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Matrix Assisted Laser Desorption Ionization Time-of-flight Mass Spectrometry

Mass spectrometry (MS) has emerged as an important analytical method used for the analysis of a variety of compounds. Since the development of soft ionization methods such as matrix-assisted laser desorption/ionization-time-of-flight (MALDI) and electrospray ionization (ESI), mass spectrometric methods have become increasingly important due to their high sensitivity for the study of modified biomolecules. Various types of mass spectrometers have been developed in the past and are now commercially available; however, here we will only refer to Matrix Assisted Laser Desorption Ionization Time-of-flight Mass Spectrometry (MALDI-TOF). From the pioneering days of Malcolm Dole, (who described the first equations, detailing the possibility to vaporize molecules in intact form into the gas phase) to the awarding of the Nobel Prize to John Fenn, for his work in mass spectrometry, there are now few biochemical labs, that do not rely on some form of spectrometry for the mass measurement of biomolecules, in particularly nucleic acids, proteins, peptides, sugars as well as combinations or modifications thereof. In a MALDI-TOF-MS instrument high-energy photons interact with a sample embedded in an organic matrix typically with subpicomole sensitivity. The mass spectrometer consists of two essential components, the ion source and the mass analyzer. In the ion source biomolecules such as protein, peptides or nucleotides are converted into intact, naked ionized molecules in the gas phase. Subsequently, in the mass analyzer, the mass-to-charge (m/z) ratios of the naked molecules ions are determined.

Various types of MALDI-TOF mass spectrometer instruments are available now. Among them are:

Linear MALDI-TOF-MS: The body of work published in 1987 by M. Karas et. al show that when a cluster of molecules on a metallic surface are ablated by a laser pulse a cluster of ions and neutrals are produced by the laser pulse. This ion cluster contains a group of ions of differing mass/charge (m/z) ratios. If they are placed in the same electric field the ions will be accelerated. This acceleration results in an ion having the same kinetic energy as any other ion that has the same charge. The velocity of the ion depends on its mass-to-charge ratio. In a time-of-flight experiment the time that it takes for the ions to reach a detector at a known distance is measured. This time will depend on the mass-to-charge ratio of the ions (heavier ions reach lower speeds). From the time differences and the known experimental parameters one can identify the mass-to-charge ratio of the ions. The MALDI instruments are calibrated by measuring the ion arrival times of known reference standards, e.g. peptides or proteins. Calibration maybe performed either externally for routine analysis or with an internal standard for higher accuracy analysis.

When an ion is accelerated in a time-of-flight tube by the voltage *U*, its potential energy is converted to kinetic energy**.** The kinetic energy of any mass can be expressed as:

$$K_{.E.} = [mv^2]/2 = zeEs$$

Where: $K_{.E.}$ = kinetic energy; m = the mass of the ion, v = velocity of the ion, z = number of charges, e = the charge on an electron in coulombs

E = electric field gradient; and s = the distance the ion travels in the drift zone of a constant electric field. The travel time of an ion is directly proportional to its m/z ratio.

An intense production of intact, naked ionized protein or peptide molecules can be achieved when dilute proteins or peptides are imbedded in a solid matrix are bombarded with intense, short duration bursts of focused ultraviolet laser light, often 337 nm from a N_2 laser. Low molecular weight organic molecules that strongly absorb the ultra violet irradiation are commonly used as matices. A whole range of compounds have

been studied but the choice of the proper matrix is still semi-empirical and may depend on the nature of the sample studied.

Delayed extraction TOF-MS: Loss of resolution is due in part to the uneven flight of identical m/z ions at the source where ions are ejected from the surface with greater kinetic energy have a higher velocity and travel farther from the surface before the accelerating voltage is turned on. It is possible to improve mass resolution by utilizing delayed pulsed ion extraction which compensates for the initial velocity distribution of the MALDI generated ion packet so that same m/z ions arrive simultaneously at the detector. Therefore, is possible to narrow ion arrival time distributions and improve mass resolution as compared to continuous ion extraction. The accelerating voltage pulse is applied after a time delay following pulsed desorption ionization from a surface. The extraction delay can produce energy focusing and improve mass resolution. With electron ionization free atoms or molecules from a gas, this is referred to as "time-lag focusing for ionization by laser desorption from a surface, also known as "delayed extraction." With delayed extraction, the mass resolution is improved due to the correlation between ion velocity and position after the ions have been ejected from the surface. Ions ejected from the surface with greater kinetic energy have a higher velocity and travel farther from the surface before the accelerating voltage is turned on. The slower ions with less kinetic energy are closer to the target surface when the accelerating voltage is applied and therefore experience a greater accelerating potential compared to the ions farther from the target. With the proper delay time, the slower ions will receive enough extra potential energy to match the faster ions. Ions of the same mass will then drift through the flight tube with close to the same velocity and will be spatially focused at the detector.

Reflectron TOF-MS: It is also possible to improve mass resolution in MALDI TOF-MS by using a single-stage or a dual-stage reflectron (RETOF-MS). The reflectron, positioned at the end of the flight tube, is used to compensate for the difference in flight times of the same m/z ions of slightly different kinetic energies of ions analyzed. The reflectron uses an electrostatic field to reflect the ion beam toward the detector. The more energetic ions penetrate deeper into the reflectron, and take a slightly longer path to the detector. Less energetic ions of the same charge and mass only will penetrate a short distance into the reflectron and take a shorter path to the detector. The detector is placed at the focal point where ions of different energies focused by the reflectron strike the detector at the same time. Furthermore, the reflectron arrangement has twice the flight path in a given length of instrument.

Matrices: A matrix is needed as a means to absorb energy form the laser pulse that will allow for the vaporization of the molecules. Most matrices consist of low molecular weight organic molecules that strongly absorb the ultra violet photons from the laser. A whole range of compounds for the use as matrices have been studied but the choice of the proper matrix is still semi-empirical and may depend on the nature of the sample studied. The most common matrices are:

Peptides/protein samples

Mass < 10 kDa CHCẠ alpha-Cyano-4-hydroxycinnamic acid

Mass > 10 kDa SA Sinapic acid

HABA 2-(4-Hydroxyphenylazo) benzoic acid

Oligonucleotide/Nucleic acids

Mass < 3.5 kDa THAP 2,4,6-Trihydroxyacetophenone

Mass > 3.5 kDa HPA 3-Hydroxypicolinic acid

Anthranilic acid

Nicotinic acid

Salicylamide

Bio-Conjugates

Peptide-DNA HPA 3-Hydroxypicolinic acid

Peptide-RNA alpha-Cyano-4-hydroxycinnamic acid

Organic molecules

DHB 2,5-Dihydroxybenzoic acid

Isovanillin

Carbohydrates

DHB 2,5-Dihydroxybenzoic acid

CHCA alpha-Cyano-4-hydroxycinnamic acid

3-Aminoquinoline

Acidic THAP 2,4,6-Trihydroxyacetophenone

Lipids DIT Dithranol

Multiple peaks: Ions can have multiple charges or a distribution of multiple charges. In MALDI-TOF-MS the singly charged ion is observed as the dominant species in most cases. Similarly, peptides, proteins and nucleotides can be singly charged or contain multiple charges. When the peptide being analyzed contains multiple charges multiple peaks will be observed. Additionally, proteins and peptides can for adducts such as dimers, trimers and sometimes even multimers. All these species can be observed in the mass spectrometer to a certain extend.

Resolution: Expressed in terms of percentages, for example an instrument with a 0.01% resolution will be able to differentiate, when the MW is say 10,000 mu, 2 species differing by 1 Dalton; whereas when the MW is 50,000 mu it will differentiate 2 species differing by 5 daltons

Practical observations: A MALDI instrumentation that has a low resolution will sometimes be able to differentiate a linear peptide from the same cyclized peptide containing a cysteine bond. In this case the cyclized peptide will have a lower mass differing by 2 Daltons.

Isotopes: A mass spectrometer separates and detects ions of different masses. Not all mass spectrometer allow to measure monoisotopic molecular weight. An instrument requires sufficient mass resolution to resolve the isotopic distribution and a sufficient signal to noise ratio to be able to identify the first peak of the envelope with confidence. For a small peptide, the first peak (often referred to as the ^{12}C peak) is also the most intense peak.

Bio-Synthesis.Inc, has been producing synthetic oligonucleotides for over 25 years; not only DNA, but RNA, other modified oligonucleotides, and provide cross-linking of various types of biomolecules using our optimized bioconjugation strategies, which have a number of applications on gene expression inhibition and related anti-sense studies. Also synthetic peptides and peptide antibodies for a number of biological relevant applications, in the areas of proteomics, epigenetics, immune regulation, post translational modifications, antisense, gene expression control, RNA interference and more. For more product information, visit: www.biosyn.com.