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Experimental Investigations of the Internal Energy of Molecules Evaporated via Laser-induced Acoustic Desorption into a Fourier-transform Ion Cyclotron Resonance Mass Spectrometer (LIAD/FT-ICR)

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Abstract

The internal energy of neutral gas-phase organic and biomolecules, evaporated by means of laser-induced acoustic desorption (LIAD) into a Fourier-transform ion cyclotron resonance mass spectrometer (FT-ICR), was investigated through several experimental approaches. The desorbed molecules were demonstrated not to undergo degradation during the desorption process by collecting LIAD-evaporated molecules and subjecting them to analysis by electrospray ionization/quadrupole ion trap mass spectrometry. Previously established gas-phase basicity (GB) values were remeasured for LIAD-evaporated organic molecules and biomolecules with the use of the bracketing method. No endothermic reactions were observed. The remeasured basicity values are in close agreement with the values reported in the literature. The amount of internal energy deposited during LIAD is concluded to be less than a few kcal/mol. Chemical ionization with a series of proton transfer reagents was employed to obtain a breakdown curve for a protonated dipeptide, val-pro, evaporated by LIAD. Comparison of this breakdown curve with a previously published analogous curve obtained by using substrate-assisted laser desorption (SALD) to evaporate the peptide suggests that the molecules evaporated via LIAD have less internal energy than those evaporated via SALD.

INTRODUCTION

The use of mass spectrometry for the analysis of nonvolatile and thermally fragile analytes (*e.g.*, peptides and oligonucleotides) has experienced increased popularity over recent years, in part due to the development and increased understanding of a variety of sample introduction techniques. Techniques such as matrix-assisted laser desorption/ionization (MALDI), laser desorption/ionization (LDI) and electrospray ionization (ESI) have proven to be very useful for the analysis of large biomolecules.^{1–4} However, due to the coupling of the desorption and ionization processes in MALDI and ESI, ionization is limited to proton attachment/detachment processes. This hinders the analysis of analytes without basic or acidic functional groups, such as hydrocarbon polymers. LD offers improvement in this regard since it evaporates predominantly neutral molecules³ (as well as ions), thus allowing a variety of post-desorption ionization methods to be employed, such as electron impact,⁵ photoionization,³ and chemical ionization.⁶ However, molecular weight information can be difficult to obtain when using electron impact ionization due to excessive analyte fragmentation. Although LD-

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photoionization has been successfully applied to the analysis of a variety of analytes,³ ionization of the analyte can be challenging due to the required resonance tuning of the frequency of the ionizing laser with a specific analyte transition. The coupling of LD with CI has been successfully performed,^{7,8} however, difficulties in obtaining molecular ion signals for larger molecular weight species⁸ prompted the development of new desorption techniques, such as substrate-assisted laser desorption⁹ (SALD) and laser-induced acoustic desorption.¹⁰

The low-energy desorption technique SALD was developed by Amster and coworkers⁹ to improve the mass range and applicability of LD/CI for analysis of thermally labile analytes in FT-ICR mass spectrometers. SALD is performed by depositing the analyte on top of a thin layer of UV-absorbing organic substrate (*i.e.*, sinapinic acid). Intact neutral molecules are evaporated from the surface and ionized by CI in an FT-ICR mass spectrometer. The desorption wavelength was optimized for absorption by the organic substrate in order to minimize the degree of analyte fragmentation upon desorption. Analytes evaporated with the aide of the absorbing substrate have been reported to have approximately 1 eV less internal energy than those desorbed by direct LD. In order to obtain additional information on the energy deposited into the evaporated molecules,¹¹ Speir and Amster determined experimental breakdown curves for several peptides desorbed via SALD and ionized by protonation. By using Brønsted acids with different acidities to protonate the peptides, various degrees of fragmentation was induced based on the different exothermicities of the protonation reactions. The internal energy of the peptides desorbed via SALD was estimated by comparing these experimental breakdown curves to calculated curves.¹¹ The results suggest that only a small amount of internal energy is transferred into the peptides when evaporated by SALD. Additionally, it was inferred through increased capture collision efficiencies that SALD-evaporated analytes have less kinetic energy than molecules desorbed with direct LD.⁹ The SALD technique is effective for the analysis of a variety of small peptides. However, obtaining ion signals for large MW analytes can be difficult. Additionally, stringent sample preparation conditions¹² are required for both small and large MW analytes (*i.e.*, analyte and substrate layers' thickness, spray parameters, etc.). Hence, the usage of SALD has been limited, prompting the development of new desorption methods.

Another technique, laser-induced acoustic desorption (LIAD),^{10,13,14} also allows the evaporation of intact neutral molecules with relatively low kinetic energies. This technique involves depositing a thin layer of analyte material (20 to 200 nmol/cm²) onto a thin (12.7 μm) Ti foil. The foil is mounted onto a specially designed probe and inserted into an FT-ICR mass spectrometer. The backside of the foil is irradiated with a series of short (3 ns) laser pulses (532 nm), resulting in the propagation of an acoustic wave through the foil which evaporates neutral analytes from the opposite side of the foil. After desorption, the neutral molecules can be ionized by a variety of ionization methods, including electron impact (EI) and chemical ionization (CI). LIAD has been successfully applied to the mass spectrometric analysis of a wide variety of analytes, including peptides,^{10,15} dinucleoside phosphates,¹⁶ saturated hydrocarbon polymers^{17,18} and petroleum distillates.¹⁹

An overall experimental characterization of the LIAD technique has been performed.^{10,14,20} However, a more detailed examination of the energies of LIAD evaporated molecules is needed. Initial studies^{10,13} of the application of LIAD to evaporate molecules for mass spectrometric analysis in an FT-ICR provide strong evidence that degradation does not occur during vaporization. This was initially indicated by the absence of protonated degradation products and the dominant presence of the protonated intact analyte (ionization via near-thermoneutral proton transfer) in these experiments. Further, examination of the reactivity of thermally desorbed thymine, the major degradation product of thymidine, toward N-phenyl-3-dehydropyridinium ion demonstrated that addition followed by methyl group elimination readily occurs for the nucleobase. This product was not observed in the reaction with

thymidine¹⁵ desorbed via LIAD, indicating that thymidine did not degrade to thymine upon desorption. Additionally, significantly different reactivity was observed for the LIAD evaporated dipeptide, ala-ala, and its major degradation product, anhydrous ala-ala, when allowed to react with the dichlorophosphonium ion (PCl_2^+).¹³ In particular, the most abundant product ion of m/z 126 from the PCl_2^+ reaction with the dehydrated peptide was not observed in the spectra measured for the intact peptide.¹³ However, this product (m/z 126) is formed for the thermally desorbed peptide when using higher desorption temperatures, thus confirming that degradation can occur under high temperature thermal evaporation conditions.

A series of velocity distribution measurements¹⁰ carried out for neutral molecules evaporated by LIAD and ionized via EI demonstrated that the translational energies of these molecules are low. These energies were determined to be lower than those of ions evaporated by direct laser desorption techniques, such as reflection and transmission geometry laser desorption.^{10,21}

Presented here is a characterization of the amount of internal energy deposited into LIAD-evaporated analytes upon desorption. Through several experimental approaches, including analyzing LIAD-evaporated material by ESI mass spectrometry, remeasuring previously established thermochemical values for LIAD-evaporated analytes, and comparison of an experimental breakdown curve for a LIAD-evaporated peptide with a previously published curve generated for the peptide desorbed by SALD, it is concluded that LIAD evaporated analytes contain a minimal amount of internal energy.

EXPERIMENTAL

Mass Spectrometers

Two similar Fourier-transform ion cyclotron resonance mass spectrometers (FT/ICR) were used in the work presented here. Both instruments have been described previously.^{17,22} The gas-phase bracketing experiments and LIAD/CI analysis of mixtures were performed in an Extrel Model FTMS 2001 dual cell FT/ICR mass spectrometer. This instrument contains a differentially pumped dual cell placed within a superconducting magnet of approximately 2.8 Tesla. A nominal base pressure of 10^{-9} Torr was maintained by two Balzers turbomolecular pumps (330 L/s), each backed with an Alcatel rotary-vane mechanical pump. The experiments used to generate the breakdown curve were performed using an Nicolet Model FTMS 2000 dual cell FT/ICR mass spectrometer. The differentially pumped dual cell is positioned within the magnetic field produced by a 3.0 Tesla superconducting magnet. A nominal base pressure of less than 10^{-9} Torr was maintained with two Edwards Diffstak 160 diffusion pumps (700 L/s), each backed with an Alcatel rotary-vane mechanical pump. Both instruments utilize two Bayard-Alpert ionization gauges located on either side of the dual cell to monitor the pressures within the mass spectrometer. In each instrument, the cells are separated by a common wall (the conductance limit) which contains a 2 mm hole in the center. Unless otherwise noted, this plate and the other two trapping plates were maintained at +2 V.

LIAD Probes

Both mass spectrometers have specially designed LIAD probes used for volatilization of neutral molecules into the mass spectrometer. Both instruments were configured with a specially made guide ring for positioning of the LIAD probe with respect to the center of the ICR cell, as described previously.^{10,14,20,21} Desorption is performed by focusing the laser beam of a Nd:YAG laser (532 nm, 3 ns pulse width) through an optical fiber (365 μm diameter, 3M Specialty Fibers, West Haven, CT) onto the back side of a Ti metal foil secured on the probe tip, as previously described.^{10,14} The two transmission geometry probes are of different diameters (1/2" and 7/8"). However, both utilize 1:1 imaging optics to focus the laser beam

from the optical fiber onto the backside of the foil. The 1/2" diameter probe²¹ was utilized for the assessment of the internal energy of acoustically desorbed molecules via the bracketing method. The larger 7/8" diameter probe^{13,23} was utilized for the internal energy assessment through generation of an experimental breakdown curve as per the work performed by Speir and Amster.¹¹

The two probes differ in the alignment of the desorption plume relative to the permanent magnetic field axis of the mass spectrometer, as described previously.¹⁴ The center of the 1/2" outer diameter probe is in alignment with the axis of the magnetic field of the instrument.¹⁰ Due to the size constraints of this probe and to enable multiple desorption sites per foil, the probe was designed with all desorption sites equidistant (1.3 mm) from the center of the ICR cell. A new sample desorption site is obtained by rotating the inner cylinder of the probe (containing the optical fiber and focusing optics) in 18° increments to obtain 22 non-overlapping sites, each with an area of 10⁻³ cm², while the outer cylinder containing the sample foil is held stationary. The 7/8" diameter laser probe^{13,23} enables the desorption of 103 discrete, non-overlapping irradiation sites, each with an area of 10⁻³ cm². This probe contains a stationary inner cylinder. Molecules are desorbed into the center of the cell and on-axis with the magnetic field of the instrument. A new sample desorption site is obtained by rotating the outer cylinder containing the sample foil into the fixed desorption axis. The inner cylinder contains the terminated fiber optic and 1:1 imaging optics which are held stationary and centered on the magnetic field axis of the instrument. Both laser probes are inserted into the instrument to within 1/8" of the source trapping plate.

Although the two probes are slightly different in their diameter, total sampling areas and desorption position (relative to the center of the ICR cell and the magnetic field axis), both probes have been shown to be useful in desorbing neutral, non-volatile molecules into the mass spectrometer.^{14,20} Alignment of the probe directly on or slightly off the magnetic field axis only influences the magnitude of ion signal generated. Increased sensitivity of LIAD analyses is achieved with on-axis desorption due to maximum overlap between the desorption cloud of neutral molecules and the stored reagent ions in the center of the ICR cell.¹⁴

All compounds were obtained from Aldrich Chemical Co. (St. Louis, MO) with the exception of methane and nitrous oxide gases which were obtained from BOC Gases (Murray Hill, NJ), and the peptide, Val-pro, which was obtained from Bachem Biosciences (King of Prussia, PA). The titanium foil (12.5 μm) was obtained from Alfa Aesar (Ward Hill, MA). All reagents and metal foils were used as received from the supplier without further purification. Samples were prepared by electrospray deposition of the analyte (~30 μL of an approx. 10 mM solution in methanol) onto the front side of the Ti foil, as described previously,²⁴⁻²⁶ to obtain sample coverage of ~120 nmol/cm² on the metal surface. Following sample preparation, the sample foil was secured to the sample support stage of a laser desorption probe. The back side of the foil, opposite to which the sample was deposited, was placed on a 200 μm thick protective glass cover. Desorption of the analyte was achieved with a series of single laser pulses from a Nd:YAG laser (Minilite II, Continuum, Santa Clara, CA) with a power density (on the backside of the foil) of ~9.0 × 10⁸ W/cm². Higher laser irradiation powers were not utilized due to possible damage to the optical fiber.

Solid, liquid and gaseous samples were introduced into one side of the mass spectrometers through a Varian variable leak valve and a batch inlet equipped with an Andonian leak valve (nominal cell pressure 6.0 × 10⁻⁸ Torr). In order to obtain the protonated reagent molecule, electron ionization (70 eV electron energy, 5–10 μA emission current) of the neutral reagent followed by Fself-chemical ionization" (2–5 s) was performed which resulted in an abundance of the desired protonated reagent, [M+H]⁺. The desired chemical ionization reagent ions were transferred into the adjacent "clean" cell by grounding the conductance limit plate for

approximately 50 – 150 μ s. Following transfer, the ions were cooled radiatively²⁷ (IR) and collisionally for $\sim$ 1 s with Ar pulsed into the cell (peak pressure $\sim 10^{-5}$ Torr). The ions were isolated by ejecting all unwanted ions from the cell through the application of a series of stored-waveform inverse Fourier-transform^{28,29} (SWIFT) excitation pulses to the excite plates of the cell (Extrel SWIFT Module). The isolated reagent ions were allowed to react with the neutral molecules evaporated by LIAD. After reaction, a broadband chirp was used to excite the ions for detection (1.9 kHz to 2.6 MHz, 200 V peak-to-peak, sweep rate 3200 Hz/ μ s). The time domain data were averaged, baseline corrected, and subjected to Hanning apodization followed by one zero-filling. Frequency spectra were obtained by Fourier-transformation of the data with magnitude adjustment. All data were obtained by collecting 64 k data points with an acquisition rate of 8000 kHz.

To evaluate sample degradation upon LIAD, 150 μ L of a methanol solution of dApdApdA (1 mg/mL) was electrospray deposited^{24–26} onto a Ti foil and placed on the end of the LIAD probe in the usual manner. Instead of inserting the probe into the instrument for mass analysis, it was inserted into a clean, dry glass vial, and the material was desorbed into the vial from the foil with multiple laser shots (approx. 200 shots per foil). Sufficient trinucleotide material for detection was obtained by evaporating the material from approximately 30 foils into the sample vial. The desorbed dApdApdA was dissolved in methanol. A blank sample of methanol was used to identify any impurities in the solvent. Fresh dApdApdA, also dissolved in methanol (1 mg/mL), was used as a reference. The reference trinucleotide solution, the methanol blank and the solution containing the LIAD evaporated dApdApdA material were analyzed in the negative ion mode by using electrospray ionization (ESI) on a FinniganMAT LCQ (ThermoFinnigan Corp, San Jose, CA) mass spectrometer system. The electrospray needle voltage was set at 4.0 kV, the heated capillary voltage was set to 10 V and the capillary temperature was 210 $^{\circ}$ C. The sample flow rate was approximately 8 μ L per minute. Typical background and operation source pressure was 1.2×10^{-5} torr as read by a Granville-Phillips ion gauge (Boulder, CO). Nitrogen was used as the drying gas. The LCQ was scanned from 50 to 2000 amu for these experiments.

In order to generate protonated nitrous oxide (N_2OH^+), a mixture of nitrous oxide and methane (each at an approximate cell pressure of $\sim 6 \times 10^{-8}$ Torr) was leaked into one side of the instrument. Electron ionization of methane to yield CH_3^+ (70 eV electron energy, 12 μ A emission current, 0.95 s) which reacts with methane to yield protonated methane and protonated ethene was used to generate protonated nitrous oxide in a reaction time of 0.5 s. Protonated ethene (C_2H_5^+) was generated by electron ionization (70 eV electron energy, 12 μ A emission, 50 ms) of pure methane gas (nominal cell pressure $\sim 6 \times 10^{-8}$ Torr) followed by 2.5 s reaction time during which one of the major fragments (CH_3^+) reacts with neutral methane within the cell to generate C_2H_5^+ and hydrogen (CH_5^+ is also formed), according to a well established reaction pathway.³⁰

RESULTS AND DISCUSSION

A series of experimental approaches involving a variety of thermally labile molecules were utilized to obtain information on the amount of internal energy deposited into LIAD-evaporated molecules upon desorption. Each approach is discussed separately below.

Degradation Upon LIAD

Evaluation of the structural integrity of a trinucleotide, dApdApdA, following evaporation via LIAD was performed. A comparison of the LIAD-evaporated trinucleotide with the original trinucleotide sample solution was made by analysis using negative ion ESI in a quadrupole ion trap (QIT) mass spectrometer. The spectra obtained (Figure 1) are nearly identical, with the base peak in both spectra corresponding to the deprotonated molecule ($[\text{M-H}]^-$, m/z 876).

These results indicate that the trinucleotide dApdApdA was desorbed as intact molecules, and further demonstrate that degradation does not occur upon desorption with LIAD.

Estimation of Internal Energies via Gas-Phase Basicity (GB) Bracketing Experiments

One approach used to characterize the internal energy of LIAD desorbed molecules was to re-measure previously determined gas-phase thermochemical values for molecules vaporized with the use of LIAD. The well-established technique of bracketing allows a relative measurement of thermochemical values and therefore can provide semi-quantitative information on the amount of internal energy introduced into the molecules through the desorption process.^{31–34} The bracketing method is typically used to obtain an approximate range for a thermochemical value before utilization of more quantitative techniques, such as the Cooks kinetic method³⁵ or equilibrium methods.³⁴ The use of more quantitative techniques is not feasible for this particular study due to the use of nonvolatile and/or thermally labile molecules.

Three common MALDI matrices (4-hydroxy- α -cyanocinnamic acid (4HCCA), 2,5-dihydroxybenzoic acid (DHB) and 1,8,9-trihydroxyanthracene (dithranol)), two peptides (Ala-ala and Val-pro) and a nucleoside (thymidine), all with well-established gas-phase basicities (GB) were desorbed by LIAD and allowed to react with reference acids in the ICR cell (Table 1). If proton transfer was observed, the GB of the desorbed molecule was concluded to be greater than that of the conjugate base of the reference acid while no reaction indicated a lower GB. If the desorbed neutral molecules contain excess internal or kinetic energy due to the desorption process, endothermic reactions may be observed.

As predicted based on the known thermochemical values, the reaction of Val-pro (GB = 224.1 kcal/mol) with protonated triethylamine reference acid (GB = 227.0 kcal/mol) does not occur due to the endoergicity ($\Delta G_{rxn} = 2.9$ kcal/mol) of the reaction. The absence of the protonated analyte molecule and fragment ions indicates that the gas-phase basicity of Val-pro is less than that of the conjugate base of the reference ion. On the other hand, the reaction of Val-pro (GB = 224.1 kcal/mol) with protonated 3,5-lutidine (GB = 220.7 kcal/mol) occurs via proton transfer ($\Delta G_{rxn} = -3.4$ kcal/mol). No fragmentation of the protonated molecule was observed. The bracketed³⁶ GB value for Val-pro (224 kcal/mol) is in agreement with the previously reported literature value of 224.1 kcal/mol for this dipeptide.¹

The GB values of several analytes were determined with the bracketing method (Table 1). All the values are in close agreement to those reported in the literature.^{32,34} Based on these results, the amount of internal energy deposited into the molecules upon desorption via LIAD is less than a few kcal/mol. This amount was determined by the difference in the gas-phase basicity for the conjugate base of the reference acid that did not (endoergic reaction) protonate the acoustically desorbed molecules and the literature GB values (Figure 2). No cases were found in which proton transfer products were detected although the reaction is endoergic. Furthermore, no unexpected reactivity was observed for the molecules desorbed by LIAD.

Estimation of Internal Energy by Comparing LIAD and SALD Breakdown Curves

To further characterize the energies of molecules vaporized via LIAD, energy dependent mass spectra (Figure 3) were obtained for the protonated peptide Val-pro through the use of post-desorption chemical ionization/activation. Through the use of chemical ionization reagent ions (Table 2) with different and known proton affinities (PA), the desorbed molecules were protonated and thereby chemically activated to induce various degrees of dissociation. To ease comparison of these results to previously published results,¹¹ ΔH (as opposed to ΔG) is used in this section only. The distributions of fragment ions obtained were used to generate an experimental breakdown curve showing the energy dependence of the various fragmentation

pathways of the protonated peptide. The internal energy of the protonated peptide was approximated by the negative enthalpy change ($-\Delta H_{rxn}$) of the proton transfer reaction. Any fragmentation beyond that allowed by the exothermicity of the proton transfer reaction likely results from internal energy deposited during the acoustic desorption process. Two scenarios exist which can contribute to an artificially high protonated peptide ion signal (Figure 4). Collisions of neutral peptides with other desorbed peptides can take place within the desorption plume prior to ionization, releasing some of the molecules' internal energy. These collisions are inevitable due to the nature of the desorption technique. Additionally, due to the use of multiple laser pulses (typically 50 – 250 laser pulses), desorption of neutral peptides from subsequent laser pulses can result in collisions with already formed protonated peptides and associated fragment ions. These undesirable secondary protonation reactions are typically of lower exothermicity than the desired reaction, resulting in less fragmentation of the protonated peptide.¹⁴ This is in contrast to the desired reaction with the selected proton transfer reagent. Consequently, attempts were made to minimize these unwanted collisions by reducing the amount of material desorbed per laser pulse through the use of a single laser pulse and utilizing relatively thin sample coverages on the foil.

The fragmentation profile of protonated Val-pro over an internal energy range of 0.42 to 4.41 eV is shown in Figure 5. This breakdown curve shows the desired correlation between the degree of fragmentation and the amount of internal energy deposited upon protonation. This is evident in the decreasing abundance of the protonated peptide as the exothermicity of the proton transfer reaction increases. The predominant fragment ions observed are the a_1 and y_1 ions (Scheme 1), each showing an increased abundance with increased internal energy.

A semi-quantitative comparison of this breakdown curve can be made with a fragmentation profile previously published for protonated Val-pro,¹¹ obtained in an analogous manner (Figure 6) but using the low-energy evaporation technique of substrate assisted laser desorption (SALD).^{9,11} The two curves indicate similar degrees of fragmentation and hence similar amounts (or none) of internal energy deposited during the desorption process. The abundance of the y_1 fragment ion in the LIAD breakdown curve does not decay to the same degree for a given reaction exothermicity as that of the y_1 fragment observed in the SALD experiments. The decay in abundance of the y_1 ion is most likely due to further fragmentation to the immonium ion (m/z 70) (data not included in the SALD curve,¹¹ Scheme 1). Data for the precursor of a_1 , the b_1 ion (m/z 100), are also shown.

Several comparisons can be made between the two breakdown curves, including comparison of the appearance energies of the fragment ions as well as their crossing point energies. Although limited by the number of data points obtained for each curve, this information provides experimental evidence as to the energetics of the two desorption techniques. The appearance energy of the a_1 fragment appears to be between 1.6 and 1.9 eV in the LIAD curve. This closely overlaps an appearance energy between 1.1 and 1.8 eV for a_1 when the peptide was desorbed by the SALD technique. The appearance energy for the y_1 fragment in the LIAD curve is between 1.2 and 1.6 eV. This range falls within a comparable appearance energy range from 0.6 to 1.8 eV for y_1 in the SALD curve. From these results, one can conclude that the energies at which the two major fragmentation pathways occur in the breakdown curves are quite similar and therefore the energetics of the desorption techniques are comparable.

The energies at which the abundances of the two major fragment ions (a_1 and y_1) are equal to the protonated peptide (MH^+) abundance can also be used for comparison of the energetics of the two desorption techniques. The energy at which the abundance of the a_1 ion equals that of MH^+ in the LIAD curve is ~2.1 eV. This is a slightly lower energy than the crossing point energy of ~2.4 eV for the abundance of the a_1 ion with that of MH^+ in the SALD curve. Similarly, the crossing point energies for the abundances of the y_1 ion and MH^+ are comparable

for the two desorption techniques. A crossing point energy of 1.6 eV for the LIAD curve, compared with 1.7 eV for the SALD curve, was observed.

The difference in fragment ion abundances at higher reaction exothermicities can be rationalized by the inherent differences in the two desorption techniques. LIAD is an optically non-invasive technique, performed in the transmission geometry wherein the energy of the laser is absorbed onto the backside of the metal foil. The laser light does not directly contact the analyte during desorption. In comparison, SALD is performed in a reflective geometry in which the laser beam contacts the substrate (and analyte). The wavelength of the laser light is adjusted to match the highest molar absorptivity of the substrate. However, the analyte may also absorb some of the laser energy, which could increase its internal energy.

The LIAD breakdown curve shows a small fractional abundance for the precursor of a_1 , the b_1 ion (m/z 100), which was observed only under low energy conditions. This fragment was not shown by the authors in the SALD breakdown curve. Although of low abundance (~ 5%), the presence of this fragment further suggests that LIAD may deposit somewhat less internal energy into the neutral molecules upon desorption than SALD.

LIAD of Mixtures Followed by Energetically Selective Ionization

In order to further establish that LIAD does not elevate the internal energy of the desorbed molecules, thus enabling endothermic processes to be observed, a mixture of two peptides (with different GB) were desorbed via LIAD and selectively ionized. The peptides Ala-ala (GB = 216.4 kcal/mol³⁴) and Val-pro (GB = 224.1 kcal/mol³⁷), were used for these experiments. Protonated piperidine (GB = 220.0 kcal/mol³⁸) was selected as one of the reference acids based on the established GBs of the peptides. Based on these values, protonated piperidine is predicted to ionize only Val-pro. Unless the LIAD evaporated Ala-ala gains excess internal energy upon desorption via LIAD, no protonated Ala-ala nor its fragment ions should be observed. A sample foil containing a mixture of the peptides Ala-ala and Val-pro was prepared by electrospray desposition of a 1:1 peptide (mol:mol) solution (methanol). The peptide mixture was evaporated by LIAD and the desorbed molecules were allowed to react with the protonated piperidine which resulted in proton transfer only to Val-pro (Figure 7a). To verify the presence of both peptides on the sample foil, another foil containing the same the peptide mixture, prepared in the same manner, was evaporated by LIAD. When the desorbed molecules were allowed to react with protonated aniline (GB = 203.3 kcal/mol³⁸) proton transfer to both Ala-ala and Val-pro was observed (Figure 7b). The ~ 2:1 ratio (Val-pro: Ala-ala) of the abundances of the two protonated peptide signals is due to the use of multiple laser shots (75 laser shots per spectrum). Secondary ionization of the desorbed Val-pro by the previously formed protonated Ala-ala occurs, decreasing the protonated Ala-ala ion signal. In conclusion, the observance of the thermodynamically favorable reaction is based solely on the favorable ΔG of the proton transfer reaction. This result further confirms that LIAD evaporated molecules do not gain excess internal energy upon desorption.

CONCLUSIONS

The ESI/QIT analysis of a LIAD-evaporated trinucleotide, dApdApdA, demonstrates that degradation does not occur during LIAD of this thermally labile species. Based on the re-measurement of the known GB values for a series of analytes desorbed by LIAD, the amount of internal energy deposited upon LIAD is less than a few kcal/mol. Further assessment of the internal energies of LIAD-evaporated molecules through a comparison of a breakdown curve of a protonated peptide with a literature curve suggests that LIAD may deposit less internal energy into the molecules than SALD. When mixtures of desorbed analyte molecules were allowed to react with selective proton transfer reagents, only thermodynamically favorable reactions were observed. These results demonstrate that LIAD does not sufficiently elevate the

internal energy of desorbed molecules to cause degradation or enable endothermic processes. Based on the uncertainties of the methods presented here, only a qualitative assessment of the energies of LIAD-evaporated molecules can be made. The results indicate that the amount of internal energy deposited upon LIAD is small and does not influence the utility of the evaporation technique.

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Figure 1.

Comparison of negative ion ESI-QIT mass spectra of a trinucleotide, dApdApdA (MW 877), for: a) original trinucleotide solution in methanol and b) LIAD evaporated trinucleotide collected into a glass vial and dissolved in methanol. Deprotonated trinucleotide ($[M-H]^{-}$, m/z 876) dominates both mass spectra.

Figure 2.

Bracketing of the gas-phase basicities of LIAD evaporated analytes (4-hydroxy- α -cyanocinnamic acid, 2,5-dihydroxybenzoic acid, 1,8,9-trihydroxyanthracene, Ala-ala, thymidine and Val-pro) by using the protonated reference compounds listed in Table 1. Solid lines denote the GB of the analytes as reported in the literature.¹ The six dashed and dotted lines indicate the brackets obtained here for GB of the analytes, *i.e.*, the GBs of the two reference acids with most similar thermochemical properties that did and did not react with the analyte, respectively. The difference between the upper bracket (endoergic reaction) and literature GB values are listed.

Figure 3. Example LIAD mass spectra of Val-pro ionized via proton transfer from (a) N_2OH^+ , (b) C_2H_5^+ , (c) H_3O^+ , (d) CH_3OH_2^+ , (e) $\text{C}_2\text{H}_5\text{OH}_2^+$, (f) $(\text{CH}_3)_2\text{COH}^+$, (g) $(\text{C}_4\text{H}_9)_2\text{COH}^+$, and (h) $\text{C}_5\text{H}_5\text{NH}^+$. * Chemical background.

Figure 4.

Diagram illustrating collisional cooling of analyte molecules upon desorption via LIAD. Two scenarios contribute to cooling of the analyte molecules. (a) Within the desorption plume and before ionization, collisions can occur between neutral analyte molecules. (b) Within the ICR cell, collisions of desorbed analyte molecules (yellow) with already protonated molecules (yellow) and their fragment ions (red) can result in low-energy proton transfer reactions. These reactions are less exothermic than reactions of the analyte with the desired reagent ion (blue) and contribute towards a larger abundance of the protonated analyte than expected based on the exothermicity of the desired proton transfer reaction.

Figure 5.

Percent (%) fractional abundance of protonated Val-pro (PA = 231.6 kcal/mol; from Table 1; evaporated by LIAD) and its fragment ions vs. the negative of the enthalpy change of the protonation reaction ($-\Delta H_{rxn}$ in eV) with reference acids $C_5H_5NH^+$, $(C_4H_9)_2COH^+$, $(CH_3)_2COH^+$, $C_2H_5OH_2^+$, $CH_3COH_2^+$, H_3O^+ , $C_2H_5^+$, N_2OH^+ (Table 2) representing an energy range from 0.42 to 4.08 eV. In addition to the y_1 and a_1 fragment ions studied in the SALD experiment (Figure 6), the b_1 and immonium ions ($m/z\ 70$) are presented for completeness. The two major fragmentation pathways of val-pro are shown in Scheme 1. Val-pro does not react with protonated triethylamine ($-\Delta H_{rxn} = -0.10$ eV) due to the endothermicity of the ion-molecule reaction (data not shown).

Figure 6.

Breakdown curve reproduced from reference.11 Percent (%) fractional abundance of protonated Val-pro (desorbed via SALD) and its fragment ions vs. the negative of the enthalpy change of the protonation reaction ($-\Delta H_{rxn}$ in eV) with CH_3NH_3^+ , NH_4^+ , $\text{C}_2\text{H}_5\text{OH}_2^+$, CH_3OH_2^+ , H_3O^+ , C_2H_5^+ , COH^+ , N_2OH^+ , CH_5^+ representing an energy range from 0.6 to 4.4 eV. Curves are shown for MH^+ (m/z 215), and y_1 (m/z 116) and a_1 (m/z 72) fragments.

Figure 7.

LIAD mass spectra of a 1:1 mixture of Ala-ala (PA = 223.9 kcal/mol; Table 1) and Val-pro (PA = 231.6 kcal/mol; Table 1) ionized by proton transfer from (a) protonated piperidine ($C_5H_{11}NH^+$, PA^{38} = 228.0 kcal/mol). and (b) protonated aniline ($C_6H_5NH_3^+$, PA^{38} = 210.9 kcal/mol). * Background.

Scheme 1.

Table 1

Comparison of bracketed gas-phase basicities (GB) with literature values and closest bracketing reference acids used.

Analyte	Bracketed ³⁶ GB Value ^{a,b} in kcal/mol	Literature GB Value in kcal/mol	Reference Acids ^c (GB Value in kcal/mol)	Reference
4-Hydroxy- α - cyanocinnamic acid (4HCCA)	196.1	194.8 ^d	Acetophenone (198.2)	38
			—	1
			Cyclohexanone (193.9)	38
2,5-Dihydroxybenzoic acid (DHB)	196.1	197	Acetophenone (198.2)	38
			—	1
			Cyclohexanone (193.9)	38
1,8,9-Trihydroxyanthracene(Dithranol)	200.5	201	Aniline (203.3)	38
			—	1
			Acetophenone (198.2)	38
Ala-ala	216.3	216.4	3-Picoline (217.9)	38
			—	34, 38
			Pyridine (214.7)	38
Thymidine	217.5	218.9	3,5-Lutidine (220.7)	38
			—	32, 38
			Pyridine (214.7)	38
Val-pro	224	224.1	Triethylamine (227)	38
			—	1
			3,5-Lutidine (220.7)	38

^a ± 3 kcal/mol^b PA (kcal/mol) values can be obtained by addition of the entropy of a proton (~ 7.6 kcal/mol at 298 K) to the gas phase basicity values.¹³^c The protonated form of each reference acid is used in the bracketing experiments. The GB values provided here are for the neutral form only.^d Average value

Table 2

Chemical ionization reagents used for generating the experimental breakdown curve and estimate of the maximum internal energy deposited upon proton transfer to LIAD evaporated val-pro.

	Reagent (PA37 in kcal/mol)	$-\Delta H_{\text{rxn}}$ (Val-pro) in eV (kcal/mol) ^a	Proton Transfer Reaction Observed
1	Nitrous oxide (137.5)	4.08 (94.1)	+
2	Ethylene (162.6)	2.99 (69.0)	+
3	Water (165.2)	2.88 (66.4)	+
4	Methanol (180.3)	2.22 (51.3)	+
5	Ethanol (185.6)	1.99 (46.0)	+
6	Acetone (194.0)	1.63 (37.6)	+
7	5-Nonanone (204.0)	1.20 (27.6)	+
8	Pyridine (222.0)	0.42 (9.6)	+
9	Triethylamine (234.0)	-0.10 (-2.4) ^b	- ^b

^aPA of val-pro is 231.6 kcal/mol (10.04 eV) *vide infra* from Table 1.

^bNo reaction