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LIF-IMAGING AND GAS-PHASE DIAGNOSTICS OF LASER DESORBED MALDI-MATRIX PLUMES

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Abstract

The first gated LIF-imaging and absorption spectroscopy has been performed on laser desorbed plumes from organic crystals which are commonly used as MALDI (Matrix Assisted Laser Desorption Ionization) matrices. These plasma diagnostic techniques, including ion probe measurements, were employed to investigate the desorbed products, densities, fractional ionization, and velocity distributions of the plume of ejecta which is typically employed as the main desorption product in the mass spectrometry analysis of large biomolecules. Ultraviolet pulsed 193-nm and 248-nm irradiation of 3-hydroxypicolinic acid (3-HPA) crystals were studied to understand the effect of very different gas-phase absorption cross sections measured here for this material. In both cases, LIF imaging revealed two plume components: a fast (maximum ~ 0.1 cm/ μ s) low-intensity component which appear to be 3-HPA fragments, and a slower component of 3-HPA expanding at 0.05 cm/ μ s. In the case of ArF-laser irradiation, optical absorption spectroscopy indicated a breaking of the intramolecular hydrogen bond in the gas-phase matrix material.

Introduction

Although MALDI has become an established technique for the analysis of large biomolecules,^{1,2} little is known of the nascent velocity distribution or the fractional ionization of the ejecta, the extent of laser interaction with the desorbed species, or the possible plume acceleration or resulting gas dynamics. A characterization of the type and dynamics of the plume species resulting from desorption of the matrix is an essential first step toward understanding³ and optimizing the MALDI technique. The spatial and temporal distributions are important for the enhancement of ion yields and resolutions for time-of-flight (TOF) mass spectrometers. This is especially true considering the recently developed pulsed-delayed-extraction (PDE) technique for resolution enhancement in which a pulsed electric field is timed to accelerate slower charged plume components for a compression of the overall ion-velocity distributions.⁴

These attempts to enhance the sensitivity of the MALDI technique require an understanding of the MALDI plumes. However plume diagnostics often used at J/cm² laser fluences have generally not been employed for the 10–50 mJ/cm² MALDI desorption experiments due to the perception of inapplicability.

Nevertheless, velocity distributions of matrix ions have been measured for some matrices. For example, desorption of sinapic acid⁵ it was found that $V_{\max} = 0.12$ cm/ μ s ($\lambda = 355$ nm, $\Phi = 10$ – 20 mJ/cm²); 2,5-dihydroxybenzoic acid (DHB),⁶ $V_{\max} = 0.27$ – 0.74 cm/ μ s ($\lambda = 266$ nm, $\Phi/\Phi_{\text{thr}} = 1$ – 5.6); and nicotinic acid⁷ $V_{\max} = 0.1$ cm/ μ s ($\lambda = 266$ nm, Φ/Φ_{thr} , where $V_{\max}$ is the distribution maximum velocity and Φ_{thr} is the threshold laser energy fluence. A little is known about the spatial, velocity, and internal energy distributions of neutral matrix molecules.

A very little has been done to image the spatial and temporal progression of MALDI plumes. However, Heise and Yeung were able to use a scanned CW-argon-ion laser to show that a transient imaging technique based on LIF is within the levels required for studying MALDI.⁸ Unfortunately, this study was carried out at atmospheric pressure where the gas-dynamics were radically changed by the ambient air.

In this paper, we report an experimental approach in which gated laser-induced fluorescence (LIF) ICCD imaging, gas-phase optical absorption spectroscopy, and ion current probe (IP) measurements are combined to study MALDI plumes from 3-hydroxypicolinic acid, the most frequently used matrix material.⁹ Ultraviolet (193-nm and 248-nm) laser irradiation of 3-HPA acid matrix crystals was studied using pulsed ArF- and KrF-lasers at low (10–70 mJ/cm²) energy fluences.

Experimental

The experimental set up has been described previously.^{10,11} It consists of a stainless steel high vacuum chamber (40 cm diameter) equipped with Suprasil quartz windows for optical diagnostics. The chamber is pumped by a turbomolecular pump to a base pressure of 10⁻⁶ Torr. A Questec (Model 2960) excimer laser operating on ArF (22 ns FWHM, 600 mJ) or KrF (28 ns FWHM, 900 mJ) was used to desorb the matrix. The unfocused beam was apertured and directed into the chamber at an incidence angle of 30° onto 1"-diameter stainless-steel substrate with 3-HPA matrix deposited on its surface using the standard dried-droplet technique. A large rectangular beam spot on the target was 2.0 cm × 1.6 cm. The maximum laser fluences at the matrix surface were 30 mJ/cm² (ArF) and 70 mJ/cm² (KrF). The target could be rotated during the experiments.

A time-delayed Lumonics (Model hyperex-400) XeCl-excimer laser pulse at 308-nm (30 ns FWHM) was used to induce fluorescence in the MALDI plume. The beam was focused to a planar sheet (dimensions 3.5 cm × 0.2 cm in the plume region) into the chamber through the bottom window with a cylindrical lens (500 mm f.l.) Oriented perpendicular to the target surface [Fig. 1(a)].

For spatially-resolved optical absorption spectroscopy within the plume of ejecta, a Xe flashlamp pulse (1.5 μs FWHM, beam width 0.5 mm) was passed parallel to the target surface, reflected backward with a small mirror placed in the chamber near the target and directed forward again with the second mirror, i.e., the probe beam passed the MALDI plume region three times to increase the optical absorption. All three Xe lamp beams were passed in one plane parallel to the target surface [Fig. 1(b)]. The lamp beam was then imaged with UV-quartz optics onto the slit of a 0.3-meter spectrometer (Acton VM-503) equipped with 600 g/mm grating under computer control. An intensified, gated diode array (Princeton Instruments IRY-700RB, 5 ns resolution) was used as a detector.

Thin microcrystalline films of redeposited 3-HPA crystals were used to measure optical absorption spectrum of the solid matrix. Optical absorption spectra were taken in situ after each desorption laser pulse by measuring the absorbance of the collecting deposit on a quartz substrate located 2 cm from the target surface [Fig. 1 (c)]. A single pass of the Xe-lamp beam was used for each spectrum. Measurable absorption was obtained approximately after 10 desorption KrF-laser pulses with energy fluence 24 mJ/cm².

A coaxial ion probe was used to measure ion current and was typically biased to -70 V (floating with respect to its shield). Transient currents from the fast ion probe were digitized (into 1 k Ω) with a Tektronix 7912 digitizer. Timing between lasers, lamp, and detector-gates was achieved via a digital delay generator (SRS DG535) and monitored on a Tektronix 7912 oscilloscope.

Gated imaging was performed with an intensified charge-coupled device (ICCD), lens-coupled camera system (Princeton Instruments) with variable gain and gate width (5 ns minimum) and a spectral range from 200–820 nm.

For matrix crystal preparation, 3-hydroxypicolinic acid (Aldrich Chem. Co., 98%) was dissolved in 1:1 solution of deionized water (produced using a Millipore cartridge system) and acetonitrile (99.9%, HPLC grade). The aqueous matrix solution (100–200 μ L, 0.3 mol/L) was placed on a 1" diameter polished stainless steel disk and dried under ambient conditions. All samples prepared in this way had complicated polycrystalline structure.

Results and Discussion

Figures 2(a) and 2(b) show typical optical microscope pictures of the solution-grown 3-HPA matrix and the microcrystalline film formed by KrF-laser generated redeposition of this target matrix onto quartz substrate. The same probe beam of the Xe-lamp pulse was used to measure gas-phase absorption spectra at different times and positions from the target surface. Figure 2(c) shows typical absorption spectra of solid- and gas-phase 3-HPA. The gas-phase absorption spectrum matches well a spectrum of 10^{-5} mol/L water solution of 3-HPA, but all three observed bands of the solid-phase spectrum are shifted to the red approximately by 10 nm.

The observed difference in solid- and gas-phase spectra of 3-HPA matrix makes it possible to realize two different regimes of laser desorption with either strong (193 nm, ArF-laser) or weak (248 nm, KrF-laser, 337 nm, N₂-laser) possible 3-HPA plume interaction with the desorbing laser radiation. Both these cases have been studied in this work.

Absorption spectroscopy using the Xe-flashlamp probe was used to analyze the composition of the propagating laser plume as well as its time-of-flight distribution. For the KrF-laser generated plume, the positions and intensity of three observed maxima at 190, 222 and 300 nm [Fig. 2(c)] taken at 3 mm from the target do not change in the time interval from 3 to 50 μ s. A comparison with the absorption spectrum of a 10^{-5} mol/L solution of 3-HPA in water shows that the species responsible for the observed gasphase absorption are 3-HPA molecules. The intensity of the 220 nm band decreases slightly when the energy fluence drops from 46 mJ/cm² to 24 mJ/cm².

According to Fig. 2(c), *gas-phase* 3-HPA molecules desorbed from the target should strongly absorb 193-nm radiation from the end of the ArF-laser pulse. Optical absorption of the ejecta in the 300-nm band region are shown in Fig. 3 for ArF-laser desorption at three times, measured 2.5 mm from the target surface. The band with a maximum at 280 nm is observed at 2 μ s delay time when the leading edge of the plume crosses the probing Xe-lamp beam. The intensity of the 280 nm-band drops rapidly and the 300 nm-band appears at 4 μ s. This spectrum evolution could be explained by breaking of the intramolecular hydrogen bond which links a hydroxyl proton and a carbonyl oxygen in 3-HPA as well as in many other compounds containing

a phenolic hydroxy group.¹² The breaking of the hydrogen bond increases the energy of the $S_0 \rightarrow S_{\pi\pi^*}$ transition responsible for the 300 nm-band in 3-HPA and related compounds by 1500–2000 cm^{-1} .¹³ The possible mechanism of hydrogen bond breaking could be gas-phase absorption of 193-nm ArF-laser radiation followed by an internal conversion of electronic energy to vibrational resulted in growth of vibrational temperature of 3-HPA molecules. The increase of vibrational temperature leads to dissociation of a relatively weak hydrogen bond and shifts the position of the 300 nm-band to the blue.

Optical absorption probing at different delays was used to measure the time-of-flight distribution of 3-HPA molecules in the MALDI plume. Figure 4 presents TOF distributions measured 2.5 mm from the target for the case of ArF-laser desorption of 3-HPA matrix. The intensity of the 280 nm-band was used to plot TOF distribution of vibrationally hot 3-HPA molecules with broken hydrogen bond. Similarly, the number of unexcited 3-HPA molecules was inferred from an integral absorbance over the 186–264 nm spectral range for each time delay used. The absorption spectrum of 10^{-5} mol/L 3-HPA in water was measured and used to derive the absolute concentrations of 3-HPA in the gasphase MALDI plume: $2.5 \times 10^{15} \text{ cm}^{-3}$ (ArF-laser, 17 mJ/cm^2) and $6 \times 10^{15} \text{ cm}^{-3}$ (KrF-laser, 68 mJ/cm^2), measured 3 mm from the target surface. The velocity distribution of the ground-state 3-HPA molecules shows a maximum at $v = 0.08 \text{ cm}/\mu\text{s}$ (ArF) and $v = 0.06 \text{ cm}/\mu\text{s}$ (KrF). As shown in Fig. 4, the vibrationally hot 3-HPA molecules with the broken hydrogen bond are propagating at the leading edge of the 3-HPA TOF distribution.

Ion probe measurement revealed that ions propagate within a relatively narrow distribution located at the leading edge of the neutral 3-HPA TOF distribution (Fig. 4). Figures 4 (a) and 4(b) show the ion-TOF distributions measured with an ion probe located 5.6 cm from the target for both ArF- and KrF-laser desorption at different laser fluences. For increasing ArF-laser energy densities, the maximum of the TOF distribution shifts to smaller time delays, indicating acceleration of the ions in the 3-HPA MALDI plume [Fig. 5(a)]. However for KrF-laser desorption, no changes in the position of the maximum of the ion TOF distribution was found in the energy fluence range 25–68 mJ/cm^2 [Fig. 5(b)]. Only a small shoulder appears at the very leading edge of the KrF-laser induced TOF distribution at the highest energy densities. At least two Maxwell-Boltzmann distributions are required to fit experimentally observed TOF distributions.¹⁴

In addition, Fig. 5(c) shows that KrF-laser irradiation produces many fewer ions compared to an equal energy density of ArF-laser irradiation, e.g., $Q(\text{KrF})/Q(\text{ArF})=10^{-3}$ at 25 mJ/cm^2 , where Q is the time-integrated ion current (from 10 to 92 μs) [Fig. 5(c)]. Using the area of the probe to estimate the ion density from the measured current at 5.6 cm, a density of $\sim 10^9 \text{ cm}^{-3}$ (ArF-laser, 23 mJ/cm^2) is determined. The ion density at a distance of 0.3 cm can then be estimated as $10^9 \times (5.6/0.3)^2 = 3.5 \times 10^{11} \text{ cm}^{-3}$ for comparison with the density of neutrals measured at the same distance. The fraction of ions in ArF-laser (20 mJ/cm^2) generated 3-HPA MALDI plumes is therefore very small, $n_i/n_0=10^{-5}$.

The most probable velocity of 3-HPA molecules ($M=139$) at the melting temperature, 493 K, is only 0.024 $\text{cm}/\mu\text{s}$. That means that 3-HPA neutrals and possibly molecular ions propagate with superthermal velocity. However, the 3-HPA neutral TOF distributions requires at least two Maxwell-Boltzmann distributions to be fitted and the most probable velocity of the second distribution is 0.025 $\text{cm}/\mu\text{s}$, which is in pretty good agreement with the 3-HPA thermal velocity at the melting temperature.

Summarizing the whole set of TOF-distributions measured (see Fig. 4) we can conclude that the neutral 3-HPA TOF distribution probably consists of hyperthermal and thermal parts. The ion TOF distribution that is also composite in nature is located at the leading edge of hyperthermal distribution of neutrals. The hot neutrals in the case of ArF-laser desorption are also created at the leading front of the hyperthermal part of the 3-HPA plume.

Figure 6 shows the first LIF images of 3-HPA MALDI plumes. Following irradiation by the expanded KrF beam, the most intense region at the bottom of the image comes from the solid-phase 3-HPA matrix which is known to emit a bright long-lived blue luminescence.¹⁵ However, no gas-phase luminescence could be detected without the time delayed LIF excitation pulse. The sheet of 308-nm XeCl-laser light was oriented parallel to, but not touching, the target surface. The camera was positioned normal to this sheet to image the induced luminescence along an interior cross-section of the 3-HPA plume.

Two different forward-propagating components can be seen in these images. A fast, low-intensity component propagates with the maximum velocity of about 0.1 cm/ μ s. A much slower, component expands at 0.05 cm/ μ s. The second bright component consists of many small separate plumes which is related to the highly nonuniform crystalline structure of the 3-HPA MALDI matrix [see Fig. 1(a)]. As described above, the species within the second component was identified by optical absorption spectroscopy as 3-HPA molecules. The nature of the fast component is not yet clear since it does not appear in the absorption spectrum. It could be that fragments of 3-HPA molecule are responsible for this component.

Although the above studies involve large areas irradiated with excimer laser pulses (not commonly employed for MALDI analysis), these same imaging techniques are applicable to the small spot sizes and wavelengths commonly employed for MALDI mass spectrometry. Using these imaging techniques, we were recently able to visualize 3-HPA plumes under actual MALDI conditions, i. e., when desorption was initiated with a 337nm N₂-laser focused to a spot diameter of a few hundreds of microns. The results of this study will be described elsewhere.¹⁶

Conclusion

Plasma diagnostic techniques of gas-phase optical absorption spectroscopy, laser-induced fluorescence imaging, and fast ion probe measurements were used to characterize the desorbed products, densities, fractional ionization, and velocity distributions of ejecta from a commonly-used MALDI matrix (3-hydroxypicolinic acid (3-HPA)). Optical absorption spectroscopy of thin redeposited films of MALDI matrices was also demonstrated as a direct in situ technique to compare gas-phase and solid-phase absorption.

The LIF imaging of ArF- and KrF-laser generated 3-HPA matrix plumes revealed two different components: a fast, low-intensity component propagating with maximum velocity of 0.1 cm/ μ s, and a much slower, component expanding at $V=0.05$ cm/ μ s. Multipass optical absorption probing has been used to identify the species within the second component, 3-HPA molecules, and measure their density, 2.5×10^{15} cm⁻³ (ArF laser, 17 mJ/cm²) and 6×10^{15} cm⁻³ (KrF-laser, 68 mJ/cm²) probed at 3 mm from the target surface. Ion probe measurements (ArF-laser, 23 mJ/cm²) revealed that density of ions is only $\sim 3.5 \times 10$ cm⁻³, (recalculated to the same distance, 3mm). The velocity distribution of neutral 3-HPA molecules shows a maximum at $V=0.08$ cm/ μ s (ArF) and $V=0.06$ cm/ μ s (KrF). Ions are propagating within a relatively narrow distribution located at the leading edge of the neutral 3-HPA TOF distribution. The ion TOF profiles show quite different behavior with increasing ArF- and KrF-laser energy fluence, i. e., the ArF-laser generated TOF

distribution demonstrates apparent acceleration while the KrF-distribution does not. Furthermore, ArF-laser desorption of the 3-HPA matrix results in intramolecular hydrogen bond-breaking while KrF-laser desorption does not.

In summary, in situ diagnostics reveal that despite the low laser energy fluences employed, MALDI matrix plumes are dense and multicomponent in character, and are composed primarily of intact matrix molecules propagating with non-thermal velocities. Fragmentation, ionization, and acceleration can result from gas-phase absorption of the laser energy. Inclusion of dye-tagged DNA molecules, as well as combined measurements with TOF mass spectrometry, should assist in the understanding of the role of gas-phase process during MALDI, help optimize pulsed delayed-extraction techniques, and clarify the desorption/ionization mechanism.

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Figure Captions

Fig. 1(a), 1(b), and 1(c). Scheme of the experimental setup. (a) Laser induced fluorescence imaging of MALDI plume, (b) spatially-resolved optical absorption spectroscopy within the plume of ejecta and (c) matrix redeposition and solid-phase absorption measurements.

Fig. 2(a), 2(b), 2(c). Optical microscope images of 3-hydroxypicolinic (3-HPA) acid matrix prepared by (a) dried-droplet method on the stainless steel substrate and (b) redeposited with KrF-laser (24 mJ/cm^2) on a quartz substrate located 2 cm from the target surface. (c) Optical absorption spectra measured in gas-phase (KrF-laser, 46 mJ/cm^2 , 250 ns gate, 6 μs delay, $D=3 \text{ mm}$) and solid-phase after 100 KrF-laser pulses.

Fig. 3. Optical absorption of the ejecta in the 300-nm band region for ArF-laser desorption at 2, 4 and 10 μs delays (ArF-laser, 20 mJ/cm^2 , 250 μs gate, $D=2.5 \text{ mm}$). Schematic energy diagram for normal 3-HPA molecule (bottom right) and molecule with broken hydrogen bond¹³ (bottom left).

Fig. 4. Time-of-flight (TOF) distributions measured for 3-HPA neutrals, 3-HPA neutrals with broken hydrogen bond (hot neutrals) with optical absorption probing at different delays, and ions (ion probe measurements at 5.6 cm recalculated to 2.5 mm).

Fig. 5(a), 5(b), 5(c). Ion time-of-flight distributions (a) ArF- and (b) KrF-laser measured at 5.6 cm from the target surface. (c) Integral ion probe current (from 10 to 92 μs) vs. energy fluence for ArF- and KrF-lasers.

Fig. 6. Laser induced fluorescence (XeCl-laser) images of KrF-laser (20 mJ/cm^2 , beam spot $20 \times 2 \text{ mm}^2$) generated 3-HPA MALDI plume. The ICCD gate was delayed by 100 ns relative to the XeCl-laser pulse.

Fig. 1 a, b, c

(a)

(b)

(c)

Fig. 2 a, b, c

Fig. 3

Fig. 4

Fig. 5 a,b c

Fig. 6