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Laser-Induced Acoustic Desorption Coupled with a Linear Quadrupole Ion Trap Mass Spectrometer

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In recent years, laser-induced acoustic desorption (LIAD) coupled with a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer has been demonstrated to provide a valuable technique for the analysis of a wide variety of nonvolatile, thermally labile compounds, including analytes that could not previously be analyzed by mass spectrometry. Although FT-ICR instruments are very powerful, they are also large and expensive and, hence, mainly used as research instruments. In contrast, linear quadrupole ion trap (LQIT) mass spectrometers are common due to several qualities that make these instruments attractive for both academic and industrial settings, such as high sensitivity, large dynamic range, and experimental versatility. Further, the relatively small size of the instruments, comparatively low cost, and the lack of a magnetic field provide some distinct advantages over FT-ICR instruments. Hence, we have coupled the LIAD technique with a commercial LQIT, the Thermo Fischer Scientific LTQ mass spectrometer. The LQIT was modified for a LIAD probe by outfitting the removable back plate of the instrument with a 6 in. ConFlat flange (CFF) port, gate valve, and sample lock. Reagent ions were created using the LQIT's atmospheric pressure ionization source and trapped in the mass analyzer for up to 10 s to allow chemical ionization reactions with the neutral molecules desorbed via LIAD. These initial experiments focused on demonstrating the feasibility of performing LIAD in the LQIT. Hence, the results are compared to those obtained using an FT-ICR mass spectrometer. Despite the lower efficiency in the transfer of desorbed neutral molecules into the ion trap, and the smaller maximum number of available laser pulses, the intrinsically higher sensitivity of the LQIT resulted in a higher sensitivity relative to the FT-ICR.

Laser-induced acoustic desorption[1−3] (LIAD) was first demonstrated in 1985 as an effective method to transfer nonvolatile, thermally labile compounds as neutral molecules into the gas phase. In 2000, the technology was implemented to a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer and combined with electron ionization (EI) and chemical ionization (CI).[4] During the following years, a variety of analytes, including oligonucleotides,[5,6] oligopeptides,[7−9] petroleum distillates,[10] base oils,[11] asphaltenes,[12] and nonpolar hydrocarbon polymers,[13,14] were successfully desorbed using LIAD and ionized with EI or CI in a FT-ICR mass spectrometer. This approach was demonstrated to be equally applicable to polar and nonpolar analytes, including analytes with and without basic or acidic functionalities. Indeed, some of these analytes could not be detected by using any other mass spectrometric technique. The LIAD technique, as implemented in the above studies, utilizes a series of short, high-intensity laser pulses to generate shockwaves in thin metal foils in order to desorb neutral analyte molecules from the opposite side of the foil into the FT-ICR mass spectrometer. Various aspects of these experiments have been examined in detail.[9,15]

During the past four years, several groups have demonstrated the utility of the LIAD method in a variety of applications by using different mass spectrometers. Peng and co-workers have used LIAD coupled with a quadrupole ion trap as a very efficient particle-counting system.[16−18] In these studies, LIAD was employed for both desorption and ionization. Zinovev et al. explored

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the mechanism of LIAD by using a LIAD/single photon ionization setup coupled with a time-of-flight (TOF) mass spectrometer.[19] Bald et al. used LIAD to study dissociative electron detachment in the gas phase by using a quadrupole instrument.[20] Recently, ambient LIAD was combined with electrospray ionization (ESI) for the direct analysis of biomolecules in solution.[21]

Inspired by the above studies, this research focuses on the implementation of the most generally applicable LIAD experiment, LIAD/CI, on one of the most widely utilized mass spectrometers, the linear quadrupole ion trap (LQIT) mass spectrometer. LQIT mass spectrometers possess several qualities that make these instruments very popular in both academic and industrial settings, such as high sensitivity, large dynamic range, and experimental versatility. Further, the relatively small size of these instruments, large ion storage capacity, and the lack of a magnetic field provide some advantages over the FT-ICR instrument utilized in the earlier LIAD/CI experiments. However, low resolution is a noticeable disadvantage of the LQIT.

In addition to the general advantages of LQIT mass spectrometry, the Thermo Scientific LTQ instrument has some distinct features that make it especially attractive for implementation of LIAD. The most notable of these features is the radial detection design.[22] Traditional quadrupole ion trap (QIT) mass analyzers,[23] as well as other LQIT instruments,[24] are aligned so that ions enter through a hole in one end and are ejected axially through a hole in the other end to be detected. Modification of this geometry for LIAD would most likely involve drilling a hole in the mass analyzer (as previously reported for a MALDI probe[25]) to allow introduction of the desorbed neutral molecules into the trap. However, the LTQ ejects ions radially through exit slits in two opposite hyperbolic rods to two external detectors. This leaves the rear of the instrument available to further modification. In fact, the back plate of the vacuum manifold in the commercially available instrument is removable for attachment of different options offered by the manufacturer, such as high-resolution mass analyzers,[26,27] and has also been modified to accept an ESI source.[28] This geometry allows for modification of this instrument for LIAD without any significant changes to the mass analyzer. We present here the first report of LIAD implemented on a commercial LQIT. The desorbed neutral molecules were ionized by CI. Some of the advantages of LIAD coupled to the LQIT are presented, including increased sensitivity compared to similar experiments performed on a 3-T FT-ICR.

EXPERIMENTAL SECTION

Sample Preparation. The tetrapeptides val-ala-ala-phe, (val-cys-OH)$_2$, and (phe-cys-OH)$_2$ were obtained from Bachem Biosciences (King of Prussia, PA), the titanium foils were obtained from Alfa Aesar (Ward Hill, MA), and methanol was obtained from Mallinckrodt Baker (Phillipsburg, NJ). All other compounds and reagents were obtained from Sigma-Aldrich (St. Louis, MO). The compounds and foils were used as received from the supplier. The radical precursor, 3-iodo-*N*-methylpyridinium iodide, was synthesized from 3-iodopyridine based on a known procedure.[29] LIAD samples were prepared via electrospray deposition[30] of analyte solutions (in concentrations of 10^{-3} M in CH_3OH) onto 1.7 cm diameter Ti foils. By varying the volume of the solution sprayed, sample thicknesses ranging from 40 to 200 nmol/cm^2 were obtained. To generate reagent ions via ESI, the appropriate sample solutions (see the Results and Discussion section) were prepared at various concentrations (10^{-6} to 10^{-4} M) in CH_3OH or 50/50 (v/v) CH_3OH/H_2O.

Instrumentation. All experiments were performed on an LQIT mass spectrometer (LTQ; Thermo Fisher, Scientific San Jose, CA) equipped with an ESI source. The instrument was modified to accept a standard 7/8 in. LIAD probe (details of this probe have been described previously[15]). The LQIT was modified for the LIAD probe by outfitting the removable back plate of the instrument with a 6 in. ConFlat flange (CFF) port, manual gate valve, and sample lock. A schematic of this instrument setup is shown in Figure 1. The sample lock was connected to a mechanical pump which allows the inlet to be pumped down to 10^{-3} Torr. Following insertion of the probe into the sample lock, the gate valve was opened to allow insertion of the probe into the LQIT vacuum manifold. This resulted in a brief spike in the pressure of the manifold, but the region was rapidly pumped back to the baseline pressure of the vacuum chamber ($\sim 7-8 \times 10^{-6}$ Torr). The probe was then pushed in until it was approximately 1/8 in. (3.2 mm) from the rear of the ion trap assembly.

The ion trap assembly consists of the LQIT mass analyzer and two lenses (one on each end) that act as conductance limits, all of which is encased in polycarbonate (Figure 2a). Each lens of the ion trap assembly consists of a solid plate with a 2 mm aperture in the center. The CFF port was placed on the backplate such that the desorption axis of the LIAD probe was aligned with the center of the ion trap assembly (and thus the center of the LQIT), which provides maximum possible overlap of the desorption plume with the stored reagent ions. Although the polycarbonate casing should adequately electrically shield the LIAD probe from the LQIT, the stainless steel cap of the sample holder on the LIAD probe was replaced with a Teflon replica.

Laser-Induced Acoustic Desorption/Chemical Ionization. Ti foils were mounted onto the LIAD probe and inserted into the mass spectrometer as described above. The LIAD probe setup employed a pulsed, Q-switched Nd:YAG laser (Minilite II; Continuum Lasers, Santa Clara, CA) whose beam (532 nm) was directed via an optical fiber[4,15] to the end of the probe and focused onto the back side of the Ti foil over an irradiation area of

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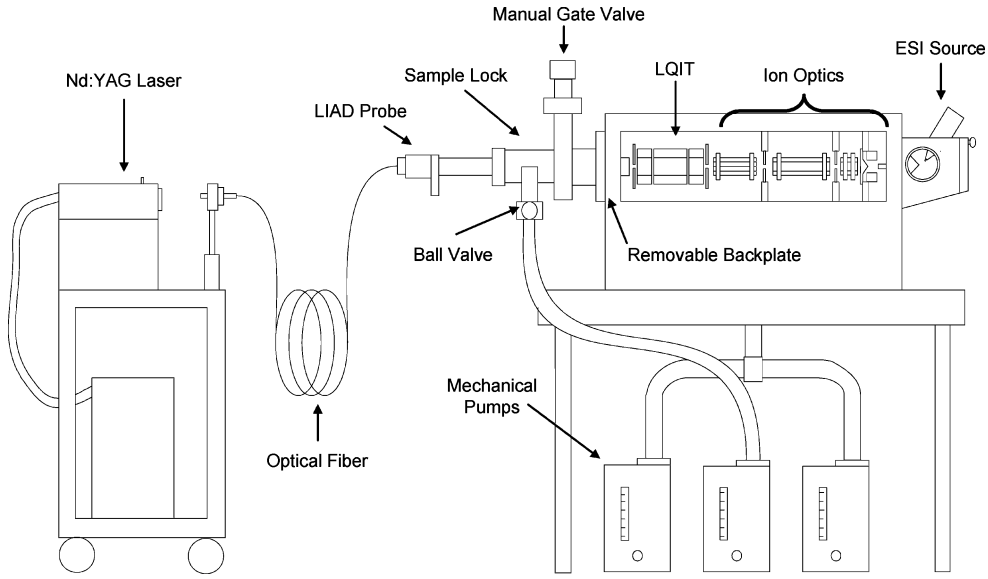

Figure 1. Diagram of the LIAD setup for the LQIT mass spectrometer.

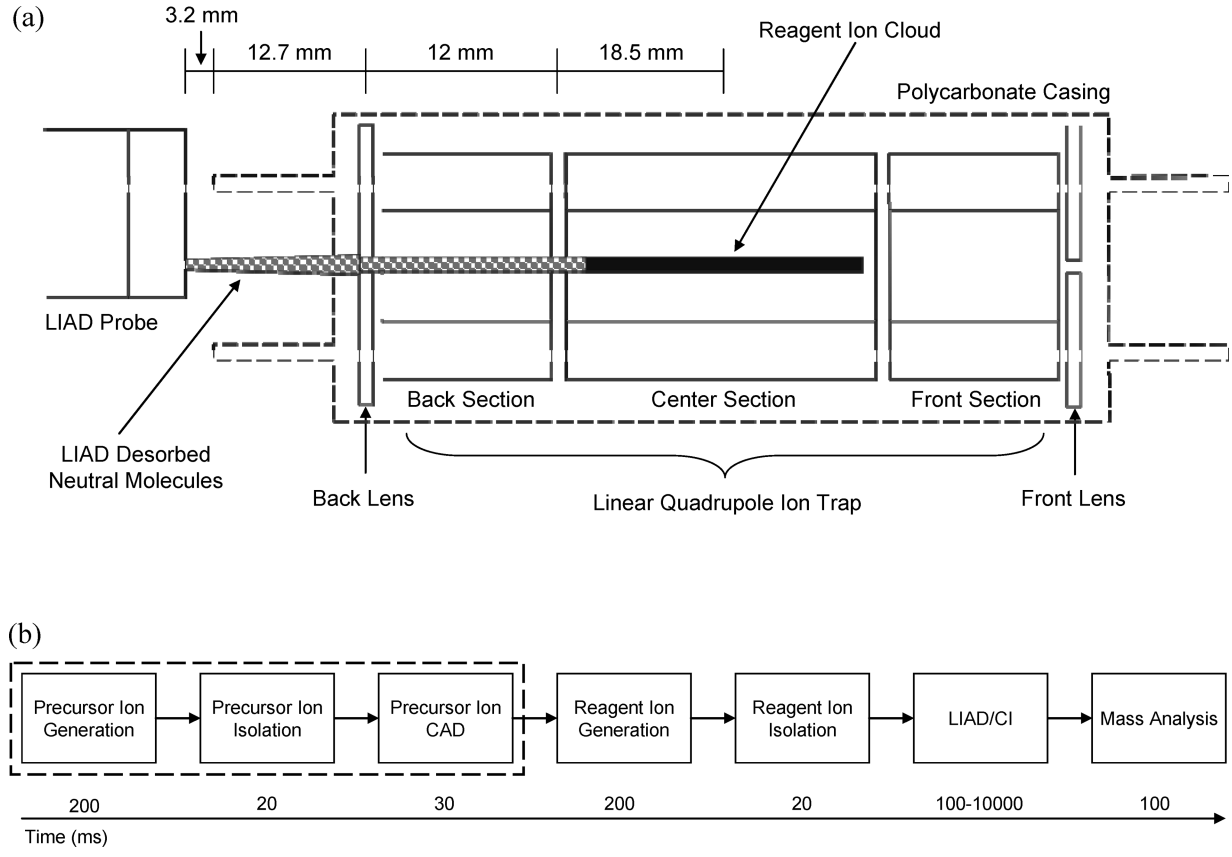

Figure 2. (a) Illustration of the LIAD/CI experiment performed in the mass analyzer of the LQIT mass spectrometer. (b) Event sequence depicting the LIAD/CI MS/MS or MS/MS/MS experiment accomplished on the LQIT, with approximate time required for each event. Some of the steps (dashed box) may not be necessary for every experiment.

approximately 10^{-3} cm^2. The foil was subjected to a series of laser pulses (~3.5 mJ/pulse, 3 ns pulse width) focused onto the back side of the foil while manually rotating the outer cylinder of the probe to sample fresh spots. The power densities were about 5×10^9 W/cm^2 at the back side of the foil.[15]

Following desorption, the neutral analyte molecules were ionized by CI via reactions with reagent ions that had been isolated and were stored in the LQIT for up to 10 s (by setting the "activation time" (in the absence of activation) in the advanced scan features of the Tune Plus interface). The chemical ionization reagent ions were generated via ESI. Typical ESI conditions were the following: needle voltage, 4.0−4.5 kV; sheath gas (N_2) flow, 5−15 (arbitrary units); auxiliary gas (N_2) flow, 0−5 (arbitrary units); capillary temperature, 275 °C. Voltages for the remaining ion optics were optimized using the tune feature of the Tune Plus interface. To generate distonic radical ions, ESI was

followed by collision-activated dissociation (CAD), either accomplished in the mass analyzer or within the preceding ion optics ("in-source" CAD[31]). The product ions formed in the LIAD/CI experiments were detected in a mass-selective manner as usual.

The event sequence for the LIAD/CI experiment in the LQIT is shown in Figure 2b. Most of the experiments described in this report represent MS/MS experiments. The first stage of mass filtering was ion isolation, and the second was the analysis of the LIAD/CI products. An exception is the examination of the radical reaction, which represents an MS/MS/MS experiment. Here, the first stage of mass filtering was precursor ion isolation, which was followed by CAD to generate the reagent ion. The second stage of mass filtering involved isolation of the ion, and the third stage involved the analysis of the LIAD/CI product ions.

Xcalibur 2.0 software was used for both data acquisition and processing. All mass spectra shown are an average of at least three scans.

Finally, we wish to point out that after about two years of carrying out LIAD experiments on the LQIT, we have not noticed any contamination of the trap from LIAD. This finding indicates that the unreacted neutral molecules desorbed via LIAD are mostly pumped away by the vacuum system or not in sufficient amount to cause noticeable contamination of the ion trap.

RESULTS AND DISCUSSION

Experimental Considerations. The setup of LIAD on the LQIT was designed based on the setup that has been utilized on FT-ICR ion trap mass spectrometers in our laboratories for the past 10 years.[4,32] A LIAD probe was inserted through a manual gate valve into the vacuum manifold of the LQIT mass spectrometer. The tip of the probe was located as close to the LQIT as possible to allow sufficient overlap of the LIAD desorbed neutral molecules and the CI reagent ions being trapped in the mass analyzer. Despite the similarities of the LQIT setup to the previous setup, there are some important differences that must be considered when performing the LIAD experiment on the LQIT.

A significant difference between the LIAD setup utilized in this research on the LQIT mass spectrometer and that previously employed on FT-ICR mass spectrometers is the distance of the probe from the center of the ion cloud in the *Z*-direction (i.e., the axis of desorption). In the FT-ICR setup, the probe was placed approximately 3.2 mm from the trapping plate of the 50.8 mm cubic ion trapping cell, and thus the desorbed neutral molecules had to travel approximately 28.6 mm (in the *Z*-direction) to reach the center of the cloud of the trapped CI reagent ions. Similarly, the probe was placed approximately 3.2 mm from the ion trap assembly in the LQIT (Figure 2). However, because the LQIT is housed in a polycarbonate casing, the probe is actually located about 15.9 mm from the back lens of the trap. Furthermore, the neutral molecules that enter the trap must travel an additional 12 mm through the back section of the analyzer before reaching the ion cloud.[22] From that point, there is another 18.5 mm into the center of the trap (and of the ion cloud). Thus, the desorbed molecules have to travel a total of 46.4 mm to reach the center of the ion cloud, which is about 1.5 times more than in the FT-ICR. Further, the pressure in the high-vacuum region of the LQIT is two orders of magnitude greater than in the FT-ICR and about five orders of magnitude greater inside the trap itself due to the helium buffer gas. The higher pressure in the LQIT results in a reduced mean free path for the desorbed molecules compared to the FT-ICR. Thus, the molecules must travel further and the mean free path is shorter in the LQIT compared to the FT-ICR. These considerations suggest that the fraction of molecules desorbed by LIAD that reach the ion cloud is much smaller for the LQIT than for the FT-ICR.

Another consideration for the LIAD experiment in the LQIT is the maximum ion trapping time. When using the Tune Plus interface, the maximum allowable ion trapping time is 10 s. Since the laser used for these experiments has a maximum repetition rate of 15 Hz, the maximum number of laser shots per LIAD mass spectrum is 150 shots. This was viewed as a potential problem since many previous LIAD experiments required the use of more laser shots (up to 300). Although both of the above issues would seem to present difficulties for successful coupling of LIAD with the LQIT, certain performance characteristics of the instrument (e.g., large ion storage capacity) should partially counterbalance them.

LIAD/CI in the LQIT. To compare the performance of the LIAD setup on the LQIT to that of the FT-ICR, several experiments that had been previously performed by using the FT-ICR were carried out on the LQIT. One of these experiments was LIAD of the tetrapeptide val-ala-ala-phe (VAAF, 406 Da) followed by ionization by proton transfer.[15,33] Various amounts of the tetrapeptide VAAF were electrospray-deposited onto Ti foils, evaporated via LIAD by using differing numbers of laser shots (each on a fresh spot on the foil), and ionized by proton transfer from protonated pyridine in the LQIT. An example mass spectrum is shown in Figure 3. The spectrum was obtained by using less than 50% of the amount of VAAF on the foil (40 vs 83 nmol/cm^2) and substantially fewer laser shots (10 vs 25) than in the FT-ICR. However, a qualitative comparison of the two spectra indicates that the spectrum obtained from the LQIT has at least as high a signal-to-noise ratio (if not higher) as the spectrum obtained from FT-ICR. Thus, despite the apparent obstacles (presented above) for coupling LIAD with the LQIT, this instrument setup appears more sensitive than the FT-ICR. Finally, preliminary CAD experiments were performed on the protonated VAAF generated upon LIAD/CI, and the results were the same as those obtained from CAD performed on the protonated VAAF generated by ESI.

Further proof of the greater sensitivity of LIAD coupled with the LQIT (compared to the FT-ICR) was provided by desorption and ionization of angiotensin IV (VYIHPF, 774 Da) (Figure 4). The same experiment required the use of higher power density (2.3×10^9 vs 9.0×10^8 W/cm^2 used here) and twice as many laser shots (200 vs 100 used here) in the FT-ICR instrument.[33] Successful desorption of this peptide was confirmed not only by ionization via proton transfer but also via sodium ion transfer and deprotonation (Figure 4). Several larger peptides (e.g., oxytocin, 1006 Da; angiotensin I, 1296 Da) were studied using

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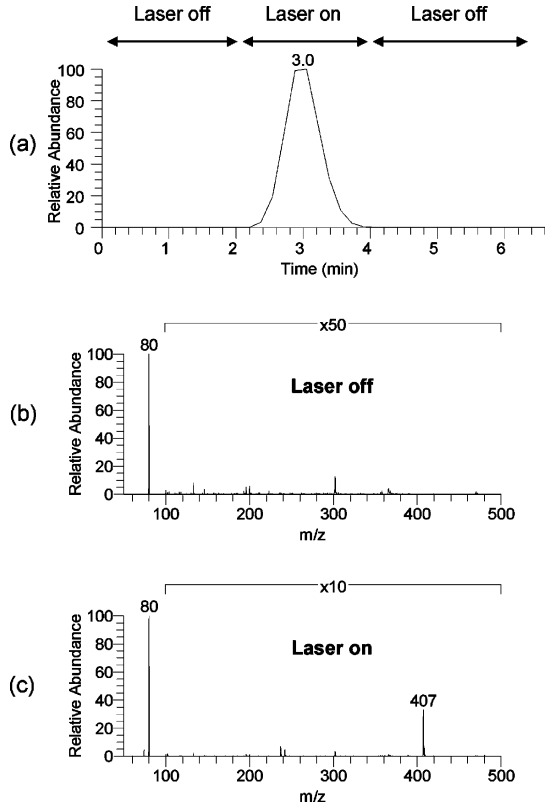

Figure 3. (a) Extracted ion chromatogram obtained by monitoring protonated VAAF (*m*/*z* 407) in real time while turning the laser on and off. VAAF was evaporated by LIAD (10 shots/spectrum) and ionized by proton transfer from isolated protonated pyridine (*m*/*z* 80) (an MS/MS experiment). The signal for the ion of *m*/*z* 407 was only obtained when the laser was on. A comparison of mass spectra obtained with (b) the laser off and (c) with the laser on confirms that VAAF was only present following LIAD.

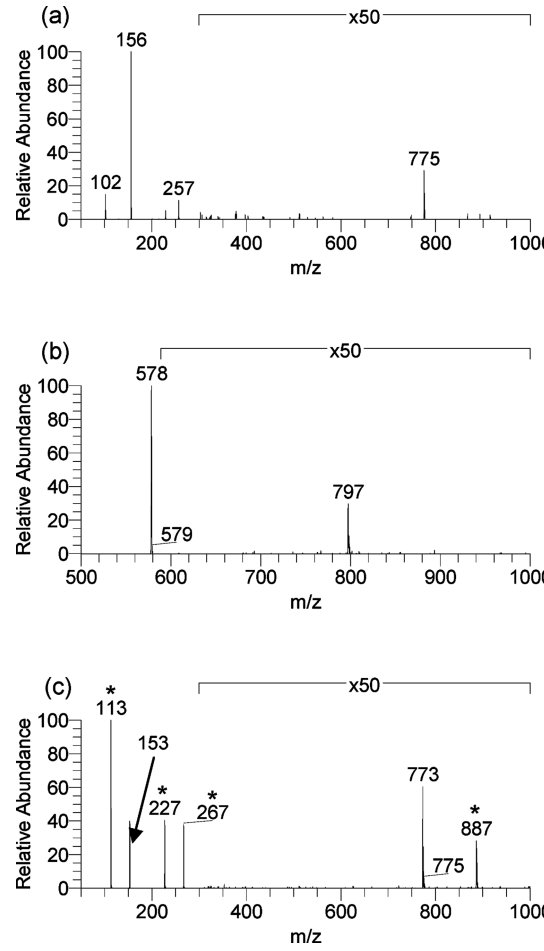

Figure 4. LIAD/CI MS/MS spectra of angiotensin IV (774 Da) evaporated by LIAD (100 shots/spectrum) and ionized (a) via proton transfer from isolated protonated histidine (*m*/*z* 156) (ionized by ESI) to form the protonated molecule (*m*/*z* 775), (b) via sodium ion transfer from isolated sodiated leucine enkephalin (*m*/*z* 578) (formed by ESI) to form the sodiated molecule (*m*/*z* 797), and (c) via deprotonation by isolated deprotonated dihydroxybenzoic acid (*m*/*z* 153) (generated by ESI) to form the deprotonated molecule (*m*/*z* 773). The negative ion mode mass spectrum (c) displays several peaks attributed to reactions of trifluoroacetic acid (denoted by *), which may be an impurity present in the angiotensin IV sample.

this setup, but no significant signals were observed. The ability to desorb these larger peptides will most likely require the use of higher laser powers in LIAD.[33]

After determining that the LIAD experiment was feasible in the LQIT, the optimal operation of the LQIT instrument for LIAD was investigated. Ion stability in the LQIT is governed by the Matthieu parameter q, just as in the QIT.[23] The q value is a function of the amplitude of the rf voltage (and other parameters, such as ion trap geometry and rf frequency) and inversely proportional to the ion's m/z value. Ions will have stable trajectories in the trap if their q value falls between 0 and 0.908. The q value that is typically employed ($q = 0.25$) in the LQIT for MS/MS experiments provides a high CAD efficiency and minimizes the low-mass cutoff, but hinders detection of ions of greater m/z values than the isolated ion. This may pose a problem in ion−molecule reaction studies, such as chemical ionization, where product ions can have m/z values much greater than the isolated ion. In fact, many LIAD/CI experiments published thus far yielded product ions of a higher m/z value than the reagent ion. The use of a q value of 0.25 for isolation of the reagent ion in these experiments would result in much smaller q values for the simultaneously trapped high-mass product ions since their m/z values are greater than that of the reagent ion. In the QIT, the efficiency of trapping ions is known to steadily decrease (evidenced by a decrease in signal) with q values below 0.15.[34] A similar situation is expected for the LQIT. Thus, choosing a higher q value than 0.25 should improve trapping of the higher mass ionic products and lead to a greater signal.

Due to the above considerations, the effect of the q value of the LQIT on the measured LIAD/CI mass spectra was investigated. Figure 5 shows mass spectra obtained after proton transfer from protonated histidine (m/z 156) to LIAD-desorbed neutral leucine enkephalin (YGGFL, 555 Da) while using different q values to isolate the protonated histidine (m/z 156) and trap the product ions. The different q values examined were 0.75, 0.50, and they 0.25 and they correspond to q values of about 0.21, 0.14, and 0.07 for protonated YGGFL (m/z 556), the reaction product. On the basis of previous studies of ion−molecule reactions in a QIT, the abundance of the protonated YGGFL would be expected to decrease as its q value decreases, especially when it is below 0.15.

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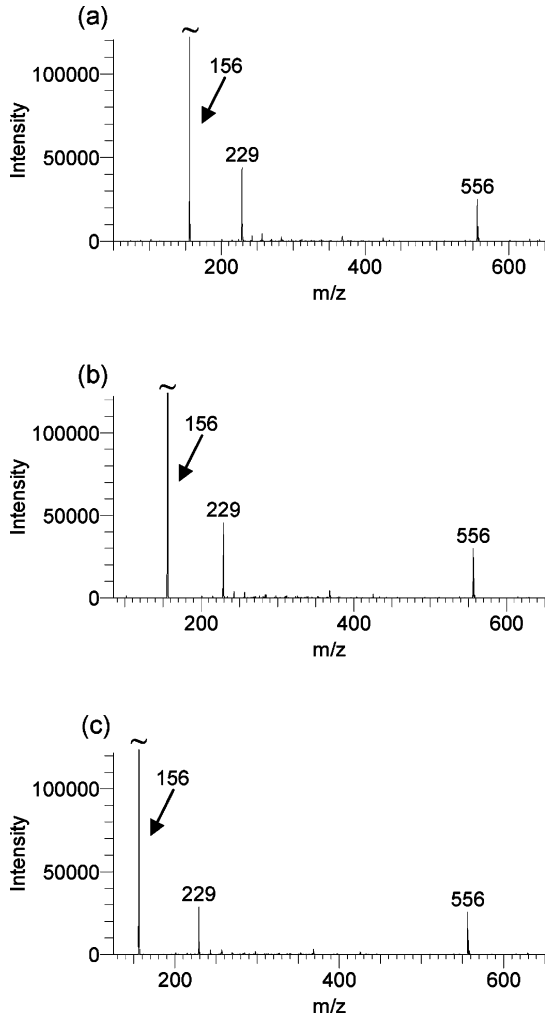

Figure 5. LIAD/CI MS/MS spectra of leucine enkephalin (555 Da) evaporated by LIAD (50 shots/spectrum) and ionized by proton transfer from protonated histidine (*m*/*z* 156) ionized by ESI and isolated with a *q* value of (a) 0.25, (b) 0.50, and (c) 0.75 prior to the reaction. The peak of *m*/*z* 229 is attributed to reactions of diethylamine, which may be an impurity present in the leucine enkephalin sample.

However, the mass spectra (Figure 5) appear identical. Thus, these results indicate a minimal dependence between the *q* value and the measured signal (and thus trapping efficiency) of the LIAD/CI product ions in this experiment.

Examination of a Gas-Phase Radical Reaction. Encouraged by the initial results, the LQIT setup was then employed to study more complex ion-molecule reactions. LIAD coupled with the distonic ion approach (i.e., the attachment of an inert charged moiety to a radical to allow its manipulation via MS[35,36]) has been shown to provide an effective method to study the intrinsic reactivity of radicals toward biomolecules, such as peptides, oligonucleotides, and their components.[5,6,8] These studies can aid in the development of more selective, less cytotoxic anticancer drugs. The feasibility of performing these studies on the LQIT was demonstrated by examining the reactions of a positively charged phenyl radical (the *N*-methyl-3-dehydropyridinium ion)

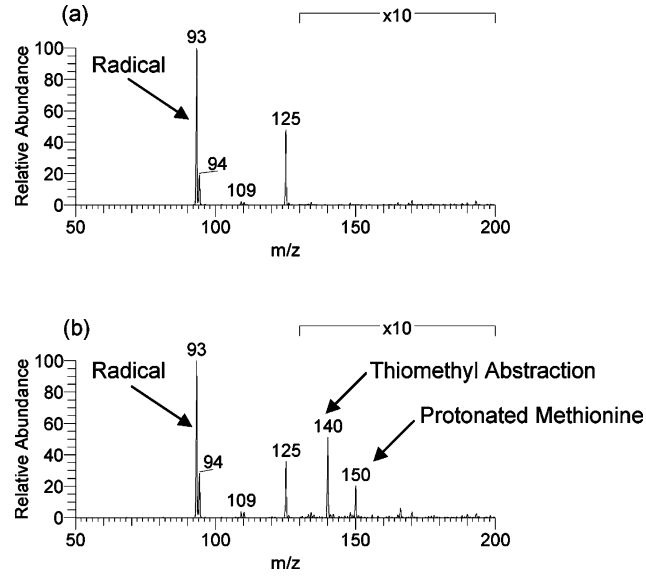

Figure 6. (a) MS/MS/MS spectrum of the isolated *N*-methyl-3-dehydropyridinium radical ion (*m*/*z* 93; generated and isolated in an MS/MS experiment as described in the Experimental Section) with the laser off. (b) LIAD/CI MS/MS/MS spectrum of the isolated *N*-methyl-3-dehydropyridinium radical ion (*m*/*z* 93) after reaction with methionine evaporated by LIAD (100 shots/spectrum).

with simple amino acids. An *N*-methylpyridinium salt of the radical precursor that is amenable to ESI was employed in these experiments, although other approaches are also feasible. For example, Barton esters can be used to generate charged phenyl radicals by ESI in the LQIT.[37,38] The *N*-methyl-3-dehydropyridinium ion was generated via ESI of *N*-methyl-3-iodopyridinium iodide salt, followed by CAD of the *N*-methyl-3-iodopyridinium ion to homolytically cleave the iodine bond and create the radical site. The radical was found to react with methionine and tryptophan (each desorbed via LIAD) via the expected side-chain abstraction pathways.[39,40] For example, the reaction of methionine and *N*-methyl-3-dehydropyridinium produced an abundant product ion (*m*/*z* 140) corresponding to abstraction of the thiomethyl side chain by the radical (Figure 6), which agrees with previously reported results.[39] However, a large peak corresponding to protonated methionine (*m*/*z* 150) was also observed. This product ion is likely the result of secondary reactions of primary product ions with methionine in the high-pressure LQIT. The peaks of *m*/*z* 109 and *m*/*z* 125 probably result from reactions of the radical and adventitious oxygen present in the LQIT,[41] since these peaks were observed without firing the laser. These initial results, sensitivity, suggest that the LIAD setup on the LQIT could serve as a powerful means of evaluating the intrinsic reactivity of radicals toward biomolecules.

CONCLUSIONS

Successful coupling of LIAD with a LQIT mass spectrometer is demonstrated through a straightforward modification, which

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makes these experiments readily accessible to many other laboratories. The instrument was shown to be capable of several LIAD/CI experiments previously performed on FT-ICR mass spectrometers. In fact, the results obtained from LIAD experiments on the LQIT indicate substantially greater sensitivity than for FT-ICR. Future research will focus on the effects of different instrumental conditions (e.g., energetics, pressure) on LIAD experiments performed in the LQIT and FT-ICR mass spectrometers. Further, the high-power LIAD technique will be implemented to the LQIT to allow for desorption of more sample per laser shot and for more efficient desorption of larger, more complex compounds.

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