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Investigation and optimization of parameters affecting the multiply charged ion yield in AP-MALDI MS

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ABSTRACT

Liquid matrix-assisted laser desorption/ionization (MALDI) allows the generation of predominantly multiply charged ions in atmospheric pressure (AP) MALDI ion sources for mass spectrometry (MS) analysis. The charge state distribution of the generated ions and the efficiency of the ion source in generating such ions crucially depend on the desolvation regime of the MALDI plume after desorption in the AP-to-vacuum inlet. Both high temperature and a flow regime with increased residence time of the desorbed plume in the desolvation region promote the generation of multiply charged ions. Without such measures the application of an electric ion extraction field significantly increases the ion signal intensity of singly charged species while the detection of multiply charged species is less dependent on the extraction field. In general, optimization of high temperature application facilitates the predominant formation and detection of multiply charged compared to singly charged ion species. In this study an experimental set-up and optimization strategy is described for liquid AP-MALDI MS which improves the ionization efficiency of selected ion species up to 14 times. In combination with ion mobility separation, the method allows the detection of multiply charged peptide and protein ions for analyte solution concentrations as low as 2 fmol/μL (0.5 μL, *i.e.* 1 fmol, deposited on the target) with very low sample consumption in the low nL-range.

1. Introduction

Matrix-assisted laser desorption/ionization (MALDI) [1,2] and electrospray ionization (ESI) [3] are key ionization techniques for the analysis of biomolecules by mass spectrometry (MS). The pulsed nature of MALDI makes it more amenable to coupling with axial time-of-flight (TOF) mass analyzers with the ionization event occurring in a high-vacuum region. On the other hand, ESI was developed at atmospheric pressure (AP) and like many other AP sources is coupled to mass analyzers designed for analysis of continuous ion beams, including tandem quadrupole and orthogonal TOF-based systems [3–5]. Although ion generation at ambient pressure requires efficient ion transportation to the high-vacuum regions of the mass analyzer, the ion source operation at elevated pressures provides more facile coupling of powerful analytical separation techniques such as liquid chromatography, and within the mass spectrometer, gas-phase techniques such as tandem mass spectrometry (MS/MS) [6] and ion mobility [7] separation. In the last few years, there has been an ongoing effort to adapt these developments for MALDI [8–14]. In 2000, Laiko *et al.* [15] reported an ion source with a common instrumental platform incorporating both ESI and AP-MALDI ion sources [15–17]. Recently it was discovered that, under certain conditions, AP-MALDI ion sources generate predominantly multiply charged ions [18–20], with similar abundances to those typically generated by ESI.

Multiply charged ions are often more suitable for MS analysis than singly charged. For instance, for microchannel plate (MCP) detectors, which are typically used in TOF MS, the ion-electron yield depends on the impact velocity and energy of the primary ion beam, which in turn depends on the charge and mass value of the ion [21], typically leading to the discrimination against ions with low charges and/or higher m/z values. In Fourier transform (FT)-based mass analyzers, such as Orbitraps and FT-ICR instruments, the measured analyte ion oscillation frequency depends on the m/z ratio [22,23], resulting in higher resolving power for the same acquisition time if the m/z value is lower. Furthermore, many commercial mass spectrometers include radio frequency (RF) ion guides or ion manipulation devices that have a limited m/z transmission range [24].

Another major advantage of multiply charged ions can be found in their superior fragmentation spectra. According to a number of

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publications [25–27], collision-induced dissociation (CID) fragmentation spectra of doubly charged peptide precursor ions provide different and often enhanced information compared to the spectra obtained by fragmentation of singly charged ions. The recently developed electron-capture dissociation (ECD) [28] and electron-transfer dissociation (ETD) [29] fragmentation methods preserve labile post-translational modifications and therefore provide complementary information to CID. The core of the ECD and ETD fragmentation process involves the capture of an electron by the precursor cation, reducing its charge state. It is therefore a requirement that the precursor ion is at least doubly charged in order to have product ions for detection.

In conventional MALDI, ions are typically singly charged and the process of ion generation is still not fully understood. However, two theories persist: the first is the gas phase protonation model [30–33] and the second is known as the 'Lucky Survivor' model [34,35]. The 'Lucky Survivor' model is based on the premise that analyte ions are preformed before ablation, while the gas phase protonation model suggests that neutral analyte molecules are protonated after ablation. Both models agree that gas phase chemistry and particularly the recombination processes with the oppositely charged species during rapid plume expansion play an important role in the ionization process and account for the observed predominantly singly charged ion yield [34,36]. Frankevich *et al.* have shown that in conventional MALDI experiments the charge state distribution of the generated analyte ion yield can be shifted to higher charge states by using a dielectric MALDI target plate and low laser fluence [37]. They attributed this shift to a decrease in the ion recombination rate due to a lower number of 'free electrons' in the MALDI plume. However, the proportion of the detected multiply protonated analytes remained inferior to the proportion of the detected singly protonated analytes [37].

Owing to the similarity of the generated predominantly multiply charged ions in the recently developed laserspray and liquid AP-MALDI approaches to ESI [19,20], it has been hypothesized that the ion formation is based on similar mechanisms. In ESI initially highly charged liquid droplets containing analyte molecules are dispersed into the gas phase. These droplets evaporate and undergo Coulomb fission upon reaching the Rayleigh limit. Two main mechanisms were proposed for the next step of gas phase ion generation, namely the charge residue model (CRM) [38] and the ion evaporation model (IEM) [39,40]. In the CRM solvent droplet fission leads to a charged progeny droplet with one analyte molecule and the ion is released when all the solvent evaporates. In the IEM, when the droplet reaches a certain radius, the applied electric field becomes sufficient to field-desorb charged analytes. Both models predict the ion release on later stages of the droplets evolution with sufficient desolvation being a prerequisite [41].

A major limitation of all AP ion sources with respect to performance is the loss of ions associated with their transfer into the analyzer's vacuum regions. Extensive research has been carried out to design more efficient interfaces with a particular emphasis on supplying additional energy to the transferred ion plume for both ESI [42–44] and AP-MALDI [15,45–47]. This can play a major role in the desolvation and declustering during the ionization process in ESI [41] and also help in declustering the generated MALDI plume [35]. Another important consideration for the design of an AP interface is the transmission of ions in the ion transfer tube (or capillary) which may be affected by the gas flow regime, transit time and temperature in the transfer region [48–51]. In this work the influence of desolvation conditions in the ion source and ion transfer region on the detected ion signal from the liquid AP-MALDI ion source is discussed. A new interface design, which allows the optimization of both the flow regime and temperature in the heated ion transfer tube is described, and the transmission biases with respect to the detected charge state similar to what has been previously reported for ESI [52,53] are discussed.

2. Materials and methods

2.1. MALDI sample preparation

A two-peptide mixture containing $[Val]^5$-angiotensin I (Sigma-Aldrich, Poole, UK) and bradykinin (Sigma-Aldrich) was prepared in water containing 0.1% formic acid (Greyhound, Birkenhead, UK) at concentrations of 50 pmol/μL for the experiments at ambient temperatures, *i.e.* without external heating, and 10 pmol/μL for the experiments with external heating. In addition, a $[Val]^5$-angiotensin I solution was prepared at a concentration of 2 fmol/μL in water containing 0.1% formic acid for assessing the limit of detection (LOD). Two matrix solutions were prepared using the

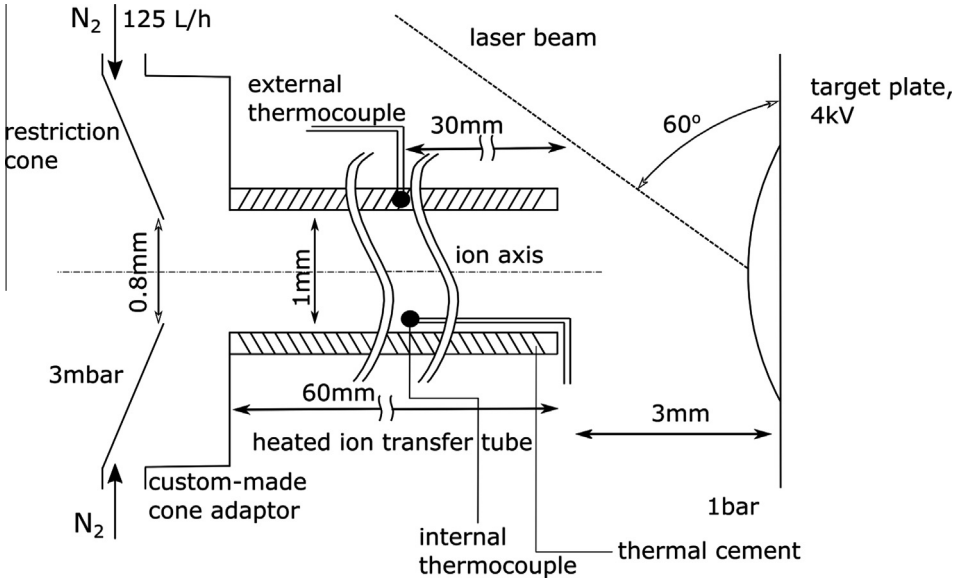

Fig. 1. Schematic of the AP-MALDI ion source and modified AP-to-vacuum interface used for the production of multiply protonated molecules. The experimental values given represent the optimum values for the detection of multiply protonated peptides in liquid AP-MALDI MS experiments.