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Matrix Assisted Laser Desorption and Ionization (MALDI)

MALDI is a soft ionization technique used in mass spectrometry. It is used for identification and spatial distribution studies of biomolecules (DNA, proteins, peptides, sugars) and large organic molecules (polymers, dendrimers, other macromolecules).

MALDI enables desorption of intact molecules, thereby facilitating accurate determination of the molecular mass of entire molecules. Subsequent controlled fragmentation studies of these molecules allow for unique identification.

Principles of the Technique

MALDI is a unique technique allowing for desorption and ionization of entire molecules, unlike more conventional ionization techniques. In a first step, a sample is mixed with a selected matrix, like α-cyano-4-hydroxycinnamic acid, and deposited on a target plate. This plate is bombarded by photon pulses from a laser resulting in ionization of the matrix (figure 1).

Subsequently, the energy is transferred from the matrix to the sample molecules. This gentle energy transfer process leaves the sample molecules intact, yielding protonated/cationized or deprotonated/anionized molecules (ions). The ions are analyzed with a Time-of-Flight Mass Spectrometer (ToF MS). Using this technique, the mass of the ions is determined by separation of the ions in time according to their mass/charge (m/z) ratio. Scans of the whole mass range are taken in ToF MS to obtain a mass spectrum. ToF MS enables one to determine the molecular masses of the ions with high accuracy.

In most cases, obtaining the mass of a specific molecule is not sufficient for unique identification. A route to acquire this information is tandem mass spectrometry (MS/MS or ToF/ToF). First, one selected ion is isolated and subsequently fragmented. The second spectrometry analysis yields a pattern of fragments, forming a characteristic fingerprint of the molecule.

Accurate Molecular Weight Measurements

A primary application for MALDI is the measurement of accurate molecular weights of compounds, especially large and fragile molecules. In this example a MALDI analysis is used to verify whether a purchased material labeled “Glu-Fibrino peptide B (Glu-Fib)” really contains Glu-Fib and to check the purity of the sample. The sample is mixed with a matrix and directly spotted on a MALDI plate.

Figure 2 shows the mass spectrum of the sample. The main peak is mass 1570.677, corresponding to the mass of Glu-Fib. Several other masses (like 1051 and 1165) are visible, meaning that the sample is contaminated. Tandem MS was applied to uniquely characterize this sample by selecting mass 1570.677 and apply subsequent fragmentation. The result of the MSMS spectrum can be seen in figure 3. The masses 175, 684, 813, 1056 and 1441 are typical for the fragments of Glu- Fib, confirming that the sample consists of Glu-Fib.

Amino Acid Sequencing

MALDI is well suited for proteomics purposes. In proteomics the structures and functions of proteins in biological systems are studied. In the present example proteins in blood plasma are identified with Liquid

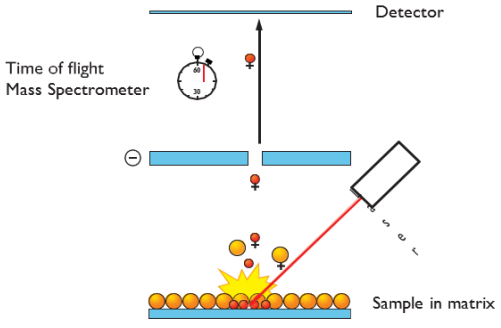

Fig. 1: Principle of MALDI

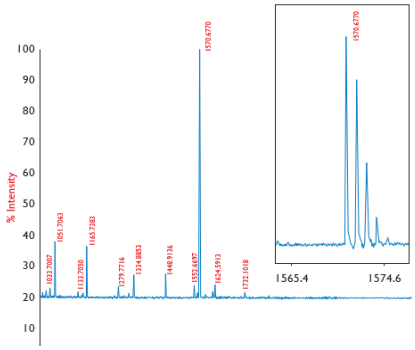

Fig. 2: mass spectrum of Glu-Fib with the isotope pattern of mass 1570.677

Chromatography (LC)-MALDI. In a first step, the proteins in the sample are cleaved in smaller parts with an enzyme, yielding peptides. These peptides can be separated with LC in an appropriate number of fractions (here 381). These fractions will be mixed in-line with a matrix and directly spotted on a MALDI plate (see small front-page image). Subsequently, the spots are ionized with MALDI and analyzed with MS and with MSMS (figure 4), which results in a huge amount of MSMS spectra. All spectra are introduced in a data search tool to identify the peptides and proteins.

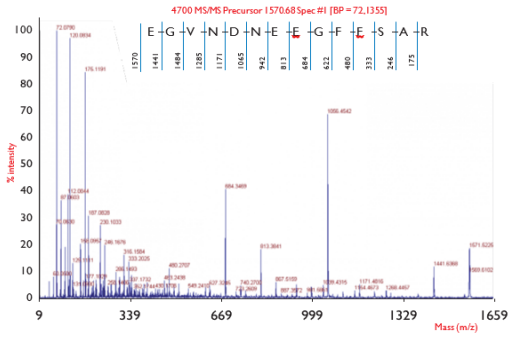

Fig. 3: MSMS spectrum of precursor 1570.677 (Glu-Fib). The inset displays the sequence of amino acids that Glu-Fib consists of (e.g. G = guanine) as well as the masses of these fragments

MALDI Imaging

A special feature of MALDI is MALDI imaging. This can be used for the determination of the spatial distribution of compounds within biological tissues or non-biological systems. Any flat surface can be analyzed for the presence and distribution of compounds using this technique. In this example a bank note is investigated for the presence and lateral distribution of different dyes (figure 5). No matrix is used because the used dyes ionize easily. A region of interest is scanned within a specific mass range (in this case 150-1000), each pixel containing the full mass spectrum.

From the data set, 2-dimensional maps of the distribution of specific masses can be generated. Figure 5 displays 2-D images of dyes with masses 575 and 378. As can be deduced from these images, the blue color is caused by a dye with mass 575 and the red color by a dye with mass 378. A mass spectrum can be extracted from the image to obtain more detailed information on the chemical nature of the different dyes. The mass spectra are shown in figure 5. This example shows how MALDI imaging makes it possible to make a fingerprint of expected and unexpected compounds of interest.

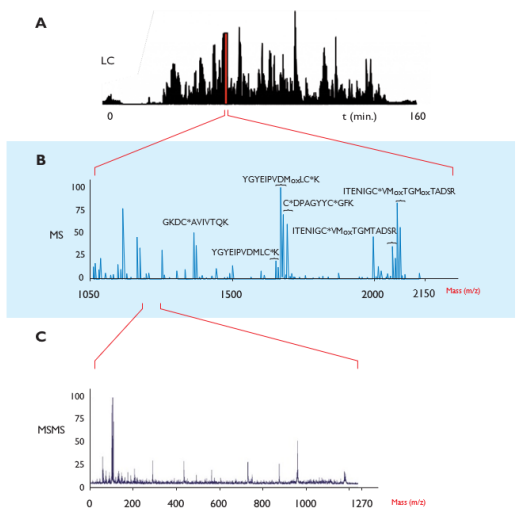

Fig. 4: Three-step analysis of proteins in blood plasma. a) Separation using LC in a large set of fractions. b) MS analysis and c) subsequent MSMS analysis of each peptide

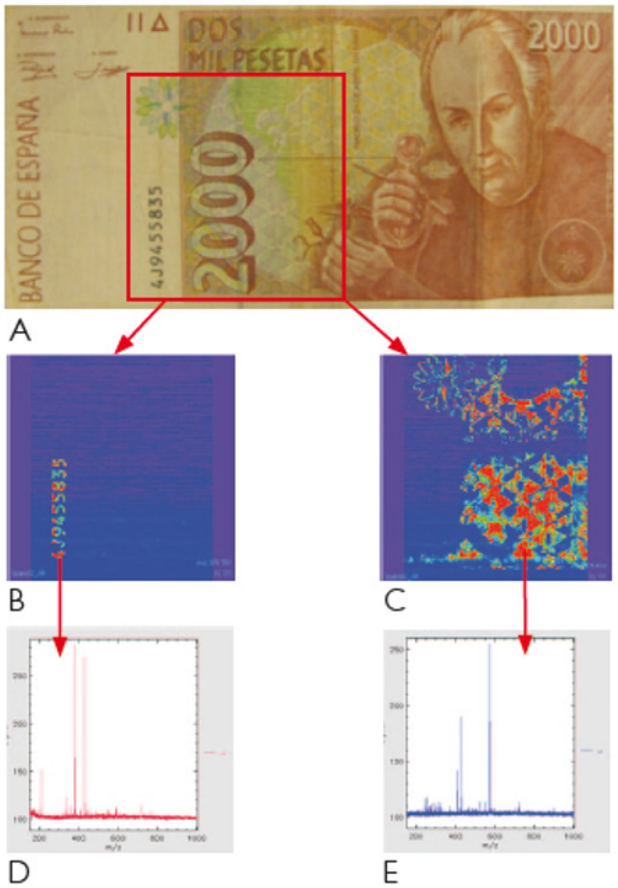

Fig. 5: MALDI imaging analysis of a bank note. b) and c) are 2-D maps of the distribution of dyes with masses 575 (blue dye) and 378 (red dye). d) and e) display spectra from specific areas on the note, indicating that on these positions different combinations of dyes are present

Characteristics

Information obtained

- Composition, molecular weight and structural information on individual components
- Qualitative and (semi-)quantitative

Sample type

- Solid or liquid (dissolved in aqueous buffers or solvents like DMF or DCM)

Mass range

- 50 – 100000 g/mol

Mass accuracy

- 0.1% (1000 ppm) for masses > 4000 g/mol
- 0.005% (50 ppm) or better for masses < 4000 g/mol
- $50*10^{-3}$ g/mol in MS/MS mode

Mass sensitivity

- Depending on compound and matrix

Lateral resolution (imaging)

- 20 x 20 µm

Applications

Accurate molecular weight measurements:

- Compound identification and verification
- Determination of the purity of a sample
- Verification of amino acid substitutions
- Verification of post-translational modifications

Reaction monitoring:

- Chemical modification
- Protein digestion

Amino acid & oligonucleotide sequencing:

- Confirmation of amino acid sequence
- De novo characterization of peptides
- Identification of proteins by database searching with a sequence “tag” from a proteolytic fragment
- Characterization or quality control of oligonucleotides

Protein structure:

- Protein-ligand complex formation under physiological conditions
- Macromolecular structure determination

Spatial distribution of compounds within biological tissues or non-biological systems