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Review Article

A REVIEW ON MATRIX ASSISTED LASER DESORPTION/IONIZATION MASS SPECTROSCOPY

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ABSTRACT

Matrix assisted laser desorption ionization mass spectroscopy (MALDI-MS) is the most important technique of MS to analyze polymer systems. It is a special case of MS using specific sample preparation methods and low fluence laser desorption to create the analyte ions. This technique is based upon an ultraviolet absorbing matrix. The matrix and the polymer are mixed at a molecular level in an appropriate solvent. The solvent helps prevent aggregation of the polymer. The sample matrix mixture is placed on the sample probe tip, under vacuum conditions; the solvent is removed, leaving co-crystallized polymer molecules homogenously dispersed within matrix molecules. When the pulsed laser beam is tuned to the appropriate frequency, the energy is transferred to the matrix which is partially vaporized, carrying intact polymer into the vapor phase and charging the polymer chains in the linear time of flight (TOF) analyzer. This review includes the detailed information of MALDI-MS, MALDI-TOF.

Keywords: Matrix assisted laser desorption ionization mass spectroscopy, Principle, Sample preparation techniques, Matrix assisted laser desorption ionization - time of flight, Matrix assisted laser desorption ionization - mass spectrometric imaging, Applications.

INTRODUCTION

The chemical structures of polymers are characterized typically by determining the repeat units, i.e., monomers, end-groups that cap the polymer chains and the molecular weight distribution. Polymer characterization with mass spectroscopy (MS) is not possible as MS requires gas phase ions for a successful analysis, and polymers are composed of large, entangled chains that are not easily converted to gas phase ions. Traditional mass spectroscopic techniques developed for polymer analysis such as pyrolysis gas chromatography use thermal energy to vaporize nonvolatile samples such as polymers. Although thermal energy also decomposed polymers into constituent parts leading to fragmentation, resulting loss of full chemical structural information during vaporization. A new technique has been developed to measure this aspect of polymer sample. This new technique is known as matrix assisted laser desorption ionization MS (MALDI-MS).

PRINCIPLE OF MALDI-MS

MALDI is a soft ionization technique in which the energy from the laser is spent in volatilizing the matrix rather than degrading the polymer. This technique is based upon an ultraviolet absorbing matrix. The matrix and the polymer are mixed at a molecular level in an appropriate solvent. The solvent helps prevent aggregation of the polymer. The sample–matrix mixture is placed on the sample probe tip, under vacuum conditions; the solvent is removed, leaving co-crystallized polymer molecules homogenously dispersed within matrix molecules. When the pulsed laser beam is tuned to the appropriate frequency, the energy is transferred to the matrix, which is partially vaporized, carrying intact polymer into the vapor phase and charging the polymer chains. In the linear time of flight (TOF) analyzer, the distribution of molecules emanating from a sample is imparted to identical translational kinetic energies after being subjected to the same electrical potential energy difference. The ions travel the same distance down an evacuated field free drift tube. The smaller ions arrive at the detector in a shorter time than the more massive ions. Separated ion fractions at the end of the drift tube are detected by an appropriate recorder that produces a signal upon impact of each ion group. The digitized data generated from successive laser shots are added up yielding a TOF mass spectrum. The TOF mass spectrum is a recording of the signal as a function of time. The TOF for a molecule of mass m, and charge z, to travel this distance is proportional to (m/z) 1/2. This relationship can be used to calculate the mass of the ions (Figs. 1 and 2).

SAMPLE PREPARATION

The sample preparation for polymer MALDI must accomplish five different roles one for the solvent and four for the matrix. The roles are as follows,

The solvent must effectively separate the polymer molecules. We need to minimize the interactions between the polymer molecules and generate individual analytes. Desorb the analyte: The matrix must convert the energy delivered by the laser to eject the molecules in the gas phase. Ionize the analyte: The matrix must provide an ionization path for the analyte molecules.

DIFFERENT METHODS OF SAMPLE PREPARATION

Dried droplet method

In this method, a dilute solution of the analyte is prepared in a good solvent. This analyte solution is then mixed with a more concentrated matrix solution in the same solvent. The solvent must be selected carefully to be a good one for both the analyte and the matrix. A relatively volatile solvent can be used. The mix ratio of the two solutions should result in a matrix to analyte ratio between 100 and 10000. For low molecular weight polymers, we typically use 5 mg/mL polymer solutions and mix them 1:7 with 0.25 M matrix solutions. About 1 μL of the resulting solution is then deposited on the target substrate and dried, and the solid mixture is then placed into the mass spectrometer.

Layer method

The matrix solution is applied to the target surface and allowed to dry. The sample solution is then applied to the dry matrix crystals (Hanston 2001). MALDI experiments are used to analyse, isolate the oligomers. Isolate the oligomers: The matrix must maintain the separation of the oligomers obtained by dissolving the polymer in a good solvent.

Fast-evaporation method

The fast evaporation method was introduced with the main goal of improving the resolution and mass accuracy of MALDI-MS measurements. Matrix and sample are handled separately. This method is similar to the layer method with a difference that the process delivers stable and long-lived matrix films that can be used to precoat MALDI targets.

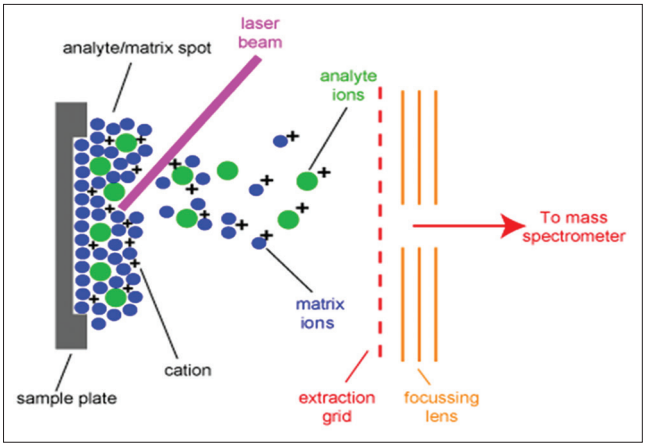

Fig. 1: Process of Ionization in MALDI-MS

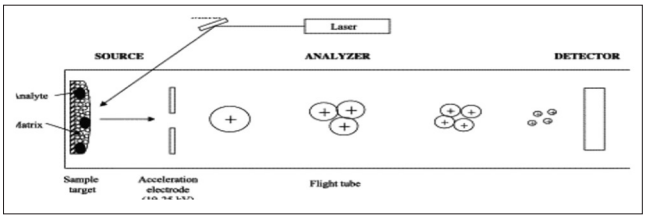

Fig. 2: Schematic diagram of MALDI-MS

Electrospray

A small amount of matrix analyte mixture is electro sprayed from a HV-biased (3-5 kV) stainless steel or glass capillary onto a grounded metal sample plate, mounted 0.5-3 cm away from the tip of the capillary. Electrospray sample deposition creates a homogenous layer of equally sized microcrystal and the polymer.

Molecules are evenly distributed in the matrix. The method has been proposed to achieve fast evaporation and to effectively minimize sample segregation effects.

Matrix-precoated layers

This sample preparation method is reduced to the straightforward addition of a single drop of undiluted sample to a precoated target spot. The advantages are faster preparation and more sensitive results than the previous methods. It also offers the opportunity to directly interface the MALDI-MS sample preparation to the output of LC and CE columns. Most efforts have been focused on the development of thin layer matrix-precoated membranes, e.g., nylon, PVDF, nitrocellulose, anion and cation modified cellulose, regenerated cellulose, or regenerated cellulose dialysis membrane.

Chemical liquid

The preparation of such samples is rather simple. An appropriate molar ratio of the analyte is dissolved in the liquid matrix, often by employing a solvent that is evaporated before the introduction of the sample into the vacuum system of the mass spectrometer.

Particle-doped (two-phase) liquid

In the case of two-phase matrices, a suspension of particles (fine metal or graphite with a diameter of £ 1 μm) in a solvent is mixed with analyte and binder. The solvent is evaporated, and the remaining paste on the sample holder is introduced in the ion source. Care should be taken about possible contamination by small particulates that are sputtered from such samples. The fine particles absorb most of the energy from the laser beam and promote the desorption. The liquid molecules provide the charge for ionization. Several combinations of particulates and liquids have been used to analyze polymers and dyes up to a molecular weight of over 10,000.

CHOICE OF THE MATRIX

We have four important roles to accomplish to produce ions: Intimacy, absorption, desorption, and ionization. Intimate contact between the matrix and the analytes is required to produce ions. The matrix should readily mix with the analyte molecules. They should have the same hydrophobicity or hydrophilicity to the analyte. As a general rule, one should try to match the solubility of the matrix and the sample, since the MALDI effect depends upon the close proximity of the two generated by co-crystallization for charge transfer to take place. The analyte molecules need to be in close proximity to the matrix molecules to be effectively desorbed. The only source of energy to induce desorption is the laser light. The matrix must absorb at the wavelength of laser. Most commercial MALDI instruments have ultraviolet nitrogen lasers with emission at 337 nm. To volatilize the analyte, the matrix must assist the desorption of the analyte from the substrate. Upon irradiation by the laser pulse, the matrix molecules absorb most of the laser's energy. The high matrix to analyte ratio ensures that most of the energy is absorbed by the matrix and minimizes direct irradiation of the analyte. The energy absorbed by the matrix molecules is utilized for electronic excitation of the matrix within the solid sample mixture, creating an instantaneous phase transition from a solid phase to the gas phase. The amount of material removed by each laser pulse is estimated to be approximately about 10-100 μm in diameter (laser spot size) and a few hundred nanometers deep. A dense gas cloud is formed and expands supersonically into the vacuum. It is believed that analyte ionization occurs in the expanding plume as a direct result of collision between analyte neutrals, excited matrix ions, and protons and cations such as silver and sodium. The matrix must also facilitate ionization of the analyte molecules. MALDI ions are not formed directly by the absorption of laser light. Primarily, we observe cat ionized species. Since most matrices are acidic, protonation is a common mechanism for analytes with basic functional groups. Another mechanism is metal cat ionization. Analytes with oxygen functionality are readily cat ionized with alkali metal salts such as LiCl, NaCl, KCl, and analytes with unsaturated hydrocarbon functionality are readily cat ionized with transition metals such as silver and copper. Addition of metal cations is a part of the sample preparation method.

MALDI-TOF

Charged ions of various sizes are generated on the sample slide, as shown in the diagram. A potential difference V_0 between the sample slide and ground attracts the ions in the direction shown in the diagram. The velocity of the attracted ions V is determined by the law of conservation of energy. As the potential difference V_0 is constant with respect to all ions, ions with smaller m/z value (lighter ions) and more highly charged ions move faster through the drift space until they reach the detector. Consequently, the time of ion flight differs according to the mass-to-charge ratio (m/z) value of the ion. The method of mass spectrometry that exploits this phenomenon is called TOF MS.

Electron spray ionization coupled to triple quadrupole and ion trap MS and MALDI coupled to TOF analyzers have been successful in obtaining very accurate mass measurements. MALDI-TOF-MS is a good tool for screening peptide masses of tryptic digests. This method is more effective because it requires relatively less intense sample preparation since the matrix is less susceptible to interferences caused by salts and detergents. Second, MALDI-TOF-MS generates peptides containing only one charge and show only one peak in the spectrum which facilitates data interpretation.

SCHEMATIC AND THEORY OF MALDI

MALDI is a very sensitive technique for determining the mass of proteins, peptides, or polymers. Protein masses are the identity of proteins, and thus help in proteomics. Thus, MALDI allows protein identification. MALDI sample preparation is relatively fast and easy. It is a first choice when it comes to protein study. Proteins, peptides, and polymers are fragile and tend to fragment when ionized by other ionization techniques (Fig. 3).

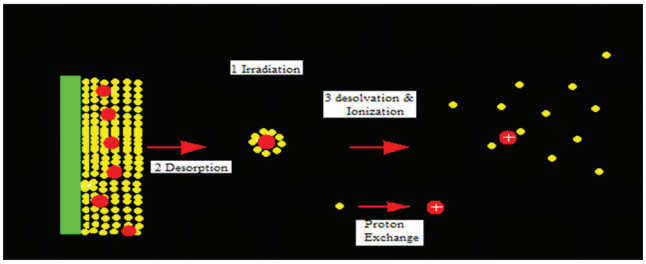

Fig. 3: Schematic process of MALDI in MS

MALDI is attached to a TOF analyzer which measures time it takes for the molecules to travel a fixed distance. MALDI is a soft ionization technique in which a short laser pulse, instead of continuous laser, of nitrogen gas usually around 237 nm is used to ionize molecules. A protein or peptide sample is placed on a target plate and mixed with an appropriate matrix on the target plate. The mixture of sample and matrix crystallizes due to the vacuum environment and then is irradiated with a short laser pulse. The sample molecules and the matrix now enter gas phase. This leads to release of matrix, samples molecules, and ions from the target plate. The ions then accelerate in TOF analyzer because they are subject to equal electric field. TOF is a field-free flight tube. The ions travel in a straight and linear direction to the detector. The mass to charge ration (m/z) of the sample ions can be calculated using the equation

$$T = C1\ (m/z)^{0.5} + C2. \quad \text{Eq. 1}$$

C1 and C2 are instrumental constants, which can be determined with compounds of known mass. This equation is derived from the fact that potential energy equals kinetic energy.

$$KE = 0.5mv^2 \quad \text{Eq. 2}$$

$$v = (2KE/m)^{1/2} \quad \text{Eq. 3}$$

Since velocity is distance/time substitute it in eq. 2 and solve for t to get eq. 3

$$t = m^{1/2} * d/(2KE)^{1/2} \quad \text{Eq.4}$$

The distance the molecules travel and their kinetic energy is constant. So, it is replaced by C1. Furthermore, since the relationship between the t and $m^{1/2}$ is linear, an intercept of C2 is added to get the equation of a line.

$$t = C1(m/z)^{1/2} + C2 \quad \text{Eq.5}$$

SAMPLE PREPARATION

Biomolecules such as proteins, peptides, sugars, and large organic molecules such as polymers, dendrimers, and other macromolecules can be analyzed using MALDI. MALDI is more tolerant to sample contaminants, but contaminants can seriously disturb incorporation of sample molecules with growing matrix crystals. This results in bad spots on the target plate, leading to low signal to noise ratio, resolution, and sensitivity.

Samples can be prepared in two different ways. One removes the contaminants before applying them onto the matrix, and one removes the contaminants after the sample is spotted onto the target plate either before or after adding the matrix.

Miniaturized chromatographic set-up is used for the first approach, while some scientists have cleaned in-gel digest of proteins using reverse phase (RP) high performance liquid chromatographic (HPLC) micro columns packed with different types of RP-HPLC beads. Now tips are also packed with RP or ion-exchange resin to remove salts and detergents from protein mixtures, and their effectiveness is shown by their recent commercialization. Tips and columns effectively remove MALDI contaminants and give a small volume of sample; this can result in possible highly sensitive MALDI analysis of the samples.

Purification of biological samples on target plates involves synthetic membranes or surfaces. Membranes go on top of the target plate, and the biological samples are spotted on top of the membranes. Biological samples interact with the membrane through strong hydrophobic forces. This enables samples to remain on the membrane while the buffers and salts are washed away. Then the MALDI-matrix solution is added to the purified samples on the target plate, ready for analysis. Perfluorosulfonated ionomer films, polyethylene membranes, nonporous polyurethane membranes, and C8 and C18 extraction disks are examples of membranes that have been used successfully in the past for biological mixtures. Self-assembled monolayer's of octadecyl mercaptan on gold-sputtered disposable MALDI probe tips have been used to concentrate the sample and to act as a purification device.

MATRIX

The function of the matrix is absorption of energy from laser pulse, and then transfer to sample thereby causing desorption of the analyte molecules in an expanding plume, to ionize the desorbed analyte molecules, and to prevent aggregation of the analyte molecules. The matrix molecules for MALDI are chosen on the basis on fulfillment of requirement that matrix molecules must be able to absorb ultraviolet wavelength of usually 237 nm, low volatility, and ability to transfer protons to the sample molecules. For proteins samples typical MALDI matrix consist of cinnamic acid and hydroxylated benzoic acid derivatives to prevent contamination from the sample.

2,5-dihydroxybenzoic acid is more tolerant to the sample contaminants because it excludes them during crystallization process. The use of specially prepared thin matrix layers uses fast evaporation setup, which not only improves the sensitivity and resolution, but also allows the samples to be extensively washed, removing salts and detergents. Since the sensitivity depends on the concentration of the sample on the target plate, samples can be concentrated using PR-HPLC or bead-peptide concentration. In bead-peptide concentration, RP-chromatographic beads are added to the proteins or peptide samples, and these samples preferentially bind to the beads through hydrophobic interactions while the contaminants such as salts and chaotropes do not. After a short incubation, the peptide-bead solution is harvested using pellets from a centrifugation, and dried in speed vacuum concentrator. In both cases, highly concentrated pellets of peptide-bound beads are obtained, which can be transferred to MALDI and left to dry. Because the beads are hydrophobic in nature, they form a cluster in highly concentrated spot (<1 mm 2) on MALDI target plate after drying. Peptides elute on the target plate by a small volume of aqueous/organic MALDI-matrix solution and become incorporated into the growing matrix crystals at the same time. This process allows 10-100 mol to be enough to be loaded on to the gels.

SENSITIVITY

Sensitivity of MALDI depends on sample preparation and the preparation of sample/matrix layer. Preparing a very thin matrix layer and apply the sample to the matrix. So that the sample is on the outer layer of matrix, gives sensitivity level of low to mole. This method makes it possible for removal of salts present in the analyte solution by a sample washing procedure away from the sample very simply. Small matrix spots using nanoliter volumes of matrix and analyte solution, combined purification, concentration, and application procedures have also given similar sensitivities. In this method, matrix is adsorbed to nanoliter bed volume reversed-phase column prepared in an Eppendorf GeLoader tip. Then the column is washed and the sample is eluted with a few nanoliter volume of matrix onto the target plate.

Sensitivity is reduced with increasing molecular weight. The sensitivity is two to three magnitudes lower for proteins than it is for peptides. So, the sensitivity of proteins is in the femtomole range.

STRUCTURAL INFORMATION

Structural information of proteins can be determined by digesting proteins with specific endoprotease such as trypsin, AspN, and GluC. MALDI is one of the best spectrometric techniques for direct analysis of peptide mixtures. Signals of peptides are suppressed because there is a competition for charge or optimal position in the matrix. Therefore, signal intensity does not necessarily reflect the quantities of different peptides in the mixtures. Complete sequences can be obtained from a combination of spectra recorded in different modes such as positive and negative matrices and different enzyme digestion.

Sequence information is also possible to get from power spectral density (PSD). This is possible by controlling the voltage of the reflector, which results in different m/z ranges on the detector and generates a PSD spectrum. A large sample amount is required since only a small fraction goes under PSD. Additionally, the fragmentation cannot be controlled since the different site of a peptide can get fragmented. This makes it very hard to get complete sequences of a peptide. Alternatively, collision cells are included to the flight tube in MALDI-TOF by some manufacturers, to have controlled fragmentation by collision-induced dissociation.

Large amounts of in-source fragmentation occurs before initiation of the acceleration voltage called in-source decay in delayed extraction (DE) equipped MALDI-TOF, which only yield long regions of sequence-specific ions. C-terminal sequence ladders can be generated by digestion of peptides with carboxypeptidase and N-terminal sequence ladders can be obtained by Edman degradation using a low percentage of phenylthiocarbamate rather than phenyoisothiocarbamate in the coupling reaction. These ladders in the mixtures of peptides can be an alternative to sequence-specific fragment ions. This process often gives a lot of sequence information.

Secondary protein modifications can also be determined using MALDI-TOF-MS. The steps involved in determining secondary modifications are measuring mass of the intact protein, knowing the protein's primary sequence, and generating site-specific information by direct mass spectrometric peptide mapping of a mixture derived by proteolytic cleavage of the proteins. In tandem (TOF/TOF) configurations, MALDI instruments can provide protein sequence data, as well.

In tandem mass spectrometry, an ion of a particular mass is selected (that's the first stage of the analysis) and fragmented. Its constituent fragment ions are then mass-analyzed a second time (that's the tandem stage) to reveal data about the molecule's structure or sequence; single-stage TOF instruments lack this capability (though some fragmentation does occur via "post-source decay" as the ions traverse the flight tube).

TOF ANALYZER

Modes

Ions can travel in a linear fashion and be detected by the detector at the opposite end as an ion source. This is called linear TOF. It is different from a reflectron TOF, in which ions are reflected to electrostatic mirror and detected by another detector. Linear TOF spectrum is limited in resolution leading to low mass accuracy. This is because initially different amount of kinetic energy can be attained by the ions with the same charge. This leads to different (m/z) ratio of ions which have different initial velocities. This is partially corrected by reflectron TOF. High energy ions penetrate deeper into the reflectron, taking longer distance and time, while low energy ions do not penetrate as deep into the reflectron and take a shorter path and time. This leads to correction of different times of ions with the same mass and charge. This leads to an increase of resolution to 10,000.

The amount of energy put into ions by the laser initially can also be corrected by a technique called DE in which acceleration voltage is applied slightly after the laser pulse. DE TOF increases resolution to 2000.

RE TOF can give full isotopic resolution for molecules up to 15 kDa. However, 12C-only ion peak will have very low intensity for molecules bigger than 5kDa. Resolution is further decreased because each ion decays after acceleration, and its TOF cannot be adjusted by RE TOF because decay has already occurred. These ions are not detected in RE TOF. However, these ions can be detected in linear TOF, but the resolution still decreases because linear TOF has no way of correcting different energy inputs to an ion with the same mass and charge. It is better to use linear mode to get spectra and determine isotopically averaged mass for molecules bigger than 5-10 kDa. Resolution depends on the size of the molecules in a sample. The greater the size that the sample molecule has, the lower the resolution of it.

MALDI-TOF can only be compared to electrospray ionization (ESI) because there are two ways of directly analyzing proteins, peptides, and polymers. MALDI-TOF samples can be reanalyzed while ESI samples cannot because ESI is connected to LC column, and the analysis is limited to the width of the chromatographic peak. MALDI-TOF can scan 10 spectra for a peak 10 seconds wide per second, while it takes ESI almost 15 seconds. MALDI-TOF-MS generates mostly ions ±1 charge while ESI generates a charge for every 8-10 amino acids. That means ESI spectra are considerably more complex than MALDI spectra. In general, MALDI is faster than ESI and enables higher throughput. But, ESI is more sensitive.

MALDI-MSI

MALDI imaging is the use of matrix-assisted laser desorption ionization as a mass spectrometry imagingtechnique in which the sample, often a thin tissue section, is moved in two dimensions while the mass spectrum is recorded.

Matrix-assisted laser desorption ionization imaging mass spectrometry (IMS) is a relatively new imaging modality that allows mapping of a wide range of biomolecules within a thin tissue section. The technology uses a laser beam to directly desorb and ionize molecules from discrete locations on the tissue that are subsequently recorded in a mass spectrometer. IMS is distinguished by the ability to directly measure molecules *in situ* ranging from small metabolites to proteins, reporting hundreds to thousands of expression patterns from a single imaging experiment. This article reviews recent advances in IMS technology, applications, and experimental strategies that allow it to significantly aid in the discovery and understanding of molecular processes in biological and clinical samples.

APPLICATIONS

In photolithographic structuring

This method was used to image an ultraviolet (UV) exposed the negative photoresist layer, which is generally used to manufacture printed circuit boards for electronic components. The negative photoresist layer consisting of the main component novolac, benzophenone as the active component, and the solvent tetrahydrofuran was mixed with the matrix dithranol and the salt additive LiTFA and spin-coated onto an indium tin oxide-conductive glass slide. To imprint an image on the created surface, a transparency with a printed wiring diagram was placed on top of it and irradiated by UV light for 15 minutes. The inspection of the efficient imprinting of the microstructure onto the photoresist layer was performed by MALDI-MSI. This unique application represents a further step toward the surface analysis of polymer films by this emerging life science imaging technique.

SYNTHETIC POLYMER ANALYSIS

Synthetic polymers which can be analyzed by MALDI are classified into the following four groups (Kuang and Odom 1998): (i) Water soluble

polymers: poly (acrylic acid) and poly (ethylene glycol); (ii) polar organic soluble polymers: acrylics and poly (methyl methacrylate); (iii) non-polar organic soluble: polystyrene, polyethylene, and (iv) low solubility polymers: cured polyimide. With the developments in MALDI methods, the technique is used to characterize the polymer reactions. MALDI data can be used to characterize both the chemical structures of the products and the rate coefficients of chemical reactions. MALDI combined with gel permeation chromatography were used to characterize the cyclic aryl ether ketone oligomer.

MALDI has been used mostly for the characterization of homopolymers created from a single monomer species. Materials created from two or more monomer species are copolymers. There has been comparatively little work done on copolymers due to problems in developing suitable sample preparation methods for them.

BIO MEDICAL

MALDI-TOF-MS is used to obtain fast and accurate determinations of molecular mass, but quantitative determinations are generally made by other techniques. In this study, we illustrate the practical utility of automated MALDI-TOF-MS as a tool for quantifying a diverse array of biomolecules covering an extensive molecular weight range, and present in biological extracts and fluids. Growth hormone was measured in rat pituitary tissue; insulin in human pancreatic tissue; homovanillic acid in human urine; and LVV-hemorphin-7, epinephrine, and norepinephrine in human adrenal and pheochromocytoma tissues. Internal standards, including compounds of similar molecular weight, structural analogs or isotopomers, were incorporated into each analysis. We report on the current practical limitations of quantitative MALDI-TOF-MS and highlight some of the potential benefits of this technique as a quantitative tool.

BIOMARKER DETERMINATION

One of the most active MALDI-TOF application areas is in the analysis of disease-specific biomarker peptides and low abundance proteins in blood, urine, and other biological fluids. These molecules are used as disease-specific biomarkers.

MALDI-TOF has demonstrated that it can be a straightforward and reproducible tool for rapidly scanning these samples, detecting and distinguishing hundreds of low molecular weight proteins and peptides in seconds.

The MALDI protocol first involves the capture and enrichment of peptides using magnetic beads or some other chromatographic method. In the second step, a portion of the enriched sample is mixed with a matrix like α-cyano-4-hydroxycinnamic acid. Next, the mixture is spotted onto a target and allowed to dry at room temperature. The final step is the desorption of bulk portions of the solid sample by a short pulse of laser light. The matrix absorbs the laser energy, causing co-desorption of the analyte and promoting analyte ionization. Liberated ions from the sample are swept by vacuum into the mass analyzer.

The resulting spectra can be recorded in the linear positive ion mode with external calibration in the range of analysis (1-15 kDa) using a mixture of peptide/protein standards. Up to 400 peptides can be detected and characterized in this mass range. This approach has been used in studies focused on the detection of biomarkers for asthma, acute lymphatic leukemia, and brain tumors, among many others. In each case, specific profiles of peptides have been observed that can be used to predict if the sample is normal or diseased.

CLINICAL DIAGNOSTIC IN MICROBIOLOGY

The development of MALDI-TOF-MS devices has revolutionized the routine identification of microorganisms in clinical microbiology laboratories by introducing an easy, rapid, high throughput, low-cost, and efficient identification technique. This technology has been adapted to the constraint of clinical diagnostic laboratories and has the potential to replace and/or complement conventional identification techniques for both bacterial and fungal strains. Using standardized procedures, the resolution of MALDI-TOF-MS allows accurate identification at the species level of most Gram-positive and Gram-negative bacterial strains with the exception of a few difficult strains that require more attention and further development of the method. Similarly, the routine identification by MALDI-TOF-MS of yeast isolates is reliable and much quicker than conventional techniques. Recent studies have shown that MALDI-TOF-MS has also the potential to accurately identify filamentous fungi and dermatophytes, providing that specific standardized procedures are established for these microorganisms. Moreover, MALDI-TOF-MS has been used successfully for microbial typing and identification at the subspecies level, demonstrating that this technology is a potential efficient tool for epidemiological studies and for taxonomical classification.

SINGLE CELL ANALYSIS BY MALDI-MS

Single-cell analysis is gaining popularity in the field of mass spectrometry as a method for analyzing protein and peptide content in cells. The spatial resolution of MALDI MS imaging is by a large extent limited by the laser focal diameter and the displacement of analytes during matrix deposition. Owing to recent advancements in both laser optics and matrix deposition methods, spatial resolution on the order of a single eukaryotic cell is now achievable by MALDI MS imaging. Provided adequate instrument sensitivity, a lateral resolution of approximately 10 μm is currently attainable with commercial instruments. As a result of these advances, MALDI MS imaging is poised to become a transformative clinical technology.

LIPIDOMICS OF INTACT MITOCHONDRIA BY MALDI-TOF-MS

A simple and fast method of lipid analysis of isolated intact mitochondria by means of MALDI-TOF mass spectrometry is described. Mitochondria isolated from bovine heart and yeast have been employed to set up and validate the new method of lipid analysis. The mitochondrial suspension is directly applied over the target and, after drying, covered by a thin layer of the 9-aminoacridine matrix solution. The lipid profiles acquired with this procedure contain all peaks previously obtained by analyzing the lipid extracts of isolated mitochondria by TLC and/or mass spectrometry. The novel procedure allows the quick, simple, precise, and accurate analysis of membrane lipids, utilizing only a tiny amount of isolated organelle; it has also been tested with intact membranes of the bacterium.

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