker Autoflex III MALDI-TOF mass spectrometer (Bruker Daltonics, Billerica, MA.) Data was acquired using reflector-positive ion mode, with an acceleration voltage of 20 kV, and delayed extraction.

Ergebnisse

The dendrimer calibrants exhibited a number of attractive advantages, including exceptional shelf-lives, broad compatibility with a wide range of matrices and solvents, and evenly spaced calibration masses across the mass range examined, 700-30,000 u. The exceptional purity of these dendrimers and the technical simplicity of this calibration platform validate their broad relevance for high molecular weight mass spectrometry.

Neuer Aspekte

Monodisperse dendrimers exhibit enhanced shelf-life and compatibility with matrix and counterion enhancing their utility as calibrants.

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SILAC-Based Secretome Analysis of Non-Small Cell Lung Cancer Cell Lines

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Stichworte: SILAC, NSCLC, secretome, cancer

Einleitung

Lung cancer is the most common cancer fatality in Europe (335,000 deaths/year) [1] with non-small cell lung cancer (NSCLC) comprising 80-85% of all cases [2]. Survival rates are poor (5-year survival rate lower than 15%) as most patients develop a metastatic disease that is too advanced for curative surgical resection. Even for these patients who undergo surgery, cure is not guaranteed. Tumor-host interaction plays a major role in carcinogenesis, but also in resistance to apoptosis- and necrosis-inducing treatment strategies. This interaction is modified by therapeutic interventions and changes substantially when tumor cells become resistant to a therapeutic agent. Analyzing the secretome of NSCLC cells may provide novel targets to overcome the therapeutic resistance of lung cancer.

Experimenteller Teil

For quantitative comparison of proteins secreted by two different NSCLC cell lines the SILAC (stable isotope labeling with amino acids in cell culture) approach was applied.

Mixed SILAC cell supernatants were concentrated via ultrafiltration. After SDS-PAGE followed by tryptic *in-gel* digestion the resulting peptide mixtures were separated by nano-HPLC (UltiMate, Dionex) and analyzed by nano-ESI-MS/MS (LTQ-Orbitrap XL, Thermo Fisher Scientific).

Eight biological replicates were performed. Differentially regulated proteins were evaluated based on their heavy/light SILAC ratios, the number of unique peptides, and their origin as secreted proteins. The interactions of selected proteins will be further investigated by chemical cross-linking combined with mass spectrometry to identify binding partners and its interface regions.

Ergebnisse

To differentially quantify NSCLC secretomes, a stable isotope label can in principle be introduced by chemical modification or by metabolic labeling [3]. The earliest stage for introducing stable isotope signatures into proteins is by metabolic labeling during cell growth and division [4]. SILAC was chosen as it is a metabolic labeling approach [4].

Heavy (^{13}C -arginine and ^{13}C -lysine labeled) and light (^{12}C -arginine and ^{12}C -lysine labeled) states of NSCLC cell lines PC9 (erlotinib sensitive) and PC9ER (erlotinib resistant) were analyzed.

On average, 900 proteins were identified in each sample with low variations in the numbers of identified proteins, indicating the robustness of our approach. Applying abovementioned criteria to identify protein candidates that are differently regulated between PC9 and PC9ER cells yielded 17 proteins, with five proteins (tissue-type plasminogen activator, epidermal growth factor receptor, urokinase-type plasminogen activator, platelet-derived growth factor d and stromal cell-derived growth factor) showing the most prominent different regulation. Tissue-type plasminogen activator (t-PA) is an already established tumor marker for other cancer types and seems to be a promising biomarker for erlotinib resistance in NSCLC cells.

Therefore different variants of t-PA were successfully expressed in *E. coli* BL21(DE) and purified by immobilized metal ion affinity chromatography (IMAC). As a next step, cross-linking experiments will be performed with purified t-PA variants and supernatants of PC9 and PC9ER cells to investigate the interactions between t-PA and proteins secreted by NSCLC cells.

Neuer Aspekte

Quantitative secretome analysis of NSCLC cell lines and interaction studies between t-PA and secreted proteins by chemical cross-linking and MS.

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Human bone marrow stromal cell-derived osteoblast matrix vesicle proteome and functions are regulated by sulfated glycosaminoglycan derivatives

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Stichworte: Osteoblasts, matrix vesicles, extracellular matrix, glycosaminoglycans

Einleitung

The bone formation and the remodeling is a highly complex process that involves among others osteoblasts. These cells synthesize the extracellular matrix (ECM) and initiate its mineralization. Osteoblast-released matrix vesicles (MV) where shown to have an outstanding role in the initiation of the ECM mineralization and additionally possess proteins with different functions [1]. It is known that mesenchymal stromal cells and osteoblasts respond to the mechanical and chemical properties of their microenvironment [2]. E.g., sulfated glycosaminoglycan (GAG) derivatives where shown to promote osteoblast functions like the ECM synthesis and mineralization [3]. To delineate the molecular effects of different GAGs on the MV biogenesis, release and composition, we isolated MV from SILAC-labeled osteoblasts and quantified their respective protein composition using nano-HPLC/nano-ESI-MS/MS.

Experimenteller Teil

For a SILAC approach Human bone marrow stromal cells (hBMSCs) were labeled for 34 days. 7,000 labeled hBMSCs/cm² were cultured on Tissue culture polystyrene and treated with different soluble GAGs: non-sulfated hyaluronan (HA), singly-sulfated hyaluronan (sHA1), or chondroitin sulfate (sCS1). hBMSCs were differentiated into osteoblasts in osteogenic medium. The medium was changed every 3-4 days and collected until day 22. MVs were enriched by a centrifugation procedure with a final ultracentrifugation at 100,000xg for 60 min. MV and cell samples were lysed and separated by 1D-SDS-PAGE. Measurements were conducted by LTQ-Orbitrap XL ETD after nUPLC peptide separation using a 1-40 % non-linear gradient of acetonitrile. To confirm the proteomics results biochemical parameters were analyzed using immunofluorescence microscopy, Westernblot, zymography, and ELISA.

Ergebnisse

Initial results showed a strong promoting effect of sHA1 on the osteoblast activity by increasing the activity of the tissue non-specific phosphatase (TNAP) – an early marker of osteogenesis - as well as the calcium and phosphate deposition. Previous studies revealed only a slight impact of sulfated hyaluronan on cellular proteome of osteoblasts [3] suggesting other processes being involved. Indeed, proteomic analyses of MV fraction revealed 385 proteins of those 273 were quantified. The unusable high proportion of about 15 % regulated proteins after sHA1 treatment ($FC \pm 0.5$, p -value < 0.05) indicates an altered MV composition to hold a key role in the promoted ECM mineralization. The purity of the MV protein fraction was evaluated based on three criteria: (i) 21 of the major 25 extracellular vesicle (EV) proteins were present, (ii) a majority of 73 % of the identified proteins were either shown or predicted to be part of EVs and (iii) only a minority of 23 % of the identified proteins include a signal peptide to be secreted by classical mechanisms. A functional clustering of regulated proteins after sHA1 treatment indicates a promoted growth factor bioavailability by LTBP2 and fibrillin as well as activation by thrombospondins. Additionally, the cell-matrix-interaction and a proper ECM organization seem to be increased by different proteins, e.g. fibronectin and periostin. The other tested GAGs do not alter the MV composition in the same extents. sCS1 seem to have an additional influence on the cell adhesion whereas HA do not show any additional bone formation-specific influence. Taken together sHA1 seem to promote the ECM mineralization in a specific way. This seems to be relied to a large extent on an altered MV composition, but the underlying mechanisms still need to be revealed.

Neuer Aspekte

We showed by quantitative proteomic and functional assays that osteoblasts MV composition and activity can be modulated by sulfated GAGs.

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Monitoring PPAR γ induced changes in glycolysis by selected reaction monitoring mass spectrometry

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Stichworte: Adipocytes, selected reaction monitoring (SRM), SIS peptides, absolute quantification, Proteomics

Einleitung

The peroxisome proliferator-activated receptor gamma (PPAR γ) is a nuclear fatty acid receptor that has been shown to be the ultimate regulator of fat cell differentiation [1,2]. The realization that a fatty acid sensor such as PPAR γ might also be an important regulator of glucose metabolism arose from the discovery that the insulin-sensitizing thiazolidinediones (TZDs) such as rosiglitazone are potent agonists for PPAR γ [3] leading to an upregulation of the glucose transporter GLUT4. Because of this regulatory function in glucose metabolism, it is of great interest to monitor the relative and absolute changes in glycolysis upon PPAR γ activation. Since PPAR γ is the metabolic master regulator of many processes, we used this model system to investigate cellular metabolic remodeling.

Experimenteller Teil

To screen and identify the glycolytic and other main regulated pathways upon PPAR γ activation, we first set up a label free quantitative global proteomics approach by using a high resolution mass spectrometry system (QExactive Plus). By applying STRING 9.1 and Cytoscape 3.2.0 we visualized the regulated proteins, pathway and network dynamics. To further investigate the regulatory effects of PPAR γ on different pathways, we performed a titration of rosiglitazone and analyzed the samples with a targeted proteomics approach by using selected reaction monitoring (SRM). This allowed for deeper investigation of each pathway by having a higher sensitivity, absolute amounts and wider dynamic range.

Ergebnisse

Here we established a quantitative proteomics approach to analyze and quantify proteins of the glycolytic and other metabolic pathways by using a global label free and a targeted proteomics setup. Our results show in general the regulation of 719 proteins quantified with at least 2 peptides. 23 enzymes and their isoforms which are involved in the glycolytic pathway could be identified and absolute quantified using stable isotope labeled standard peptides (SIS peptides). The rosiglitazone titration experiment revealed that certain key players such as the ADP-dependent Glucokinase, Hexokinase (initial steps) and Phospho-fructo-kinase (rate limiting step) display a significant upregulation during the PPAR γ activation while the level of regulation and abundance is reverse correlated with their enzymatic activity. A first closer look at those regulated proteins revealed them as potential downstream targets of PPAR γ . Moreover there is an evidence for a metabolic shift from the glycolysis pathway to other metabolic pathways which is not controlled by PPAR γ . With this set of methods and system we want to further investigate the networks of lipid metabolism controlled by PPAR γ activation to reveal the detailed dynamics of protein and pathway regulation and possible metabolic shifts.

Neuer Aspekte

Absolute quantification of abundance changes in the glycolytic pathway upon PPAR γ activation using SIS peptides and SRM.

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Proteomic study of the impact of Magnesium implants on osteoblasts

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Stichworte: Magnesium, Implant, Proteomic, mass spectrometry

Einleitung

Magnesium implants for bone healing offer the opportunity getting degraded by corrosion and thus avoiding a second surgery for removing them, because the implants in the best case will have been disappeared after bone healing will have been finished (1 and 2). By the corrosion process Mg^{2+} ions are formed which will increase the Mg^{2+} concentration significantly above physiological levels (1). Thus, the question arise, how these elevated Mg^{2+} levels affect bone cells. Therefore, we studied the effect of elevated Mg^{2+} levels on differentiated and proliferated osteoblasts by proteomics.

Experimenteller Teil

In this study, we investigated the proteomes of cultured proliferating and differentiating osteoblast, in absence (control) and presence of 5mM $MgCl_2$, and Mg-alloy extracts.

Cell pellets were lysed; proteins of these cells were extracted, reduced, alkylated and finally incubated with trypsin. Tryptic peptides were subjected to a nano-UPLC-column coupled to a hybrid orbitrap system (Orbitrap-Fusion, Thermo Fisher scientific). Data analysis was performed with Proteome Discoverer (Thermo Fisher scientific) and a first data interpretation for a rough preliminary comparison of relative protein abundance done by using spectral counting.

Ergebnisse

370000 spectra were recorded in total yielding approximately 4700 identified proteins including more than 10000 peptides. The levels of hundreds of proteins increased respectively decreased in response to elevated concentrations of Mg^{2+} . The level of Neuroblast differentiation-associated protein (AHNAK), a 700-kDa scaffold protein, was massively associated with the increase of Mg^{2+} concentrations and thus attracted attention. AHNAK is involved in regulation of voltage-gated calcium channel activity, thus obviously being an important protein in regulating the physiological actions of osteoblasts. The level of AHNAK is decreased in differentiating osteoblasts in presence of elevated Mg^{2+} levels in comparison with differentiating osteoblast in absence of Mg^{2+} . In contrast, the AHNAK abundance in proliferating osteoblasts is increased in presence of elevated Mg^{2+} compared with proliferating osteoblast in absence of Mg^{2+} .

In summary, the investigation of the response of osteoblasts towards elevated Mg^{2+} with proteomics tools gave deep insights in the Mg^{2+} induced changes in the protein composition of the osteoblasts. Linking these changes to molecular physiology of the osteoblasts the overall response of osteoblasts to elevated Mg^{2+} seems to be beneficial for bone healing.

Acknowledgements

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Neuer Aspekte

Comprehensive proteomics study of the effect of magnesium implants on bone cells

Referenzen

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Uncontrolled modification of redox-active cysteines in proteins and peptides

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Stichworte: artifactual protein modifications, redox-active cysteines

Einleitung

Modifications of proteins caused by sample handling interfere with the detection of posttranslational modifications. Phosphorylation is a case in point, as it may easily be confused with artifactual sulfonation (Δ 9.5 mDa). Moreover, both real and artificial modifications are typically of low abundance increasing the analytical difficulties further. It has been, e.g., shown that artifactual sulfation of hydroxyamino acids can occur during silver staining [1]. S-sulfonation of cysteine has also been described for transthyretin when incubated at 37 °C under alkaline conditions for one week [3]. Here we present evidence that S-sulfonation alongside other modifications of redox-active cysteines can occur as a result of standard proteomic work-up procedures, such as tryptic digest and SDS-PAGE.

Experimenteller Teil

Commercial standard proteins (Sigma-Aldrich) and synthetic peptides (Thermo Scientific) were subjected to proteomic routine procedures. Proteins were purified by SDS-PAGE using 12 % SDS-polyacrylamide gels and stained by blue silver staining [2]. Gel pieces were destained and digested with trypsin (Serva) in NH_4HCO_3 -solution (50 mM, pH 8.7, Fluka) at 37 °C overnight. Alternatively, the proteins were directly trypsinized without prior purification steps. Synthetic peptides were heated to 95 °C for 5 min (pH 6.8) and incubated overnight at 37 °C in NH_4HCO_3 -solution (50 mM, pH 8.8) with 0.05 % $(\text{NH}_4)_2\text{S}_2\text{O}_8$ (w/v, USB Corporation) to simulate an SDS-PAGE. LC-MS/MS was performed using C18 nanoUPLC-tandemMS (nanoAcquity, Q-TOF Premier, Waters Corp.).

Ergebnisse

Peptides of interest (containing “redox-active” cysteines) were selected based on the close proximity of a basic amino acid to cysteine. The addition of nominal 80 Da was detected after SDS-PAGE or tryptic digest in every examined sequence. Additional cysteine modifications were also found (+76 Da, +64 Da, +48 Da, +32 Da, -34 Da). The formation of dehydroalanine and thiocysteine (-34 Da, +32 Da) has been suggested before [3]. Disulfide formation with β -mercaptoethanol (+76 Da) has also been discovered by other authors [4]. This modification must be expected in commercial products and synthetic peptides even when the use of β -mercaptoethanol is not explicitly stated as it is a common agent in protein purification procedures. The addition of +48 Da might either be attributed to oxidation to cysteic acid or to S-sulfenation whereas the +64 Da modification most likely represents S-sulfination. Only when adding 10 mM DTT to every step of the work-up including the sample buffer, modifications of +76/80 Da were eliminated.

Neuer Aspekte

Proteomic procedures cause artifactual modifications of redox-active cysteines

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Low abundant N-linked glycosylation in wild-type hen egg white lysozyme is localized at non-consensus sequons

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Stichworte: Hen egg white lysozyme, non-consensus N-glycosylation sites, glycan heterogeneity, LC-MS/MS, GlycoQuest bioinformatics

Einleitung

Hen egg white lysozyme (HEL) is one of the most extensively studied glycoside hydrolase enzymes, and was the first enzyme, for which a crystal structure had been published. Although wild-type HEL is lacking the consensus sequence motif NX(S/T), in 1995 Trudel et al.¹ proposed the existence of a low abundant *N*-glycosylated form of HEL. However, the identity of active glycosylation sites in HEL remained a matter of speculation. For the first time since Trudel's initial work, we report here a comprehensive characterization by means of mass spectrometry of the proposed low abundant *N*-glycosylation in wild-type HEL aiming for the unambiguous identification of active glycosylation sites and for profiling the compositional heterogeneity of the modifying *N*-glycans.

Experimenteller Teil

HEL was digested in solution using trypsin as a cleaving enzyme. From the resulting digest, the *N*-glycopeptides were enriched by means of ZIC-HILIC. Subsequently, the enriched *N*-glycopeptides were enzymatically deglycosylated by ¹⁸O/PNGase F and various endoglycosidases (F1, F2, F3, H). Both the intact and deglycosylated *N*-glycopeptides were analyzed by LC-MS/MS employing multiple mass spectrometric techniques (MALDI-TOF/TOF, ESI-iontrap using either CID or ETD for fragmentation).

Data analysis was performed using Bruker's ProteinScape 3.1 bioinformatics platform featuring a novel data interpretation workflow, consisting of MS/MS spectra classification and glycan database searching, enabling the efficient automatized detection and characterization of *N*-glycopeptides in LC-MS/MS datasets obtained from glycoprotein digests.

Ergebnisse

LC-MS/MS data obtained from the intact *N*-glycopeptides enriched from HEL trypsin digest enabled the identification of the asparagine-rich *N*-glycopeptide [34-45] FESNFNTQATNR containing three Asn residues (N37, N39 and N44) as potential glycosylation sites. The automated detection of this *N*-glycopeptide was facilitated by the novel GlycoQuest data interpretation workflow featured in the ProteinScape 3.1 software used here.

Furthermore, the intact *N*-glycopeptide data were utilized to profile the heterogeneity of the *N*-glycans modifying HEL. The novel GlycoQuest search engine was employed to identify the composition of the glycan moieties. Overall, the heterogeneity profile of *N*-glycans in HEL comprised at least 26 different glycan compositions.

Additional deglycosylation experiments were required to achieve the unambiguous identification of the active sites of *N*-glycosylation in HEL. LC-MS/MS results obtained after deglycosylation with ¹⁸O/PNGaseF and endoglycosidase F/H, respectively, provided clear evidence for Asn residues N44 and N39 as active glycosylation sites in HEL. These sites are part of the non-consensus sequons NXN and NXQ, respectively, which have only been reported in very few cases before for glycoproteins of eukaryotic origin.

Neuer Aspekte

Identification of previously unknown *N*-glycosylation sites in HEL; profiling the *N*-glycan heterogeneity; novel bioinformatics workflow for glycopeptide analysis

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A closer look at the quality of microwave digests

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Stichworte: Microwave protein digestion, rel. quantification, MALDI-MS

Einleitung

Protein digestion and digestion time is still a bottleneck in proteolytic analyses. For accelerating digestion microwave-assisted protein digestion has been reported as a promising technique with digestion times of even < 30 min. Preliminary results presented on the 2014 DGMS meeting showed that there are no distinct differences between digests in microwave system compared to a water bath under comparable temperature and time conditions. These data were in accordance with published results. In most cases the sequence coverage or the number of identified peptides was used as measure for comparison. [1] Some authors used the quantity of single marker peptides or the absence of the intact protein as indicator for the completeness of the digest. [2]

Experimenteller Teil

For evaluation of the microwave digests we used a CEM Discover System (CEM, Matthews, USA) with a maximum power output of 300 W and a frequency of 2.45GHz and compared it with a thermoshaker. Apo-transferrin and myoglobin were taken as standard proteins and digested with the commonly used highly specific serine protease trypsin (E:P = 50). As an internal standard for relative quantification [Glu¹]-fibrinopeptide (Glu-Fib) was used and mixed with α -cyano-4-hydroxycinnamic acid (CHCA). The experiments covered a digestion time range of 30 min to 18h and were performed with and without prior reduction and alkylation. For optimization of the digest results the anionic detergent sodium deoxycholate was added to some samples.

Ergebnisse

When comparing microwave and thermoshaker at equal time points and temperatures, again no superiority of the microwave could be detected with respect to sequence coverage. While the sequence coverage often reaches a maximum after short times and stays constant over a long period it is expected that the total peptide number should increase over a longer time interval until the protein is completely digested. For a detailed comparison of microwave and shaker digests a relative quantification of digestion peptides was performed using Glu-Fib as internal standard.

Evaluation of all peptides of the protein used for Mascot search without missed cleavages and modifications over the time shows that the intensity and accordingly the quantity of the peptide not necessarily increases with time. Different peptides show different time profiles. The shape of these profiles is comparable between microwave and shaker with a slight superiority of the thermoshaker.

The results show, that it is not meaningful to take a random peptide as a quantitative marker over time and that the sequence coverage is not a reliable marker for digestion efficiency.

The addition of sodium deoxycholate prior the digestion increases the sequence coverage - especially for non-reduced and alkylated samples. The quantitative evaluation of samples with addition of sodium deoxycholate shows a higher amount of some peptides.

Neuer Aspekte

Quantification of many peptides to compare microwave and shaker digests.

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New Workflows for Identification and Profiling of Disulfide Bonds in Biopharmaceuticals

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Stichworte: Disulfide bond, ETD, ion trap, protein identification, biopharmaceutical

Einleitung

The spatial structures of biologics are crucial for their safety and efficacy and fundamentally determined by disulfide bonds (DSBs). We evaluated alternative approaches to the classical comparison of reduced and non-reduced digests using ESI LC-MS³ and MALDI LC-MS³ and non-reduced protein digests.. These workflows were applied to various model proteins (α -lactalbumin, RNase, adalimumab)

Experimenteller Teil

Digests of were performed with a mixture of LysC and trypsin to obtain a good yield. Free cysteins were blocked with N-ethylmaleimide to avoid disulfide scrambling . The samples were separated using nano-LC followed by MS analysis. An ion-trap equipped with a CaptiveSpray ionization source was used in the ETD workflow, and a MALDI-TOF/TOF instrument was used in the ISD workflow. Software was developed to identify characteristic fragmentation patterns of disulfide-linked peptides in MS² spectra and generate target lists for the MS³ step.

Ergebnisse

To improve ETD efficiency, sheath gas was acetonitrile-enriched : the average charge state distribution (CSD) of the analyzed DSB-peptides was shifted by 30% towards the higher values, and their average intensity was doubled.

In this approach, we utilized a new targeted MS³ experiment to profile DSB-peptides by applying ETD in MS² followed by targeted CID in MS³. we acquired retention time dependent full scan MS³ spectra in the IT, enabling simultaneous detection of many peptide fragments. .With this targeted workflow, 7 of 8 expected adalimumab DSB-peptides were identified.

MALDI-ISD was used to partially reduce LC separated DSB-peptides. For the identification of DSB-peptides, MS spectra were screened for triplets of the DSB-peptide and their two characteristic ISD fragments. All peaks were subjected to LC-MALDI-MS/MS analysis. For adalimumab, all DSB-peptides with one disulfide bond were identified with this method.

Neuer Aspekte

Protein disulfide bond analysis was performed using either an ETD-MRM workflow with an ion-trap or a new MALDI-ISD workflow

Exploring the potential of the last generation UHR-Q-TOF for rapid generation of accurate information on proteoforms distribution and relative abundance

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Stichworte: Proteoform, top-down, QTOF, intact proteins

Einleitung

All along their life cycle, proteins undergo various transformations that can alter their functions while keeping a good part of their primary sequence intact. These multiplications of PTM patterns, alternative splicing forms or products of proteolytic processing cannot be simply resolved with a bottom-up approach, as very few peptides are specific from the given proteoform. However, the information relative to the distribution of different proteoforms is encoded in their intact masse.

In this study we were willing to evaluate how the latest generation UHRQ-Tof capabilities of delivering resolution, mass accuracy and isotopic fidelity even in highly complex mixtures separated with Fast LC could help to quickly resolve proteoforms in complex mixtures, in order to map their distribution and relative abundancies.

Experimenteller Teil

Undigested protein mixtures of E.Coli ,Yeast and non-depleted plasma were separated on a Phenomenex Aeris Widepore, 3.6 μ , C4, 150x2.1 mm column. LC was performed on a Dionex RSLC system coupled to an impact II (Bruker) or maXis II UHR-Q-Tof (Bruker) , operating in MS, Auto MS/MS or targeted MS/MS acquisitions.

Mixtures of yeast intact protein (Promega) spiked with various ratios of UPS2 Proteomics Dynamic Range Standard Set (Sigma) were separated on a 250 mmX100 μ m monolithic pepswift PS-DVB column after preconcentration on a monolithic pepswift 200 μ m x 5mm trap. LC was performed on an Ultimate nano-RSLC system (Thermo) coupled to an impact II (Bruker) via a CaptiveSpray nanoBooster ion source (Bruker), and operated in MS and auto MS/MS modes.

Ergebnisse

Using High-Quality threshold protein detection, we could easily detect more than 800 proteoforms (from doubly charges peptides up to 35Kda proteins) out of the E.Coli mixture, and more than 1500 proteoforms out of 50 μ g of the Yeast mixture, using a 15 minute gradient (20 minutes method). The separation of yeast over a 35 minutes gradient (45 minutes method) enabled to distinguish more than 1000 proteoforms from a 1 μ l injection of non-depleted plasma.

The spectral quality observed for single compounds was preserved while measuring these highly complex mixtures : the average mass error for the monoisotopic peak of the RS 19 protein (10,2 Kda) over 4 injections was $0,07 \pm 0,2$ ppm. The isotopic fidelity (express as shift of the theoretical abundance, relative to the most abundant isotope) was always better that 2%, and the resolution exceeded 50 000. An Auto-LC MS/MS run enabled the identification of RS 19 using CID from several charge states. In the absence of on-the flight deconvolution, automated LC-MS/MS enabled to fragment the high abundance low MW (<20KDa) proteins. Lower intensity proteins could be fragmented and identified using a targeted MS/MS Strategy.

A proteoform distribution profile could be obtained for glycoproteins spiked in the sample, which was similar to the one observed for the isolated glycoprotein, therefore suggesting that the approach enables to give a relative quantitation information for the compounds present in the initial mixture. Using unsupervised statistics approaches, we have been able to separate the yeast extracts spiked with various amount of the UPS II mixture.

Neuer Aspekte

High-quality proteoform distribution information was delivered which is complementary to bottom-up approaches.

High quantification efficiency for discovery and validation approaches on a Q-TOF platform

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Stichworte: Quantification, proteomics, QTOF, biomarker

Einleitung

Shotgun mass spectrometry is a popular tool for candidate protein biomarker discovery. This global profiling method, typically performed on high-resolution instruments, is used to gain qualitative and quantitative information of several thousands of proteins in complex proteomic digests. Subsequent validation/verification of detected biomarkers requires accurate measurements of protein target abundances in biological samples. These tasks are typically performed through a targeted quantitative proteomic approach involving MRM. This approach requires a priori knowledge of the target precursor/product ion pairs and time-consuming method preparation, but prevents post-analysis data mining. Actual high-resolution systems are now able to address these limitations while providing comparable selectivity. We report here the evaluation of a QTOF for exact quantification as well as for targeted quantification of peptides.

Experimenteller Teil

To evaluate quantification performance for proteomics, different complex tryptic digests were mixed with stable isotope labeled peptides or digests of standard proteins at known concentrations. Samples were analyzed with nano-flow UHPLC and a CaptiveSpray ion source connected to the impact II Q-TOF instrument. For peptide identification and label-free quantitative analysis the MaxQuant software package was used (Nature Biotechnology 26, 1367 - 1372 (2008)). Targeted quantification data was processed using Skyline.

Ergebnisse

For the label-free quantification experiment a defined model system for complex proteomics samples, consisting of a mixture of 48 standard proteins spanning a concentration range of five orders of magnitude (UPS-2, Sigma) was spiked into samples of 500ng Yeast digest at a concentration of 1:2. During separation in 2h gradients, the UPS-2 peptides could be quantified based on the MS full scans at levels from 500fmol down to the low attomole range.

For targeted quantification plasma tryptic digests samples were spiked with stable isotopically labelled standard peptides, spanning a 10000 fold concentration range. Samples were measured using a high resolution extracted ion chromatogram (HR-XIC) or Data Independent Analysis (DIA) mode. Results clearly show high quantification efficiency in undepleted plasma covering a dynamic range of 4 orders of magnitude. Comparison with MRM-based approach revealed excellent correlation displaying high versatility of the Q-TOF instrument for targeted quantification.

Neuer Aspekte

The evaluated high-resolution mass spectrometer platform provides high efficiency both for discovery and targeted approaches

MeCAT - Using light for cleavability

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Stichworte: Proteomics, metal labeling, lanthanide, DOTA, photocleavable, MALDI-Imaging

Einleitung

As part of proteomics, the need for reliable protein quantification methods steadily increases. Thereby, the use of stable isotopes for protein and peptide labeling including ICAT and iTRAQ are increasingly applied. Labels are based on a chemical labeling reaction with a specific reagent. Mass differences of differently labeled samples are then detected by mass spectrometry (MS) to gain 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 can be employed. Here, we introduce a new photocleavable MeCAT-IA reagent, carrying an iodoacetamide moiety [2].

Experimenteller Teil

A new cleavable linker including the MeCAT-reagent was synthesized. Proteolysed model proteins were labeled with this photocleavable MeCAT-reagent, which contains a cysteine-reactive group for quantitative labeling, an elemental tag, loaded with a lanthanide ion for quantification and a photocleavable group for formation of characteristic fragments that can be used for quantification as well. For separating labeled peptides from other sample components a HPLC was used coupled online to electrospray ionization (ESI)-MS. Furthermore, irradiation experiments with labeled peptides were performed, using an UV-lamp. Moreover, first experiments of analysing labeled peptides via MALDI-MS and MALDI-Imaging were conducted.

Ergebnisse

The investigated proteolysed model proteins have at least five cysteine residues in its sequence. The completeness of the labeling was examined in dependence of the presence of cysteine containing peptides. The obtained spectra of labeled peptides from HPLC/ESI-MS were compared with the native peptide spectra. No unlabeled peptides were found after the labeling reaction with the photocleavable MeCAT-reagent. This was shown for all investigated model proteins. Hence, an important requirement for reliable quantification was fulfilled. In further experiments, the photocleavability of the new MeCAT-reagent was investigated. For that reason, an UV-lamp was used irradiating the labeled peptides, followed by HPLC/ESI-MS. A characteristic photofragment, which can be used for quantification in prospective experiments, was detected. Furthermore, the fragmentation behavior of labeled peptides was investigated. Here, the fragmentation techniques CID and IRMPD were applied.

In order to increase the analytical options with the MeCAT-reagent, labeled peptides were also investigated by MALDI-MS and MALDI-Imaging.

Neuer Aspekte

Synthesis of a new MeCAT-reagent, which can be cleaved from peptides by light.

Referenzen

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Displacement chromatography as enrichment step in phosphoproteomics

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Stichworte: phosphoproteomics, mass spectrometry, displacement chromatography, enrichment of phosphopeptides

Einleitung

Protein phosphorylation plays a major role in regulation of cellular signaling pathways. Thus phosphoproteomics is a major tool for investigating signaling pathways. Due to the low abundance of phosphoproteins in cells in comparison with non-phosphorylated proteins, detection and identification of phosphoproteins are considerably difficult. Therefore, enrichment of phosphoproteins or tryptic phosphopeptides is inevitable for analysis of phosphoproteomes [1,2]. The aim of this study was to optimize existing enrichment techniques to increase the yield of phosphopeptides by applying displacement chromatography in order to have a deeper insight into the phosphoproteome.

Experimenteller Teil

The enrichment of phosphopeptides of tryptic-digested α -, β -Caseins via displacement chromatography was performed in a monolithic cation-exchange chromatography (CEX) column with spermine as a displacer followed by subsequent mass spectrometric analysis. Displacement chromatography was carried out with the HPLC system: Äkta Explorer using a Uno S polishing column at a flowrate 20 μ L/min. The collected fractions were analyzed by MALDI-MS.

Ergebnisse

With displacement CEX tryptic phosphopeptides were separated from non-phosphorylated peptides. Analysis of the CEX fractions showed that not only most of non-phosphorylated peptides eluted late, whereas most of the phosphopeptides eluted early as expected. Furthermore, the yield of the number of multi-phosphorylated peptides was higher compared to conventional gradient chromatography. The results show that CEX displacement chromatography provides advantages for enrichment of phosphopeptides and non-phosphorylated peptides.

Neuer Aspekte

phosphopeptide enrichment by displacement chromatography

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Mass spectrometric based approaches for studying oligomerization of β -amyloid peptide at amino acids level

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Stichworte: A β aggregation, Alzheimer disease, mass spectrometric approaches

Einleitung

A frequent disease, especially among the aging population, is the Alzheimer disease (AD), a neurodegenerative disorder characterized by memory loss and cognitive decline. Very often AD is associated with the formation and accumulation of fibrillar plaques of β -amyloid peptide (A β) in the brain [1]. Many methods such as circular dichroism, atomic force microscopy, electron microscopy, light scattering have been employed in order to elucidate the aggregation mechanism of β -amyloid peptides, but still a detailed understanding of this pathophysiological process is needed. In this work, the aggregating A β (1-40) peptide is investigated as model system by a footprinting strategy consisting of a combination of FPOP (fast photochemical oxidation of proteins) [2] with mass spectrometry.

Experimenteller Teil

The oligomerization of A β model peptide was investigated by a mass spectrometric based approach, namely by the combination of FPOP (fast photochemical oxidation of proteins) with high resolution mass spectrometry. FPOP is a chemical footprinting method whereby exposed amino acid residues are covalently labeled by oxidation using hydroxyl radicals produced by the photolysis of H₂O₂ in presence of scavengers. The modified peptide is digested with trypsin and the proteolysis mixture is analyzed by LC/MS/MS, providing information about solvent accessibility within the peptide structure.

Ergebnisse

Here, we report the FPOP data of β -amyloid (1-40) peptide for different aggregation times (from hours to days). In order to obtain the oligomeric conformation, experimentally, A β (1-40) peptide was used right after dissolution in HFIP followed by dilution with PBS at a concentration of 20 μ M. Periodically the aggregating solution was exposed to hydroxyl radicals produced in FPOP experiment, followed by quenching, trypsin digestion and mass spectrometric measurements using an Thermo LTQ Orbitrap MS. Using Mathcad, a mathematic algorithm program, FPOP data gave us indications of every single amino acid modification within different times of A β aggregation process. We observed that several C-terminal amino acids were not exposed to the oxidation after 4 to 7 days of β -amyloid (1-40) peptide incubation indicating that C-terminal part is not flexible and rigid in the aggregates structure. Also we observed several β -amyloid (1-40) amino acids having a high dynamic during the aggregation process.

Neuer Aspekte

Better understanding of aggregation mechanism of A β peptides could offer new direction for developing aggregation modifiers.

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A top to bottom approach for in-depth characterization of therapeutic monoclonal antibodies

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Stichworte: monoclonal antibody, Orbitrap, high resolution, top down, bottom up

Einleitung

By 2016, eight of the top ten medicines will be therapeutic proteins. Their manufacture is accomplished in bacterial or eukaryotic expression systems, requiring extensive purification of the target product. In order to ensure highest-level safety and efficacy of the drug compounds, a rigorous control of a large set of chemical, physical, and biological properties is obligatory.

Because of their high information content and versatility, characterization methods based on high-performance liquid chromatography and mass spectrometry are among the most powerful protein characterization methods. The latter can be performed on the intact protein level, which delivers information related to the intact protein including all its modifications. Alternatively, proteins can be enzymatically digested to obtain peptides enabling their analysis by means of generic methods.

Experimenteller Teil

High-resolution ion-pair reversed-phase high-performance liquid chromatography employing an organic monolithic stationary phase in combination with high-resolution quadrupole-Orbitrap mass spectrometry were employed to separate intact antibody, reduced antibody, and digested antibody on a single analytical platform with gradients of acetonitrile in 0.05% trifluoroacetic acid. In depth analysis of the mass spectrometric data was aided through computational tools such as Protein DeconvolutionTM, ProSightPCTM, and PepFinderTM.

Ergebnisse

The technologies were employed to the in-depth characterization of monoclonal antibodies in intact form, after FabRICATOR digestion, after chemical or electrochemical reduction to yield light and heavy chains, as well as after proteolytic digestion to peptides. The intact mass and fragmentation data obtained from different optimized workflows allowed for the determination of the molecular weight of the intact proteins, the confirmation/verification of their amino acid sequence, the detection and relative quantification of oxidation or deamidation variants, and the identification and evaluation of the relative abundance of various glycovariants of the recombinant proteins. Details of data computational workflows for evaluation are presented and discussed.

Neuer Aspekte

Demonstrating the benefits of a generic analytical platform employing highly efficient chromatographic protein separations in combination with high-resolution quadrupole-Orbitrap MS

Targeted quantification of myofibrillar myopathy aggregate related proteins via PRM

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Stichworte: PRM, laser micro dissection, quantification, myofibrillar myopathies

Einleitung

Myofibrillar myopathies (MFM) are a group of autosomal dominant inherited muscle disorders characterized by focal disintegration of myofibrils and by the formation of intramyoplasmic protein aggregates. Known diseases related genes encode proteins that are located at or associated with the Z-disc. Beside, Z-disc and Z-disc-associated proteins, especially desmin, filamin C and their binding partners our previous label free based proteomic analysis in MFM identified novel disease-relevant proteins that accumulate in aggregates. Moreover, subgroup analysis revealed specific proteomic profiles in different MFM subtypes. Here we present the setup of the PRM method for absolute quantification of proteins from muscle tissue laser micro dissection.

Experimenteller Teil

In order to validate the discovered protein candidates for their suitability as predictive/diagnostic biomarkers in MFM we established an independent targeted mass spectrometric method based on parallel reaction monitoring (PRM). Therefore, tissue from muscle biopsies were collected by laser microdissection. Samples were processed and the optimal spike in point for heavy peptides was determined followed by reproducibility studies and adaption of the PRM method. The final method was applied to samples of aggregates and control tissue from MFM patients.

Ergebnisse

The PRM data confirmed a highly over-representation of MFM specific proteins like desmin and FilaminC in aggregates. Furthermore, we were able to detect a peptide carrying a point mutation specific form MFM, which was not detected using a label-free mass spectrometry approach. This is due to the fact that PRM is a highly sensitive and specific quantification method based on the selection of a defined precursor ion followed by fragmentation and detection of all transitions in a single analysis. The here presented method for absolute quantification of proteins from muscle tissue laser micro dissection shows that PRM qualifies as a powerful validation method. Furthermore, due to the possible analysis of various of peptides from a complex sample within one experiment it enables the development of a multiplexed quantification method for diagnostic approaches especially for the definition of MFM subtype-specific.

Neuer Aspekte

Targeted protein quantification of proteins from muscle tissue laser micro dissection

UPLC Ion Mobility Mass Spectrometry: A New Approach to Authentication and Routine Screening of Ginsenocide Isomers in Functional Food Products.

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Stichworte: CCS, UPLC, ion mobility, natural products

Einleitung

Directive 2004/24/EC, came into full effect on 30 April 2011. Hundreds of traditional herbal remedies were banned, as EU law aims to protect consumers from possible damaging side-effects of over-the-counter herbal medicines. New regulations allow only long-established and quality-controlled medicines to be sold. Products that have been assessed by the Medicine and Healthcare products Regulatory Agency (MHRA) may go on sale. Manufacturers have to prove that their products have been made to strict standards and contain a consistent and clearly marked dose.

Globally the popularity of nutraceutical products continues to increase, such natural product remedies are found in foods, roots and herbs. The legislative focus has resulted in a growth in method development to analyse active compounds in such products.

Experimenteller Teil

Korean ginseng, ginko biloba, red panax extracts and ginsenocide standards were analysed using ultra performance liquid chromatography (UPLC). A UPLC® HSS T3 (100mm x 2.1mm, 1.8mm) analytical column was utilised. The chromatographic conditions consisted of a 20 minute water (0.1% Formic Acid)/acetonitrile (0.1% Formic Acid) gradient at 0.6 ml/min, using a sample injection volume of 1µl. Negative ion electrospray with ion mobility data acquisition was performed using a Synapt G2-S mass spectrometer.

Ergebnisse

Non targeted UPLC ion mobility mass spectrometry has been used to generate collision cross sections (CCS), precursor ion accurate mass, accurate mass fragment ions and retention times to profile ginsenocide standards Rb1, (Rb2, Rc), (Rd, Re), (Rf,Rg1) and Rg2. This data was utilised to create a scientific library incorporating the expected CCS values. Three extracts, ginko biloba, red panax and korean ginseng were analysed and routinely screened against the created ginsenoside CCS library, to determine the presence/unequivocal identification of the ginsenoside isomers. For marker ginsenoside isomer pairs (Rb2,Rc), CCS measurements of $361.5 \text{ \AA}^2/350.4 \text{ \AA}^2$ have been determined. For (Rd,Re), $329.1 \text{ \AA}^2/333.1 \text{ \AA}^2$ resulted. Where as for (Rf,Rg1) $306.2 \text{ \AA}^2/296.2 \text{ \AA}^2$ were obtained. This proved that it is possible to distinguish the marker isomer pairs of ginsenocides from the extracts of the specified products analysed using CCS measurements. When comparing the expected against the measured collision cross sections determined (for the all eight ginsenocides profiled in the extracts), the CCS measurement errors were typically <0.5%. In addition it has been possible to acquire the cleaned up fragmentation spectra, which are mobility resolved from co-eluting components.

This approach offers a unique selectivity in profiling complex mixtures. The results obtained clearly show the benefits of using the collision cross section measurements and the combined peak capacity of UPLC and ion mobility. Co-eluting analytes and isomers have been resolved as well as unequivocally identified in the three extracts profiled. In this case the rationale for such a screening approach is to permit characterization of the distribution and content of mono- di- and tetra-glycosides in the raw material or processed functional food/nutraceutical products, in order to assess quality, potency and consistency of the final product, incorporating ingredients such as ginseng and korean red panax. The approach changes the scope of authentication profiling.

Neuer Aspekte

Routine collision cross section ion mobility screening to profile ginsenoside isomers of korean ginseng, red panax and ginko biloba.

The Combining of an Integrated Microfluidic Device with CCS Ion Mobility Screening for the Analysis of Pesticide Residues in Food.

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Stichworte: CCS, screening, pesticides

Einleitung

Full spectra acquisition and the specificity of accurate mass measurement is well characterised. It is used in combination with time tolerances, isotopic matching, fragment ions/ratios and response thresholds to help reduce false positive and false negative identifications in screening assays. Advances in mass spectrometry have vastly improved sensitivity for full spectral analysis, but further sensitivity enhancements would improve the mass spectral data quality. This is especially important to avoid compromised precursor ion or fragment ion information, and ensure high mass accuracy below the legislation levels. The integrated microfluidic device was interfaced to a Synapt G2-S mass spectrometer operating in ion mobility data acquisition mode, enabling enhanced sensitivity and selectivity to be obtained for the sample acquisitions.

Experimenteller Teil

The assay is based on the analysis of sample extracts, matrix matched calibrants (pear, ginger, leek and mandarin) and quality control samples generated for an EU-RL proficiency test. These samples were analysed using an integrated microfluidic device containing an analytical channel (150µm x 100mm) along with the ionisation emitter, coupled with ion mobility mass spectrometry. All microfluidic, gas and electrical connections are automatically engaged when the integrated microfluidic device is inserted into the source enclosure. The chromatographic gradient was provided from a nanoUPLC system with all separations occurring on the integrated microfluidic device. The integrated microfluidic device was interfaced with a Synapt G2-S operating in HDMS^E data acquisition mode.

Ergebnisse

Initially, ion mobility data was acquired using the integrated microfluidic device, for a series of solvent standard mixtures. These were utilized to generate retention time information and collision cross section (CCS) measurements for the pesticide library of the scientific information system. This subsequently enabled the correct identification of the pesticide residues in the matrix matched samples and proficiency samples. The results were compared to those previously obtained, where analysis was performed using conventional UPLC. Initial results have shown gains in both sensitivity and signal to noise with excellent linearity correlation coefficients being obtained for the matrix matched calibrants ($r^2 \geq 0.95$). Improvements in sensitivity have enabled matrix dilution to be performed and detection of 1pg on column to be obtained. In addition the CCS measurements obtained during the UPLC ion mobility acquisitions, were used to rapidly determine the retention times of the pesticide solvent standards and identify the residues present in a previous proficiency sample. The integrated microfluidic device ion mobility data, was screened using accurate mass measurement of precursor ion and fragments (10ppm tolerance), since the integrated microfluidic device retention times were not known, a 20 min retention time window was applied. Thereafter the processed data was filtered using a 2% CCS measurement tolerance, hence, the integrated microfluidic device retention times for the pesticide solvent standards were rapidly identified as well as the residues present in the FV-13 proficiency sample.

Neuer Aspekte

CCS measurement, improved sensitivity and matrix suppression reduction for screening pesticide residues in food using an integrated micro fluidic device.

Screening and Quantitation of About 350 Pesticides in Fruit Juices with Positive/Negative Switching LC/MS/MS

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Stichworte: Pesticides, polarity switching, MRM, food testing, triple quad

Einleitung

Liquid chromatography coupled with tandem mass spectrometry operated in multiple reaction monitoring (MRM) mode with electrospray ionization (ESI) is widely used for polar, semi-volatile, and thermally labile pesticides in food testing. Many contract labs currently perform multi-residue analysis of pesticides using separate positive and negative methods due to instrument limitations especially for methods with hundreds of MRM transitions. This requires twice the sample and twice the analysis time. Recently, the Bruker EVOQ Elite LC-triple quadrupole system has been introduced to the market; thereby providing fast positive/negative switching allowing for simultaneous determination of positive and negative co-eluting compounds numbering in the hundreds.

Experimenteller Teil

The measurements were conducted by dilute-and-shoot without sample enrichment. The fruit juices were centrifuged and diluted 10-fold before injection. An YMC-Pack ODS-AQ, 3 μm , 150 mm x 3 mm (I.D.) column with mobile phases (A) 5 mM ammonium fluoride in water, and (B) methanol were used. The total run time was 18 minutes including re-equilibration.

Ergebnisse

A study using the EVOQ analyzed about 350 pesticides in apple juice, cranberry juice, grape juice, orange juice and V8 vegetable juice using only one method with positive negative switching for about 500 MRM transitions. The preliminary results showed that both positive and negative co-eluting peaks have $R^2 > 0.99$ with linear range 0.1 to 100 $\mu\text{g/L}$.

Neuer Aspekte

Fast polarity switching for 500 MRM transitions for pesticide analysis in fruit juices

Screening und Quantifizierung abwasserbürtiger Spurenstoffe in einer Uferfiltrationstransekte mittels UHPLC-HRMS

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Stichworte: LC-HRMS, Online SPE, Screening, Uferfiltration, Transformationsprodukte

Einleitung

Trinkwasser wird in Berlin zu 100% aus Grundwasser mit überwiegenden Anteilen an Uferfiltrat und künstlicher Grundwasseranreicherung gewonnen. In der vorliegenden Arbeit wurde die natürliche Barrierefunktion der Uferfiltration entlang einer Grundwassertransekte am Tegeler See untersucht. Die Transekte, bestehend aus mehreren Grundwassermessstellen zwischen dem See und einem Rohwasserbrunnen, wurde mittels eines UHPLC-HRMS Screeningverfahrens auf „neue“ bisher nicht im Berliner Wasserkreislauf detektierte Verbindungen untersucht. Der Tegeler See enthält durch die Vorflut des Klärwerks Schönerlinde je nach Saison einen Klarwasseranteil von ca. 10-30 %. Abwasserbürtige Verbindungen, die über die Uferfiltration bis ins Rohwasser gelangen, standen im Fokus [1]. Ausgewählte Substanzen wurden quantifiziert. Durch die Darstellung des Konzentrationsverlaufes der Spurenstoffe über die verschiedenen Redoxbereiche innerhalb der Transekte konnten Rückschlüsse auf das Abbauverhalten gezogen werden.

Experimenteller Teil

Die Messungen erfolgten an einem EQuan Max Plus™ Chromatographie-System, gekoppelt mit einem Exactive Plus™ Massenspektrometer (Thermo Fisher Scientific). 1mL Probe wurde injiziert, online angereichert und auf der analytischen Säule chromatographisch getrennt. Die Einstellungen des MS sind für die Screening Methode in [2] und für die Quantifizierungsmethode in [3] beschrieben. Die Datenauswertung erfolgte mit den Softwareprogrammen SIEVE 2.1 und TraceFinder 3.1 (beide Thermo Fisher Scientific) in zwei verschiedenen Ansätzen: Suspect Screening und Non-Target Screening. Das Suspect Screening erfolgte über eine selbst erstellte Datenbank (>2000 Einträge). Das Verfahren basierte auf der Suche nach Suspects, die über die exakte Masse ermittelt und anschließend über Isotopenmuster und Fragment-Ionen bestätigt wurden. Für das Non-Target Screening wurden online Datenbanken wie STOFF-IDENT oder das Biotransformationsvorhersagemodell EAWAG-BBD herangezogen.

Ergebnisse

Mit Hilfe des Suspect Screening konnten 93 Verbindungen ermittelt werden. Davon wurden 60 Verbindungen erstmalig im Projektgebiet detektiert: 32 Arzneistoffe, neun Arzneistoff-Metabolite, zwei Pestizide, drei Pestizid-Metabolite und 14 sonstige Spurenstoffe. 58 Verbindungen wurden insgesamt quantifiziert, 21 Verbindungen dabei erstmalig in dem Projektgebiet. Im See hatten 46 Verbindungen eine Konzentration größer der Bestimmungsgrenze, sechs sogar mit einer Konzentration größer 1,0 µg/L. Im Rohwasserbrunnen wurden noch 31 Verbindungen detektiert. Hier lagen 11 Verbindungen vor, die den allgemeinen Vorsorgewert des Umweltbundesamt (0,1 µg/L) überstiegen.

Mit den Quantifizierungsdaten wurde das Transportverhalten in der Transekte untersucht. Die Messstellen haben unterschiedliche Teufen. Dadurch können innerhalb der Transekte die verschiedenen Redoxbereiche abgedeckt werden, um mikrobielle und chemische Prozesse zu charakterisieren. Einige der mit dem Suspect Screening neu detektierten Verbindungen zeigten sich während der Uferfiltration persistent, was im Hinblick auf die Gefährdung der Trinkwasserressourcen relevant ist. So konnte für die Blutdrucksenker Candesartan und Olmesartan ein redoxabhängiges Abbauverhalten nachgewiesen werden. Diese verhielten sich im Aeroben persistent, während im Anaeroben ein Abbau stattfand. Das Antiepileptikum Gabapentin und das Transformationsprodukt der Sartane, die Valsartansäure, verhielten sich dagegen unabhängig vom Redoxmilieu persistent. Während Verbindungen wie das Analgetikum Tramadol und das Diuretikum Hydrochlorothiazid in allen Redoxmilieus Abbau zeigten.

Über das Non-Target Screening konnten des Weiteren drei potentielle Transformationsprodukte mit dem EAWAG-BBD vorhergesagt werden. Diese stellen vermutlich Derivate des Phenazons (Acetylierung), des Propyphenazons (Hydroxylierung) und des PDPs (Formylierung des Abbauprodukts von Propyphenazon) dar. Eine Verifizierung dieser Verbindungen konnte aufgrund fehlender Referenzstandards nicht vorgenommen werden. Ein anderes Transformationsprodukt, das Gabapentin Lactam, wurde ebenfalls über das Screening identifiziert. Gabapentin Lactam resultiert aus Gabapentin durch Wasserabspaltung und intramolekularem Ringschluss und konnte sogar mittels eines Referenzstandards verifiziert werden.

Neuer Aspekte

Spurenstoffcharakterisierung einer Uferfiltrationstransekte mittels UHPL-HRMS Screening und Quantifizierung zur Bewertung des Berliner Grundwassers.

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A DESI MS based screening method for phthalates in consumer goods

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Stichworte: DESI, Phthalates, toys, screening, quantitation

Einleitung

Phthalates are used as plasticizers in many everyday items, but they are also recognized as hormone disruptors being especially harmful during childhood [1]. The European Union therefore restricted their use in children's toys and certain food packaging [2]. Due to the ever-increasing number of polymer-containing consumer goods, rapid screening methods are needed to ensure and improve consumer safety in the future. In this study we evaluated the performance of desorption electrospray ionization (DESI) mass spectrometry (MS) for rapid quantitative screening of phthalates in consumer goods.

Experimenteller Teil

Analysis of matrix-matched reference materials and authentic samples was carried out using a custom-built DESI source attached to an Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific GmbH, Bremen, Germany). Reference materials were prepared by mixing PVC powder and plasticizer, followed by heating of the gel for 20 min to 200°C in a mold. Authentic samples were analyzed without further sample treatment. Samples and reference materials were fixed to a motorized 2D stage and measured in an automated fashion, with the MS operating in positive ion mode. Additional experiments were performed with the DESI source being attached to a portable MS Mini 11 [3] (Purdue University, USA). Determined calibration curves show the TIC-normalized sum of phthalate ion signals against true concentrations.

Ergebnisse

In this study a DESI MS method for rapid identification and quantification of phthalates in consumer goods was developed. DESI allowed for direct surface sampling of the consumer goods under atmospheric conditions with minimal sample preparation, while the high-performance mass spectrometer provided for high sensitivity and reliable identification via accurate mass measurements, high mass resolving power and MS/MS capabilities. External calibration curves for six banned phthalates (DBP, BBP, DEHP, DNOP, DINP and DIDP) were obtained from matrix-matched reference materials. Coefficients of determination were greater than 0.985, LOQs ranged from 0.02 to 2.26 %_w (legislative limit: 0.1%_w) and the relative standard deviation of calibration-curve slopes was less than 7.8 % for intraday and 11.4 % (DEHP) for interday comparisons. Measurement of a calibration curve (12 points) was achieved within three minutes. The phthalate contents of eleven authentic samples were determined in a proof-of-concept approach using DESI MS, and results were compared to those from confirmatory methods. Sample preparation for authentic samples involved the attachment of a small piece to a sample holder and less than a minute for analysis. The phthalate contents were correctly assigned for the majority of samples within a range of -20 to +10 % relative deviation to the results obtained with confirmatory methods. Compared to confirmatory methods such as gas and liquid chromatography coupled with spectroscopic and spectrometric detection, the DESI MS method constitutes a major reduction in total analysis time, due to shorter measurement times and omitted sample preparation. Given further optimization and automation, DESI MS might become a useful tool for rapid and accurate phthalate screening in the future. Currently a field-portable instrumental setup is tested in on-site screening experiments.

Neuer Aspekte

Quantitative DESI MS based method for screening of phthalates in consumer goods.

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Identifizierung von intrazellulären Cisplatin-Protein Addukten mithilfe des fluoreszierenden Cisplatin-Analagon CFDA-Cisplatin

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Stichworte: Cisplatin, CFDA-Cisplatin, 2D-Gelelektrophorese, CFDA-Cisplatin Addukte

Einleitung

Cisplatin ist ein breit eingesetzter Wirkstoff in der Krebstherapie. Derzeit erfolgt eine Behandlung mit Cisplatin bei Plattenepithelkarzinomen an Kopf und Hals, Bronchial-, Darm-, Harnblasen-, Hoden- und Ovarialkarzinom [1-3]. Seine Wirksamkeit ist aber durch toxische Nebenwirkungen sowie durch eine Resistenz von Tumorzellen gegenüber Cisplatin begrenzt.

Die zytotoxische Wirkung von Cisplatin beruht auf der Platinierung der DNA [4], aber nur ein geringer Anteil des zellulären Cisplatins gelangt zum Zielort [5]. Es wird angenommen, dass Cisplatin eine höhere Affinität zu schwefelhaltigen Aminosäuren wie Cystein und Methionin hat [6]. Im Inneren einer Tumorzelle gibt es für Cisplatin eine Reihe von möglichen Bindungspartnern. Daher kann für Cisplatin die Wechselwirkung mit Proteinen ein wichtiger Faktor für dessen intrazelluläre Verteilung, Elimination und Zytotoxizität sein [7].

Experimenteller Teil

Zur Identifizierung und Auftrennung solcher intrazellulären Cisplatin-Protein Addukte wurde mithilfe des fluoreszierenden Cisplatin-Analagon CFDA-Cisplatin [8] eine 2D-Gelelektrophorese etabliert. Dieses fluoreszierende Cisplatin-Analagon ermöglichte die Detektion bzw. Unterscheidung von intrazellulären Cisplatin-Protein Addukten.

Um die Komplexität und Auflösung von sauren, neutralen sowie basischen Proteinen zu erhöhen, erfolgte die Auftrennung mithilfe der 2D-Gelelektrophorese in teilweise überlappende immobilisierte pH-Gradienten (pH 4-7 und 6-10).

Die detektierten CFDA-Cisplatin-Protein Addukte wurden mittels ESI-MS/MS identifiziert.

Ergebnisse

Mit dieser Strategie konnte der Elongation factor 1-alpha 1, die D-3-phosphoglycerate dehydrogenase und einige Protein-Disulfid-Isomerasen identifiziert werden.

Neuer Aspekte

Dies sind die ersten Ergebnisse zur Detektion von intrazellulären Cisplatin-Protein Addukten.

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Veränderungen des organischen Bodenkohlenstoffs bei Waldbränden

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Stichworte: ESI-FTICR-MS, Kendrick-Diagramm, Neubildung, stickstofforganische Verbindungen, Van Krevelen-Diagramm

Einleitung

Thermisch entstandene, hocharomatische Kohlenstoffverbindungen, sogenannter „Black Carbon“ wird aufgrund seiner Klimarelevanz seit Jahren intensiv untersucht. Aufgrund seiner schweren Abbaubarkeit reichert er sich in Böden an – Anteile am Gesamt-C variieren zwischen 1-6 % im europäischen Waldboden und bis zu 60 % in den Schwarzerden - und stellt somit eine Senke im globalen Kohlenstoffkreislauf dar. Unbekannt ist, inwieweit Anteile des Black Carbon wasserlöslich sind und den Pool an mobilen gelösten organischen Substanzen qualitativ und quantitativ beeinflussen. In der Präsentation wird dargestellt, wie sich der (wasserlösliche Teil des) organischen Bodenkohlenstoffs unter Hitzeeinwirkung als Simulation von Waldbränden verändert.

Experimenteller Teil

Nach der Elementaranalyse der erhitzten sowie der unbehandelten Kontrollproben aus Freiland-Lysimetern wurden Heißwasserextraktionen durchgeführt. Nach Zentrifugation und Festphasenextraktion zur simultanen Entsalzung und Aufkonzentrierung der Proben wurden diese mittels Fließinjektion in ein ESI-FTICR-MS (LTQ-FT Ultra, ThermoFisher Scientific) infundiert und als gemittelte Spektren aus 6 Scans mit jeweils 50 Transienten unter einer Auflösung von 400.000 (bei $m/z = 400$ Da) und mit einer Genauigkeit <1 ppm gemessen.

Die 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 statistischen Auswertung und grafischen Darstellung als Kendrick- bzw. Van Krevelen-Diagramm übertragen.

Ergebnisse

Die Elementaranalyse der erhitzten Bodenproben zeigt erwartungsgemäß eine Abnahme der Kohlenstoff-, Wasserstoff- und Schwefel-Gehalte.

Die ESI-FTICR-Spektren der Extrakte sind typisch für gelösten organischen Kohlenstoff (DOC) mit einer breiten Verteilung über den Massebereich von 200-800 Da. Auffallend sind zunächst die ca. doppelt so intensiven, geradzahligigen Peaks in den erhitzten Proben. Diese Peaks sind eindeutig CHO(S)N-Verbindungen zuzuordnen, ^{13}C -Isotopenpeaks der CHO-Verbindungen werden ebenfalls, aber mit deutlich geringer Intensität detektiert.

Darüber hinaus sind in den erhitzten Proben die Peaks im unteren Massebereich deutlich intensiver im Vergleich zu den Referenzproben – Hinweis auf pyrolytische Effekte, gut erkennbar sowohl in den Spektren selbst sowie in den Kendrick Diagrammen.

Van Krevelen-Diagramme (als zweidimensionale Darstellung H/C über O/C) zur Charakterisierung von Polarität und Aromatizität der organischen Komponenten werden für CHO, CHON und CHOSN-Verbindungen getrennt dargestellt. Auffallend ist die quantitative Zunahme der stickstoff-organischen Verbindungen bei gleichzeitiger Intensitätserhöhung. Ähnliche, aber schwächere Effekte sind in den Diagrammen der CHOSN-Verbindungen zu finden.

Wir vermuten, dass unter erhöhten Temperaturen erwartungsgemäß ein Teil des organischen Bodenkohlenstoffs verflüchtigt und/oder oxidiert wird, gleichzeitig jedoch in einen Teil der CHO(S)-Verbindungen atomarer Stickstoff eingebaut wird, analog der Neubildung von Organika beim Plasmaätzen mit Stickstoff als „Inertgas“.

Dies würde erklären, warum die Bodenfruchtbarkeit nach Brandrodung kurzzeitig zunimmt (polarere, wasserlösliche Verbindungen, die über Wurzel Aufnahme entsprechend schnell bioverfügbar sind), langfristig aber abnimmt, denn

besonders unter tropischen Starkregen-Bedingungen würden diese Verbindungen entsprechend schnell ausgewaschen werden.

Neuer Aspekte

Thermisch behandelte Bodenproben zeigen Neubildung von Verbindungen durch Einbau von Stickstoffatomen.

Detection of interfering substances of enzyme-based glucose measurement in complex matrices by Flowprobe™ micro extraction mass spectrometry

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Stichworte: glucose measurement, interference, dried blood spots DBS, liquid microjunction surface sampling probe (LMJ-SSP), ambient mass spectrometry

Einleitung

Self-monitoring of blood-glucose levels is essential for managing and improving diabetes treatment. However, endogenous and exogenous interfering substances can cause erroneous readings. Common interferents are coexisting medications, contaminations or metabolites. As an example, the analgesic antipyretic drug acetaminophen can interfere with enzymes of the test system and lead to false-positive blood-glucose measurements [1]. A subsequently increased insulin dosage is inadequate and could lead to hypoglycemic coma. Exogenous interfering substances can be contaminations during the production process of test-systems, resulting in products with inferior quality. Identification and characterization of interfering substances is therefore necessary to optimize products for blood glucose monitoring and thus therapy for diabetes patients.

Experimenteller Teil

The Flowprobe™ *in situ* micro extraction system (Prosolia) represents a new approach for direct analysis of interfering substances out of complex matrices [2]. In the set-up we used, the continuous flow liquid microjunction surface sampling probe is coupled to an Orbitrap™ mass-spectrometer (Thermo Fisher Scientific). As an initial application, we optimized the system to detect acetaminophen in blood samples. For higher precision the internal standard [²H₄]-acetaminophen was used. Dried blood spots (2.5 µl) on glucose measurement tests of the Accu-Chek® Mobile glucose meter (Roche) were analyzed by direct extraction.

Ergebnisse

In our hands at least an acetaminophen concentration of 0.025 mg/dL was detected in blood, with a reproducibility of sampling with CV% = 5.7 for n=5. A linear calibration curve within a concentration range from 0.032 – 20 mg/dL was obtained with R² > 0.98. For method validation, the performance of the Flowprobe system will be further compared with a cobas® 6000 analyzer series (Roche) measurement as a reference method.

As a second application and an example for detection of exogenous interfering substances, contamination with the hydrophobic reagent dioctyl sulfosuccinate sodium (DONS) were analysed on irregularities on semi-finished products (coated foil) of glucose measurement tests.

Taken together, our results suggest that the Flowprobe™ method is applicable for on-line extraction and detection of acetaminophen in dried blood spots on test strips as well as a contamination on coated foils. The novel Flowprobe™ system has the potential to directly analyse small molecules out of complex matrices. This can facilitate detection of interfering substances for enzyme-based glucose measurements. The main advantage of this method is a very small sample volume (< 2.5 µl) and a direct analysis from used test strips without any sample preparation. Additionally, the system can be used for detection of exogenous interfering contaminations on surfaces like a coated foil. This approach could be a rapid alternative for time-consuming in-process controls during production of test systems.

Neuer Aspekte

analysis of acetaminophen in dried blood on glucose test-fields and detection of DONS on foils of test-stripes as in-process control

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Improved Determination of Allergenic Fragrances in Detergents and Personal Care Products in Multiple Reaction Monitoring GC-MS/MS

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Stichworte: allergy, MRM, database, triple quad, PCP

Einleitung

Many compounds added to improve the aroma of cleaning agents and personal care products (PCP) are classified as allergenic. Quantifying the risk of these to personal safety is complicated as even sub-ppb levels may invoke a serious immunogenic response in hypersensitive individuals. Therefore EU Directive No. 76/768/EC stipulates that allergens should be labelled if they exceed 0.001% (w/w) for rinse-off products and 0.01% (w/w) for stay-on products. Developers therefore require analytical techniques that can accurately and reliably screen for low level fragrance residues. GC-MS/MS in MRM mode is regarded as the gold standard for low level multiple residue analysis, particularly in matrices containing cleaning agents and PCPs. However MRM setup and method development is a complicated and time intensive process.

Experimenteller Teil

GC-MS/MS techniques based on compound based scanning (CBS) greatly simplify this process, combining enhanced sensitivity, reliability and robustness with rapid method development and simple sample preparation.

GC: Bruker GC 456 SCION TQ; S/SI-injector temp: 250°C; Split: 1:20; Column: BR-5ms, 30 m x 0.25 mm x 0.25 µm; Oven program: 50°C (1 min) -> 250°C (12°C/min); 3.33 min; Injection volume: 1 µL; MRM measurements with optimized transitions for all compounds (at least 2 transitions per compound); Data system: Bruker MSWS 8.0 SP2

Ergebnisse

To illustrate the speed and sensitivity of employing this method for allergen screening, a GC-MS/MS was used to screen for allergenic compounds within a number of commercially available cleaning agents. CBS software automatically built the screening method while advanced GC-MS/MS hardware delivered excellent sensitivity at trace ppb levels.

After a small number of initial runs to locate the retention time window for each compound, CBS software used a comprehensive MRM database to automatically select optimal scan times for the 27 allergen compounds.

Calibration curves from 5 ppt to 250 ppb could be measured. The MRM traces clearly identified the presence of ten prohibited allergenic compounds screened for using the CBS method. Analysis was also performed in single quadrupole SIM mode to illustrate the improvements in selectivity in matrix samples. Usage of a triple quadrupole MS/MS offers greater s/n ratio and improved peak shapes.

The majority of allergenic compounds listed in the EC regulation have been optimized in CBS scanning, which provided fast and robust method development, showing the method to be ideal for product developers and quality control (QC) laboratories.

Neuer Aspekte

GC-MS/MS for quantification of trace-levels of allergenic compounds in personal care products (PCP)

Direct Analysis of Degradation Products in Lithium-ion Batteries Using Low-temperature Plasma Ambient Desorption/Ionization High-Resolution Mass Spectrometry

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Stichworte: LTP-MS, Lithium-ion battery, volatile compounds

Einleitung

Lithium ion batteries (LIB) are important energy storage devices due to their high energy density, relatively low self-discharge, and low maintenance needs. LIB devices help to realize time independent and delocalized energy storage on a large scale. One limitation, however, is aging of LIBs due to degradation processes within the batteries. To improve the performance and long-term stability of these devices, it is critical to understand these degradation processes.

Typically, analysis of degradation products is performed with analytical instrumentation including ion chromatography or gas chromatography coupled to mass spectrometry, respectively [1,2]. These methods, however, require rather complex sample pretreatment and include a time-consuming separation step.

Experimenteller Teil

In this work, we eliminate sample preparation and perform direct analysis of model LIB samples by using ambient desorption/ionization high-resolution mass spectrometry (ADI-HR-MS). Based on previous work by our group [3], desorption/ionization of electrolytes is facilitated with a home-built low-temperature plasma probe (LTP, based on the original work by Harper *et al.* [4]) followed by mass spectrometric detection and identification. Commercially available electrolytes LP30 (composed of ethylene carbonate, dimethyl carbonate, and LiPF_6) and LP50 (composed of ethylene carbonate, ethyl methyl carbonate, and LiPF_6) were investigated in thermal degradation experiments and results will be discussed.

Ergebnisse

After careful optimization of LTP-HR-MS and controlled aging of model electrolytes, new chemical species are identified that were not observed in our previous work. In addition, the influence of the electrolyte-additive vinylene carbonate on the LIB thermal aging process is investigated. Finally, a cooling system for the HR-MS sampling/ionization source stage will be presented. Advantages of temperature-controlled sampling will be discussed with a focus on very volatile compounds. Based on our results, LTP-HR-MS is considered a useful and complementary tool for future degradation studies on LIB electrolytes and electrodes.

Neuer Aspekte

Temperature-controlled degradation studies of electrolyte/additive mixtures for LIB with LTP-HR-MS; cooling/heating sampling plate for temperature-controlled sampling of volatile compounds.

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Automated top-down mass spectrometry of hemoglobin for a clinical application

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Stichworte: Hemoglobin, ion trap, top-down, ETD

Einleitung

Hemoglobin (Hb) is a tetrameric protein present in red blood cells. Healthy adults have HbA (~97%), HbA₂ (~3%) and HbF (<1%), which are composed of two alpha and two beta, delta or gamma chains, respectively. Hb disorders (hemoglobinopathies) are divided into two groups: Hb variants (structurally abnormal Hb) and thalassemias (deficient synthesis of one globin chain). Hemoglobinopathy diagnostic is currently based on several methods: hematological tests, chromatography, electrophoresis and molecular biology. We aimed to establish an automated workflow for the identification of most common Hb variants (HbS, HbC, HbE, HbD-Punjab, HbO-Arab) and the quantification of HbA₂ and HbF by top-down mass spectrometry, suitable for clinical laboratory practice.

Experimenteller Teil

A sample set containing blood specimens from healthy patients and HbS or HbC carriers was analyzed in an integrated system composed of a nanoflow liquid chromatograph hyphenated to a 3D-ion trap with ETD capabilities. The system is fully managed by an open access software which allows for an automated execution of the analysis. This includes 1) creation of a sample table with associated methods, 2) acquisition of top-down LC-MS/MS data, and 3) automated data interpretation. This last point comprises quality control parameters (total ion counts, presence of the alpha chain), automated detection of specific ETD fragment ions of Hb variants, and calculation of the delta/alpha chain ratio. A report is subsequently generated for each sample.

Ergebnisse

Preliminary results show that this integrated system can confidently detect the presence of HbS and HbC. The generated report shows an extracted ion chromatogram with specific ETD transitions for each Hb chain. If a chromatographic pic is detected, the presence of the corresponding Hb variant is listed in a summary table. From a total of 45 analyses, corresponding Hb chains were correctly detected in 39. The current false negative rate for detection of Hb variants is 2% and the false positive rate is 11%. To decrease the number of false negatives and false positives, we are working on the fine tuning of detection parameters, ion abundance thresholds and reporting parameters. We have to point out that for a screening method, the aim is to not miss a patient carrying the disease, therefore the priority is to avoid false negatives and minimize false positives. The analysis cycle time was nine minutes *per* sample. Work is in progress to include the detection of HbE, HbO-Arab and the relative quantification of HbA₂ delta chain and HbF gamma chain. Finally, a comparative assay between this integrated LC-MS/MS system and the conventional diagnostic workflow is planned.

Neuer Aspekte

Automated hemoglobin analysis of clinical samples by ETD LC-MS/MS.

Electrospray ionization of aromatic amino compounds

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Stichworte: ionization efficiency, mass spectrometry, pH dependence, aromatic amino compounds, electrochemical oxidation

Einleitung

Many aromatic amines are carcinogenic and mutagenic causing DNA damage; therefore, monitoring the exposition with these compounds by chemical analysis is an important topic in occupational health.

The chemical analysis of amines is, however, often troublesome. Among other critical factors such as their stability, the high polarity of the amines which do not easily dissolve in classical organic solvents restricts the choice of analytical methods. On the other hand, their basicity makes them a promising target for analysis using electrospray ionization mass spectrometry (ESI-MS) for detection.

For analysis of aromatic amino compounds, we tested signal dependency on type of the substituents, pK_b , solvent pH and electrolyte concentration; parameters influencing the ESI-MS response beyond the extent of protonation are also discussed.

Experimenteller Teil

ESI analyses were performed using an API 2000 Triple Quadrupole MS equipped with the Analyst 1.4.2 Software from AB Sciex coupled to a JASCO 900 HPLC at a flow rate of 20 $\mu\text{L}/\text{min}$ and an Esquire 3000⁺ from Bruker equipped with the Data Analysis 3.3. Each of the aromatic amines was measured at a concentration of 10 μM in ACN/H₂O 1/1 at a pH of 7 or 3, alternatively. To achieve a pH value of 7, different types of degassing were tested in order to remove CO₂, pH 3 was adjusted by adding formic acid. The influence of the electrolyte concentration was tested in presence of 1 mM HCl, NaCl, formic acid, ammonium formate and sodium formate compared to pH7.

Ergebnisse

The results of the degassing experiments showed that heating the water under reflux for 30 min and allow to cool down under nitrogen lead simply and quickly to a pH of 7.

Data evaluation of the signal intensities of the aromatic amino compounds revealed that amines with electron donating groups, such as 2-Methoxy or 2-Methyl aniline have higher intensities compared to anilines with electron withdrawing groups, such as 2-Nitro and 2-Fluoro aniline. Electron donating groups, releasing electrons into a reaction center and as such stabilizing electron deficient carbocations, increase the basicity of the molecule by stabilizing the positive charge, while electron withdrawing groups draw electrons away from a reaction center and consequently decrease the basicity of the molecule. Thus, the signal response of the anilines followed their basicity; i.e. the more basic compounds exhibited an increased ESI-MS signal intensity. Of the electron donating groups, the mesomeric donating effect of the methoxy group seemed to improve the ESI-MS signal intensity of the anisidine $[\text{M}+\text{H}]^+$ ion to a higher degree compared with the other electron donating and withdrawing groups of aniline.

Increasing the electrolyte concentration showed a loss in signal intensity for all electrolytes except for formic acid. This indicates that the non-volatile electrolytes hinder the evaporation of the droplets during the ionization process.

Apart from this, we noticed that the anilines may undergo oxidation during the electrospray process. In close succession, they may react with solvents such as methanol to yield imines and other by-products by the Eschweiler-Clarke reaction providing evidence for the electrochemical nature of the ESI process.

Neuer Aspekte

We present a model approach for optimizing signal intensities in (LC)-EI-MS analysis.

Multiple mass spectrometric imaging analyses from single tissue section with Desorption Electrospray Ionization (DESI) on a oa-TOF mass spectrometer

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Stichworte: DESI, imaging, SYNAPT G2-Si, ambient ionization

Einleitung

Over the years Desorption Electrospray Ionization (DESI), an ambient ionization technique, has been applied to Mass Spectrometry Imaging (MSI) to allow for the direct analysis of surfaces at atmospheric pressure. Here we demonstrate that sufficiently low gas and solvent flow rates, the desorption was a soft even and therefore the same tissue section could be analyzed more than once without modification or exhaustion of the surface molecules. It allows the potential to carry out dual polarity analysis on the same section to access to a wealth of molecular information from a single tissue section. Furthermore, it gives the capability to run one experiment at low spatial resolution, followed with a higher spatial resolved experiment, specifically to a region of interest.

Experimenteller Teil

Snap frozen tissues of porcine liver were sectioned on a cryo-microtome to 15µm thickness and thaw mounted onto conventional glass slides; the samples were stored at -80°C. Immediately prior to analysis the samples were brought to room temperature and placed onto the stage, no further sample preparation is required. The 2D DESI source was mounted onto a SYNAPT HDMS G2-Si. DESI spray conditions were set at 1.5µl/min, 90:10 MeOH: water at 100psi N₂ gas pressure and a voltage of 5kV for both polarities. The pixel size was determined in the X-direction by the speed of the stage movement and acquisition rate of mass spectra. The Y-direction was defined by the distance between two lines of acquisition.

Ergebnisse

Dual polarity DESI imaging experiment on the same tissue section

Initially imaging experiment on porcine liver were performed using DESI with the MS operating in negative mode, subsequently followed by another DESI imaging experiment on the same tissue section with the MS operating in positive mode. In both modes of ionization, plentiful lipids and endogenous metabolites were detected, giving intense peaks.

A second experiment was designed to evaluate whether the first passage of the DESI spray alters the chemical information that is obtained from the same tissue. Comparing spectrum that was generated from a single DESI imaging experiment in positive mode and the spectrum that was generated from a consecutive tissue section also in positive mode after a first experiment was carried out in negative mode. Identical peaks were observed in both spectra with very similar relative intensities.

Different spatial resolution DESI imaging experiment on the same tissue section

In the first DESI imaging experiment, a raster pattern was defined over the whole tissue with a pixel size of 150 µm for the porcine liver. The second experiment was carried out onto a specific region of the same tissues, both at 50 microns.

Spectra obtained from a single pixel acquired with a spatial resolution of 150 µm from pristine tissue section gave comparable relative lipid intensities with spectra obtained from single pixel acquired with the second DESI imaging experiment at 50 µm specific to a region of the same tissue section.

Further experiments will be carried out using a human clinical liver samples, followed by the Hematoxylin and Eosin (H&E) staining for accurate characterization of the tissue regions.

Neuer Aspekte

Multiple DESI imaging analyses of the same tissue section with different experimental conditions to obtain maximum information.

Enhancement of weak analyte signals in human disease-related MALDI MS images by applying fast randomized denoising and compression algorithms

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Stichworte: MALDI imaging, enhancement, PCA, randomized

Einleitung

Conventional mass spectrometry image pre-processing methods used for denoising typically do not only remove noise but also weak signals. They do not consider single-pixel spectra in their local context and do not use information from spectra of adjacent pixels for denoising or signal enhancement. Recently, memory-efficient principal component analysis (PCA) in conjunction with random projections (RP) has been proposed for analysis and compression of large mass spectrometry imaging data sets [1,2]. We provide detailed quantitative data for comparing this promising approach with conventional methods and demonstrate how to enhance signals of low intensity amyloid- β peptide isoforms in sparsely distributed Alzheimer's disease-related β -amyloid plaques and imaging of multiply acetylated histone H4 isoforms in response to pharmacological histone deacetylase inhibition *in-vivo* [3].

Experimenteller Teil

Tissue samples were sprayed using a SunCollect (SunChrom) system and measured in linear positive mode using an Autoflex III TOF/TOF (Bruker Daltronics) to acquire the MALDI-MSI datasets. The resulting images contain ~0.3 billion elements (5.000 pixel x 60.000 bins). MATLAB R2014a and flexImaging 3.0 software were used for data processing. All datasets were preprocessed with baseline correction and total ion count (TIC) normalization. For the RP-PCA algorithm two crucial parameters were optimized (number of random projections k , number of components for reconstruction L). To evaluate the performance of the method, comparative algorithms like SGF (savitzky golay filtering) and DWT (discrete wavelet transformation) were applied to the dataset. The signal noise energy was used for objective comparison.

Ergebnisse

We chose to study two examples to identify weak signals. The first MSI dataset represents the distribution of β -amyloid plaques in the brain of a mouse model of Alzheimer's disease. The second dataset investigated the pharmacodynamic effect of the histone deacetylase (HDAC) inhibitor drug LBH-589 on histone H4 acetylation on gastric tumors of a mouse model. Conventional PCA as a well-known option for noise removal is frequently not feasible on raw unreduced MSI datasets. In contrast, RP-PCA can be applied but has not been evaluated in-depth for its ability to denoise mass spectra and the corresponding images. We show that by using the RP-PCA algorithms with carefully selected parameters, low abundant and sparsely distributed $A\beta$ species such as $A\beta$ 1-26 were significantly enhanced in the first dataset in combination with noise reduction and improved image quality with respect to the comparative algorithms. The colocalization to the more abundant isoform $A\beta$ 1-42 may support the view that weak signals in low-intensity single-pixel spectra, which are visible only after RP-PCA enhancement, are real. In the second dataset the RP-PCA smoothing outperforms SGF and DWT on the image level and shows very similar effects by enhancing less abundant features like weak H4 3Ac and H4 4Ac signals. Furthermore we provided a method to adapt the parameters L and k of the RP-PCA algorithm to the specific biological and technical conditions of an experiment to avoid information loss.

Neuer Aspekte

RP-PCA shows promising results for the processing of complete MALDI-MSI datasets for enhancement, denoising and compression.

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Imaging mass spectrometry (IMS) to discriminate breast from pancreatic cancer metastasis in FFPE tissues

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Stichworte: Imaging, cancer, MALDI, FFPE

Einleitung

Diagnosis of the origin of metastasis is mandatory for adequate therapy.

In the past, classification of tumors was based on histology (morphological expression of a complex protein pattern), while supportive immunohistochemical investigation relied only on few “tumor-specific” proteins. At present, histopathological diagnosis is based on clinical information, morphology, immunohistochemistry, and may include molecular methods. This process is complex, expensive, requires an experienced pathologist and may be time consuming.

Currently, proteomic methods have been introduced in various clinical disciplines. MALDI IMS combines detection of numerous proteins with morphological features, and seems to be the ideal tool for objective and fast histopathological tumor classification.

Experimenteller Teil

To study a special tumor type and to identify predictive patterns that could discriminate metastatic breast from pancreatic carcinoma MALDI IMS was applied to multi-tissue paraffin blocks. A statistical classification model was created using a training set of primary carcinoma biopsies.

Ergebnisse

This model was validated on two testing sets of different breast and pancreatic carcinoma specimens. We could discern breast from pancreatic primary tumors with an overall accuracy of 83.38 %, a sensitivity of 85.95 % and a specificity of 76.96 %.

Furthermore, breast and pancreatic liver metastases were tested and classified correctly.

Neuer Aspekte

Applying MALDI imaging for discrimination of different cancer metastasis forms in FFPE tissue

3D MALDI Imaging of Mouse Heart after Myocardial Infarction

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Stichworte: imaging, 3D, MALDI, myocardial infection

Einleitung

MALDI Imaging (MSI) is now maturing into a tool for three-dimensional analyses. We integrate magnetic resonance imaging (MRI) and 3D MSI and apply it to a mouse model of myocardial infarction. The entire MS data set was co-registered with MRI data and histological images to construct a 3D representation of the multi-modal data.

Experimenteller Teil

Myocardial infarction was induced in a C57BL/6J mouse. After sacrifice, MRI data was acquired on the heart which was then embedded in paraffin, sectioned and mounted onto conductive glass slides. After de-paraffinization, low-res optical images were acquired and MSI analysis was performed on a MALDI-TOF mass spectrometer operating in linear mode. After MS acquisition, all sections were stained with H&E for detailed histological examination.

Ergebnisse

Combining classical histology, MRI data and MSI, we elucidated proteomic signatures specific to the different areas of the infarcted myocardium. In practice, we encountered several challenges to this approach.

Standard deposition of sinapinic acid matrix proved to be insufficient for small structures such as mouse heart. We show how α -CHCA matrix, applied with a modified protocol, allowed us to analyze the sample in sufficient detail.

Our previously established registration method to reconstruct a 3D object proved insufficient for handling the fine detail of the mouse heart. We refined the procedure and show a process of initial stacking, rigid and elastic registration of the images. Due to the large amount of data generated by even a single 3D analysis, we have developed new approaches for data. In particular, we show the application of a novel, spatially-aware peak picking algorithm to 3D MSI data.

Neuer Aspekte

Detection of proteomic signatures of a myocardial infarct in 3D MALDI Imaging data.

LA-ICP-MS to Study the Distribution of Copper in Wilson's Disease Liver Samples

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Stichworte: Laser Ablation, Quantification, Wilson's Disease, Copper, Iron

Einleitung

Copper is an essential element and part of several enzymes in the human organism. However, an excess of copper may lead to various changes and disturbances within the organism. Wilson's disease is an autosomal recessive inherited dysfunction of the copper metabolism. It causes the accumulation of copper in different organs, including the liver, the central nervous system and cornea. This way, Wilson's disease may initiate hepatitis or neurological symptoms like movement disorders and behavioral abnormalities.

To learn about the distribution of copper in Wilson's disease liver samples, different human liver samples were analyzed by Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS).

Experimenteller Teil

Within this work, the distribution of copper in human liver tissue sections from patients suffering Wilson's disease was studied. Additionally, the distribution of iron was monitored. These human liver samples were collected by a liver biopsy of Wilson's disease patients within a medical investigation.

To study the elemental distribution, LA-ICP-MS was applied as imaging technique. The analysis by LA-ICP-MS was performed with a 213 nm Nd:YAG laser using a spatial resolution of 10 μm and a scan rate of 20 $\mu\text{m/s}$. In a next step, copper and iron were quantified by LA-ICP-MS using homemade matrix-matched standards made of gelatin.

Ergebnisse

Results showed an inhomogeneous copper and iron distribution in human liver with hotspots of very high intensities. Thereby, the two elements showed an inverse correlation within the hepatocytes. Copper and iron were quantified successfully by an external calibration using matrix-matched standards made of gelatin. Copper and iron concentrations up to several thousand micrograms per gram liver within the hepatocytes were determined.

In medicine, rhodanine staining is routinely used to determine the copper distribution in liver tissues. Nevertheless, this method does not provide results with a high sensitivity in comparison to LA-ICP-MS. Therefore, the presented method offers a new possible diagnosis. These results may contribute to a better understanding of Wilson's disease and its development.

Neuer Aspekte

The copper and iron distribution in human Wilson's disease liver samples were determined by LA-ICP-MS.

Improving ion–signal reproducibility in MALDI MSI using an internal standard

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Stichworte: MALDI MSI – Internal standard – Inkjet printer - Normalization

Einleitung

MALDI MSI proved to be an excellent tool for the spatial analysis of endogenous compounds in tissue sections. However, the signal intensity of a given molecule suffers irreproducibility as it is affected by several factors. The heterogeneity of tissue surface microenvironment affects both extraction and ionization efficiencies resulting in an unpredictable bias source for the analyte of interest. In addition, the application of MALDI matrix represents a key source of ion-signal irreproducibility, and hence it is crucial to carefully optimize its concentration, method of deposition, and the solvent system. In the current work, we present an approach for the standardization of ion-signal intensity through improved matrix deposition via inkjet printing, and the use of an internal standard

Experimenteller Teil

An inkjet printer was used to apply the matrix and the internal standards, which are non-endogenous lipids closely resembling the chemical identity of the target phospholipids. In addition, the internal standards were simultaneously deposited with the matrix to account for probable inconsistency during deposition and crystallization. Preliminary experiments were done to optimize the composition of the matrix-internal standard mixture to be compatible with the inkjet printer. In addition, the effect of the printing density and the number of printing cycles were monitored in the initial MALDI MSI measurements. The distribution and abundance of proton, sodium, and the potassium adducts of the internal standard showed an interesting behavior on the tissue surface, and enrichment of the potassium adduct was attempted

Ergebnisse

The inkjet printer is used to deposit the internal standard and the MALDI matrix to reduce matrix deposition heterogeneity and analyte delocalization commonly associated with air brush nebulizers. Simultaneous application of the internal standard and the MALDI matrix was useful to compensate for irreproducibility resulting upon matrix-tissue unspecific interactions along tissue surface. Initial measurements used the commercial yellow ink as a solvent for better adhesion to tissue slides and to facilitate visual monitoring of deposition, however this was discontinued due to high background signals in MALDI spectra, and hence an appropriate solvent for the matrix-internal standard mixture that is compatible with the printer's cartridges was chosen. Concentrations between 100 and 300 ppm of the internal standards were tested for optimum signal intensity. Regular purging of the printer injectors is crucial for improved printing performance. In addition an online printing control is needed to demonstrate the reproducibility of deposition. The preliminary ion distribution images of the matrix and the internal standard according to the optimized experimental parameters revealed homogenous deposition of both all over the tissue surface. The distribution behavior of the internal standard outside the tissue showed a remarkable difference, however this had no correlation to the lipids of interest. The ion images of the lipids of interest which were normalized to the internal standard show improved ion signal reproducibility in comparison to images normalized to the matrix signals or the TIC

Neuer Aspekte

The developed method presents a promising approach for the standardization of ion-signal intensities of endogenous lipids in kidney sections

Pre-separation of ions in an atmospheric-pressure MALDI ion source based on differential mobility in a frequency-asymmetric alternating electrical field

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Justus-Liebig-Universität Giessen

Stichworte: FAIMS, MALDI imaging, nano-ESI

Einleitung

MALDI imaging is one of the new, powerful methods used in bioanalysis. Due to the complexity of biological tissue it is often not possible to unequivocally and comprehensively interpret the resulting large datasets of MALDI imaging experiments. Ion suppression effects and a large number of compounds, closely neighboring in mass, lead to misinterpretations and a loss of analytical sensitivity. In most cases even a very high mass resolving power does not allow to circumvent these problems. A very fast and easy-to-use differential ion mobility filter, connected to the mass spectrometer inlet can be used to overcome some of these limitations.

Experimenteller Teil

A commercial differential ion mobility filter (UltraFAIMS, Owlstone Ltd, Cambridge, UK) was attached to a high resolution atmospheric-pressure MALDI imaging source (AP-SMALDI10, TransMIT GmbH, Giessen, Germany). An integrated static nano-ESI device, as a continuous ion source, was used to characterize and optimize the ion separation efficiency and resolution of the FAIMS device. Nano-ESI was also used to calibrate the compensation field of the FAIMS device for different compound classes and ions. Subsequently, after optimization and calibration, MALDI measurements of angiotensin II standards were performed with fixed compensation field values for specific ions.

Ergebnisse

Using FAIMS allowed to separate different compound classes such as proteins, lipids and peptides prior to m/z analysis by means of their differential ion mobility. Selective transmission of only one compound or of a specific charge state of the same compound could be achieved.

A major improvement was achieved regarding an increase of the FAIMS resolution for different charge states of the same compound. With an optimized airflow and slower ion velocities, the resolution of the FAIMS device for singly and doubly charged angiotensin II could be doubled. A larger capillary diameter and a higher inlet temperature were used to slow down the ion flow.

MALDI imaging measurements of angiotensin II showed a sensitivity enhancement when using the FAIMS device as a pre-separator, as a result of a reduced interference of ions in the mass spectrometer. Furthermore, data size per measurement was strongly decreased, due to a reduction of spectral complexity.

Neuer Aspekte

Direct coupling of a differential ion mobility device with an atmospheric-pressure MALDI MSI source

Chromatographic resolved mass defect plots: A new analytical tool for the interpretation of high-resolution MS data.

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Stichworte: mass defect plots, time-of-flight, petrochemistry, odor chemistry

Einleitung

Accurate MS data could be visualized due to application of mass defect plots. Mass related features of a series of compounds could be detected and interpreted in a very convenient way. However, isobaric compounds sharing the same accurate mass (isomeric compounds) are not considered. In such cases complementary analytical methods like gas chromatography are indicated to reveal the full complexity of the sample matrix. Due to the introduction of multireflectron technology, commercial available systems are now able to provide high resolution as well as accurate MS spectra information at very high acquisition frequencies. This will allow the hyphenation of even very fast chromatographic separation processes. Hence, classical mass defect plots could now be completed by chromatographic information's targeting the isomeric composition.

Experimenteller Teil

Recently introduced fast and high resolution time-of-flight mass spectrometry in combination with one- and comprehensive two-dimensional gas chromatography has been used for the profiling of petrochemical and odor active samples. The unique characteristics of the multi-reflecting time-of-flight instrument (Folded Flight Path® (FFP) technology) with a resolution of up to 50.000, a mass accuracy of below 1ppm and an acquisition rate up to 200 Hz provide a novel tool to determine the exact mass information and combine it with retention time information. Resulting mass defect plots will be augmented by an additional dimension, providing isomeric resolution.

Ergebnisse

The unique acquisition speed of the multi-reflecting time-of-flight instrument allows the hyphenation to very fast chromatographic processes. Mass accuracy and mass resolution of the instrument are sufficient to generate meaningful mass defect plots from mass spectral data. Retention time data could be linked to retention index and added as complementary independent axis to the mass defect plots. The mass defect plot will be completed by one additional dimension in case of one-dimensional gas chromatography and two independent axes in case of comprehensive two-dimensional gas chromatography.

Neuer Aspekte

chromatographically separated mass defect plots, fast and accurate time-of-flight mass spectrometry,

Metal labelling of antibodies

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Stichworte: ICPMS, MeCAT, Antibody

Einleitung

Nowadays, antibodies are widely used in the field of biotechnology, diagnostics and therapeutic medicine. Despite numerous approaches for antibody labeling, developing new detection tools for antibodies is still of great importance. Electrospray ionization Mass spectrometry detection (ESI-MS) is proved to be a good option for the detection of proteins as well as antibodies. However, in many cases both accurate quantification and identification are desired. Metal tags have been reported as ones that allow to probe this aspect. As far as quantification is concerned, inductively coupled plasma mass spectrometry (ICP-MS) is known to be a perfect tool for this purpose. In the given work, we present an approach for Metal-Coded Affinity Tags (MeCAT) labeling of antibodies through pre-click reaction procedure.

Experimenteller Teil

In this study, a 3-step strategy for antibody labelling with further two-dimensional gel electrophoresis separation and ICPMS quantification is utilized. In the first step, MeCAT is attached to trans-cyclooctene (TCO) derivative through NHS chemistry reaction. The second one includes antibody labelling with bis-sulphone reagent functionalized with tetrazine group. In the last stage, MeCAT is attached to antibody through click chemistry reaction. The crucial difference from reported MeCAT labelling approaches of antibodies is that bis-sulphone reagent can restore a covalent linkage between the two cysteines, unlike maleimide based conjugates. Click chemistry is known as efficient tool for MeCAT labeling of proteins. However, tetrazine ligation is considered to be a very attractive option because of its unprecedented reaction kinetics and good selectivity.

Ergebnisse

A novel approach for antibody labelling with MeCAT has been developed. This approach is based on 3 step strategy including labelling with bridging bis-sulphone reagent followed by conjugation via Inverse electron demand Diels–Alder reaction. The stage of attaching MeCAT to TCO derivative was optimized to enhance the yield of coupling product and suppress the hydrolysis of N-Hydroxysuccinimide which results in undesired side products. The important factors for optimization were finding appropriate solvent composition, establishing the most reasonable time of conversion and ratio of chemicals. It is known that addition of organic base such as Triethylamine (TEA) influences the yield of reaction. It was found out that not only the concentration of TEA, but also the solvent used for it, both have a significant impact on the reaction yield. Labelling efficiency of this method for several antibodies has been evaluated with ICPMS quantification. It is also planned to try the approach for labelling of proteins with disulfide bridges. First preliminary results will be demonstrated.

Neuer Aspekte

The first time click chemistry approach, namely tetrazine ligation was utilized for MeCAT labelling of antibodies.

Reactivity of Hydrated Monovalent First Row Transition Metal Ions $M^+(H_2O)_n$, $M = Cu$ and Zn toward C_6H_5Cl , C_6H_5Br , C_6H_5I and C_3H_7I

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Stichworte: Reactivity, Metal ions, Benzenehalogenide, Oxidation, FT-ICR

Einleitung

Hydrated singly charged metal ions $M^+(H_2O)_n$ in the gas phase can be considered as a model system for aqueous solutions of transient species. They have been studied over the last two decades by a combination of experiment and theory [1]. Uptake of HCl by $M^+(H_2O)_n$ ($M = Cr, Mn, Fe, Co, Ni$ and Cu) leads to the precipitation of a single MCl molecule in a protonated water cluster [2]. Furthermore several transition metal ions, like Sc^+ , Ti^+ and V^+ , show dehydrohalogenation with phenyl halides [3]. In the present study, reactions of $M^+(H_2O)_n$ ($M = Cu$ and Zn ; $n < 40$) with C_6H_5Cl , C_6H_5Br , C_6H_5I and C_3H_7I were studied by FT- ion cyclotron resonance (FT-ICR) mass spectrometry in the gas phase.

Experimenteller Teil

The experiments were performed on a modified Bruker/Spectrospin CMS47X FT-ICR mass spectrometer with a 4.7 T superconducting magnet. $M^+(H_2O)_n$ ($M = Zn$ and Cu) ions were generated in a laser vaporization source using an Nd:YAG laser and transferred to the ICR cell. The reaction gases were introduced into the ultrahigh-vacuum region ($p = 10^{-9}$ - 10^{-7} mbar) through a needle valve at constant pressure. Mass spectra were recorded at different reaction times in the range of 0-500 s to monitor reaction pathways.

Ergebnisse

Depending on the metal and reaction gas different reactions occurred. Ligand exchange of C_6H_5Cl , C_6H_5Br , C_6H_5I and C_3H_7I was observed for $Cu(H_2O)_n^+$. At longer reaction times the uptake of more than two molecules was observed. However, at longer reaction delays (approximately 500 s) evaporation of the halogenated hydrocarbon ligand takes place. For $Zn(H_2O)_n^+$ the reaction with C_6H_5Br , C_6H_5I and C_3H_7I proceeds by forming $[ZnX(H_2O)_m]^+$ ($X = Br, I$). Furthermore the uptake of C_6H_5X ($X = Br, I$) by the product $[ZnX(H_2O)_m]^+$ ($X = Br, I$) was observed. In contrast the reaction with C_6H_5Cl occurred only at 10^{-7} mbar and showed the formation of $[ZnC_6H_5(H_2O)_m]^+$ with elimination of a Cl radical. By analysing the kinetics the reaction rate constants were determined for all observed reactions. $Zn(H_2O)_n^+$ shows a higher reactivity compared to $Cu(H_2O)_n^+$.

Previous studies at our working group show the high reactivity for oxidation reactions of $Zn(I)$. An earlier study with NO shows the oxidation of zinc and iron by forming $[MOH(H_2O)_n]^+$ [4]. The new results show the oxidation of $Zn(I)$ exposed to halogenated aromatic and aliphatic compounds. The activation of the C-Cl bond is difficult due to the high bond dissociation energy ($BDE(C_6H_5-Cl) = 95.5$ kcal/mol) [5]. Therefore the reaction between $Zn(H_2O)_n^+$ and chlorobenzene is observed only at relatively high pressure. Presumably due to the large radius of $Cu(I)$, up to three molecules are taken up by the clusters. However, as soon as all H_2O molecules are evaporated, black-body radiation induces the loss of the non-covalently bound ligands.

Neuer Aspekte

Investigation of the oxidation reaction of $Zn(H_2O)_n^+$ by C_6H_5Cl , C_6H_5Br , C_6H_5I and C_3H_7I and determination of the reaction rate constants..

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Untersuchung der Fragmentierungen von auf Cyclen basierenden Ligandensystemen mittels ESI-MS/MS und MS³

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Stichworte: ESI-MS, makrozyklische Verbindungen, Fragmentierungsverhalten, Ringverengungen

Einleitung

In der medizinischen Diagnostik werden auf Cyclen (1,4,7,10-Tetraazacyclododecan) basierende Metallkomplexe als MRT- oder PET-Kontrastmittel eingesetzt.^[1] Ihre Spezialisierung zur Aufklärung bestimmter Fragestellungen, z.B. in der Tumordiagnostik, ist eine große Herausforderung. Neue Ligandensysteme werden synthetisiert und charakterisiert. Die Strukturaufklärung größerer neuer Ligandensysteme und ihrer Metallkomplexe stellt jedoch oftmals ein Problem dar, da die NMR-Spektroskopie aufgrund zahlreicher Signalüberlagerungen nur bedingt geeignet ist. Im Falle paramagnetischer Metallionenkomplexe (Einsatz in der MRT) ist mittels NMR gar keine Strukturaufklärung möglich. Hier können jedoch Fragmentierungsstudien mittels ESI-MS nähere Erkenntnisse liefern.^[2] Für neue Studien wurden sowohl das reine Cyclen, das 1-Formyl-1,4,7,10-tetraazacyclododekan, ein mit einem fluorierten Benzoylrest monosubstituiertes System, sowie die tert-Butyl-geschützten Verbindungen 1,4,7,10-Tetraazacyclododecan-1,4,7-triessigsäure (DO3A) und 1,4,7,10-Tetraazacyclododecan-1,4,7,10-tetraessigsäure (DOTA) herangezogen.

Experimenteller Teil

Alle Substrate mit Ausnahme des Cyclens wurden frisch synthetisiert. 1-Formyl-1,4,7,10-tetraazacyclododekan erhielt man in einer Zweistufensynthese durch Reaktion von Cyclen mit *N,N*-Dimethylformamid-dimethylacetal (DMF-DMA) und anschließender Hydrolyse zum 1-Formylderivat.^[3] Die fluorierte Verbindung wurde über eine Reaktion von Cyclen mit 3,5-Bis(trifluormethyl)benzoylchlorid dargestellt. Die beiden tert-Butyl-geschützten Verbindungen erhielt man durch Reaktion von Cyclen mit tert-Butylbromessigsäure. Neben der NMR-spektroskopischen Analyse erfolgte eine ausführliche massenspektrometrische Untersuchung der Zielsubstrate, wobei die Massenspektren an einem Elektrospray-Ionenfallen-Massenspektrometer (Esquire-LC, Bruker Daltonik), aufgenommen wurden. Die Probenzufuhr erfolgte mittels Spritzenpumpe (Fluss: 3 µL/min), wobei Methanol als Lösungsmittel diente.

Ergebnisse

Die Fragmentierung der einzelnen Substrate im positiven Ionenmodus ergaben je nach Substituent am Stickstoff unterschiedliche Fragmentationen. Beim kleinsten der untersuchten Moleküle, dem Cyclen, wird neben einer NH₃- (-17 u) und einer Ethenaminabspaltung (-43 u) auch die Abspaltung von Piperazin (-86 u) unter Bildung neuer cyclischer Verbindungen beobachtet.

Das 1-Formyl-1,4,7,10-tetraazacyclododekan zeigt darüber hinaus die erwartete Abspaltung von Wasser bzw. CO. Des Weiteren wird die Bildung von 1,4,7-Triazon mit $m/z = 130$, 1,4-Diazocan mit $m/z = 115$ und Piperazin mit $m/z = 87$ beobachtet. Eine NH₃-Abspaltung wie im Fall des reinen Cyclens tritt jedoch nicht auf.

Befindet sich eine fluorierte Benzoylgruppe an der Stelle des Aldehyd-Wasserstoffs, so ist ausgehend vom Molekülion neben einer Wasserabspaltung (-18 u) der Verlust von Piperazin (-86 u) zu beobachten. Darüber hinaus erfolgt eine Abspaltung von C₆H₁₂N₂ aus dem Cyclengrundgerüst unter Bildung eines Fragmentions mit $m/z = 283$.

Die MS/MS- und MS³-Massenspektren der tert-Butyl-geschützten Cyclenderivate, wie z.B. DO3A oder DOTA, zeigen erwartungsgemäß zunächst die Abspaltung der Schutzgruppen, im Fall des DO3A-Derivats dreimal, im Fall des DOTA viermal den Verlust von 56 u (= Isobuten). Anschließend konnten zahlreiche heterocyclische, zum Teil bicyclische Fragmentationen charakterisiert werden. Es wird auch in diesen Beispielen deutlich, dass zunächst eine Spaltung des Cyclengrundgerüsts bis zum Piperazinderivat erfolgt, bis die am N-Atom gebundenen Essigsäurereste abgespalten werden.

Neuer Aspekte

Die Studien geben neue Erkenntnisse betreffend Ringverengungen in makrozyklischen Systemen bei MS-Experimenten nach Einführung verschiedener Substituenten (Art und Anzahl).

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Kinetic Energy Release und Fragmentierungswege substituierter Aniline

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Stichworte: Anilinderivate, Kinetic Energy Release, Fragmentierungswege

Einleitung

Der Verlust von Propan aus der Diethylaminoseitengruppe wurde am Beispiel von Rhodamin B mit Elektrosprayionisation (ESI) und FT-ICR-Massenspektrometrie gezeigt und ein möglicher Reaktionsmechanismus vorgeschlagen [1]. Um weitere Informationen über diese Fragmentierung zu gewinnen, wurden Anilinderivate mit Diethylaminoseitengruppe unter anderen Reaktionsbedingungen untersucht.

Experimenteller Teil

Alle Messungen wurden mit einem Micromass ZAB-2F der Firma Vacuum Generators durchgeführt. Die Proben wurden in die Gasphase überführt und mittels chemischer Ionisation ionisiert. Mass-Analysed-Ion-Kinetic-Energy-(MIKE)-Spektrometrie der gebildeten Ionen lieferte Werte für den Kinetic Energy Release (KER). Informationen über den Fragmentierungsweg wurden mit MIKE-Spektrometrie und linked-scan Techniken wie B²E erhalten.

Ergebnisse

Bei Messungen mit einem Sektorfeld-Massenspektrometer laufen Fragmentierungsreaktionen deutlich schneller und mit höheren Energien ab als bei Messungen mit einem FT-ICR-Massenspektrometer.

Die untersuchten Diethylanilinderivate zeigten nach chemischer Ionisation ein nahezu identisches Fragmentierungsmuster. In den MIKE-Spektren der protonierten Molekülonen traten sowohl neutrale als auch radikalische Fragmente auf. Der vorherrschende Verlust eines Methylradikals aus dem protonierten Molekülon ist eine Verletzung der Even-Electron-Regel [2]. Für diese Fragmentierungsreaktion wurde ein kleiner Wert für den KER bestimmt, der auf einen einfachen Bindungsbruch hindeutet. Neben den radikalischen Fragmenten trat der Verlust von 44 Da (Propan) aus den protonierten Molekülonen auf. Für die Propan-Verluste konnten erhöhte KER-Werte bestimmt werden, was einen Hinweis auf eine Umlagerungsreaktion liefert. Die Vorläufer des Fragmentions, das aus dem Verlust von 44 Da aus dem protonierten Molekülon entstand, wurden mittels der B²E-Messungen bestimmt. Die Beobachtung unterschiedlicher Vorläufer führt zu der Vermutung, dass der Verlust von 44 Da aus dem protonierten Molekülon auf unterschiedlichen Fragmentierungswegen zustande kommt.

Neuer Aspekte

Es wurden Werte für den Kinetic Energy Release von Alkylfragmenten bei der Fragmentierung diethylsubstituierter Anilinderivate bestimmt.

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The Extraction of Maximum Information from Individual Ion Arrivals And Its Application to Extending the Dynamic Range of IMS-*oa*TOF-MS Data

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Stichworte: ADC Detection, Dynamic Range Extension, IMS-MSe

Einleitung

The emergence of orthogonal-acceleration time-of-flight mass spectrometry has been made possible in great part by the rapid development of high speed digital electronics. These include increasing speed and dynamic range of analogue-to-digital converters, large speed rapidly accessible memory and high-performance field programmable gate arrays (FPGAs). The latter allows for sophisticated data processing to be applied for individual ion arrivals, enhancing signal-to-noise ratio and extraction and storage of information about each ion arrival individually. We have examined these features of detection in final spectra for extending the linear range of the ion mobility coupled multiplexed fragmentation technique (IMS-MSe).

Experimenteller Teil

All data were acquired using a Waters Synapt G2Si instrument coupled to an IClass UPLC. An Acquity BEHC18 1.7 μ m 2.1x50mm column was used with 0.1% formic acid and 0.1 %formic acid in acetonitrile mobile phases. The gradient used for small molecule mix (verapamil, sulphadimethoxine, leucine enkephalin, caffeine and acetaminophen) was 10-98% B in 5min. Propranolol in human plasma was analysed on Cortecs UPLC C18 1.6 μ m, 2.1x50mm column in a stepwise gradient of 2-60% B in 4mins, followed by 60-95% B in 3min. The FPGA was positioned between the ADC and detector on-board memory.

Ergebnisse

From the following metadata which were stored for further data processing: peak width at base, peak area, skew and standard deviation of width, centre of mass, kurtosis and FWHM, calculation of the ration of these values could be calculated. For example, the number of ADC digitisation values exceeding the vertical dynamic range of the detection (saturated points) could be recorded and this information was stored for each time point in the summed spectra. Distortion in intensity and time of flight measurement would result in inaccurate quantification and mass measurement. It was found out, that no significant ion distortion occurs below 20 ions/push, where 35% of arrivals are saturated.

The transmission switching method uses a dynamic range extension electrostatic lense (DRE) positioned between quadrupole and first gas cell. By fast on/off pulses it can control transmission of ion packets which remix into continuum beam by ion cooling in the gas cell. This method been used for dynamic range extension in TOF data. Previously, this method was restricted to centroid data up to 2000 spectra/second. We applied this method for alternating low and high transmission continuum data scans in IMS-TOF-MSe. These scans were then stitched into single wide dynamic range spectra in each MS function replacing saturated peaks with corresponding peaks from the low transmission data scaled approximately to 1/20th of maximum transmission. We could achieve an approximately 10 fold increase in dynamic range with highly accurate mass measurement (3.5ppm).

In summary, continuous development of FPGAs and new spectrum storage algorithms will facilitate more complex processing of individual ions arrivals, leading to higher quality time-of-flight data.

Neuer Aspekte

Further developments of processing ADC detectors. Enhancing linearity of ion mobility coupled mass analysis in various application fields.

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Expediting Peptide Mapping with High Resolution LC-MS

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Stichworte: Peptide mapping, Orbitrap, high resolution, increased speed, Q Exactive HF

Einleitung

In the area of biopharma peptide mapping is an important and routine application for the verification of the correct sequence of a recombinantly synthesized protein. In large proteins such as monoclonal antibodies chances are very high that amino acid sequences are composed in a way that enzymatic cleavage with trypsin produces some very large peptides but also a number of small, peptides such as di- or tripeptides and also single amino acids which are not possible to identify by MS/MS. Thus a current valid and accepted approach is the sequence confirmation based on retention time and accurate peptide masses alone or a combination of accurate precursor masses and supporting MS/MS data in order to achieve the required 100% sequence coverage.

Experimenteller Teil

An enzymatic digest of a monoclonal antibody was analyzed on a UHPLC system with a 10 cm x 2.1mm Accucore™ column coupled online to the Q Exactive HF™ high field quadrupole Orbitrap™ mass spectrometer. Analyses were performed with shortening the actual LC gradient from 45min all the way down to 6 min. Data analysis was performed with PepFinder™ 2.0 software.

Ergebnisse

A series of analyses of the enzymatic digest of a monoclonal antibody was performed by decreasing the actual gradient time from 45min all the way down to 6 min.

All data were analyzed with the software PepFinder which considers both the precursor masses of the peptides as well as MS/MS fragment ion information as far as available. The software not only allows for the identification of neat peptides but also of their modified forms of the peptides even at very low abundance. Both expected as well as unexpected modifications are picked up and even sequence variants can be identified and quantified.

This poster will present all results: chromatograms at different gradient lengths, peaks shapes, quality of single scan MS/MS spectra as well as summary reports of the data analysis displaying qualitative and quantitative aspects.

Neuer Aspekte

Expedited peptide mapping with an ultrafast high resolution LC-MS system

Analysis of multiple toxins by LC-MS/MS: In-depth analysis of column selectivity

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Stichworte: mycotoxin analysis, LC-MS/MS

Einleitung

Mycotoxins are toxic secondary metabolites produced by fungi, which can exist in food as a result of fungal infection of crops. Their strong resistance to decomposition and digestion cause mycotoxins to remain in the food chain. The analysis of mycotoxins in food and animal feed has been a challenge mainly due to the complexity of food matrices and desired low detection limits.

In recent years, significant advances in the analytical techniques have been applied to detection of mycotoxins. There has been an increasing need for a method to detect multiple mycotoxins with a single sample preparation and analysis method.

Recent Mycotoxin Sample Preparation

- Involves QuEChERS cleanup
- Simplified extraction for various matrices
- Multicomponent assays
- LC-MS and LC-MS/MS detection
- Single assays for multiple mycotoxins

Experimenteller Teil

This study investigated the selectivity for over 15 common mycotoxins on a variety of Solid-Core HPLC columns with different stationary phase chemistries. The results of these analyses were evaluated for optimum resolution and selectivity. The separation of analytes from matrix was also important as often no sample cleanup is performed during analysis and matrix effects are highly probable.

Comparison of Solid-Core analysis to sub-2 μm porous particle analysis was also performed.

Ergebnisse

Solid-Core Analysis

Varying elution orders were obtained with the three different phases.

The biphenyl phase allowed for greater retention of the mycotoxins but less resolution for the later eluting compounds. Slightly better resolution was obtained for the isobaric acetyl-DON peaks but not baseline separation.

The Phenyl-Hexyl column offered the best resolution for the majority of the peaks including the acetyl-DON compounds.

Fused-Core versus Porous Sub-2 μm

Varying elution orders were obtained with the two different C18 phases. Greater retention was obtained for most analytes on the porous sub-2 μm column because of the even particle distribution allowing for tighter packing of the smaller particles.

Separation of the isobaric acetyl-DON analytes were not achieved on either C18 column.

Similar analyte peak asymmetries were observed with the sub-2 μm porous and solid-core columns. Some analyte peak shapes were improved on the sub-2 μm porous column.

Neuer Aspekte

Using phenyl-hexyl Fused-Core technology, improved separation of the 15 mycotoxins was possible.

Sensitive and fast analysis of aflatoxin M1 in milk at picogram levels using LC-MS/MS

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Stichworte: aflatoxin m1, milk, LC-MS/MS

Einleitung

Aflatoxins are secondary metabolites produced by fungi of the *Aspergillus* genus(1). They are potent natural hepatocarcinogens and have been classified by the International Agency for Research on Cancer (IARC) as group 1 carcinogens (carcinogenic in humans) (2,3). Of several aflatoxin producing species, *Aspergillus flavus* and *Aspergillus parasiticus* commonly infect cereal grains including maize, as well as ground and tree nuts. Many of these commodities are important human food staples and are also used in animal feeds. If the grains become infected with *Aspergillus*, aflatoxin contamination may result.

Experimenteller Teil

Milk preparation, protein crash, solvent partitioning, sample clean-up and the LC-MS method are presented

Ergebnisse

Silane treated glass and polypropylene vials were used to prevent possible binding of AFM1 to untreated glass surfaces.

SPE cleanup using Supel Tox AflaZea was fast and efficient. Conditioning, equilibration and elution steps were not required. Samples passed through the cartridge followed by a simple rinse to ensure a maximum recovery of AFM1.

AFM1 peak was well resolved in LC: There was no significant background interference with the compound of interest when analyzing AFM1 at the low concentration of 0.025 µg/kg (25 ppt.)

During LC-MS analysis some matrix suppression was present in the samples at the retention time of the AFM1 peak. The matrix suppression effect was measured at 40%.

The use of Matrix-matched calibration standards was required due to sample matrix effects.

Using Ascentis Express column provided short HPLC analysis time. The total run time for the LC-MS analysis including a required column wash, was 7 minutes.

The sample preparation procedure developed in this application facilitates the extraction and analysis of low trace (0.025-0.3 µg/kg) concentrations of AFM1 from milk, and appears to be robust with 10% RSD values or less.

Sample recoveries were good

– 76% for 0.250 µg/kg spiked samples

– 103% for 0.025 µg/kg spiked sample

– within the ranges for recovery and precision required by European Union specifications for its treaty members, which are among the most stringent requirements currently written(6,7).

The use of interference removal SPE (Supel Tox AflaZea) cleanup was rapid, less than 5 minutes for 6 samples.

The procedure produced sample cleanliness that was acceptable for LC-MS/MS analysis.

The LC-MS analysis used superficially porous HPLC column (Ascentis Express C18) and was also fast with a run time of 7 minutes.

Neuer Aspekte

Sample Preparation using Interference Removal Solid Phase Extraction

TLC-MALDI-MS investigation of a multi-compound flu medication

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Stichworte: thin layer chromatography, mass spectrometry, TLC-MS, TLC-MALDI-MS, small molecules

Einleitung

Thin layer chromatography (TLC) and mass spectrometry (MS) are well established and appreciated for their reproducibility, sensitivity and stability regarding the separation and identification of analytes present in a given mixture. With TLC complex sample mixtures can be separated and impurities can be detected, but no information about mass is obtained. Therefore reference substances, staining methods or UV activity are required. Mass spectrometry in addition to TLC allows an excellent classification of substances by their mass. The combination of these two powerful techniques leads to a new level of information based on chromatographically separated and mass selected analytes as an orthogonal technique to LC-MS but with the advantage of visualization [1].

Experimenteller Teil

After chromatographical separation of all compounds, a mix of matrix with 2,5-dihydroxybenzoic acid and α -cyano-4-hydroxycinnamic acid was applied via spraying the solution homogeneously onto the TLC plate. By using a spraying method in comparison to a standard dipping process, the wash-out of spots is reduced. The mass spectra of the bands were recorded using matrix assisted laser desorption/ionization (MALDI)-MS imaging in reflectron positive mode.

Ergebnisse

We show an application for TLC-MS on newly developed HPTLC plates with a reduced separation layer thickness [2]. The characterization of small molecules via MALDI-MS in a multi-compound flu medication is presented. The single compounds of the drug like paracetamol, caffeine and methylisothiazolinone could be identified with MS and UV detection. These molecules are detected in the mass range of about m/z 150 - 300. This is a rather unusual mass range for MALDI-MS as most matrices ionize at $m/z < 700$, and the matrix signals overlap the compound masses and cause complex spectra [3]. With the new plate type a good reproducibility and sensitivity could be achieved and even analyses in this small mass range lead to high quality mass spectra.

Neuer Aspekte

Direct coupling of thin layer chromatography with mass spectrometry via a TLC-MALDI-MS adapter using newly developed thinner TLC plates.

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Influence of the silica gel layer thickness on the quality of TLC-MALDI mass spectra of lipids

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Stichworte: thin layer chromatography, mass spectrometry, TLC-MS, TLC-MALDI-MS, lipids

Einleitung

Coupling thin layer chromatography (TLC) with mass spectrometry (MS) is a relatively new field of great interest [1].

Matrix assisted laser desorption/ionization (MALDI) mass spectra can be recorded directly from a developed TLC plate. The matrix is applied on the TLC plate after separation and then the TLC plate is mounted onto a TLC-MALDI adapter followed by direct measurement. Limitations of TLC like spot capacity and sensitivity of UV detection or staining methods can be overcome by the spatial resolution and high sensitivity using MALDI-MS.

Here we present how the quality of TLC-MALDI mass spectra of lipids is influenced by the thickness of the separation layer.

Experimenteller Teil

TLC plate: HPTLC silica gel, stationary phase 60 µm, 100 µm and 200 µm

Matrix application: two times dipping into a solution (200g/L in acetonitrile / 10 mM aqueous NH₄H₂PO₄ (90/10, v/v)) of 2,5-dihydroxybenzoic acid (DHB)

Mass spectrometer: Ultraflextreme & TLC-MALDI adapter (Bruker Daltonics)

MALDI-Imaging-MS: Reflectron positive mode, m/z 500-1500, DHB as Matrix, 500µm spatial resolution

Ergebnisse

TLC-MALDI-MS analysis of lipids benefits significantly from a reduced silica gel separation layer thickness.

It will be shown that the intensity of the matrix background signals (for instance m/z 551.0 in the case of positive ion mode spectra recorded in the presence of DHB) can be significantly reduced and a sensitivity gain can be achieved if the silica gel layer thickness is decreased from 200 µm to 60 µm. This will be illustrated by using selected lipid mixtures with the focus on phosphatidylcholines (PC) and phosphatidylethanolamines (PE) which are abundant in biological samples and present in nearly all samples from biological origin.

The positive effect of reduced layer thickness can be explained by the reduced amount of DHB on thinner layers (2.1, 2.9 and 5.1 mg/cm² of DHB on the 60, 100 and 200 µm plates determined by weighing the plate before and after matrix application) and improved detectability because the analyte is closer to the surface [2].

Neuer Aspekte

Reduced stationary phase layer thickness increases the signal intensity and S/N-ratio for the lipid analysis via TLC-MALDI-MS.

Referenzen

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Exploring Impact of Dynamic Accumulation for Improving MS/MS Quality of QqTOF Data

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Stichworte: TripleTOF, dynamic accumulation, MSMS

Einleitung

Recent innovations in QqTOF instrumentation has resulted in a large increase in MS and MS/MS acquisition speed providing deeper coverage of complex proteomes. Some workflows, such as iTRAQ[®] reagent quantitation or PTM characterization, benefit more from higher spectral quality than traditional data-dependent workflows. Here, a QqTOF acquisition strategy that uses precursor intensity to adapt the MS/MS accumulation time (dynamic accumulation) was explored for its utility in improving these proteomic datasets.

Experimenteller Teil

Analysis of protein digests was performed using nanoflow LC/MS analysis on a TripleTOF[®] system. Data collection was done in data dependent mode to explore a range of acquisition rates and precursor intensity combinations for optimal coverage and spectral quality. Protein identification data was processed using ProteinPilot[™] Software and results assessment was performed using Excel tools. A number of areas of improvement were investigated, e.g. impact on MS/MS quantitation for iTRAQ reagents, effect on number of acquired spectra, protein identification rates, and generation of SWATH[™] ion libraries.

Ecoli lysate was labeled with 8plex iTRAQ[®] reagents and mixed with equal loading in all channels. The sample was analyzed using different acquisition strategies and the identification yields were characterized for ID and quantitation.

Ergebnisse

The dynamic accumulation approach provided a small increase in total protein/peptide identifications and significant improvements in the quantitation quality. The median reporter ion intensity was shifted higher by 34%, and the variance of protein ratio distributions was reduced (16% improvement in quality). The peptide variation about the protein was constant across the peptide intensity range, indicating improved quantitation of lower signal peptides.

Neuer Aspekte

improving spectral quality of fragment ion spectra

Design and Development of a New, High Performance 20kV HED Detector with Improved Efficiency and Low Noise Characteristics

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Stichworte: HED, EM

Einleitung

Ion detectors which use a high energy conversion dynode (HED) in combination with an electron multiplier (EMT) have attained widespread use in both MS quadrupole and ion trap-based mass analyzers. Although signal increases with applied HED voltage, concomitant noise, primarily from field emissions, increases in a near-exponential manner. We report on a successful HED detector design for the triple quadrupole mass spectrometer which overcomes this operational barrier while delivering enhanced sensitivity, particularly in negative ion mode.

Experimenteller Teil

SIMION simulations were used to model and optimize the new detector geometry and determine primary and secondary beam trajectories. A series of experiments and measurements were carried out to determine the optimal HED position, EMV horn position, iris and HED voltage settings in correlation with the simulation results. Optimization of detector performance in regards to overall gain and secondary ion beam geometry, was performed by adjusting HED positions in the orthogonal plane. Predictable shifts in optimal horn position were observed (refer to next plot). The final detector geometry optimization was found to be universal for operation over the entire range of HED voltages (10 to 20kV).

Ergebnisse

The specific MRM transitions (precursor & product ions), collision energies, etc.) were determined by product ion scans and optimized for each thyronine analog. Operation at higher HED voltages correlated with lower RSD values. Instrument detection limits obtained for the thyronine analogs were: Chloramphenicol (CAP) was analyzed at 0.5fg, 1fg, 2fg, 10fg, 100fg, 1pg levels utilizing a ballistic LC gradient of H₂O and methanol. CAP yielded improved instrument detection limits with an average of 0.5fg with 10% RSD

Serotransferrin peptides from a tryptic digest were analyzed in positive ion mode at -10kV and -20kV with detector gain kept constant (adjusted for m/z 622 calibrant ion). Relative area increases in the range of 0-60% were obtained for product ions at the higher HED setting.

Neuer Aspekte

high energy conversion dynode (HED) electron multiplier (EMT) in combination, in positive and negative ESI Modus in QQQ

Coupling of Acoustically Levitated Droplets with Ion Mobility Spectrometry

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Stichworte: Ion Mobility Spectrometry, Acoustically Levitated Droplet

Einleitung

The list of applications for ion mobility spectrometry (IMS) is steadily growing quickened by advances in instrumental design, experimental methods and accompanying theory. The performance of IMS is greatly influenced by the ionization source and the introduction of the analyte, hence new ionization schemes and sampling strategies recently received particular interest.[1] An overall new approach is the coupling of IMS to an acoustically levitated droplet and combined sample desorption/ionization by a laser. In this setup the acoustically levitated droplet works as a wall-less micro reactor.[2] The latter opens the way towards quality control of fast reactions. Generally, the combination of acoustically levitated droplet and IMS bears a wide range of applications from routine analysis to microfluidic optimization of chemical reactions.

Experimenteller Teil

A novel coupling of an acoustically levitated droplet with a homebuilt drift tube ion mobility spectrometry is presented. A new prototype of inlet was customized according to design constraints yielding in maximum ion transmission. Moreover, the commonly used plate repeller was replaced with a point electrode to form a concentric electric field between the droplet and the IMS, geometrically allowing space for the acoustically levitated droplet. The desorption/ionization out of the droplet was performed with an optical parametric oscillator at 2,94 μm wavelength with pulse duration of FWHM = 7 ns. Primary evaluation of the setup was achieved using tetra-n-butylammonium bromide, promazine, perphenazine and L-arginine in direct comparison with the results obtained with a μ droplet IR-MALDI IMS.

Ergebnisse

The coupling of acoustically levitated droplet with IMS was successfully realized giving further possibilities to utilize it as detection method to interrogate the fast kinetics of reactions performed directly in the droplet. The presented modifications simplify the assembly of the setup and allow for a sufficient isolation between the high voltage, applied on the individual electrodes of the IMS, and the acoustic field sonotrode. The implemented point electrode produces a concentric steep potential field around the acoustically levitated droplet forcing the flow of nascent ions towards the drift tube inlet region. Additionally, it minimizes spatial restrictions giving space for a better accessibility for the sampling laser beam. The recorded ion mobility spectra confirm that the entire arrangement results in an efficient desorption, ionization and ion transfer of the sample liquid. First results show a sensitivity of the IMS comparable to that of the previously introduced μ droplet IR-MALDI. However, the main advantage of acoustically levitated droplet is the reduction of long memory effect typically observed for the μ droplet source. These memory effects are attributed to wash-out times of the sample volume and to contamination of surfaces that get into contact with the sample. The contribution can therefore positively demonstrate that by avoiding sample-surface interfaces, the instrument response function can be drastically shortened. This not only significantly decreases the time necessary to clean the system between subsequent measurements but mostly opens the path towards fast kinetic monitoring of chemical reactions within the microdroplet reactors.

Neuer Aspekte

The combination of acoustically levitated droplets and ion mobility separation provides a tool for direct online detection of reaction kinetics.

Referenzen

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Identification of human milk derived proteolytic glycopeptides by use of ion mobility separation (IMS) and subsequent low energy collision-induced dissociation

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Stichworte: nanoESI MS, glycopeptides, structure elucidation, ion mobility, CID

Einleitung

The outright elucidation of protein *N*-glycosylation with respect to the glycan moieties and the corresponding glycosylation sites requires the inspection of intact glycopeptides. However, a direct analysis of proteolytic glycopeptides is frequently hampered by their low ionization efficiencies, signal suppression caused by the vast excess of non-glycosylated peptides, and the relatively low abundance of individual glycopeptide species due to the microheterogeneity of the glycan moieties. Therefore, in the present study a multi separation strategy i.e. a combination of glycoprotein enrichment by use of phenylboronic acid, proteolytic digestion, ZIC-HILIC SPE, and direct infusion nano electrospray ionization (ESI) MS including IMS and subsequent low energy collision-induced dissociation (CID) was applied to elucidate the site specific *N*-glycosylation of whey proteins from human milk.

Experimenteller Teil

ESI MS experiments were carried out by use of a SYNAPT G2-S mass spectrometer (Waters, Manchester, UK) equipped with a Z-spray source in the positive ion sensitivity mode. Typical source parameters were: source temperature: 80 °C, capillary voltage: 0.8 kV, sampling cone voltage: 20 V, and source offset voltage: 50 V. For low energy CID experiments, glycopeptide precursor ions were selected in the quadrupole analyser, subjected to IMS (wave velocity 800-1200 m/s, wave height 40 V, nitrogen gas flow rate 90 ml/min, and helium gas flow rate 180 ml/min), and fragmented in the transfer cell using a collision gas (Ar) flow rate of 2.0 ml/min and collision energies up to 120 eV (E_{lab}).

Ergebnisse

More than 50 glycopeptides derived from the heavy-chain constant regions of immunoglobulin α -1 and α -2 (IgHA1 and IgHA2), the secretory component (polymeric immunoglobulin receptor, PIgR), lactotransferrin (TRFL), and immunoglobulin J chain (IgJ) were identified. Ions with different charge states and/or considerably distinct m/z values could be readily separated as demonstrated exemplarily for the ions derived from a tryptic digestion in the m/z range 1242 to 1245. Two glycopeptide ion species (m/z 1242.56, triply charged, IgHA1 aa₃₃₂₋₃₅₃, GlcNAc₂Man₆ and m/z 1243.90, six-fold charged, IgHA2 aa₁₅₆₋₂₀₈, GlcNAc₂(Fuc)Man₃(GlcNAc)GlcNAc₂) were separated, fragmented and their glycan structures as well as the corresponding glycosylation sites were determined from a single IMS-CID experiment. Due to the reduced background noise of IMS-CID spectra even low abundant fragment ions are detectable. Thus, e.g. the identification of three triply charged tryptic/chymotryptic glycopeptide species whose isotopologue signals superimposed each other from one and the same fragment ion spectrum was successful (m/z 1308.94, PIgR aa₄₆₆₋₄₈₇, GlcNAc₂Man₃(GlcNAc)GlcNAc₂; m/z 1309.29, IgHA1 aa₁₃₀₋₁₄₉, GlcNAc₂Man₈; m/z 1309.62, TRFL aa₁₅₅₋₁₇₀, GlcNAc₂(Fuc)Man₃GlcNAc₂(Fuc)Gal₂NeuAc). The above examples show that ion mobility is a powerful tool for gas-phase separation of glycopeptide ions exhibiting the same or close m/z values. Combined with direct infusion that facilitates a broad variation of collision energy by an extended time frame for fragmentation experiments, IMS clearly improves the analysis of glycopeptides derived from a complex glycoprotein mixture.

Neuer Aspekte

The use of IMS helps to break down the complexity of glycoprotein mixtures and assists deciphering of *N*-glycosylation.

Enrichment of Glycoproteins from Human Breast Milk and Structural Characterization of N-Glycosylation

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Stichworte: proteomics, human breast milk, N-glycosylation, de novo-sequencing

Einleitung

Despite serving as an amino acid source, proteins of human milk contribute essentially to the development of the infant's immune system and to the defence against pathogens [1]. Especially glycoproteins have been shown to possess antimicrobial, antiviral or immune-enhancing properties repeatedly [2]. The present study aims at the development of a practicable methodology that enables the characterisation of milk protein glycosylation, i.e. to obtain both information on glycan structure and individual glycan-protein linkage site.

To this end we used a phenyl boronic acid (PBA)-based resin for specific enrichment of glycoproteins and a ZIC-HILIC solid phase extraction (SPE) for enrichment of glycopeptides. We relied on manual inspection and de novo-sequencing of N-glycopeptides for structure elucidation and information on glycosylation sites.

Experimenteller Teil

The current experimental approach comprises a two-step protocol to isolate the whey fraction from human milk samples. The milk was first delipidated by high-speed centrifugation, resulting in a cream layer on top and cellular fragments as a pellet followed by removal of caseins by calcium chloride precipitation. Glycoproteins were enriched from the whey fraction by SPE employing PBA. The resulting mixture was submitted to proteolytic digestion employing proteases with varying specificities and glycopeptides were specifically enriched by use of ZIC-HILIC SPE and submitted to mass spectrometric analysis. Gas-phase ions were formed by nanoESI and measurements were performed with a Waters Synapt G2-HDMS instrument.

Ergebnisse

SPE of whey fraction proteins from human milk using PBA furnished a mixture of proteins mainly comprised of glycoproteins. The latter were submitted to enzymatic degradation using either trypsin, chymotrypsin or a mixture of both proteases. Peptides resulting from the mixed digestion typically exhibit a relatively short backbone and thus a higher hydrophilicity which allows their specific enrichment employing ZIC-HILIC SPE [3]. The resulting glycopeptides were analysed by direct infusion nanoESI mass spectrometry (MS) and tandem MS/MS experiments. Inspection of CID spectra allowed the identification of several N-glycopeptides from different milk glycoproteins. In many cases the glycan structure, of complex as well as high mannose type, was unambiguously identified and assigned to a specific glycosylation site.

Our results show that selective enrichment of glycoproteins followed by enzymatic digestion and selective separation of N-glycopeptides with ZIC-HILIC is a powerful and fast method for the exploration of glycosylated proteins from complex samples such as human milk.

Neuer Aspekte

De novo-sequencing of N-glycopeptides in human milk

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Enhanced Confirmation Criteria for Reducing False Positive Rates (FPR) in Toxicology Screening using High Resolution QToF Accurate Mass Analysis

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Stichworte: Toxicology, QTOF, screening, false positive rates, cocaine

Einleitung

Toxicological drug screening based on the measurement of exact precursor m/z values, isotopic pattern and retention time alone is unable to distinguish isomers or interfering matrix compounds. As well due to the large numbers of structural and stereoisomers for a molecular formula, false positives are common in real life screening. We describe the application of diagnostic ions (isotopes, adducts and fragment ions) for confirmation by rapidly alternating full scan ToF-MS acquisitions and broad-band Collision Induced Dissociation (bbCID) to significantly reduce the FPR and increase the degree of confidence in screening applications.

Experimenteller Teil

61 compounds covering several compound classes based on their relevance in post-mortem and routine drug screening were used in this study. After acetonitrile precipitation, urine and serum samples were spiked with toxicological mixes at 4 levels (10-500 ng/ml) and analyzed in a QTOF (impact HD, Bruker) in full scan and bbCID modes using a 14 minute RP-UHPLC gradient. In the bbCID mode, additional detection criteria mandated at least one diagnostic ion with a retention time difference <0.05 minutes. The data were compared to the number of expected identifications to assess the FPR. Authentic samples from two forensic laboratories were investigated using the same workflow.

Ergebnisse

In full scan mode, all compounds were detected at all concentration levels in the spiked samples, no false negatives were encountered. A few additional plausible compounds were also detected such as caffeine, or degradation compounds such as cocaine in a mix with cocathylene. However, the full scan ToF-MS data produced 333 false positives in serum, i.e. more than the 274 expected identifications and 547 false positives in urine. These typically arose due to the low detection threshold of 750 counts for traces with high noise levels or from low intensity peaks within the ± 0.5 min RT window. For the enhanced detection criteria, ToF MS and bbCID were used for confirmation. Here at least one diagnostic ion must be detected within a ± 0.05 min RT detection window. Diagnostic ions include bbCID fragment ions, isotopes and adducts. After applying the enhanced criteria, the false positives were completely removed with the exception of tramadol which cannot be removed as a positive in presence of o-desmethylvenlafaxine. These compounds have the same RT, identical mass and the same main fragment ions.

Neuer Aspekte

Diagnostic ions provide an enhanced level of confidence of detection and identification.

Introducing ion mobility mass spectrometry in glycomics of Schindler's disease

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Stichworte: Ion mobility mass spectrometry, α -N-acetylgalactosaminidase, Schindler disease, glycopeptides, isomers.

Einleitung

Schindler disease is a rare inherited metabolic disorder characterized by the deficient activity of the lysosomal enzyme α -N-acetylgalactosaminidase (α -NAGA). The enzyme defect leads to the abnormal accumulation of certain complex compounds (glycoproteins, oligosaccharides), which have terminal or preterminal N-acetylgalactosaminyl residues in many tissues of the body and in urine. The specific symptoms and severity of Schindler disease can vary from one person to another. Schindler disease may be diagnosed after birth by a thorough clinical evaluation and a variety of specialized tests. Urinary analysis (e.g., oligosaccharide and glycopeptide profiles) may reveal increased levels of certain complex compounds in the urine [1,2]. Reduced activity of the enzyme may be confirmed by conducting enzyme tests on leukocytes and/or fibroblasts from affected individuals.

Experimenteller Teil

An integrative approach based on electrospray ionization (ESI) and ion mobility mass spectrometry (IM-MS) was optimized and applied to discover the conformation of O-glycopeptides in the urine of a Schindler disease patient. IMS-MS experiments were performed on a Synapt G2-S HDMS (Waters).

A mixture of O-glycosylated sialylated amino acids and peptides, denoted QS6, was previously purified from the Schindler disease type I patient urine. The patient's urine was submitted to gel filtration chromatography on Biogel P2. The glycans were separated by gel filtration chromatography on Fractogel TSK HW50 and pre-fractionated by anion-exchange chromatography using a monoQ column. The sixth migrating fraction (QS6) was desalted and dried. For IMS-MS analysis aliquots having a concentration of about 2 pmol/ μ L were used.

Ergebnisse

Mass spectrometry based on microfluidics has revealed in the last decade the ability to discover in Schindler disease patient urine O-glycosylated amino acids and O-glycopeptides as disease markers useful for a rapid diagnosis [3,4].

To test the possibility to correlate glycan and glycopeptide conformation with diseased state, we examined a highly complex mixture of O-glycosylated sialylated amino acids and peptides previously purified from Schindler disease patient urine.

With this ESI IM-MS approach, the mobility distributions and mass spectra associated with all ions that are transmitted through the source are simultaneously obtained. The IMS separation reduced spectral congestion by separating components into mobility families. One outcome of this separation is a reduction of background chemical noise, which is dispersed across a wide range of drift times and can be recognized easily. According to the IM-MS results, we have observed that the attachment of only one or two amino acids to the carbohydrate backbone has a minimal influence on the general molecule conformation, which limits the discrimination of the free oligosaccharides existing in the mixture from the glycopeptides. Another important outcome is the possibility to perform structural analysis of different molecules that present the same m/z ratio and different charge states. The structural characterization achieved after ion mobility separation demonstrated the presence of different conformations for some

of the Schindler disease biomarkers. The presence of more than one conformation can be correlated with this pathological state.

Although this work is still at an early stage, the methodology appears to be relevant to studies related to glycan and glycopeptide mixtures extracted from various human matrices since particular isomeric forms may exhibit structures that are readily separated and distinguished based on differences occurring in three-dimensional geometries.

Neuer Aspekte

Electrospray ionization in combination with ion mobility mass spectrometry was successfully introduced in glycomics of Schindler disease.

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The maturation of sperm is accompanied by changes of the (phospho)-lipid composition – A MALDI-TOF MS Investigation of murine epididymal spermatozoa

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Stichworte: MALDI-TOF MS, epididymal maturation, murine epididymal sperm, phospholipids, lysophospholipids, plasmalogens

Einleitung

After leaving the testis and upon the passage through the epididymis, spermatozoa undergo important steps of maturation which is needed to increase their motility and fertilizing ability. These changes include also modifications of the phospholipid composition and this is assumed to be particularly important for the achievement of motility and fertilizing capacity.

The epididymis consists of caput, corpus and cauda, through which the spermatozoa are transported and undergo distinct maturation stages. Using MALDI-TOF (matrix-assisted laser desorption/ionization time-of-flight) mass spectrometry, we established three striking differences in the lipid compositions of murine spermatozoa from the different epididymal regions: in comparison to the caput sperm, sperm from the cauda are characterized by a higher extent of unsaturation, an enhanced plasmalogen and lyso(phospho)lipid content.

Experimenteller Teil

Twenty (18 C57BL/6N and 2 BALB/c) mice were used in this study.

After orchietomy, the caput and cauda of epididymis were dissected from the testis and spermatozoa isolated from these tissues. The motility of caput and cauda sperm was checked by microscopy. Afterwards, sperm concentration was determined.

The lipid extraction was performed according to the procedure by Bligh & Dyer [1]. The spermatozomal lipid extract of the epididymis was immediately analyzed by MALDI-TOF MS using 2,5-dihydroxybenzoic acid (DHB, 0.5 M in methanol). To overcome assignment problems, analyses were repeated with a DHB matrix that has been saturated with cesium chloride (CsCl) as described in [2]. All mass spectra were recorded on a Bruker Autoflex MS device.

Ergebnisse

Spermatozoa from the epididymal head and tail are characterized by significantly different lipid compositions. For both parts of the epididymis high quality (positive ion) mass spectra with intense phosphatidylcholine (PC) as well as sphingomyelin (SM) signals could be recorded. The discrimination of differently matured spermatozoa based on their lipid mass spectra is, thus, possible.

The most abundant signals in the spectra of the caput sperm extract can be assigned to PC 18:0/20:4, PC 18:0/18:1 and PC 18:0/18:2. Considerable changes regarding the contents of highly unsaturated fatty acyl residues are obvious if the spectrum of caput spermatozoa is compared with that of the cauda: in the cauda PC 18:0/22:5, PC 18:0/22:6, PC 18:0/18:2, PC 16:0/22:5 and PC 16:0/18:1 predominate. Unfortunately, some peaks in the MS¹ spectra cannot be unequivocally assigned: for instance, the H⁺ adduct of PC 16:0/20:4 and the Na⁺ adduct of PC 16:0/18:1 result in isobaric ions. However, this problem can be easily overcome even at limited mass accuracy: when the used DHB matrix is saturated with Cs⁺, this leads to a significant mass shift (132.9 u) that makes assignments simple.

These data suggest that spermatozoa membranes become more fluid during epididymal maturation due to the higher content of double bonds, which is also expressed by an increase of the PC (18:0/22:5 and 18:0/22:6) / PC (18:0/18:1 and 18:0/20:4) ratio from caput to cauda. This ratio increased from 0.1 to 3.61 and these changes of the fatty acyl compositions are also reflected by changes of the sperm motility, which increases (as well as further biochemical

parameters) upon the transition from the caput to the cauda [3]. Finally, the fertilizing ability of sperm does also correlate with the observed changes of the lipid composition [4]. It is suggested that the lipid composition of sperm may be useful as fertility marker.

Neuer Aspekte

MALDI-TOF MS represents a method to estimate different maturation states of murine sperm and maybe sperm quality.

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Phospholipid-Profilierung mittels HILIC-ESI-MS/MS

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Stichworte: Phospholipide, HILIC, Electrospray, MS/MS, Bis(monoacylglycero)phosphat

Einleitung

Die bedeutende biologische Funktion der Lipide ist seit Langem bekannt. Trotzdem sind ihre System-Funktions-Beziehungen in Organismen nicht annähernd so detailliert untersucht worden wie die der DNS, RNS und der Proteine. Wichtige Vertreter dieser Klasse sind die Phospholipide, die in biologischen Systemen mannigfaltige Funktionen übernehmen.[1] Die Einteilung erfolgt in Phospholipidklassen anhand der polaren Kopfgruppe, die an einen Phosphatrest gebunden ist. Die wichtigsten Vertreter sind Phosphatidylcholin, Phosphatidylethanolamin, Phosphatidylserin, Phosphatidylglycerol, Phosphatidylinositol, Diphosphatidylglycerol und Bis(monoacylglycero)phosphat. Jede Phospholipidklasse umfasst eine Vielzahl verschiedener Molekülspezies, die sich durch die zwei am Glycerin gebundenen Fettsäurereste unterscheiden. Diese Diversität erfordert eine leistungsstarke Analysenmethode. In diesem Beitrag wird eine LC-MS/MS-Methode vorgestellt, die auf der hydrophilen Interaktionsflüssigkeitschromatographie (HILIC) basiert und auf die Identifizierung der Phospholipide in Extrakten von Brustkrebszellkulturen angewendet wird.

Experimenteller Teil

Für die Trennung der Phospholipidklassen aufgrund ihrer polaren Kopfgruppe sowie zur Trennung von isobaren Verbindungen, wie das Phosphatidylglycerol und das Bis(monoacylglycero)phosphat, wurde eine HILIC-basierte Trennmethode unter Verwendung einer amin-modifizierten stationären Phase entwickelt. Als mobile Phase kam ein binäres Gradientensystem aus Acetonitril und Ammoniumacetat-Puffer zum Einsatz. Die Detektion erfolgte mittels eines ESI-Ionenfallenmassenspektrometers im negativen und positiven Ionisationsmodus. Die Trennmethode wurde mit Phospholipidstandards entwickelt und zur Identifizierung der Phospholipide in Lipid-Extrakten von Brustkrebszellkulturen angewendet.

Ergebnisse

Durch den Einsatz der amin-modifizierten HILIC-Trennsäule konnten die folgenden Phospholipidklassen separiert werden: Phosphatidylcholin, Phosphatidylethanolamin, Phosphatidylserin, Phosphatidylglycerol, Phosphatidylinositol, Diphosphatidylglycerol und Bis(monoacylglycero)phosphat. Die Detektion der Phosphatidylcholone konnte trotz der positiv geladenen Kopfgruppe im negativen ESI-Modus erreicht werden. Aufgrund des verwendeten Acetat-Puffers konnte das Phosphatidylcholin als einfach negativ geladenes Acetat-Addukt detektiert werden.

Bei der Betrachtung von MS/MS-Spektren der isobaren Phospholipide Phosphatidylglycerol und Bis(monoacylglycero)phosphat im negativen ESI-Modus sind keine Unterschiede bei den m/z -Verhältnissen der Produktionen zu erkennen[2][3]. Kleine Unterschiede in den Intensitäten der Produktionen sind vorhanden, die jedoch bei gleichzeitiger Messung beider isobarer Phospholipide keine Hilfe bei der Identifikation leisten. Somit ist eine chromatographische Trennung der Konstitutionsisomere der beiden Phospholipidklassen mit übereinstimmenden Fettsäureresten für eine sichere Identifizierung der einzelnen Lipide unerlässlich.

Die Anwendbarkeit dieser Lipidprofilierung-Methode wird an Extrakten von Brustkrebszellkulturen demonstriert. Diese wurden vom Leibniz-Institut für Arbeitsforschung an der TU Dortmund zur Verfügung gestellt.

Neuer Aspekte

HILIC/ESI-MS/MS-Charakterisierung von Phospholipiden, insbesondere der Klasse der Bis(monoacylglycero)phosphate.

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Metabolomics Profiling using Atmospheric Pressure Gas Chromatography-MS

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Stichworte: Atmospheric pressure GC, APGC, metabolic fingerprinting, metabolomics, structural elucidation

Einleitung

Gas chromatography coupled with mass spectrometry is a well established analytical approach to metabolomics. The most widely used ionization technique is electron ionization (EI), which produces library searchable spectra dominated by fragments. The molecular ion in an EI spectrum is often of very low abundance or absent. The lack of molecular ion information, especially for derivatized compounds, can lead to incorrect identification, if using spectral matching alone. Atmospheric pressure GC (APGC) is a 'soft' chemical ionization technique that generates a spectrum conserving the molecular ion species with minimal fragmentation. Here we explore the use of APGC-MS in metabolomics applications.

Experimenteller Teil

Biological samples including plant, animal tissues and microorganisms were prepared by protein precipitation. The supernatant was evaporated to dryness and derivatized: Methoxymation to protect the carbonyl groups, followed by trimethylsilyl (TMS) derivatization. The GC was coupled to a quadrupole-time-of-flight(Q-ToF)-MS fitted with an atmospheric pressure chemical ionization chamber. Data was collected using positive mode in data-independent acquisition mode, alternating between low and elevated collision energy to acquire precursor and product ion information. Sample data generated with the low energy function (precursor ion spectrum) were searched against the custom APGC library created from reference compounds of known metabolites. To add confidence to compound identification, collision energy was ramped (20-40 eV) in the high energy function to generate maximum information from fragment ions.

Ergebnisse

APGC provided abundant molecular ions with minimal fragmentation at low collision energy. Due to the use of charge exchange chemical ionization the elevated collision energy data resulted in a spectrum similar to the traditional EI data. High energy spectra (fragment ion spectrum) were searched against both, custom and commercial mass spectrum libraries. The i-FIT confidence percentage obtained for each library hit based on accurate mass and isotope ratios was used as indication of the reliability of peak assignment. As structurally similar metabolites often co-elute, the concurrent acquisition of an intact molecular ion along with fragmentation data for sub-structural determination resulted particularly useful. In combination with accurate mass measurement, the molecular ion helps determine the limits of chemical composition which can subsequently be used along with the fragmentation data for more detailed and specific structural elucidation of both known and unknown metabolites. Such an APGC-MS approach was used for metabolic fingerprinting of various biological samples, including plants, animal tissues and microorganisms.

Neuer Aspekte

Atmospheric pressure gas chromatography-MS provides molecular ion information, which is typically missing when traditional vacuum source GC-MS is employed

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A novel high resolution MS/MS Human Metabolite Spectral Library enabling rapid and accurate metabolite identification in human metabolomics studies

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Stichworte: Metabolite library, QTOF, metabolomics, human

Einleitung

Human biofluid metabolomics is being increasingly used for the discovery of metabolite biomarkers of diseases. However, metabolite identification remains to be a major analytical challenge in metabolic profiling. Here, we describe a workflow for rapid and accurate metabolite identification based on the use of a high resolution MS/MS spectral library.

Experimenteller Teil

An MS/MS spectral library of 800 human endogenous metabolites from the Human Metabolome Database (HMDB) was created by running individual standards in a high resolution impact QTOF mass spectrometer (Bruker Daltonics). All spectra were manually curated and possible structures and/or chemical formula of the fragment ions were manually deduced with the assistance of the SmartFormula 3D software. Human urine after filtering was analyzed directly by LC-QTOF-MS and MS/MS.

Ergebnisse

We used ~800 human endogenous metabolites from HMDB to create a high resolution MS/MS spectral library using a QTOF-MS instrument. For each metabolite, at least 5 different collision energies were used to generate a set of standard MS/MS spectra, which provides flexibility for spectral match in a real sample analysis with different instrumental settings. We found that the high mass accuracy we defined as part of the Standard Operation Procedure (SOP) for adding spectra to the library (<2 ppm) was important for narrowing the match list and improving match specificity.

Preliminary work to apply this library for rapid identification of metabolites present in human urine was performed. Searching over 1500 MS/MS spectra from a urine sample measured by LC-QTOF-MS against the spectral library resulted in matches of many different metabolites to the standard metabolites. We are currently in the process of manually examining these matches in order to confirm their identifications. We will report the specificity and sensitivity of this approach for analyzing urine metabolome.

Neuer Aspekte

A high resolution MS/MS library of 800 human metabolites was created and applied for urine metabolomics.

Quantifizierung von TMAO und verwandter Metabolite im Plasma mit HILIC-MS/MS im Kontext ernährungsphysiologischer Studien

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Stichworte: TMAO, Ernährung, Quantifizierung, HILIC-MS/MS

Einleitung

Trimethylammonium-N-oxid (TMAO) ist aus zwei Gründen relevant für ernährungsphysiologische Studien. Zum einen wird es als quantitativer Marker für Fischkonsum diskutiert, da einige Fischarten hohe TMAO-Konzentrationen enthalten. Zum anderen entsteht TMAO aus Cholin und Carnitin, die ebenfalls mit der Nahrung aufgenommen werden und im Darm mikrobiell abgebaut werden. Hauptquelle für Cholin sind Phosphatidylcholone (Lecithin), z.B. aus Milch oder Ei, Carnitin stammt vor allem aus rotem Fleisch. TMAO ist zunehmend im Fokus klinischer Studien weil hohe Plasma-Konzentrationen mit einem erhöhten kardiovaskulären Risiko assoziiert sind (1,2), weshalb TMAO ein möglicher Marker sein könnte. Zudem könnte die TMAO-Bildung neue Erklärungsansätze für den Einfluss von Lebensmitteln (z.B. hoher Fleischverzehr) auf das Arteriosklerose-Risiko bieten. Für Phospholipide ist eine präventive Wirkung in der Diskussion (3).

Experimenteller Teil

Zur Beantwortung von Fragestellungen in diesem Kontext wurde eine Methode zur Bestimmung niedermolekularer alkylierter Aminoverbindungen etabliert. Die Methode umfasst neben TMAO die beiden Vorläufer Cholin und Carnitin sowie einige vom Glycin abgeleitete Metabolite (Betain, Dimethylglycin, Sarcosin).

Methode: UPLC-MS/MS im HILIC-Modus (Acquity H-Class + TQD, Waters)

Chromatographie: polare Amid-Säule 150x2.1mm (Acquity BEH Amide, Waters), inverser Gradient, Acetonitril + Ammoniumformiat-Puffer 50mM

MS-Bedingungen: ESI positiv, MRM 1-2 Übergänge je Analyt, deuterierte interne Standards für 6 Analyte

Probenvorbereitung: Proteinfällung und Verdünnung 1:10 (Acetonitril)

Kalibration und Kontrollen: matrixadaptiert (gespiktes Humanplasma); Standard-Addition zur Ermittlung der Konzentrationen im ungespikten Plasma

Ergebnisse

Für die Trennung bot sich die Hydrophilic Interaction Chromatography (HILIC) an, da kleine, polare Analyte auf gängigen RP-Säulen nur eine sehr geringe Retention haben. Die chromatographische Laufzeit beträgt nur 4.5 Minuten. Aufgrund der Konzentrationen im μ -molaren Bereich und der stark basischen, z.Tl. quarternären Aminogruppen ist eine Verdünnung bei gleichzeitiger Proteinfällung ausreichend für die Probenvorbereitung. Zentrifugierte Proben können direkt ohne Eindampfen injiziert werden.

Der Matrixeffekt ist gering und beträgt je nach Analyt -10% bis +20%. Wiederfindungen wurden mithilfe der deuterierten Analoga bestimmt, da kein analytfreies Plasma zur Verfügung stand, und lagen bei 81-93% (2 Level). Die Präzision (Serie, Tag-zu-Tag) ist gut und lag bei 2-8% (2-3 Level), mit Ausnahme von Sarcosin (10-15%). Der errechnete Bias (Richtigkeit) betrug -8% bis +10%. Die Bestimmungsgrenzen liegen je nach Analyt bei 0.4 bis 9.4 μ M, der lineare Bereich umfasst für die meisten Analyte mehr als 2 Größenordnungen. Die Stabilität in Plasma wurde ebenfalls untersucht, die maximalen Abweichungen betragen je nach Analyt und Lagerung zwischen -11% und +19%.

Die Methode wurde bereits in einigen Studien erfolgreich eingesetzt, so etwa in der Humanstudie KarMeN, in der der Einfluss von Ernährung und körperlicher Aktivität auf das Metabolom untersucht wurde. In zwei Interventionsstudien wurde der Einfluss von Milch-Phospholipiden auf physiologische Parameter untersucht, wobei hier ein möglicher präventiver Effekt im Vordergrund stand (3). In einer toxikologischen Risikostudie an Ratten wurden mögliche

Effekte einer Carnitin-Supplementierung untersucht (4). Beispielhaft werden Ergebnisse aus diesen Studien gezeigt. Die Methode ist einfach, schnell und robust, und damit gut geeignet für eine Routineanalytik in Diagnostik oder Forschung.

Neuer Aspekte

Schnelle und robuste Quantifizierungs-Methode für TMAO und verwandte Verbindungen, geeignet für Ernährungsstudien und klinische Studien

Referenzen

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An extraction strategy for systems biology

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Stichworte: Systems biology, multi 'omics', PPAR signaling

Einleitung

Recent achievements in mass spectrometric technology enabled the comprehensive characterization of biological systems, fostering a wide range of metabolism-related disorders. Typically, systems are described by investigating individual molecular components and thus generating molecular snapshots of biological relevance. The disadvantages of such approaches are that they do not account for global interconnectivity of the system [1], require high amount of sample and increased handling time, and can introduce additional experimental errors. Here we demonstrate how to overcome these limitations by extracting lipids, metabolites and proteins from one sample and therefore advancing to a systems biology approach. Integrating multiple 'omics' strategies along with the corresponding data, provides valuable information regarding how components of a complex network contribute to specific biological functions.

Experimenteller Teil

To accomplish our aim, we have used methyl-tert-butyl-ether (MTBE) as part of a biphasic extraction [2] that allows us to isolate lipids in the upper organic phase, metabolites in the lower polar phase and proteins in the pellet. Overall, three independent molecular classes could be extracted out of one sample and further analyzed as follows: the upper phase by shotgun lipidomics, the lower polar phase by an LC-SRM/MS approach while, the protein pellet was subjected to nano-LC-MS/MS and LC-SRM/MS. To corroborate our strategy we compared the established protocol with already published extraction procedures for metabolomics and proteomics.

Ergebnisse

Since no single 'omics' analysis can entirely decipher the complexity of a biological system, we introduce here a protocol that contributes to resolving this challenge by acquiring a more precise picture of the system through the detection of more than one molecular class. By applying this protocol to a stromal stem cell model we were capable to quantitatively analyze 360 lipids, 70 metabolites and 3327 proteins with an excellent reproducibility. The efficiency of the protocol was assessed by the high correlation coefficients (> 0.97) obtained from the comparison of the established extraction protocol with standard extraction protocols. To further demonstrate the robustness of the developed protocol we investigated the PPAR γ signaling, a deeply intertwined system at multi molecular layers. This is of special interest since PPAR γ is a metabolic master regulator [3] and plays a key role in adipogenesis and inflammation. The analysis elucidated major regulatory effects at the protein, metabolite and lipid level and revealed a tight control on LPA, a lipid intermediate and possible PPAR γ antagonist. In spite of this being a challenging task, the results have demonstrated that the multi 'omics' approach is a powerful tool for understanding the functional principles and dynamics of biological systems.

Neuer Aspekte

A novel extraction strategy towards integrative multi 'omics'

Referenzen

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Characterization of a Cisplatin-DNA-Antibody and its Corresponding Antibody-Antigen-Complexes by high-mass Q-ToF MS and MS/MS

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Stichworte: antibody, cisplatin, oligonucleotides, antibody-antigen-complex

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, mass spectrometry, especially in combination with soft ionization techniques like electrospray ionization, has been employed 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 by mass spectrometry has been investigated and first results are presented here.

Experimenteller Teil

We analyzed the rat antibody R-C18 specific for cisplatin-DNA adducts that was developed by Thomale *et al.* [1] The detection of this antibody in its native state was successfully accomplished by using a nano-ESI High-Mass Q-ToF mass spectrometer. [2] To gain further insight into the structure the antibody was treated with various enzymes like PNGase F and Endo S for deglycosylation.

With regards to analyses of antibody-antigen-complexes, we used 30 and 50 base pair synthetic oligonucleotides to produce the specific platinated antigens. The sequences of these oligomers were chosen that only one of the single strands contains two adjacent guanines to yield the supposed binding pattern G*G*-cisPt(NH₃)₂.

Ergebnisse

We were able to identify up to 8 fine structures for the native antibody, probably referring to post translational modifications. The mass of the unmodified antibody was determined to 146.0 kDa. CID-based experiments resulted in fragments and yielded in a partial amino acid sequence of the antibody.

After incubation of the DNA with cisplatin, we were able to detect the unmodified and the singly platinated single and double stranded oligonucleotides.

The whole antibody-antigen-complex containing these synthetic platinated oligomers could be successfully analyzed in positive ionization mode. Surprisingly, the antibody does not only bind platinated double stranded, but also the corresponding platinated single stranded DNAs. That means that the binding pattern of this special antibody might either not be restricted to cisplatin modified DNA double strands, or the until now assumed binding pattern of G*G* **intrastrand** cisplatin adducts is not correct.

Dissociation of the complex in MS/MS mode was successfully performed by applying a collision energy of 150 eV. As a result, we could detect signals for the free intact antibody and the above mentioned specific antibody fragments, while we did not detect any specific signals of the DNA part. This suggests a high stability of the complex and also of the antibody itself in contrast to the oligomers, which easily undergo fragmentation.

In conclusion, we have shown the successful detection and specific fragmentation of the antibody and its antibody-antigen-complexes under native conditions *via* nano-ESI High-Mass Q-ToF-MS and MS/MS.

Neuer Aspekte

In the future we are aiming to determine the precise epitope-binding structure of DNA antigens and their diagnostically relevant antibodies.

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Mass-to-charge-dependent suppression of trapped ions

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Stichworte: Ion trapping, charge densities, nano-ESI, imaging

Einleitung

A m/z -specific ion discrimination was observed for high charge densities, in a quadrupolar ion trap of an orbital trapping mass spectrometer. Above a certain trapped-charge density only a narrow m/z window was found to be trapped and subsequently analyzed. A high ion density in the trap led to a complete suppression of the low m/z range. The observed suppression effect was depending on the injection time as well as on the ion transmission into the trap, affected by the position of the nano-ESI needle in front of the inlet capillary. Both, the injection time and the position of the needle directly correlate with the number of ions entering the trap.

Experimenteller Teil

Static nano-ESI was implemented in an atmospheric-pressure scanning microprobe matrix-assisted laser desorption/ionization imaging ion source (AP-SMALDI10, TransMIT GmbH, Giessen, Germany). The nano-ESI needle was mounted on the three-dimensional moving stage for imaging the nano-ESI plume. The ion source was coupled to an orbital trapping mass spectrometer (Q Exactive, Thermo Fisher Scientific GmbH, Bremen, Germany). The automatic gain control of the instrument was switched off in order to obtain position-correlated absolute numbers of ions for each image pixel. Two-dimensional images of the ion distribution were generated with the imaging software package MIRION.

Ergebnisse

The observed changes of relative intensities of the various trapped ion species were found to directly correlate with the total number of ions in the trap. Increasing the injection time led to a mass-to-charge-specific ion suppression. Ion loss is an expected behavior, resulting from an overfilling of the ion trap and coulomb repulsion effects. It was unexpected, however, that a sharp m/z cut-off, accompanied by a complete loss of ion signals, was obtained for ions below a certain m/z value under overfilling conditions. The m/z -cutoff value was found to correlate with the total charge in the trap. As a result, reducing the total ion current that enters the trap, by measuring low-density areas of the nano-ESI plume, led to a shift of the m/z cutoff to lower m/z values.

Ion suppression images of the three-dimensional nano-ESI plume will be presented to visually describe the overfilling behavior of the quadrupolar C-trap of an orbital trapping mass spectrometer. It will also be presented that controlling the ion density in the C-trap by using the automatic gain control (AGC) method results in a perfect description of the true and homogeneous ion density distribution of the nano-ESI plume.

Acknowledgement

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Neuer Aspekte

Mass-to-charge-dependent suppression of trapped ions

Analysis of crude oil mixtures by Atmospheric pressure photoionization Fourier Transform Mass Spectrometry (APPI-FTMS)

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Stichworte: APPI, FTMS, crude oil, PCA

Einleitung

FTMS is a very powerful technique to study oil on the molecular level and a well-received method in petroleomics. Routinely a resolving power of > 800.000 with ppb mass accuracies can be achieved by FTMS. Since the chemical composition of oil can be extracted from ultrahigh resolution mass spectra, the mass spectrum is very specific, comparable to a fingerprint for the analysed oil. Oils can not only be classified by statistical methods by FTMS, also mixtures of oils can be identified and quantified based on the chemical composition.

However, even with this technique not all compounds in oils can be detected due to ion suppression, therefore various ionization modes are needed. Here we present data with APPI.

Experimenteller Teil

Two crude oils were mixed in different ratios and analysed by Atmospheric Pressure Photoionization (APPI) FT-ICR mass spectrometry on the molecular level. The relative abundances of the compound classes of the oil mixtures were calculated and correlation plots were generated. Repetitive measurements were performed to check the reproducibility of the results and to calculate errors based a) sample preparation and b) mass detection. The correlation was based on the calculated compound classes detected in the APPI mass spectra.

Ergebnisse

The correlation of the detected compound classes based on the mixing ratios have shown linear trends with very good regression factors. The mixing ratios of crude oils were calculated based on the detected compound classes in good agreement with the known ratio. It has to be taken into account that a sample preparation error exists beside the error of reproducibility of the mass measurement using repetitive measurements. The reproducibility of the abundant compound classes have shown a good relative reproducibility based on compound class calculation. The spectra of the mixed crude oils were also analysed by the statistical method Principle component analysis (PCA). The samples with different mixing ratios were well separated in the scoring plot of PCA. Mixing ratios of two crude oils even with small differences can be measured by APPI FT-ICR mass spectrometry. The calculation is based on the accurate detection of compound classes. Due to the very high complexity of crude oils and crude oil mixtures especially in APPI mass spectra, ultrahigh mass resolution achieved by FT-ICR mass spectrometry is needed to detect all compound classes accurately.

Neuer Aspekte

APPI FTMS is used to identify the relative ratios in crude oil mixtures.

Characterisation of SIL universal antibody and SIL human proteins for quantitative mass spectrometry

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Stichworte: SILUMab, stable isotope labeled antibody, stable isotope labeled proteins

Einleitung

For accurate protein quantification in the clinical setting, a prerequisite is that experimental variations in protein extraction, fractionation, enrichment, proteolysis and analysis need to be minimized. The ideal way of optimizing the reproducibility is to add a full length stable isotope labeled (SIL) recombinant protein, that is equivalent to the native target protein to the sample, at the initial stage of sample preparation. To this end, we have developed a stable-isotope-labeled universal monoclonal antibody (SILUMab) standard which contains conserved regions of IgG1 heavy chain and lambda light chain as well as SIL human proteins. A representative SIL human protein, APOA1, and SILUMab are characterized to show that they are similar to the native protein and can serve as excellent internal standards in quantitative MS workflows.

Experimenteller Teil

SILUMab was expressed in CHO cells and human SIL proteins were produced in HEK293 cells. Both cell lines were grown in serum-free $^{13}\text{C}_6$ $^{15}\text{N}_4$ Arg/ $^{13}\text{C}_6$ $^{15}\text{N}_2$ Lys enriched media. The SIL proteins and SILUMab were analyzed at the intact protein level and after trypsin digestion. The sequence coverage and isotope incorporation were determined. Intact mass analysis (SEC-MS) was used to confirm the sequence of the protein and level of glycosylation. For quantification, SILUMab was spiked into a complex biological sample with different amounts of native mAb, and a calibration curve was generated by LC-MS/MS MRM.

Ergebnisse

UV trace and deconvoluted mass spectra resulting from intact mass analysis of the SILUMab standard

Incorporation of SIL for a selected peptide of SILUMab

Extracted ion chromatogram (XIC) of six SILUMab peptides

Calibration curves of three representative peptides obtained by spiking 20 $\mu\text{g}/\text{mL}$ of SILUMab as an IS into canine plasma containing 1-1000 $\mu\text{g}/\text{mL}$ target antibody

Incorporation of $^{13}\text{C}_6$ $^{15}\text{N}_2$ labeled Lys in DYVSQFEGSALGK from the Sigma R&D batch and a commercially sourced “heavy” Apo-A1

Binding kinetics for SIL-APOA1 and native APOA1 isolated from human serum with a goat polyclonal Ab

Neuer Aspekte

Stable isotope labeled full-length monoclonal antibody and SIL human proteins have been produced with high purity and isotopic incorporation >98%.

Evaluation of native cross-links in elastin

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Stichworte: native elastin, cross-linking, desmosine, lysinonorleucine, diagnostic ions

Einleitung

Elastin is a major protein of the extracellular matrix in gnathostomata and provides resilience and elasticity to numerous tissues including lung, skin and large blood vessels [1]. At the beginning of life, it is secreted as the soluble precursor protein tropoelastin followed by extensive cross-linking through reactive allysine residues yielding a highly hydrophobic biopolymer. So far the exact pattern of cross-linking remains unknown. Recent development allows the direct detection of peptides carrying the tetrafunctional cross-linking amino acids desmosine (DES) and isodesmosine (IDES) in an LC-MS based workflow [2]. This study aims to determine exact domains, which are connected to each other via bi- and tetrafunctional cross-linking amino acids. This provides indirect insight into the structural organization of mature elastin.

Experimenteller Teil

Isolation of bovine aortic elastin from one adult animal was carried out as described previously [3]. Purified elastin was digested by adding pancreatic elastase. Enzymatic digests were subsequently analyzed on a nanoHPLC - Orbitrap Fusion Tribrid mass spectrometer. For enriching cross-linked peptides, fractions were further collected applying a linear gradient on a reversed phase Agilent 1100 system. Relevant fractions were measured on an Orbitrap Velos Pro mass spectrometer. For detection of bi- and tetrafunctionally cross-linked peptides, processed data files were exported in MGF-format and analyzed with StavroX software [4] and PolyLinx software [5], respectively.

Ergebnisse

A total number of 310 linear elastin peptides were identified after digestion with PE, which corresponds to a sequence coverage of 78 %. It is interesting to note that many of the assumed cross-linking domains were found to be non-cross-linked as well. Search algorithms specifically designed for the detection of bi- and tetrafunctional amino acids allowed the detection of 41 bifunctionally cross-linked peptides. They were found to be inter- and intramolecularly cross-linked via lysinonorleucine (LNL) and allysine aldol (AA). It became apparent that some domains were cross-linked via LNL, whereas other domains were exclusively connected via AA. Since the digestion of elastin with site specific proteases, e.g. trypsin, is not possible, sequencing is more complicated. Thus the results were confirmed by manual spectrum interpretation. Interestingly, DES/IDES were found to connect two or three different peptide chains, although it was stated that they only may link two chains together.

Moreover in an energy resolved study, it was found that the cross-linking amino acid LNL, one of the most abundant cross-linking amino acids in elastin, does not yield any diagnostic ions in fragment spectra but only fragment ions, which may also be produced upon fragmentation of lysine. This further impedes identification of those peptides. Mass spectrometric identification of peptides putatively cross-linked by LNL, therefore, requires high mass accuracy in terms of both MS and MS/MS mode.

Neuer Aspekte

Modern mass spectrometric methods allow insight into exact cross-linking sites in native elastin

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Influence of glycoforms on the tryptic digestion efficiency of immunoglobulin G based biopharmaceuticals

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Stichworte: Bottom-up proteomics, Glycoform profiling, Biopharmaceuticals, nanoLC-MS, Tryptic digestion biases

Einleitung

Bottom-up proteomics is employed frequently in biological and clinical research. Nowadays, it is becoming increasingly popular for the analysis of biopharmaceuticals in discovery, development and batch control. The digestion of the target protein by proteases, generally by trypsin, into more easy to analyse peptides is a key step in bottom-up proteomics. Thus, a robust analysis needs to incorporate an efficient and unbiased tryptic digestion step. It has been observed previously that incomplete tryptic digestion can introduce biases into glycoform profiles[1].

Therefore, we investigated the preferential tryptic digestion of glycoforms of two biopharmaceutical formats: 1. an immunoglobulin G1(IgG1) monoclonal antibody(mAb) and 2. Flebogamma, an intravenous IgG(IVIG) from healthy donor pools. Via denaturation experiments, we collected evidence for the 3D-structure dependence of the biases.

Experimenteller Teil

The tryptic digestion of the target protein into glycopeptides was followed over time. The digestion of protein with an intact 3D-structure was compared to that of protein denatured by various methods. These included denaturation, reduction and alkylation as a gold standard as well as a novel, save and efficient protocol for IgG-denaturation. Glycopeptides from the conserved glycosylation site in the F_C-part of IgG were detected by a nanoLC-ESI-q-TOF-MS method[2]. In addition, the glycoproteins and glycopeptides of partially digested samples were separated by gel electrophoresis and the residual glycoproteins were reanalysed after complete in-gel tryptic digestion. By relative quantitation of the glycopeptides and estimation of their absolute abundances, we could visualize a preference of trypsin for the digestion of certain glycoforms.

Ergebnisse

In general, we proved that the glycoforms of the IgG1 mAb containing high mannose and hybrid type glycans in the Fc portion were preferentially digested into the glycopeptides compared to those glycoforms containing complex type glycans. The biases observed in IVIG were smaller, because this formulation did not contain high mannose or hybrid glycans. The main bias in IVIG was created by preferential digestion of bisected species. Interestingly, the α 2-3 linked sialic acid containing species in the IgG1 mAb were amongst the first to be digested while the α 2-6 linked sialic acid containing species in IVIG were not preferred. Comparing the tryptic digestions of non-denatured and denatured protein revealed that the biases are much less pronounced in denatured samples. Additional experiments proved that this effect is independent of the increased digestion efficiency. A significant part of the digestion biases must therefore be caused by the differences in antibody 3D-structure induced by the different glycoforms.

Our simple, novel pre-treatment method — acid incubation and evaporation — exhibited a denaturation efficiency comparable to the gold standard (see *Methods*) for IgG while significantly reducing the hands-on time and the toxicity of the reagents involved. It additionally offers the advantage of performing the tryptic digestion under optimal buffer conditions.

In conclusion, proper denaturation and digestion completeness are key to avoiding biases in bottom-up proteomics based glycoform profiling. However, the digestion biases have implications beyond glycoproteomics. For example, relative protein quantitation (even with isotopically labelled standards) in two samples could be biased by differences in glycoform profiles, if complete tryptic digestion is not achieved.

Neuer Aspekte

Trypsin preferentially digests certain glycoforms of IgG. This preference is extensively induced by the specific 3D structure of the glycoform.

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Seeking marker peptides for tracking age-related changes in elastic tissues

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Stichworte: elastin, cleavage susceptibility, aging, label free quantification

Einleitung

Elastic fibers are extracellular assemblies that provide elasticity and resilience to many tissues such as aorta and skin [1]. Reduced fiber strength and disorganization as a consequence of aging is an important cause of morbidity and mortality [2,3]. Elastin, as the core component of elastic fibers, is an insoluble and extremely durable protein that undergoes very little turnover. However, elastin isolated from skin samples of differently aged individuals shows striking differences in ultrastructure and cleavage susceptibility [4]. To understand the processes that lead to these structural changes, it is necessary to gain insight into cleavages that take place in vivo. In the present study, label-free quantification (LFQ) was applied to identify marker peptides that allow monitoring the aging of elastin.

Experimenteller Teil

Elastin was isolated from non-sun-exposed skin samples of differently aged individuals using a recently developed method [4]. Purified elastin samples were dried, weighed and digested using leukocyte (HLE) or pancreatic elastase (PE). The digests were analyzed under identical experimental conditions by LC-MS using an Ultimate nanoHPLC system (Thermo Fisher, Germany) coupled to a Q-TOF-2 mass spectrometer (Waters, UK). The raw files were analyzed using the LFQ module of the proteomics software PEAKS 7 (Bioinformatics Solutions Inc., Canada).

Ergebnisse

Elastin isolated from skin samples of all ages was readily digested by PE, as proven by LC-MS/MS analysis. Interestingly, HLE was only able to degrade elastin derived from elderly people even though it is considered to be an elastase. Digestion of intact fibers was not possible as indicated by the presence of a remaining yellowish elastin pellet in the reaction tube at the end of the incubation time. Moreover, no peptides were identified in the supernatant. Increasing the concentration of HLE and the incubation time of the digestion, respectively, did not change these findings. This suggests that the presence of impairments in the elastin fibers in matured tissues enhances the susceptibility of elastin to HLE.

PE digests of elastin derived from differently aged people were further used to investigate whether differences may exist in the relative abundance of certain peptides. Preliminary results gained by LFQ analysis show age-related differences in the peptide patterns. Such differences include the complete absence of certain peptide species as well as distinct quantitative variations and are most likely a result of cleavages introduced by extracellular proteases throughout life. Therefore, such peptides can serve as marker molecules to follow the age-related breakdown of elastin.

Neuer Aspekte

LFQ enables the identification of marker peptides that provide insights into the aging of elastin

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Protein structure prediction guided by crosslinking constraints – a systematic evaluation of the impact of the crosslinking spacer length

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Stichworte: crosslinking, 3D-MS, mass spectrometry, computational biology

Einleitung

Driven by the mass spectrometry (MS) instrument development chemical crosslinking combined with MS becomes a powerful approach to obtain structural information about proteins and protein complexes [1]. Even though the number and resolution of distance restraints is typically insufficient to unambiguously determine a protein's structure, it has been shown frequently that distance restraints obtained by chemical crosslinking can be used to refine protein models, restrict the protein fold space, guide protein/protein docking, and help distinguishing accurate from inaccurate structural models [2, 3]. However, the questions (i) what is the most useful crosslinker length to study a given protein, (ii) which model accuracy can be expected, and (iii) to which extent do crosslinks improve model selection are not yet fully evaluated.

Experimenteller Teil

In this study the number of crosslinks as well as the discriminative power of the derived distance restraints was systematically analyzed *in silico* on a set of 2055 non-redundant protein folds considering homobifunctional amine reactive crosslinkers between 1 and 60 Å [4]. Depending on the size of the proteins an optimal crosslinker length was predicted taking the total number of helpful crosslinks – i.e. crosslinks that significantly reduce search space – as well as the ratio of helpful vs possible crosslinks into account. Two protein structures had been *de novo* modeled with experimental restraints and our proposed optimal crosslinks and the enrichment as RMSD of both data sets compared.

Ergebnisse

We found that in average the data obtained by the optimal crosslinker improved the *de novo* modelling accuracy from 6.6 Å to 5.6 Å whereas utilization of the data of all five crosslinkers improved the modelling accuracy to 5.2 Å. Furthermore, restraints from crosslinking allowed for the selection of more native-like models. Whereas only 11% of the most accurate models could be selected with the default BCL::Fold scoring function, the value almost doubled to 21% when crosslinking data with the optimal crosslinker length were included. By including data obtained from all crosslinker lengths this value could be further improved to 24%. Finally, to study the effects of experimentally derived chemical cross-links on *de-novo* folding Cytochrome C and Oxymyoglobin were studied using published crosslinking results ranging from 7.7 to 11.4 Å. Even though just a fraction of the theoretical and experimental crosslinks coincided model accuracy and discriminative power the scoring function improved significantly. In conclusion, for >70% of the low molecular weight proteins (<25 kDa) homobifunctional amine reactive cross-linkers with a length of 13 Å performed best. Their application resulted in a moderate improvement of the modelling accuracy and a significantly improved discrimination of incorrect models.

Neuer Aspekte

Presentation of a molecular weight based optimal crosslinker spacer length and their impact on *de novo* modeling.

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A data independent strategy for a multi-omic approach to investigate obesity treatment within a mouse model

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Stichworte: Proteomics, Lipidomics, Multi-Omis, LC-MS, DIA

Einleitung

Obesity is one of the risk-factors associated with metabolic syndrome, causing excess body fat to be accumulated to the extent that it adversely affects health and life expectancy. It has previously been demonstrated that glycosphingolipids play a crucial part in such metabolic syndromes. The manipulation of the function of glycosphingolipids with small molecule drug compounds within mouse models has shown that symptoms can be negated. Knowledge relating to the proteome, metabolome and lipidome during development is still to be fully explored. The work presented here is to provide a multi-omic analysis of protein and lipid liver extracts from control and obese mouse models undergoing treatment to prevent or revert obesity.

Experimenteller Teil

Lipid and protein extracts were generated from liver tissue originating from 3 control and 3 obese mice models. Protein extracts were proteolysed with trypsin and the resulting peptides separated over a 90 minute linear reversed-phase nanoscale LC gradient, whilst the extracted lipids were separated over a 20 minute reversed-phase LC gradient. Data were acquired using a data independent acquisition approach, whereby the collision energy was switched between a low and elevated energy state during alternate scans. Proteomic acquisitions also utilized ion mobility in the acquisition scheme. The acquired data were processed and searched using Progenesis QI and dedicated protein sequence and lipid compound databases, providing normalized label-free quantitation results for both datasets.

Ergebnisse

Proteomic samples were based on 100 ng loadings and analyzed as triplicate technical replicates in a randomized order. Processing and searching the data using Progenesis QI resulted in over 1250 curated proteins being identified, across all technical replicates and biological conditions. Over 300 proteins exhibit a fold change greater than 2 with significant analysis of variance. Unique peptides were used for relative label-free quantitation with median abundance normalization performed across all samples. Lipid extracts were prepared using 500 µL IPA/water (50:50), of which 2 µL were injected on-column and analyzed in triplicate. Samples were also acquired in a random order with a QC comprised of all samples in equal amounts and injected every 5 injections. Lipid data analysis was conducted in a similar manner with Progenesis QI used for processing and searching. Interrogation of the data revealed over 500 potential identifications for combined positive and negative ion acquisitions with mass errors less than 2 ppm. Compound searches provided a range of lipid classes including free fatty acids, ceramides, triglycerides, sphingomyelins and glycosphingolipids. Identification scores are based on mass accuracy, isotopic fit and fragmentation. Unsupervised multivariate analyses showed clear distinction between obese and control groups in both proteomic and lipidomic experiments. Pathway analysis tools were used to review the complimentary datasets and hence provide an understanding of the underlying biology of differentially expressed proteins and lipids.

Neuer Aspekte

A multi-omic, biochemical and network investigation for the study of obesity using a mouse model.

MS optimization for identification and quantification of TMT labeled peptides

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Stichworte: Proteomics, TMT Labeling

Einleitung

In quantitative proteomics, the aim is to quantify as many peptides/proteins as possible in a given cell type or cell line. The stable isotope labeling of peptides using Tandem Mass Tag (TMT) reagents facilitates the quantification of peptides. After protein digestion, the peptides in each sample are labeled with a different TMT reagent that has the same nominal mass and chemical structure. This means that the labeled peptides will co-elute during the LC separation and will be co-isolated during the MS scan. During MS2 scan, simultaneously to the generation of peptide fragment ions which are used for peptide identification, each isobaric tag fragments produces a unique reporter ion mass that is used for peptide quantification by comparing their relative intensities.

Experimenteller Teil

We tried to optimize the MS parameters for the identification and quantification of TMT labeled peptides. Mouse macrophages were activated by Salmonella and treated with the toxic compound benzo[a]pyrene at various concentrations, leading to 6 different samples. Proteins were extracted and digested with Trypsin. The peptides of each 6 samples were labeled with a different TMT tag and were then combined into 1 single sample. This sample was then analysed by LC-MS using an Orbitrap-Fusion MS with different parameters:

(i) HCD activation with stepped collision energy (ii) a dual method using LIT-CID for peptide identification and HCD at high collision energy for TMT quantification and (iii) a combination of HCD at low (for identification) and high (for quantification) collision energy.

Ergebnisse

The different methods showed significant difference in the number of peptides identified and quantified. It is key to find a right balance for collision energy to ensure good fragmentation of peptides, but setup too high and the fragments will be too small for identification purpose and too low it would lead to too few fragments for identification and also for quantification using the reporter ions. When comparing HCD with CID it was clear that better accuracy and variability were obtained using HCD.

The best method allowed the quantification of more than almost 5000 peptides which led to important information on macrophages treated by BaP with and without additional activation by Salmonella.

Neuer Aspekte

TMT labeling of peptides to improve peptide/protein quantification of complex cell lysate sample

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Complex Regional Pain Syndrome: Targeted Resolvin and Neuropeptide Analysis

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Stichworte: CRPS, neuropeptides, resolvines, skin, LC-MS/MS

Einleitung

Complex Regional Pain Syndrome (CRPS) is a severe and often disabling syndrome, which develops after trauma (~5% of all cases); most often after distal radius fractures.^[1] CRPS is characterized by a variety of clinical features including spontaneous pain and hyperalgesia. Increased neuropeptide release from peripheral nociceptors has been suggested as a possible pathophysiological mechanism triggering the symptoms.^[2] Resolvins are endogenous anti-inflammatory lipid mediators generated during the resolution phase of acute inflammation. Emerging evidence also points to a potent anti-hyperalgesic role of resolvins of inflammatory pain.^[3] Here, LC-MS/MS is used to analyze marker substances in patient biopsies and possibly serum. Resolvins D1/D2 and neuropeptides substance P, bradykinin (BK), vasoactive intestinal peptide, endothelin I and calcitonin gene-related peptide (CGRP) were of interest.

Experimenteller Teil

Resolvin extraction was performed according to an optimized protocol of Giera et al. (2012)^[4] with the goal of allowing subsequent peptide extraction (using 0.1% formic acid, 5% acetonitrile, water) from the same biopsy. Peptides were purified with solid-phase extraction (ZipTip Omix C18, Agilent), because residual biopsy material presented a clogging problem in the LC. The complex analyte composition in particular endangered the nanoLC set up so that a capLC (Poroshell 120 EC-C18, Agilent HP1100) coupling to ion trap Esquire3000 (Bruker Daltonics) was used. For resolvins, the sodiated ion was targeted by automatic MS/MS. In case of the neuropeptides the extract was tryptically digested in order to generate peptides of suitable size for distinct fragment ion formation and nonambiguous identification.

Ergebnisse

Initially, breast tissue containing commercially obtained standards was used to develop the extraction and analysis procedure. In the process, three of the neuropeptides were eliminated from the program, because they could not be detected reliably. Substance P showed fast (5 min) adsorption to the vessel wall (glass). Endothelin I and vasoactive intestinal peptide did not respond well to enzymatic treatment, partially due to disulfide bridge formation. Therefore, the work focused on BK and CGRP peptide 25-35, although, in turn, BK showed a tendency to stick to the chromatographic material. β -nerve growth factor (β -NGF) was added to the project, because it has been found to sensitize pain processing.^[5] β -NGF peptide 1-9 was targeted. Extraction procedures generated two one-shot samples for autoLC-MS/MS (fatty acid measurement with adapted gradient and peptide analysis). Although the instrumentation is capable of f/pmol detection limits in both cases, losses during work-up are considerable. Sensitivity limits in the pmol-range are, however, not satisfactory. An additional filtering step prepares samples for nanoUPLC-Q-TOF MS.

Neuer Aspekte

Target analysis of inflammatory marker substances

Referenzen

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CoFGE mapping of flour proteins for protein aeroallergen database

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Stichworte: Comparative fluorescence gel electrophoresis (CoFGE), protein reference grid, protein aeroallergens, 2D-PAGE database

Einleitung

Proteins inhaled with dust particles may cause allergic reactions in sensitive persons. They can also be an occupational hazard as is the case, e.g. in baker's asthma. 2D-PAGE mapping of aeroallergens has been recognized as valuable tool for diagnostic evaluation of workplaces [1]. However, this idea was hampered so far by the method-inherent problem of gel-to-gel variability. With the advent of CoFGE [2], [3], it became possible to reproducibly assign protein spot coordinates on 2D-gels so that their storage in a corresponding database became feasible again. We are developing such repository for flour proteins.

Experimenteller Teil

Using two related fluorescent dyes (G-Dye200 and G-Dye300, NH DyeAGNOSTICS), flour proteome (wheat, buckwheat, rye) were separated on HPE FlatTop Tower (precast 2D HPE™ large gel, Serva) accompanied by a marker grid of 140 nodes. Sample grids were individually mapped to an ideal theoretical grid using Delta 2D (Decodon). The resulting match vectors were applied to the sample proteome. Subsequently, fluorescently labelled gels were scanned using Typhoon 9400. Spots were picked with Ettan Dalt Spot Picker (Amersham Biosciences). The picked spots were digested with trypsin and analyzed with C18 nanoUPLC/nanoESI-Q-TOF-MS.

Ergebnisse

Horizontal CoFGE is emerging as a robust tool for proteome mapping. We have archived three different flour proteomes so far as a reference database for the analysis of airborne protein allergens (spot coordinates and identified proteins). The Repository of Gel-Separated Proteins (ReGeSeP) is continuously developed as a web-based database with search and match tools to allow comparison of field samples to the reference pool. In addition to the storage of 2D-PAGE image and MS data, it will also host the results of 1D-PAGE proteome separation experiments.

Neuer Aspekte

Repository of Gel-Separated Proteins (ReGeSeP)

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Metal labelling for quantification of post translational sugar modifications of proteins

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Stichworte: Metal labeling, Glycoprotein, Quantification

Einleitung

Quantitative analysis has become more important for understanding the dynamics of the proteome. Metal Coded Affinity Tagging (MeCAT) uses chelate complexes of lanthanides for relative and absolute quantification with elemental mass spectrometry together with sequence elucidation of peptides and proteins by molecular tandem mass spectrometry. The aim of this present work is to extend MeCAT for quantification of post translational modified proteins, i.e. sugar modifications.

Experimenteller Teil

A MeCAT reagent containing hydrazinamide reactive group was used for quantitative labeling of sugars harboring a lanthanide ion for quantification. This reagent was coupled to post translational sugar modifications of proteins, i.e. α 1-Acid Glycoprotein (*human*), after treatment with β -N-Acetylglucosaminidase. The labeled protein was separated from buffer and excess of reagent with SDS-PAGE. After in-gel proteolysis labeled peptides could be detected using HPLC/ESI-MS and identified with -MS/MS. In addition, ICP-MS of the labeled proteins was used for quantitative analyses after mineralization of the gel protein bands.

Ergebnisse

A linker with an hydrazinamide reactive group can be attached for labeling using 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid . All intermediates were examined using ESI-MS/MS. Qualitative data can be obtained by relative quantification using the cysteine tag MeCAT-IA with a different lanthanide and HPLC/ESI-MS. Absolute quantification of sugar modification sides is done by ICP-MS and external calibration using standard salt solutions.

Neuer Aspekte

Quantitative and qualitative analysis of post translational sugar modifications of proteins using lanthanide containing metal labels.

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Improved glycopeptide analysis using acetonitrile enriched sheath gas and oxonium ion dependent ETD

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Stichworte: Glycosylation, ETD, ion trap, oxonium ions, glycan database

Einleitung

Mass spectrometric analysis of protein glycosylation is a major topic. The analysis of glycopeptides in complex biological samples is due to their low ionization efficiency and high heterogeneity challenging. This study uses solvent-enriched sheath gas to boost glycopeptide signal intensities for improved detection. Site-specific glycosylation profiling is based on the most efficient combination of CID and fragment triggered ETD.

Experimenteller Teil

A tryptic digest of Universal Proteomics Standard (UPS1-Sigma) was separated on an UltiMate™ 3000 nanoRSLC system. A quadrupole ion trap MS (amaZon speed ETD, Bruker Daltonics) equipped with a CaptiveSpray nanoBooster source was used for fragment triggered ETD experiments. Acetonitrile-enriched nitrogen was used as sheath gas to enhance glycopeptide intensities and increase charge states. Data processing was performed using ProteinScape 3.1 software (glycopeptide characterization and identification).

Ergebnisse

The main challenges when analyzing glycopeptides in complex mixtures are the low concentrations combined with their low ionization efficiency compared to non-glycosylated peptides. A significant improvement of detection sensitivity can be achieved by acetonitrile-enriched sheath gas.

Low energy CID was used for both, the identification of non-glycosylated peptides and the characterization of the glycan moiety of glycopeptides. Mascot was used for protein identifications, and the glycopeptide CID spectra were searched against CarbBank using GlycoQuest in order to identify the glycan moiety.

As ETD is mandatory for sequencing of the glycopeptide backbone, we used an advanced acquisition strategy in which the ETD acquisition is only triggered if oxonium ions have been observed in the former CID spectrum ("Fragment Triggered ETD"). This acquisition method allows for an efficient analysis of glycopeptides in complex mixtures. Moreover, ETD spectra quality is significantly improved by the acetonitrile-enriched sheath gas, as it does not only increase the signal intensity but also the charge state of glycopeptides.

Neuer Aspekte

The described approach generates new opportunities for glycosylation site profiling without laborious sample preparation

Comparison of peptide separation techniques using strong cation exchange materials

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Stichworte: PeptideSeparation, Strong Cation Exchange, Proteomics

Einleitung

In mass spectrometry-based proteomics the complexity of digested cell lysate is generally too high for a mass spectrometric analysis. Different methods reducing this complexity by peptide separation have been introduced successfully including 2-dimensional liquid chromatography (2D-LC). In the first dimension strong cation exchange (SCX) is often applied. SCX separation has several advantages compared to other separation techniques: efficient separation, orthogonality to reversed phase, high reproducibility and high loading capacity. The instrumental requirements are nonetheless quite high. The work presented here aims at examining the separation quality of some commercially available and self-made spin columns. The potential of SCX spin columns as a separation technique is compared with conventional SCX chromatography.

Experimenteller Teil

Materials: Acetonitril (ACN), KCl, Formic Acid (FA), Bovine Serum Albumin (BSA), Trypsin (Sigma Aldrich). SCX spin column (Mini), C18 spin column (Pierce, Thermo Fisher Scientific), POLYSULFOETHYL A column and bulk material (100x2.1 mm; 5µm; 200 Å; PolyLC).

Standard protocol: Spin columns. Equilibrating with solution A (30% CAN, 0.05% FA), loading sample (BSA digest, 33 µg), eluting with solvent A + [0.025, 0.05, ..., 0.8] M KCl. Centrifugation: 1min, 2000 x g. Desalting sample with C18 spin columns.

LC. Buffer: Solvent A (30% CAN, 0.05% FA); Solvent B (Solvent A + 0.5 M NaCl). Gradient: 0-80% in 30 min, 80-100% in 2 min, 100% for 5min. One-minute fractions were collected.

Ergebnisse

There are different ways to assess the quality of separation. First, the separation quality of commercially available spin columns was investigated by correlating the salt concentration of the buffer to the average charge of the detected peptides in the single fractions. The peptide's charge was calculated by counting the number of histidine, arginine and lysine residues + 1, for the N-terminal amino group. Conventional SCX-chromatography results in a well-defined separation of peptides of different charge states for low-complex samples and also yields at a low overlap of charge states even for more complex samples, whereas an elution from spin columns only leads to a gradual elution of peptides with the same charge state by stepwise increasing salt concentration. While over 75 % of the peptides elute in ≤ 3 fractions by using hp chromatography, the results for spin columns are inferior and most of the peptides elute in more than four fractions. The higher presence of carryover for spin columns could be caused by its different ion-exchange material. Sulfonic acid groups are bound to a membrane in SCX columns, while they are bound to silica in a conventional column. In order to elucidate the effect of ion exchange material, spin columns using SCX chromatography material have been constructed and results will be reported.

Neuer Aspekte

Vergleich verschiedener Säulenmaterialien im Bezug auf ihre Trenngüte.

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Investigation of the composition of protein mixtures extracted from muscle tissue with a picoseconds infrared laser

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Stichworte: Picosecond Infrared laser (PIRL), classical extraction, LC-MS/MS

Einleitung

Recently, Prof. Dwayne Miller and his group have been developed a picosecond infrared laser (PIRL) [1] [2]. With PIRL biomolecules can be extracted via desorption by impulsive excitation (DIVE) of water molecules which is present in the cells of the tissue. PIRL guarantees a soft extraction of proteins without changing their chemical composition [3]. In this study the efficacy of the extraction of proteins from muscle tissue with DIVE was investigated with respect to the composition of the proteins and compared with the protein composition of extracts yielded by a classical method.

Experimenteller Teil

Porcine muscle tissue was either cut, frozen, lyophilized, grinded to a powder, mixed with PBS buffer, including protease inhibitors, homogenized, and centrifuged or irradiated by PIRL, the ablation plume collected, mixed with PBS buffer including protease inhibitors, sonicated, centrifuged, filtrated by 10 KDa cut-off centrifuge filter (for removing fragmented DNA). The resulting samples were subjected to SDS-PAGE. The bands in both SDS-PAGE were cut and digested by trypsin. The resulting tryptic peptides were analyzed by LC-MS/MS. Protein identification was performed by processing the mass spectrometric data by proteome discoverer and searching against sus-scrofa protein database.

Ergebnisse

By looking at the bands in SDS-PAGE from classical and PIRL extraction we noticed that the total number of abundant bands was 16 in both gels. This result indicates on the first view that PIRL and the classical method yielded similar proteins.

We compared these two extraction methods more closely by cutting the SDS-PAGE bands, digested the proteins, and analyzed the resulting peptides by mass spectrometry. Approximately the same amounts of proteins were identified in both extracts. In detail some differences were noticed: A comparison between the SDS-PAGE bands of both extracts revealed that PIRL yielded some proteins which were not detected in the classical extract. For example, at the band located in the range of 25 KDa, 3 proteins were identified: These proteins are dihydropteridine reductase (25.7 KDa), carbonyl reductase (31.7 KDa), and high mobility group protein B1 (24.9 KDa).

In summary, the results showed that PIRL yields a similar composition of proteins compared with the classical extraction.

Neuer Aspekte

Extraction of proteins from tissues by PIRL.

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Proteome analysis of beewolf-associated symbiotic 'Streptomyces philanthi' revealed bacterial factors essential for survival under in vivo stress conditions.

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Stichworte: Proteome, Streptomyces, symbiosis, beewolf, stress

Einleitung

'*Streptomyces philanthi*' is a defensive bacterial symbiont tightly associated with digger wasps (Crabronidae, Hymenoptera) over the last 68 millions of years (1). These bacteria populate female-specific antennal reservoirs. Females secrete these bacteria into the brood chamber; later, larva applies them on its cocoon where bacteria produce antibiotic cocktail protecting beewolf larvae from fungal infection (2). The aim of this work was to analyze protein profiles of bacteria excreted from antennal reservoirs and to compare it to the profile of bacteria grown *in vitro* in order to identify core protein machinery and to reveal bacterial factors essential for survival under the stress conditions in a brood chamber.

Experimenteller Teil

Soluble proteins were analysed from *in situ* bacterial samples ('white substance' – bacteria and matrix secreted from female antenna); soluble and membrane proteins from *in vitro* samples were separated by 1 and 2D gel electrophoresis; protein spots were excised from the gel matrix, tryptically digested and analysed using data-dependent (DDA) and data-independent acquisition (DIA) on Synapt HDMS mass spectrometer (waters).

Ergebnisse

Totally, 4140 protein hits were identified from *in vitro* samples representing 951 individual proteins (12% of all CDS in the genome). These proteins correspond to bacterial central metabolic pathways such as carbohydrate (15), energy (7), lipid (7), nucleotide (2), amino acid (18), glycan (3) and vitamins and cofactors biosynthesis and metabolism (11 pathways). Besides that, piericidin biosynthesis proteins were detected there. Stress proteins like chaperones and superoxide dismutase were highly abundant in both *in vitro* and *in vivo* samples suggesting that these proteins play an important role in functionality of cellular machinery and could also enable survival under stress conditions in brood chamber.

Neuer Aspekte

Proteome analysis provides insight into stress response and central metabolism of unique bacterial symbiont.

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Application of Metal Coded Affinity Tagging to *Bdellovibrio bacteriovorus*

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Stichworte: Multiplexing, Bdellovibrio bacteriovorus, quantitative proteomics

Einleitung

Bdellovibrio bacteriovorus is a gram-negative bacterium that actively hunts other gram-negative bacteria. It has a unique dimorphic life cycle, which consists of a motile attack phase and an intraperiplasmic growth phase.[1] Since there is a potential for the future application of *B. bacteriovorus* as a living antibiotic, it is essential to determine the mechanisms that lead to the recognition and penetration of the prey cells. With Metal Coded Affinity Tagging our group developed a versatile tool for the quantification of proteins.[2] Quantification can be achieved both with molecular and elemental mass spectrometry analyzing lanthanide chelate complexes bound to thiol groups of cysteine residues.[3,4] Here, we applied MeCAT to *B. bacteriovorus* to quantify proteins that are involved in that life cycle.

Experimenteller Teil

Synchronized *B. bacteriovorus* cells were treated with azide to achieve and preserve different states of the life cycle. *B. bacteriovorus* cells were lysed with the freeze/thaw lysis. Obtained lysates were reduced, labeled differentially with MeCAT and then mixed. For purification one-dimensional gel electrophoresis was performed. After in-gel proteolysis resulting peptides were analyzed with liquid chromatography and electrospray ionization mass spectrometry (ESI-MS).

Ergebnisse

Since quantification with MeCAT is based on lanthanide chelate complexes, the method is highly suitable for multiplex analysis that is only limited by the number of metals employed. Therefore, a multiplex approach can be used for the quantification and determination of proteins in *B. bacteriovorus*. Due to the complexity of the samples and also due to the multiplexing a software-based (Thermo Proteome Discoverer) identification and quantification method that we developed recently was used for the processing of the spectra. For further validation of the results, there are also plans to quantify the proteins both on protein and on peptide level with inductively coupled plasma (ICP)-MS. By measuring the metal signals, aside from relative quantification even an absolute quantification of the proteins is possible with elemental MS.

Neuer Aspekte

Using multiplex quantifications with MeCAT, proteins of *Bdellovibrio bacteriovorus* are determined with molecular and elemental MS.

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Comparison of Thermolysin and Neutrophil Elastase for Peptide Mapping (LC-MS/MS) to fill the Gaps in the Tryptic Digest

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Stichworte: peptide mapping, LC-MS/MS, trypsin, thermolysin, neutrophil elastase

Einleitung

Trypsin is often a preferred protease for peptide mapping as it cleaves the carboxyl side of Arg and Lys with high specificity. The selectivity of this protease has long been proven beneficial for reproducible protein digestions and mass spectrometry based methods for the identification of heterogeneities on the peptide level. To increase the sequence coverage and to fill the gaps in the tryptic digest, we often use thermolysine as a second protease although its cleavage selectivity is less specific. The serine endoprotease neutrophil elastase has been reported to preferentially cleave at the carboxyl side of valine and to a lesser extent after alanine.

Experimenteller Teil

Performing short digestion times (30 min incubation) of a human IgG1, a 90 min UPLC gradient and MS/MS on an Orbitrap Fusion, we evaluated and compared the use of thermolysin and neutrophil elastase to be used as a second gap filling protease. The data were evaluated using Mascot searches (Matrix Science) and PeptideAnalyzer, an in house MS data evaluation software.

Ergebnisse

Without any cleavage specificity defined and without setting threshold values for peptide ion intensities or Mascot ion scores, we obtained close to 100 % sequence coverages with both proteases when performing non-error tolerant searches. In average, neutrophil elastase and thermolysin resulted in peptide length of 20 aa (STD 12 aa) and 16 aa (STD 10 aa), respectively. The optimal total sequence coverage was evaluated for each protease in combination with trypsin by applying a full scan intensity cut off for peptide ion intensities. In addition, MS/MS based Mascot ions score cut offs were used to select for good MS/MS spectra quality.

Neuer Aspekte

Data will be presented of which protease complements the tryptic digest the best.

An assessment of label-free quantification approaches for protein quantification in complex samples

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Stichworte: Label-free quantification, proteomics, high-resolution mass spectrometry, Proteome Discoverer, OpenMS

Einleitung

Label-free quantification attracts increasing attention in proteomic studies. While today's fast high-resolution mass spectrometers can identify several thousands of proteins in a single LC-MS experiment, the comparison of protein quantity in a set of samples remains challenging. Since there is no immediate value which is related to protein abundance in bottom-up analyses, different estimators are commonly being used, including^[1]:

- the number of identified peptides of the protein (*peptide counting*)
- the number of identified MS/MS spectra from peptides of the protein (*spectral counting* or *PSM counting*)
- the mean area under the extracted ion chromatogram of the peptides' precursor ion, including
 - a) the three most intense peptides (*top three* or *high flier*) or
 - b) all peptides

Experimenteller Teil

100 ng of a HeLa digest were spiked with a BSA digest at concentrations from 3 fmol to 100 fmol, and with a yeast enolase digest at a constant concentration of 100 fmol. Each sample was analyzed twice with an Orbitrap Fusion LC-MS instrument. A gradient of 120 minutes was used. Peptides were identified with Sequest HT and quantified with the EventDetector in Proteome Discoverer and the FeatureFinder in OpenMS.

The number of peptides, the number of PSMs, top three area and top all area of BSA, enolase, and three HeLa proteins were correlated to their actual concentrations. Areas were normalized for equal mean values. Each method was tested for linearity and variance.

Ergebnisse

Interestingly, good linearity ($R^2 > 0.95$) was achieved by all methods for the BSA concentration series, with the best correlation given by OpenMS top three ($R^2 = 0.99$). Also, relative standard deviations were < 0.1 on the log scale ($< 25\%$ on linear scale) for enolase and the three HeLa proteins for all methods.

The EventDetector in Proteome Discoverer found a significantly higher number of features (2704) than the FeatureFinder in OpenMS (724). However, the percentage of low-variance features was higher with the OpenMS algorithm (78%; standard deviation < 0.1) compared to the Proteome Discoverer algorithm (48%). With the latter, a higher number of features will have to be disregarded due to their high variance in the replicates so the actual numbers will approximate.

All methods tested were able to give a good estimate for the protein abundance in the model mixture. However, in clinical samples, the outcome of the methods was observed to vary considerably. Further studies should be performed to carefully assess and optimize the performance of the different label-free quantification methods available, so that reliability and reproducibility of these kinds of experiments will improve.

Neuer Aspekte

Comparison of label-free quantification approaches on a defined mixture of proteins.

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Investigation of amino acid mutations in the plasma protein C1-Esterase-Inhibitor in a human population

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Stichworte: Glycopeptide, C1-inhibitor, Population Proteomics, Off-Gel

Einleitung

In the past years LC-MS/MS (especially triple-quadrupole mass spectrometer) is used to quantify protein disease markers. Here usually a unique peptide of the target protein is selected for monitoring (SRM)[1]. A further advancement in this field is the SISCAPA[2] process. Using immobilized anti-peptide antibodies target peptides are enriched resulting in a detection limit of proteins in the low ng/mL range. A problem that should be addressed is the variety of protein species in the population[3]. Some of these species include different PTMs and/or point mutations, which result in different m/z values. Tryptic peptides containing these changes are lost for SRM. This study investigates if these variances are critical for the quantification of defined proteins in human blood plasma by SRM.

Experimenteller Teil

The therapeutic protein “Berinert[®]” from CSL Behring, containing C1-Esterase-Inhibitor, was dissolved to obtain a stock solution of 6.5 mg/mL.

Enrichment of Glycopeptides using HILIC: Tryptic peptides of C1-Esterase-Inhibitor were fractionated on a self made mini column packed with TSKGel[®] Amide 80.

Peptide fractionation: Tryptic peptides of C1-Esterase-Inhibitor were incubated with PNGase F for removing N-glycans and then subjected to a gel-free isoelectric focussing (OFF-gel).

Mass spectrometric analysis of the fractions: All fractions were analysed on a LC-MS/MS instrument (Thermo Fusion[™] using an HCDpdETD setting with prior online separation on an Acclaim[®] PepMap 100 C18 Nano-Trap column). Data processing and interpretation was performed with several algorithms (ProteomeDiscoverer, Sequest[™], X!Tandem and Byonic[™]).

Ergebnisse

After measuring the flowthrough and the eluate of the HILIC enrichment, using HCDpdETD setting, it could be shown that the glycopeptides were quantitatively separated from the non-glycopeptides. Using Byonic with 2 allowed missed cleavages, 10 ppm precursor mass tolerance and 0.1 Da and 0.6 Da fragment mass tolerance for HCD and ETD respectively, carbamidomethyl (C) as fixed and oxidation (M) as common modifications and a list of common human plasma N-glycans and 98 common O-glycans a total of 4 N-glycans and 1 O-glycan (with some degree of microheterogeneity) were identified. It was hypothesised that using isoelectric focussing peptides including an amino acid exchange because of a mutation will be detectable, resulting in signals for which no sequence assignment is possible by search engines. After analyzing the 24 fractions of the isoelectric focusing with several search engines nearly all signals above a threshold of 5% were assigned to peptides of the C1-Inhibitor yielding a sequence coverage of 83.8% (semi-tryptic search).

For identification of peptides of the C1-inhibitor containing mutations, ProteomeDiscoverer[™] was used to export all unidentified MS/MS-spectra. These spectra were then analyzed using Byonic[™] with a list of all possible amino acid mutations. By this approach 349 mutations were identified.

Considering that the C1-Inhibitor was purified from a pool of more than 6000 donors and that the intensities of the peptides with amino acid mutations were less than 1% of those corresponding to the wild-type it seems fair to say that these mutations will not have much influence on the quantitation of protein marker but at the same time, to circumvent this statistical problem, two or more unique peptides should be used to quantify a target protein by SRM.

Neuer Aspekte

Screening for mutation present in tryptic peptides of C1-Esterase-Inhibitor yielded from a pool of more than 6000 human individuals.

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Discovery of Pesticide Protomers Using Routine Ion Mobility Screening

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Stichworte: ion mobility, protomers, pesticides

Einleitung

Criteria to instill confidence in identification include acceptable product ion ratio tolerances and relative intensities of the detected ions, expressed as a percentage of the intensity of the most intense (abundant) ion or product ion, which should correspond to those of the calibration standard at comparable concentrations and measured under the same conditions. Ion ratio performance can vary with instrumentation, matrix and is affected by sample concentration. SANCO/12571/2013 guidance document describes the method validation and analytical quality control requirements to support the validity of data used for checking compliance with maximum residue limits, enforcement actions, or assessment of consumer exposure to pesticides in the EU. Here we use ion mobility mass spectrometry to gain greater understanding of ion ratio variation.

Experimenteller Teil

The assay is based on the analysis of sample extracts and matrix matched calibrants of pear, ginger, leek and mandarin, as well as quality control samples generated for an EU-RL proficiency test. These samples were analysed using ultra performance liquid chromatography using a UPLC® BEH C₁₈ (1.7µm, 2.1x100 mm) analytical column. In addition a series of mixtures of pesticide solvent standards were injected on column. The chromatographic conditions were comprised of a 17 minute water/acetonitrile (0.1% Formic Acid) gradient at 0.45 ml/min and sample injection volumes of 5µl were used. Positive ion electrospray with ion mobility mass spectrometry data acquisition was performed using a Synapt G2-S mass spectrometer.

Ergebnisse

Empirically isobaric pesticide protomers have been identified and characterised using ion mobility. It has been possible to separate the protomers (ions different only by their protonation site), determine their respective collision cross section and individual protomer fragmentation dissociation pathways. This has enabled unique visibility of product ion formation information, enabling the product ions to be selected that will result in improved product ion ratio reproducibility. For the assay, UPLC-HDMS^E experiments were performed on a Synapt G2-S using a series of standard solutions, spiked matrices and a previous proficiency test.

For indoxacarb determined to be present in proficiency sample FV-13, two mobility separated species, with CCS values of 136.49 Å² and 147.94 Å² were obtained. From the structure of indoxacarb it can be seen that protonation is possible on more than one site and that multiple sites of protonation may have been observed, and two protomers have been mobility resolved. The fragmentation spectra generated from the mobility separated protomers of indoxacarb allowed the distinctive different fragments of each respective protomer to be identified, as well as common fragments (m/z 190 and m/z 249). A further example of protomer formation observed, is for fenpyroximate with measured CCS values of 147.15 Å² and 158.33 Å². The protomers of fenpyroximate each respectively produce one of the two most abundant fragment ions. If conventional MRM analysis was performed the ion ratio measure would be affected by protomer formation. It has previously been observed that protomer formation is affected by experimental parameters such as flow rate, matrix, cone voltage and capillary voltage. As a result the fragments observed will be affected by these parameters and will affect the reproducibility of the fragments and their intensities observed. This in turn could have impact upon assays where by MRM transition is the method of choice for confirmation analysis

Neuer Aspekte

Identification and collision cross section measurement of empirically isobaric pesticide protomers in residue analysis.

Using the Routine Separation Dimension and Identification Criteria of UPLC Ion Mobility to Enhance Specificity in Profiling Complex Samples.

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Stichworte: CCS, ion mobility, passiflora, glycosides

Einleitung

The combined peak capacity of UPLC/ion mobility and collision cross section measurements (CCS) can be used to produce routine unequivocal identification of marker flavonoid isomers in complex mixtures such as herbal tea products (functional foods)/ phytomedicines). The genus *Passiflora* comprises of approximately 450 species, but only a few are commercially exploited. Several *Passiflora* (Passifloraceae) species are utilized as phytomedicines (sedative/tranquillising), the species contain flavonoids, mainly C-glycosylflavones (apigenin and luteolin derivatives; frequently occurring as isomers). Flavonoids are one of the largest and most wide spread classes of compounds and possess diverse pharmacological and biological properties. "Natural" is not necessarily safe, but such products can be if produced to legislative standards e.g. (Directive 2004/24/EC, which came into full effect 30 April 2011).

Experimenteller Teil

Hydroethanolic extracts of *P. incarnata*, *P. edulis*, *P. caerulea* and *P. alata* were analysed using ultra performance liquid chromatography. A UPLC® BEH C₁₈ (1.7µm, 2.1x100 mm) analytical column was utilised. The chromatographic conditions were comprised of a 17 minute water + (0.1% Formic Acid) /acetonitrile + (0.1% Formic Acid) gradient at 0.75 ml/min with a sample injection volume of 2µl. Negative ion electrospray with ion mobility HDMS^E data acquisition was performed using a Synapt G2-S mass spectrometer.

Ergebnisse

Collision cross sections (CCS), accurate mass, fragment ions and retention time have been used to profile the hydroethanolic extracts of *P. incarnata*, *P. alata*, *P. edulis* and *P. caerulea*, grown in Brazil. This approach offers a unique selectivity in profiling complex mixtures. Results obtained clearly show the benefits of using the collisions cross section measurements and the combined peak capacity of UPLC with ion mobility. The enhanced peak capacity enabled more information to be extracted from fragmentation studies and the individual fragmentation spectra have been obtained for flavonoid isomers which are co-eluting. From the extracts characteristic assignment for 6-C and 8-C flavonoid glycosides isomers (vitexin and isovitexin) (orientin and isoorientin) were obtained.

Collision cross section measurements were obtained for the marker flavonoid standards, and this information was used to create a scientific library incorporating the expected CCS values. The four *Passiflora* extracts were analysed and routinely screened against the flavonoid CCS library, to determine the presence/unequivocal identification of the 6-C and 8-C flavonoid glycosides isomers. CCS measurements for marker glycoside pairs (vitexin and isovitexin) 188.8 Å²/195.5 Å² have been determined. For (orientin and isoorientin) 187.7 Å²/198.1 Å² were obtained. This proved that it is possible to distinguish the marker isomer pairs for the extracts analysed using CCS measurements. When comparing the expected and the measured collision cross sections, the CCS measurement errors were typically <0.5%. In addition, it has been possible to acquire the cleaned up fragmentation spectra, which are mobility resolved from co-eluting components. In the case of isoorientin/orientin (which co-eluted chromatographically and had the same fragment ions), ion mobility resolution enabled unique fragment ion ratios to be observed. For the first time unique collision cross section measurements and corresponding isomer fragmentation spectra have been obtained.

Neuer Aspekte

Using ion mobility individual collision cross section measurements and unique fragmentation spectra were obtained for flavonoid isomers that chromatographically co-elute.

Enhanced reduction of matrix effects using LC-MS/MS with online extraction for the rapid quantitation of antibiotics in milk

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Stichworte: Antibiotics, MRM, triple quad, clean-up

Einleitung

Matrix effect is a major issue of LC-MS analysis when handling complicated samples. For example, milk is a very complicated matrix with various carbohydrates, proteins, etc. Testing milk for antibiotics is a routine LC-MS/MS analysis, which typically involves off-line sample clean-up steps to minimize matrix interference. The clean-up steps are labor-intensive, tedious, and time consuming. A quantitative method was developed to quantify 8 antibiotics in milk matrix using an LC-MS/MS system with an integrated online extraction option. The milk matrix clean-up was fulfilled online using an innovative UHPLC integrated with a binary pump and one loading pump. The analytical method used short run liquid chromatography (LC) coupled with mass spectrometry (MS) and using electrospray ionization (ESI) as an ionization technique.

Experimenteller Teil

Organic milk was purchased from a local organic store. Proteins were precipitated and removed from the blank milk. The supernatant was collected as the blank milk matrix. 7 cephalosporin and penicillin G antibiotics were spiked in the milk matrix to prepare quantitative curve solutions. Online extraction was set up on the Bruker EVOQ LC-MS/MS system to clean up the milk matrix prior to LC-MS/MS analysis. The MRM transitions of each antibiotic were optimized as well as the chromatography conditions. 2% ACN in water with 0.1% formic acid was used to clean up the matrix at 1mL/min. The entire analysis time including matrix clean-up is 15 min, including a 3-min online clean-up and a 12-min LC run.

Ergebnisse

The spiked milk matrix samples were directly injected to the LC-MS/MS system without online clean-up. The results were compared with the analysis of the same solutions with online clean-up. Without online clean-up, some antibiotics, such as cefsulodin, exhibit high matrix effects, for example, ~ 50%, versus < 5% (with online clean-up). The integrated UHPLC-OLE system was able to minimize the matrix effect by washing the loading column at a high flow rate (1 mL/min) to remove minerals, sugars, and other interfering compounds in milk. During elution, the UHPLC gradient back-flushed the loading column to elute analytes to the analytical column for separation, followed by MS/MS quantitative analysis. The turn-around time for each sample was 15 min, including 3-min clean-up time and 12-min analysis time. The online sample preparation is under complete software control and allows setting up timed events in one run, such as clean-up, elution, and column conditioning. The calibration range of this method is from 0.02 – 100 ng/mL. The linear response for each antibiotic is above 0.996, demonstrating excellent linearity. Reproducibility of the online-cleanup LC-MS/MS method was also evaluated. At 0.5 ng/mL level, the peak area RSD% of all 8 antibiotics is less than 9%, and 4 of them even have a RSD% < 4%.

Neuer Aspekte

A rapid LC-MS/MS quantitative assay using a novel online extraction system with a completely integrated UHPLC pump

Verfolgung der photochemisch induzierten Bildung gebundener Rückstände von Benzotriazol mit gelöstem organischem Material mittels ultra-hochauflösender Massenspektrometrie

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Stichworte: gelöstes organisches Material (DOM), FTICR-MS, bound residue, Benzotriazol

Einleitung

Benzotriazole werden verbreitet als Korrosionsschutzmittel eingesetzt und zählen aufgrund ihrer hohen Wasserlöslichkeit und ihrer unvollständigen Entfernung in Kläranlagen mittlerweile zu den omnipräsenten organischen Schadstoffen [1]. Gelöstes organisches Material (DOM) kann als Sensibilisator und Quencher in der Photooxidation verschiedener Verbindungen agieren [2]. In vorangegangenen Experimenten konnten wir zeigen, dass DOM die indirekte Sonnenlicht-Photolyse von Benzotriazol verstärken kann. Es ergab sich daraus die Frage, ob Benzotriazol oder seine Photolyse-Produkte Anilin und Aminophenol auch einen Einfluss auf die Struktur von DOM haben und womöglich in der Lage sind, gebundene Rückstände mit dem DOM zu bilden. Dies wäre auch ein interessanter Aspekt für die Bildung neuer gelöster organischer Stickstoff-Verbindungen durch anthropogene Einflüsse [3].

Experimenteller Teil

Als gut beschriebenes gelöstes organisches Material (DOM) wurde der Fulvinsäure-Standard Suwannee River Fulvic Acid (SRFA) verwendet. Dieser wurde für 24 h mit einem QSUN Xe-1 Sonnensimulator bestrahlt. Die Bestrahlung von SRFA erfolgte sowohl allein als auch in Gegenwart von 45 µM Benzotriazol (1H-BT) sowie mit jeweils 12 µM Anilin bzw. Aminophenol. Die Proben wurden mittels ultra- hochauflösender Massenspektrometrie analysiert (FTICR-MS, Bruker, solariX XR, 12 Tesla).

Ergebnisse

Nach Sonnenlicht-Photolyse von Benzotriazol in Gegenwart von DOM konnten mittels FTICR-MS mehr als 100 neu gebildete stickstoffhaltige Moleküle über einen weiten Massenbereich des DOM gefunden werden. Diese Verbindungen konnten weder in separat bestrahltem DOM noch in bestrahltem 1H-BT detektiert werden. Im Carbon-versus-mass (CvM) Diagramm [4] können die Serien neu gebildeter Moleküle aus der Reaktion der Sonnenlicht-Photolyseprodukte von 1H-BT und DOM anschaulich dargestellt werden.

Weitere Laborexperimente mit nachfolgender FTICR-MS Analyse dienen zur Klärung des Reaktionsweges, der Reaktionsbedingungen und der Reaktionspartner. So wird bei der gemeinsamen Bestrahlung von DOM mit Anilin ein ähnliches Produktmuster gefunden, nicht jedoch nach Bestrahlung mit Aminophenol. Somit ist Anillin sehr wahrscheinlich das reaktive Photolyseprodukt des Benzotriazols, das mit chinoiden Gruppen im DOM reagiert [5]. Weitere Untersuchungen, auch mit anderen Kontaminanten, sollen Aufschluss über die strukturellen Voraussetzungen für die Bildung gebundener Rückstände sowie über deren Relevanz in der Umwelt geben. Ferner sollen weitere massenspektrometrische Experimente die Struktur der Reaktionsprodukte erhellen.

Neuer Aspekte

Die Bildung gebundener Rückstände von Benzotriazol mit gelöstem organischem Material unter Sonnenlicht-Photolyse konnte auf molekularer Ebene mittels FTICR-MS nachgewiesen werden.

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Eliminating Matrix Effects During Multi-residue Pesticide Analysis by Extensive Dilution Using A New Triple Quadrupole MS With Enhanced Sensitivity

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Stichworte: Quantification, LC-QQQ, Pesticides, Sensitivity

Einleitung

The screening and quantitation of pesticide residues in food products is one of the most important applications in food safety. European Commission regulation (EC) 396/2005 and its annexes set maximum residue limits (MRLs) for more than 170,000 matrix-pesticide combinations for food. Most pesticides are analyzed with multi-residue methods covering hundreds of compounds which are applied to various food commodities for both screening and quantitation. Matrix effects in electrospray ionization present a significant challenge to the accurate quantitation of pesticides. Sample dilution is one approach to minimize matrix effects but requires the use of highly sensitive analytical instruments due to the need to detect contaminants below the maximum residue levels stipulated by the EU.

Experimenteller Teil

Tea samples were obtained from a local grocery store and extracted according to the citrate buffered QuEChERS protocol using Agilent BondElut QuEChERS kits. Two grams of tea were wetted with 8 mL ultrapure water and extracted with 10 mL acetonitrile. Raw extracts were cleaned up by dispersive SPE using primary secondary amine and graphitized carbon black. Final extracts of blank samples were spiked in five relevant concentrations with the comprehensive pesticide working solution and were then diluted 1:5, 1:10, 1:20, 1:50 and 1:100 with acetonitrile. Matrix matched standards and dilutions thereof were prepared immediately before injection and were measured with 5 technical replicates.

Ergebnisse

A UHPLC/MS/MS based multi-residue method for the determination of more than 250 pesticides and pesticide metabolites has been developed. The method benefits from the highly sensitive Agilent 6495 Triple Quadrupole LC/MS system and from the versatile ionization capabilities of the Agilent Jet Stream ionization source.

The method was applied to the analysis of pesticides in complex matrices like black tea. Enhanced sensitivity allowed extensive dilution of sample extracts resulting in minimized matrix effects. The increased sensitivity of the 6495 LC/MS system enabled the quantitation of most targeted pesticides below the maximum residue limits specified by the European Commission even in the 1:100 diluted extracts with improved precision and excellent robustness.

Neuer Aspekte

Quantification by LC-QQQ, Matrix Effects, High Sensitivity by Matrix dilution

Influence of Different Buffer Cations on Charge-State Distribution and Collisional Cross Sections of Native-Like Protein Ions in Native Mass Spectrometry

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Stichworte: native mass spectrometry, buffer cation, charge state, ion mobility

Einleitung

The ability of “native” MS in combination with nano-ESI to preserve native fold and assembly status of proteins in the gas phase relies largely on solution parameters, including the choice of volatile buffer. Changes of protein structures during the electrospray process and interactions with buffer components determine the final charge state distribution, collisional cross section and stability of protein complexes in the gas phase. The influence of salts, i.e. sample impurities, on charge state distribution and collisional cross sections of proteins and protein complexes has been investigated in a number of studies [1,2]. Nevertheless, data on the influence of buffer components, buffer concentration and the tuning of the mass spectrometer are so far incomplete.

Experimenteller Teil

Four model proteins (cytochrome C, human serum albumin, bovine hemoglobin, alcohol dehydrogenase) and six buffers (ammonium acetate, ammonium bicarbonate, triethylamine acetate, triethylamine bicarbonate, ethylenediamine diacetate, *N,N*-dimethylcyclohexylamine acetate) were used to record native mass spectra. A NanoMate autosampler (Advion) and pulled gold-coated glass needles were used. Mass spectra were recorded with a high mass modified Q-TOF2 system (Micromass) [3]. Ion mobility measurements were recorded with a Synapt G2 (Waters). Ion mobility spectra were calibrated with a calibration framework of native-like ions to calculate collisional cross sections [4]. Solvent accessible surface areas and collisional cross sections were calculated from crystal structures. Measured charge state distributions and collisional cross sections were compared with predicted values.

Ergebnisse

In general, better desolvated spectra were obtained using pulled gold-coated glass needles than using a chip-based electrospray source (NanoMate). The amount of intact protein complexes and desolvation was improved and the total ion count was higher.

Bicarbonates yielded good spectra only for the smallest protein (cytochrome C), while for larger proteins and protein complexes badly resolved spectra were obtained, showing intense signals of denatured protein as has already been described in the literature [5]. The charge state distribution of native-like protein ions was mainly determined by the buffer cation and correlated with the gas phase basicity of the cationic buffer component. The nearly equal gas phase basicities of triethylamine and dimethylcyclohexylamine lead in all cases to comparable charge state distributions. Generally, the highest protein charge states were observed when using ammonium salts, middle charge states for ethylenediamine, and lowest for triethylamine/dimethylcyclohexylamine. In case of alcohol dehydrogenase, this behaviour was found for both the tetrameric and for the monomeric species. Ammonium and ethylenediamine showed an improved desolvation of the protein ions. As expected, higher collisional cross sections were found for higher charge states, while only minor differences were observed in collisional cross sections of cytochrome C in different buffers for the same charge states. On the other hand, differences between the collisional cross sections of comparable charge states were observed for albumin and alcohol dehydrogenase in triethylamine and dimethylcyclohexylamine. It was shown that estimations of collisional cross sections and charge state distributions lack correctness and fit best with measurements for ammonium acetate that give Rayleigh-Limit-like charge states.

Neuer Aspekte

The influence of different amine buffers on protein charge states and collisional cross sections in native MS was tested.

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Lysosomal Storage Diseases: Development of ESI- ion trap diagnostic assay for MPS II and MPS VI. Multiplexing possibilities

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Stichworte: Lysosomal storage diseases, clinical diagnosis, MS-MRM, enzyme activity, multiplex determinations

Einleitung

The activity loss of a specific lysosomal enzyme is a characteristic for lysosomal storage diseases (LSDs), a group of genetic metabolic disorders (e.g., mucopolysaccharidosis, Fabry's Disease; Gaucher's Disease etc.).[1] The enzyme substrates resulting from inherited genetic defects or loss of function can no longer be processed and are accumulating in the lysosome causing severe disease symptoms leading to multiple organ failure (e.g., skeletal malformations, pulmonary deficits, short stature, retarded growth etc.) and finally death. [2] State of art now in LSDs diagnostic uses in first stage enzymatic determinations by fluorimetry or by mass spectrometry (in few cases) based on different substrates-structures with terminal sugar moieties.

Experimenteller Teil

In this report we describe a specific and sensitive diagnostics on dry blood spots (DBS) for single and multiplex assay of MPS II and MPS VI by multiple reactions monitoring mass spectrometry (MRM-MS). Multiple structures of umbelliferyl standards were synthesized using a modified Pechmann condensation, and used as basis for development of new single and multiplex assays. [3] Rehydrated dried blood spots were incubated with an extraction buffer and then with the enzyme substrates. Clinical diagnostics assays were developed by comparative fluorimetric and MS-MRM determinations (Bruker 3000+ ion trap-MS).

Ergebnisse

The quantification of accumulated byproduct in DBS by tandem mass spectrometry can be used to monitor more than 40 genetic disorders in newborn-screening programs. We have developed a high-throughput single and multiplexing assay for MPS II and MPS VI in DBS. In our studies, the enzymatic activity levels in DBSs were determined by fluorimetry or multiple reactions monitoring mass spectrometry in the presence of an internal standard (4-ethyl umbelliferone) showing a good statistical correlation in single assays.

The method described here, may be applied to almost any enzyme that retains activity in a rehydrated DBS. We analyzed dried blood spots for single and multiplexing assay from MPS II and MPS VI patients and in all patient cases the enzyme activities were below the minimum activities measured in healthy controls. The data obtained suggest that the new mass spectrometric assay is fast, reliable and can be successfully used in clinical trials for quantification of metabolites.

Neuer Aspekte

Clinical diagnosis of LSDs using an optimized method for fluorimetry and MRM-MS determinations.

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