editorial

A "new" thematic series: mass spectrometry-based proteomics of lipid biology

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Proteomics, a global approach to understanding protein expression, regulation, and function, transcends analysis of individual proteins. One of its major tools is mass spectrometry (MS), which can detect and quantify hundreds or even thousands of proteins in one sample. Indeed, it has already made major contributions to important biological and clinical problems [reviewed in (1–3)]. MS measures molecular weight and therefore can detect and characterize posttranslational modifications of proteins (4). Its ability to identify disease-related biomarkers is also a powerful advantage (5).

This issue inaugurates a Thematic Series highlighting the application of MS to the study of proteins important in lipid metabolism and biology. We first provide a brief overview of MS, illustrating two of the major approaches used to identify proteins and posttranslational modifications, and then we summarize the topics that will be reviewed in the series.

MASS SPECTROMETRIC IDENTIFICATION OF PROTEINS

All mass spectrometers have three essential components: *i*) an ion source, *ii*) a mass analyzer, and *iii*) a detector that registers ions. The ion source introduces molecules of interest into the gas phase, enabling the mass analyzer to separate them by mass-to-charge (m/z) ratio. The detector registers the number of ions of each m/z, producing a mass spectrum. To identify the proteins or peptides that produce the spectral peaks, MS uses various algorithms and ways to search databases. Each of these aspects has been discussed in recent reviews (1–3).

Mass analysis is performed at very low pressures. It is often impossible to convert large biomolecules to gaseous ions without altering their properties. Therefore, MS was initially applied only to volatile materials and small biomolecules that are resistant to the high temperatures and vacuum required for ionization (6). Its application to protein analysis was made possible in the late 1980s by the discovery of ESI and MALDI. These two distinctly different ionization methods introduce large biomolecules into the gas phase without damaging them. Their importance was recognized by the 2002 Nobel Prize in Chemistry, which was awarded to two investigators who pioneered their development (7, 8).

MALDI is the premier method for examining biomolecules in the solid state by MS (2). A peptide or protein of interest is cocrystallized with excess matrix material that absorbs at the frequency of a rapidly pulsing laser. When the intense light strikes the matrix analyte crystal on the target plate, the matrix absorbs energy, introducing the peptide or protein into the gas phase. This analytical method is robust and sensitive. When used in preparation for time-of-flight (TOF) MS, it typically detects subpicomolar quantities of analyte. Moreover, MALDI-TOF-MS can be interfaced with high-throughput liquid chromatography systems that permit many individual samples to be analyzed in a short time.

MALDI-TOF-MS is widely used to identify unknown proteins by peptide mapping or peptide mass fingerprinting (2). A protein is digested with a protease that cleaves the peptide bond at a known amino acid residue, and the peptide digest is analyzed by MALDI-TOF-MS. The parent protein is identified by matching the masses of the detected peptides with a list of calculated peptide masses in a theoretical digest of the organism's proteins.

MALDI-TOF-MS is well suited for peptide mapping because its high mass accuracy and resolution greatly facilitate matching experimental data with database information. However, the presence of multiple proteins in a sample greatly increases the complexity of peptide mapping, which therefore must be applied to relatively pure proteins that are typically obtained by one- or two-dimensional gel electrophoresis.

An example of peptide mapping is shown in **Fig. 1**. Human monocyte-derived macrophages were exposed to high concentrations of glucose and free fatty acids, and lysates of the cells were then subjected to two-dimensional gel electrophoresis. A single protein exhibited a striking 5-fold increase (151 ± 35 vs. 711 ± 29 arbitrary units; n = 3) in

Abbreviations: CID, collision-induced dissociation; HOCl, hypochlorous acid; LC, liquid chromatography; MMP, matrix metalloproteinase; MS, mass spectrometry; MS/MS, tandem mass spectrometry; TOF, time-of-flight.

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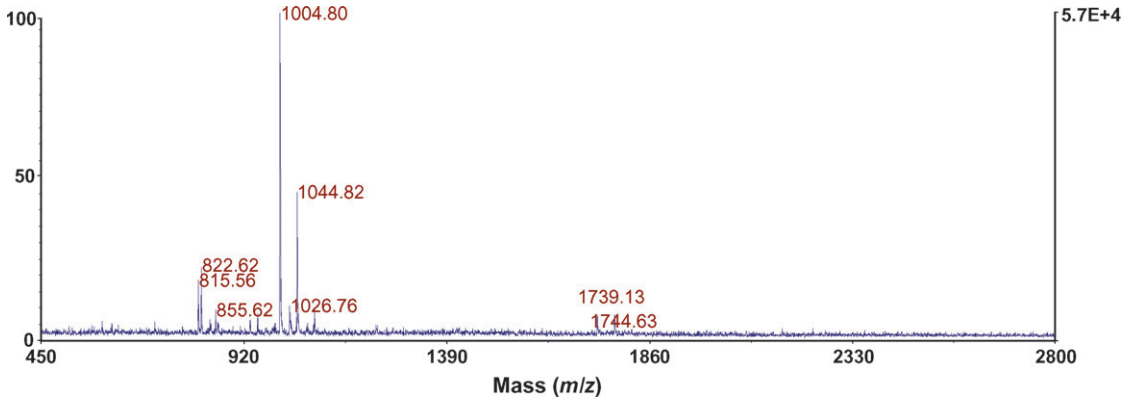

Fig. 1. Peptide map analysis of a macrophage protein. A protein that exhibited a marked increase in human macrophages incubated in medium containing a high concentration of glucose and free fatty acids bound to albumin was identified, quantified, and isolated by two-dimensional gel electrophoresis. The protein spot was excised, digested with trypsin, and analyzed with MALDI-TOF-MS. The resulting MS spectrum is termed a peptide map or peptide fingerprint.

relative abundance. We excised that spot from the gel, digested it in situ with trypsin, desalted and concentrated the peptide digest, and analyzed the material with MALDI-TOF-MS. Peptide mapping identified eight peaks (Fig. 1). Five of the peptides closely matched the *m/z* ratios of the protonated molecular ions $(M + H)^+$ of peptides predicted to be present in manganese superoxide dismutase (**Table 1**); the other major peaks were derived from the gel or matrix.

ESI is the key method for introducing biomolecules in solution into the gas phase for mass spectrometric analysis. It offers subpicomolar sensitivity and can interface with liquid chromatography (LC). ESI is typically applied to tryptic digests of complex mixtures of proteins that are separated by LC and then analyzed with instruments capable of performing tandem mass spectrometry (MS/MS). In MS/MS, the peptide ion of interest is induced to fragment by a process termed collision-induced dissociation (CID), which provides a powerful source of sequence information (1–3).

USING MS TO IDENTIFY POSTTRANSLATIONAL MODIFICATIONS

MS/MS analysis is also a powerful technique for detecting posttranslational modifications of proteins (4, 9), such as oxygenation of thiol residues. This type of modification has been implicated in the regulation of matrix metalloproteinases (MMPs) by myeloperoxidase, which generates a powerful oxidizing agent, hypochlorous acid (HOCl). A thiol group in MMP's prodomain keeps the protease inactive because it binds to the zinc atom at the active site (10). The thiol, which belongs to cysteine, is part of a highly conserved regulatory region of pro-MMP called the cysteine switch. When it can no longer bind to zinc, the pro-MMP becomes proteolytically active.

TABLE 1. Peptide map of a macrophage protein yields tryptic peptides $[(M + H)^+]$ derived from human mitochondrial superoxide dismutase

	(M + H)+	
Peptide	Predicted	Observed
124-130	815.4	815.6
69-75	822.4	822.6
195-202	1004.6	1004.8
54-68	1738.9	1739.1
203-216	1743.9	1744.6

Note the excellent correspondence of the predicted mass of the singly protonated peptides, $(M + H)^+$, with those observed in the protein digest (Fig. 1).

To test the proposal that HOCl can oxygenate the prodomain of MMPs, we used the synthetic peptide PRCGVPDVA, which duplicates the cysteine switch (10). Analysis of this peptide by ESI in the positive ion mode revealed a major peak at *m/z* 913.6, the predicted molecular mass of the protonated cysteine switch peptide $[\text{peptide} + H]^+$. This peak disappeared almost completely after the cysteine switch peptide was exposed to HOCl, and two new peaks appeared, at *m/z* 945.3 and 961.3, respectively. The masses of the new materials were 32 and 48 units greater than the mass of the precursor, respectively, strongly suggesting the acquisition of two or three oxygen atoms (16 atomic mass units for each oxygen).

To identify the site that was covalently modified by HOCl, we subjected the modified peptide to CID and MS/MS (10). In this case, the mass spectrometer selects a precursor ion of interest (e.g., the protonated molecular ion, $[M + H]^+$), which is activated by CID and induced to fragment into product ions. The latter are detected with the mass spectrometer. Peptides characteristically fragment at the amide bond, yielding a ladder of ions that differ in *m/z* by the masses of the individual amino acids (1–3). The fragmentation pattern therefore predicts the peptide's amino acid sequence. Using this approach, we confirmed the native peptide's sequence (**Fig. 2A**) and demonstrated that it became oxygenated on the cysteine residue (Fig. 2B). These results suggest a mechanism by which myeloperoxidase could activate MMPs.

Using ESI, it is possible to directly analyze complex mixtures of peptides in solution (1–3). For high sensitivity, ESI

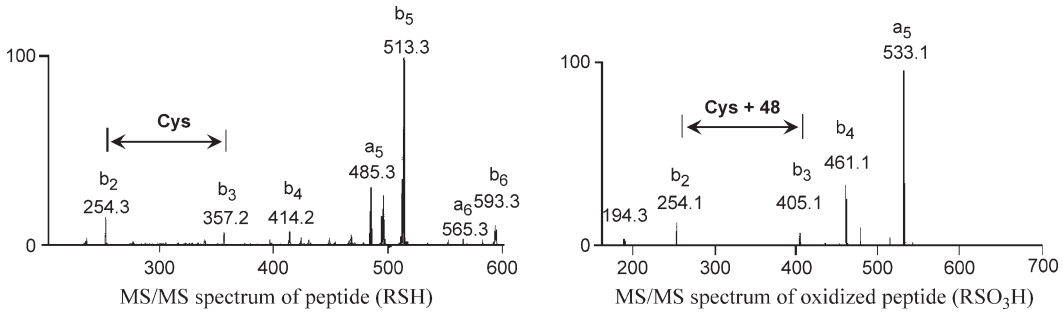

Fig. 2. Sequence analysis by MS/MS of the cysteine switch peptide (RSH) and the same peptide modified by HOCl (RSO_3H). The MS/MS spectrum demonstrates that the cysteine residue (Cys; difference in mass of the b_2 and b_3 ions) in the modified peptide had gained 48 atomic mass units, indicating that it was the site at which three oxygen atoms (16 atomic mass units each) were added. Note that one can also infer the peptide's amino acid sequence from the difference in mass of other ions in the mass spectrum (e.g., b_3 and b_4 ions, and b_4 and b_5 ions).

typically is interfaced with a LC system that operates at low flow rates. Nanoelectrospray MS separates molecules by LC at a very low flow rate (typically 200–300 nL/min) and is exquisitely sensitive, often capable of detecting attomoles of material. LC-ESI-MS/MS analysis, often termed shotgun proteomics, can detect hundreds or even thousands of proteins in one analysis (3).

Protein identifications using protein or peptide MS/MS spectra are often more definitive than those achieved by peptide mass fingerprinting because they use information about peptide sequence (1–3). However, the spectra do not provide unambiguous sequence information for most peptides. Instead, MS/MS spectra are searched against protein sequence databases using various algorithms. Peptide and protein identifications are thus probabilistic (11, 12), and they depend in part on the algorithm used.

TOPICS OF THE THEMATIC SERIES

MS analysis of proteins that associate with lipid presents a number of technical challenges. In the first article of the series, Tomas Vaisar (University of Washington) provides an overview of the theoretical, technical, and practical aspects, offering insights into critical features of the proteomic analysis of integral membrane proteins and lipoproteins.

In the second article in this series, Edward Dennis and colleagues (University of California) review the eicosanoid class of signaling molecules, which are of great interest in systems biology. More than a hundred different eicosanoids have been identified, and these molecules are implicated in a vast number of physiological and pathophysiological events.

In the third article, Andy Hoofnagle and Jay Heinecke (University of Washington) review the rapidly emerging field of lipoprotein proteomics. Lipoproteins are lipid-protein complexes that circulate in blood, transporting cholesterol, triglycerides, and other lipids. However, they also have been linked to atherosclerosis, the immune system, and macrophage biology. Moreover, recent studies suggest that the proteome of one plasma lipoprotein, HDL, might serve as a biomarker for cardiovascular disease.

In the fourth article, Leonard Foster and Emma Zheng (University of British Columbia) discuss how quantitative proteomics is changing our view of what proteins really exist in lipid rafts and how the rafts' composition changes in response to stimuli. Lipid rafts play a central role in cell signaling by acting as scaffolding platforms for a variety of signaling molecules. Therefore, proteomic analysis of these compartments may lead to novel insights into the control of signal transduction.

Finally, Christine Wu (University of Colorado) will discuss the proteomics of lipid droplets, while a complementary article by Joel Goodman (University of Texas, Southwestern) will review the role of lipid droplet proteins in the regulation of lipid metabolism and trafficking. All cells have the capacity to package excess triglycerides and other neutral lipids into droplets, which were traditionally considered to be simple storage depots. We now realize that lipid droplets are dynamic organelles that participate in many cellular functions.

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