MALDI-TOF Mass Spectrometry

by Reena Zutshi

I. Introduction

Mass spectrometry is a powerful tool used for studying the masses of atoms, molecular fragments, and molecules. In general, molecules in the gas phase (or species desorbed from a condensed phase) are ionized, and the ions are then accelerated by an electric field and separated on the basis of their mass-to-charge ratio (m/z). For an ion with a charge of +1, m/z will be numerically equal to the mass. The electron ionization that converts molecules to ions can not only remove electrons, but may impart so much energy that the molecule fragments. The molecular ions are deflected by a magnet as they travel through the analyzer tube toward the detector. A mass spectrum is a chromatogram presenting the signal intensity (y-axis) versus m/z (x-axis). See Figure 1 below. The peak intensities are expressed as a percentage of the most intense signal (the base beak). A time-of-flight (TOF) mass spectrometer separates ions with identical kinetic energy but different m/z, since lighter ions travel faster than heavier ones. Interpretation of the fragmentation patterns and isotopic peaks can provide valuable clues for the structure determination of organic molecules.

Mass spectrometry of proteins is primarily used for determination of molecular mass; therefore, high-energy ionization that leads to fragmentation is not desirable. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) emerged as an effective analytical tool about twelve years ago. MALDI provides a 'soft' ionization source that prevents decomposition of fragile biomolecules, producing primarily singly charged, intact protein ions. In MALDI, the sample (such as an aliquot of purified peptide) is embedded in an excess of matrix, which is a solution of an ultraviolet absorbing compound. When the liquid evaporates, the sample consists of dry crystals of sample mixed with matrix. When this mixture is irradiated with a laser, the matrix assists in the volatilization and ionization of the analyte. The ions produced with the MALDI technique are analyzed using a time-of-flight (TOF) mass spectrometer, which is characterized by a high ion throughput and therefore high sensitivity. By knowing the molecular mass of your peptide or protein, you can determine whether it is present in a given sample by examining the mass spectrum for peaks with the right mass.The Chemistry department has a MALDI-TOF mass spectrometer located in the instrument center. Chemical Biology students will be trained by the instrument center staff and their TA. As always, your TA will provide specific guidance and suggest modifications to the protocol below as necessary.

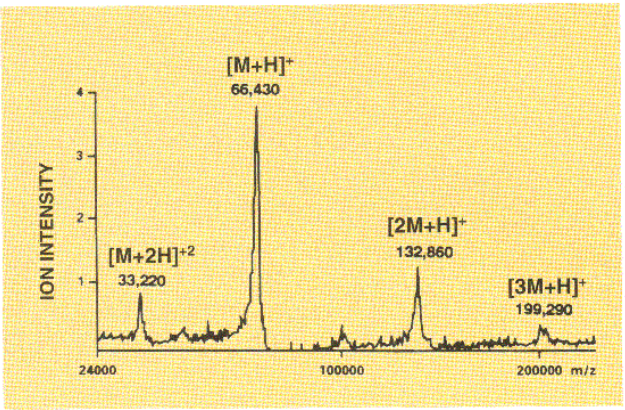

Figure 1. Mass Spectrum of bovine serum albumin obtained using MALDI-TOF. Figure adapted from the American Society for Mass Spectrometry website (http://www.asms.org/whatisms/p12.html).

II. Sample preparation for MALDI-TOF

The general protocol for preparing samples for MALDI is as follows:

Select matrix → Prepare matrix → Prepare sample → Mix sample and matrix → Load sample/matrix on a clean sample plate → Dry

A. Selecting a matrix

Selection of proper matrix is important for getting a good mass spec, since the matrix plays a key role in ionization. The chart below suggests guidelines to consider.

Matrix	Application
Sinapinic Acid (3,5-dimethoxy-4-hydroxy cinnamic acid)	Peptides and proteins greater than 10 kDa in mass
CHCA (a-cyano-4-hydroxycinnamic acid)	Peptides and proteins less than 10 kDa in mass
THAP (2,4,6-Trihydroxyacetophenone)	Small oligonucleotides less than 3.5 kDa in mass
HPA (3-hydroxypicolinic acid) in diammonium citrate	Large nucleotides greater than 3.5 kDa in mass

I have found that aPP derived molecules and other hydrophobic peptides work well in sinapinic acid. For smaller peptides (18-24 mer), CHCA can be used.

B. Preparing stock solutions of matrix

1. Sinapinic Acid:

In an eppendorf tube, weigh out 10 mg of sinapinic acid. Add 600 µL of deionized water, 100 µL of 3% TFA and 300 µL of acetonitrile to the matrix. Vortex for 1 minute to disslove, then centrifuge for 1 minute to precipitate any undissolved sinapinic acid. Use only the supernatant for applications.

Note: Buffers, salts and detergent retard the ionization of the matrix. As far as possible, avoid using the last two (salts and detergents). If you must use a sample prepared in a buffer, increase the concentration of TFA in the matrix stock to enhance sample ionization.

2. CHCA:

In an eppendorf tube, weigh out 10 mg of CHCA. Add 400 µL of deionized water, 100 µL of 3% TFA and 500 µL of acetonitrile to the matrix. Vortex for 1 minute to disslove, then centrifuge for 1 minute to precipitate any undissolved sinapinic acid. Use only the supernatant for applications.

Note 1: Buffers, salts and detergent retard the ionization of the matrix. As far as possible, avoid using the last two (salts and detergents). If you must use a sample prepared in a buffer, increase the concentration of TFA in the matrix stock to enhance sample ionization.

Note 2: If the dry matrix is a mustard-yellow color instead of bright yellow, it may contain impurities. To purify, dissolve CHCA in warm ethanol. Filter and add 2 volumes of deionized water. Let the solution stand in the refrigerator for 2 hrs. Filter and wash the precipitate with cold water.

3. THAP:

Make a 50 mg/mL solution of diammonium citrate in deionized water. Dissolve 10 mg of THAP in 50% acetonitrile/deionized water. Combine 8:1:: THAP solution:diammonium citrate solution.

Note: For oligonucleotide applications, do not use HPLC grade water for sample preparation. Use deionized water only.

4. HPA:

Make a 50 mg/mL solution of diammonium citrate in deionized water. Dissolve 50 mg of THAP in 50% acetonitrile/deionized water. Combine 8:1:: THAP solution:diammonium citrate solution.

Note: For oligonucleotide applications, do not use HPLC grade water for sample preparation. Use deionized water only.

C. Preparing Sample Stock for MALDI

Stock solutions of samples should be at the following concentrations before adding to the matrix solution:

Peptides and proteins: 1-100 pmol/µL (Lower concentration for smaller peptides and higher concentration for proteins and larger peptides, i.e. > 5 kDa)
Oligonucleotides: 10-100 pmol/L

Samples should be preferably dissolved in water. If insoluble in water, add acetonitrile to the solution (up to 50%) and then 0.1% TFA to increase solubility. Consult your TA for specific guidelines on sample stock solutions.

1. Mixing Sample and Matrix

Mix 1 µL of sample and 9 µL of matrix in a microcentrifuge tube for a final concentration of 0.1 - 10 pmol/µL. Your particular sample may require a different ratio of sample to matrix; consult your TA for guidance.

D. Loading sample/matrix on the plate

Load 1 - 2 µL of the sample/matrix solution onto the plate. Make sure that the sample is placed in the center of the cell you are loading onto and note the cell # for future reference (a mass spec sample record table is provided at the end of this section for your convenience). Allow the sample plate to dry.

E. Cleaning the sample plate

Wipe the sample plate with a Kimwipe soaked in ethanol, then with soap/water, then with water and finally with ethanol again (for each of these I suggest using a Kimwipe soaked in the appropriate solution to wipe the top of the plate only - a wet sample plate can damage the instrument).

III. Troubleshooting

If you do not see any signal on the mass spec, it could be due to the following reasons:

Possible Cause	Possible Solution
Sample concentration too low	a) Use a 0.1-10 pmol/µL (final conc) of peptides and proteins b) Use at least 1:1 ratio of sample :

	matrix
Sample concentration too high - sample signal may be suppressed	1. Dilute the sample 2. Use at least 1:1 ratio of sample : matrix
Sample made in buffer - poor crystallization on sample plate	🕐 If you must use buffer, use a low ionic strength nitrogen containing buffer - avoid PBS or sulfate buffers 🕐 Use a higher conc of TFA (upto 1% final conc) to enhance ionization
Sample contains salt or detergent - poor crystallization on sample plate	Get rid of salt/detergent on desalting column or by dialysis
Matrix is old	Make fresh matrix

Be sure to avoid:
Using organic solvents to dissolve samples - causes the sample to spread out and not crystallize properly.
Touching the surface of the sample plate with the pipet tip - causes uneven crystallization.

Mass Spec Sample Record
Your Name: _____ Date: _____

Peptide ID	*Sample/Cell #*	*Matrix*	*Expected mass*

Mass Spec Sample Record
Your Name: _____ Date: _____

Peptide ID	*Sample/Cell #*	*Matrix*	*Expected mass*