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TABLE OF CONTENTS

AGENDA

7:30 – 8:25 am **Registration & Continental Breakfast**

8:25– 8:30 am **Opening Remarks/Welcome**
Arpad Somogyi, The Ohio State University

8:30 – 9:50 am **Session I**
Akiko Tanimoto (The Ohio State University)
Mass-spectrometry-based Footprinting to Map the Precursor tRNA Binding Sites in a Protein-only RNase P Variant
Whitney Houser (University of Cincinnati)
Improving Mass Spectral Analysis of Oligoribonucleotides Obtained from Ribonuclease U2 Digests
Mei Lu (Ohio University)
Probing Gold-Catalyzed Alkyne Hydration Reaction by Mass Spectrometry: Evidence for Dinuclear Gold Intermediates and Dual Activation of Substrates
Ferdinando Cerciello (The Ohio State University)
Selected Reaction Monitoring Based Targeted Proteomics for Clinical Investigation of Malignant Pleural Mesothelioma Biomarkers in Serum

Ten Minute Break

10:00 – 10:30 am **Thermo Fisher Scientific**
Application of Quantitative Proteomics and RNA-Seq to Study the Evolution of Primate Transcript and Protein Expression Levels

Ten Minute Break

10:40 – 12:00 pm **Session II**
Yun Zhang (The Ohio State University)
Surface Induced Dissociation (SID) and Collision Induced Dissociation (CID) Characterization of Human Nucleosomes
Yu Liu (Case Western Reserve University)
Bioinformatics Tools to Analyze Phosphoproteomics Data
Yang Jiao (University of Cincinnati)
A Labeling Method for Multiplex Comparative Analysis of RNA Samples with T4 RNA Ligase
Jae Kwak (Air Force Research Laboratory)
Comparison of Sampling Probe and Thermal Desorber in HAPSITE ER for Analysis of TO-15 Compounds

12:00 – 12:40 pm **Complementary Lunch**

12:40-2:00 pm **Session III**

Cindy James (The Ohio State University)
Proteome and Proteomics - Sample Preparation and Processing
Robert Ross (University of Cincinnati)
Inductive Based Fluidics Mass Spectrometry for the Analysis of RNAs Containing Modified Nucleosides
Mehmet Atakay (University of Akron)
Characterization of Noncovalent Complexes of Ployelectrolytes by Ion-mobility Mass Spectrometry
Pengyuan Liu (Ohio University)
Development of Solvent-Free Ambient Mass Spectrometry for Green Chemistry Applications

Ten Minute Break

2:10 – 2:40 pm **Waters**

Novel Tools to Facilitate Translational Medicine Research

Ten Minute Break – Afternoon Refreshments Available

2:50– 3:50 pm **Session IV**

Jianzhong Chen (The Ohio State University)
Absolute Quantitation of Major Neutral Lipid Species in Human Meibum by Direct Infusion ESI-MS
Qiuling Zhang (Ohio University)
A New Cross-linking Electrochemical Mass Spectrometry for Probing Protein Structures
Xiaoyu Cao (University of Cincinnati)
An Exclusion List Strategy to Improve Detection of Modified Oligonucleotides from RNA

Ten Minute Break – Afternoon Refreshments Available

4:00– 5:20 pm **Session V**

Yang Song (The Ohio State University)
Effect of Conformational Flexibility on Gas-phase Unfolding of Noncovalent Protein Homodimers Probed by CID and SID
Yi Cai (Ohio University)
Coupling of Ultrafast LC Separation with Mass Spectrometry by DESI
Royston Quintyn (The Ohio State University)
Using Surface Induced Dissociation (SID) to Distinguish Various Conformational States of a Source-activated Precursor Ion
Si Cheng (Ohio University)
Fast Enrichment and Detection Using Trap Column Combined with Desorption Electrospray Ionization Mass Spectrometry

2014 OMSS ORAL ABSTRACTS

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Mass-Spectrometry-Based Footprinting to Map the Precursor tRNA Binding Sites in a Protein-Only RNase P Variant

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Introduction

RNase P catalyzes 5'-maturation of precursor tRNAs (pre-tRNAs) in all three domains of life. RNase P functions either as a ribonucleoprotein (RNP) complex powered by a catalytic RNA or a protein-only enzyme – the need for two such divergent active sites to catalyze the same reaction remains unclear. The RNP form has been studied for four decades, but the proteinaceous RNase P (PRORP) found only in eukaryotes has been studied only recently. Although a high-resolution structure of plant PRORP1 is available, we do not understand how substrate recognition is accomplished by this ~60 kDa polypeptide. Here, we present the results of a high-resolution mass spectrometry approach in which lysine modification of PRORP1 with and without its substrate was used to deduce the pre-tRNA substrate contact sites.

Method

A recombinant version of *Arabidopsis* PRORP1 was used for all our studies. Pre-tRNA substrate variants were generated by *in vitro* transcription. Free PRORP1 and PRORP1−pre-tRNA complex (assembled in Ca^{2+}, allowing the formation of complex but not cleavage) were modified with N-hydroxysuccinimide-biotin (NHS-biotin) prior to separation on SDS-PAGE; the free and RNA-bound forms were in-gel digested using trypsin and Glu-C (Promega). The proteolytic digests were then analyzed using reversed-phase liquid chromatography (NanoAcquity, Waters) coupled to a Thermo Scientific Velos Pro Linear Ion Trap mass spectrometer. The MS/MS spectra were analyzed using Proteome Discoverer (Thermo Scientific). Each identified peptide was semi-quantified by spectral counting, thereby allowing a direct measure of which PRORP1 lysines were protected by the pre-tRNA substrate (either wild type or mutant derivatives).

Preliminary Data

Despite the long-standing use of trypsin in MS/MS proteomics, we found that its utility for the specific goal described above was limited. Although the tryptic digest gave a PRORP1 sequence coverage of 97%, data analysis (especially comparing different treatments) was somewhat complicated by the degree of biotinylation since trypsin cannot cleave C-terminal to biotinylated lysines. We addressed this shortcoming by using endoproteinase Glu-C, which cleaves C-terminal to glutamic acid residues – the expectation was that Glu-C should be insensitive to the presence of modified lysines in a protein substrate. Indeed, this switch proved beneficial for our studies. Since Glu-C is less efficient than trypsin, and glutamates are less abundant than lysines

in PRORP1, it was not surprising that the Glu-C digest only afforded only 65% sequence coverage of PRORP1. However, we observed a three-fold increase in the coverage of modified residues (7 to 22 lysines), and we could identify reproducibly three lysines that were protected from modification in the presence of a pre-tRNA substrate. Moreover, by making deletions in the pre-tRNA, we have also begun to map which regions in PRORP1 contribute to recognition of specific parts of the pre-tRNA. To further extend the utility of this method, we are now exploring proteases other than Glu-C, which are also unaffected by lysine biotinylation. Overall, we have gained valuable insights into the PRORP1-pre-tRNA contact sites (especially, the contributions of different RNA-binding domains), and the dynamic changes in PRORP1 that occur upon substrate binding.

Novel Aspect
Use of chemical modification and high-resolution mass spectrometry for mapping RNA-protein interactions.

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Improving Mass Spectral Analysis of Oligoribonucleotides Obtained from Ribonuclease U2 Digests

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Introduction

Ribonuclease (RNase) mapping is a biochemical approach for sequence and structural analysis of ribonucleic acid (RNA). A specific RNase such as RNase U2, which will cleave 3' of any unmodified purine residues in the RNA, is used to digest a larger RNA into smaller digestion products. As shown initially by McCloskey and coworkers, these digestion productions can be separated and analyzed using liquid chromatography tandem mass spectrometry (LC-MS/MS). One drawback of RNase U2 digestion is that it can leave both a 2',3'-cyclic phosphate and a linear phosphate at the 3' end of its digestion products. Here we examine enzymatic approaches for removal of these residual cyclic phosphates and document the improvement in mass spectra of RNase U2 digestion products.

Methods

All tRNAs were digested using RNase U2 at 65° C for 30 min and phosphodiesterases at various temperatures and times to identify ideal conditions. Chromatographic separation was conducted using a Thermo Surveyer HPLC system. A Waters Xbridge™ C18 column with mobile phase A (MPA) of 400 mM hexafluoroisopropanol (HFIP), 16.3 mM triethylamine (TEA) in water, pH 7.0 and mobile phase B (MPB) of 50% MPA and 50% methanol at a flow rate of 30 µL min-1 was used for separation of oligonucleotides. A Thermo LTQ-XL was used for MS and MS/MS analyses. A capillary temperature of 275 °C, spray voltage of 4 kV, and 25, 14, and 10 arbitrary flow units of sheath, auxiliary and sweep gas, respectively, were used.

Preliminary Data

Lambda protein phosphatase (LPP) removes phosphate groups from Ser, Thr, and Tyr residues. Previous work in our lab found this enzyme also has hydrolysis activity towards cyclic phosphate-ending ribonucleotides. The goal of this study is to identify appropriate conditions for LPP treatment of RNase U2 digestion products from model transfer RNAs. RNase U2 typically generates oligonucleotides ending in –A>p and –G>p (where >p represents a cyclic phosphate). Upon treatment with LPP, these cyclic phosphates should be catalyzed to convert into products ending in –Ap and –Gp (linear phosphates).

Lambda protein phosphatase treatment of cyclic-ending oligonucleotides shows a marked decrease in the amount of cyclic phosphates detected, with a considerable increase in the number

of digestion products ending in 3'-phosphates. Before treatment with LPP, U2 digestion results in ~98% of the products ending in 2',3'-cyclic phosphates. Post LPP-treatment, the amount of 2,3'-cyclic phosphates is reduced to ~25% of the total digestion products detected. Efforts are currently underway to reduce cyclic phosphate levels even further, as well as examining the ability to incorporate isotopic labels into 3'-linear phosphate products during LPP-catalyzed conversion from 2',3'-cyclic phosphates.

Novel Aspect
Improving MS and MS/MS characterization of RNA after digestion with RNase U2 by removing 2',3'-cyclic phosphates.

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Probing Gold-Catalyzed Alkyne Hydration Reaction by Mass Spectrometry: Evidence for Dinuclear Gold Intermediates and Dual Activation of Substrates

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Introduction

Gold can effectively catalyze the reactions of unsaturated compounds to form complex products, which is fascinating to organic synthesis chemists. The reactive intermediate was proposed to include gold–π complexes, gold–vinyl species, and gold–carbene species. Recently, the dinuclear gold intermediate is given much more attention. But the exact role of the dinuclear gold species in gold-catalyzed reactions is unclear and the mechanistic study of the reaction is still in need. In this paper, the mechanism of Ph3PAuNTf2 catalyzed propargyl acetate hydration reaction was investigated by mass spectrometry and an important dinuclear intermediate was detected, then isotope labeling experiment were performed to testify the proposed mechanism. The reaction is also applicable to similar alcohol nucleophiles.

Method

The propargyl acetate alkyne hydration reaction was performed in small scale by mixing 20 mM Ph3PAuNTf2, 100 mM alkyne in acetone which contains water. At different time, an aliquot of reaction mixture was diluted by acetone and then infused for ESI detection with 2 μL/min. Isotope labeling experiment was performed using H2O18 and D2O, also alcohol nucleophile MeOH and d4-MeOH was tested. The instrument used was a LCQ DECA mass spectrometer (Thermo Finnigan) and Xevo QTof mass spectrometer (Waters).

Preliminary Data

Monitoring of the reaction mixture at different time showed an interesting dinuclear ion [L2Au2+S+H2O-H]+ (*m/z* 1109) (L=Ph3P, S= propargyl acetate alkyne), it contained all the reactant: the gold catalyst, the substrate and the water nucleophile. Furthermore, the intensity of 1109 decreased soon with time while the protonated product peak with *m/z* 193 was detected. All this indicated 1109 might be a very interesting reactive intermediate ion. When H2O18 was used, the isotope of *m/z* 1111 increased, confirmed the composition of 1109 contained water. Also, in the case of D2O, the mass shift is 1 Da; for MeOH and d4-MeOH, the similar dinuclear ions at *m/z* 1123 and *m/z* 1126 were detected, respectively, strongly indicating that the proton loss is from the nucleophile instead of from the alkyne substrate. Based on these results, we propose a dual activation mechanism for gold catalyzed alkyne hydration reaction. The first step is the coordination of active gold catalyst with the alkyne substrate which undergoes addition with gold-activated nucleophiles H2O or MeOH, [LAuOH] or [LAuOCH3] to form the dinuclear intermediate ion.

Novel Aspect

A di-nuclear gold intermediate ion in alkyne hydration reaction was detected by mass spectrometry.

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Selected Reaction Monitoring based Targeted Proteomics for Clinical Investigation of Malignant Pleural Mesothelioma Biomarkers in Serum

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Introduction
Recent advances in the treatment of malignant pleural mesothelioma (MPM) have raised growing interest in the identification of low-invasive diagnostic biomarkers for the disease. Here, we apply targeted proteomics approaches based on the mass spectrometry (MS) technology selected reaction monitoring (SRM, also known as multiple reaction monitoring - MRM) for clinical investigation of protein derived MPM candidate biomarkers in serum.

Method and Preliminary Data
The potential of SRM for MPM diagnostics in serum was explored in our previous work [1]. SRM allowed for multiplexed screening of cell lines derived candidate biomarkers in patient serum samples enriched for N-linked glycopeptides. Quantitative SRM in training and independent validation set of serum samples from more than 150 MPM and non-small cell lung cancer subjects as well as healthy donors (HD) revealed a multiplexed glycopeptide signature in serum which reproducibly discriminated MPM from HD (area under the curve, AUC, of 0.94). Measurements of the MPM marker mesothelin using an FDA approved ELISA kit confirmed robustness of the approach and accuracy of the signature. Currently we are establishing serum processing and quantitative SRM analysis protocols for large scale investigation in clinical cohorts. These will allow clinical assessments of MPM candidate biomarkers in serum samples from more than 500 individuals collected at the James Thoracic Center and from international collaborations.

Novel Aspect
SRM based targeted proteomics allows for multiplexed quantitative investigation of protein derived MPM candidate biomarkers in serum. At the James Thoracic Center we are establishing an SRM based platform for large scale clinical assessment of candidate biomarkers in serum cohorts.

1. Cerciello, F., M. Choi, A. Nicastri, D. Bausch-Fluck, A. Ziegler, O. Vitek, E. Felley-Bosco, R. Stahel, R. Aebersold and B. Wollscheid, *Identification of a seven glycopeptide signature for malignant pleural mesothelioma in human serum by selected reaction monitoring.* Clin Proteomics, 2013. **10**(1): p. 16.

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Surface Induced Dissociation (SID) and Collision Induced Dissociation (CID) Characterization of Human Nucleosomes

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Introduction

Nucleosomes consists of 145–147 base pairs of DNA wrapped around a histone octamer containing one histone $(H3–H4)_2$ tetramer and two histone H2A–H2B dimers. Nucleosomes are stable DNA–histone protein complexes. They must be unwrapped and disassembled for genome expression, replication, and repair. The molecular mechanisms associated with posttranslational modifications (PTMs) of the histone proteins are not well understood. Previous studies have shown that the histone octamer is released as one entity, while a recent view is that H2A-H2B dimers dissociate before the $(H3-H4)_2$ tetramer. In this study, we applied surface induced dissociation (SID) and collision induced dissociation (CID) for the characterization of human nucleosomes using ion mobility mass spectrometry (IM-MS).

Method

A commercial quadrupole/IM/time-of-flight (TOF) mass spectrometer, fitted with a nano-electrospray ionization (nano-ESI) source, and modified to integrate a custom SID device was used in this study. The SID device is located between the truncated trap traveling wave ion guide (TWIG) and ion mobility cell (SID-IM). This allows separating the MS/MS product ions in the IM cell. Human nucleosome wild type (WT) was prepared by Dr. Michael Poirier's group (The Ohio State University). Nucleosome was washed extensively to 75 mM ammonium acetate and was concentrated to ~10 μM. Triethylammonium acetate (TEAA) was added to reduce the charge states of the nuclesome to compare CID/SID fragmentation behaviors of different charge states.

Preliminary Data

The human nucleosome WT sample was ionized using nano-ESI. The charge states of +28~+32 were observed. The measured collisional cross sections (CCSs) of the nucleosome examined under the experimental conditions are slightly (4%) smaller than the calculated CCS based on the crystal structure (pdb file 3AFA) without unstructured terminal tails of histones. Therefore, the nucleosome may exist as a compact conformation with folded tails.

Both CID and SID were employed to dissociate the nucleosome in order to compare the dissociation behaviors of DNA/protein complexes. Both activation methods produced the four histone monomers. However, H3 top-down covalent fragments were also observed in CID spectra, but absent in SID spectra. Because CID is known to produce an unfolded subunit from

the complexes, whereas SID can cause the dissociation of folded fragments before protein unfolding, this may explain why covalent bond cleavages for the unfolded H3 were observed in CID. In addition, the covalent bond cleavages only occur on H3 where only one double strand DNA is present. This might be the reason why we observed the covalent bond cleavages for H3.

An energy-resolved SID plot of the charge reduced nucleosome shows the order of histone monomers ejected from nucleosome with increasing SID acceleration voltages H2A was ejected first, followed by H2B, H4, and H3. This is consistent with the recent view for the nucleosome disassembly pathway that H2A-H2B dimers dissociate before $(H3\text{-}H4)_2$ tetramer. For the energy-resolved SID plot of the charged nucleosome produced in ammonium acetate, we observed H2A ejection first, followed by H4. Thus, nucleosomes with the lower charge states may preserve more native-like conformations, which may provide more information about the nucleosome disassembly pathway.

The MS/MS results show that CID and SID dissociation patterns of nucleosomes are different. It is possible to apply SID on DNA/protein complexes for more structural information and disassembly pathway investigation.

Novel Aspect
Nucleosome structural information and gas-phase disassembly pathway studied by SID-IM.

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Bioinformatics Tools to Analyze Phosphoproteomics Data

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Protein phosphorylation is one of the key regulation mechanisms in many important cellular processes, such as cell signaling, growth, and proliferation; abnormal phosphorylation can lead to serious diseases, such as cancer and diabetes. Mass spectrometry (MS) based technologies have become major platforms to systematically study the phosphoproteome through the ability to to identify and quantitate thousands of phosphopeptides and thus provide a powerful tool to detect signaling alterations in diseases. However, due to the complexity of the datasets, there is no well-established bioinformatics tool to extract and annotate valuable information from phosphoproteomics data.

We developed web-based bioinformatics tools to process and analyze phosphoproteomics data, including identification of important proteins that are phosphorylated, such as transcription factors, and kinases. Our tool can also predict functionally important phosphosites based on their positions. Moreover, we developed two approaches for identifying pathways with altered phosphorylation patterns: Protein Set Enrichment Analysis (PSEA) and Protein Interaction Enrichment Network Analysis (PIENA). PSEA, through pathway scoring, identifies pathways that show dysregulated phosphorylation changes, while PIENA is designed to identify pathways enriched with altered phosphoprotein interactions by comparing the differences between phosphopeptide pairs for one given pathway.

We will demonstrate our tools by applying them to phosphoproteomics data from two lung adenocarcinoma cell lines, treated with either control or a novel anti-cancer compound. The results demonstrate the advantage of our online phosphoproteomic analysis tools in providing unbiased and global identification of key drivers of signaling.

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A Labeling Method for Multiplex Comparative Analysis of RNA Samples with T4 RNA Ligase

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Introduction

T4 RNA ligase can be used to join two single-stranded oligonucleotides together. As such, this enzyme may be a potential tool for site-specific labeling of oligonucleotides in analytical methods that benefit from differentially labeled samples. The donor molecule in ligation reaction must contain a 5'-phosphate group, and the acceptor molecule must contain a 3'-hydroxyl group. The previous labeling method used in our lab is based on introducing ^{18}O label during RNA hydrolysis, and this method can only differentiate two samples. This ligation method can use an oligonucleotide, which has multiple potential sites for isotope labeling, as a carrier to introduce isotope labels into the analyte, so this method is able to label more than two samples at the same time.

Methods

In this research, a model reaction is used to optimize ligation conditions, and this reaction has an acceptor of a 10-mer oligonucleotide and a donor of nicotinamide adenine dinucleotide (NAD^+). After the ligation reaction is optimized, the RNase T1 digestion products of yeast $tRNA^{phe}$ are used as acceptor to investigate this labeling method on real RNA samples.
Liquid chromatography mass spectrometry (LC-MS) is used to monitor all the reactions. Enzyme kinetic analysis and design of experiment (DOE) method are used to optimize the ligation yield.

Preliminary Data

Variables in the model ligation reaction, including reaction time, ratio between acceptor and donor, T4 RNA ligase amount and dimethyl sulfoxide (DMSO) concentration, were investigated and optimized, and the labeling yield is about 95%.
The optimized conditions were applied on $tRNA^{phe}$ RNase T1 digestion products and ligation product was observed, however, the yield was only about 25%. Then ligation conditions were modified for digestion products and finally yield is over 90%.
For future easier optimization, enzyme kinetic analysis and DOE method are used to understand kinetic parameters and contribution of reaction variables.
Future work is focusing on synthesizing isotope labeled NAD^+ analog and applying this labeling method on comparative analysis of tRNA samples.

Novel Aspect

A labeling method can offer a multiplex comparative analysis ability on RNA samples by using T4 RNA ligase.

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Comparison of Sampling Probe and Thermal Desorber in HAPSITE ER for Analysis of TO-15 Compounds

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The Hazardous Air Pollutants on Site (HAPSITE), a portable gas chromatograph-mass spectrometer (GC-MS), has been used to detect, identify, and quantify volatile organic compounds (VOCs) from environmental samples, providing on-site analysis to aid in operational risk management. HAPSITE is equipped with a hand-held sampling probe in which an air sample is delivered into a concentrator, and the VOCs collected in the concentrator are transferred, separated, and identified in the GC-MS. An upgraded version, HAPSITE ER, has recently been introduced with additional sampling capability for solid phase microextraction and thermal desorption (TD). To our knowledge, however, no study has yet evaluated the performance of the thermal desorber accommodated in HAPSITE ER. In this study, therefore, we analyzed EPA Method TO-15 compounds with two different sampling methods (probe and thermal desorber for TD tubes) in a HAPSITE ER, and compared their results against each other. A major finding was that the peak intensities of the TO-15 compounds, particularly those with high boiling point (bp), were substantially higher in the results obtained with the thermal desorber than in those with the sampling probe. The lower peak intensities of the compounds observed in the probe analysis are likely due to the condensation of the VOCs in the probe (transfer) line that is 6 feet long and maintained at 40°C as they are delivered from the probe to the concentrator, whereas the thermal desorber is directly connected to the HAPSITE (no transfer line is used), thereby eliminating the condensation of VOCs. In conclusion, our study suggests that for the analysis of VOCs with high bp up to 220°C, the use of TD tubes followed by desorption in the thermal desorber offered by the newer version of HAPSITE is recommended.

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Proteome and Proteomics - Sample Preparation and Processing

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The human genome is composed of about 26,000 human genes from which we are conservatively estimating over 1,000,000 proteins from only a fraction of the mRNA produced. Genomic studies cannot tell us how much of the mRNA population of the sampled cells is translated into functional protein molecules or whether these proteins are modified after translation for effective functioning. The goal of Proteomics is to provide rapid, reliable protein purifications, separations, protein-protein interaction studies, 2D and protein identifications, along with technical support to researchers and clinicians in the biomedical field. Biological research can very rapidly and accurately determine the state of the proteome and the metabolite flux in selected tissues, patient samples and cells. The data produced gives insight on how proteins interact with each other to form complexes that enable pathway functions not seen by the examination of individual complex members. The common factor amongst all proteomics techniques is to isolate the proteins of interest from thousands of cellular proteins in order to study them alone and in combination with other proteins and/or cofactors at the time of sampling. Cutting edge science requires delicate and complicated customized approaches to isolate proteins and can insure, if necessary, that biological activity is maintained. In order to achieve these goals, it is imperative to examine issues that scientists deal with concerning achieving dependable and reproducible data from biological experiments, and treatment of the samples regarding the feasibility of a given approach and/or advice on most experimental design to enhance the probability of success.

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Inductive Based Fluidics Mass Spectrometry for the Analysis of RNAs Containing Modified Nucleosides

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Post-transcriptional chemical covalent modification of adenosine, guanosine, uridine and cytidine occurs frequently in all types of ribonucleic acids (RNAs). In ribosomal RNA (rRNA) and transfer RNA (tRNA) these modifications make important contributions to RNA structure and stability and to the accuracy and efficiency of protein translation. These modifications can be present at very low levels and their analysis can be challenging. The focus of this study is to examine the utility of Inductive Based Fluidics (IBF) as a sample introduction method for modified nucleosides and oligonucleotides. The utility of IBF-based sample introduction is compared to standard microspray electrospray ionization (micro-ESI) methods. The goal of this study is to determine whether IBF-based sample introduction provides any advantages in the ionization and detection of modified nucleosides found in RNA. To conduct this study, standard tRNAs were enzymatically digested using the guanine-specific endonuclease RNase T1. These RNase T1 digestion products can be chromatographically separated and analyzed by IBF- or ESI-MS and MS/MS.

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Characterization of Noncovalent Complexes of Polyelectrolytes by Ion-mobility Mass Spectrometry

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Introduction

Poly-L-lysine (PLL) is a cationic poly(amino acid) that is commonly used as a biomolecule carrier in various fields of research. The positively charged amine pendants in PLL provide an interaction interface for species with more negative surface charge. Comprehensive characterization of noncovalent complexes of poly-L-lysine is an important prerequisite for the determination of any structural changes in its structure during the formation of noncovalent interactions. In this study, the non-covalent complex formation of poly-L-lysine (PLL) with an anionic polymer, polystyrene sulfonate (PSS), was monitored using ion mobility-mass spectrometry.

Methods

Poly-L-lysine with a molecular weight range of 500-2000 Da and polystyrene sulfonate sodium salt with Mw ≈ 1100 Da were used in experiments. PLL and PSS solutions at a concentration of 100 μM were prepared in 7:3 (v/v) mixture of water and methanol. Polymer solutions were mixed in several molar ratios and all samples were directly injected into the electrospray ionization (ESI) source. Analyses were carried out using a SYNAPT HDMS hybrid quadrupole/time-of-flight (Q/ToF) mass spectrometer equipped with traveling wave ion mobility mass spectrometry (TWIM-MS) capabilities. Mass spectra were acquired in positive ion mode using the following main settings: capillary voltage 2.5 kV, sampling cone voltage 45 V, extraction cone voltage 2.7 V, source temperature 60 ^{o}C and desolvation temperature 100 ^{o}C.

Preliminary Data

The major ion distribution was identified as protonated PLL with H- and -OH end groups, i.e. $[H\text{-}(C_6H_{12}N_2O)_n\text{-}OH + H]^+$. Minor distributions were observed for the PSS sodium salt, C_4H_9–$[CH_2CH(C_6H_4–SO_3Na)]n–H$ as well as the PLL-PSS noncovalent association product. The differently charged ions obtained after electrospray ionization were separated according to their drift times in the ion mobility drift tube of the instrument. This way, the complexity of the ESI mass spectrum could be minimized by evaluating the data for each charge state separately. The molecular shape of polymers used for biomolecular delivery is critical, as it determines the extent of interactions between polymer carrier and biomolecular load. For example, the accessibility of amine groups on the side chains of PLL may vary depending on the structural shape (conformation) of the whole molecule. IM-MS enabled us to probe this kind of molecular differences. It combined with MS/MS by comparing the conformations of native PLL to those of

PLL that was bound to PSS. The IM-MS and MS/MS data also unveiled insight about the influence of the PLL and PSS chain lengths on the stabilities of the PLL-PSS complexes.

Novel Aspect
The non-covalent complex formation between poly-L-lysine and polystyrene sulfonate was monitored using ion mobility-mass spectrometry.

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Development of Solvent-Free Ambient Mass Spectrometry for Green Chemistry Applications

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Introduction

Green chemistry minimizes chemical hazard in many ways, including eliminating traditional solvent or using alternative recyclable solvents like ionic liquids. Compared with the booming in chemical synthesis, green chemical analysis in mass spectrometry (MS) field develops slowly, due to the difficulty of eliminating solvent usage for analysis. Meanwhile, current atmospheric pressure solvent-free MS methods are limited and still require complex sample preparations or complicated instrumentation. To address the issues, a simple and convenient solvent-free ionization approach, probe electrospray ionization (PESI), is employed for monitoring solvent free reactions as well as analyzing ionic liquids, solids, and catalysts, with the advantages of good sensitivity, high throughput, and opportunity to analyze moisture-/air-sensitive compounds. We believe this solvent-free PESI-MS method would impact green chemistry field.

Methods

The commercial ion source of the employed mass spectrometer was removed for the accommodation of home-made PESI probes. Two different home-made PESI probes, a regular PESI and a thermally assisted-PESI, were designed, which simply consisted of a stainless steel needle and a soldering iron, respectively, as the probes. The former probe was used for ionizing liquid samples by applying a high voltage to the probe. The latter probe was used for analyzing higher melting-point solid samples, on which loaded analytes were heated to melting (30^{o}C ~ 300^{o}C) and then ionized by PESI immediately. In thermally assisted-PESI, the soldering iron tip was polished to a small triangle shape for promoting spray ionization because of the high electric field on the tip.

Preliminary Data

Major types neat viscous room-temperature ionic liquids (RTILs, including imidazolium, pyridimium, and phosphonium) in trace amount (e.g., 25 nL) could be directly analyzed without sample carryover effect, thereby enabling high throughput analysis. With thermally assisted-PESI, ionic solid compounds such as organometallic complexes as well as a variety of neat neutral solid chemicals (e.g., amines) can be ionized. Perylene, a representative polycyclic aromatic compound which is insoluble in traditional ESI solvents can also be ionized by thermally assisted-PESI to a radical cation, showing the the strength of PESI method. More importantly, a moisture-sensitive sample, 1-butyl-3-methylimidazolium tetrachloroaluminate, could be successfully ionized. The moisture-sensitive sample analysis ability shows a big advantage compared to solvent-based ionization methods.

This solvent-free PESI ionization was applied for green chemistry analysis purpose, such as to analyze catalysts in ionic liquids and to monitor solvent-free reactions. In the former case, organometallic catalysts (including air-sensitive [Rh-MeDuPHOS][OTf]) in ionic liquids with typical catalyzing concentrations (10^{-3}-10^{-4} folds of magnitude lower than the ionic liquid) could be directly detected using PESI, showing the strength of PESI because such analysis is a traditionally challenging task due to strong ion suppression effect from ionic liquids. In the latter case, solvent-free PESI was employed for monitoring many solvent-free reactions, including the alkylation of amines with alcohols, the conversion of pyrylium into pyridinium, the preparation of arylhrazones, and the condensation of aldehyde with indoles as well as air- and moisture-sensitive reactions such as the oxidation of ferrocene and the condensation of pyrazoles with borohydride. For all these analysis, solvent-free PESI-MS works fast, sensitively, and effectively. More interestingly, besides the expected reaction products, some reaction intermediates, especially a moisture-sensitive intermediate, monopyrazolylborate ion, were also observed. Such observation makes PESI a valuable tool for reaction mechanism illustrations.

Novel Aspect
Solvent-free PESI-MS is powerful and versatile with unique green chemistry applications as analyzing air-/moisture- sensitive samples and monitoring solvent-free reactions.

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Absolute Quantitation of Major Neutral Lipid Species in Human Meibum by Direct Infusion ESI-MS

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Introduction

Tear film is composed of three layers: a lipid layer, an aqueous layer and a mucous layer. The lipid layer contains various types of lipids that originate from meibum, the secretion of meibomian glands. Changes in meibum lipid composition can lead to eye diseases including dry eyes, therefore the knowledge of lipid composition in meibum will inform our understanding of the etiology of eye diseases and aid in the development of treatments. The major components of meibum have been identified and include wax esters (WEs), cholesteryl esters (CEs), diesters (DEs), triacylglycerols (TGs), free fatty acids (FFAs) and (O-acyl)-hydroxy fatty acids (OAHFAs). However, reliable quantitative information of these lipids was still lacking. Therefore, this work focused on achieving reliable quantitation of neutral meibum lipids detected in positive mode mass spectrometry (MS).

Methods

Human meibum samples were collected in 0.5 μL x 32 mm Drummond Scientific Microcaps with the length of 1–3 mm (~17–46 nL) and stored at -80°C. Meibum working solutions contained ~1.34–13.4 μg/mL lipids and 10 μM–1 mM sodium iodide additive. The samples were analyzed by direct infusion electrospray ionization (ESI) mass spectrometry (MS) in positive mode on a Q-TOF II mass spectrometer (Waters). Absolute quantitative analysis of neutral meibum lipids was obtained by comparing meibum spectra in the presence and absence of spiked internal standards. Three adjustments in the final quantification were made to correct for the effect of peak overlapping, isotopic distribution and ionization efficiency.

Preliminary Data

The MS analysis of WE and CE standards suggested that the corresponding ionization efficiencies of these lipids were primarily dependent on the specific lipid class and saturation degree of the lipids while independent of their carbon chain length. This observation permitted quantification of lipids within the same class but containing different chain lengths by using only a few representative lipid standards.

The sample solutions containing a maximum of 0.27 μg meibum lipids (100 μL working solutions of 1.4–2.7 μg/mL lipids) were analyzed by direct infusion MS and the spectra were acquired for 1–5 minutes. The absolute amount (μmol/mg) for each of a total of 145 lipid species

(including 51 WEs, 31 CEs, 42 α,ω Type II DEs, and 21 ω Type I-St DEs) were quantified; the ionization efficiency correction was determined for WEs and CEs and estimated for DEs from the responses of WE and CE standards. The summed masses for WEs, CEs, α,ω Type II DEs and ω Type I-St DEs accounted for 48 ± 4%, 40 ± 2%, 3.4 ± 0.1% and 4.3 ± 0.1% respectively, of the total meibum lipids.

Novel Aspect

We have developed a sensitive, reliable, rapid and comprehensive approach to quantify the absolute amounts of the molecular species of major neutral lipid classes in human meibum.

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A New Cross-Linking Electrochemical Mass Spectrometry for Probing Protein Structures

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Introduction

Chemical cross-linking combined with mass spectrometry is a powerful method, while several experimental obstacles limit its usefulness, such as the identification of cross-linked peptides and the complexity of the fragmentation patterns of cross-linked peptides. We present an electrochemistry-assisted chemical cross-linking method employing a cleavable cross-linker dithiobis[succinimidyl propionate] (DSP) carrying a disulfide bond. This method provides a rapid way to identify cross-linked peptides in the protein digest, based on their abrupt intensity changes before and after electrolytic reduction. The type of cross-links could also be determined by mass shift before and after electrolytic reduction. Furthermore, electrochemical reduction of disulfide bond cleaves the cross-link, yielding linear peptides whose structures are more easily elucidated by tandem MS analysis than their cross-linked precursor peptides.

Methods

Intact proteins in buffer reacted with access amount of DSP, followed by trypsin digestion to get peptide mixture. The mixture was injected by syringe pump at rate of 5 μl/min, and flowed through a thin-layer μ-PrepCellTM electrochemical flow cell for electrolytic reduction, finally ionized by DESI-MS. A magic diamond (MD) electrode was used as working electrode and a RoxyTM potentiostat (Antec BV, Netherlands) was used to apply reduction potential to the cell. The DESI spray solvent was methanol/ water (1:1 by volume) containing 0.5% formic acid at injection rate of 5μL/min. A Thermo Finnigan LCQ DECA ion trap mass spectrometer (Thermo Scientific, San Jose, CA), or Waters Xevo QTOF mass spectrometer (Milford, MA) were employed for ion detection and tandem MS analysis.

Preliminary Data

Ubiquitin and calmodulin-melittin were chosen as sample protein and protein complex. The protein/protein complex was dissolved in PBS buffer and reacted with access amount DSP. After desalting and trypsin digestion, a peptide mixture containing cross-linked products was monitored by online electrolytic reduction coupling with DESI-MS. By observing the relative intensity decrease of disulfide-bond containing peptides among the peptide mixture, cross-linked products can be recognized rapidly. Disulfide-bond containing peptides' electrochemical behaviors also provide us important information about the type of cross-links. For those intra-peptide cross linking products, new ions with 2 Da mass increment arose. For those inter-peptide products, which disulfide bond links two peptide chains, two new peptides can be observed after

electrolytic reduction. For those dead-end products, a new ion was generated with a mass decrement of 88 Da. Upon CID, more fragments were produced from reduced peptides than the precursor cross links and modification sites of precursor peptides were well determined. Dead-end products helps to determine lysine residues which are most solvent accessible. Intra-peptide products provide information about distance of two lysine residues in the protein 3-D structure. In addition, inter-peptide products are used to identify two lysine residues which are in close proximity in solution.

Novel Aspect
A new method for rapidly analyzing protein cross-link products has been developed by combing electrochemistry and mass spectrometry.

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An Exclusion List Strategy to Improve Detection of Modified Oligonucleotides from RNA

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Transfer RNAs (tRNAs) were used for method development as these tRNAs contain more modifications per base than any other biological RNA. The identification and localization of modified nucleosides are important to understand their biological significance. The goal of this study is to identify improvements to the standard RNase mapping approach for localizing RNA modifications. Using the 47 expected *Escherichia coli* tRNAs as our model system, an in silico digestion of these tRNA sequences (with modifications) predicts 71 mass unique RNase T1 digestion products that contain at least one modification. By the traditional DDA-based LC-MS/MS RNase mapping approach, we can typically detect 75% of these expected T1 digestion products containing a modified nucleoside. As an attempt to overcome the limitations of a traditional DDA-based approach, we created exclusion lists based on *m/z* values obtained by taking the genomic tRNA sequences. In this manner, we are excluding any RNase digestion product that would not contain a modification. This exclusion list strategy enabled us to detect up to 95% of the expected T1 digestion products from *E. coli* tRNAs that contain modifications.

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Effect of Conformational Flexibility on Gas-Phase Unfolding of Noncovalent Protein Homodimers Probed by CID and SID

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Introduction

Mass spectrometry has become a powerful tool in the structural analysis of noncovalent protein complexes. Tandem mass spectrometry aids in the determination of the topology by releasing substructures in the dissociation. However, CID leads to ejection of highly charged monomers for many protein complexes, thus providing limited direct interaction information. This disproportional charge partitioning has been observed in CID of intra-disulfide bond reduced protein dimers, while not in their counterparts with disulfide bonda intact. Thus, one of the reasons as to why asymmetric charge partitioning occurs has been ascribed to the conformational flexibility of the monomers. Our group has observed more symmetrical charge partitioning by surface induced dissociation (SID). Herein, we analyze the effect of structural rigidity on SID behavior.

Methods

We studied three disulfide containing proteins including α-lactalbumin (type I, L5385, Sigma-Aldrich), β-lactoglobulin (L3908, Sigma-Aldrich), and lysozyme (L6876, Sigma-Aldrich). The protein samples were dissolved and buffer exchanged into 100mM ammonium acetate (AmAc) solution. The concentration was 150μM for monomer (in equilibrium with its oligomers). In order to reduce the disulfide bonds, the protein solutions were mixed with 0.5M dithiothreitol (DTT) solution at a volume ratio of 10:1 and reacted at 70ºC for 5 minutes. For the controls, DTT solution was replaced with 100mM AmAc solution. Samples were sprayed via a nano-electrospray source into a Waters SYNAPT G2S mass spectrometer (Manchester, UK) with a custom SID device incorporated before the ion mobility cell.

Preliminary Data

There are 123 amino acids and 4 intramolecular disulfide bonds in the protein bovine α-lactalbumin. The +11 charged homodimer (D11) was chosen from mass spectrum full scan for further study. CID of α-lactalbumin D11 results in symmetric charge partitioned monomers, i.e. the major monomer products are +6 and +5 charged. While the +7 and +4 charged monomer products exist, they are present in a much lower abundance. Similarly, SID of α-lactalbumin D11 shows symmetric charge distribution, but with a lower average charge state (+5 dominant). In contrast to the disulfide-intact dimer, charge partitioning in the CID of α-lactalbumin D11 without disulfide bonds is asymmetric, with +7 and +4 charged monomers present as the major dissociation products. However, +6 and +5 monomers dominate in SID of disulfide-reduced α-lactalbumin D11, showing the same symmetric charge partitioning character of SID to the

disulfide-intact dimer. An observation of the change in collision cross section of D11 with increasing CID energy reveals that unfolding occurs as CID energy increases and the disulfide-reduced D11 has a more flexible structure than the disulfide-intact D11. The low abundance of remaining D11 even at a low SID energy made it difficult to evaluate, thus the extent of α-lactalbumin D11 unfolding in SID is not clear.

To further confirm that the symmetric charge partitioning of SID is independent of conformational flexibility, we did similar experiments on two noncovalent additional homodimers , namely β-lactoglobulin and lysozyme. Triethylammonium acetate (TEAA) was added to the β-lactoglobulin solution to reduce the charge, thereby increasing the abundance of remaining precursor after dissociation. It was observed that the extent of unfolding in SID is less than in CID for disulfide-reduced D11.

In this study, CID and SID show a different behavior for intra-disulfide reduced protein homodimers, with less precursor unfolding in SID.

Novel Aspect
Higher conformational flexibility, which causes the asymmetrical charge partitioning in CID, does not cause asymmetrical charge partitioning in SID products.

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Coupling of Ultrafast LC Separation with Mass Spectrometry by DESI

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Introduction

Monolithic columns have a low hydraulic resistance and provide a higher efficiency at a higher flow rate (up to 9 mL/min) compared with particle-packed columns1-3, which are designed for high-throughput analysis. But the high flow rate would exceed the limit of MS tolerance. To solve this issue, we presented a DESI interface to combine LC with MS. In this study, we further examined several real-world applications using our new LC/DESI-MS approach. These include LC/MS analysis of metabolites in human urine, drugs in Pepsi drink, and acidic anti-inflammatory drugs. Furthermore, as we miniaturize the orifice, the splitting ratio can go down to 1:99 or less, leading to "nearly non-destructive" MS detection with simultaneous online collection of purified analytes.

Method

The experiments were performed with an apparatus of a PEEK tubing(i.d. 510 μm; wall thickness: 530 μm; length: 3 cm) with micro-drilled orifice (i.d. 50 or 100 μm) coupled LC with DESI-MS. The splitting ratio of the orifice is around 4:96. The orifice was located in the tube 2 cm downstream from the LC column and the tube outlet was bent downward to facilitate sample collection. The sample eluent flowing out of the orifice underwent interactions with the charged microdroplets generated from DESI spray for ionization. The spray solvent for DESI was CH3OH/H2O/FA (50:50:1 by volume) for positive mode and CH3OH/H2O/NH4OH (50:50:2 by volume) for negative mode. The spray solvent was injected at 10 μL/min with 5 kV applied.

Preliminary Data

First, this work demonstrates detection of drug metabolites in biological matrices with minimum sample clean up and straightforward analysis. Dopamine, 3-methoxytyramine, L-tryptophan and L-kynurenine were dissolved in human urine. Then the urine sample was diluted 1:1 with water to 60 μM, and then went through syringe filter with 0.2 μm Nylon Membrane for injection. The metabolites were well separated just in 1.6 min using monolithic column at flow rate of 4 mL/min. The resulting DESI-MS spectra also clearly display without influenced by the urine. The 3-methoxytyramine was also collected for NMR. For comparison purpose, ESI was also used to detect the LC separated analytes at this condition after splitting. The signal of the four EIC peaks of the metabolites recorded using LC/DESI-MS are 10-fold higher than that using LC/ESI-MS. A drug mixture of cocaine, codeine, and flunitrazepam (15 μM) in Pepsi (diluted with water) was chosen as a test sample. The Pepsi sample was also treated using 0.2 μm nylon membrane, and then was directly loaded into the LC/DESI-MS system. The three drugs were well separated in 3 min at 3 mL/min. So this device coupled monolithic column with MS could

provide a quick way for the drug-of-abuse detection for forensic use. Besides the applications to LC separation using positive mode, we also examined acidic anti-inflammatory drugs including ketoprofen, fenoprofen and iburopfen in negative mode. For LC/DESI-MS, they were all separated and eluted within 2.5 min. While for LC/ESI-MS, the iburopfen could not be detected in such condition, probably for that the formic acid in the mobile phase suppressed the signal in the negative mode. Using LC/DESI-MS, the spray solvent MeOH: H2O: NH4OH=50:50:2 could neutralize the mobile phase and make for the ionization of the target compounds. We also test the coupling of UPLC and MS using this DESI interface.

Novel Aspect
The splitting interface for Ultrafast-LC/DESI-MS allows fast MS detection of LC-separated analytes and online collection of purified analytes.

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Using Surface Induced Dissociation (SID) to Distinguish Various Conformational States of a Source-activated Precursor Ion

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Introduction

The dissociation of protein complexes in the gas-phase via tandem mass spectrometry gives information on protein complex stoichiometry and topology. Studies conducted within the Wysocki lab reveal that surface induced dissociation (SID) gives more "native-like" products than obtained using conventional collision induced dissociation (CID). Within recent years, the coupling of mass spectrometry with ion mobility (IM) has emerged as a vital tool in structural biology as it not only utilizes the advantages of mass spectrometry, but it enables one to study the conformational state of that protein complex. Here, we describe a novel method, which utilizes SID after ion mobility separation to gain insight into the conformational state of a number of protein complexes activated in the source.

Methods

A modified Waters Synapt-G2 (Manchester, UK) quadrupole/IM/time-of-flight (TOF) mass spectrometer with a custom SID device was used in this study. The SID device is located between the IM cell and a truncated transfer traveling wave ion guide (TWIG). This allows for the separation of the precursor ion based on shape and size, followed by the dissociation of each conformation via SID. Alternatively, CID was done in the transfer region as a means of comparing CID and SID. C-reactive protein, streptavidin and concanavalin A were purchased from Sigma-Aldrich (St. Louis, MO). Protein complexes were buffer exchanged into 80:20 100 mM ammonium acetate: 100 mM triethylammonium acetate and sprayed at a concentration of ~10 μM using a nano-electrospray ionization (nano-ESI) source.

Preliminary Data

A series of proteins ranging in molecular weight from 52.8kDa to 115kDa were sprayed using gentle source conditions (capillary voltage of 1.0-1.3kV, cone voltage of 50V and source temperature of 20°C). The following charge state distributions were observed for streptavidin (+10 to +12), concanavalin A (+13 to +16) and C-reactive protein (+16 to +20). A specific charge state was then selected for each complex and the experimental collision cross section (CCS) calculated from the drift time obtained from ion mobility experiments. The experimental CCS of streptavidin (34.3nm2), concanavilin A (54.4nm2) and C-reactive protein (64.7nm2) were found to be very similar to their theoretical CCS. Both CID and SID were subsequently employed to dissociate the precursor ion. For each protein complex, the precursor ion was then activated in the source by increasing the cone voltage and CID and SID experiments were repeated at cone voltages of 160V and 200V. CID produced mainly monomer and the

complementary (n-1)-mer at 50V, 100V, 160V and 200V. However, SID produced a variety of fragments, with more native-like products at low cone voltages and more CID-like products at higher cone voltages.

Novel Aspect
Here we describe a method of distinguishing precursors with different conformations by monitoring the fragments produced after dissociation es of major neutral lipid classes in human meibum.

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Fast Enrichment and Detection Using Trap Column Combined with Desorption Electrospray Ionization Mass Spectrometry

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Introduction

Biological samples often occur in trace amount in complicated matrices. Thus direct analysis of them by mass spectrometry (MS) is challenging and desalting, enrichment of samples and simplified sample preparations are highly demanded. Our group recently use trap cartridge column cartridge (opti-guard, ID 1mm, Optimize technologies) coupled with Liquid Sample DESI could achieve an online desalting, enrichment of sample at the same time. And due to the material of the trap column, a simple separation could be also complied.

Method

Trap cartridge column were purchased from Optimize technologies. A low concentration protein in 0.1 M phosphate buffer is loaded to the C4 column; the column can retain the protein and let the phosphate buffer washed out with water. Then an organic solvent was injected to elute the protein out which can be detected by DESI directly from the tip of trap column. A C18 trap cartridge column was applied for peptides and small compounds analysis. A protein is digested, injected into a trap-cartridge which pre-concentrates the digested protein from buffers used for digestion. The detection of enriched analytes in the cartridge can be straightforward and fast as DESI ionization occurs on the tip of the cartridge.

Preliminary Data

As the trap column could function as a pre-concentration device, a low concentration drug contained sample could be introduced to the trap column. The ESI-MS spectrum of 50 nM flunitrazepam in Diet Pepsi drink shows that dominant peak is m/z 295 which corresponds to aspartame while caffeine (m/z 195) could also be seen. However, no drug peak was observed, indicating that either the concentration was lower than LOD or the signal was suppressed. Another ESI-MS spectrum of a higher concentration flunitrazepam (50 μM) in Diet Pepsi showed that even the concentration was increased 1000 times the peak of flunitrazepan still could not be seen. Then, 5mL 50nM FLU in Diet Pepsi which was introduced to C18 trap column. Then washed with 1 mL distilled water followed an elution with 100% ACN. With these "desalting" and enrichment steps, aspartame has almost been removed, probably due to its high solubility in water and at the same time signal of flunitrazepam (m/z 314) was clearly detected. The sample was confirmed by a MS/MS at 35% collision energy. The fragment ion m/z 268 represents a loss of 46 Da, which indicates a loss of neutral NO2. The other fragment ion m/z 286 correlates with the loss of CO and the contraction of the seven membered ring to six membered one. With the same column, trace amount angiotensin II and digested ubiquitin were

applied and a good enrichment result could be seen. Thus a series tests have been done with C4, cation exchange and anion exchange trap catridge column and, trace amount protein desalting, enrichment; simple peptides separation and phosphopetides enrichment from digested β-casein were achieved respectively.

Application of Quantitative Proteomics and RNA-Seq to Study the Evolution of Primate Transcript and Protein Expression Levels

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Introduction:
The last decade has seen many studies of gene expression evolution across primates and other mammals. These studies have revealed substantial differences across primate transcriptomes and have led to the identification of putatively adaptive changes in transcript expression levels. To date, however, essentially all such studies have focused on mRNA levels rather than protein levels, which are likely more important in determining phenotypic differences. The general assumption of the field has been that between-species differences in mRNA levels are a sound indication of between-species differences in protein levels, which are likely more important in determining phenotypic differences. Here, we report our work combining transcriptomic and proteomic approaches to study expression data from human, chimpanzee, and rhesus macaque lymphoblastoid cell lines.

Methods:
Using a SILAC (stable isotope labeling by amino acids in cell culture), we acquired a large data set consisting of over 7 million LTQ-Orbitrap Velos Pro mass spectra to measure peptide expression levels in LCLs from 5 human, 5 chimpanzee, and 5 rhesus macaque individuals. In addition, we collected RNA-seq data using an Illumina Hi-seq 2000 from the same samples and estimated mRNA expression levels (using sample ratios, to be consistent with the protein measurement data) from RNA sequencing reads that map to the orthologous exons. RNA-seq reads were mapped to each species genome using BWA SILAC mass spectra were analyzed using PVIEW (http://compbio.cs.princeton.edu/pview). Crucially, due to the amino acid sequence similarity between the species, we used peptides with the same underlying sequence to quantify protein levels across species.

Preliminary Data:
We analyzed the peptide expression data in the context of orthologous gene models from the three species to obtain comparative protein expression measurements for an average of 4,853, 4,760, and 4,218 proteins from humans, chimpanzees, and rhesus macaques, respectively. Of these, 4,157 proteins were quantified in at least three human and three chimpanzee individuals and 3,688 proteins were quantified in at least three individuals from each of the three species. In addition, we collected RNA-seq data from the same samples and estimated mRNA expression levels (using sample ratios, to be consistent with the protein measurement data) from RNA sequencing reads that mapped the orthologous exons. With this combination of approaches, we

obtained both mRNA and protein expression levels for 3,390 genes in at least 3 individuals from each of the three species. We restricted all subsequent analyses to these 3,390 genes. Focusing on differences between human and chimpanzee, we classified 1151 genes as differentially expressed (DE) between species at the mRNA and/or protein expression levels independently (FDR = 1%, LR test). We found that the number of inter-species DE genes at the mRNA level is higher than the number of DE proteins. We found a similar pattern for species pairwise comparisons that include the rhesus macaque data. We additionally accounted for incomplete power to detect inter-species differences in expression by estimating the proportion of tests at the protein level that are true under the null, conditional on the observation of DE at the mRNA level for the same genes. Using this approach, we arrive at a conservative estimate of 266 genes (266/815 = 32.6%) that are DE at mRNA but not at the protein level.

Novel Aspect:
First application of transcriptomics (RNA-Seq) and Quantitative proteomics (SILAC) to study the evolution of protein expression levels in primates

Novel Tools to *Facilitate Translational Medicine Research*

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Translational Medicine has been defined in *Science* as an effort to carry scientific knowledge "from bench to bedside". Translational medicine builds on basic research advances, studies of biological processes using cell cultures, or animal models and uses them to develop new therapies or medical procedures.

Metabolomics, lipidomics and proteomics are key player in translational medicine. They represent a paradigm shift in research, away from approaches that focus on a limited number of enzymatic reactions or single pathways, to approaches that attempt to capture the complexity of biological networks. The high-throughput nature of these approaches is also well suited to biomarker discovery. Mass spectrometry is highly discriminatory for a large range of pathological processes making it the principal analytical method for these studies. The large amount of data derived from “omics” based mass spectrometry studies requires new methods and computational tools. We will describe some of our work in metabolomics and lipidomics and illustrate the utility of our novel methods and our new Progenesis QI software with examples from studies employing animal models to better understand the metabolic syndrome.