International Journal of Mass Spectrometry xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Ambient molecular imaging by laser ablation electrospray ionization mass spectrometry with ion mobility separation

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ARTICLE INFO

Article history:
Received 29 March 2014
Received in revised form 21 June 2014
Accepted 27 June 2014
Available online xxx

Keywords:
Laser ablation electrospray ionization
LAESI
Ambient ionization
Ion mobility separation
Mass spectrometry imaging

ABSTRACT

Mass spectrometry imaging (MSI) by laser ablation electrospray ionization (LAESI) enables the lateral mapping of molecular distributions in untreated biological tissues. However, direct sampling and ionization by LAESI–MSI limits the differentiation of isobaric ions (e.g., structural isomers) in a complex sample. Ion mobility separation (IMS) of LAESI-generated species is sufficiently fast to be integrated with the MSI experiments. Here, we present an imaging technique based on a novel combination of LAESI–MSI with IMS that enables in vivo and in situ imaging with enhanced coverage for small metabolites. Ionized molecules produced at each pixel on the tissue were separated by a traveling wave IMS and analyzed by a high performance quadrupole time-of-flight mass spectrometer. Plant (*Pelargonium peltatum* leaves) and animal tissues (frozen mouse brain sections) were imaged under atmospheric pressure. In LAESI–IMS–MSI, a multidimensional dataset of *m*/*z*, drift time (DT), ion intensity, and spatial coordinates was collected. Molecular images for the *P. peltatum* leaf illustrated that the distributions of flavonoid glycoside ions are aligned with a vein pattern in the tissue. Differentiation of isobaric ions over DT reduced the chemical interferences and allowed separate imaging of these ions. Molecular images were constructed for selected ions in the sagittal sections of the mouse brain, and isobaric species were distinguished by differences in drift times corresponding to distinct molecular structures or conformations. We demonstrated that IMS enhanced the metabolite coverage of LAESI in biological tissues and provided new perspective on MSI for isobaric species.

1. Introduction

Mass spectrometry imaging (MSI) has emerged as an important tool to visualize the spatial distributions of multiple molecules across sample surfaces. Various ionization techniques have been implemented for molecular imaging of biological specimens [1–5]. Secondary ion mass spectrometry (SIMS) [6] and matrix-assisted laser desorption ionization (MALDI) [7] mass spectrometry (MS) are the most widely used MSI methods, both of which require vacuum conditions. SIMS has demonstrated advantages in imaging of inorganic compounds at relatively low masses with very high resolution (~100 nm) [1], whereas MALDI-MS excels in imaging of large molecules, particularly proteins, with a typical spatial resolution of tens of microns [8,9]. In the past decade, the implementation of ambient ionization techniques for MSI has grown rapidly, enabling operation under native conditions with minimum or no sample preparation [1]. With the advent of ambient ionization sources, such as desorption electrospray ionization (DESI) [10–13], laser ablation electrospray ionization (LAESI) [14–16], desorption atmospheric pressure photoionization (DAPPI) [17], probe electrospray ionization (PESI) [18], and nanospray desorption electrospray ionization (nanoDESI) [19,20], *in vivo* and *in situ* MSI have become accessible. Ambient MSI facilitates the simultaneous imaging of small metabolites, lipids, polypeptides, and large proteins in biological samples. In most of these techniques, soft ionization yields minimum or no fragmentation, due to their relatively low energy deposition [21]. Ambient MSI has been extensively applied in tissue analysis,

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http://dx.doi.org/10.1016/j.ijms.2014.06.025
1387-3806/

including drug development [22], forensic sciences [23], clinical research, and biomarker discovery [1].

Designed to utilize the natural water content of cells and tissues as a matrix, LAESI uses mid-IR laser pulses at 2940 nm to excite the O—H vibrations in the sample for efficient energy deposition [14]. Sudden energy deposition leads to ablation, driven by phase explosion and ejection of particulates by the recoil pressure [24,25]. The ablated particles are captured by the electrospray, and sample-related ions are produced. Two-dimensional LAESI–MSI has been demonstrated for the mapping of metabolite and lipid distributions in live plants and frozen rat brain sections [15,26]. The feasibility of three-dimensional LAESI–MSI has also been shown for plant leaves with a depth resolution of ~30 μm [16]. The obtained molecular images exhibit good correlation with visually observed features (e.g., variegation pattern) and known biological functions (e.g., localization of chlorophyll).

As a direct ionization technique, conventional LAESI-MS lacks a separation step, known to improve molecular coverage for bioanalytical methods. Likewise, direct ionization exhibits an inherent limitation in the imaging of, for example, isobaric compounds in complex samples. In conventional LAESI-MS, a number of ions, especially isobaric or isomeric structures, may interfere with each other resulting in distorted molecular images [27,28]. For example, lipids often interfere with multiply charged peptides in the m/z 600–1200 range [29]. Therefore, it seems beneficial to integrate an on-line separation step with LAESI–MSI.

Ion mobility spectrometry was first introduced in the early 1970s [30]. Gas phase ions are separated based on their differences in collision cross sections by driving them through a buffer gas using electric fields [27,29,31,32]. Different from traditional separation methods, e.g., liquid chromatography, capillary electrophoresis, and gel-based separations, that take place on the time scale of many minutes [28], IMS is sufficiently rapid (separation takes typically less than 1 s) to be coupled with direct ionization, MS and MSI experiments. It had been combined with MALDI–MSI under vacuum conditions and was demonstrated for the imaging of biological samples [29,32–39]. The combination of IMS with MS enables a multi-dimensional gas phase separation, based on the mass, charge states, and molecular structures [29].

Here we present an IMS–MSI technique by integrating it with a LAESI ion source that enables in vivo and in situ investigations under ambient conditions. For imaging, ions generated by LAESI from the tissue at multiple locations are separated by IMS within milliseconds according to their molecular structures and conformations. The separated ions are analyzed by a quadrupole time-of-flight (Q-TOF) mass spectrometer for identification. Distribution of metabolites, lipids, and polypeptides represented by m/z combined with drift time (DT) are mapped across the tissue surface.

2. Materials and methods

2.1. LAESI–IMS–MSI

Imaging experiments were performed by LAESI–IMS-MS using a high performance quadrupole time-of-flight (Q-TOF) mass spectrometer (Synapt G2 S, Waters Co., Milford, MA, USA) equipped with a home-built LAESI ionization source, and a programmable xyz-translation stage. In the LAESI event, Q-switched 5 ns mid-IR laser pulses at 2940 nm wavelength (IR Opolette 100, Opotek Inc., Carlsbad, CA, USA) were focused by a single plano-convex lens (Infrared Optical Products, Farmingdale, NY, USA) onto the sample surface to produce an ablation plume. The diameter of the focal spot was ~150 μm. Particles from the generated plume combined with the charged droplets in an electrospray. A spray solution of 50% methanol with 0.1% acetic acid (v/v) was used for positive ion mode analysis. A syringe pump supplied this solution at a flow rate of 0.3 μL/min to a metal capillary (MT320-50-5-5, New Objective, Woburn, MA, USA) held at 3100 V to produce a stable electrospray. The ions generated by LAESI entered the traveling wave ion guide of the mass spectrometer through an orifice. Ions were initially confined to a traveling wave trap where, if needed for structural identification, collision-induced dissociation could be performed. They were then released into a traveling wave drift tube for

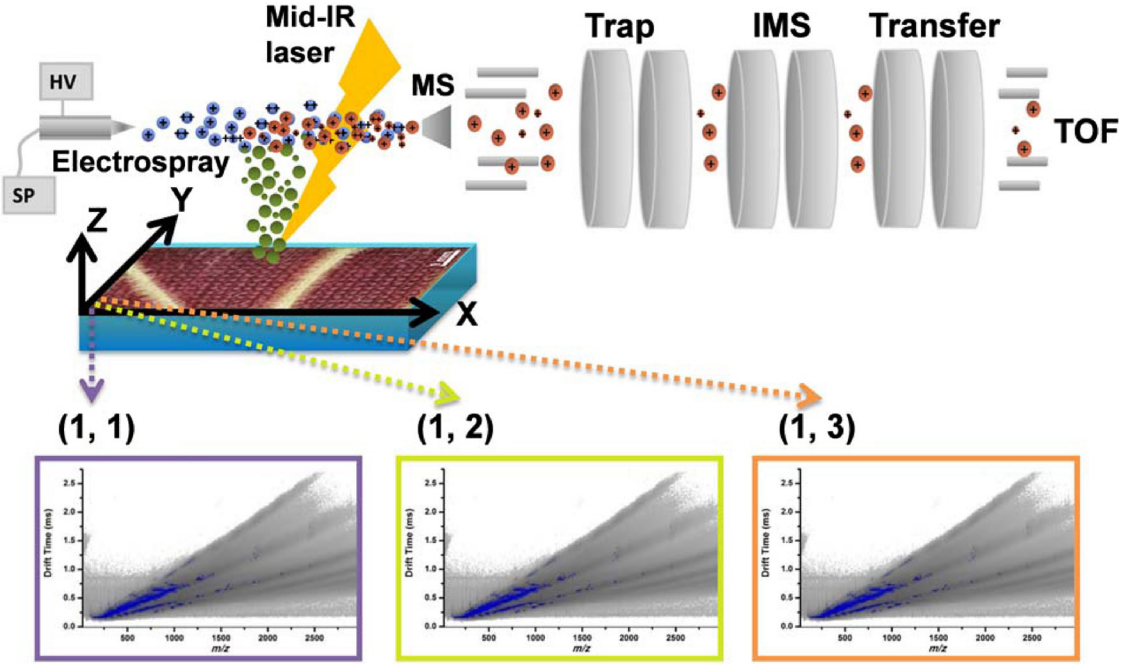

Fig. 1. Schematic of LAESI–IMS–MSI system, including laser ablation sampling, electrospray ionization, sample x–y positioning for surface mapping, traveling wave IMS, and time-of-flight MS. A DT vs. m/z plot is recorded and analyzed for each (x, y) position. Three examples of these plots are displayed for the (1, 1), (1, 2) and (1, 3) coordinates.

Please cite this article in press as: H. Li, et al., Ambient molecular imaging by laser ablation electrospray ionization mass spectrometry with ion mobility separation, Int. J. Mass Spectrom. (2014), http://dx.doi.org/10.1016/j.ijms.2014.06.025