

MALDI-TOF Mass Spectrometry and Protein Purification

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DESCRIPTION

In clinical and laboratory diagnostic settings, Matrix-Assisted Laser Desorption/Ionization- Time-of-Flight (MALDI-TOF) mass spectrometry has been used for several decades as an approach for protein-centered analysis of samples in medical chemistry.

Matrix-Assisted Laser Desorption/Ionization- Time-of-Flight Mass Spectrometry (MALDI-TOF MS) is an analytical technique in which particles are ionized, separated according to their mass-to-charge ratio, and measured by determining the time it takes for the ions to travel to a detector at the end of a time-of-flight tube. The MALDI-TOF mass spectrometry profiling methodology is an effective technique that was created and is largely used for the quick and precise identification of microorganism species.

Systems (instrument, processing method, and databases) were developed for this purpose by at least three commercial manufacturers, with findings that were basically interchangeable but not totally identical. These techniques were developed for species level identification, but it was also explored with a few changes for expression profiling applications. Mass spectrometry has brought possibilities in the field of proteomics which are unexpected by the researchers.

Outstanding improvements in the previous ten years have made data comparison and acquisition faster and more accurate. MALDI-TOF-MS is primarily used to identify proteins using Peptide Mass Fingerprinting (PMF). Many researchers have demonstrated that MALDI-TOF MS can be used for early detection of bacteria in blood cultures, Urinary Tract Infections (UTIs), respiratory tract infections, stool samples, and more. The major advantages of MALDI-TOF are its rapid turnaround time (<10 min) and 95% overall accuracy at the species level, enabling more rapid and accurate treatment of patients. Another advantage of MALDI-TOF in diagnostics field is the low cost of consumables and technician turnaround time. The advantages of time-of-flight mass spectrometers over quadrupole measurements are clear. Samples can be measured faster and without spectral contortion. For the same mass range, TOF analyzers measure each ion with greater sensitivity. The MALDI-TOF can be used to profile and image proteins directly from

thin tissue sections, known as MALDI Imaging Mass Spectrometry (MALDI-IMS). It provides specific information on the local molecular composition, relative abundance, and spatial distribution of peptides and proteins within the analyzed section. MALDI-IMS can together analyze multiple unknown compounds in biological tissue sections through a single measurement that can obtain a molecular map of the tissue while maintain the cellular and molecular integrity within the tissue.

MALDI-TOF mass spectrometry can analyze a wide variety of biomolecules such as peptides, proteins, carbohydrates and oligonucleotides. Due to the fact that the ions formed have low internal energies, a major advantage of MALDI-TOF is that the process of soft ionization allows observation of ionized molecules with less analyte fragmentation. This allows even molecular ions of analytes in mixtures to be discriminated. The fast data collection also makes it easy to use and maintain properly. Selection of an appropriate matrix material is very important for successful MALDI-TOF mass spectrometry. The determination of intact mass is fundamental and important to protein characterization, since the exact molecular mass of a protein can indicate the intact structure. MALDI, a gentle ionization technique, is suitable for proteins that tend to break easily and fragment when ionized by other ionization methods. The performance of MALDI-TOF MS is less affected by buffer components, detergents and contaminants. In addition, it allows mass determination of intact proteins with sufficient accuracy (≤ 500 ppm) for sequence confirmation. After proteolysis, the MALDI-TOF MS can also be used to analyze the resulting peptides to further confirm the primary sequence by mass imprinting of the peptide. More recently, MALDI-TOF MS has been used in research settings for the detection and identification of viruses, protozoa and arthropods. MALDI-TOF MS is significantly less prone to contamination and results are available within minutes. Also, MALDI-TOF MS is no longer restricted to high-income countries as it is increasingly available in reference laboratories in every low-income country as well. Preliminary data suggest that MALDI-TOF MS can hold promise as a future diagnostic tool for direct and rapid identification of pathogenic helminthes in clinical specimens with sufficient accuracy.

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