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# Laser-induced acoustic desorption/chemical ionization in Fourier-transform ion cyclotron resonance mass spectrometry

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## Abstract

Laser-induced acoustic desorption (LIAD) of neutral molecules coupled with electron and chemical ionization was examined as an analysis method for nonvolatile organic and biomolecules in Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometry. LIAD involved the production of a high amplitude acoustic wave by laser ablation of a copper or titanium foil from the opposite side of where the sample was deposited. The experiment was carried out with a simple probe designed for transmission mode laser desorption. Large amounts of neutral molecules were desorbed this way, but ions were not detected. The desorbed neutral molecules were ionized by 70 eV electron ionization or by reactions with reagent ions that were generated, isolated, and trapped in the ICR cell. Strong, reproducible signals were obtained in these experiments. The applicability of the method was demonstrated for a wide variety of molecules, including an organic salt, steroids, sugars, oligopeptides, nucleic acid bases, nucleosides, and synthetic polymers. For example, the tetrapeptide val-ala-ala-phe was volatilized by LIAD and ionized by a proton transfer reaction. The ions were stored in the FT-ICR cell for up to 30 s before detection. The base peak in the spectrum obtained corresponds to the protonated peptide, which indicates that the neutral peptide was desorbed intact. In contrast, thermal desorption of this peptide leads to substantial degradation. Based on this and other results obtained, LIAD combined with postdesorption ionization in an FT-ICR shows promise as a practical method for the analysis of thermally labile, nonvolatile molecules. A special advantage of this approach is that it allows better control of the ionization step (through selection of reagent ions) and a broader choice of ionization modes (e.g. group transfer, addition/elimination) than laser desorption/ionization methods, such as matrix-assisted laser desorption/ionization (MALDI). A disadvantage is lower sensitivity. (Int J Mass Spectrom 198 (2000) 173–188) © 2000 Elsevier Science B.V.

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## 1. Introduction

Since its introduction by Karas and co-workers [1] in 1987, matrix-assisted laser desorption/ionization (MALDI) has become a powerful and popular tool for the study of thermally labile, nonvolatile samples by

mass spectrometry [2–5]. The ionization efficiency of MALDI depends on the properties of the analyte and matrix, the sample preparation conditions, and the deposition method [2,6]. Since the role of the matrix in the ionization (and desorption) process is poorly understood, it is not straightforward to find a suitable matrix and sample preparation protocol for analysis of samples with widely varying properties. Because of

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these limitations, several researchers have continued to pursue alternative desorption/ionization methods [7–11].

The feasibility of using laser-driven shock waves in desorption/ionization mass spectrometry was first demonstrated by Lindner and Seydel in 1985 [12]. The experiments involved the deposition of relatively thick layers ( $\sim 20\ \mu\text{m}$ ) of small sugars and oligosaccharides (of  $m/z$  values up to 1982) onto formvar coated copper grids (mesh size  $100\ \mu\text{m}$ ). Samples were then subjected to backside laser irradiation inside a laser microprobe mass analyzer (LAMMA). The authors added alkali metal salts into the sample, which resulted in the detection of quasimolecular ions formed by alkali metal ion attachment onto the analyte molecules.

Following Lindner and Seydel, three other laser-induced acoustic desorption (LIAD)-ionization studies have appeared in the literature. In those cases, the authors employed thin metal films, such as copper [13] or mercury [7,14], to shield the samples from the high intensity laser. Samples were deposited either on the metal surface opposite to that irradiated by the laser, or on the surface of a thermally insulating layer of material (e.g. sapphire) that was in physical contact with the metal. However, ionization of the analytes in these experiments proved to be generally difficult. For example, Lindner attempted LIAD of oligosaccharides that were electrodeposited (with added alkali metal salts) onto copper foils [13]. In contrast to his earlier work (where samples were directly exposed to the laser light), no analyte ions were detected by a time-of-flight mass spectrometer. However, LIAD of the organic salt carbofuchsin resulted in detectable ion signals. Lindner observed evidence of material removal at the deposit surface in both cases. Hence, he concluded that most of the mechanical energy provided by the acoustic wave was coupled into the desorption stage and that insufficient energy was available for the ionization process. Furthermore, he predicted that the ejection of neutral species will predominate in LIAD, unless the analyte molecules are precharged in the deposit.

In one of the studies, 3-hydroxypicolinic acid was used as a matrix in the preparation of samples. This

approach resulted in the desorption of ions indicative of cytochrome c and insulin in the time-of-flight instrument [7]. However, since a matrix was employed in the preparation of samples, this LIAD approach does not offer a significant improvement to MALDI because the ionization process remains uncertain. Aside from this, no further LIAD studies have been reported in the literature.

LIAD has not developed into a practical desorption/ionization method. However, the predominant desorption of neutral molecules predicted by Lindner prompted us to explore the combination of LIAD with postdesorption electron ionization (EI) and chemical ionization (CI). CI is an especially attractive approach because it offers a wide range of ionization modes (e.g., electron transfer, atom transfer, group transfer) that depend on the reagent ion and the functionalities in the analyte. Further, the energetics of the ionization process can be controlled by choice of the reagent ion [15], so that molecular weight as well as structural information can be obtained. However, chemical ionization of neutral molecules evaporated by laser-based methods is not straightforward. The typically high forward velocities of these molecules result in low ionization efficiencies due to a short interaction time with the reagent ions [16]. Another limitation that is especially severe in scanning-type mass spectrometers (e.g., magnetic sector, quadrupole) is that the time necessary to collect a mass spectrum is much longer than the duration of the pulse of neutral molecules generated by laser desorption.

Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR) offers several advantages over other instruments [17–20] in laser desorption/chemical ionization (LD/CI) experiments. First, all ions are detected simultaneously in FT-ICR. Secondly, FT-ICR allows the use of several consecutive reaction/ion isolation steps to form reagent ions that can be stored in the cell prior to the introduction of the neutral analyte [21–24]. Finally, the stored reagent ions can be exposed to a large population of neutral molecules desorbed by several laser pulses.

The first successful LD/CI study in FT-ICR involved the use of trapped reagent ions  $\text{CH}_5^+$  and  $\text{C}_2\text{H}_5^+$  to protonate thymidine and 9-pentadecylacridine

evaporated by direct laser irradiation [25]. Samples were deposited as neat thin films onto a polished gold or platinum surface. A very large number of reagent ions had to be generated and trapped for observable signals of analyte ions. Therefore, prior to detection of the analyte ions, the reagent ion population had to be ejected from the cell to avoid space-charge effects (Coulomb repulsion among the ions). This approach resulted in a reasonable ionization efficiency for the two analytes studied. However, the authors concluded that the desorption process probably caused dissociation of thymidine.

The substrate-assisted laser desorption (SALD) technique proved more successful [26–29]. In those experiments, a thin layer of sample was applied over an ultraviolet-absorbing organic substrate and irradiated with 248 nm laser light that is not strongly absorbed by the analyte molecules. The generation and storage of an excess population of reagent ions was not necessary to obtain ion signals, and detection of protonated molecules indicated volatilization of intact neutral peptide and amino acid molecules. Although SALD is not widely used, these studies established FT-ICR as a useful tool for postdesorption chemical ionization experiments.

## 2. Experimental

The experiments were performed in an Extrel Model 2001 Fourier transform ion cyclotron resonance mass spectrometer, described previously [21–24]. The instrument contains a differentially pumped dual cell that is placed within the magnetic field produced by a 3.0 tesla superconducting magnet operated at about 2.8 tesla. The nominal base pressure is less than  $10^{-9}$  Torr, as maintained by two Balzers turbomolecular pumps. The pressure is measured with two ionization gauges, located on each side of the dual cell. The two cells are separated by a common wall (the conductance limit) that contains a 2 mm hole in the center. Unless otherwise noted, the conductance limit and the other two trapping plates were maintained at + 2 V.

Laser-induced acoustic desorption was accomplished using a novel probe that was designed to

facilitate transmission mode laser desorption in a FT-ICR equipped with a manual insertion probe inlet (Fig. 1) [30]. The apparatus consists of an optical fiber (3M Specialty Fibers, West Haven, CT) and 1:1 imaging optics, all assembled in a self-contained manual insertion probe. The probe includes the capability to irradiate 22 discrete, nonoverlapping sites ( $10^{-3}$  cm<sup>2</sup>) at the sample backsurface by rotating the inner cylinder at 18° increments over one full rotation. Alternatively, a total area of about  $3 \times 10^{-2}$  cm<sup>2</sup> can be irradiated by continuous rotation of the inner cylinder while the laser is fired repeatedly. Further details can be found elsewhere [30].

Application of the laser desorption probe for the volatilization of neutral molecules in FT-ICR requires alignment of the probe tip with respect to the center of the ICR cell. For this purpose, a home-built aluminum centering ring was installed in the insertion path of the probe, within the high vacuum region of the cell (on the cell flange rods approximately 4 in. before the ICR cell). The ring insures optimum alignment of the probe when inserted to within 1/8 in. from the source trap plate. Consequently, all of the desorption sites on the front surface of the metal foil are equidistant (1.3 mm) from the center of the ICR cell.

All compounds were obtained from Aldrich Chemical Co. (St. Louis, MO), except for polyethylene glycol 610 (American Polymer Standards, Mentor, OH) and *trans*-estriol (Steraloids Inc, Newport, RI). Titanium (10 μm) and the thinnest copper (2.5 and 5 μm) foils were obtained from Goodfellow Corp. (Berwyn, PA). Thicker copper foils (10, 12.5, and 25 μm) were obtained from Alfa Aesar (Ward Hill, MA). The reagents and metal foils were used as received from the supplier. Samples (10 mM solutions in methanol or tetrahydrofuran) were deposited on one side of the metal foils by using the electrospray method [31–33]. The spray volumes were adjusted to obtain a sample coverage of  $\sim 75$  nmol/cm<sup>2</sup> on the metal surface.

After sample deposition, the foil was transferred to the sample support stage of the laser desorption probe. The side of the foil that was coated with sample was exposed to the mass analyzer while the opposite surface rested on a thin (200 μm) glass support. Light

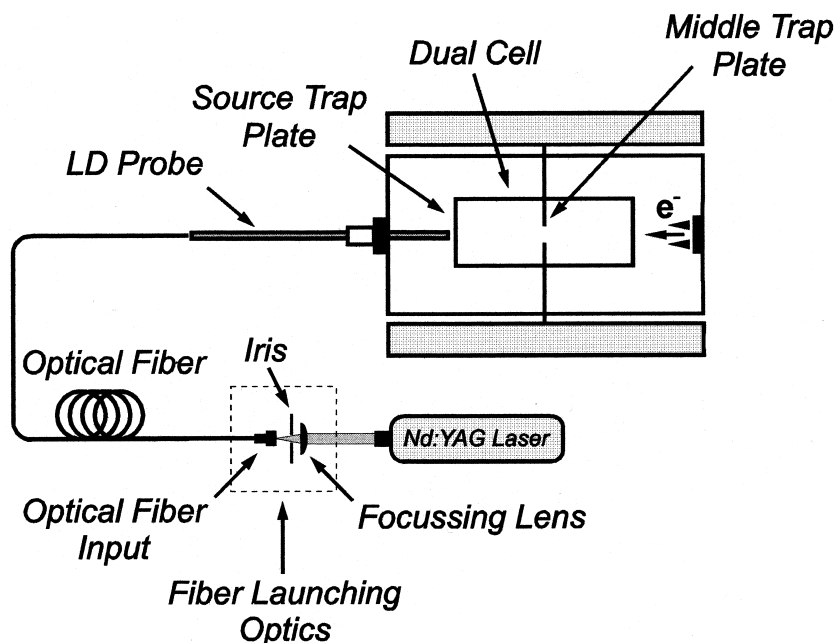


Fig. 1. Diagram of the transmission geometry laser desorption setup for a dual cell FT-ICR.

(532 nm wavelength, fluence between  $5 \times 10^8$  and  $1 \times 10^9$  W/cm<sup>2</sup> at the metal surface) from a Continuum Minilite II Nd:YAG laser (Continuum, Santa Clara, CA) was transmitted through the fiber optic and focused to an area of about  $10^{-3}$  cm<sup>2</sup> on the opposite foil surface. At  $10^9$  W/cm<sup>2</sup>, the laser ablation of copper was possible for at least 100 shots at one given spot without perforation of the foil. Higher laser irradiation intensities were not employed because of possible damage to the optical fiber.

Electron ionization of the desorbed neutral molecules was achieved by switching the bias of a grid to allow entry of the ionizing electrons (70 eV electron energy, 6–20  $\mu$ A emission current) into the cell during the laser trigger event ( $\sim 1$  ms). Chemical ionization (CI) was accomplished by reaction of the desorbed neutral molecules with either protonated methanol ( $m/z$  33), or anilinium ( $m/z$  94), hydronium ( $m/z$  19), ammonium ( $m/z$  18), or 1,3-dioxolane-2-phosphenium ions ( $m/z$  91) stored in the cell. The phosphenium ions were generated by electron ionization of 2-chloro-1,2,3-dioxaphospholane. The other reagent ions were produced by a multistep procedure.

Methanol, aniline, water, or ammonia was introduced into the source cell via a batch inlet system (nominal pressure in the cell was  $6.0 \times 10^{-8}$  Torr) and ionized by electron ionization (typically 70 eV electron energy, 8  $\mu$ A emission current, 50 ms ionization time). The resulting radical cations and fragment ions were allowed to react for 2–5 s with the neutral precursor, which resulted in an abundant protonated molecule (MH<sup>+</sup>). In all cases, the desired reagent ions were isolated by ejecting all unwanted ions from the cell through the application of a series of stored-waveform inverse Fourier transform (SWIFT) [34,35] pulses to the excitation plates of the cell (Extrel SWIFT module). The reagent ions were then allowed to react with neutral molecules evaporated by LIAD (LIAD/CI). The LIAD/CI spectra shown are the result of a single scan that included 100–500 laser shots. The laser pulses were applied at 70 ms intervals (due to the 15 Hz maximum trigger rate of the laser). Each laser shot resulted in a population of neutral molecules with a residence time of about one ms in the cell [36]. The LIAD/EI spectra are the result of one laser pulse.

The average flight times (from the sample stage to

near the center of the ICR cell) of  $\alpha$ -cyano-4-hydroxycinnamate ions evaporated by direct front- or backside laser irradiation were measured previously in our laboratory [30] by using a literature procedure. This procedure was adapted here to measure the average flight time of neutral  $\alpha$ -cyano-4-hydroxycinnamic acid evaporated by LIAD. In these experiments, the ion population in the cell was monitored as a function of the time duration of the electron ionization event (70 eV electron energy, 10  $\mu$ A emission current) after application of the laser trigger.

A broadband chirp excitation sweep (from 1.9 kHz to 2.6 MHz; 200 V peak-to-peak; sweep rate 3200 Hz/ $\mu$ s) was chosen for excitation of ions prior to detection. The time domain data were subjected to Hanning apodization, followed by augmentation of the data set by an equal number of zeroes. Frequency domain spectra were obtained by magnitude mode Fourier transformation of the time domain data set. All spectra were produced by collecting 64 k data points at an acquisition rate of 8000 kHz. Each spectrum is the result of a single scan except where otherwise noted.

The energetics of several reactions were investigated using AM1 semiempirical molecular orbital calculations (SPARTAN version 4.1.4) [37]. The ion pairing energy of brilliant green bisulfate was computed as the difference in the heat of formation of the brilliant green-bisulfate ion pair (lowest energy configuration) and the individual heats of formation of the two counter ions. The proton affinity of val-ala-ala-phe was estimated via an isodesmic reaction [38] comparing its proton affinity to that of alanine, for which a good experimental value exists. All structures were determined to be local energy minima at the AM1 level of theory by the absence of imaginary frequencies.

### 3. Results and discussion

#### 3.1. Desorption of neutral molecules by laser-induced acoustic waves

The first goal of this work was to determine whether LIAD is applicable to a wide range of neutral

organic molecules of varying properties. The analytes chosen for this study are cytosine (molecular weight (MW) 111), ribose (MW 150), guanine (MW 151),  $\alpha$ -cyano-4-hydroxycinnamic acid (MW 189), gly-gly-val (MW 231), thymidine (MW 242), cytidine (MW 243), guanosine (MW 283), *trans*-estriol (MW 288), val-ala-ala-phe (MW 406.5), heptacosanoic acid (MW 410), brilliant green bisulfate (MW 482), tetracontane (MW 563), polyethylene glycol 610, oxytocin (MW 1007), angiotensin I (MW 1296), polyisobutylene 1600, and polystyrene 2500. Each sample was deposited on the surface of a metal foil. 10  $\mu$ m Copper foils were used predominantly because they are relatively inexpensive and because previous studies [13] suggest that LIAD of organic analytes deposited on a copper surface may result in the evaporation of neutral molecules. The opposite surface of the metal foil was exposed to one or more laser pulses. The intensity of the laser beam ( $10^9$  W/cm<sup>2</sup>) was high enough to initiate laser ablation on the irradiated surface of the metal without perforation of the foil. Based on visual inspection of the coated foil surface after LIAD, all the samples were successfully volatilized and depleted at the surface by the energy of the acoustic wave generated during the ablation process. Ions were not detected by FT-ICR without postdesorption ionization, which supports Lindner's [13] observation that ions are generally not formed by the LIAD process alone.

Experimental variables, such as metal foil thickness (2.5–25  $\mu$ m), sample deposition coverage (35 nmol/cm<sup>2</sup>–4.5  $\mu$ mol/cm<sup>2</sup>), and laser irradiation intensity ( $10^8$ – $10^9$  W/cm<sup>2</sup>), were investigated to optimize LIAD conditions. Employing metal foils that were thicker (12.5 and 25  $\mu$ m) than 10  $\mu$ m resulted in little or no desorption of the analytes. Desorption was more effective with the thinnest copper foils studied (2.5 and 5  $\mu$ m). However, the thicker 10  $\mu$ m foil was chosen for most experiments to minimize possible sample overheating by the laser. Desorption was most effective by using a laser irradiation intensity of  $10^9$  W/cm<sup>2</sup> at the back surface of copper. The sample coverage applied at the front surface did not have a substantial effect on the observed desorption efficiency. Nonetheless, relatively high sample coverages

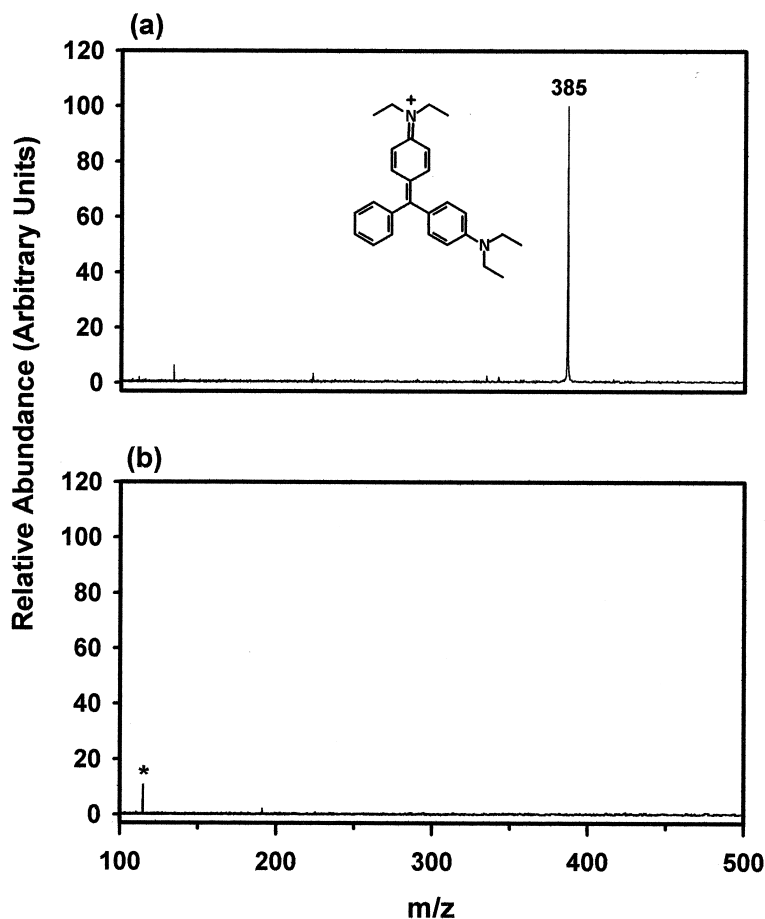


Fig. 2. Evaporation of neutral brilliant green bisulfate (MW 482) by LIAD. (a) Ionization by 70 eV electron impact results in the dissociation of the ionic bond, as demonstrated by the detection of  $m/z$  385. This ion corresponds to the positively charged moiety of the salt. (b) Spectrum resulting from the same experimental conditions except without electron ionization. The signal at  $m/z$  122 arises from electronic noise.

( $\sim 75$  nmol/cm<sup>2</sup>) were used in most of the experiments (especially those involving chemical ionization) to yield a large population of volatilized molecules.

Lindner's prediction that precharged molecules in the deposit are necessary to observe ions in LIAD experiments was probed by performing LIAD of brilliant green bisulfate. This species is an organic salt that consists of an alkyl ammonium moiety (MW 385) with a bisulfate counterion. The ion pairing energy of brilliant green bisulfate was estimated by semiempirical molecular orbital calculations to be about 58 kcal/mol, which is less than most covalent bonds. Based on Lindner's previous observations involving

LIAD of similar organic salts [13], the energy from the LIAD process alone was expected to result in dissociation of the fragile ionic bond and subsequent detection of the positive ion of  $m/z$  385. However, brilliant green was desorbed with its counterion as a net neutral species (Fig. 2). This finding was encouraging, as it indicates that LIAD is a gentle evaporation method. However, the apparent disagreement of our result with Lindner's requires a comment. These two studies employed different copper foil thicknesses. The energy of the acoustic wave after transmission through the 10  $\mu$ m foil in our study is likely to be lower than that following delivery through the 2.5  $\mu$ m copper foil used by Lindner. Indeed, when we em-



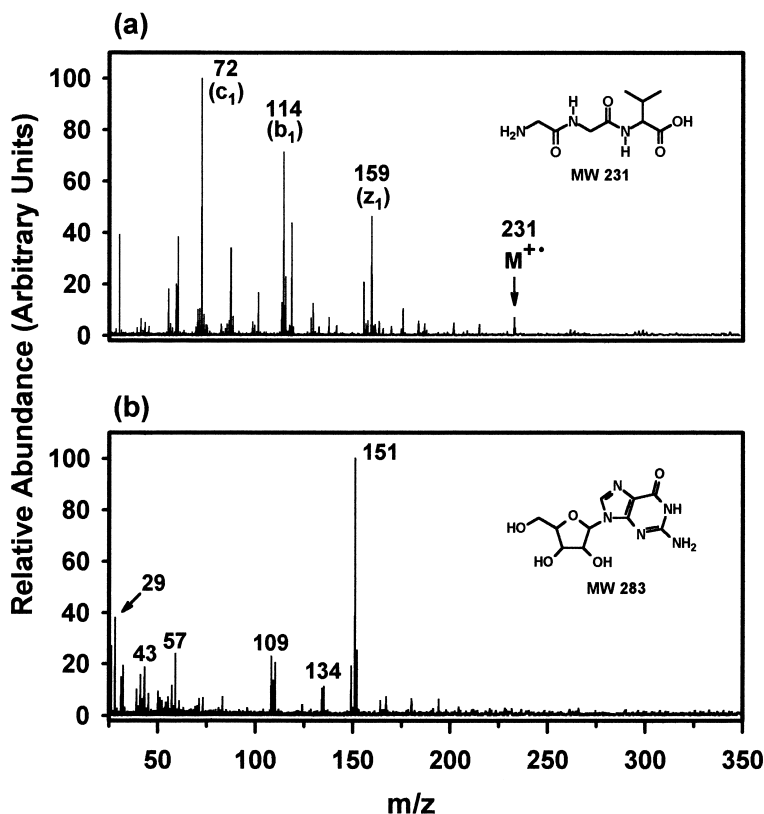


Fig. 3. LIAD/EI mass spectra of (a) gly-gly-val and (b) guanosine. The major peaks observed in the spectrum (a) correspond to those typically formed upon fragmentation of peptides [45]. The major peaks in spectrum (b) are in agreement with EI mass spectra of guanosine presented in the literature [39]. The major peak in (b) ( $m/z$  151) corresponds to the guanine radical cation, which results from dissociation of the glycosidic bond in guanosine radical cation.

ployed a 2.5  $\mu\text{m}$  copper foil, LIAD of brilliant green bisulfate yielded detectable ion signals. This finding demonstrates that LIAD from thin copper foils ( $\approx 5 \mu\text{m}$ ) results in decomposition of the samples from sample overheating by the laser.

### 3.2. Postdesorption electron ionization

All analytes desorbed by LIAD were subjected to 70 eV electron ionization (LIAD/EI). One laser pulse was sufficient to generate relatively intense signals for all of the low-mass analytes studied (i.e., molecules of  $\text{MW} \leq 1000$ ) (for example, see Figs. 3 and 4). The LIAD/EI spectra obtained compare favorably with thermal desorption/EI mass spectra available in the literature, as demonstrated by the comparison shown

in Fig. 4 for *trans*-estriol [39]. It is concluded that the number of optoacoustically desorbed molecules is large enough for efficient ionization by electron bombardment, and that the translational energy of the molecules is low enough to allow effective trapping of the ions resulting from electron ionization.

Volatilization of the high-mass analytes (oxytocin, angiotensin I, polyisobutylene 1600, and polystyrene 2500) was readily achieved by LIAD. However, electron ionization of these compounds did not yield detectable ions in the FT-ICR. Similar results were obtained when these molecules were subjected to chemical ionization by several reagent ions, such as protonated aniline, protonated acetone, and carbon disulfide radical cation. We are currently investigating alternative ways of sample deposition and ion trap-

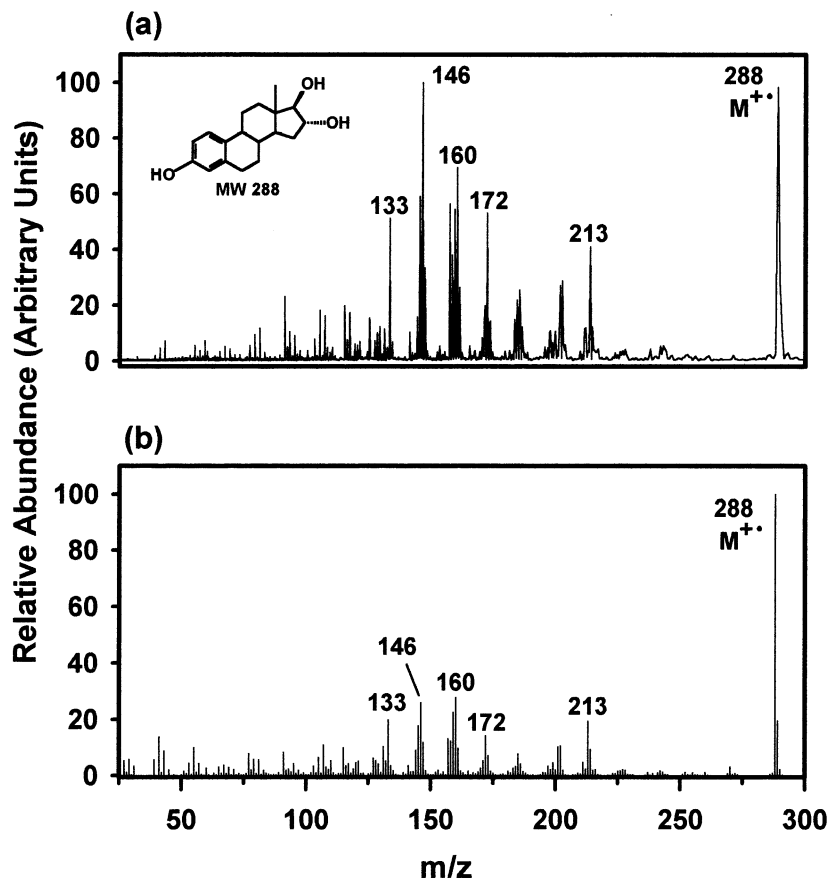


Fig. 4. (a) LIAD/EI mass spectrum of *trans*-estriol (MW 288). (b) A *trans*-estriol mass spectrum reproduced from data obtained from the NIST mass spectral libraries [39].

ping for these substances, as LIAD of high-mass analytes may result in the ejection of particles and clusters [7] instead of molecules.

### 3.3. Postdesorption chemical ionization

Under normal operating conditions, the number of ionizing electrons during EI (70 eV, 6  $\mu$ A, 1 ms) is five orders of magnitude greater than the maximum number of reagent ions that can be simultaneously stored in the FT-ICR cell. The typical ionization cross sections for EI and CI are comparable ( $\sim 10^{-15}$  cm<sup>2</sup>) [40]. Therefore, a greater number of desorbed molecules will be necessary for CI than EI to achieve a given ion signal. Speir and Amster suggested that

laser-based desorption of at least 100 pmol of neutral analyte is necessary to produce useful signals (signal-to-noise (S/N)  $\sim 10$ ) upon proton transfer chemical ionization in FT-ICR [26].

The number of molecules desorbed per laser shot upon LIAD was estimated by monitoring the relative abundance of the ions resulting from LIAD/EI of  $\alpha$ -cyano-4-hydroxycinnamic acid at one irradiation spot over a series of consecutive laser shots (irradiation intensity:  $\sim 10^9$  W/cm<sup>2</sup>). In this experiment, sample coverage was about 75 nmol/cm<sup>2</sup> and the irradiation area  $10^{-3}$  cm<sup>2</sup>. Consistent and reproducible signal intensities were observed for up to 60 laser shots before the signal decreased sharply upon sample depletion. Thus, approximately 1.4 pmol of  $\alpha$ -cyano-



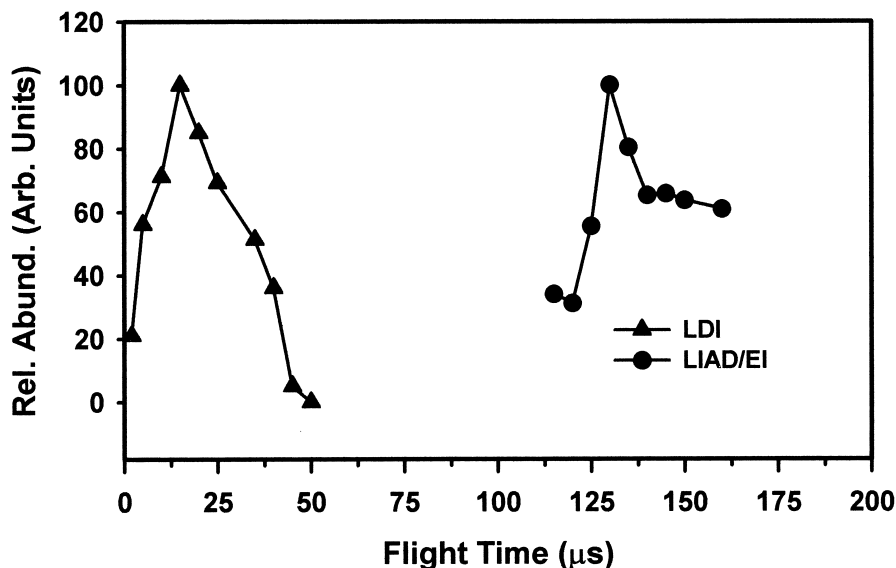


Fig. 5. Flight time of  $\alpha$ -cyano-4-hydroxycinnamate from the sample stage to near the center of the ICR cell. The ions were generated by direct laser irradiation of neat deposits of  $\alpha$ -cyano-4-hydroxycinnamic acid LDI or by electron ionization of the neutral acid desorbed by LIAD (LIAD/EI).

4-hydroxycinnamic acid was volatilized per laser pulse. Hence, approximately 75 laser pulses are necessary to achieve the  $\sim 100$  pmol threshold suggested for CI by Speir and Amster [26]. To achieve this, multiple spots must be irradiated at the metal foil.

Chemical ionization of molecules that are desorbed by direct laser irradiation (LD/CI) is complicated by their typically high velocities ( $\sim 300$ – $1200$  m/s) [41], which cause a reduction in the capture collision rate, and hence reduce the ionization efficiency. As mentioned above, Amster et al. compensated for this by performing LD/CI with an excess of stored reagent ions in a FT-ICR [25]. Other LD methods (i.e., substrate-assisted laser desorption) appear to generate gas-phase molecules with lower kinetic energies, which result in higher efficiency for CI under normal FT-ICR operating conditions [26].

We assessed the velocities of neutral molecules evaporated by LIAD and by LD in the FT-ICR by adapting [30] a previously reported gated ion trapping technique [42–44]. The mean forward velocity of the desorbed molecules is related to the optimum gated trapping time, i.e., the time necessary for the greatest population of ions (determined by the absolute inten-

sity of the measured ion signal) to arrive to the center of the ICR cell after desorption [30]. The gated trapping time was measured for  $\alpha$ -cyano-4-hydroxycinnamic acid (MW 189) desorbed by LIAD and subsequently ionized by EI. The results were compared to those obtained for  $\alpha$ -cyano-4-hydroxycinnamate ( $m/z$  188) evaporated and ionized by direct laser irradiation (LDI) of the neat deposit (Fig. 5) [30]. The data demonstrate significantly lower velocities (and hence, lower kinetic energies) for molecules desorbed by LIAD than for the analogous ions that are desorbed by direct laser irradiation. Based on these measurements, an increase in the chemical ionization efficiency can be expected for LIAD as compared to LD. As demonstrated below, this feature of acoustic desorption is key for the success in coupling LIAD to chemical ionization.

We chose  $\alpha$ -cyano-4-hydroxycinnamic acid (a commonly used MALDI matrix) as the first analyte to be studied by LIAD/CI because of its ease of handling. The sample coverage was about  $75$  nmol/cm<sup>2</sup>, and the back surface of the metal was irradiated by 100 laser pulses that were applied at multiple spots over a total irradiation area of  $3 \times 10^{-2}$  cm<sup>2</sup>. Ionization of the evaporated molecules by protonated meth-

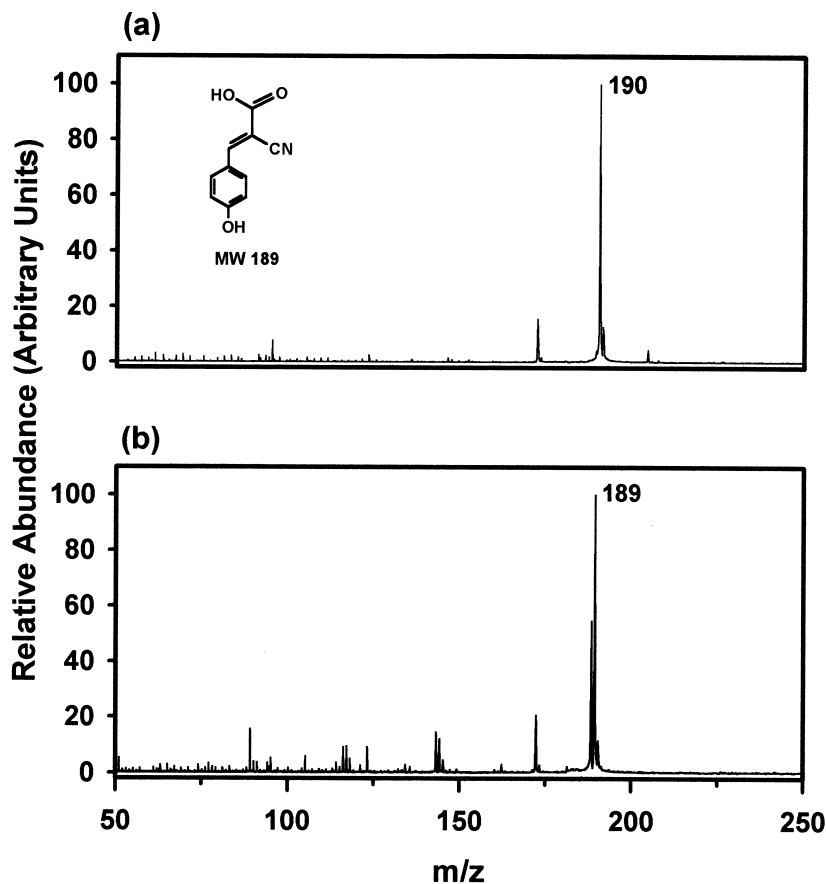


Fig. 6. LIAD/CI of  $\alpha$ -cyano-4-hydroxycinnamic acid (MW 189) (a). Chemical ionization was achieved by reaction of the desorbed neutral molecules with protonated methanol. (b) Electron ionization (70 eV, 6  $\mu$ A) mass spectrum of LIAD desorbed  $\alpha$ -cyano-4-hydroxycinnamic acid.

anol resulted in a large signal intensity for the protonated  $\alpha$ -cyano-4-hydroxycinnamic acid with little fragmentation (Fig. 6(a)). The experiment was reproducible from spot to spot, sample to sample, and day to day. Therefore, the feasibility of LIAD/CI was demonstrated. For comparison, Fig. 6(b) shows the LIAD/EI (one laser pulse) spectrum of  $\alpha$ -cyano-4-hydroxycinnamic acid (same sample coverage). The base peak in the LIAD/EI spectrum corresponds to the molecular ion ( $M^+$ ). Hence, both LIAD/CI and LIAD/EI experiments indicate desorption of intact analyte molecules into the mass spectrometer.

In general, it was found that the conditions discussed above for LIAD/CI were necessary to obtain useful signals ( $S/N \geq 10$ ). Under these conditions,

large signals were measured upon LIAD/CI (proton transfer) for cytosine,  $\alpha$ -cyano-4-hydroxycinnamic acid, sinapinic acid, gly-gly-val, thymidine, cytidine, guanosine, *trans*-estriol, val-ala-ala-phe, and polyethylene glycol 610. The additional experiments discussed below illustrate several performance factors of the LIAD/CI technique. (1) LIAD/CI of the tetrapeptide val-ala-ala-phe demonstrates the applicability of the technique to thermally labile compounds. The same analyte was used to compare the performance of copper and titanium foils. (2) LIAD of thymidine followed by ionization by two different protonation reagents with different acidities shows that the energetics of the ionization step can be controlled in LIAD/CI by choice of the reagent ion. (3) Finally, a

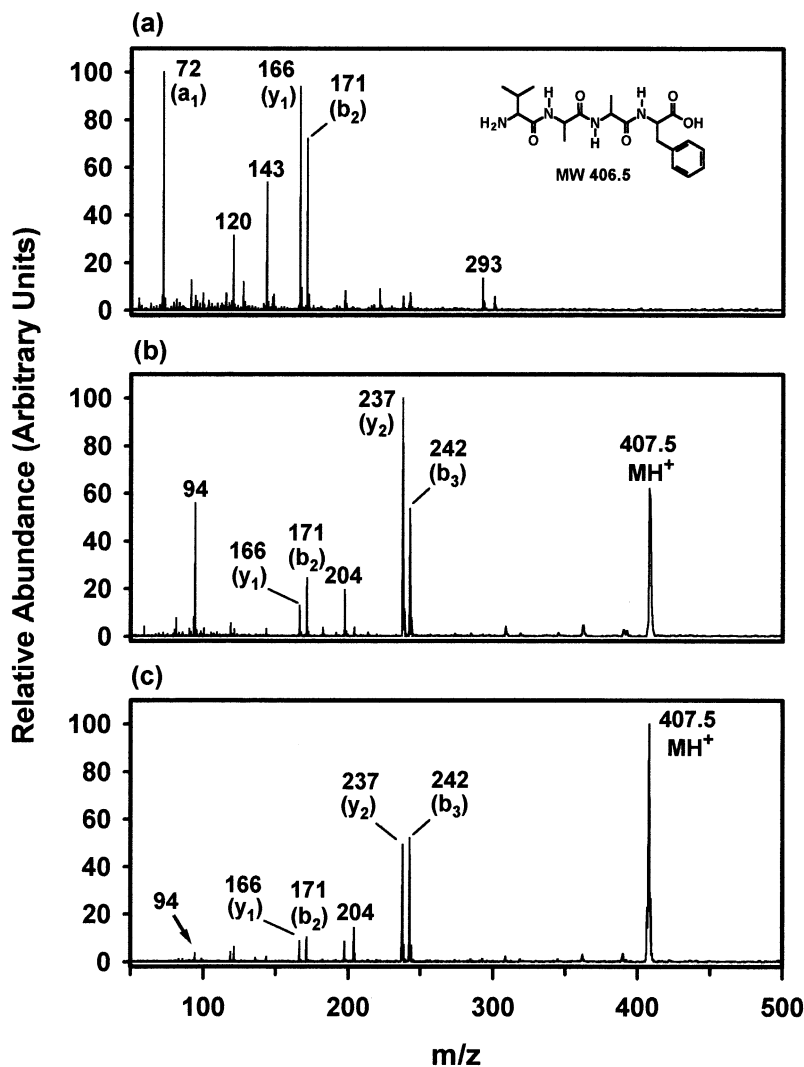


Fig. 7. LIAD of the tetrapeptide val-ala-ala-phe (MW 406.5) ionized by (a) 70 eV electron ionization and (b) chemical ionization by reaction with protonated aniline ( $m/z$  94). (c) The same experiment as in (b) was conducted by using titanium foil instead of copper.

different ionization mode than proton transfer was employed to ionize neutral d-Ribose evaporated by LIAD as well as thermal desorption.

### 3.3.1. Val-ala-ala-phe

The tetrapeptide val-ala-ala-phe (MW 406.5) was evaporated by LIAD and subsequently ionized either by electron ionization [Fig. 7(a)] or by proton transfer (Figs. 7(b),7(c)). The LIAD/EI spectrum [Fig. 7(a)] is the result of a single laser pulse. The peaks in the

spectrum can be rationalized based on known fragmentations of ionized peptides [45]. In a separate experiment, the same peptide was introduced into the FT-ICR via a heated solids probe and subjected to 70 eV electron ionization. This spectrum indicates partial decomposition of the peptide presumably caused by the heating process. Hence, the spectrum in Fig. 7(a) is likely to be the first demonstration of an electron ionization mass spectrum of the intact val-ala-ala-phe.

The LIAD/CI spectra in Figs. 7(b) and 7(c) re-

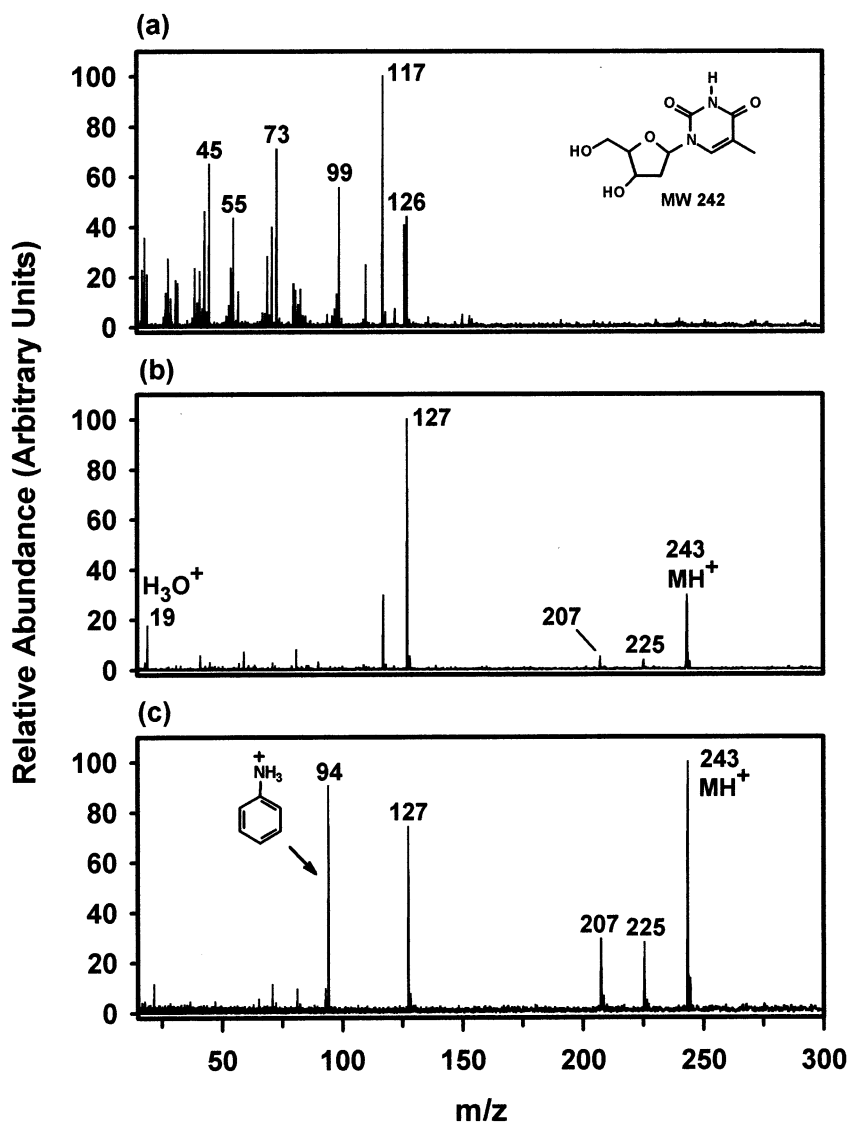


Fig. 8. LIAD of thymidine (MW 242) followed by (a) 70 eV electron ionization, and by (b) proton transfer from the hydronium ion and (c) from the anilinium ion.

sulted from 500 laser pulses applied to copper and titanium metal foil, respectively (total irradiation area  $\sim 3 \times 10^{-2} \text{ cm}^2$ ). Ionization was accomplished by proton transfer from the anilinium ion. Protonation of val-ala-ala-phe with the anilinium ion is about 12 kcal/mol exothermic (the proton affinity of the peptide was estimated by semiempirical calculations to be  $\sim 223 \text{ kcal/mol}$ ; proton affinity of aniline [46] =

210.9 kcal/mol). Therefore, some fragmentation of the protonated peptide molecules was expected and observed. However, a major peak corresponding to the protonated peptide is present in each spectrum. The fragment ion peaks are analogous to those observed for protonated peptide molecules upon collision-activated dissociation [47]. This study suggests that LIAD provides a feasible means to evaporate

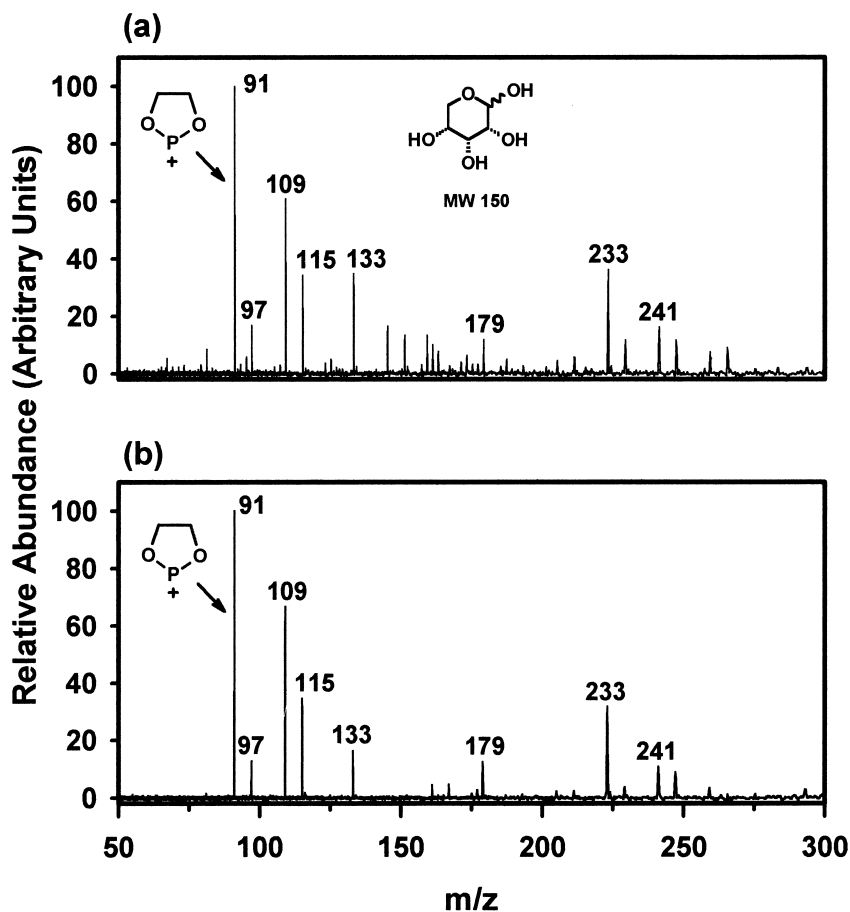


Fig. 9. Ribose (MW 150) was evaporated by (a) LIAD and (b) thermal desorption. Desorbed neutral ribose was allowed to react with the 1,3-dioxolane-2-phosphonium ion ( $m/z$  91). The product ions result from complex addition/elimination pathways rather than simple atom or group transfer reactions (see text for details).

intact neutral oligopeptides for mass spectrometric analysis.

The val-ala-ala-phe experiment was employed to compare the performance of 10  $\mu\text{m}$  thick copper and titanium foils. Titanium was of interest to us because it has a similar absorption coefficient toward 532 nm light, but a lower thermal conductivity and a higher expansion coefficient than copper [48]. Therefore, better transmission of the laser-induced acoustic wave was expected for titanium [7,49]. Also, this metal is likely to transfer less thermal energy to the sample layer upon irradiation with the laser. The spectrum of val-ala-ala-phe in Fig. 7(c) obtained by using titanium

Table 1

Product distributions measured for 1.5 s reaction of 1,3-dioxolane-2-phosphonium ion with thermally desorbed neutral D-ribose that was introduced into the source cell at a pressure of  $2 \times 10^{-8}$  Torr

Product ( $m/z$ )	Branching ratio (%)	Reactivity
97	7	$\text{OH}^-$ abstraction, $2 \times \text{H}_2\text{O}$ loss
109	35	$\text{H}_2\text{O}$ abstraction
115	22	$\text{OH}^-$ abstraction, $\text{H}_2\text{O}$ loss
133	12	$\text{OH}^-$ abstraction
179	10	Addition-( $\text{HOCH}_2$ ) <sub>2</sub>
223	13	Addition- $\text{H}_2\text{O}$
241	2	Addition

foil shows somewhat less intense fragment ion peaks and more intense protonated tetrapeptide peak than that obtained by using copper foil. This observation suggests that LIAD from titanium foil may result in the generation of cooler gas phase neutral molecules than by using copper. However, it should be noted that these results are preliminary and that further studies are needed.

### 3.3.2. Thymidine

Fig. 8 shows mass spectra of thymidine (MW 242) evaporated by LIAD and ionized by electron impact (Fig. 8(a)) or by transfer of a proton from either the hydronium (Fig. 8(b)) or the anilinium ion (Fig. 8(c)). As before, the LIAD/EI spectrum resulted from one laser pulse, while the LIAD/CI spectra resulted from application of 200 laser pulses. The EI spectrum (Fig. 8(a)) is similar to thymidine EI mass spectra presented in the literature [39]. The two main peaks in the LIAD/CI spectra (Figs. 8(b), 8(c)) correspond to protonated thymidine ( $m/z$  243) and protonated thymine ( $m/z$  127). The latter is the charged product of cleavage of the glycosidic bond in protonated thymidine likely induced by the exothermicity of the proton transfer reaction. The two minor products observed at  $m/z$  207 and  $m/z$  225 correspond to consecutive water losses from protonated thymidine. Protonation of thymidine with the hydronium and anilinium ions is 62 and 16 kcal/mol exothermic, respectively [46]. Comparison of the spectra reveals that the extent of fragmentation of the analyte ions is greater when the more aggressive acid, hydronium ion, was used as the CI reagent. Hence, the energetics of the ionization process can be controlled by the LIAD/CI method. Similar capabilities have been demonstrated previously for chemical ionization of neutral molecules desorbed by direct laser irradiation methods (LD/CI) [28,50].

### 3.3.3. Ribose

The spectrum shown in Fig. 9 provides an example of LIAD/CI where ionization is effected by a reaction other than simple proton transfer. This spectrum resulted from reaction between acoustically desorbed

ribose (MW 150) and the 1,3-dioxolane-2-phosphonium ion ( $m/z$  91). Phosphonium ions have been studied in our laboratory for their use as novel chemical ionization reagents that can target, for example, hydroxyl groups in gaseous neutral substrates [51]. Indeed, hydroxide abstraction from ribose results in the product ion of  $m/z$  133 in the spectrum. Other peaks in the spectrum correspond to product ions that result from addition/elimination pathways. For example, the product ion of  $m/z$  223 results from addition of ribose to the 1,3-dioxolane-2-phosphonium ion, followed by elimination of a water molecule. The same experiment was performed for thermally desorbed ribose (Fig. 9(b)). Both spectra (Figs. 9(a) and 9(b)) contain the same peaks ( $m/z$  97, 109, 115, 133, 179, 223, 241) at about the same relative intensities (see Table 1). It is concluded that the collision complex formed between trapped reagent ions and acoustically desorbed neutral molecules is long lived enough to undergo multistep reactions.

## 4. Conclusions

Laser-induced acoustic desorption from metal foils has been demonstrated to successfully volatilize large amounts of intact, thermally labile molecules into an FT-ICR mass spectrometer. Furthermore, the energetics of the acoustic desorption process have been determined to be well suited for chemical ionization experiments. LIAD coupled to postdesorption chemical or electron ionization results in strong, reproducible signals for a wide variety of compounds, including sugars, steroids, oligopeptides, nucleosides, and synthetic polymers. The physical separation of the evaporation (LIAD) and ionization (EI or CI) events allows the use of a variety of additional ionization reactions (e.g., group transfer reactions, addition/elimination) than desorption/ionization methods such as MALDI (proton and electron transfer). Furthermore, segregation of the desorption and ionization steps allows independent control of each process. However, this method appears to be less sensitive than MALDI. Ongoing studies in our laboratory are



aimed at understanding the size dependence of LIAD. These studies will also contribute to the present understanding of the laser-induced acoustic desorption process.

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