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2016 OMSS ORAL ABSTRACTS

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Catalytic Dehydrogenation Reactions in Confined Micro-reactors

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Introduction

The objective of this study is to investigate new catalytic pathways for achieving accelerated dehydrogenation reactions in ambient air, without the use of metal oxidants such as manganese dioxide and lead tetraacetate. Two novel catalytic platforms will be discussed: one, based on visible light photo-catalytic reactions using off-the-shelf $Ru(bpy)_3Cl_2•6H_2O$ photo-catalyst, and the other, based on plasma-chemical reactions using graphite as catalyst. Both platforms utilize droplets as micro-reactors to achieve reagent confinement, and enhance reaction yields. Chemical transformations of interest include the dehydrogenation of (i) tetrahydroquinolines and tetrahydroisoquinolines to give the corresponding quinolines, and (ii) primary amines into the corresponding nitriles. Only picomoles (10^{-12} mol) of reactants is required. The combination of the micro-reactor platforms with mass spectrometry (MS) enabled the capture of transient reaction intermediates and subsequent elucidation of reaction mechanism.

Methods

The visible light photo-catalytic reaction platform is made by combining a portable laser source with nano-electrospray ionization MS. The plasma-chemical reactions, on the other hand, were performed on a hydrophobic paper substrate coated with pure graphite (from commercial pencil). The hydrophobic paper was cut into a triangular sharp tip to facilitate on-surface product characterization by paper spray ionization MS. All reactions were performed under ambient conditions, outside the high vacuum environment of the mass spectrometer.

Preliminary Results

Catalytic dehydrogenation is one of the most common reactions in the manufacturing of commodity chemicals (e.g., pharmaceuticals and agrochemicals). Dehydrogenation reactions are particularly attractive because they offer high atom economy. However, progress in this area is beset with long reaction times and high catalytic loading. The use of oxygen as oxidant is a recent advancement, and our motivation is to fundamentally advance aerobic oxidation of amines by implementing catalytic strategies that enable (i) accelerated reaction rates (reactions achieved in seconds), (ii) low pressures of O_2, (ambient air) (iii) avoidance of costly or toxic additives. In pursuit of this large goal, we have developed two real-time reaction screening platforms that attempt to use photon and electrical energies as renewable, clean and sustainable technologies for chemical synthesis. Three chemical systems are presented: (i) first, by using the unmodified $Ru(bpy)_3Cl_2•6H_2O$ and a blue visible laser light a novel photo-catalytic pathway was discovered that enabled rapid dehydrogenation of tetrahydroquinolines into the corresponding quinolines via

the removal of 4 hydrogen atoms; (ii) under the same $Ru(bpy)_3Cl_2 \cdot 6H_2O$ and a blue visible laser light reaction condition, tetrahydroisoquinolines underwent dehydrogenation through the abstraction of only 2 hydrogen atoms. This high dehydrogenation energy bottleneck was overcame by manipulating the electron density of the nitrogen atom through N-phenyl substitutions; and (iii) under the photo-chemical reaction conditions, primary amines ($R\text{-}CH_2NH_2$) typically underwent self-cross coupling reaction yielding the corresponding imine (R-N=C-R) via the loss of ammonia. The use of corona discharge created from DC voltage allowed a smooth transition of the primary amine into the corresponding nitrile ($R\text{-}C\equiv N$) through the loss of 4 hydrogen atoms. A common mechanism is identified for both the real-time photon- and plasma-energy driven reactions that involved superoxide anion radicals.

Novel Aspect

Accelerated dehydrogenation reactions are achieved in ambient air using photon and electrical energies.

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Effects of Molecular Gas Addition on a Helium Flowing Atmospheric-Pressure Afterglow (FAPA) Ambient Desorption/Ionization (ADI) Source

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Introduction

Plasma-based ambient desorption/ionization (ADI) sources generally use helium as the discharge gas due to the large reaction cross section of excited helium species with atmospheric gases, such as N_2 and H_2O, which lead to substantial reagent-ion densities. While most plasma-based ADI sources utilize pure discharge gases, some work has been performed with mixed-gas plasmas. Previously, it has been shown that the addition of hydrogen in low mole fractions to a helium plasma can greatly enhance analyte-ion signal. Here, we explore changes in discharge processes and ionization chemistry with the addition of various molecular gases to the helium discharge gas of a flowing atmospheric-pressure afterglow (FAPA) source.

Methods

The FAPA source used consisted of a discharge sustained between a pin cathode and plate anode. Electrodes were held in place within a quartz chamber that sealed the discharge from the open air. A hole in the plate allowed discharge species to mix with ambient gases leading to desorption and ionization of analytes. Small fractions of molecular gases were precisely mixed with the helium plasma gas via mass-flow controllers. A linear ion-trap and an Orbitrap mass spectrometer were used to obtain reagent-ion and analyte-ion mass spectra. Changes in reagent-ion populations, analyte ions, and current-voltage characteristics of the plasma were monitored to understand the effects of molecular gas addition on the FAPA.

Preliminary data

Recently, it was found that the abundance and type of reagent ions produced by the FAPA source, and the corresponding ionization pathways of analytes, can be altered by changing the source working conditions (*e.g.*, discharge current and helium flow rate). Here, discharge gas composition was changed by adding <1.0%, v/v, of a molecular gas to the helium discharge. It was found that the addition of oxygen increased the abundance of protonated water clusters ($[H_2O]_nH^+$ where n = 1, 2, 3, etc.) by five times. The greatest abundance of protonated water clusters was found to occur with 0.1% (v/v) oxygen in helium. Correspondingly, an increase in protonated analyte signal was also observed for simple polar analytes, such as methanol and acetone.

Interestingly, ionization of analytes containing aromatic constituents, such as benzene or acetaminophen, exhibit significant chemical modification of the aromatic system. Under He-FAPA conditions, these analytes are detected as molecular ions, $M^{+\cdot}$ or MH^+. When these species are desorbed/ionized with a He:O_2-FAPA, the dominant species was always $(M+3)^+$. These unique $(M+3)^+$ ions were further characterized through exact mass measurements revealing that the

$(M+3)^+$ ion corresponds to the formation of pyrylium ions; for example, the formation of pyrylium ($C_5H_5O^+$, *m/z* 81.033) from benzene (C_6H_6). The formation of pyrylium-based ions was confirmed by tandem MS of the $(M+3)^+$ ion of 2,4,6-trimethylbenzene compared to that of a commercially available 2,4,6-trimethylpyrylium salt where both spectra exhibited a major fragment at m/z 95. In addition, MS^3 spectra of the major fragment ion was the same for both approaches. Lastly, rapid and efficient production of pyrylium in the gas phase was used to convert ammonia and benzene into pyridine with $He:O_2$ FAPA. Preliminary findings of the effects of added nitrogen and hydrogen gas to the FAPA will also be discussed.

Novel aspects

Addition of molecular gases to helium FAPA can lead to enhanced ionization efficiency or unique chemical reactions.

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Coupling Microdialysis (MD) with Desorption Electrospray Ionization Mass Spectrometry (DESI-MS)

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Introduction

The new coupling of microdialysis (MD) with desorption electrospray ionization mass spectrometry (DESI-MS) is presented. Due to the semi-permeable membrane of the MD, detection of small molecules such as drugs in the blood sampling is realized. Furthermore, the membrane of the MD can be modified by changing the function group of the membrane which can do the desalting of the large molecules such as proteins. In addition, MD is a technique to monitor chemical events in specific tissues, without systemic involvement. This allows investigators to measure free analyte concentrations along with temporal and spatial release and concentration patterns by coupling with the DESI-MS. This study reveals the versatility of MD/DESI-MS which can have great contributions in bioanalysis fields.

Methods

The experiments were performed with a Finnigan LCQ Deca Mass Spectrometer System (LDE 00368; 355 River Oaks Parkway San Jose, CA 95134, USA). All of the DESI experiments were carried out using an electrosonic spray ionization(ESSI) source to generate charged microdroplets. The spray voltage was set at +5kV for the positive ion mode and -5kV for the negative ion mode and the nebulizing gas(N_2) pressure for ESSI was 160 psi. A tube is used to connect the syringe and the MD for the sample introduction. The sample will flow out of the membrane of the MD. The ESSI sprayer was aimed to the outlet of the membrane of the MD with an optimal spray impact angle of 30°-45°. The distance between the membrane, ESSI sprayer tip and the mass spectrometer inlet varied from 0.5 to 1.0 cm. The ions generated from MD/DESI-MS were collected and detected.

Preliminary Data

To demonstrate the feasibility of this technique, whether the membrane of the MD can block the large molecules (such as proteins) and let the small molecules (such as drugs) go through was proved first. In this experiment, 10μM ibuprofen and 10μM lysozyme dissolved in water was prepared as the sample. Methanol, water with 1% acetic acid was prepared as a spray solvent for the positive ion mode detection and methanol, water and 1% ammonia was prepared for the negative ion mode detection. Then the sample was injected to go through the MD and the spray solvent was ionized via applying a high voltage in both positive and negative ion mode. In a control experiment, the MD was replaced by the silica capillary to introduce the sample solution. In the acquired MS spectrum, the ion at m/z 205 was observed under negative ion mode in both experiments, corresponding to the ibuprofen. The ion at m/z 1605 and 1803 was also observed

under positive ion mode in the control experiment, corresponding to the lysozyme, while these peaks could not be seen when using the MD. These result demonstrated that the membrane can block the proteins successfully. Furthermore, the ibuprofen dissolved in serum was also detected. With the MD, the intensity of the signal of m/z 205 was almost four times compared to the control experiment (without MD). During this experiment, the MS inlet was blocked by the solid when the MD was not used, which also revealed the advantages of the MD/DESI-MS.

Novel Aspect

The drugs in the blood sampling has been detected by the MD/DESI-MS. Also, the desalting of the protein can be realized.

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Evaluating the Specificity of RNase U2 Variants by Mass Spectrometry
Introduction

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Introduction

Ribonuclease U2 (RNase U2) is an endoribonuclease that generates purine-ending products for the analysis of RNA sequences. Digestion using this enzyme coupled with digests of RNase T1 shows higher sequence coverage than using a single enzyme. Our goal is to alter the binding site of RNase U2 to generate only adenosine-ending products by mutagenesis to simplify data analysis. Evaluation of the specificity of RNase U2 variants is complex due to a large variety of possible digestion products generated by these variants. To aid our data analysis, we also aim to develop a program that searches and locates CID fragments that represent the possible 3'-end of a digestion product.

Methods

In silico alanine scanning of RNase U2 was used to determine amino acids that affect its binding affinity to the ligand, 3'-AMP. Site saturation mutagenesis was employed a plasmid containing the RNase U2 gene to generate various mutations at E49 of the protein. Active cells were determined through a modified Toluidine-blue indicator plate method. Cells were grown at 37oC where expression of the mutant proteins was induced by addition of IPTG. The enzymes were then purified through a nickel-Nta column.

Binding affinities are distinguished by UV absorbance monitoring at 630nm of oligonucleotides containing alternating adenosine or guanosine. The Thermo LTQ-XL mass spectrometer was used to analyze digestion products generated from mutant digests of a short oligonucleotide and yeast tRNAPhe .

Preliminary Data

In silico alanine scanning results showed E49A and E62A will increase the affinity of the enzyme to 3'-AMP compared with the wild type. As E62 is part of the RNase U2 catalytic site, we targeted E49 initially to minimize effects on enzyme activity. An E49 variant was generated through site saturation mutagenesis. 54 individual transformed clones were formed. Activity screening for the other E49 variants was employed after optimization of an indicator plate containing toluidine blue was employed.

Mutant E49A was used to digest a short oligonucleotide and *S. cerevisiae* tRNAPhe and was found to be active. Since de novo sequencing of the digests of RNase U2 variants is required and as the

number of mutants increases the complexity of data analysis also increases, we developed a program that searches for MS/MS masses that represent possible ends of the RNA digests. Evaluation of this program as well as the other RNase U2 variants is ongoing.

Novel Aspect
Determining specificity of various endoribonucleases through unbiased CID searching m/z of 3'- or 5'- ends of RNA digests

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Surface induced dissociation (SID) of protein complexes in a hybrid FT-ICR

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Introduction

Mass spectrometry has become a useful tool in the analysis of proteins and protein complexes. In the native-like protein studies, surface-induced dissociation (SID) has been successfully applied in quadrupole time-of-flight (Q-TOF) instruments and shown to be able to provide structural information on non-covalent protein complexes. Ion mobility provides useful separation in Q-TOF instruments, but the mass resolution can limit the information that can be obtained for protein complexes by SID. Fourier transform ion cyclotron resonance mass spectrometers (FT-ICR MS) provide ultrahigh resolution, ultrahigh mass accuracy measurements. In this study, an SID device was designed and successfully installed in a hybrid FT-ICR instrument (external to ICR cell). The SID-FT-ICR platform has been tested with several protein complex systems.

Methods

All MS and MS/MS experiments were performed on a Bruker SolariX XR 15 T FT-ICR mass spectrometer at OSU, with a customized SID device installed in the collision cell region, replacing the original CID cell. The SID device consists of DC electrodes and a rectilinear quadrupole for trapping ions with four extra asymptotic electrodes for trapping and pulsing ions into the ICR cell. The protein samples were buffer exchanged into 100 mM ethylenediamine diacetate (EDDA) and were diluted to a monomer concentration of 20 μM. The samples were ionized by using a home-made nano-ESI source.

Preliminary data

The SID device was fabricated and has been successfully installed in the FT-ICR. Streptavidin (53 kDa tetramer), cholera toxin B (CTB, 58 kDa pentamer) and C-reactive protein (CRP, 115 kDa pentamer) were chosen as model systems to test the performance of SID. The protein ions were tuned to collide with the surface and the fragment ions were trapped in the trap region after the SID. The fragment ions were then pulsed into the ICR cell for *m/z* detection. The oligomeric state of the fragments can be clearly distinguished using the high resolution capabilities of the FT-ICR. As expected, the SID of CRP pentamer ions with an average charge state of +20 at an acceleration voltage of 35 V shows the production of monomers with +4 as the predominant charge state indicating symmetric charge distribution on CRP pentamer (as expected with SID). SID of streptavidin tetramer ions with a charge state of +12 at an acceleration voltage of 35 V shows the

production of dimers with +6 as the predominant charge state which is consistent with the relatively weak interaction between dimers in streptavidin. The SID spectra of charge reduced streptavidin under different acceleration voltages show the dissociation of dimers to monomers when higher internal energy being involved in the surface collision process. The isotopic resolution of the FT-ICR analyzer distinguished multiple oligomeric species that have the same nominal mass and overlap in low resolution spectra (e.g.+8 tetramer, +6 trimer, +4 dimer and +2 monomer of CTB).

Novel aspect

SID has been successfully installed in a hybrid FT-ICR instrument and tested for protein complexes.

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Surface Composition of Films Made from Partially Functionalized Polymer Blends

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Introduction

An important question concerning the formation of solid films from blends is the degree to which end group functionalization can influence surface segregation. Improved methods for understanding surface segregation in polymers will allow greater advances in many industries such as adhesives, coatings, membranes, and biomaterials. Surface segregation can allow for surface properties that differ from the bulk without the loss of mechanical strength that accompanies phase separation. It has been shown that functionalization with groups of lower surface energy such as a group of fluorinated units can drive enrichment at air and substrate interfaces. Conversely, high surface energy groups should deplete from the surface to lower the overall free energy of the system; this premise is examined here.

Method

Surface layer matrix-assisted laser desorption time-of-flight mass spectrometry (SL-MALDI-ToF-MS), which probes only the top layer of solid surfaces, is applied to a film prepared from a polystyrene blend. The two blend components differ only by the terminal end group (< 0.5 wt. % of the entire chain). The primary blend component is terminated with hydrogen, and the secondary component is terminated with a hydroxymethyl group. Differences in repeat unit, end groups, and molecular weight can be observed simultaneously in SL-MALDI-ToF-MS.

Preliminary Data

Conventional MALDI-MS analysis showed that the blend used, contained 9 mol % hydroxymethyl terminated polystyrene. Using SL-MALDI-MS, we observe complete depletion of this component from the surface of films prepared by spin coating of the blend. The amount of chain-end functionalized chains remaining on the film-air interface is below noise level (< 1 mol %), providing strong evidence that the blend component of high surface energy, viz. hydroxymethyl polystyrene, segregated away from the surface during spin coating. Such depletion is very drastic. Assuming that the hydroxyl-methyl end groups are strongly repelled from the hydrophobic surface-air boundary, our result is in reasonable agreement with self-consistent mean-field lattice theory calculations that were originally developed by Scheutjens and Fleer. The study of such segregation phenomena by other spectroscopic methods usually requires labelling and thus is more complicated and cumbersome.

Novel Aspect
SL-MALDI-MS makes possible the straightforward characterization of film surfaces without the need of labelling or other derivatization.

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Beam-Type Energy-Resolved Collision-Activated Dissociation in a Linear Quadrupole Ion Trap/Orbitrap Mass Spectrometer for Differentiation of Aromatic Ions Derived From Petroleum Samples

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Introduction

The extreme complexity of crude oil poses constant challenges for mass spectrometric analysis. High-resolution mass spectrometry can be used to obtain elemental compositions of the molecules in crude oil. However, these experiments do not provide structural information. Furthermore, each ion of a given elemental composition derived from a petroleum sample may correspond to a complex mixture of isomeric ions. We report here on an energy-resolved CAD method using the "HCD" cell of a linear quadrupole ion trap/orbitrap mass spectrometer for structural analysis of isomeric aromatic compounds in petroleum samples.

Methods

Model compounds were obtained from Dr. Murray Gray or were synthesized in our laboratories. Asphaltene samples were obtained from Conoco Phillips and crude oil samples containing aromatic molecules were obtained from Nested Oil. All samples were dissolved in CS_2 and ionized by APCI (+) to form stable molecular ions. The ions were isolated in the linear quadrupole ion trap by using a mass window of m/z 1 (petroleum samples) or m/z 5 (model compounds). The isolated ions were subjected to beam-type collision-activated dissociation at 25, 50, 75 and 100 normalized collision energy in the "HCD" cell of the mass spectrometer before they were transferred into the orbitrap for high-resolution detection.

Preliminary Results

Ionized model compounds with island structures lost their alkyl chains in a consecutive manner as the normalized collision energy in the "HCD cell" was increased. Model compounds with four alkyl chains demonstrated faster alkyl group losses as collision energy was increased compared to model compounds with only two alkyl chains. Ionized archipelago model compounds fragmented by loss of an aromatic group, resulting in decreased double bond equivalence. It is notable that extensive fragmentation was observed in one single activation step in the "HCD" cell as opposed to CAD performed in the quadrupole ion trap.

Model compounds with different aromatic core structures but no alkyl chains were also investigated. Ionized 9,10-dihydroanthracene demonstrated one methyl radical loss at a normalized collision energy of 50. The product ion was stable until the normalized collision energy was raised to 100, which caused the loss of one additional methyl radical. Ionized benzothiophene began to lose H_2S at a normalized collision energy of 75. Similarly, ionized dibenzothiophene

began to lose H_2S at a normalized collision energy of 75. Hence, degradation of ionized aromatic cores requires more energy than dealkylation.

Examination of ions derived from petroleum samples revealed the presence of ionized molecules with archipelago structures based on their fragmentation patterns. Some ions with island structures lost all of their alkyl chains at a normalized collision energy of 25 while others required more energy. The high-resolution mass spectral data obtained at different normalized collision energies were analyzed by plotting double bond equivalence (DBE) versus carbon number. These DBE versus carbon number plots offer compositional and structural information on petroleum samples.

Novel Aspect
Beam-type high-resolution energy-resolved tandem mass spectrometry approach utilizing the "HCD" cell of a linear quadrupole ion trap/orbitrap was demonstrated to be valuable in structural elucidation of aromatic ions derived from petroleum samples.

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Probing UV-induced Effects on RNA and RNA-Modifications

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Introduction

Identification of ultraviolet radiation (UVR)-induced RNA damage and changes in RNA modification profiles are critical for understanding of the cellular stress responses to UVR. UVR exposure is the main etiological factor for melanoma, and the response to UVR varies with pigmentation. Little is known about the damaging effects of UVR on RNA in the presence of melanin and the contribution of such damage to melanoma. Our initial goal is to document a list of UVR-induced RNA photoproducts by LC-MS and to investigate whether melanin sensitizes or protects RNA from the induction of RNA damage by UVR. Such information will be helpful for analysis of the differential generation of RNA photproducts, when melanocytes with varied pigmentation are exposed to UVR.

Methods

To understand the effects of UVR, E. coli tRNA (20 μg) was exposed to UVR at 375 nm for 20 min in the presence of 100 μM riboflavin with or without melanin (synthetic eumelanin and pheomelanin). The exposed RNA was digested to nucleosides and subjected to LC-MS analysis. Nucleosides were analyzed by Phenomenex Synergi Hydro RP column (800A, 4 μm, 2.0 x 250 mm) coupled to an LTQ-XL mass spectrometer. In addition to nucleoside data, we have also analyzed the effect of UVR at 375 nm in in the presence of 100 μM riboflavin using a synthetic oligonucleotide, CUUAAUCCGG.

Preliminary data

Exposure of total tRNA to UVA in the presence of riboflavin resulted in significant changes to the nucleoside profiles. The most significant changes include the emergence of oxidized adenosine and guanosine (8-oxo-rA and 8-oxo-rG), loss of sulfur containing modifications (s2C, mnm5s2U, s4U and ms2i6A), and other modifications present in the tRNA anticodon loop such as Q, and i6A upon UV exposure compared to unexposed sample. Although the exact nature of the conversion products is not clear, potential damage products can be identified by analyzing unique nucleoside m/z values. When tRNA was exposed in the presence of melanin, a reduced the amount of 8-oxo-rG and 8-oxo-rA were found although sulfur containing modifications were still affected. The LC-MS analysis of UVA exposed oligonucleotide indicated that guanine sites were the most photoreactive.

Novel Aspect

LC-MS based detection of UVR-induced photoproducts of tRNA

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Confirmation of Subunit-Subunit Connectivity and Topology of Computationally Designed Protein Complexes using Surface Induced Dissociation/Ion Mobility

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Introduction

The declining price of gene synthesis provides motivation for the expression of large sets of proteins, as for instance calculated by computational biology. A current major limitation of the research lies in the subsequent structural characterization of those proteins. The most commonly employed technique, X-ray crystallography, is time- and resource- consuming and heavily relies on sample quantity and quality. This is particularly true for protein-complex samples, where a single quaternary assembly as starting material is the prerequisite for diffraction-quality crystals. In this work, we demonstrate the applicability of mass spectrometry as a structural biology screening tool. We show that information about sample quality, sample suitability for X-ray crystallography, stoichiometry, complex shape, and inter-connectivity is rapidly gained by our methodology.

Methods

Protein complexes were designed at the University of Washington by using the Rosetta protein modeling suite. The subunits were co-expressed with one subunit carrying a histidine tag in *Escherichia coli*. Complexes were purified by metal chelate affinity chromatography followed by size exclusion chromatography. Fractions containing sufficiently pure complex were desalted / buffer exchanged using BioSpin columns. Accurate masses of denatured proteins were determined on an EMR Orbitrap (Thermo Scientific) instrument at the Ohio State University. Mass spectrometry under native-like conditions was done on an in-house (The Ohio State University) modified Waters Synapt G2 (Manchester, UK) ion-mobility mass spectrometer with a surface induced dissociation (SID) device located in between a truncated trap traveling wave ion guide and the IM cell.

Preliminary Data

Protein subunits were designed to form homo-dimers and homo-trimers that assemble to hetero-dodecamers. Within the hetero-dodecamers, each homo-dimer was designed to connect the homo-trimers by interacting with one subunit of each homo-trimer. Samples D32_B03 and D32_D04, which are based on this design, were analyzed by mass spectrometry. Accurate mass determination of the denatured subunits confirmed the protein identity and sample purity. A truncation of the N-terminal methionine was observed for a small proportion of the subunits. Samples D32_B03 and D32_D04 were sprayed from ammonium acetate buffer / TEAA at different ionic strengths under native-like conditions. Different ionic strengths are required for hetero-dodecamer formation of D32_B03 and D32_D04. The experimentally determined collision cross sections (CCS) for the

formed hetero-dodecamers are in good agreement with the CCSs calculated from the intended design structures. Furthermore, the determined molecular weights match the intended subunit composition. In a next step, we selected a single charge state hetero-dodecamer and dissociated it by surface induced dissociation (SID). With this technique, sub-complexes are generated. In contrast to collision induced dissociation (CID), no significant unfolding is induced by SID. As a consequence, the SID-generated sub-complexes are reflective of the structure of the complex. We found that the assigned composition of all SID generated sub-complexes confirm the intended designs. Thus the generation of hetero-dodecamers with two trimers connected by three dimers was successfully achieved by computational design.

In the future, information on the ionic strength requirements for complex formation will be utilized to guide the crystallization and to yield crystal structures of the complexes.

Novel Aspect
We established a structural biology screening methodology, which we used to confirm the quaternary structure of two designed protein complexes.

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Study of Interactions between Lignin Model Compounds and Metals Ions by Using a Tandem Mass Spectrometer Coupled with Electrospray Ionization

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Introduction

Catalytic conversion of biomass to fuels and high value chemicals is of increasing interest. One of the primary components of biomass is the complex polymer known as lignin, which is composed of aromatic rings joined together via varying linkages. To facilitate catalytic lignin degradation, various metal ions have been studied. However, a better understanding on the interactions between lignin and metal ions is needed to rationally design catalysts for lignin degradation. Herein, an ESI/tandem mass spectrometry approach has been utilized to study the interactions between metal ions and lignin model compounds in solution. Collisionally activated dissociation (CAD) was used to provide structural information for the complexes.

Methods

Lignin model compounds (vanillin, 2-ethoxyphenol and guaiacylglycerol- β-guaiacylether) were dissolved in methanol to make approximately 2 mM solutions. Metal chlorides/sulfates were added into lignin model compound solutions to make the molar ratio between lignin model compounds and metal ions = 2:1. All experiments were performed in a Thermo Scientific LTQ OrbiTrap XL mass spectrometer equipped with an electrospray ionization (ESI) source. Samples were introduced into the instrument via the integrated syringe drive of the LTQ at a flow rate of 20 μL/min unless otherwise stated.

Preliminary Data

Vanillin was found to act as both monodentate and bidentate ligand in the coordination with Mg^{2+}, Zn^{2+}, and Al^{3+}. The oxygen in the aldehyde group interacts with the metal ion when vanillin acts as a monodentate ligand. Both oxygen atoms from phenol and methoxy groups interact with the metal ion when vanillin acts as bidentate ligand. 2-Ethoxyphenol only acts as a bidentate ligand and uses both oxygen atoms to interact with metal ions. Guaiacylglycerol-β-guaiacylether (GGE) can act as a mono-, bi-, or tridentate ligand, depending on the number of molecules involved in the coordination. As a tridentate ligand, GGE uses the oxygen atoms in its β-O-4 linkage to interact with metal ions.

In the mixtures of Mg^{2+}/vanillin and Zn^{2+}/vanillin, vanillin and different solvent molecules (*e.g.*, methanol) participate in the coordination. Both singly and doubly charged ions were observed. CAD of $Mg(vanillin)_4^{2+}$ produced both $Mg(vanillin)_3^{+}$ and $[vanillin+H]^{+}$. Similar fragmentation was also observed for $Zn(vanillin)_4^{2+}$. These result indicate that for the doubly charged complex ions formed in Mg^{2+}/vanillin and Zn^{2+}/vanillin mixtures, one of the positive charges is located on

the metal atom and the other one on the protonated aldehyde group of a vanillin ligand. No doubly charged ions were observed for the Mg^{2+}/2-ethoxyphenol mixture.

In the mixture of Mg^{2+}/GGE, both GGE and negative ions (*e.g.*, Cl^- and SO_4^{2-}, depending on the metal salt being used) participated in the coordination. Both singly and doubly charged ions were observed. Upon CAD, $Mg(GGE)_4^{2+}$ produced $Mg(GGE)_3^{2+}$ as well as $Mg(GGE)_2^{2+}$ without formation of any singly charged complex ions. CAD of $Mg(GGE)_3^{2+}$, however, produced both $Mg(GGE)_2^{2+}$ and $Mg(GGE)_2^+$ at the same time. This indicates the existence of different types of coordination linkages in $Mg(GGE)_4^{2+}$ and $Mg(GGE)_3^{2+}$.

Novel Aspects
Coordination chemistry, charge sites and fragmentation patterns were determined for lignin model compounds interacting with metal ions.

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Automatic Analyte-Ion Recognition and Background Removal for Ambient Mass Spectrometry Data Based on Cross-Correlation

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Introduction

The emergence of ambient desorption/ionization mass spectrometry (ADI-MS) enabled convenient, direct analyses of samples with minimal sample preparation. Unfortunately, chemical background and matrix ions can preclude detection of low-abundant analytes. Furthermore, conventional background subtraction approaches are difficult to implement in ADI-MS analyses due to random variation in background signals.

One potential treatment approach is cross correlation, which has been used in MS data processing, but typically for spectral library matching to identify analytes. In those cases, however, the time-domain information of the signals is discarded. Here, we propose cross-correlation of ion chronograms to gauge the similarity of all ions in a mass spectrum; differentiation of ions stems from chemical information encoded in the time-domain signal variation, often viewed as "noise."

Methods

A program was developed in-house to automatically perform cross-correlation of all extracted ion chronograms from ADI-MS analyses of samples containing many analytes. Spectra were obtained with either a Direct Analysis in Real-Time (DART) or Flowing Atmospheric-Pressure Afterglow (FAPA) source coupled to a high-resolution Orbitrap or a low-resolution linear ion trap mass analyzer. The degree of similarity between ion signals in the time domain was assessed based on the displacement of the maximum of cross-correlation function (τ_{max}). Analysis of a pesticide mixture with the DART ID-Cube source was used to characterize this cross-correlation approach. The fixed sample introduction duration of 30 seconds provided by the ID-Cube led to reproducible and predictable ion chronograms to evaluate this approach.

Preliminary Data

Initial investigation of this data-treatment approach focused on correlation of an analyte-ion chronogram with three other chronograms: that of the same analyte (autocorrelation), that of another analyte in the sample, and that of a chemical-background ion. A maximum in the cross-correlogram of zero seconds indicates the ions came from the same chemical species, a maximum within the sample-introduction duration (±30 s) indicated unrelated ions from the sample, and maxima outside sample-introduction window were considered chemical background. Any ions identified as chemical background from this approach were automatically removed from the mass spectrum. In one example, the protonated molecular ion for the pesticide carbaryl was used as an

analyte reference where ion signals with τ_{max} outside the interval of ±30 seconds were discarded from a spectrum. As a result, the complex mass spectrum originally containing 1447 MS-peaks was simplified to only 284 MS-peaks, more than 80% reduction in spectral complexity.

Furthermore, ions which produced a τ_{max} of zero seconds with respect to the reference ion were classified as ions that stem from the same chemical species (e.g., fragments, adducts, isotopes, etc.). In the case of the analysis of a pesticide mixture, 17 of the 1447 detected ions were found to directly stem from the carbaryl pesticide. Majorities of this ion category had signals below the ones of chemical background. Exact mass measures were used to confirm the identity of these associated ions as isotopic species, fragment ion(s), adducts ion(s), and analyte clusters. Though, the ion recognition and classification process was optimized with an Orbitrap mass spectrometer, similar results were obtained with a unit-resolution MS. Finally, prospects for this cross-correlation approach to distinguish ionization pathways and even isomers will be discussed.

Novel Aspect
Automatic and rapid approach to simplify and group ion signals from direct mass spectrometry analyses.

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The Comparison of Glycosphingolipid Profiles in Tumorigenic and Nontumorigenic Epithelial Ovarian Cell Lines using MALDI-MS and MS/MS

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Introduction

Glycosphingolipids (GSLs) are biologically important molecules, which are linked to cancer development. The expression of GSLs may be altered in ovarian cancer cells in comparison to nontumorigenic ovarian cells. Here, GSL profiles were studied in an epithelial ovarian cancer cell line SKOV3 and a nontumorigenic epithelial ovarian cell line T29 using MALDI-MS and MS/MS. GSLs were isolated using Folch partition and detected by MALDI-MS in both Folch partition upper and lower phases. These GSLs were identified using MALDI-MS/MS, and SimLipid and LIPID MAPS databases. The results indicate that some neutral and acidic GSLs are differentially expressed in SKOV3 in comparison to T29 cells.

Methods

SKOV3 and T29 cells were cultured in appropriate media. GSLs were extracted from ~0.25 mL of packed cells by Folch partition and these GSLs were purified using a published protocol (*Proc Natl Acad Sci* **2013**, *110*, 4968-4973). Extracted GSLs in Folch partition lower (organic) phase and upper (aqueous) phase were analyzed by MALDI-MS and MS/MS using a MALDI TOF/TOF mass spectrometer in positive and negative ion modes, and DHB as the matrix. These GSLs were structurally assigned by a comparison of their MALDI-MS/MS spectra with MS/MS data found in SimLipid and some GSLs were assigned manually.

Preliminary Data

MALDI-MS of GSLs found in Folch partition lower phase (LP) of SKOV3 cells detected singly-charged sodiated GSL ions (e.g., *m/z* 806.6, 832.6, 834.6, 884.5, 968.6, 994.6, 996.6, 1046.6, 1130.6, 1156.7, 1158.7, 1197.7, 1199.7, 1249.6, 1277.7, 1333.7, 1359.7, 1361.7, 1387.8, 1389.8, 1411.7, 1495.8, 1523.8 and 1549.8). These ions were identified as neutral GSLs as confirmed by the analysis of their MS/MS spectra using SimLipid database. Singly-charged ions of neutral GSLs were also identified in Folch LP of T29 cells as sodiated ions. While most of the GSL ions mentioned above were detected in Folch partition LP of T29 cells, GSL ions with *m/z* values of 1277.7, 1389.8, 1411.7, 1495.8, 1523.8 and 1549.8 were found only in SKOV3 cells. The MALDI-MS and MS/MS analyses of GSLs in Folch partition upper phases (UP) of SKOV3 and T29 cells showed that most of neutral GSLs also partitioned in this aqueous phase, but the intensities of these ions were lower than in the mass spectra acquired in the lower phase. In addition to neutral GSLs, MALDI-MS analyses of GSLs in Folch partition UP of SKOV3 cells detected singly-charged sodiated ions with *m/z* values of 1462.8, 1674.8 and 1836.9 which were identified by MALDI-

MS/MS as monosialylated gangliosides (acidic GSLs). Singly-charged sodiated ions with *m/z* 1175.7, 1285.8, 1378.7, 1462.8 and 1488.8 were detected and identified as gangliosides in Folch partition UP of T29 cells. These gangliosides were also detected in Folch partition upper phase of SKOV3 and T29 cells by MALDI-MS analyses in negative ion mode as deprotonated molecules.

Novel Aspect

Differentially-expressed neutral and acidic GSLs were identified in tumorigenic and nontumorigenic epithelial ovarian cell lines using MALDI-MS and MS/MS.

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Multidimensional Mass Spectrometry of Peptides and Proteins Conjugated with Poly(ethylene glycol)

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Introduction

Poly(ethylene glycol) (PEG) conjugation (PEGylation) of proteins increases their circulatory half-life, provides protection against enzymatic degradation and decreases immunogenicity. It also reduces the protein's tendency to denature and self-associate into fibrils. All of these properties and outcomes of PEGylation are crucial for efficiency of biopharmaceutical applications. The performance of a conjugate depends on the number and location of its PEG chain(s). Here, we present a multidimensional mass spectrometry (MS) approach for ascertaining these important properties.

Methods

Substance P and Insulin were PEGylated by using a monofunctionalized methoxy-PEG (Mn = 5,000 Da) with succinimidyl carboxymethyl ester. The composition and the primary structure of the PEGylated substance P and the different isoforms of the PEGylated insulin conjugates were determined by utilizing various combinations of polarity-based fractionation via ultrahigh performance liquid chromatography (UPLC), MALDI- and/or ESI-MS, in-source dissociation (ISD), and orthogonal separation by shape and charge via ion mobility mass spectrometry (IM-MS).

Results

PEGylation was performed with succinimide-functionalized PEG, which reacts with free amino groups. The conjugation was carried out under near physiological pH values (pH = 8.4), therefore, only the exposed amino groups on the tertiary structure of the peptide or protein were expected to be PEGylated. LC-MS and IM-MS of PEGylated substance P (one Lys) showed the addition of two PEG chains, providing evidence that the N-terminus was also derivatized. With insulin, which contains one Lys and two N-termini, LC-IM-MS had to be used to resolve unreacted PEG from mono- and multi-PEGylated protein. ESI-MS analysis of the intact conjugates required the addition of triethylamine (directly in to the sample during direct injection or post-column during LC-MS) to lower the charge state. Presence of different isoforms of mono-PEGylated insulin was observed and site-specific characterization of these different architectures was carried out by combination of ISD and LC-IM-MS. Results were also confirmed by investigating individual A- and B- chains, after the cleavage of disulfide bonds on PEGylated insulin.

Novel Aspect

Multidimensional MS was utilized for site-specific characterization of PEGylated peptides and proteins and their isoforms.

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Characterization of recombinant cusativin for its cytidine-specific cleavage of RNA

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Introduction

A variety of chemical modifications increase the alphabets of RNA to more than 120 alternative nucleotide forms. These RNA modifications could be related to a number of human disease through their structural and functional role. Nucleoside specific ribonucleases are useful in mapping the location of these chemical modification in nucleotide sequence. RNase Cusativin, a member of the RNase T_2 family, exhibits cytidine-specific/preferential cleavage of RNA. Here, we report the inducible expression, purification and characterization of recombinant cusativin from *E. coli*. After ascertaining the enzyme's purity, its activity and associated Km and Vmax values were estimated from enzyme-mediated change in UV-absorbance. The cytidine specific-cleavage properties of cusativin was confirmed by treating tRNA under defined conditions for subsequent analysis by liquid chromatography coupled with mass spectrometry (LC-MS).

Methods

We have identified the optimal condition for overexpression of Cusativin gene in *E. coli*. Following purification of enzyme by affinity chromatography, the enzyme activity was assayed by monitoring the change in UV absorbance following incubation of RNA with the purified protein. The cleavage specificity of purified protein was determined through analysis of enzyme-treated RNA by ion-pairing reverse phase liquid chromatography coupled with mass spectrometry (IP-RP-LC-MS).

Preliminary Data

Overexpression of Cusativin in *E. coli* using autoinduction medium resulted in higher protein yield compared with other media. Our efforts also resulted in identification of optimal temperature for its nucleobase-specific ribonuclease activity. Using tRNA as substrate, we have estimated the K_M and Vmax values of purified enzyme. LC-MS analysis of tRNA digest strongly indicated the cytidine-specific cleavage of RNA by Cusativin.

Novel Aspect

Cytidine-specific ribonuclease is a useful biochemical tool in RNA modification mapping.

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Enhancing Performance of Liquid Sample Desorption Electrospray Ionization Mass Spectrometry Using Trap and Capillary Columns

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Introduction

In this paper we present the improvement of liquid sample DESI-MS by replacing the sample transfer silica capillary with a trap column filled with chromatographic stationary phase materials (e.g., C4, C18). This type of trap column/liquid sample DESI can be used for trace analysis of organics and biomolecules such as proteins/peptides (in nM concentration) in high salt content matrices. Furthermore, when the sample transfer capillary is modified with enzyme covalently bound on its inside capillary wall, fast digestion (<6 min) of proteins such as phosphoproteins can be achieved and the online digested proteins can be directly ionized using DESI with high sensitivity. The latter is ascribed to the freedom to select favorable spray solvent for the DESI analysis.

Methods

For enrichment of trace amount sample, 5 mL of test sample in low concentration (either flunitrazepam in Diet Pepsi or proteins/peptides in phosphate buffer) is loaded into the trap column; the column can retain the sample and allow the sample matrix or buffer to be washed away with water. Then an organic-containing solvent is injectedto elute the sample out, which can be monitored by DESI-MS with a spray solvent of MeOH/H2O/1%HOAc aiming at the exit surface of the trap column. For flunitrazepam sample desalting, a C18 trap column was first equilibrated with 1 mL water, and then 5 mL of 50 nM flunitrazepam in Diet Pepsi was infused to the trap column followed by washing with 1 mL of water. Elution was carried out use ACN. For fast online tryptic digestion, the enzyme trypsin is immobilized on the inner wall of the silica capillary. When protein is infused through the capillary, digestion can be achieved. The resulting peptides can be directly detected by DESI-MS.

Preliminary Data

By applying the trap column coupled with DESI-MS, 50 nM flunitrazepam in Diet Pepsi, 50 nM angiotensin II in 0.1M phosphate buffer and 40 nM insulin in 0.1M phosphate have been desalted and enriched, finally have been detected by using DESI-MS. For fast protein digestion, β-casein

and myoglobin have been used as models for online tryptic digestion. With 6 minutes, proteins have been digested and detected by using DESI-MS.

Novel aspect: Trace amount samples in complex matrix could be desalted, enriched and detected. Fast online tryptic digestion could be achieved with 6 minutes.

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Mass Spectrometry for Paper-based Immunoassays: Towards On-demand Diagnosis

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Introduction

Current analytical methods, either point-of-care or centralized detection are not able to meet recent demands of patient-friendly testing and increased reliable results. Here, we describe a two-point separation on-demand diagnostic strategy based on a paper-based mass spectrometry (MS) immunoassay platform that adopts stable and cleavable ionic probes as mass reporter; these probes make possible sensitive, interruptible, storable and restorable on-demand detection. In addition, a new touch paper spray (TPS) MS method was developed for on-chip, sensitive and cost-effective analyte detection. This concept is successfully demonstrated via i) the detection of *Plasmodium falciparum* histidine-rich protein 2 antigen and (ii) multiplexed and simultaneous detection of cancer antigen 125 and carcinoembryonic antigen.

Methods

The experimental workflow consists of three specific steps: i) fabrication of paper test zones and immobilization of capture antibody (cAb) with aldehyde-functionalized paper; ii) immunoreaction on the prepared paper surface, and iii) MS analysis. For example, to detect malaria *Pf*HRP2 antigen, a paper surface immobilized with anti-*Pf*HRP2 antibodies was used, where a solution containing *Pf*HRP2 is added and the antigen is selectively captured. Then add a solution of detection antibody conjugated to the cleavable ionic probes to complex with the captured *Pf*HRP2 antigen. Finally, the positive charge-tags can be released by the addition of NH_4OH solution to the antigen/antibody complex. Quantitation of the intensities of released charge-tags is achieved using nESI or TPS tandem MS analysis.

Preliminary data

The use of the ionic probes for disease diagnosis via MS was first applied in the detection of malaria *Pf*HRP2 antigen in undiluted human serum. 2-(4-isothiocyanatophenethoxy)-*N*,*N*,*N*-trimethyl-2- oxoethanaminium chloride (ITEA)- and 4-(4-isothiocyanatophenethoxy)-*N*,*N*,*N*-trimethyl-4-oxobutan-1-aminium chloride (ITBA)-conjugated anti-*Pf*HRP2 antibodies (ITEA-dAb and ITBA-dAb) were used for complexation and reporting, whereas nESI or TPS MS/MS was first employed for the detection. The fragment ions m/z 59 from the hydrolysis products (carboxymethyl) trimethylammonium chloride (CMTA) and m/z 87 from (3-carboxypropyl) trimethylammonium chloride (CPTA) were chosen for quantification. Good linearity was obtained for analytes spiked into serum, with LOD of 2.8 ng/mL and 37 ng/mL for using ITBA-dAb and ITEA-dAb, respectively, which correspond to only 1.5 fmol and 50 fmol each test zone. For the TPS MS analysis approach, the LODs are 3.7 ng/mL and 37 ng/mL in serum, respectively. The sensitivity of the proposed paper-based MS immunoassays with ITBA-dAb is comparable to that

of the enzyme amplified ELISA methods recorded in our hands (LOD: 1 ng/mL for *Pf*HRP2 in serum) although no amplification is adopted for the MS method. These results indicate that the proposed MS immunoassay can be used to diagnose malaria infection for blood parasite densities of 200 parasites μL^{-1} (mean antigen concentration is 9.1 ng/mL), which is the WHO recommended lowest density for diagnosis. The results are stable and reproducible even after 30 days of storage from the completion of immunoreaction, which enable the combination of onsite sample operation and centralized detection.

Novel aspect

A novel Touch Paper Spray mass spectrometry-based immunoassay platform was developed to enable on-chip, on-demand disease diagnosis.

2016 OMSS GUEST SPEAKER ABSTRACTS

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Label-free Protein Quantitation by top-down Proteomics for Biomarker Discovery

Introduction

Recent advances in high-throughput genomics have allowed deep characterization of cancer at the DNA and RNA level. Despite a detailed catalogue of mutations and polymorphisms in cancer, it is still uncertain how they may translate into proteome variation and which of those genomic abnormalities drive tumor biology. Here, we utilized both top down (TD) and bottom-up (BU) proteomic approaches to detect cancer-specific aberrations at the peptide and proteoform levels and to measure differential expression of proteins and proteoforms. Using genomically well-characterized patient-derived xenograft (PDX) models of basal and luminal B breast cancer, we compared the performance of TD and BU proteomics for gene identification, whole proteoform characterization, and detection of differential expression.

Methods

PDX samples established as Comparison Reference (CompRef) samples within the Clinical Proteomic Tumor Analysis Consortium (CPTAC) were used. Tumors were harvested, subjected to cryopulverization and the pulverized tissue from each CompRef sample (WHIM16, P33, luminal B) and (WHIM2, P32, basal) was solubilized into an SDS-containing buffer. The proteome below 30 kDa was isolated by GELFrEE separation and analyzed by TD on an Orbitrap Elite (Thermo) and BU on a TripleTOF 5600+ (AB Sciex) mass spectrometer. Data were searched against tumor-specific protein sequence databases that included variants detected in whole genome sequencing (WGS) of the xenografts and the corresponding germline and alternative splice forms detected by RNA-Seq.

Preliminary Data

The analysis of PDX proteomes by TD resulted in the identification of over 1000 gene products and >5000 proteoforms with an estimated false discovery rate <5%. The number of proteoforms per protein (unique accession number) identification varies significantly. About half of all proteins identified, were detected as a single proteoform, ~30% of proteins were represented by two proteoforms, and the rest were detected as 3 or more proteoforms. Using our recently established label-free top-down quantitation platform, we were able to quantify >1000 unique masses, of which 538 were unambiguously identified using MS/MS information.

A comparison of TD and BU for protein identification and quantitation was performed. BU resulted in a greater number of protein identifications than TD. However, as tryptic digestion may sever the linkage between modifications co-occurring on the same molecule, information about PTM stoichiometry was lost. An example of this was the detection of alpha-endosulfine, whose phosphorylation affects secondary structure and its corresponding protein-protein interactions. While the protein was detected by both methods and was found to be differentially expressed in the two samples, only TD was able to detect changes in phosphorylation levels. Several translated single nucleotide polymorphisms (SNPs) were detected by both TD and BU, but TD uniquely

identified SNPs co-occurring with PTMs. In addition, several truncated forms of type 2 cytoskeletal keratin 8, a variable diagnostic tool in differentiating lobular and ductal breast cancers, were detected by TD. In BU, protein truncations are difficult to detect since intact protein length information is lost.
Of those proteins detected by both methods and mapping to the same accession number, the TD and BU quantitation agreed 60% of the time. However, the ability to detect and quantify proteoforms makes TD more sensitive at determining changes in PTM and variant expression that may be relevant in cancer biology.

Novel Aspect
First large-scale integration of genomic, bottom-up and top-down proteomic data for comparative analysis of tumor xenografts.

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Full Spectrum Molecular Imaging: Multimodal Imaging MS for Biomedical Applications

In 1997, Mass spectrometry imaging (MSI) was introduced by Caprioli *et. al1*. Since then, MSI has been gaining importance in clinical, 'omics and pharmaceutical research areas aided by the significant technological improvements achieved over the past decade or more. In the last few years, several ambient ionization techniques have been developed to ionize molecules directly from tissue, such as desorption electrospray ionization (DESI).

Full Spectrum Molecular Imaging is the combination of two ionization techniques (DESI and MALDI) on a single instrument platform, with the possibility to integrate ion mobility separation and powerful informatics workflows. This collaboration of technology allows for the extraction of maximum levels of molecular distribution directly from the surface of a single, or multiple samples.
This presentation will discuss the principle of MS imaging using MALDI and DESI ionization, applied to a variety of biomedical samples, ultimately demonstrating the complementary chemical information that can be obtained.

1: Caprioli, R.M; Farmer, T.B; Gile, J. *Anal. Chem*. **1997**, 69, 4751

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Biomarker Discovery and High-Resolution mass spectrometry is possible on a MALDI-FTMS imaging system

MALDI imaging coupled with a high-resolution mass spectrometer such as FTMS is typically considered an ideal platform for mapping small endogenous and exogenous compounds ranging up to a few hundred m/z in thin tissue sections. Performance features such as sub-ppm measurement accuracy and mass resolution of >500,000 make it possible to clearly separate compounds that differ in mass by 1-2 mDa while accurate mass and isotopic fine structure often can assign a single formula identification to each compound.

Recent advances in sample preparation strategies and software combine with the high-performance instrumentation to extend the utility of MALDI-FTMS imaging to mapping larger and more complex analytes, opening the door for new and novel biomarker discoveries. Applying proteolytic enzymes such as trypsin directly onto the tissue sections makes possible to measure peptides from thousands of proteins. The high mass resolution resolves many unique peptides at each nominal m/z allowing them to be mapped and their abundance correlated with tissue state. Once digested, the peptides can be extracted and analyzed by LC-MS to provide identified proteins which we have assigned to the images. Other enzymes such as PNGase F liberate N-linked glycans from glycoproteins in the tissue allowing the abundance and nature of various glycosylations to be correlated to tissue or disease state. Finally, so-called super charging matrices can be employed to produce highly-charged intact protein ions that would otherwise not be measurable when using common matrices that produce predominantly singly-charged ions.

2016 OMSS POSTER ABSTRACTS

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A Multi-model Statistical Approach for Proteomic Spectral Count Quantitation

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Introduction

The rapid development of mass spectrometry technologies has solidified shotgun proteomics as the most powerful analytical platform for large-scale proteome interrogation. The ability to map and determine differential expression profiles of the entire proteome is the ultimate goal of shotgun proteomics. Our novel approach to determine differential expression from spectral counts in shotgun proteomics leverages multiple statistical platforms to assess differential expression from spectral count data derived from multiple proteomic pipelines. This analytical approach allows for *in silico* cross-validation of proteomic results and increases the depth of the experiment with the ultimate goal of understanding the biological system of interest.

Methods

The dataset described by Chen *et al*. was used to validate that the use of spectral counts generated from three independent proteomic pipelines (MassMatrix, MyriMatch and Proteome Discoverer) is a robust and acceptable approach to estimate fold-changes. This dataset consisted of 36 human proteins spiked into a *Pyrococcus furiosus* (Pfu) lysate. Once these pipelines were determined to be appropriate for spectral counting, they were then used to highlight the ability of our approach. We used this approach, MultiSpec, to identify unknown proteomic changes from a murine model of multiple sclerosis (EAE/Sham) previously described by Dagley *et al*.

Preliminary Data

Count data derived from RNA sequencing experiments is routinely analyzed with edgeR, DESeq and baySeq. These statistical approaches leverage the negative binomial distribution to perform hypothesis tests on multivariate data from discovery-based experiments. In these hypothesis-generating experiments, biological replicates are often limited and the ultimate goal is to deepen the understanding on how sets of proteins/genes are being influenced by a given, often unknown, stimulus. MultiSpec is our approach to evaluating differential expression. The analysis is compiled in R and is executable from command line. The automated output consists of several diagnostic plots to evaluate data quality from multiple proteomic pipelines. By leveraging multiple search engines, protein identifications from each pipeline are confirmed and the depth of the shotgun proteomics experiment is enhanced. However, due to the presence of protein groups introduced by each protein inference mechanism, there is an inherited discordance regarding the overall dataset. This ambiguity between proteomic pipelines is removed through using an iterative protein group-collapsing mechanism and a single ranked list of differentially expressed proteins is obtained.

Novel Aspect
We developed an automated approach to integrate differential expression analyses from spectral count data obtained from multiple proteomic platforms.

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Native Mass Spectrometry as a Structural Biology Tool

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Introduction

A rapid and comprehensive characterization of proteins is desirable to determine the effects of point mutations on the protein structure, ability to interact with ligands and on the propensity to form oligomers. Native mass spectrometry is a particularly suitable tool for the structural characterization of proteins and protein complexes due to its sensitivity, speed, low sample consumption and possibility to analyze even large complexes in the mega Dalton range. In combination with ion mobility measurements and gas phase dissociation techniques, information on stoichiometry, ligand binding, shape, complex topology and interconnectivity of subunits can be gained. In this work, we focus on pentameric C-reactive protein (CRP), which is a host-defense molecule and a biomarker for some inflammatory diseases.

Methods

We mutated the three critical amino acids at the phosphocholine-binding site of CRP and an amino acid at the inter-subunit interaction surface of CRP. Using native mass spectrometry techniques, we determined the effects of these mutations on the ligand-binding property and oligomerization of CRP. Prior to mass spectrometric analysis, proteins were buffer exchanged to 200 mM ammonium acetate. Samples were analyzed using an Exactive Plus EMR orbitrap mass spectrometer (Thermo) and a Synapt G2 quadrupole ion mobility time-of-flight mass spectrometer (Waters) with a surface induced dissociation (SID) device placed after a truncated Trap cell.

Preliminary Data

Titration studies showed that CRP binds Ca^{2+} ions with high affinity and that the mutation of three amino acids prevents the binding of phosphocholine to CRP. The interaction between monomer subunits is considerably weakened by low pH as well as by an amino acid mutation at the inter-subunit surface.

Novel Aspect

The effect of amino acid mutations on the ligand-binding property and oligomerization of CRP can be studied by native mass spectrometry.

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Direct Analysis of Capsaicinoids by Thread Spray Ambient Mass Spectrometry

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Introduction

Direct analysis of capsaicinoids in pepper spray is achieved with a novel thread spray ionization method. Pepper sprays are available to both law enforcement personnel and the general public for riot control and self-defense purposes. The presence/absence of pepper spray on an evidentiary garment may help establish the realities of the incident. Colored dyes are sometimes added to the pepper spray mixture to assist in locating the position of capsaicinoids found in pepper spray for subsequent extraction and characterization. When no stains are visible, testing is limited to random sampling, which may be tedious. The current study explores a mass spectrometric method that is capable of using fabric threads for rapid in-situ determination of probative value of evidentiary garments.

Method

100% cotton, 100% polyester, and 65:35 cotton:polyester fabrics were purchased from Jo-Ann Fabric and used without treatment. Pepper spray (Pepper Power by UDAP Industries) was sprayed onto the fabrics and allowed to dry. Two experiments were performed to analyze the capsaicinoids (i) thread spray MS – a single thread was pulled from the sprayed fabric and placed inside of a glass capillary (length = 5 mm; ID = 0.8 mm). The glass capillary was filled with 10 µL of methanol, and a DC voltage (3 kV) was applied to the thread. This glass/thread/liquid assembly was set in front of a LTQ mass spectrometer for analysis; (ii) a piece of the fabric was cut into triangles and used for direct capsaicinoids analysis.

Preliminary Data

Pepper spray mixtures typically contain five different capsaicinoids, including capsaicin (69%; *MW* 305), dihydrocapsaicin (22%; *WM* 307), nordihydrocapsaicin (7%; *MW* 293), homodihydrocapsaicin (1%; MW 321), and homocapsaicin (1%; *MW* 319). The capsaicinoids share a common basic structure consisting of a linear alkyl group of varying lengths connected to a phenolic head group via an amide bond. The weak acidic nature of the phenolic moiety allows MS detection in the negative mode through the generation of $[M-H]^-$ ions. All five capsaicinoids were sensitively and simultaneously detected during the thread spray experiment, without the need for sample pre-treatment. Negative ions from capsaicin (*m/z* 304) and dihydrocapsaicin (*m/z* 306) were the most abundant ions. All ions fragmented through a diagnostic elimination of a neutral phenolic head group (*MW* 136). This common fragmentation pattern made it easy to confirm the presence of the low abundant capsaicinoids from all fabric types tested. These preliminary results suggest that a thread found at a crime scene has potential to become useful physical evidence. In addition, the thread spray ionization method can enable reduction in backlogged evidence if combined with portable mass spectrometers for in-situ material analysis. Data from optimized

thread spray will be presented that involves the use of different solvents and voltages. The performance (i.e., sensitivity and accuracy) of the method will be characterized by using well defined thread sizes and purified capsaicin and dihydrocapsaicin. The robustness of the method will also be tested; here, fabrics coated with capsaicin and dihydrocapsaicin will be stored and analyzed at different times. We expect the sensitivity to decrease if the capsaicinoids decompose for fabric stored under different conditions. Also, we will investigate the effect of washing the fabric using various detergents.

Novel Aspect

A novel thread spray ionization method enables direct and in-situ analysis of capsaicinoids in pepper spray coated on different fabrics.

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2D Wax-Printed Paper Substrates with Extended Solvent Supply Capabilities Allow Enhanced Ion Signal for Analysis of Water Samples using Paper Spray Mass Spectrometry

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Introduction

Solid wax printing represents an efficient approach to create microfluidic channels on paper in which the working hydrophilic regions are surrounded by hydrophobic wax barriers. We hypothesized that the well-defined hydrophilic channels generated from the wax-printing methodology can be utilized to confine DC potentials in narrow regions of the paper for efficient use of electrical power. The resultant wax-printed paper substrates enabled (i) the use of lower voltages (0.5 kV) for analyte ionization, and (ii) extended signal lifetime which permits both improved signal averaging and provides an increased timeframe to monitor multiple fragmentation/reaction pathways in tandem MS (MS/MS) experiments. The new wax-printed paper spray method was applied for the analysis of corrosion inhibitors (e.g., Duomeen) and pesticides (metaldehyde) in water samples.

Methods

The wax-printed paper substrates were created by solid wax printing using a commercial Xerox ColorQube wax printer. The paper (Whatman grade 1) on which the wax has been printed was then heated to 95°C for 3 minutes to allow the wax to permeate the paper fibers. The wax is patterned in such a way to allow a hydrophilic channel for the sample and spray solvent that lead to the tip of the paper triangle. Five microfluidic channels were created and optimized for paper spray (*Analyst*, DOI: 10.1039/C6AN00168H). By confining sample and solvent to a smaller area, the electric field is altered at the tip of the paper, increasing ionization efficiency of small organic molecules. Compared to regular un-waxed paper, sample analysis time was greatly increased. For example, when 20 μL 1:1 MeOH/H_2O v/v was sprayed from wax and un-waxed paper, the total ion current (TIC) revealed analysis time was 10 minutes and 1.5 minutes, respectively.

Preliminary Data

The motivation of this study was to develop a rapid and simple analytical method for water analysis in the field. Analytes of interest include Duomeen and metaldehyde. Duomeen is a widely used chemical substances for controlling corrosion in high pressure water-tube boilers. Direct detection and motoring is necessary for boiler maintenance. Use of low spray voltages will facilitate field analysis. With the new wax-printed paper substrates, Duomeen is sensitively detected with calculated limits of detection (LODs) of 0.09 pg/mL and 0.68 pg/mL for 3 and 1 kV spray voltages, respectively using spray solvent of 4:1 MeOH/H2O v/v. Excellent linearity (R^2 = 0.9997) and precision (RSD = 7.5%) were achieved without internal standards. This performance is due in part to the high ionization efficiency of the duomeen (a diamine) and the occurrence of a long lasting stable spray from the wax-printed paper substrate which enables ensemble averaging of many

spectra. Analysis of metaldehyde is necessitated by recent reports of metaldehyde poisoning and death in both animals and humans. The molluscicide is used in agriculture to control slugs in order to protect crops. However, large residues of metaldehyde are mobilized during heavy rainfalls, which end up in rivers and groundwater and finally in drinking water. In fact, the European Commission and U.S. Environmental Protection Agency have both issued instructions on permissible level for metaldehyde, restricting its use as a pesticide. At 3 kV, LOD was determined to be 4.9 ng/mL with an LOQ of 6.2 ng/mL using protonated ions, and at 1 kV, LOD was 5.2 ng/mL with a LOQ of 8.2 ng/mL for sodiated ions.

Novel Aspect
The use of wax-printed paper substrates in paper spray ionization achieved extended analysis time with lower DC voltages.

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Electrospray-based Photo-catalytic screening method for Studying Electronic Effects on Oxidative dehydrogenation of N-heterocycle Compounds

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Introduction

Photocatalytic reactions have many advantages over thermal reactions including the ability to overcome large activation barriers, formation of thermodynamically disfavored products and eco-friendliness. Therefore photo-catalytic screening platforms are essential in discovering new reaction pathways; the ability to achieve on-line optimize in order to gain efficient product generation will be an added advantage. Catalytic oxidative dehydrogenations of N-hetorocycles are important as they are structural components of natural as well as pharmacologically active substances. The current methods of oxidative dehydrogenations require sophisticated/expensive catalysts and high pressures of O_2. Here, we describe a new elctrospary-based photo-catalytic analytical platform capable of studying the electronic effect (e.g., inductive and pi-conjugation effects) on oxidative dehydrogenation of N-derivatives of 1,2,3,4-tetrahydroquinoline (**THQ**) and 1,2,3,4-tetrahydroisoquinoline (**THiQ**) into its respective (iso)quinolines under atmospheric O_2 using a unmodified Ruthenium catalyst.

Method

Experiments were performed on a newly developed picomole-scale photoreaction screening platform, which involves the coupling of portable laser source with nano-electrospray ionization (nESI) emitter. The secondary/tertiary amine reactant and the $Ru(bpy)_3Cl_2$ (bpy= 2,2'-bipyridine) photo-catalyst were contained in a single barrel nESI glass capillary; application of ~1.2 kV DC voltage to reaction mixture produced charged droplets, containing the reactants, which were transported to mass spectrometer (MS). In-situ exposure of these charged droplets to the blue coherent laser light (wavelength 452 nm, power 5 mW) initiated photochemical reaction under ambient conditions, and products were promptly characterized by MS in real time.

Preliminary Data

In the presence of visible light, $[Ru(bpy)_3]^{2+}$ is excited after photon absorption, which subsequently react with N-hetorocycle (amine) through an one electron transfer to produce amine radical cation. This species then loses 2 hydrogen atoms (H_2 elimination) to form the iminium product. In the presence of the laser light, the THQ/$[Ru(bpy)_3]^{2+}$ reaction mixture yields protonated species at *m/z* 134 during nESI-MS analysis. After a brief exposure to the visible light, the on-line nESI-MS analysis showed the presence of protonated 3,4-dihydroquinoline at *m/z* 132 via the loss of two hydrogen atoms. Increasing the exposure time to 1 min produced the expected quinoline at *m/z* 130 (72% yield, through four hydrogen abstractions). It was hypothesized that tautomerization of 3,4-dihydroquinoline to 1,4-dihydroquinoline is an important step to allow the formation of this quinoline product. To investigate this expectation, THiQ, a constitutional isomer of THQ was used

in the photo-reaction. Here, continuous exposure of THiQ/$[Ru(bpy)_3]^{2+}$ to light for >5 min did not give the expected isoquinolines product at *m/z* 130. Instead, protonated 3,4-dihydroisoquinoline species at *m/z* 132 were observed. The inability of THiQ to undergo complete dehydrogenation was attributed to the localization of the double bond at allylic position, which restrains the delocalization of nitrogen electrons after the tautomerization step and prevents further dehydrogenation. To further test this hypothesis, N-phenyl derivatives of THiQ were employed. Results showed that 2-Phenyl-1,2,3,4-tetrahydroisoquinoline (2-phe-THiQ) was susceptible to full dehydrogenation after exposure to blue light and $[Ru(bpy)_3]^{2+}$ catalyst. Results from other derivatives that can induce conjugation and/or induction will be presented. MS data will be correlated with computer simulations.

Novel Aspect

Electrospray-based photoreaction screening platform facilitates the study of electronic effects in dehydrogenation reactions

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Elucidation of the Droplet Reactivity Source in Contained-electrospray Ionization

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Introduction

Contained-electrospray ionization (ESI) is a new method for controlling charged micro-droplets reactivity. It has proven to be powerful for elimination of matrix effects during ESI. When compared to conventional electrospray, significant improvements in accuracy, precision, and sensitivity were achieved. Contained-ESI has also shown promise for online folding/unfolding of proteins in microseconds time scale. These performance characteristics have been ascribed to the generation of fine reactive droplets in the presence of excess of protons. The current study seeks to understand the source/nature of the protons that impact the droplets with the observed reactivity; specifically, we are interested in studying gas-phase contributions in ESI. This objective is accomplished through H/D exchange reactions, which revealed that exchange-out phenomenon is achievable in contained-ESI.

Methods

A cross Swagelok unit was used to construct contained-ES apparatus that consists of three inlets for nebulizer gas, head space vapor of modifying reagent and analyte solution. The apparatus has one outlet for continuous generation and modification of electrospray droplets after applying a high DC voltage to a movable ES emitter that contains the analyte solution. The emitter is inserted into outer capillary allowing operation in two modes: (i) Type I mode – the ES emitter is pushed slightly outside (~0.5 mm) of the outer capillary (for short droplet modification on microseconds time scale) and (ii) Type II mode – the ES emitter is pushed inside (~5 mm) the outer capillary (for extended droplet modification with vapor of modifying reagent).

Preliminary Results

A recent innovation in mass spectrometry is the ability to use charged micro-droplets as reaction vessels. Surface protons are suggested to contribute predominantly to droplet acidity and reactivity. We have observed an enhanced electrospray droplets reactivity by exposing them to HCl vapor. The current study evaluates the contribution of the HCl vapor through H/D exchange. The contained-ESI apparatus was used for analysis of illicit drugs prepared in various D_2O/CD_3OD solvents, and comparing resultant mass spectra with those obtained in non-deuterated solvents droplets (treated with deuterated solvent vapor) to investigate gas-phase contribution in the contained-ESI. Results show that $[M+D]^+$ ions were formed only when the analyte was prepared in deuterated solvent; charged droplet environment was unable to induce D^+ generation from D_2O/CD_3OD vapors. When using HCl vapor in the contained-ESI ion source, $D^+ \rightarrow H^+$ exchange-out process was not observed for cocaine $[M+D]^+$, irrespective of droplet reaction time used. These results support the general hypothesis that redox reactions involving electrospray solvents are responsible for surface charges in ESI. Furthermore, given that HCl has a modest gas-phase acidity

(1395 kJ/mol) suggests that it must be captured and dissolved into the droplet bulk (at low N_2 pressure) in order to influence ESI performance. We propose that both the droplet surface and bulk can be equally reactive.

Evidence for droplet bulk reactivity is observed when fully deuterated amphetamine underwent an exchange-out process in the presence of HCl vapor (Type II mode). Unlike cocaine, amphetamine has two exchangeable hydrogens, and these exchangeable sites (not same as solvent D^+) are recovered. The ability to distinguish between actual H/D exchange and D^+ adduction in ESI can provide a unique approach to study reaction mechanisms. Results will be validated by using DCl vapor, and by cyclization reactions, that typically involve internal hopping of protons.

Novel Aspect
Contained-electrospray ionization enable efficient differentiation between H/D exchange and D^+ adduction.

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Detection of Metal Ions with Flowing Atmospheric-Pressure Afterglow-Mass Spectrometry (FAPA-MS)

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Introduction

Atomic spectrometry is one of the most established analytical fields and is widely practiced in a range of scientific disciplines such as environmental control, forensic analysis and medicine. Conventional methods for metal-ion detection often require extensive sample preparation and instruments that are expensive to operate and maintain. As such, a faster, simpler approach for screening metals is needed with minimal compromise in sensitivity. Further, it would be advantageous if such a tool could be made field deployable for *in situ* analyses. The present study explores the utility of the flowing atmospheric-pressure afterglow (FAPA) ambient desorption/ionization (ADI) source as an approach to directly and quickly screen solid and liquid samples for various elemental species.

Methods

The FAPA source used here was based on a helium atmospheric-pressure glow discharge. Trace amounts of elemental species were deposited on a mesh positioned between the FAPA and the inlet capillary of the mass spectrometer. Samples were introduced to the afterglow either as solids or as solutions. Metal ions have low vapor pressures and cannot be thermally desorbed at the temperatures offered by the FAPA afterglow. To aid in the desorption of metal ions, a common metal chelating agent, acetylacetone (AcAc), was co-deposited with the sample prior to analysis. The possibility of elemental speciation, particularly for Cr(III) and Cr(VI), was explored through a rapid complexation reaction with 1-pyrrolidinecarbodithioic acid ammoniate (APDC).

Preliminary Data

Several elemental species were subjected to the FAPA to explore the capabilities of the source for elemental mass spectrometry. Copper(II) salts were found to be the only metal species that could be directly detected as solids, while uranyl and lead salts could be detected in aqueous solutions without additional complexation agents. Interestingly, pH was found to have a significant effect on the detection of uranyl nitrate with greater signals at lower pH values; ultimately, a detection limit of 4 fmol was determined at pH 1.

For other metal cations, rapid complexation with AcAc was necessary for detection. Metal-AcAc ions were observed for over 20 elemental species, mainly in the p and d-blocks of the periodic table along with lanthanides and actinides. The two most abundant ions in many of the mass spectra corresponded to the addition of a number of AcAc ligands equal to the charge of the solution-phase metal ion with the addition of a proton (*e.g.*, $M^{2+}+2(AcAc)^{1-}+H^{+}]^{+}$) and the addition of one

less AcAc ($[M^{2+}+(AcAc)^{1-}]^{+}$). Even in the presence of AcAc, bare metal ions were observed for thallium(I) and copper(II).

Initial studies to speciate the forms of metals in samples have been explored for Cr(III) and Cr(VI) by FAPA-MS. It was found that Cr(III) ions were readily detectable with the use of AcAc, while Cr(VI) was reduced to Cr(III). Speciation of Cr ions by inductively coupled plasma and high-performance liquid chromatography techniques have been reported in literature via complexation with APDC to prevent the reduction of Cr(VI) during analysis. Traditionally this method required an hour of heating and stirring to synthesize a stable solid. However, we have shown that exposure of the two solids to the afterglow of the FAPA produced signals indicative of a Cr(VI)-APDC complex without the need for the extensive sample pretreatment.

Novel Aspect
The presented method illustrates the ability of FAPA-MS to be used as a direct analysis technique for bare elemental species.

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Investigation of Protein-Protein Interactions using Surface Induced Dissociation (SID) MS

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Introduction

Proteins have well defined tertiary structures and form quaternary assemblies via non-covalent interactions. Understanding protein structures and assemblies is necessary to understand how proteins accomplish their physiological functions. One important tool for structural analysis is native mass spectrometry (MS), which is commonly employed to determine the stoichiometry and topology of protein complexes. However, currently it is not evident whether native MS in combination with gas phase disruption techniques is suitable to obtain detailed quantitative information on the number and nature of non-covalent interactions between constituent subunits of a protein complex.

Methods

To answer this question, a set of homologous protein complexes with distinct non-covalent interaction between constituent subunits was used. Complexes were analyzed by a modified Synapt G2 mass spectrometer (Waters) that has a surface induced dissociation (SID) device installed between a truncated Trap cell and the ion mobility cell.

Preliminary Data

SID data was obtained for six homologous tryptophan synthase β dimers and αββα tetramers. For all cases, we observed a dissociation into the constituent subunits after collision of the corresponding complexes with a surface (SID). Interestingly, homolog complexes differ in the energy necessary to cause dissociation by SID. This indicates that similar protein-protein interactions with distinct inter-subunit connections can be differentiated by this gas-phase disruption technique.

Novel Aspect

Surface induced dissociation (SID) is suitable to distinguish between similar protein-protein interactions within sets of homologous protein complexes.

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Differential Mass Spectrometry Analysis of Transfer RNA by Stable Isotope Labelling

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Introduction

Post-transcriptional modification is one of the unique features of cellular RNAs and is a necessary step in the RNA maturation process. Transfer RNAs harbor a large set of chemical modifications that are obtained post-transcriptionally. Chemical modifications can vary from simple methylation to a complex chemical arrangement arising from multi-step pathways involving many enzymes. Because of the fundamental role tRNAs play in protein synthesis, it is important to know what tRNAs are present and how much of the tRNA population is modified. We have developed a differential mass spectrometry based method where a target tRNA is compared with a transcript synthesized using isotopically enriched nucleotides.

Methods

Reference and sample: A transcript of the tRNA of interest is synthesized from a PCR amplified gene block template. The gene of interest is downstream the T7 RNA polymerase promoter sequence. All guanosines in the sequence are isotopically enriched with ^{13}C and ^{15}N. The sample tRNAs are *E Coli* tRNA tyrosine (GTA) and isoleucine (GAT).

Chromatography and Mass spectrometry: Waters Xbridge™ C18 column; mobile phase A (MPA) of 200 mM hexafluoroisopropanol, 8.15 mM triethylamine (pH 7.0); mobile phase B (MPB) of 50% MPA and 50% methanol for separation. LTQ XL mass spectrometry conditions: negative polarity, 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.

Preliminary data

The reference, which is the unmodified version of the tRNA of interest, serves two purpose for this method. Firstly, it is used to create a baseline set of RNase T1 digestion products' *m/z* values. RNase T1 is known to cleave RNA at any unmodified guanosines, generating digestion products containing a single 3'-guanosine. If the sample and the reference have the same sequence, a doublet is observed, separated by a +15 Da mass shift due to the isotopically enriched guanosine in the digestion product. If the two digestion products have different sequence or modification status, a singlet will be revealed. MS/MS will confirm the sequence and location of any modifications. Secondly, it will serve as an internal standard for direct quantitative purposes. A predicted list of all singlets and doublets (*m/z* values) facilitate data analysis. In addition to demonstrating the identification of chemical modifications in the sample, a calibration curve can be created using the

isotopically enriched reference with the goal of providing absolute quantitative information on chemical modifications at specific sequence locations in the sample.

Novel Aspect
Comparative analysis of RNA digests by isotope labelling; improvement in mass spectral identification for reduced data processing.

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LDI-MS of Peptides Labeled on Carboxyl Groups with Fluorescent Dyes

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Introduction

Fluorescence labeling has been employed to improve detection and sequencing of peptides by different MS techniques. Most peptide derivatization methods are based on the labeling of N-termini, but labeling of peptide C-termini with a fluorophore can also be useful in sequence interpretation and detection of peptides. To label peptide carboxyl groups with fluorophores, appropriate synthetic protocols have been developed. In this study, carboxyl groups of peptides were labeled with fluorophores using modified labeling procedures, and such samples were analyzed by LDI-MS to study influence of labeling on peptide ionization and sequencing.

Methods

Derivatizations of the carboxyl groups of bradykinin (RPPGFSPFR) and a cyclic heptapeptide microcystin-LR (MC-LR) with fluorescent probes 4-(1-Pyrene)butanoic acid hydrazide (PBH) and rhodamine 110 (R110) were performed by modifying literature protocols (Kaminski et al., J. Am. Chem. Soc., 2005, 127, 16912-16920 and Hayama et al., Anal. Chim. Acta., 755, 2012, 93-99). Peptide solutions were prepared in methanol (1 mg/mL), and equal amounts of fluorescent probe and 4,6-dimethoxy-1,3,5-triazin-2-yl-4-methylmorpholinium (DMTMM) were added. DMTMM was used as the coupling reagent. The mixture was vortexed and incubated at 60^{o}C for 4 h. The samples were purified by solid phase extraction (SPE) using C18 cartridges before LDI-MS analyses, which were performed using a MALDI TOF/TOF-MS (Bruker) in reflectron positive ion mode. Additionally, LC-ESI-MS analysis of derivatized MC-LR was performed using an HPLC from Shimadzu and an Orbitrap Fusion MS from Thermo.

Preliminary Data

In this study, carboxyl group of the peptides were successfully labeled with PBH and R110. The peptide derivatization conditions were optimized by changing the concentrations of the peptide, fluorophores, and coupling reagent as well as reaction time and temperature. The C-terminus of bradykinin was derivatized with PBH and R110, and fluorescently labeled samples were analyzed by LDI-MS. LDI-MS spectra showed intense peaks at *m/z* 1344.83 and 1372.81 corresponding to singly-charged protonated ions of PBH and R110 derivatized bradykinin, respectively. In addition, MC-LR, which contains aspartic and glutamic acid residues, was labeled with PBH. The components of the reaction mixture were then separated by HPLC and analyzed by ESI-MS. PBH-labeled and unlabeled MC-LR were separated and detected as singly-charged ions with *m/z* values of 1563.93 and 995.56. Fluorescently-labeled MC-LR will be collected and analyzed by LDI-MS. Further experiments will also aim to improve C-terminal labeling and to simplify MALDI and LDI-MS/MS spectra of peptides.

Novel Aspect

The study of the ionization and fragmentation of peptides that were fluorescently labeled on carboxyl groups using LDI-MS and LDI-MS/MS.

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Mass Spectrometry Characterization of Isomeric Biodegradable Polyesters

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Introduction

Biodegradable synthetic polymers are promising bioactive materials in tissue engineering applications [1]. Polyesters based on fumaric acid, in particular poly(propylene fumarate) (PPF), have received attention in bone tissue engineering due to their suitability as inert cross linkable resins for the preparation at scaffolding materials by additive manufacturing (3-D printing). The well-defined molecular weight (MW) PPF oligomers needed for this purpose are not synthesized directly but by post polymerization isomerization of poly(propylene maleate) (PPM), which is prepared by ring opening polymerization of maleic anhydride and propylene oxide [2]. Here we report the detailed molecular structure characterization of initial (PPM) and final (PPF) product by MALDI-MS, ESI-MS, ion mobility mass spectrometry (IM-MS), as well as tandem mass spectrometry (MS/MS).

Methods

The samples analyzed were PPM (cis form) and PPF (trans form) with a putative average molecular weight around 2000 Da. PPM was isomerized to PPF using diethylamine as catalyst [2]. MS and MS/MS studies of these samples were carried out on a Bruker Ultraflex III MALDI-ToF/ToF mass spectrometer. The IM-MS experiments were performed on a Waters Synapt instrument equipped with an ESI source. The samples were dissolved in $CHCL_3$ (MALDI) or $CHCl_3$:MeOH 3:7 v/v (ESI) at final concentration of 10 mg/mL and 0.01 mg/mL respectively. For MALDI analysis, the sandwich method was used, with trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene] malononitrile (DCTB) serving as matrix and NaTFA as cationizing salt.

Preliminary Data

The MALDI mass spectra of PPF and PPM show one major and two minor distributions arising from different end groups (EGs) and having the composition $[Mx+EGs+Na]^+$. The mass difference between two consecutive peaks within each distribution is 156 Da, which matches the mass of the two alternating comonomers ($C_7H_8O_4$). End groups with masses of 46 Da, 104 Da, and 162 Da are observed, corresponding to C_2H_6O, $C_5H_{12}O_2$ and $C_8H_8O_3$ moieties, respectively. MS/MS analysis provided detailed information about the connectivity and individual (α and ω) end groups of these polymers. Fragmentation occurs through random 1, 5-H rearrangements over the polyester backbone. These reactions unveil the polyester sequence. The MS and MS/MS data confirm the successful synthesis of the two isomeric copolymers with the synthetic strategy used. Furthermore, ESI coupled with IM-MS was used to distinguish the isomeric polyesters. IM-MS analysis separated PPM and PPF ions into two dimensions (m/z and drift time) according to their mobilities in the traveling wave field. PPM and PPF ions were separated based on their charge state (from +1

to +3) into unique 2-D locations with specific m/z ratios and drift times. Comparison between the isomeric species revealed differences in their drift times which can be used to distinguish them. For example, the singly charged ion at m/z 1249 from PPM and PPF was observed at a drift time of 8.75 ms and 9.03 ms, respectively. The IM-MS spectra also confirmed that the post polymerization of PPM to PPF proceeds with quantitative yield.

Novel Aspect

Multidimensional MS provides rapid structural information on biodegradable polyesters including composition and end groups, and readily differentiates isomeric chain sequences.

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Cleavable Isobaric Peptides as Mass Reporters for Mass Spectrometry-based Immunoassays

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Introduction

Molecular diagnostics based of antibodies and complementary DNAs is the method of choice for high-throughput protein biomarker detection. Most of these methods rely on colorimetric detection via enzymatic reactions or fluorescent probes. Though effect, there are some restrictions regarding probe stability and the number of probes that can be included in the assay, especially for multiplexed detection. Herein, we propose novel mass spectrometry-based diagnostic platform for rapid and multiplexed detection of protein disease biomarkers. The core of this approach is the use of readily available cleavable isobaric peptides as mass reporter in immunoassays. All peptides probes are rationally designed to have the same molecular weight (i.e., isobaric) but fragment to give specific daughter ions when subjected to collision-induced dissociation (CID), thus providing well-defined spectra resolution in a single experiment for multiple biomarkers when compared with colorimetric experiments.

Methods

Selected isobaric peptides probes will be conjugated to antibodies specific to the protein biomarker of interest, and used for immuno-reaction, which will be performed on wax-printed paper substrates. Current project has three main aspects: (i) optimization of conditions for peptide ion generation and fragmentation in order to obtain a highly sensitive mass reporters with characteristic fragment ions, (ii) design and incorporation of the selected mass reporter peptides into a cleavable probe unit and (iii) development of paper-based immunoassays. The cleavable probe unit will feature three functional characteristics: (a) sulfur-containing (-NCS) groups for coupling to antibodies, (b) easily ionizable peptide molecule for sensitive MS detection, and (c) a cleavable linkage allowing release of the peptide.

Preliminary Data

To achieve these objectives, native nano-electrospray ionization mass spectrometry (nESI-MS) was first used to characterize selected peptides. Here, an electrolyte rich solution of ammonium acetate (5 mM) containing the peptide is sprayed at low voltage. For this preliminary evaluation, we designed and synthesized three peptides: AKRRG, RRGKA, and GARKK, all having molecular weight of 586 Da. Native nESI-MS analysis of these peptides showed a predominant peak at *m/z* 294 for doubly protonated $[M+2H]^{2+}$ ions and a minor signal at *m/z* 587 as singly charged $[M+H]^{+}$ species. All peptides can be sensitively detected at 10 nM concentration levels, in tandem MS (MS/MS) mode. Most importantly, fragment ions from the three isobaric peptides produced in MS/MS are different, providing a simple way to differentiate them as mass tags for

different diseases. For example, CID of $[M+H]^+$ species from GARRK produced a signature fragment ion at *m/z* 441 via elimination of lysing (K) residue. Similarly, CID of $[M+2H]^{2+}$ ions yielded signature fragment ions at *m/z* 230. CID data for all peptides tested will be discussed. Due its abundance, linear calibrations curves are easily generated for $[M+2H]^{2+}$ peptide ions in 10 – 50 nM concentration range using the characteristic fragment ions. To turn these peptides into pH-sensitive probes for immunoassay, the isobaric peptides will be linked to phenyl thiocyanate (-NCS) moiety through an ester functional group. Results on pH sensitivity of this peptide-based probe will be presented.

Novel Aspect

Cleavable isobaric peptide probes provide unlimited possibility for multiplexed biomarker detection.

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Targeted Metabolomics of Alternatively Activated Macrophages Modified by Metallothionein 3 Protein

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Introduction

Macrophages play an important role in immunity and defense against viruses and other pathogens. Classically activated macrophages (M1) are involved in pro-inflammatory processes and kill pathogens, while alternatively activated macrophages (M2) are involved in the healing process and have anti-inflammatory properties. Recently, certain metallothionein proteins have been found to be involved in macrophage immunity processes. Due to the difference in phenotype between M1 and M2 macrophages, targeted metabolomics can be used to verify the specific role of metallothionein 3 (MT3) in regulating the change in macrophage phenotype.

Methods

Bone-marrow macrophage samples were treated with siRNA and then IL-4 followed by extraction of metabolites with methanol before quantification. Ultra high performance liquid chromatography was paired with electrospray ionization and high-resolution mass spectrometry for detection and quantification of metabolites. A 10-µL sample was injected onto a Hydro-RP column (100 mm x 2 mm, 2.5 µm) and separated with a mobile phase flow rate of 300 µL/min. The mobile phase consisted of a gradient of 97:3 H2O: methanol with 10-mM tributylamine and 15-mM acetic acid and pure methanol.

Preliminary Data

Here, an ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS) method was developed to separate and quantify six metabolites (fumarate, succinate, malate, glyceraldehyde-3-phosphate (G3P), glucose-6-phosphate (G6P), fructose-6-phosphate (F6P)), with tributylamine as an ion-pairing reagent. Changes in column and solvent gradient were then varied until separation and peak shape were achieved for each metabolite. Concentrations of fumarate, succinate, and malate were found to be higher in the M2 phenotype compared to other macrophage samples due to the expression of MT3. These three metabolites were more abundant in the M2 macrophage as this phenotype harvests energy through the citric acid cycle. In contrast, macrophages with a silenced Mt3 gene, were expected to produce a greater amount of G3P, F6P, and as M1 macrophages utilize the glycolysis pathway for energy conversion. The M1 samples verified this increase in G3P, but were found to be close to equal in the amounts of G6P and F6P as that of the M2 sample. Further studies will be conducted on different samples to confirm the

quantification of the metabolites. By silencing the Mt3 gene in macrophage samples, M2 macrophages reverted back to a M1 phenotype as shown by the change in metabolite concentrations. Based on this targeted metabolomic study, metallothionein 3 protein was found to have a direct role in the regulation of the M2 phenotype.

Novel Aspects

Metabolomic studies revealed the previously unknown function of metallothionein 3 protein, which could lend insights into the role of MT3 in immune processes.

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Picomole-scale Real Time Photoreaction Screening: Discovery of Visible-light Promoted Dehydrogenation of Tetrahydroquinolines under Ambient Conditions

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Introduction

The identification of new photocatalytic pathways expands our knowledge of chemical reactivity and enables new green synthetic applications. Miniaturized screening procedures/platforms for expediting photoreaction discovery remains challenging. Herein, we demonstrate, for the first time, a picomole-scale, real time photoreaction screening platform by coupling a handheld laser source with nano-electrospray ionization mass spectrometry. This method is successfully utilized for the discovery of an accelerated dehydrogenation pathway for the conversion of tetrahydroquinolines into the corresponding quinolines. This transformation is readily brought about using an off-the-shelf $Ru(bpy)_3Cl_2 \cdot 6H_2O$ complex and performing the experiment in air at ambient temperature in direct sunlight or with the aid of an energy saving lamp. Moreover, radical cations of the tetrahydroquinolines and trans-dihydride intermediates captured in the screening platform provided direct evidence for the mechanism of the new visible light photoredox reaction.

Methods

The photoreaction screening platform involved the use of a readily available handheld laser source (laser pointer), which allowed the generation of high lighting power density and coherent blue visible light. This light was directed toward miniscule volumes of reaction mixture contained in the transparent glass capillary of the nESI emitter. Charged micro-droplets containing reactants/products are generated when a direct current (DC) voltage of 1.0 kV is applied to the solution. Synchronized application of the laser source and the DC voltage enables analysis of the photo reaction in real time. In addition, the MS detection method allows both qualitative (via MS/MS fragmentation pattern) and quantitative (by measuring ion intensity) assessment of reaction progress.

Preliminary data

With this screening platform, we discovered an effective photocatalytic pathway involving the dehydrogenation of 1,2,3,4-tetrahydroquinolines to the corresponding quinolines, which are surprisingly catalyzed by common visible light harvesting complex $Ru(bpy)_3Cl_2$ under ambient conditions. The scale-up dehydrogenation reactions of 1,2,3,4-tetrahydroquinolines to quinolines afforded excellent yields in 2-4 h at ambient conditions, either under irradiation with an energy-saving lamp or by exposure to sunlight, all showing significant advantages over current methods. In addition, when combined with tandem mass spectrometry (MS/MS), this method enabled the rapid analysis and characterization of the structure of low abundant ruthenium complexes. Furthermore, this real time MS method enable the capture of radical cations and trans-dihydride

intermediates involved in the photo-redox reaction and provided direct evidence for the reaction mechanism.

Novel aspect
A picomole-scale real time photoreaction screening platform was established, and a novel visible-light promoted dehydrogenation of tetrahydroquinolines under ambient conditions was discovered.

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Quantitative Analysis of Microcystin-LR Using an HPLC-Orbitrap MS System

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Introduction

Cyanobacteria are naturally occurring prokaryotic organisms that often release harmful cyanotoxins, such as microcystin-LR (MC-LR). The World Health Organization has set a provisional guideline limit for the maximum MC-LR concentration of 1 ppb in drinking water. As a result, robust and sensitive techniques are needed to detect sub-ppb level concentrations of MCs in water samples. LC-MS and ELISA are two common quantification techniques used for MCs in water samples. The development of a LC-ESI-MS method using an HPLC-orbitrap MS system for the quantification of MC-LR is described in this work.

Methods

HPLC system (Shimadzu) was coupled to an Orbitrap Fusion Tribrid mass spectrometer (Thermo). Phenomenex Kinetex C8 HPLC column (2.1 mm x 100 mm) packed with 2.6 μm C8 solid phase particles was used. 20 μL of the MC-LR sample was injected and a binary gradient with a flow rate of 0.3 mL/min was used for analyses [EPA Method 544, **2015**]. In addition, a reproducible and efficient solid-phase extraction method was developed for the purification and preconcentration of MC-LR (Cayman). Quantification of MC-LR was performed by SIM of the singly-charged protonated MC-LR ion (*m/z* 995.56) and calibration curves were constructed based on the peak intensity.

Preliminary Data

An HPLC system was coupled to an orbitrap mass spectrometer for the identification and quantification of MC-LR using LC-ESI-MS. The SIM method parameters were optimized for ESI-MS detection of a MC-LR standard before LC-MS quantitation. The SIM scans detected the singly-charged protonated ion of MC-LR (*m/z* 995.56). Quantitation of MC-LR was performed by LC-MS by integrating the MC-LR peak (t_R~18.5 min). The peak intensities of MC-LR standards were very reproducible, and were used to quantify MC-LR standards in water samples. A calibration curve was constructed for the concentration range between10 ppt and 10 ppb without any preconcentration before the analyses. The LOQ of MC-LR in water samples was 10 ppt, which is comparable to LOQ obtained using other similar instruments previously. A SPE method was also developed that allowed for high recovery of MC-LR during the purification and preconcentration steps before LC-MS analyses. The preconcentration improved the LOQ to 500 ppq. More complex samples and other MC standards were also analyzed and quantified in order to validate the developed method.

Novel Aspect

Development of a sensitive and reproducible LC-ESI-MS method for identification and quantification of Microcystin-LR using an Orbitrap Fusion mass spectrometer.

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Validating the Roles of FraD and FraB in the Metabolism of F-Asn by *Salmonella*

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Introduction

Salmonella is a major cause of foodborne illness, with tens of millions of human cases reported globally. Gens responsible for fructose-asparagine (F-Asn) utilization, an Amadori product from food, were recently found to be essential for *Salmonella* growth in the inflamed intestine. The possibility that this nutrient is used specifically by *Salmonella* provides a novel drug target. Among the genes of the *fra* operon, *fraD* and *fraB* encode a putative kinase and deglycase, respectively. The goal of this study is to validate the role of FraD and FraB in the metabolism of F-Asn.

Methods

In vitro, the FraD reaction entailed adding purified recombinant FraD to fructose-aspartate(F-Asp) and ATP, incubating at 37°C, and terminating the reaction after a defined time interval by boiling.^{13}C labelled F-Asn spiked as an internal standard. The abundance of F-Asp and the proposed product of the FraD reaction, 6-phospho-fructose-aspartate(6-P-F-Asp) were quantified by using selective reaction monitoring (SRM) on a Xevo TQ-S mass spectrometer (Waters). Using recombinant FraB, we tested and quantitated the conversion of its proposed substrate(6-P-F-Asp) to aspartate and glucose-6-phosphate. For our *in vivo* studies, the *Salmonella* wild type, *fra* island deletion, and *fraB*::kan strains were cultured and lysed to permit metabolite extraction. By spiking defined amounts of 6-P-F-Asp into the material extracted from wildtype, a standard curve was generated.

Preliminary Data

In the *in vitro* FraD reaction, the normalized relative abundance of F-Asp decreased by 41% at 15 min and 57% at 30 min, compared to 0-min control. The normalized relative abundance of 6-P-F-Asp increased by 27-fold at 15 min and 45-fold at 30 min. Thus, we confirmed and validated the kinase activity of FraD, i.e., the phosphorylation of F-Asp to 6-P-F-Asp. With a collision induced dissociation (CID) voltage of 15 volts in the negative mode, the m/z 259 precursors of standard glucose 6-phosphate and fructose 6-phosphate show slightly different fragmentation patterns. In the FraB reaction system, m/z 259 precursor gives a fragmentation pattern similar to standard glucose-6-phosphate, confirming that glucose-6-phosphate is the product of FraB-catalyzed deglycation of 6-P-F-Asp. The normalized relative abundance of 6-P-F-Asp decreased by 11% at 20 min and 28% at 60 min. The normalized relative abundance of glucose-6-phophate increased by 8% at 20 min and 17% at 60 min. The normalized relative abundance of aspartate increased by

13% at 20 min and 100% at 60 min. Thus, we verified *in vitro* deglycation of 6-P-F-Asp to glucose-6-phosphate and aspartate by FraB. *In vivo*, the abundance of 6-P-F-Asp in *Salmonella* was also measured. Using the transition 376→125, the abundances of 6-P-F-Asp in *Salmonella* wild type, *fra* island deletion, and *fra*B::kan were calculated to be 0.02 ± 0.01, 0.02 ± 0.01 and 90 ± 10 nmol, respectively. Using the transition 376→242, the results were somewhat similar in terms of 6-P-F-Asp levels: 0.002 ± 0.002 nmol, 0.02 ± 0.01 nmol, 80 ± 5 nmol. The R^2 is 0.99 for the standard curve. Thus in the presence of *fraD* and absence of *fraB*, 6-P-F-Asp (the product of FraD and the substrate of FraB) accumulates in *Salmonella*, consistent with the proposed model for the F-Asn metabolic pathway.

Novel Aspect

The roles of FraD and FraB were confirmed in Salmonella, and establishes prospects for pursuing FraB as a drug target.

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RNA Modification Mapping - Software Development

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Introduction

Transfer ribonucleic acids (tRNAs) play a crucial role in protein biosynthesis. The main purpose of tRNAs is to decode messenger RNA during protein synthesis. Biologically active tRNAs are post-transcriptional modified, and these modified nucleosides are important for tRNA structure, stability and decoding. A typical experimental approach for modification mapping involves digestion of total tRNAs with a base-specific ribonuclease, and these digestion products are separated and analyzed by LC-MS/MS. One challenge of this strategy arises in the interpretation of tandem mass spectra data of modified digestion products. Here we develop a computer program to perform sequence and modification mapping and sequence annotation.

Methods

Escherichia coli total tRNA(10µg) was prepared and digested by RNase T1. The digestion products were separated on an XBridge BEH130 C18 3.5 µm column. Mobile phase A consisted of 8.2 mM TEA/200 mM HFIP at pH 7.0 in water; mobile phase B was produced by equal amount of mobile phase A and methanol. MS analysis of digestion products was done using a Thermo LTQ XL instrument. The MS/MS spectra were analyzed by in-house software, with the mass tolerance of precursors and fragments set to 1 Da and 0.8 Da, respectively. The binomial probability based scoring function was used to score matched digestion products according to the number of matched c-type and y-type ions.

Preliminary Data

There are 232 predicted RNase T1 digestion products in *E. coli* total tRNA sequences, and 83 of these digestion products have modifications. A typical MS/MS analysis results in a dataset of ~1800 mass spectra. From the MS/MS data and modified sequences (which were adopted from Modomics database), the program was able to obtain 55% coverage of total modified sequences, and the sequence mapping was done within 1 minute. The false discovery rate was ~1.5%.To examine site-specific modification mapping, 27 known *E. coli* total tRNA modifications were chosen as a test set. The program was able to obtain 35% coverage of total sequence, and it took ~8 hours to finish modification mapping. The false discovery rate was ~3%.We also tested the modifications mapping speed dependence on the numbers of modifications. We found 15 modifications in the basis set required ~3 hours for modification mapping; with 8 modifications the program could accomplish mapping in ~20 minutes. On-going efforts are directed at improving program speed, with improved accuracy and reduced false positives.

Novel Aspect
An automatic ribonucleic acids sequence and modification mapping program was developed.

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Evaluating the Impact of Soy Biodiesel and Petrodiesel Combustion Ultrafine Particles to Gut Bacterial Metabolism by Targeted LC-MS/MS Metabolic Profiling

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Introduction

Exposure to airborne particulate matter (PM), especially fine PM and ultrafine particles (UFPs) has given rise to pulmonary and cardiovascular diseases. However, emerging evidence suggests that the gastrointestinal (GI) tract is exposed to high concentrations of pollutant PM as well, either by mucociliary transport of inhaled PM from lungs into intestines, or by PM-contaminated food and drinks. Recent studies found the changes in the gut microbiome with GI tract diseases, but effects of PM and UFPs on the gut microbiome and their metabolism are largely unknown. So the health and environmental effects of UFPs are necessary to be studied. We conducted this proof of concept study to evaluate the impact of environmental ultrafine particles to gut bacterial metabolism *in vitro* using LC-MS/MS-based targeted metabolic profiling techniques.

Methods

Two types of UFPs collected from light-duty diesel engine emission tests by running either pure petrodiesel (B0) or petrodiesel blended with 20% soy biodiesel by volume (B20), were used to expose to bacteria in this study. Three intestinal bacterial strains, *Streptococcus salivarius* (*S.salivarius*), *Lactobacillus acidophilus* (*L.acidophilus*) and *Lactobacillus fermentum* (*L.fermentum*) were purchased from ATCC and incubated based on their growth protocols. The particles were dissolved into sterilized water and then mixed with bacterial culture for 4-hour co-incubation. Bacterial metabolites were extracted by using cold methanol extraction prior to targeted metabolic profiling. A Thermo Scientific Ultimate 3000 HPLC system (with a hydrophilic interaction chromatography column) coupled with a Thermo Scientific Quantiva mass spectrometer was used in this study. Targeted data acquisition was performed using selected reaction monitoring in positive mode. Statistical analysis and metabolic pathway analysis were performed for metabolic pathways impact evaluation.

Preliminary data

We have successfully established a targeted metabolic profiling panel to accurately and specifically detect 221 metabolites from more than 30 metabolic pathways simultaneously. In our preliminary test, these three strains were each exposed 20μg/ml exhaust UFPs from either petrodiesel (B0) or 20%V/V blend of soy-based biodiesel (B20) fuel combustion. In these 221 targeted metabolites, we successfully detected 141 metabolites for *S.salivarius*, 92 metabolites for *L.acidophilus*, 82 metabolites for *L.fermentum* in B0, B20 or the control group. Mann-Whitney U tests suggested that for the *S.salivarius* test 14 metabolites (e.g., D-glucono-1,5-lactone, N-acetyl-D-galactosamine, maleamate, L-homocysteine-thiolactone, 5-aminolevulic acid) showed

significant statistical difference (with *p-value* <0.001) between B0 and control, 32 metabolites showed significant statistical difference between B20 and control, 14 metabolites showed significant statistical difference from B0 to B20 comparison. Furthermore, metabolic pathway analysis are conducted and the UFPs effect to important metabolic pathways are observed for all three strains in this study. Take *S. salivarius* for example, the data showed the B0 UFPs exposure had heavy impact on several significant metabolic pathways such as pantothenate and CoA biosynthesis, D-alanine metabolism, taurine and hypotaurine metabolism, pentose phosphate pathway and so on.

Novel aspect

This study demonstrated that environmental UFPs effect on multiple intestinal bacterial strains can be successfully evaluated by targeted metabolic profiling.