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Laser induced post-desolvation of MALDI clusters

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ABSTRACT

Despite being controversial for many years, it is now widely accepted that the Matrix Assisted Laser Desorption/Ionization (MALDI) process leads to the ejection of aggregates or clusters in addition to isolated particles and ions. Clusters composed of analyte surrounded by matrix molecules exhibit a distribution in size and are relatively stable. Several studies have demonstrated that these clusters do not necessarily fully desolvate before ion extraction, but rather progressively along the ion path. This leads to a decrease in analytical performances since these non-desolvated clusters do not contribute to the ion signal but also generate an important chemical background noise. Therefore, proper cluster desolvation before ion extraction in order to release naked analyte ions in the gas phase holds great promise of improved mass spectra sensitivity. This work presents a set-up allowing cluster desolvation using nanosecond pulses of a near-infrared laser that intercepts the expanding MALDI plume before ion extraction.

1. Introduction

Matrix-Assisted Laser Desorption Ionization (MALDI) has revolutionized the field of biomolecules analysis thanks to the pioneering work of Hillenkamp and Karas [1,2]. Although very high analytical performances have now been reached, fundamental mechanisms governing the MALDI process are still not fully understood. One illustration is the very different charge distribution shown between MALDI and ElectroSpray Ionization (ESI) [3] despite very similar performances in terms of both sensitivity and molecular weight/polarity of the accessible analytes. It is then clear that a better understanding of these mechanisms is necessary when aiming at optimizing the MALDI source.

Several models have been proposed to explain the features observed in the MALDI spectra. Certain models have focused on the desorption/ablation event [4], others have addressed in more detail the ionization stage [5–7], while some have considered the desorption/ionization event as a continuous integrated process [8–10]. Currently, the model on which instruments are designed is based on the hypothesis that gas phase molecules, including ions produced in MALDI, are individualized and separated from each other. However, because the analyte is diluted in matrix crystals during co-crystallization and is further ejected together with the surrounding matrix molecules, a significant number of analyte-matrix clusters might be present in the plume. Fundamental studies [11–15] and numerical simulations [16–21] have gradually converged towards a comprehensive MALDI model including cluster formation. Numerical simulations have demonstrated the transition from a thermal vaporization regime, characterized by the exclusive ejection of neutrals, to a phase explosion regime with emission of charged compounds and clusters when laser fluence is increased. These results are supported by a series of experiments demonstrating the existence of these clusters using repulsive fields in the source during the delayed extraction [11,12]. If cluster formation has become a model accepted by the community, its impact on the ionization mechanisms is still under discussion [5,13]. According to the simulations, clusters are already present in the early desorption phase (<1 ns after the laser shot) where they have a broad size distribution. The main problem raised by their presence is that some (possibly many) analyte ions are trapped in these complexes and are being solvated by matrix molecules (by analogy with the mechanisms associated with ESI). Various experiments have shown that the electric field applied to transfer the ions in the mass analyzer (e.g. ion traps IT, Orbitrap, Ion Cyclotron Resonance (ICR) or Time-of-Flight (TOF)) contributes to the desolvation of these complexes, which nevertheless remains incomplete. Studies using atmospheric pressure MALDI (AP-MALDI) sources [22] have demonstrated that clusters can be stabilized by AP relaxing collisions. Relaxation is then sufficient to allow for the observa-

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tion of analyte molecules (M) clustered with n matrix molecules (m), resulting in the presence of $[M+nm]^+$ peaks in the mass spectra. In fact, stabilized clusters were shown to desolvate inside the heated capillary of the instrument under AP-MALDI conditions, making possible the observation of multiply charged ions and thus reinforcing the similarity between MALDI and ESI models [23–25]. However, for high-vacuum MALDI, which is still the most sensitive configuration, the experiments have demonstrated that collisions neither effectively stabilize the clusters nor provide their full desolvation. This phenomenon remains a major problem for the quality of the spectra. The naked analyte ions, formed before the ion extraction, will be detected at a time fully defined together by the ejection parameters and the analytical conditions (geometry of the instrument, applied voltages, delayed extraction...). In contrast, there is no direct correlation between the time-of-flight and the m/z for clusters which are not fully desolvated before their extraction but continue to desolvate during their acceleration. Clusters with a broad size distribution and long desolvation time appear as unresolved signals and contribute to increase the ("chemical") background noise in MALDI-TOF spectra. This phenomenon has been described by several authors. In particular, the work of Krutchinsky and Chait [26] has pointed out the existence of matrix ions or clusters in the background "noise" when performing MS/MS studies. Specifically, the authors demonstrated using a MALDI-IT instrument the presence of clusters for almost all selected mass-to-charge (m/z) values and with a lifetime significantly higher than that expected in MALDI-TOF. Sachon et al. [14] studied the desolvation of clusters in MALDI-TOF linear mode by varying the beam deflection voltage. These experiments highlighted, for two different matrices, the existence of a slow component compatible with the hypothesis of desolvation occurring in the source. The same authors showed that the analyte ion was the main fragment released when portions of "chemical background noise" above the analyte m/z value were selected for subsequent MS/MS studies. All these studies have demonstrated the limitation of a gradual and partial cluster desolvation during analysis, which results ultimately in a reduced spectral quality due to a concurrent increase in background noise and decrease in analyte signal, especially for high-mass analytes such as proteins. Therefore, achieving full cluster desolvation before their transfer into the analyzer (especially during the delayed extraction occurring before the acceleration for conventional MALDI-TOF systems) should improve spectral quality. More specifically, spectral sensitivity should be increased because of the rise in analyte signal intensity accompanied by the reduction in background noise.

The aims of this study are twofold: first is to develop and test a system that achieves more efficient cluster desolvation during the MALDI process, and second is to observe the effects of such a post-desolvation on MALDI signals. Taking into account the prominent role of MALDI-TOF systems for analytical purposes, we have developed a system based on a commercial instrument. To achieve effective cluster desolvation on a short time scale (shorter than the extraction delay, i.e. few hundred nanoseconds at most) we use a second pulsed laser beam which intercepts the expanding plume perpendicularly in the vicinity of the sample plate surface. The effect of various experimental parameters on the analyte, matrix and cluster signals has been investigated.

2. Material &Methods

2.1. Chemicals

HPLC grade water, ACN and TFA were purchased from BioSolve (Valkenswaard, The Netherlands). Standard calibration peptides Bradykinin (Mw = 1060.2 u) and ACTH 18–39 (Mw = 2465.7 u), as well as the α-cyano 4-hydroxycinnamic acid matrix (HCCA, Mw = 189.2 u) were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France) and used without further purification.

2.2. Sample preparation

Peptides were dissolved in water to prepare solutions at 5.10^{-6} M for both analytes. The HCCA matrix solution was prepared just before the experiments by dissolving 10 mg/mL of HCCA in 0.1% TFA in water:ACN (7:3, v/v). The sample was prepared according to the dried droplet method. Briefly, 1 μL of matrix solution and 1 μL of analyte were mixed onto the MALDI sample plate. The sample was then left at room temperature and normal pressure conditions until co-crystallization of the matrix/analyte system.

2.3. MALDI-TOF MS instrument

The laser post-desolvation setup was installed on a commercial MALDI-TOF (Voyager Elite system upgraded to STR, PerSeptive Biosystems, Framingham, MA). The instrument is equipped with a delay extraction system and a single stage reflectron, and can be operated both in linear and reflectron modes. The laser used on this instrument is a pulsed N_2 laser (λ = 337 nm) with a 3 ns pulse width at a repetition rate of 3 Hz. Two adjacent stages are used for delayed extraction-ion acceleration in the instrument [27]. The N_2 laser is focused at 45° incidence, with an irradiation spot of roughly 200 μm size on the sample plate. A neutral density filter placed on the laser beam path outside the chamber insures the control of the delivered laser energy. The maximum output laser energy is 200 μJ/pulse, this is decreased to 5–15 μJ/pulse using the filter for MALDI experiments. Inside the vacuum chamber the sample plate is inserted in a support mounted on an automatic (x, y) plate allowing sample motion under the N_2 laser beam. A camera (placed at 45° with respect to the TOF axis) is used to monitor the sample during experiments.

Experiments are performed by working just above the laser energy threshold allowing analyte ion observation. Conventional MALDI MS spectra of the standard peptides are recorded using both the delayed extraction and reflectron in positive mode. During the delay time t_d, the first (sample plate) and second electrodes are fixed to 23 kV. After t_d, the first electrode is switched to a total acceleration of 25 kV and the second electrode is kept to 23 kV. t_d is adjusted in the range 0–200 ns to provide the highest spectral resolution for the used analyte. It must be noticed that a supplementary electronic delay of 180 ns must be taken into account. Signal at the detector is monitored during the acquisition using a 500 MHz digitizing oscilloscope (2 ns resolution, Tektronix TD520). Recorded spectra are the average of a fixed number of individual spectra (500 laser shots) obtained while moving regularly the sample under the laser beam to achieve good statistics. Recorded spectra are then processed using the Data Explorer v5.0 software.

2.4. Setup of the desolvation laser

A second laser was added on the commercial MALDI-TOF instrument for the purpose of matrix/analyte cluster desolvation. The laser has to be chosen so that it induces the dissociation of matrix clusters and avoids gas phase post-ionization in the meantime [28,29]. Accordingly, we used a ns pulsed Nd:YAG laser emitting at 1064 nm (Brillant B, Quantel). Taking into account the ionization potential of the irradiated species (typically 8–9 eV), eight photons are generally required to ionize them, which is highly unlikely in the fluence range used (~320–3200 J/m^2, corresponding to 1–10 mJ/pulse). The second laser was implemented on the side of the instrument allowing the laser beam to enter the source chamber through a newly mounted quartz window. Beam align-