International Journal of Mass Spectrometry 416 (2017) 61–70

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

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

Generation of multiply charged peptides and proteins from glycerol-based matrices using lasers with ultraviolet, visible and near-infrared wavelengths and an atmospheric pressure ion source

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ARTICLE INFO

Article history:
Received 3 August 2016
Received in revised form 19 October 2016
Accepted 4 November 2016
Available online 9 November 2016

Keywords:
Multiply charged ions
Laser wavelength
Spallation
Atmospheric pressure ion source
Glycerol matrix
OPO laser

ABSTRACT

Conventional matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is characterized by the predominant generation of singly charged analyte ions. Recent studies demonstrated that high charge states of peptides and small proteins can be produced with an atmospheric pressure (AP) MALDI ion source if liquid glycerol-based matrix systems are utilized and the AP-vacuum transfer is realized via a heated ion inlet tube [R. Cramer, A. Pirkl, F. Hillenkamp, K. Dreisewerd, Angew. Chem. Int. Ed. 52 (2013) 2364–2367]. Here, we used this AP ion source and employed an optical parametric oscillator (OPO) laser to study the wavelength dependence of the ion generation between 260 and 1080 nm. Three matrix systems with different optical absorption characteristics were investigated: non-absorbing glycerol mixed with trifluoroacetic acid (TFA) and glycerol mixed with either 2,4- or 2,5-dihydroxybenzoic acids as two classical UV-MALDI matrices. The highest ion yields of multiply charged peptides were consistently obtained at laser wavelengths for which the chromophore-containing matrix systems exhibited the lowest optical absorption. Best sensitivities were even achieved with the transparent glycerol/TFA mixture and by use of a Nd:YAG-laser operated at 1064 nm (or with the OPO laser tuned to a similar wavelength). Experiments with absorbing and transparent sample substrates demonstrated the involvement of substrate absorption. In contrast to the results obtained with the AP ion source, using the same matrix systems in combination with a standard oMALDI2^{TM} ion source, operated at a fine vacuum of ~1 mbar and without an inlet tube, the inverse wavelength dependence was observed. Or in other words, like in standard UV-MALDI under these conditions the highest analyte ion yields were obtained for laser excitation wavelengths corresponding to a high absorptivity of the matrix. Our findings thus point to completely different desorption/ionization processes with the two ion source geometries and laser excitation regimes. We hypothesize that for the AP case the material ablation comprises low-energy laser spallation and that rapid evaporative ionization of small droplets in the heated transfer capillary is responsible for charge production. From an analytical point of view, the production of multiply-charged peptide and protein ions with charge states that for the tested analytes (ranging by mass from 1 to 17 kDa) almost fully resembled those generated by nano-electrospray ionization (ESI) could facilitate the coupling of laser-based ion sources with mass analyzers, such as orthogonal-extracting time-of-flight (QTOF) instruments or orbitraps which exhibit only limited m/z ranges.

1. Introduction

Conventional matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is characterized by the predominant generation of singly-charged gaseous ions [1–3]. Only particularly large biomolecules, e.g., proteins are typically also detected with higher abundances as multiply charged species [4]. The prevalence of singly charged MALDI ions simplifies interpretation of the mass spectra, but this also comes with a few notable disadvantages. For example, structurally more informative tandem mass spectra are generally obtained if multiply charged precursor ions are subjected to collision-induced dissociation (CID) [5] and electron capture and electron transfer dissociation (ECD/ETD), two tech-

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http://dx.doi.org/10.1016/j.ijms.2016.11.007
1387-3806/

niques that are exceedingly useful for top-down sequencing of proteins [6], cannot be applied to singly charged precursor ions. A third limitation is given by the restricted m/z ranges of some important mass analyzer types. For example, orbitrap and Paul ion trap mass spectrometers have typical upper m/z limits of a few thousand, and although providing a wider acceptance window upon use of suitable driver electronics, also hybrid orthogonal time-of-flight (QTOF) and Fourier-transform ion cyclotron resonance (FTICR) mass spectrometers generally exhibit a highest sensitivity in a similar m/z range [7]. As a consequence, the coupling of these mass analyzers with electrospray ionization (ESI) sources is much more common than the coupling with MALDI.

Since the introduction of MALDI by the Hillenkamp group in the 1980ies [8,9] the observation of increased yields of multiply charged ions has been occasionally reported for different experimental conditions. Those included, for example, the employment of a pulsed CO_2 infrared (IR) laser for IR-MALDI in combination with particularly suited matrices (e.g., caffeic acid [10]), and the use of special sample preparation protocols for ultraviolet (UV)-MALDI-MS (e.g., by electrospraying of multiply-charged analytes onto matrix surfaces [11]). However, while being mechanistically interesting, these approaches could not compete with the performance characteristics of conventional MALDI, nor that of standard ESI-MS.

In the last years a more robust and consistent enhancement of the yields of multiply charged ions generated from laser-ablated material was reported in several studies. By and large, these approaches can be grouped into two categories. In the first, multiply charged ions are generated "within" the convoluted laser-induced phase transition process, i.e. desorption and ionization processes like, e.g., in MALDI. In the second group, a more separated postionization process following the initial laser ablation of material occurs.

An example from the first group is IR-MALDI with a water ice matrix [12,13]. The fine-vacuum MALDI source used in these two works from our own group contained a cooling sample stage and a 6 ns-pulsed OPO laser, tuned to the O-H stretch vibration band of (liquid) water at 2.94 μm. Average charge states between 2 and 3 were obtained for a range of biomolecules including disaccharide glycosaminoglycans [13] as well as peptides and proteins, the latter ranging in molecular weight (MW) from ~ 1 to 150 kDa [12]. A second example is given by work of Muddiman and coworkers in which liquid matrices were used in combination with an AP ion source. Multiply charged peptides and proteins with ESI-typical charge states were generated both from analyte-doped water droplets by use of a pulsed CO_2 IR-laser [14] and from aqueous solutions containing a classical UV-MALDI matrix (2,5-dihydroxybenzoic acid, 2,5-DHB) and use of a Nd:YLF laser (λ = 349 nm) [15]. Samples were prepared on metal plates held at an electrical potential of 0.5–4 kV. The researchers assigned the generation of the high charge states to the laser ablation of positively charged droplets and subsequent ESI-like desolvation processes. It should be noted that an ion transfer capillary (inlet tube), that formed the interface between the ambient side and the first vacuum stage of the mass spectrometer, was used in these experiments.

In the second group of techniques, (heated) inlet tubes play a critical role in postionization processes [16,17]. Using a liquid composite matrix, consisting of a glycerol base and 2,5-DHB as chromophore, we have recently demonstrated the generation of peptide and protein ions with mean charge states close to ESI-typical values [16]. Samples were irradiated with the beam of a Q-switched Nd:YAG laser of 355 nm wavelength. Notably, (multiply charged) ions were only observed if the inlet tube, forming the interface between the AP side and the first vacuum stage of a Qstar pulsar i (AB Sciex) QTOF mass spectrometer, was heated to temperatures in excess of ~ 150 °C. A long-lasting and stable ion current was obtained from microliter volumes of analyte-matrix solutions at MALDI-typical fluences of a few 100 J/m^2. In preceding work, peptide mass spectra with high charge states were also obtained by using an IR-laser in combination with a glycerol/trifluoroacetic acid (TFA) mixture, albeit with an overall lower sensitivity [18]. By coupling an UV-laser based AP ion source of similar design to a Synapt G2-Si ion mobility mass spectrometer (Waters), Ryumin et al. recently demonstrated a sensitivity in the low fmol range for the analysis of [Val^5]-angiotensin I [17], with a base peak detected for the $[M+2H]^{2+}$ species. These authors also conducted a series of systematic experiments to study the influence of parameters such as temperature of the inlet tube, counter gas flow, and extraction field strength. These experiments showed the importance of establishing an optimal desolvation regime in the AP-to-vacuum inlet, which in turn was shown to be affected by the inlet temperature and residence times (length of the tubing) and, possibly, the type of achieved gas flow. Also the research groups of Trimpin and McEwen introduced a large number of desorption/ionization schemes that enabled the production of multiply charged ions. Most notably, these included both laser-based and non-laser based methods [19–23]. The two groups also performed a set of fundamental studies to elucidate the underlying ionization mechanisms [22,24]. Taking all studies together, in our view a rather 'universal' picture arises: As long as suitable (rapid) phase transition conditions are met, the generation of multiply charged ions appears possible from a multitude of different matrix systems. However, the most reproducible and sensitive results were so far obtained if liquid samples and heated inlet tubes were used [16,25].

Our hypothesis that initiated the present study was that the plume composition (e.g., number and mean size of laser-generated droplets) could form a key factor in these processes. Secondly, we speculated that this could to some extent be controlled by adjustment of the excitation laser wavelength (i.e., in particular by control of the penetration depth into the sample and the laser fluence). To investigate these potential effects we employed an optical parametric oscillator (OPO) laser and tuned it to individual wavelengths between 260 and 1080 nm, thereby spanning the UV, visible (VIS), and near-IR wavelength ranges. In addition to the OPO, a Nd:YAG-laser operated at its fundamental frequency of 1064 nm was also tested. Apart for a few adjustments made to the sample plate geometries and for coupling of the lasers the used AP ion source was essentially the same as used in our previous study [16]. Pure glycerol, mixed with ~ 0.2% of TFA as protonation-enhancing agent, and a mixture of glycerol and either 2,4- or 2,5-DHB as classical MALDI matrices and UV-chromophores were investigated. Peptides and proteins with increasing MW were utilized as analytes. For comparison with the AP-based results, equivalent matrix systems were moreover investigated using a standard elevated pressure oMALDI2^{TM} ion source [26], operated at ~ 1 mbar and without a heated inlet tube.

2. Materials and methods

2.1. Chemicals

Glycerol was purchased from Riedel-de Haën (Seelze, Germany), melittin from Serva (Heidelberg, Germany), and methanol from Roth (Karlsruhe, Germany); all other chemicals and ITO slides (surface resistivity 70–100 Ω/sq) were from Sigma-Aldrich (Schnelldorf, Germany). All chemicals were used as supplied.

2.2. Sample preparation

Two different matrix systems were used. In the first, glycerol was mixed with TFA in a 1000:2 (v/v) ratio. Before adding ana-