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Piezoelectric matrix-assisted ionization

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Abstract

We have developed a new actuation method for matrix-assisted ionization with good temporal and spatial resolution using piezoelectric cantilever. A strike from the piezoelectric bimorph cantilever on a thin metal foil was used to remove materials deposited on the opposite side facing the mass spectrometer inlet. Highly charged ions of peptides and proteins were generated from dried droplet deposits and sampled into the inlet of the mass spectrometer. A lateral resolution of 1 mm was obtained with the piezoelectric sampling configuration. Singly charged lipids and gangliosides were detected from tissue with piezoelectric matrix-assisted ionization using a silica nanoparticle co-matrix.

Keywords

Ambient ionization, cantilever, matrix-assisted ionization, piezoelectric, tissue

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Introduction

Matrix-assisted ionization (MAI) is a novel method of ionization where the interaction of an analyte molecule with specific matrix compound enhances the formation of highly charged ions.[1,2] The method is in general similar to MALDI[3] in that a matrix is mixed with the analyte on a sample target to form a dry deposit. Ions can be formed by a laser as with MALDI; however, the resulting ions are highly charged in contrast to MALDI where they are typically singly charged. Ions can also be formed by application of mechanical shock or simply by sublimation of the matrix. In all cases, MAI produces highly charged ions through a mechanism that appears to be closely associated with the production of fine particulate.[4,5]

Shock-generation of ions for MAI can be implemented in a number of ways. The simplest is to strike a target near the inlet of the mass spectrometer.[6,7] Other methods for particle production include devices such as a pellet gun[6] or mouse trap[4] to produce a mechanical shock. We recently developed a pulsed valve method for precise temporal and spatial control of MAI in which a high-speed pulsed valve was used to direct a high-pressure gas pulse at the back side of a thin foil with a MAI sample on the opposite side facing the MS inlet.[8] The shock from the gas pulse creates a plume of ions that are sampled into the mass spectrometer. The approach of gas-pulse-driven MAI was demonstrated for the ionization of peptide and protein molecules from ambient conditions. Compared to tapping methods, the pulsed valve provides better temporal resolution; however, the spatial resolution achieved with the pulsed valve is limited.

In this work, a piezoelectric cantilever-based method was developed for temporally and spatially localized ion formation for MAI that uses a voltage pulse and does not require a high-pressure gas. Here, a piezoelectric bimorph cantilever with a sharp tip attached to the arm was used as an electrically-driven striker on a thin metal foil with an MAI sample on the opposite side. When the cantilever is actuated, the needle strikes the foil and the material ejected from the other side forms ions when introduced into the mass spectrometer inlet. The piezoelectric cantilever configuration was used for ionization of peptides and protein standards as well as lipids and gangliosides from thin tissue sections at atmospheric pressure.

Experimental

A piezoelectric bimorph cantilever (PB4NB2S, Thorlabs, Newton, NJ) was used to remove samples

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deposited on a thin metal foil. The piezoelectric cantilever has a length of 32 mm, width of 7.8 mm, and a 28 mm free length with maximum displacement of 0.45 mm. The resonant frequency of the bare cantilever is 370 Hz. A 4-mm section from the tip of a 100-μm diameter sewing needle was attached to the end of the arm of a cantilever with cyanoacrylate glue which added a mass of approximately 40 mg to the cantilever. The modified cantilever was operated at 300 Hz, which was the highest frequency possible with the added mass; higher frequencies caused overheating and damaged the cantilever. Aluminum foil (13, 25, and 50 μm; Reynolds Wrap, Pittsburgh, PA), tungsten foil (50 μm), and titanium foil (13 and 25 μm; Alfa Aesar, Ward Hill, MA) were used for the experiments described below. The foil containing the sample was mounted between two 0.64-mm thick, 5-cm square stainless plates with a central 25-mm hole (Kimball Physics, Wilton, NH), similar to that previously described.[8] The foil was held approximately 250 μm from the tip of the needle with the sample side 4 mm from the inlet of the mass spectrometer. Figure 1 shows the schematic of the ion source and the modified cantilever.

The mass spectrometer used for this experiment is an ion trap mass spectrometer (Amazon Speed ETD, Bruker, Bremen, Germany). The captive spray interface was removed for inlet ionization operation and the glass capillary inlet was heated to maximum of 350°C. Before removing the interface, the voltage was turned off and the gas was disconnected. Samples were analyzed in Ultrascan mode between 100 m/z and 3000 m/z at 32,500 m/z per second in positive ion mode. Data collected were analyzed using the instrument control software (Bruker Compass 4.1).

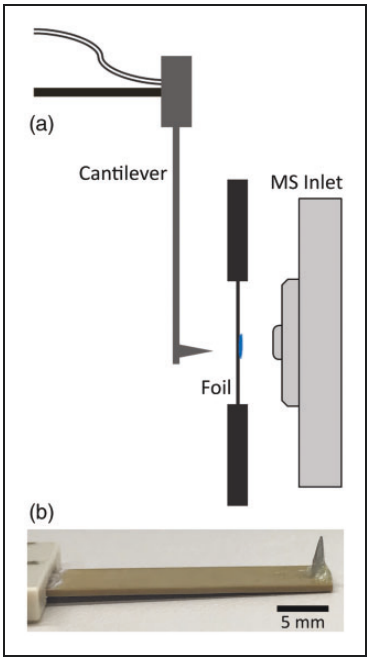

Figure 1. Piezoelectric matrix assisted ionization instrument (a) schematic and (b) photograph of the cantilever with attached needle tip (right).

Stock solutions of protein standards were prepared in HPLC grade water (Sigma–Aldrich, St. Louis, MO) and diluted to 10 μM. Additional solvents were lab grade ethanol, HPLC grade acetonitrile (ACN) and trifluoroacetic acid (TFA; Thermo Fisher Scientific, Waltham, MD), and ammonium bicarbonate (ABC; Sigma-Aldrich). Protein standards insulin, cytochrome C, myoglobin, ubiquitin, matrix compounds 2-nitrophloroglucinol (2-NPG), 3-nitrobenzonitrile (3-NBN), and 2-nitrobenzonitrile (2-NBN) were obtained from Sigma-Aldrich. The three matrices were selected because of their ability to produce high-intensity ions with ESI-like charge states.[5,8] Stock solutions at 1 mM concentration were prepared for all proteins. Ubiquitin, cytochrome C, and myoglobin were prepared in HPLC grade water whereas insulin had 0.1% TFA added. Matrix solutions were prepared by dissolving 10 mg of 2-NPG in 200 μL of 1:1 ACN: water with 0.1% TFA, and 10 mg of 2-NBN or 3-NBN in 100 μL of ACN with 0.1% TFA. The sample target was a thin metal foil and sample deposits were formed by depositing 0.5 μL analyte on the foil followed by 0.5 μL matrix solution and mixing on the foil using the pipette tip. An additional 0.5 μL of matrix was deposited on top of the spot and left to dry. The resulting sample spots approximately 1 mm in diameter were used for the experiments described below.

Mouse brain tissue was obtained from the LSU School of Veterinary Medicine Division of Laboratory Animal Medicine (DLAM) as described previously using procedures approved by the LSU Institutional Animal Care and Use Committee (IACUC).[9] Sections 10-μm thick were prepared from frozen tissue with a cryostat (CM1850, Leica Microsystem, Wetzlar, Germany), thaw-mounted on foil, and stored at -80°C. Prior to analysis, the sections were thawed and dried under rough vacuum for 10 min to remove moisture from the tissue. After drying the tissue, silica nanoparticles 20 nm in diameter (US Research Nanomaterials, Houston, TX) were sprinkled from a spatula onto the tissue to form a distributed layer. The matrix solution was then deposited using a micropipette onto nanoparticle-treated tissue and allowed to dry.

Results and discussion

The cantilever and foil were mounted at the inlet of the mass spectrometer. Initially, a bare cantilever was used to strike the surface either parallel to the surface or at a 45° angle. However, the flat edge of the cantilever distributed the force over a large area and was not efficient at material removal. To concentrate the force of the strike, approximately 4 mm of a sewing needle tip was attached to the end of the arm. The distance between the foil and the MS inlet was optimized for the highest signal intensity at a distance of 4 mm. Driving the cantilever with the added mass at frequencies above 300 Hz of the needle tip resulted in overheating and failure of the bimorph. Tungsten, aluminium, and titanium were

tested and it was found that it was difficult to remove material from the relatively thick and inflexible 50-μm tungsten foil and ionization was not observed. Aluminum (13, 25, and 50 μm) and titanium (13 and 25 μm) foils produced ions. Material removal from the thinner foils was more efficient; however, the 13-μm-thick foils were susceptible to damage by tearing. Though the goal was to remove material efficiently with each strike, care was taken such that the striker did not penetrate the foil. All the experiments described below were performed with 25-μm-thick aluminum foil.

A comparison of piezoelectric-driven MAI and manual tapping is shown in Figure 2. A 0.5-μL volume of a 10-μM ubiquitin solution was deposited on the aluminum foil followed by two deposits of 0.5 μL of 2-NPG matrix which was mixed and allowed to dry. To create ions by manual tapping, the foil target was tapped once on the inlet capillary. For the piezoelectric configuration, 20 pulses at 300 Hz were used to remove material from the foil. Figure 2(a and b) shows the ion signal obtained by manual tapping and piezoelectric cantilever, respectively, as a function of time (strike at 20 s). The signal obtained from tapping (Figure 2(a)) was slightly higher than from the cantilever (Figure 2(b)) in a triplicate measurement. In the corresponding mass spectra, the charge distribution of peaks in the mass spectra from tapping (Figure 2(c)) is similar to that from piezoelectric strike (Figure 2(d)), with the maximum peak intensity observed for the +8 charge state in both cases. Mass spectra of cytochrome C and myoglobin (data not shown) also contained peaks from highly charged ions.

The MAI matrices 2-NPG, 2-NBN, and 3-NBN were tested and compared using the cantilever striker. A capillary inlet temperature of 350°C was used for 2-NPG whereas 200°C was used for 2-NBN and 3-NBN. The total ion signal and mass spectra obtained using 20 pulses at 300 Hz are shown in Figure 3 for each matrix. Of the three matrices, 2-NPG had the lowest peak signal intensity and longest signal duration with a decay time of 25 s obtained by fitting a single exponential to the data. The 2-NBN and 3-NBN had comparable peak signal intensity and had decay times of 9 and 6 s, respectively. The 2-NBN had a larger integrated signal intensity that was approximately four times larger than 3-NBN and 250 times larger than 2-NPG.

The number and frequency of cantilever strikes for efficient removal of sample material was assessed using 2-NBN and insulin. To determine the number of pulses required for complete removal of material, the cantilever was operated at a frequency of 1 Hz and number of strikes was varied. Figure 4(a) shows the total ion current for all insulin charge states as a function of the number of strikes. Three trials were done for each experiment and the error bars represent one standard deviation from three replicate experiments. The signal reached its maximum after approximately ten strikes, suggesting that this number is required to completely remove the deposit from the foil. Similar experiments were performed for 3-NBN and 2-NPG and it was found that ten strikes were required for the former and five for the latter to completely remove the deposit from the foil.

To assess the effect of the cantilever driving frequency, a burst of ten pulses was applied to the foil at a range of frequencies. A new spot was analyzed for each strike and the total ion intensity was recorded. Results for 2-NBN and insulin are shown in Figure 4(b). The observed signal increases up to a frequency of 300 Hz; the cantilever could not be operated at higher frequencies without damage. Similar results were obtained for 3-NBN and 2-NPG (data not

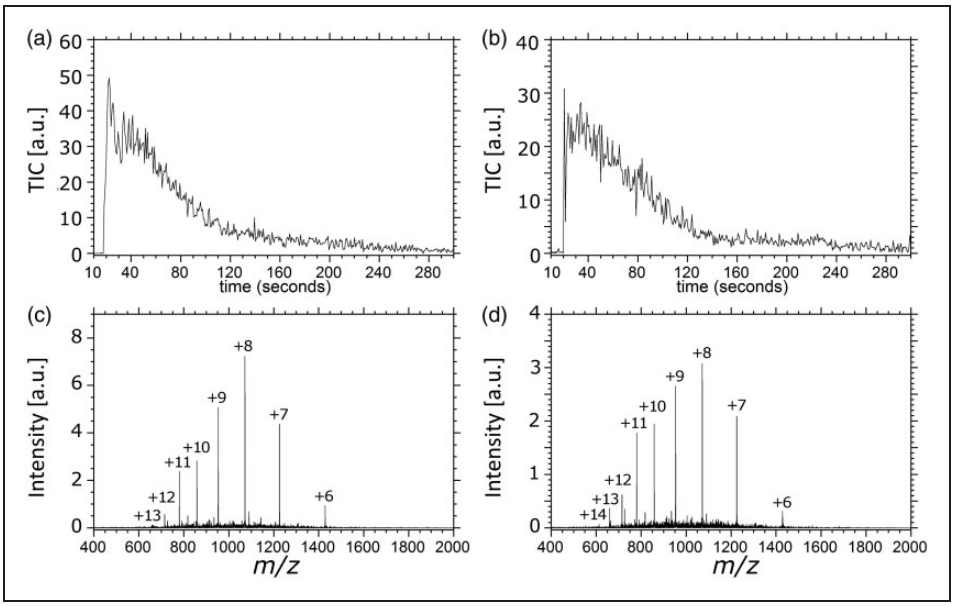

Figure 2. Matrix-assisted ionization mass spectra of ubiquitin protein using 2-NPG matrix: (a) total ion current tapping, (b) total ion current cantilever, (c) mass spectrum tapping, and (d) mass spectrum cantilever.

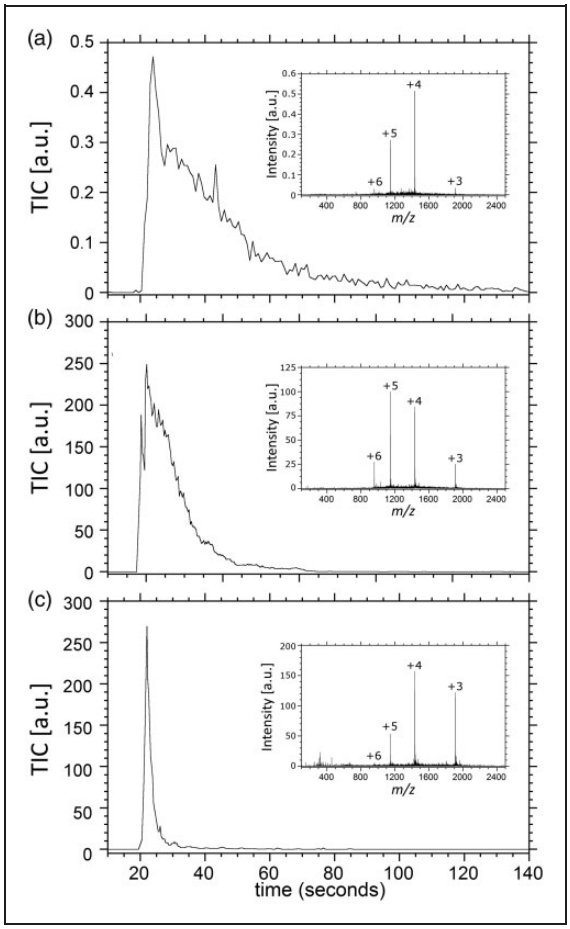

Figure 3. Total ion current for piezoelectric matrix-assisted ionization of insulin using matrices (a) 2-NPG, (b) 2-NBN, and (c) 3-NBN; the insets show the mass spectra for each matrix.

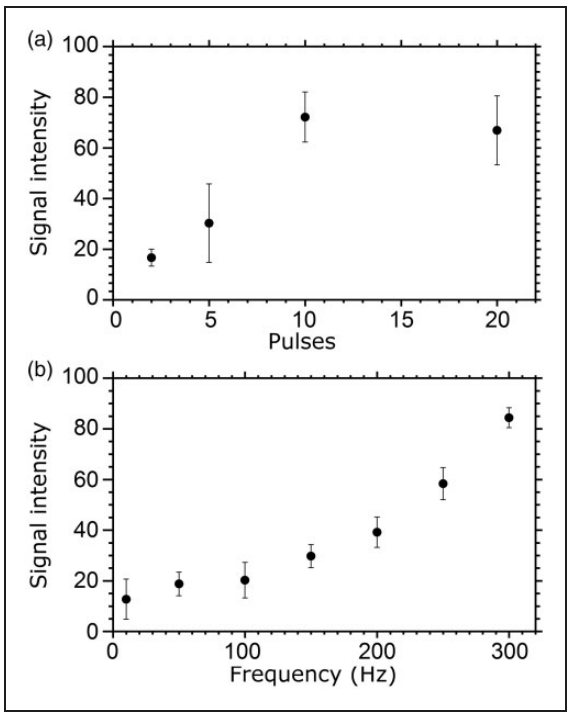

Figure 4. Piezoelectric matrix-assisted ionization ion signal for insulin with 2-NBN matrix as a function of (a) cantilever strikes at 1 Hz and (b) cantilever frequency.

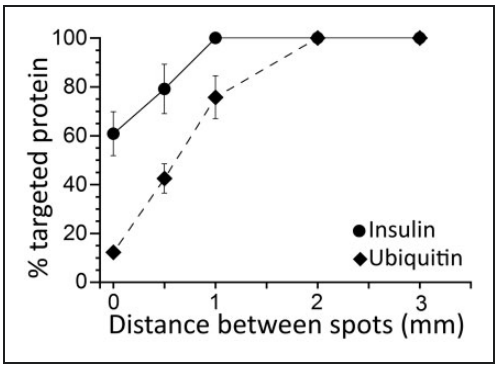

Figure 5. Fractional signal for targeted protein from spots separated by the indicated center-to-center distance for insulin (●) and ubiquitin (◆). Peaks of + 5 and +8 charges were used for insulin and ubiquitin, respectively.

shown). For the remaining experiments described below, the cantilever was operated with ten strikes at 300 Hz frequency for optimum removal of material from the foil.

An assessment of lateral resolution of the system was performed using pairs of deposited sample spots of proteins ubiquitin and insulin. Individual deposits of ubiquitin and insulin that were separated by 0 (overlapping), 0.5, 1, 2, or 3 mm were created on the aluminum foil. The goal was to strike one spot and determine how close the second spot could be without producing signal from the second protein. The cantilever was set to strike the center of either the ubiquitin spot or the insulin spot with ten strikes at 300 Hz. Figure 5 shows the signal intensity for the insulin +5 peak and the ubiquitin +8 peak for striking either the ubiquitin spot or the insulin spot plotted as a function of the center-to-center distance between the spots. When the distance from the strike point (at spot center) to the center of the adjacent spot is 1 mm or more, primarily the targeted protein is observed. At 1 mm center-to-center distance between spots, more than 75% of the signal corresponds to ubiquitin when the ubiquitin spot is targeted and struck and close to 100% of the signal corresponds to the insulin when the insulin spot is targeted and struck. This suggests that the piezoelectrically driven tip can remove material from a region localized to approximately 1 mm.

The piezoelectric cantilever striker was tested for ionization of biomolecules from tissue using a 10-μm mouse brain tissue section mounted on aluminum foil. After sectioning, the tissue was stored at –80°C and was thawed, dried under vacuum, washed with 70% ethanol followed by 90 % ethanol, and dried again under vacuum.[9] Matrix was deposited on the tissue as a 1-μL spot and allowed to dry. No signal from the tissue could be observed using the above sample preparation either with or without washing. To create a more easily displaced sample deposit, silica nanoparticles were deposited on the tissue after washing and prior to matrix addition. It was found that these particles produced a deposit at the surface of the tissue that could be

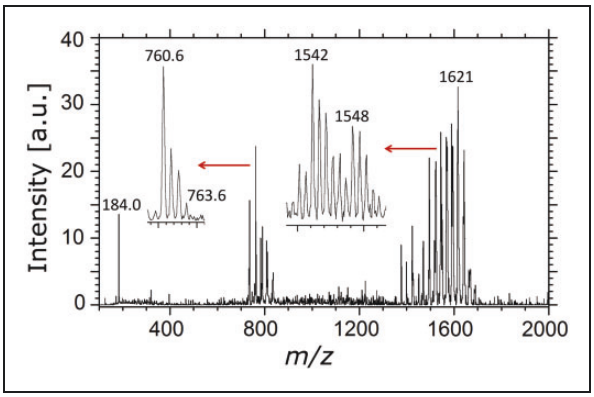

Figure 6. Mass spectrum obtained from mouse brain tissue using 2-NBN matrix.

removed by the piezoelectric striker. Approximately 0.5 mg of 20-nm silica NPs were sprinkled over an area of approximately 3 mm^2 on the tissue followed by a 1 µL volume of matrix solution. A mass spectrum resulting from 10 strikes on tissue with a matrix and nanoparticle co-matrix deposit is shown in Figure 6. Phospholipids and gangliosides were detected from the tissue; no signals from peptides and proteins were observed. The molecules detected were identified as phosphocholine head group at 184 m/z, PC (34:1) at 760.6 m/z, and ganglioside (GM1a) at 1548 m/z by comparison with results from previous studies.[10–12] No multiply charged ions of these species were observed.

The mass spectra obtained with the piezoelectric cantilever are similar to those obtained previously with a high-pressure pulsed valve.[8] The decay time constant obtained by fitting to a single exponential curve was less than 5 s for all three matrices using the pulsed valve, whereas it ranged from 6 to 25 s with the piezoelectric striker. This may be due to the relatively localized piezoelectric strike which may not remove the entire sample and could result in delayed emission from the surrounding area. Contrastingly, the pulsed valve rapidly ejects all of the material in a short period of time leaving no residual.

Mass spectra of mouse brain tissue obtained using 337 nm laserspray MAI in negative ion mode reported the observation of both singly and doubly charged gangliosides.[13] Proteins have been observed from tissue samples using MAI in vacuum in which the tissue sample is subjected to a vacuum source and ions are formed during the sublimation process.[14] In the results reported above for the piezoelectric striker at atmospheric pressure, singly charged ganglosides were observed in positive ion mode. Although widely different ionization configurations and mass spectrometers, the MAI matrixes have the ability to produce ions from tissue without fragmentation.

Conclusions

A new method for sample introduction for MAI has been developed that uses a piezoelectric cantilever striker. It was found that 25 µm thick aluminum foil provided an excellent target surface for the striker: thicker foils did not produce ions and thinner foils tended to tear. A needle tip attached to the cantilever allowed localization of the striking force to a zone of approximately 1 mm in diameter. The duration of the ion signal following the strike ranged from 10 to 40 seconds. The matrix 2-NBN was found to give the best overall performance for the system under present conditions. It was found that a silica nanoparticle co-matrix assisted in producing singly-charged ions from thin tissue sections using the striker. The piezoelectric cantilever system has potential applications for imaging using MAI. The piezoelectric device used for this study is relatively large with a low-resonant frequency, yet was able to achieve relatively good spatial precision. Future work will aim at improving quantification using internal standards and on decreasing the spatial extent and duration of ion signal using smaller actuators. The use of one or more small high-frequency piezoelectric devices could potentially improve both the spatial precision as well as the speed of data acquisition for this approach.

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Declaration of conflicting interests

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