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Pulsed valve matrix-assisted ionization†

Bijay Banstola and Kermit. K. Murray *

We have developed a new ionization approach for matrix-assisted ionization with high temporal resolution using an electrically actuated pulsed valve. Matrix and analyte samples are deposited on a thin metal foil and placed at the inlet of an ambient ionization mass spectrometer. When the pulsed valve is actuated, a short puff of high pressure gas impinges on the foil and ejects particulate from the sample on the opposite side. Highly charged ions are formed from the particles at the mass spectrometer inlet. Using this source, multiply charged protein ions are produced within a selectable 4 second time window.

Introduction

Matrix assisted ionization (MAI) is a general term used to describe a mass spectrometer ion source in which ions are formed by the interaction of an analyte molecule with specific matrix compounds that promote the formation of ions.[1–3] As with matrix-assisted laser desorption ionization (MALDI),[4] the matrix is mixed with the analyte and deposited and dried on a sample target. Ion formation is associated with the production of particles by laser ablation, mechanical shock, solvent boiling, or sublimation.[5,6] Some matrix compounds that have been developed for MALDI can also be used for matrix-assisted ionization, but there are many compounds that are unique to MAI.[7] Unlike MALDI, MAI tends to produce ions that are highly charged.[8]

MAI has some potential advantages for mass spectrometry imaging due to its simplicity, low fragmentation, and tandem mass spectrometry facilitated by highly charged ion formation. For imaging in laserspray mode, a pulsed laser is directed at a thin tissue section in transmission mode (back side irradiation) to create ions by MAI.[9,10] Matrix-assisted ionization in vacuum (MAIV) can be used for the analysis of tissue by spotting matrix on selected areas and applying vacuum to the entire tissue section.[11] Precision spotting can limit the exposed tissue area to several hundred μm. An alternative approach uses a glass melting point tube to sample from tissue under ambient conditions for MAI.[12] Better temporal and spatial control of ion formation could add significant utility to these imaging approaches.

Precise control of material removal from a metal sample surface for mass spectrometry analysis can be achieved using a locally directed shock pulse. For example, laser induced acoustic desorption (LIAD)[13] uses a pulsed nanosecond laser that is directed in transmission geometry at a thin metal foil, which ejects material from the opposite side. Post-ionization can be accomplished using electron ionization,[14] electrospray ionization,[15] and photoionization.[16–18] A similar approach that does not require a laser nebulizes liquid samples from piezoelectric ally driven targets using surface acoustic wave nebulization (SAWN).[19,20] Here a high frequency piezoelectric device is used to nebulize a thin film of liquid from a surface and bare ions are formed upon solvent evaporation and sampled into a mass spectrometer ion source.

In this work, we present a method for temporally and spatially localized sampling for matrix-assisted ionization using a solenoid pulsed valve. Here, a high-speed pulsed valve is directed at the back side of a thin foil with a MAI sample on the opposite side facing the inlet of a mass spectrometer. When the valve is actuated, the gas pulse creates a plume of particles, forming ions that are detected in the mass spectrometer. The pulsed valve matrix-assisted ionization source was demonstrated for ionization of peptide and protein molecules under ambient conditions.

Experimental

The modified mass spectrometer ion source comprises a pulsed valve that is aimed at the back side of a metal foil that has an inlet ionization matrix and analyte deposited on the front. Ions created at ambient pressure are sampled by the

Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana, 70803, USA. E-mail: kkmurray@lsu.edu

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inlet of the mass spectrometer. A diagram of the ion source is shown in Fig. 1. The pulsed valve was a Parker Series 9 solenoid valve with an orifice diameter of 0.51 mm and a nitrogen gas backing pressure of 90 psig (600 kPa gauge pressure). The valve was actuated with a 280 V high voltage pulse of 500 μs duration provided by a high voltage switch (Model GRX-3, Directed Energy, Fort Collins, CO) and high voltage power supply (RR3-15R, Gamma, Ormond Beach, FL) driven by a pulse and delay generator (DG 535, Stanford Research Systems, Sunnyvale, CA).

The sample target was a 250 μm thick sheet of aluminium foil (Reynolds Wrap, Alcoa, Pittsburgh, PA) that was mounted between two 0.64 mm thick 5 cm square stainless steel plates with a central 25 mm hole (Kimball Physics, Wilton, New Hampshire). The foil was held 1 mm from the pulsed valve orifice with the opposite side 9 mm from the inlet of an ion trap mass spectrometer (Amazon Speed ETD, Bruker, Bremen, Germany). Both the valve and the sample holder were placed on a translation stage to adjust the distance from the mass spectrometer inlet. The electrospray interface was removed for inlet ionization operation and the inlet was heated to 350 °C. Samples were analyzed in Ultrascan mode at 32 500 *m*/*z* sec.

The reagents 2,5-dihydroxyacetone phosphate (2,5-DHAP), 2-nitrophloroglucinol (2-NPG), 3-nitrobenzonitrile (3-NBN), formic acid (FA), bovine insulin, and bovine erythrocytes ubiqui-tin were purchased from Sigma-Aldrich (St Louis, Missouri). HPLC grade acetonitrile (ACN) and water were purchased from Honeywell (Morris Plains, New Jersey). A solution of 10 μM bovine insulin was prepared in 1 : 1 ACN : 0.1% FA and ubiqui-tin in HPCL grade water. Saturated solutions of 2,5-DHAP, 2-NPG, matrix solutions were prepared in 1 : 1 acetonitrile : water and 3-NBN was prepared in ACN. To create a sample deposit, 1 μL of analyte was deposited on the aluminium foil followed immediately by 2 μL of matrix solution and air dried.

Results and discussion

The pulsed valve matrix-assisted ionization configuration was installed at the inlet of the ion trap mass spectrometer in nanospray configuration (CaptiveSpray) with the commercial spray source removed and the interlock defeated. The foil was held vertically and placed as close as practical to the mass spectrometer with the MAI deposit facing the inlet. The pulsed valve was placed just behind the foil and backed with high pressure nitrogen gas. It was found that the highest gas pressure gave the highest signal. Conventional MAI was accomplished by removing the pulsed valve and foil and tapping a microscope slide with a matrix and analyte deposit against the side of the inlet.

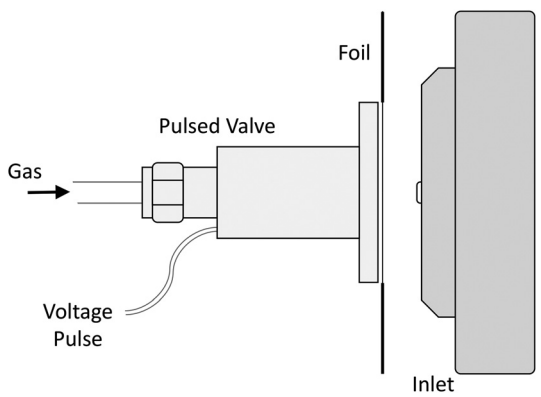

Fig. 1 Pulsed valve matrix assisted ionization schematic.

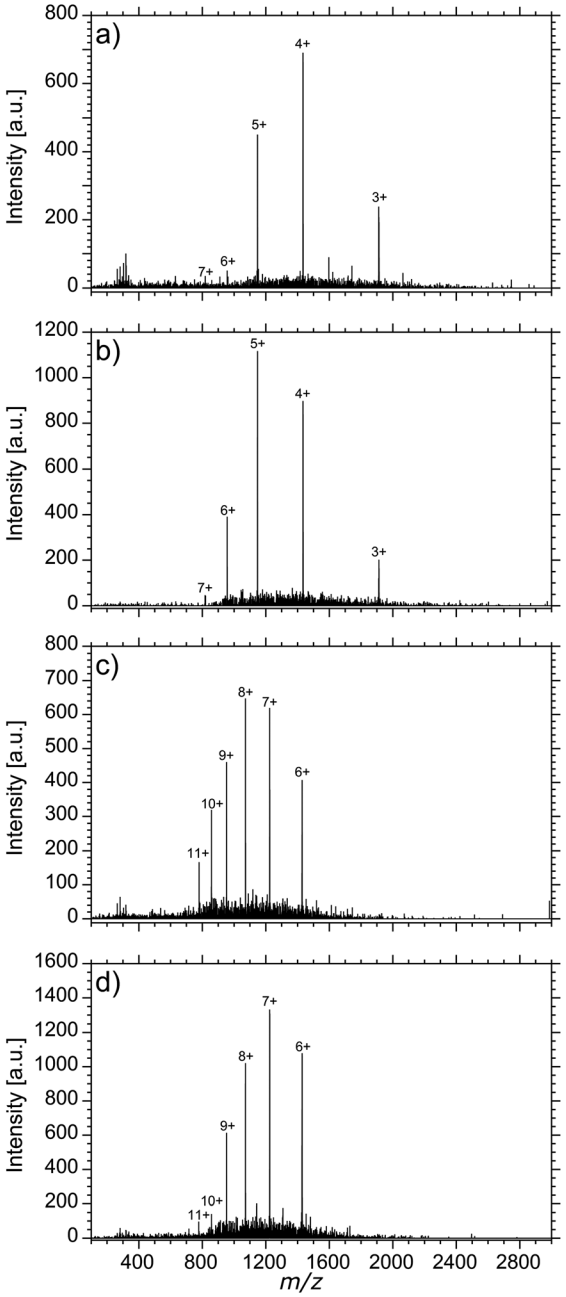

Fig. 2 Mass spectra for 2-NPG matrix-assisted ionization of (a) insulin using tapping, (b) insulin using pulsed valve and (c) ubiquitin tapping, and (d) ubiquitin with pulsed valve.

MAI mass spectra of the proteins insulin and ubiquitin are shown in Fig. 2. A 1 μL volume of 10 μM protein was droplet dried on the foil target with 2 μL of 2-NPG matrix and allowed to dry. The resulting spot was approximately 3 mm in diameter. The mass spectrum shown in Fig. 2a results from insulin deposited on a glass slide and tapped against the inlet of the mass spectrometer. The pulsed valve matrix-assisted ionization of the same solution is shown in Fig. 2b. A total of 5 pulses at 1 Hz repetition rate were used. Fig. 2c is the mass spectrum of the protein ubiquitin from microscope slide tapping and Fig. 2d is the corresponding pulsed valve matrix-assisted ionization mass spectrum of ubiquitin. The mass spectra are comparable, although the pulsed valve matrix-assisted ionization spectra are approximately a factor of two larger than the mass spectra obtained by tapping.

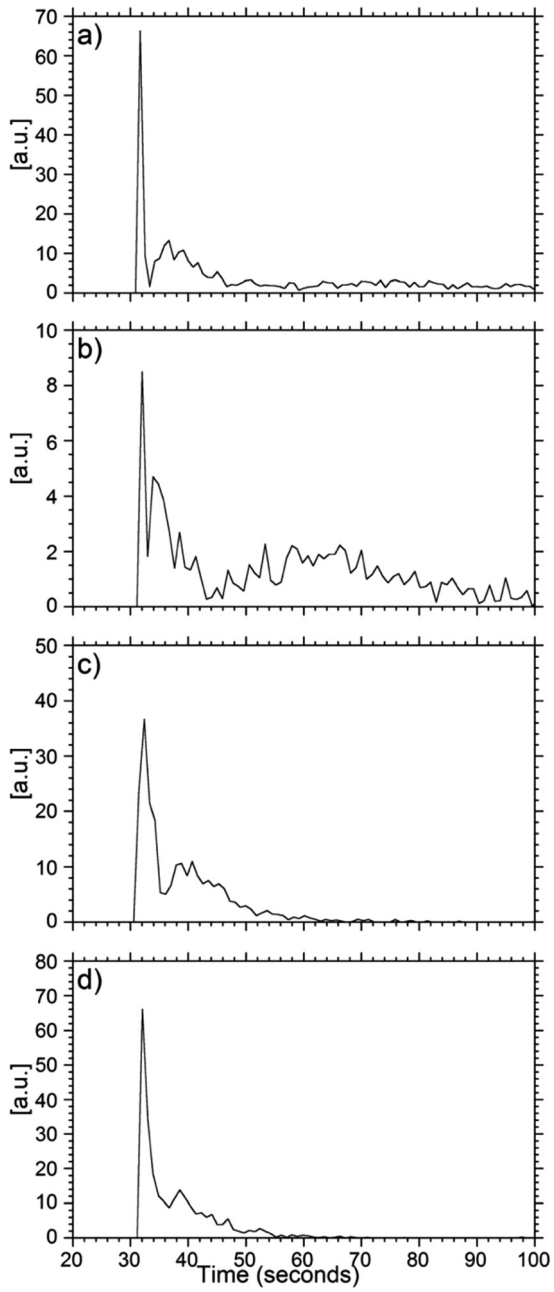

Fig. 3 Total ion current as a function of time for 2-NPG matrix-assisted ionization of insulin using (a) tapping, (b) 1 valve pulse, (c) 2 pulses and (d) 5 pulses at 1 Hz repetition starting at 30 s.

Mass spectra obtained using other inlet ionization matrix compounds produced similar results: pulsed valve matrix-assisted ionization mass spectra showing a comparison of 2-NPG, 3-NBN and 2,5-DHAP matrix compounds is shown in ESI Fig. S1.† The 3-NBN produced the largest signal and the analyte signal intensity was more than 150 times as intense as with 2-NPG and 300 times more intense than 2,5-DHAP, consistent with previously reported results.[7] The 2-NPG produced analyte with the highest charge state.

To assess the number of valve pulses required to deplete the sample was performed using 2-NPG matrix. Fig. 3 shows the total ion current as a function of time for MAI of insulin. Fig. 3a results from striking a glass slide with sample deposit on the mass spectrometer at 30 s elapsed time. Fig. 3b shows the TIC as a function of time for 1 valve pulse, Fig. 3c for 2 pulses at 1 Hz, and Fig. 3d for 5 pulses at 1 Hz. In all cases, the maximum signal is achieved after approximately 2 s and decayed rapidly with an approximately 2 s time constant. The integrated ion signal for 2 and 5 pulses (and for 10, 20 and continuous pulses not shown) was similar and approximately twice the total intensity of a single pulse. This suggests that approximately half of the available particulate was removed with the initial pulse and nearly all of the remainder with the second pulse. A second broad ion signal maximum is observed between 40 and 60 s in the Fig. 3 plots, which suggests two modes or regions of ionization and may be related to the bimodal particle size distribution for inlet ionization matrices that has been observed previously.[21]

A comparison of the time response of the pulsed valve MAI signal for 2-NPG, 3-NBN and 2,5-DHAP is shown in ESI Fig. S2.† In these plots, the pulsed valve was fired five times at a repetition rate of 1 Hz at a time of 30 s. For all of the matrix compounds, maximum signal was observed about 2 s after the valve was fired, decreased rapidly with a 2 s time constant and a lower intensity tail returning to baseline within 30 s.

Conclusions

We have developed a new ion source for matrix-assisted ionization with high temporal resolution. A high-pressure electric solenoid pulsed valve directed at a thin metal foil was capable of ionizing the available material in the sample within 5 seconds of valve actuation. This source has potential applications in matrix-assisted ionization imaging both at ambient pressure and under vacuum and shock wave technology that has been developed for biomedical applications[22–24] has potential applications for precision MAI imaging. We are currently developing a temporally and spatially focused system capable of selectively producing inlet ionization from an array of on tissue samples. Additional studies will be aimed at

investigating the role of high voltage applied to the sample foil in determining the analyte charge distribution and measuring the size distribution of the ejected particulate.

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