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Review

Spatial neuroproteomics using imaging mass spectrometry ☆

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ABSTRACT

The nervous system constitutes arguably the most complicated and least understood cellular network in the human body. This consequently manifests itself in the fact that the molecular bases of neurodegenerative diseases remain unknown. The limited understanding of neurobiological mechanisms relates directly to the lack of appropriate bioanalytical technologies that allow highly resolved, sensitive, specific and comprehensive molecular imaging in complex biological matrices. Imaging mass spectrometry (IMS) is an emerging technique for molecular imaging. The technique is characterized by its high chemical specificity allowing comprehensive, spatial protein and peptide profiling in situ. Imaging MS represents therefore a powerful approach for investigation of spatio-temporal protein and peptide regulations in CNS derived tissue and cells. This review aims to provide a concise overview of major developments and applications concerning imaging mass spectrometry based protein and peptide profiling in neurobiological and biomedical research. This article is part of a Special Issue entitled: Neuroproteomics: Applications in Neuroscience and Neurology.

1. Introduction

The human nervous system is characterized by its immense complexity. It constitutes the most complex and heterogeneous cellular network and is involved in a vast variety of body function ranging from somatic and autonomous motor control, cognition, sensory perception to hormone regulation and many more. However, the complex mechanisms that underlie intercellular communication in the nervous system are not fully understood. The nervous system maintains its functionality through a vast number of chemical substances orchestrating neurological processes. Complex biological and pathological processes in the nervous system involve the translocation of a wide range of chemical species. It is therefore imperative to probe the spatio-temporal dynamics of ongoing biochemical processes in order to deepen our understanding of e.g. signal transduction processes within distinct regions of the central nervous system (CNS) as well as the CNS with peripheral systems.

In current biological and biomedical research, molecular mechanisms and interactions at subcellular levels are studied and visualized by the acquisition of molecular images. Here microscopy, tomography, or chemical imaging tools are employed to retrieve topographical and temporal information of molecular abundance distributions. The main challenge in molecular imaging is to achieve appropriate temporal and spatial resolution with high precision, specificity and sensitivity. However, this is significantly hampered due to the lack of effective research tools available to study the chemistry of these complex systems. The development of analytical technologies that facilitate subcellular analysis with high molecular specificity and at high spatial resolution is therefore of essential relevance in order to deepen our understanding of inter- and intra-cellular molecular processes.

Over the last two decades, mass spectrometry (MS) has transformed biomedical research, especially for large scale, comprehensive protein profiling, termed proteomics [1]. This was, in particular, enabled by the introduction of soft ionization techniques such as matrix assisted laser desorption ionization (MALDI) [2] and electrospray ionization (ESI) [3] that allow fast, sensitive, and specific detection and characterization of intact large biomolecules including proteins and peptides. Whereas proteomic and peptidomics approaches for tissue extracts facilitate sensitive peptide and protein identification and quantitation, spatial information within the respective tissue compartment is not obtained. Given the complexity of the human nervous system, the spatial information of protein and peptide distribution is of major interest in order to resolve ongoing molecular mechanisms. This is particularly relevant for neuroactive peptide species that are involved in numerous neuronal processes and their localization is therefore of essential relevance for identifying their role in various neuronal signaling pathways.

Imaging mass spectrometry (IMS) is a powerful approach for spatial profiling of molecular species in biological tissue and single

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cells [4]. In contrast to common molecular biology imaging techniques, including chemical staining, antibody based imaging approaches (immunohistochemistry, confocal laser scanning microscopy) and nucleotide detection (in situ PCR), IMS does not require any a priori knowledge of the potential target species. It is furthermore not dependent on antibody or primer availability and specificity. IMS features high molecular specificity and allows comprehensive, multiplexed detection, identification, and localization of hundreds of proteins, peptides and lipids in biological tissue samples [4]. Over the last decade, IMS has slowly evolved as a relevant, alternative approach in biomedical research for studying proteins, peptides, lipids, drugs and metabolites in disease pathology, pharmacotherapy, drug metabolisms as well as fundamental biological processes [5,6]. The technique allows matching of histological features of a biological tissue sample to molecular localization patterns and can therefore also be referred to as molecular histology [7,8]. Although theoretically, one can carry out un-targeted studies with IMS, in general, it is necessary to have target molecules in mind in choosing the best suited sample preparation protocol to provide the required sensitivity. This is particularly relevant for protein and peptide analysis, where typically several tissue-washing steps are performed for analyte precipitation and lipid and salt removal.

Imaging mass spectrometry can be performed with different probes to desorb and ionize molecular species directly from a biological sample (Fig. 1a). The most prominent approaches include, laser desorption/ionization (LDI) based techniques including MALDI, and time of flight secondary ion mass spectrometry (ToF-SIMS), in which an ion beam is employed to sputter molecular species into the gas phase [9]. In addition, another MS based imaging technique, desorption electrospray ionization (DESI) [10], has become rather popular recently allowing image data acquisition at atmospheric pressure though at larger spatial resolution.

Different imaging MS technologies have various strengths and limitations particularly with respect to spatial resolution and molecular information [9]. Other aspects include sample throughput, sample preparation, economical, and experimental parameters as well as data handling challenges. A major consideration in MS based imaging, is the issue of matrix effects including e.g. ion-suppression that will affect ionization yield and thereby bias the quantitative information obtained. Appropriate internal and external controls are necessary to account for these limitations, some of which are presented and discussed below. All of these different features have to be taken into account when designing an IMS based experimental study.

The aim of the present review is to provide a concise overview of MALDI and SIMS based IMS techniques and their suitability for protein and peptide imaging in complex biological samples. Different methodological concepts and advances as well as challenges with respect to

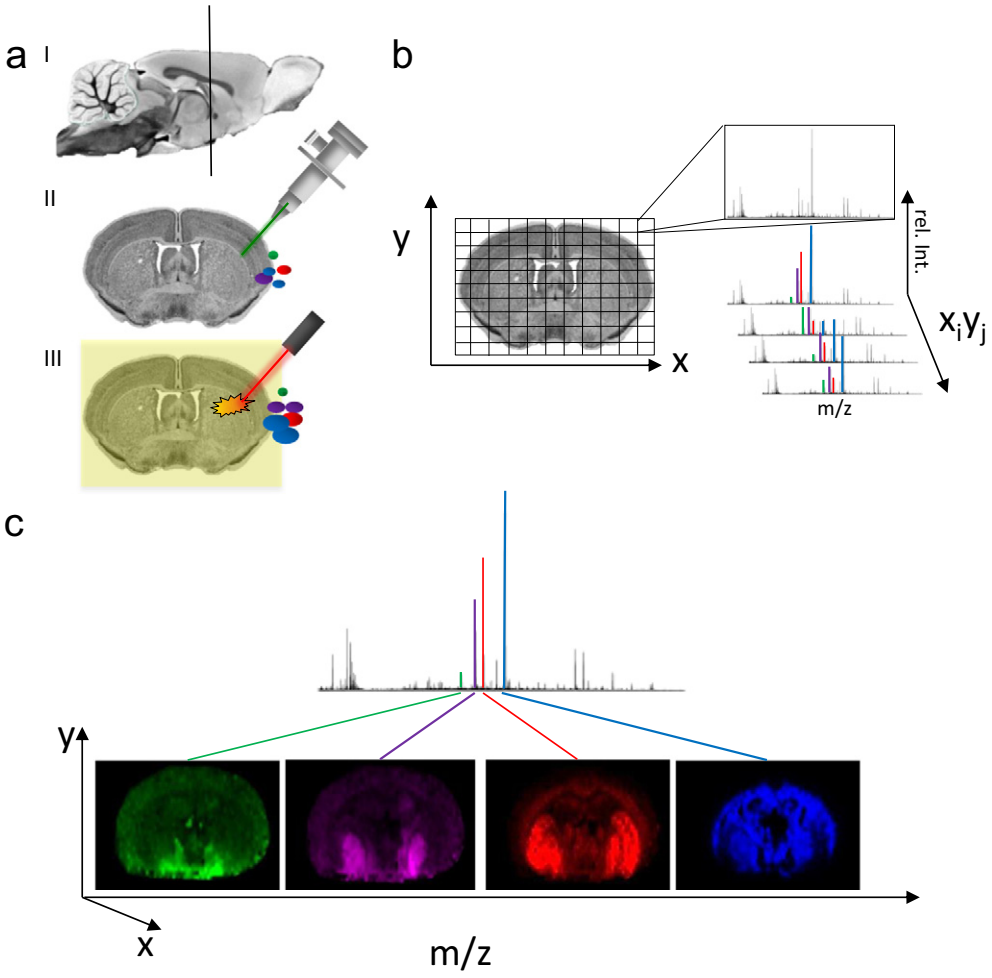

Fig. 1. Principle of imaging mass spectrometry. (a) I, Tissue sections are collected and mounted on a target for imaging MS. II, For SIMS IMS, tissue sections are probed with an ionbeam, generating low molecular weight secondary ions (m/z > 1000 Da). III, In contrast, MALDI IMS requires precoating with matrix (indicated in yellow) before systematic scanning with a laser probe. MALDI based ionization generates larger intact molecular species, including peptides and proteins. (b) One mass spectrum is acquired for every x_i,y_j coordinate of the scanned tissue section. (c) Single ion images are generated by mapping the intensity of an individual ion signal (m/z;rel.Int) over a the whole tissue slide.