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Review Article

Advances in Protein Characterization by High-Resolution Mass Spectrometry

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Abstract

High-resolution mass spectrometry has become an indispensable tool for a variety of biological research areas, including protein chemistry. The coupling of electrospray ionization to the MS has revolutionized the approaches for the identification of new proteins. Some examples of these applications include the identification of proteins involved in the virulence of pathogenic bacterial strains. MS played an important role in advancing protein folding studies, identification of new biomarkers for the detection of diseases in early stages. A recent development in MS technique called fast photochemical oxidation of proteins significantly advanced the protein structural analysis.

Key words: High-resolution mass spectrometry, Virulence, Protein analysis

INTRODUCTION

Mass spectrometry (MS) has become an indispensable tool for biological and analytical chemistry research. It is a powerful analytical tool with high sensitivity and high mass accuracy. Mass spectrometers measure the mass/charge ratio of ions, whether positive or negative. It is presented as a mass spectrum which is a plot of the intensity of the ions as a function of mass/charge ratio of the ions. These spectra are used to determine the elemental or isotopic pattern of a sample, the masses of particles and of molecules, and to elucidate the chemical identity or structure of molecules and other chemical compounds. In a typical MS procedure, a solid, liquid, or gaseous sample is ionized by ion source, ions are sorted based on their mass and charge by the mass analyzer and then detected by a detector, and the results are displayed in a chart.

The first mass spectrometer was built by Sir Joseph John Thomson at the Cavendish Laboratories in Cambridge, UK, in the early 20th century. From 1897, the work carried out by Thomson *et al*. receives seven Nobel prizes in physics and chemistry. Sir J.J. Thomson is regarded as the "grandfather of the MS." In the 1940s, the applications of MS started spreading away from mostly academic work into more practical fields such as nuclear isotope enrichment and the analysis of the components of petroleum. The world's first commercial instrument became available in 1948. The coupling of gas and liquid chromatography to the MS was the major breakthrough. This allowed, for the 1st time, the analysis of mixtures of analytes without laborious and time-consuming separation of its components. High-resolution MS (HRMS) and their few applications in protein analysis are discussed below.

HRMS

HRMS allows detection of analytes to the nearest 0.001 atomic mass unit.[1] One of the most popular HRMS has Orbitrap as an ion trap. Ions are electrostatically trapped in an orbit around a central, spindle-shaped electrode. The electrode confines the ions so that they both orbit around the central electrode and oscillate back and forth along the central electrode's long axis. This oscillation generates an image current in the detector plates which is recorded by the instrument. This ion current is converted to mass spectrum by Fourier transform of the frequency signal. Because of their high sensitivity and high mass accuracy, Orbitrap-based mass spectrometers are used in proteomics, metabolomics, environmental science, and food safety

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analysis.[2] In this review, I will focus on the role of MS in protein-related studies.

MS IN PROTEIN-FOLDING STUDIES

HRMS has been used widely to understand the protein folding.[3] Eyles and Kaltashov have published a very thorough article on methods to study protein dynamics and protein folding by MS.[4] It was useful in determining the folding pathways of methylenetetrahydrofolate reductase oligomers.[5] Another classic paper describing the use of MS methods to study the protein architecture and dynamics was published in protein science.[6] Near amino acid resolution step-by-step protein folding was elucidated by hydrogen exchange and MS.[7] A recent useful review highlights the role of MS in understanding protein folding by hydrogen–deuterium exchange and fragment separation.[8]

MS IN CANCER BIOMARKER DISCOVERY

A biomarker is a measurable indicator of severity or the presence of a phenomenon such as a disease, infection, or environmental exposure. The use of biomarkers in basic and clinical research as well as in clinical practice has become so commonplace that their presence as primary endpoints in clinical trials is now accepted almost without question. MS played an important role in the discovery of a large number of cancer biomarkers. A recent review article on the advances in the MS-based cancer biomarker discovery summarizes the advances in this field.[9] Some notable examples for cancer biomarkers include biomarker for liver cancer detection in early stages, pancreatic cancer, and genitourinary cancer.[10-12]

MS IN THE STUDY OF BACTERIAL VIRULENCE

The study of the bacterial virulence is of immense importance to understand host–pathogen interaction and finding ways to treat multidrug-resistant virulence strains. In recent years, MS has played an important role in understanding the role of virulence proteins in pathogenic bacteria. MS has played a very important role in elucidating the role of these key proteins and identified new proteins involved in virulence in *Listeria monocytogenes*, *Mycobacterium tuberculosis,* and *Streptococcus pneumoniae*.[13-16] The catalytic role of urease, converting urea into carbon dioxide and ammonia, has been well studied and shown to protect this bacterium in the low pH environment of the stomach lumen. Mass spectrometric studies on the *Helicobacter pylori* urease revealed the non-catalytical role of this enzyme in quenching oxidant and improved the understanding of virulence factor.[17] A recent review by Perez-Llarena and Bou details the role of MS-based proteomics on the study of bacterial virulence and antibacterial resistance.[18]

MS AND FAST PHOTOCHEMICAL OXIDATION OF PROTEINS (FPOP)

FPOP is an emerging MS-based technique for the high-resolution characterization of protein structure. It offers an ultrafast method for free radical generation using an ultraviolet laser to generate high concentrations of hydroxyl radicals by photolyzing hydrogen peroxide. These free radicals react with protein, buffers, and other components in a sample mixture on roughly a microsecond time scale. The hydroxyl radical is very reactive, and they have broad reactivity. Hence, the ligands, buffers, and excipient present in the sample will all react with hydroxyl radicals. The recent development in this field (JASMS paper, Analytical Biochemistry paper, and Analytical Chemistry paper) offers exciting opportunities to the researchers in probing the structural characterization of proteins and various applications of this technology in the diverse biological research fields.[19-21] For example, the conformation of biosimilar adalimumab was compared in phosphate and citrate buffers, using FPOP and HRMS, and adalimumab was found to have different conformations in these buffers. The protective role of polysorbate-80 in aggregate formation for this monoclonal antibody was also shown utilizing FPOP and HRMS.[22]

CONCLUSION

The MS-based proteomics has seen a tremendous application in characterization of protein higher-order structure, the study of the role of key proteins responsible for bacterial pathogenesis and their importance in developing the disease and searching for new means to control the bacterial infectious diseases. The clinical research field has significantly advanced after the MS-based discovery of biomarkers that can accurately indicate the onset of cancer in the early stages and the outcome of treatment.

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