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Mass Spectrometery as an Analytical Method for Determining Protein Structures

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Abstract

Mass spectrometry (MS) has become a powerful technology in the discovery and development of protein therapeutics in the biopharmaceutical industry. This review article describes the two main types of mass spectrometry methods for determining the protein structure. Further the role of tandem mass spectrometry in diagnostics has also been highlighted. Mass spectrometry based proteomics is a central life science technology that has realized great progress towards identification, quantification and characterization of the proteins that constitute a proteome.

Keywords: Mass spectrometry; Matrix assisted laser desorption ionization; Electro spray ionization; Tandem mass spectrometry

Introduction

Mass spectrometry (MS) has replaced the Edman technique as the principal method for determining the sequences of peptides and proteins owing to its superior sensitivity, speed and versatility. MS entered the laboratories during 1960s. Initially chemists used them for mass determination and structural investigations of volatile molecules. Since the 1990s, MS has played an increasingly significant role in biological sciences.

MS is an analytic technique that utilizes the degree of deflection of charged particles by a magnetic field to find the relative masses of molecular ions and fragments. It is a powerful method because it provides a great deal of information and can be conducted on tiny samples. MS can detect comparatively subtle physical change in proteins that can occur during the life cycle of a cell or organism.

The analysis of biomolecules by MS initially was hindered by difficulties in volatizing the molecules, while small organic molecules could be readily vaporized by heating in a vacuum, proteins, oligo nucleotides etc., were destroyed under these conditions. Under high energetic electron bombardment they would disintegrate into countless components. With a trick it was possible to let high molecular ions jump into the vacuum. Matrix-assisted laser desorption ionization (MALDI) and electrospray (ES) are the ionization techniques that should be credited most for the success of mass spectrometry in the life sciences. Major advances were also made in sample preparation for MS, a crucial area for overall feasibility and sensitivity of analysis.

The last few years have seen development of even more powerful instrumentation and algorithms for protein characterization, a trend that shows no signs of slowing down.

The general operation of a mass spectrometer is:

1. Create gas-phase ions

2. Separate the ions in space or time based on their mass-to-charge ratio

3. Measure the quantity of ions of each mass-to-charge ratio [1].

Ionization Methods

Matrix assisted laser desorption ionization-Time of flight: (MALDI-TOF)

In MADLI-TOF sample is ionized by bombarding sample with laser light. Sample is mixed with UV absorbent matrix (sinapinic acid for proteins, 4 hydroxycinnaminic acid for peptides). Light wavelength must match that of absorbance maximum of matrix so that the matrix transfers some of its energy to the analyte.

When MALDI starts to work it results in a gas of positively charged protein ions. An electric field accelerates these ions toward a slit. All ions fly the same distance: namely the length of the TOF. They then hit the detector. However, because they have different mass-to-charge ratios, and hence different speeds, they reach the detector at different times. These flight times are measured. By the heights of a MALDI-TOF spectrum the protein concentration can be measured [1,2] (Figure 1).

Electro spray ionisation (ESI)

In a second and equally successful method, macromolecules are forced directly from liquid to gaseous phase. This involves the production of ions by spraying a solution of the analyte into an electrical field. This is a soft ionisation technique and enables the analysis of a large intact biomolecules such as proteins and DNA. The electrospray (ES) creates very small droplets of solvent containing analyte. The essential principle in ES is that a spray of charged liquid droplets is produced by atomistaion. Solvents (typically water and organic solvent) are removed as the droplets enter the mass spectrometer. As the solvent evaporates in the high vacuum region, the droplet size decreases and eventually charged analyte remains (Figures 2 and 3).

With ESI MS, the ion mass is determined with quadrupoles. Several

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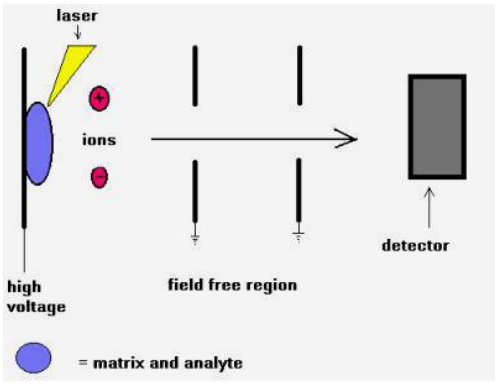

Figure 1: MALDI-TOF (Matrix assisted laser desorption ionization)-Time of flight.

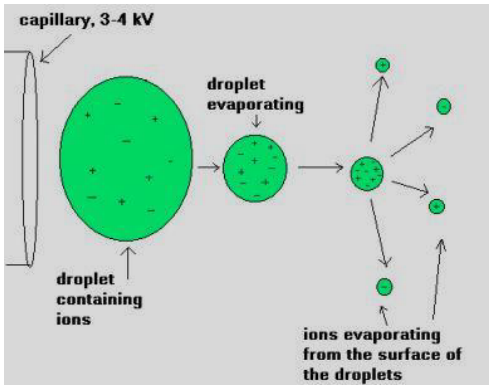

Figure 2: Electrospray ionization.

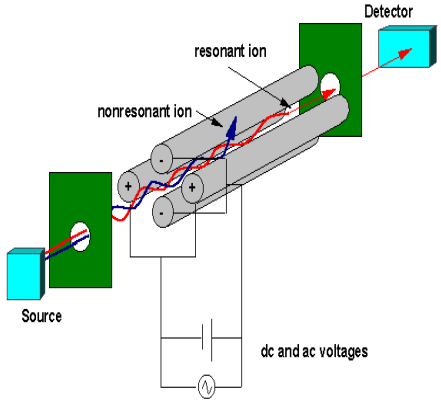

Figure 3: Quadrapoles connected to an ESI mass spectrometer.

quadrupoles, one after the other, allow the fragmentation of individual protein via collision with rare gases and to determine the molecular weights of the fragments. Electrospray is most often combined with a quadrupole MS. The quadrupole is a mass filter, which consists of four rods to which an oscillating electric field is applied and which lets only a certain mass pass through (the other masses are on unstable trajectories and do not reach the detector). By scanning the amplitude of the electric field and recording the ions at the detector, one obtains a mass spectrum. To date, most peptide sequencing experiments have been performed on triple quadrupole instruments that consist of three sections: two mass separating quadrupole sections separated by a central quadrupole (or a higher multipole) section whose function is to contain the ions during fragmentation.

Tandem Mass Spectroscopy in Diagnosis

Complex peptide mixtures can be analyzed without prior purification by tandem mass spectrometry, which employs the equivalent of two mass spectrometers linked in series.

A solution containing the protein under investigation is first treated with a protease or chemical reagent in order to hydrolyze it to a mixture of shorter peptides. The mixture is then injected into a device that is essentially two mass spectrometers in tandem.

The first spectrometer separates individual peptides based upon their differences in mass. By adjusting the field strength of the first magnet, a single peptide can be directed into the second mass spectrometer, where fragments are generated and their masses determined. Tandem mass spectrometry can be used to screen blood samples from newborns for the presence and concentrations of amino acids, fatty acids and other metabolites. Abnormalities in metabolic levels can serve as diagnostic indicators for a variety of genetic disorders. The sequence of peptides can be determined by interpreting the data resulting from fragmenting the peptides in tandem mass spectrometers [3,4].

In this technique, one peptide species out of a mixture is selected in the first mass spectrometer and is then dissociated by collision with an inert gas, such as argon or nitrogen. The resulting fragments are separated in the second part of the tandem mass spectrometer, producing the tandem mass spectrum, or MS/MS spectrum. In the instruments in use today, multiple collisions impart energy onto the molecule until it fragments. (This is low-energy fragmentation, in which any single hit is not sufficient to break the peptide bond. In high-energy fragmentation, the molecules have higher velocity and a single hit can break bonds).

Most peptide sequencing is performed on electro-sprayed ions. These ions generally have a charge state corresponding to the number of positively charged amino acids plus the charge formally localized at the N terminus of the peptide. Tandem mass spectra are usually interpreted with computer assistance, or matched against databases directly.

Tandem mass spectrometry (MS/MS) has been used for several years to identify and measure carnitine esters in blood and urine of children suspected of having inborn errors of metabolism. Indeed, acyl carnitine analysis is a better diagnostic test for disorders of fatty acid oxidation than organic acid anaylsis because it can often detect the conditions when the patient is not acutely ill [5].

MS has been used in pilot programs to screen newborns for these conditions and for disorders of amino and organic acid metabolism as well.

MS/MS thus permits very rapid, sensitive and with appropriate internal standards, accurate measurements of many different types of metabolites with minimal sample preparation and without prior chromatographic separation. Because many amino acidemias, organic acidemias and disorders of fatty acid oxidation can be detected in 1 to 2 minutes, the system has adequate throughput to handle the large number of samples that are processed in newborn screening programs.

Amino acid quantitation by MS/MS is more accurate than most methods now in use for new born screening and would thus provide more specific and sensitive screening for phenylketonuria [6], maple syrup urine disease [7] and homocystinuria [8].

New born screening (NBS) primarily aim at early detection and treatment of clinically important disorders in order to minimize morbidity and mortality in early childhood. With early diagnosis and appropriate treatment some problems can be avoided, these include biochemical disturbances such as hyper ammonemia in patients with urea cycle that present after the new born period, severe metabolic acidosis in patients with disorders of organic acids and hypoketolic hypoglycaemia , cardiomyopathy or rhabdomyolysis in patients with disorders of fatty acid oxidation, if left untreated these disorders may lead to brain damage or other organ damage or death [9].

Future Directions

Understanding the biochemical processes that constitute life requires the detailed comprehension of the participating proteins and metabolic substrates. Mass spectrometry is the core technique of proteomics. Progress in instrumentation continues to be made at a fast pace. Automation also makes it possible to obtain rates of data generation that exceeds those of genomics. Quantitative proteomics will most likely be achieved by stable isotope methods in combination with mass spectrometry. Applications of MS-based proteomics range from descriptive to quantitative, providing insight into emergent biological properties through systems biology initiatives and driving biomarker discovery efforts for the development of new diagnostics.

A part from the pressing areas of automation of data acquisition and interpretation. Areas for future research will be the analysis of protein modification on a large scale. Cross-linking studies will tell us not only about the composition but also about the spatial organization of protein complexes. There is much scope for creativity in connecting cell and molecular biological strategies with the powerful mass spectrometric capabilities to solve queries that were not previously is addressed.

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