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Review

In situ metabolomic mass spectrometry imaging: Recent advances and difficulties☆

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ABSTRACT

MS imaging (MSI) is a remarkable new technology that enables us to determine the distribution of biological molecules present in tissue sections by direct ionization and detection. This technique is now widely used for *in situ* imaging of endogenous or exogenous molecules such as proteins, lipids, drugs and their metabolites, and it is a potential tool for pathological analysis and the investigation of disease mechanisms. MSI is also thought to be a technique that could be used for biomarker discovery with spatial information. The application of MSI to the study of endogenous metabolites has received considerable attention because metabolites are the result of the interactions of a system's genome with its environment and a total set of these metabolites more closely represents the phenotype of an organism under a given set of conditions. Recent studies have suggested the importance of *in situ* metabolite imaging in biological discovery and biomedical applications, but several issues regarding the technical application limits of MSI still remained to be resolved. In this review, we describe the capabilities of the latest MSI techniques for the imaging of endogenous metabolites in biological samples, and also discuss the technical problems and new challenges that need to be addressed for effective and widespread application of MSI in both preclinical and clinical settings.
This article is part of a Special Issue entitled: Imaging Mass Spectrometry: A User's Guide to a New Technique for Biological and Biomedical Research.

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1. Introduction

Understanding the complex biochemical processes that occur within living organisms requires not only the elucidation of the molecular entities involved in these processes, but also their spatial distribution within the organism. Analytical technologies for elucidating multiple molecular dynamics in the micro-region that retain the spatial information of the target tissue are thought to be important for understanding biological complexity of disease progress. Chemical stains, immunohistochemical tags and radiolabels are common methods for visualizing and identifying molecular targets. However, there are limits to the sensitivity and specificity of these methods and to the number of target compounds that can be monitored simultaneously. Thus, the simultaneous multiple molecular imaging with high sensitivity will be a technical breakthrough for pathophysiological research.

Metabolites are the result of the interactions of a system's genome with its environment, and are the end products of gene expression. The metabolome is defined as the total quantitative collection of small-molecular-weight metabolites present in a cell, tissue, or organism, that participate in the metabolic reactions required for growth, maintenance, and normal function [1–3]. Unlike the transcriptome and proteome that represent the processing of information during the expression of genomic information, the metabolome more closely represents the phenotype of an organism under a given set of conditions and can be defined as the "compound-level phenotype" of the genomic information. Metabolomics, the measurement of the global endogenous metabolite profile from a biological sample under different conditions, can lead us to an enhanced understanding of disease mechanisms, the discovery of diagnostic biomarkers, the elucidation of mechanisms for drug action, and an increased ability to predict individual variation in drug response phenotypes [4,5]. Thus, this rapidly developing discipline has important potential implications in the field of biomedical research.

To date, MS coupled with pre-separation techniques such as LC-MS or GC-MS has been known to be a conventionally used strategy for metabolomics [6–8]. However, these methods have a drawback in the analysis of tissue samples because of the requirement of metabolite extraction, which causes the loss of information on the spatial localization of the metabolites. In contrast, imaging techniques capable of determining the spatial localization of molecules have revolutionized our approach to diseases by allowing us to directly examine the pathological process, thereby giving us a better understanding of the pathophysiology. In most cases, however, there is a tradeoff among sensitivity, molecular coverage, spatial resolution, and temporal resolution. For example, magnetic resonance imaging (MRI), positron emission tomography (PET), and fluorescence microscopy can visualize the spatial localization of targeted molecules with high sensitivity, but these techniques have low molecular coverage (only a few molecules at a time) [9]. The simultaneous and spatially resolved detection with high sensitivity of a broad range of molecules is still a challenging issue.

MS imaging (MSI) is an emerging technology that makes it possible to determine the distribution of biological molecules present in tissue sections by direct ionization and detection. MSI has received considerable attention as a potential imaging technique for a molecular *ex vivo* review of tissue sections from an animal or plant based on label-free tracking of endogenous molecules with spatial resolution and molecular specificity [10–12]. The MSI technique was initially developed as a tool for intact protein imaging from the tissue surface using MALDI-MS [12–16]. In current research, proteins or peptides are still the main targets. However, the analysis of a wide variety of low-molecular weight compounds, including endogenous metabolites, using MSI combined with several soft ionization methods is emerging as a research target. In this review, we describe recent advances and difficulties in developing an analytical platform for MSI of endogenous small metabolites.

2. Ionization platform for MSI of endogenous metabolites

2.1. MALDI

MALDI is one of the laser desorption ionization (LDI) methods that can softly ionize several biological molecules. This ionization technique is usually used combined with TOF MS. A conventional MALDI source is equipped with a UV laser such as a nitrogen laser (337 nm) or Nd-YAG (355 nm). Spatial resolution is dependent mostly on the diameter of the laser; the diameter is usually more than 5 μm [17]. However, because MALDI-MSI requires a matrix application step, potential limitations in spatial resolution can be introduced by the matrix. The main limitations are the diffusion of metabolites within the tissue during matrix application and the heterogeneous size of crystal formation.

At the first decade of development, MALDI-MS has been used for synthetic polymer or protein (peptide) analysis. In the post-genomic era, the dramatic progress of bioinformatics research accelerated the use of MALDI-MS in proteomics research for identifying vast numbers of proteins [18]. MALDI-MS is known as a highly sensitive analytical method that can be used to analyze low concentrations (~fmol) of tryptic peptide. Sensitivity is extremely important characteristics for MSI because numerous biological molecules exist in a very small amount on the thin tissue section. However, MALDI-MS has rarely been used for low-molecular-weight metabolite analysis because many kinds of matrix and/or matrix-analyte cluster ion peaks are observed in the low-mass range ($m/z<700$), and the strong peaks that they