Metabolomics and Metabolite Profiling using Mass Spectrometry Imaging (MSI): A New Biochemical Tool for Drug Discovery

High resolution mass spectrometry imaging has been used to study endogenous metabolites from biological tissues. The localization and the identification of small molecules involved in the main metabolic pathways can be achieved thanks to a FTICR mass spectrometer and a MALDI ionization source. It allows the possible discovery of new therapeutic targets, the obtention of valuable pharmacodynamics data or the characterization of most of the metabolism pathways in animal models.

Introduction

By definition, metabolites are intermediates and products from different metabolisms. More commonly, they are small compounds found in organisms and have an important role in cells life and survival. Each of them can have a specific function and represents a marker of a biochemical process. They can be nucleotides, amino or organic acids, lipids... Their identification permits the deconvolution of major biological pathways (TCA or Urea Cycle, Purine metabolism, Glycolysis…) and the better understanding of pathologies mechanism (Cardiovascular disease, Diabetes, Cancer, Inflammatory diseases…).

Different techniques such as Nuclear Magnetic Resonance (NMR) or Mass Spectrometry (MS) can be used to characterize these molecules. Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR-MS) is commonly chosen to study complex biological mixtures thanks to unmatched mass resolution, high mass accuracy and structural characterization. Therefore, FTICR is the instrument of choice for metabolite profiling in tissue in combination with MALDI ionization source[1]. The application of Mass Spectrometry Imaging (MSI) in the study of endogenous metabolites from biological tissues is a quite recent but promising technique which offers to simultaneously monitor several compounds (drug and metabolites) with spatiotemporal information about molecular behavior[2,3]. In this respect, it can be a valuable tool for pharmacodynamics (the study of biochemical and physiological effects of drugs on organism) to understand drug efficacy and potential toxicity in drug preclinical development.
Figure 1

Distribution of ribonucleotides in different organs using MSI (negative mode, 110 µm of lateral resolution)

Figure 2

Representation of main metabolites from different tissues (rat kidney and brain) involved in Krebs cycle (Citrate cycle and Glycolysis) using MSI ((negative mode, 110 µm of lateral resolution)
Brain, Liver and kidney tissue sections from control rat were carried out with a Microm cryostat HM560 (Thermo Scientific, USA), at 10 µm thickness. All sections were mounted on conductive ITO glass slides, and then dried. The matrix was adapted to negative ionization polarities; the 9 amino-acridine (9AA) was choice and applied using SunCollect Automated Sprayer (SunChrome, Friedrichsdorf, Germany).

MS images were acquired with a Solarix 7.0T MALDI-FTICR mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with a SmartBeam II laser used at a repetition rate of 1000 Hz. All instrumental parameters were optimized before the imaging experiment on adjacent tissue sections. Negative mass spectra were acquired within the 100- to 1200-m/z range. The mass spectrometer was operated in the fullscan mode (with on-line data reduction and the accumulation during detection mode) and the mass spectrum obtained for each image position corresponds to the averaged mass spectra of 500 consecutive laser shots at the same location. MALDI Images were performed on the different organs at a spatial resolution of 110 µm (≈ 10000 voxels depending of the tissue dimension). MS Images were visualized using Quantinetix software (ImaBiotech, France). Cryosections of tissue were stained with hematoxylin and eosin (H&E) solution after MSI analyses in order to localize fine histological structures.

**Results**

Endogenous metabolites are involved in different diseases as biomarkers of a pathology or readout molecules used to ensure the efficacy of a treatment. MSI gives access to a wide range of metabolites depending on ionization properties, small molecules (AMP or ATP) or lipids (phosphatydilcholine, ceramides, triglycerides…) are easily detected by mass spectrometry. As example, we present here a study of nucleotides in tissue which play an essential role in the organism and disease mechanism (Lesch-Nyhan syndrome, xanthinurie or rena llithiase).

The Figure 1 shows molecular images of specific ribonucleotides observed in tissue section using MSI. They are involved in the synthesis of nucleic acids (DNA or RNA) but also in the entire metabolism. These nucleotides consist of a phosphate group, the sugar ribose, and the nucleobase, the three metabolites chosen in the present study were:

- **AMP or Adenosine monophosphate** (m/z 346.0575); AMP could be produced during ATP synthesis using adenylate enzyme by combining two ADP molecules. Energy-state of cells or tissues which control the entire metabolism can be directly assessed using these metabolites.

- **GMP or Guanosine monophosphate** (m/z 362.0506); GMP is essential in the activation of G proteins which is involved in signal transduction.

- **IMP or Inosinic acid or inosine monophosphate** (m/z 347.0396); IMP has a central position in Purine Metabolism, (the first nucleotide formed during the synthesis of purine). IMP act as the common intermediate of AMP/GMP synthesis.

AMP, GMP and IMP are linked in purine metabolism which maintains a desired and constant composition of the nucleotide pool of the organism. Their triphosphate forms (also detected using MSI, data not shown) are the substrates of cyclic nucleotides (cAMP, cGMP…) which are secondary messenger for a large numbers of significant reaction in the organism (Kinase protein activation, cellular signals induction,…). All these molecules have been characterized by tandem mass spectrometry (MS/MS) experiment to validate their identification directly in tissue. The method applied to fragment these species was the collision induced dissociation abbreviated “CID”. These ions have specific fragmentation pattern which exhibit approximately the same daughter ions especially the phosphate moiety at m/z 94.9696. We can observe that IMP is mainly distributed in medulla region of the kidney or in the Glisson's sheath (GS) of the liver but is quite spread over the entire brain with some variation. Its specific localization in kidney and liver (two organs with a high metabolism activity) reflects the importance of IMP in the metabolic pathways. In this case, MSI gives precise data that usual analytical techniques without spatial information couldn’t provide. AMP and GMP are also partly localized in the medulla or GS region. These metabolites are closely related in biological pathways as well as within tissue. In addition, AMP is concentrated through the outer structure of the brain; in
High resolution mass spectrometry imaging was successfully applied to study in-situ metabolism and to characterize specific biological pathways. With this technique, hundreds of endogenous metabolites from different classes could be monitored simultaneously in the same experiment and could give, at the same time, their precise localization. At ImaBiotech, we have generated a database of a few thousands of endogenous metabolites to accelerate the metabolite research in biological samples. In conclusion, high resolution MSI might become a tool of choice for better understanding of the metabolic pathways in biological tissues and for pharmacodynamics studies, for biomarker or therapeutic target discovery.

**Conclusion**

High resolution mass spectrometry imaging was successfully applied to study in-situ metabolism and to characterize specific biological pathways. With this technique, hundreds of endogenous metabolites from different classes could be monitored simultaneously in the same experiment and could give, at the same time, their precise localization. Also at ImaBiotech, we have generated a database of a few thousands of endogenous metabolites to accelerate the metabolite research in biological samples. In conclusion, high resolution MSI might become a tool of choice for better understanding of the metabolic pathways in biological tissues and for pharmacodynamics studies, for biomarker or therapeutic target discovery.

**Advantages**
- Discover new therapeutic target
- Obtain valuable pharmacodynamics data
- Correlate Drug Distribution and readout variation
- Characterize the metabolism of animal model

**Benefits**
- Save time
- Reduce costs
- Accelerate research

Thanks our new service “ImaMet” combining Metabolomics and MSI, ImaBiotech provides valuable data for drug discovery development by offering new insight into biochemical process, biomarkers, drug efficacy or toxicity.
References


Keywords

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