[22] Matrix-Assisted Laser Desorption Ionization Mass-Spectrometry of Proteins

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Introduction

In this chapter, we provide a practical guide to the application of matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS) for the analysis of peptides and proteins. We describe in detail the best methods that are currently available for preparing samples for MALDI-MS, because good sample preparation is the key to successful mass analysis. We consider aspects of the method that are important for obtaining high-quality data. Finally, we describe a selection of strategies for studying proteins with this powerful new technique.

Pulses of laser light have been employed since as early as 1976[1] to produce intact gas phase peptide ions from solid samples. The resulting peptide ions could then be analyzed by mass spectrometry. These early investigations and subsequent measurements over the following decade produced useful mass spectra from only a few short peptides. In addition, the probability for obtaining a useful mass spectrum depended critically on the specific physical properties of the peptide (e.g., photoabsorption spectrum, volatility) under study. This situation changed dramatically with the development by Karas and Hillenkamp.[2] MALDI-MS provides the means to volatilize proteins readily and to make the conditions for volatilization largely independent of the specific physical properties of the protein. This effect is achieved in two steps. The first step involves preparing an appropriate sample by dilutely embedding proteins in a matrix of small organic molecules that strongly absorb ultraviolet wavelength laser light. The second step involves ablation of bulk portions of this solid sample

[1] M. A. Posthumus, P. G. Kistemaker, and H. L. C. Meuzelaar, *Anal. Chem.* **50,** 985 (1978).
[2] M. Karas and F. Hillenkamp, *Anal. Chem.* **60,** 2299 (1988).

METHODS IN ENZYMOLOGY, VOL. 270
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by intense, short-duration pulses of the laser light. In the ablation step, molecular components of the solid are put into the gas phase and ionized, producing intact protein ions. The molecular masses of these protein ions are easily determined by time-of-flight mass analysis. The marvelous development by Karas and Hillenkamp has been steadily refined and has become a method of choice for characterizing peptides and proteins.[3-8]

MALDI–MS is versatile and effective for the analysis of peptides and proteins because of the special properties and capabilities of the technique. Some of these capabilities and properties are as follows.

Biological samples can be examined without extensive purification.

Common biochemical additives such as buffers, salts, glycerol, chelating agents, chaotropic agents, and certain detergents do not interfere with the analysis.

Most classes of proteins can be examined, provided that the protein can be dissolved in appropriate solvents.

Posttranslationally modified proteins can be measured.

Useful mass spectra can be obtained from complex mixtures of peptides and proteins.

Proteins with masses ranging to greater than 100 kDa can be analyzed.

The total amount of protein required for an analysis is usually in the range of 1–10 pmol.

Protein molecular masses can be determined with mass accuracies as high as 1 part in 10,000.

Complete analyses can be made in a matter of minutes.

It is instructive to compare the properties of MALDI–MS for the analysis of proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE), a method widely used in biological research. SDS–PAGE is a universal technique for separating and analyzing proteins because of the effectiveness of the detergent SDS for dissolving proteins and for converting them into entities that migrate on electrophoretic gels with relative velocities that depend on their size. By contrast, there are certain restrictions (see below) on the detergents and additives that can be used in the preparation of samples for MALDI–MS. However, the more complex and expensive instrumentation required by MALDI–MS is justified because MS provides much higher mass accuracy determination (typically three to four orders

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