J. Am. Soc. Mass Spectrom. (2017) 28:1091–1098
DOI: 10.1007/s13361-017-1684-1

FOCUS: HONORING R. G. COOKS' ELECTION TO THE NATIONAL ACADEMY OF SCIENCES: RESEARCH ARTICLE

Laser-Induced Acoustic Desorption/Electron Ionization of Amino Acids and Small Peptides

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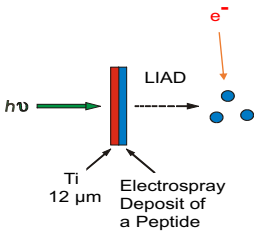

Abstract. Laser-induced acoustic desorption (LIAD) allows for desorption of neutral nonvolatile compounds independent of their volatility or thermal stability. Many different ionization methods have been coupled with LIAD. Hence, this setup provides a better control over the types of ions formed than other mass spectrometry evaporation/ionization methods commonly used to characterize biomolecules, such as ESI or MALDI. In this study, the utility of LIAD coupled with electron ionization (EI) was tested for the analysis of common amino acids with no derivatization. The results compared favorably with previously reported EI mass spectra obtained using thermal desorption/EI. Further, LIAD/EI mass spectra collected for hydrochloride salts of two amino acids were found to be similar to those measured for the neutral amino acids with the exception of the appearance of an $HCl^{+\bullet}$ ion. However, the hydrochloride salt of arginine showed a distinctly different LIAD/EI mass spectrum than the previously published literature EI mass spectrum, likely due to its highly basic side chain that makes a specific zwitterionic form particularly favorable. Finally, EI mass spectra were measured for seven small peptides, including di-, tri-, and tetrapeptides. These mass spectra show a variety of ion types. However, a_n type ions are prevalent. Also, electron-induced dissociation (EID) of protonated peptides has been reported to form primarily a_n type ions. In addition, the loss of small neutral molecules and side-chain cleavages were observed that are reminiscent of other high-energy fragmentation methods, such as EID. Finally, the isomeric dipeptides LG and IG were found to produce drastically different EI mass spectra, thus allowing differentiation of the leucine and isoleucine amino acids in these dipeptides.

Keywords: LIAD, EI, Amino acids, Peptides

Received: 14 October 2016/Revised: 5 April 2017/Accepted: 7 April 2017/Published Online: 12 May 2017

Introduction

Electron ionization (EI) is one of the oldest and best characterized ionization methods, providing highly reproducible and structurally characteristic mass spectra [1, 2]. Furthermore, EI is the only known universal ionization method (i.e., the only method that ionizes all organic compounds) even for mixtures [1, 2]. EI often yields stable molecular ions as well as fragment ions for organic compounds, thus allowing the determination of the molecular weight (MW) of the analyte as well as various structural features. The most serious limitation of this ionization method is that until recently, only volatile (including derivatized amino acids and peptides) or nonvolatile but thermally stable analytes (including some volatile peptides [3, 4], and even some volatile salts of amino acids [5]) could be analyzed as vaporization of nonvolatile samples occurred via the use of a heated desorption probe or other heated device [3–8]. However, heating is not feasible for thermally labile analytes that decompose upon heating, such as oligopeptides and oligonucleotides. Therefore, EI mass spectra are not readily available for these compounds.

Laser-induced acoustic desorption [9] (LIAD) involves evaporation of neutral molecules from a thin metal foil (often 12 μm thick titanium) when the opposite side of the foil is

Electronic supplementary material The online version of this article (doi:10.1007/s13361-017-1684-1) contains supplementary material, which is available to authorized users.

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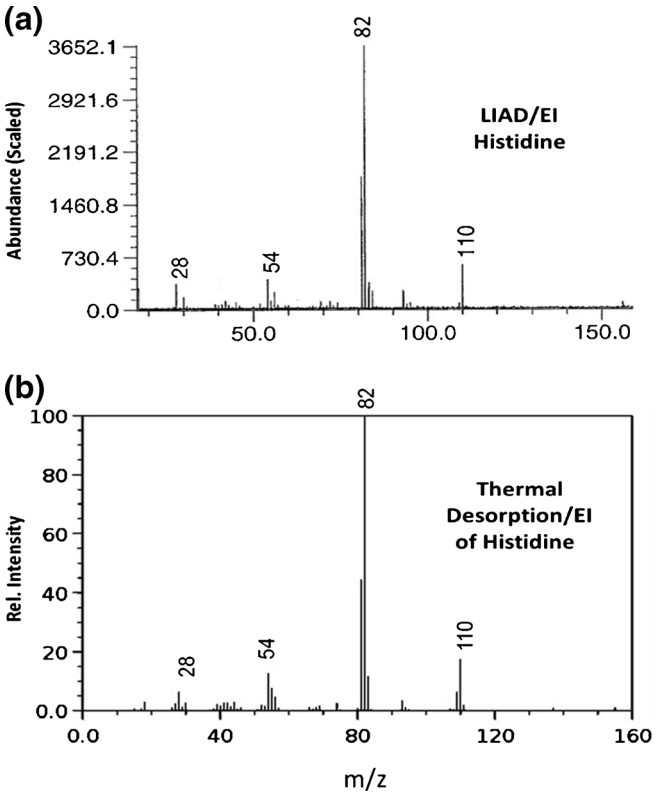


Figure 1. Mass spectra measured by (**a**) LIAD/EI and (**b**) thermal desorption/EI (reference [21]) for histidine (MW 155 Da)

irradiated by a pulsed laser beam. LIAD allows for desorption of nonvolatile and thermally labile molecules into the gas phase with no fragmentation and with unprecedentedly low internal and kinetic energies [10–13]. LIAD can be coupled to various ionization methods [9], including EI [14, 15]. Hence, combining LIAD with EI allows the measurement of EI mass spectra for many compounds, the EI mass spectra of which are not currently available, such as peptides.

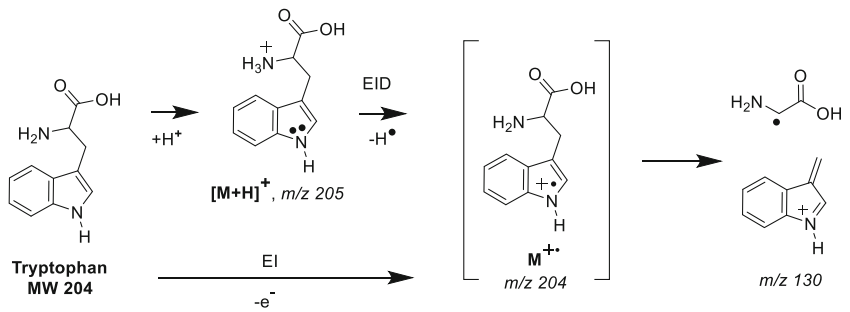
Peptide ions are typically generated through the use of "soft" ionization techniques, such as electrospray ionization [16] (ESI) and matrix-assisted laser desorption/ionization [17, 18] (MALDI). These methods involve nearly simultaneous evaporation and ionization of the peptides. Most commonly, they produce protonated (sometimes multiply protonated) peptides. The resulting mass spectra allow the determination of the MWs of the peptides. In order to obtain structural information, tandem mass spectrometry (MS/MS) experiments have to be utilized. The most common methods used to characterize the structures of protonated peptides include collision-activated dissociation (CAD) [19], electron transfer dissociation (ETD), and electron capture dissociation (ECD) [20–22]. However, perhaps most relevant to the current study due to the use of electron ionization is electron-induced dissociation (EID) [23, 24]. EID involves irradiation of a protonated (or multiply protonated) molecule with electrons (kinetic energies 10–30 eV) that can induce electron and hydrogen atom loss, thus producing a molecular ion and its fragment ions [24]. Therefore, the fragmentation upon EID may be comparable to EI-induced fragmentation. EID has been reported to form primarily a_n type ions for protonated peptides [24].

In this study, LIAD/EI was explored as a means for nearly simultaneous ionization and fragmentation of eight amino acids and seven small peptides in a single step (MS experiment). The results are compared to those obtained using thermal desorption/EI (MS experiment) and EID (MS/MS experiment).

Experimental

Methanol was purchased from Mallinckrodt Baker (Phillipsburg, NJ, USA) and used without further purification. Amino acids and di- and tripeptides were obtained from Sigma Aldrich (>98% purity). Ti foil (12.7 µm thick) was purchased from Alfa Aesar (Ward Hill, MA, USA).

Experiments involving EI as the ionization method were performed on a Nicolet dual-cell Fourier-transform ion cyclotron resonance mass spectrometer (FT-ICR) with a 3 T magnet. A high vacuum was maintained by two diffusion pumps (Edwards Diffstack 160) operating at 700 L/s.



Scheme 1. Proposed parallel fragmentation pathways for the formation of ions of *m/z* 130 from tryptophan via electron-induced dissociation [24] and electron impact ionization

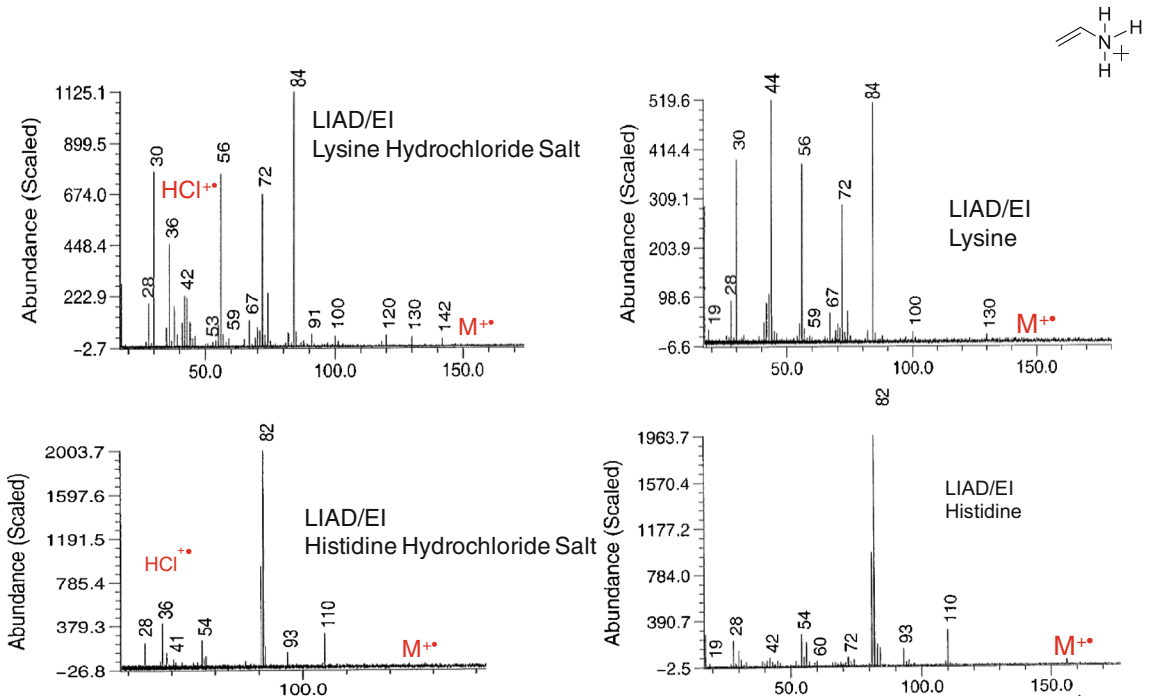


Figure 2. Mass spectra measured by LIAD/EI for lysine (MW 146 Da) and its hydrochloride salt (top) and for histidine (MW 155 Da) and its hydrochloride salt (bottom)

The diffusion pumps were backed by two dual rotary-vane pumps (Alcatel 2010) operating at 3.2 L/s. Baseline pressure in the vacuum chamber of the FT-ICR was maintained below 10^{-8} Torr. Changes in pressure were monitored at either side of the differentially pumped dual cell by Bayard-Alpert ionization gauges placed at each end of the vacuum chamber. Chemical reagents and analytes were introduced via various inlets, including a heated solids probe, a LIAD probe, Varian leak valves, pulsed valves, and batch inlets utilizing Andonian leak valves.

As described in previous work [9, 12, 13, 25, 26], samples were dissolved in MeOH at a concentration of 1 mg/mL, and 25 µL of these solutions were sprayed onto 12.7 µm thick Ti foils by using electrospray deposition. Each foil was mounted on a custom-built LIAD probe [9, 12] to be introduced into the high vacuum environment of the mass spectrometer. The laser shots (3–5 ns pulse width at 532 nm with a 10 Hz repetition rate and power density ~10^9 W/cm^2) obtained from a Nd:YAG laser (Minilite II, Continuum, Santa Clara, CA, USA) were directed either by using a mirror system or an optical fiber to the back side of a foil mounted on the tip of the probe to induce desorption of the sample on the opposite side of the foil [10, 11]. The laser beam was shot in a circular pattern on the foil. After the molecules were desorbed into high vacuum, they had a ~1 ms residence time within the cell before they were removed by the vacuum system. This residence time allows for ionization by electron bombardment. The following conditions were used: EI time of 50 µs, electron beam energy of 70 eV, and filament current of 7 µA.

Results and Discussion

LIAD/EI mass spectra were measured for ten different types of free amino acids, i.e., polar, nonpolar, aromatic, acidic, and basic (histidine, lysine, arginine, isoleucine, phenylalanine, glutamic acid, proline, cysteine, valine, and tryptophan), and compared to literature EI mass spectra. In addition to the free amino acids, the hydrochloride salts of lysine, histidine, and arginine were also investigated. The lack of volatility of the hydrochloride salts typically prevents desorption via thermal methods; therefore, the EI mass spectra of these compounds are unknown. Finally, LIAD/EI mass spectra were measured for seven small peptides, including three dipeptides (GV, GA, and AF), three tripeptides (VGG, FGG, and AGG), and a tetrapeptide (FGGF). These peptides were selected as they contain nonpolar (G, A, V) as well as aromatic (F) and highly basic (A) amino acid residues. Finally, the isomeric dipeptides LG and IG were found to produce drastically different EI mass spectra, thus allowing differentiation of the leucine and isoleucine amino acids in these dipeptides.

LIAD/EI Mass Spectra of Amino Acids

The LIAD/EI (70 eV) mass spectrum measured for each amino acid was found to be similar to the EI (70 eV) mass spectrum obtained earlier by using heat to evaporate the amino acids (previously reported in the NIST database [27]). For example, Figure 1 shows the LIAD/EI mass spectrum obtained here for histidine, together with the NIST EI mass spectrum [27]. The

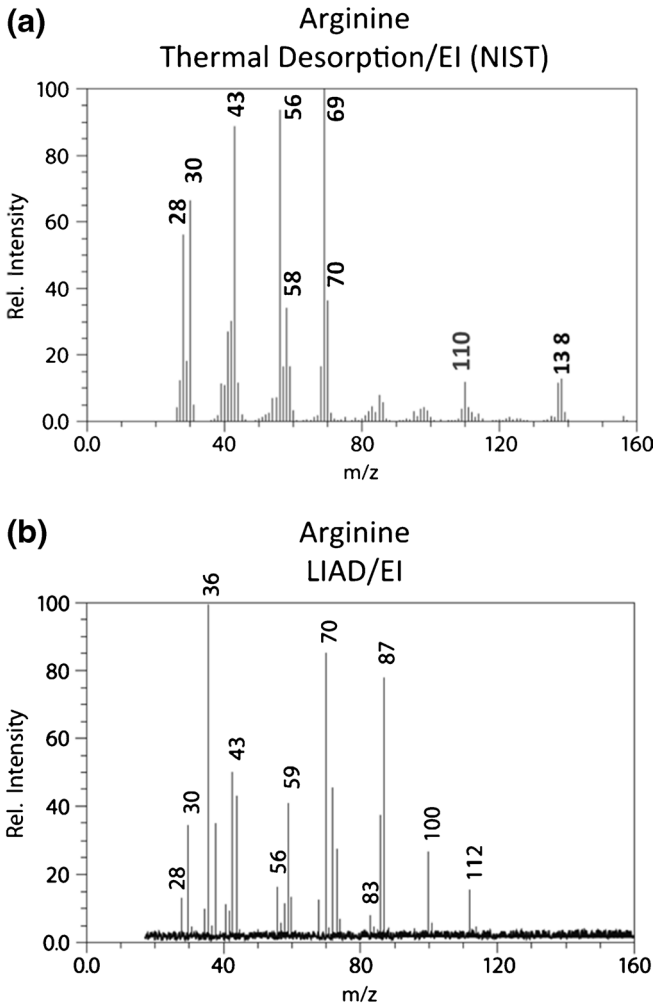


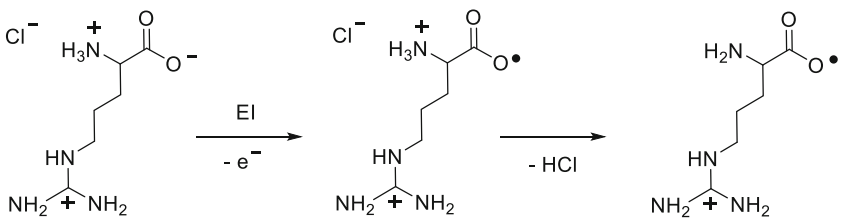
Figure 3. Mass spectra measured by (**a**) thermal desorption/EI (reference [27]) and (**b**) LIAD/EI for arginine. The molecular ion (*m/z* 174) was not observed. Note the formation of the HCl ions of *m/z* 36 and 38 in the bottom mass spectrum

LIAD/EI mass spectra measured for the remaining seven amino acids, together with the corresponding NIST EI mass spectra, can be found in Supporting Information (Supplementary Figures S1–S9). This finding demonstrates that the evaporation method (heating or LIAD) does not influence the EI mass spectra measured.

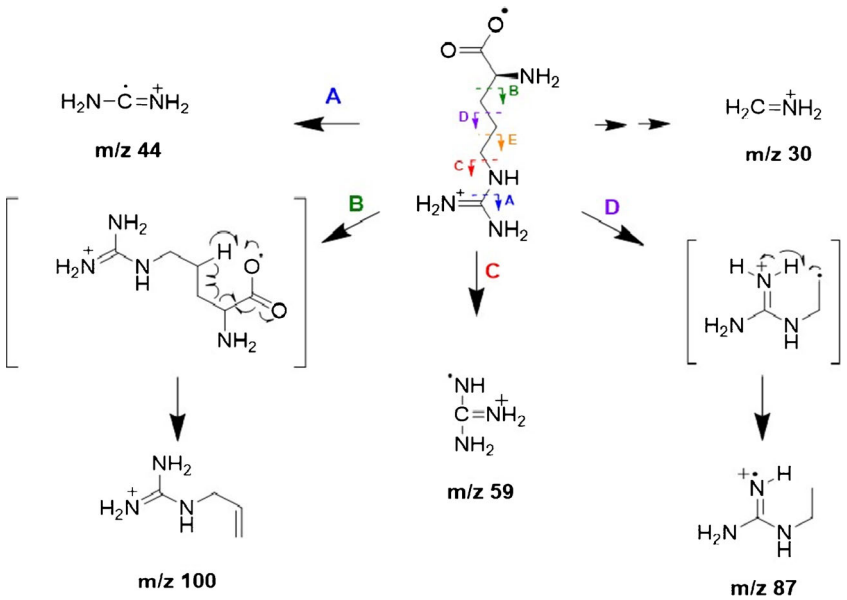
Electron-induced dissociation (EID) of protonated peptides has been proposed [24] to follow, in part, a similar fragmentation mechanism as EI, in that EID leads to a hypervalent radical that is unstable and often expels a hydrogen atom, thereby forming an energized radical cation. This ion generates many of the observed fragment ions (Scheme 1) [24]. The LIAD/EI mass spectra reported here show many of the same fragment ions that have been reported for protonated amino acids exposed to EID. For example, upon EID, protonated tryptophan yields predominantly a resonance stabilized ion of *m/z* 130 resulting from the loss of the glycyl radical [24], which is also the dominant ion upon LIAD/EI of tryptophan (Scheme 1, Supplementary Figure S9). However, the relative abundances of many of the less abundant fragment ions in these two experiments differ [24]. Further, EID produces some fragment ions that were not observed upon LIAD/EI, such as a fragment ion of *m/z* 188 resulting from the loss of NH_3 for tryptophan. This fragmentation has been proposed to occur without formation of an intermediate excited molecular ion [24]. Hence, it is not surprising that it was not observed upon LIAD/EI.

Finally, the EI mass spectra of the hydrochloride salts of the basic amino acids histidine, lysine, and arginine were measured using LIAD. Due to the low volatilities of amino acid salts, their EI mass spectra cannot generally be measured (for exceptions, see reference [5]). However, LIAD allows evaporation of intact nonvolatile salts with their counter ions and involves no heating of the analyte. The fragmentation patterns observed for the hydrochloride salts of histidine and lysine were mostly the same as for pure amino acids. Two exceptions were discovered. The hydrochloride salts showed $HCl^{+\bullet}$ ion of *m/z* 36 and its isotope (*m/z* 38) in their mass spectra (Figure 2). Further, the dominant fragment ion of *m/z* 44 (possibly with the structure shown in Figure 2) observed for lysine is almost entirely missing from the mass spectrum measured for its hydrochloride salt. This finding suggests that the protonated α-amino group likely interacts with the chloride anion in the hydrochloride salt, and that this structure fragments predominantly by formation of $HCl^{+\bullet}$ instead of the pathway that eventually yields the ion of *m/z* 44.

The hydrochloride salt of arginine provides an example of LIAD/EI fragmentation patterns that are distinctly different from previous EI experiments [27, 28] on net-neutral arginine evaporated using thermal desorption (Figure 3). When using thermal desorption, arginine decomposes before it evaporates [28]. Therefore, some differences may be attributed to ionization of neutral degradation products in this experiment. However, the *m/z* values of the larger LIAD/EI fragment ions differ by 1–3 units from those produced upon thermal desorption/EI (Figure 3).



Scheme 2. Proposed generation of a distonic radical cation upon LIAD/EI of an arginine hydrochloride salt. Note that the distonic ion fragments spontaneously as no stable distonic ions were observed in the mass spectra



Scheme 3. Proposed pathways and some mechanisms for fragmentation of the distonic molecular ion of arginine upon LIAD/EI. The proposed mechanism for the formation of the ion of *m/z* 59 via pathway C involves a simple homolytic cleavage of the indicated C–N bond. Formation of the ion of *m/z* 87 via pathway D is probably also initiated by a simple homolytic cleavage of the indicated C–C bond, followed by the rearrangement shown above

For example, while thermal desorption mass spectrum shows major ions of *m/z* 56 and 69, LIAD mass spectrum shows a major ion of *m/z* 59 and a minor ion of *m/z* 56, and a major ion of *m/z* 70 and a minor ion of *m/z* 69. Furthermore, some new fragment ions appear in the LIAD mass spectrum, such as ions of *m/z* 87 and 100.

The above findings may be explained by elimination of neutral HCl after electron ejection from the carboxylate functionality (requires 3.3 eV for acetate anion [29]) in the hydrochloride salt of arginine upon LIAD/EI (Scheme 2). The hydrochloride salt contains a zwitterionic arginine molecule that is protonated both at the guanidinium side chain and the amino group but deprotonated at the carboxylic acid group. The guanidinium side chain is more basic than the amino functionality [30, 31]. Hence, when HCl is eliminated, it likely contains the proton from the protonated amino functionality instead of the guanidium side chain, thus generating a distonic arginine radical cation (Scheme 2) instead of a traditional molecular ion. This distonic ion is unlikely to be stable [32] but instead fragments by elimination of CO_2 as well as other groups. This likely explains the differences between the EI mass spectra measured by using thermal desorption and LIAD. Pathways are proposed in Scheme 3 for the formation of some of the fragment ions from the distonic radical cation via well-accepted EI fragmentation pathways [33].

LIAD/EI Mass Spectra of Small Peptides

LIAD/EI mass spectra were measured for three dipeptides (GV, GA, and AF), three tripeptides (VGG, FGG, and AGG), and a tetrapeptide (FGGF) (Figure 4) to probe the fragmentation behavior of the molecular ions of these larger systems. Only in one instance, a stable molecular ion was observed (Figure 4b). In all cases, LIAD/EI produced a predominant a_1 fragment ion. Formation of predominant a_1 fragment ions has been reported also for EID of protonated peptides [24].

In addition to the a_1 fragment ion, molecular ions of peptides containing only aliphatic side chains (Figure 4a, b, d, and f) underwent cleavages across the entire backbone of the ion. Further, side-chain cleavages were observed for these ions, e.g., formation of the 2-methylpropenylium ion (*m/z* 55) from the valine side chain (Figure 4a and d). In contrast, EI mass spectra measured for peptides containing phenylalanine did not provide full backbone coverage (Figure 4c, e, and g). Fragmentation of these ions predominantly involved the bonds in phenylalanine, as evidenced by the formation of benzyl cations and elimination of benzyl radicals. For some peptides, losses of small neutral molecules (e.g., H_2O, CO_2, and NH_3) from the main fragment ions were also observed (Figure 4b, c, d, and f). Similar losses of small molecules have been observed during fragmentation of protonated peptides via EID (e.g., losses of CO_2 and NH_3) [22, 34–36].

The isomeric dipeptides LG and IG were selected for this study to probe whether leucine and isoleucine residues in dipeptides can be distinguished from each other by using EI mass spectrometry. The mass spectra measured for these two dipeptides are drastically different (Supporting Information, Supplementary Figures S10 and S11).

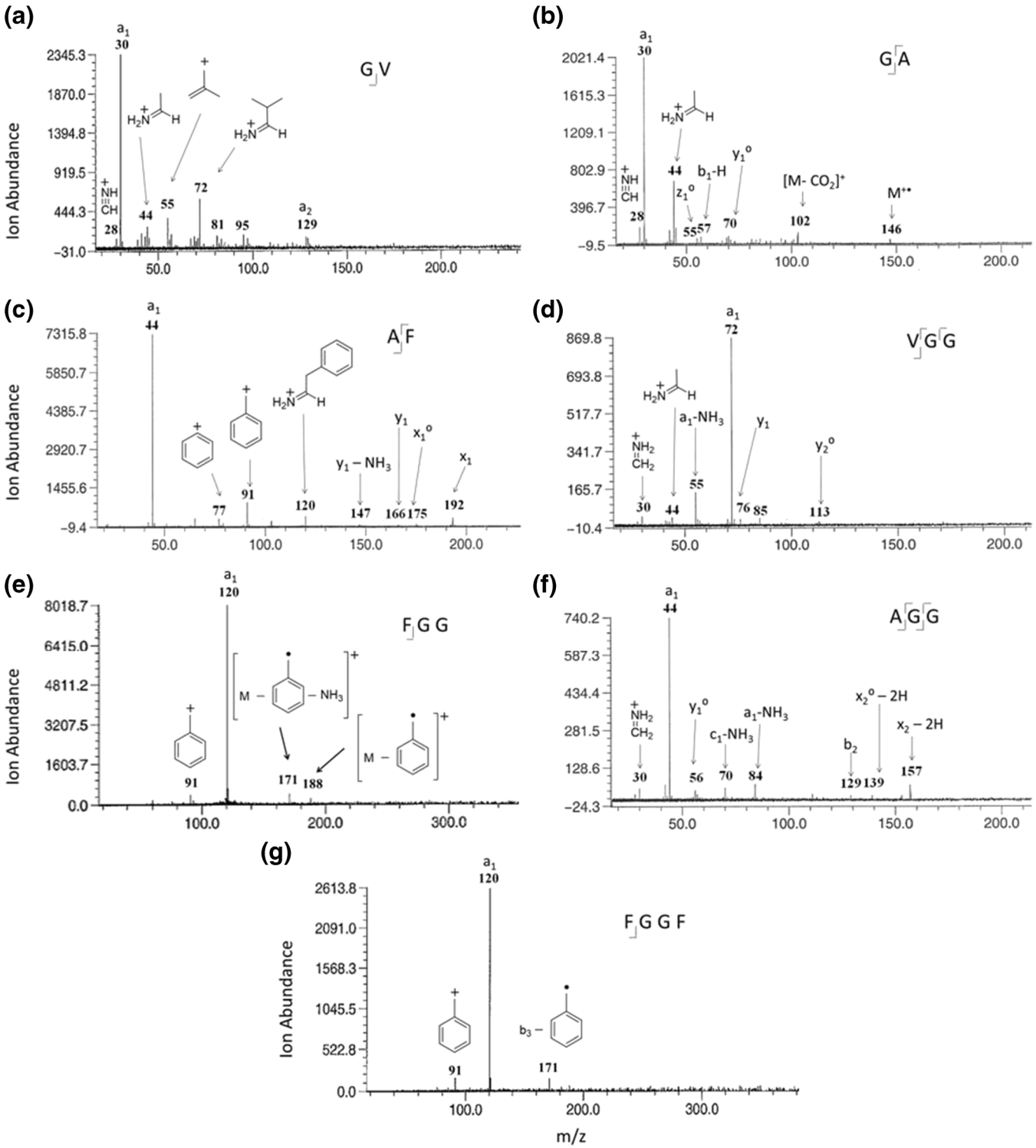


Figure 4. LIAD/EI mass spectra of (**a**) GV, (**b**) GA, (**c**) AF, (**d**) VGG, (**e**) AGG, (**f**) FGG, and (**g**) FGGF

A key difference is the observation of the losses of COOH and C_3H_6 for LG (also observed for leucine) but not for IG (nor for isoleucine). On the other hand, both isoleucine and IG produce a fragment ion C_4H_9 that is not observed for either leucine or LG. These findings suggest that EI mass spectrometry may allow differentiation of leucine and isoleucine residues in peptides.

Conclusions

LIAD has been previously shown to be an invaluable tool for the analysis of a variety of nonvolatile and thermally labile molecules [4, 6, 19]. The application of LIAD coupled with EI for the measurement of previously unattainable EI mass spectra for nonvolatile hydrochloride salts of amino acids and for

nonvolatile thermally fragile peptides is demonstrated here. The validity of LIAD/EI was first demonstrated by comparing LIAD/EI mass spectra measured for several net-neutral amino acids to previously published EI mass spectra generated for the same amino acids desorbed thermally. The mass spectra were remarkably similar. The same is true for the mass spectra measured for the hydrochloride salts of the basic amino acids histidine and lysine, with the exception of observation of a characteristic $HCl^{+\bullet}$ fragment ion for the salts. However, the hydrochloride salt of arginine behaved quite differently, which is explained by its unusually basic side chain.

LIAD/EI mass spectra were also measured for nine small peptides. These MS experiments were found to yield different information than traditional peptide sequencing methodologies based on CAD of protonated peptides that typically yield b- and y-type ions. EI-induced fragmentation predominantly produces a-type ions, similarly to EID on protonated peptides [18]. Furthermore, drastically different mass spectra were measured for isomeric dipeptides LG and IG, suggesting that this approach may allow the distinction of leucine and isoleucine residues in peptides. Unlike EID, which requires singly protonated peptide ions and MS/MS experiments, LIAD/EI is applied to neutral peptides and does not require MS/MS experiments. These features make LIAD/EI unique among the mass spectrometric techniques used to characterize peptides.

Acknowledgements

This material is based upon work supported by the National Science Foundation under grant number CHE-1464712.

References

1. de Hoffmann, E., Stroobant, V.: Mass spectrometry principles and applications, 3rd edn. John Wiley and Sons, Ltd, West Sussex (2007)
2. Vestal, M.L.: Method and apparatus for dissociating ions by electron impact. US Patent 06/918,932, March 15 (1988).
3. Lüderwald, I., Przybylski, M., Ringsdorf, H., Silberhorn, D., Kalbacher, H., Voelter, W.: Untersuchung von Isomeren Tripeptiden und Derivaten des Thyrotropin- "Releasing" Hormons durch Elektronenstoß- und Fielddesorptions- Massenspektrometrie. Zeitschrift Naturforschung B **33B**, 809–817 (1978)
4. Barrett, G.C., Elmore, D.T.: Amino acids and peptides. Cambridge University Press, Cambridge (1998)
5. Biemann, K., McCloskey, J.A.: Mass spectra of organic molecules. Amino acids. J. Am. Chem. Soc. **84**, 3192–3193 (1962)
6. Skoog, D.A., Holler, F.J., Crouch, S.R.: Principles of instrumental analysis. Thomson Brooks/Cole, Belmont (2007)
7. Cappiello, A., Famiglini, G., Palma, P.: Electron ionization for LC/MS. Anal. Chem. **75**, 1173–1179 (2003)
8. Seemann, B., Alon, T., Tsizin, S., Fialkov, A.B., Amirav, A.: Electron ionization LC-MS with supersonic molecular beams – the new concept, benefits, and applications. J. Mass Spectrom. **50**, 1252–1263 (2015)
9. For a brief review on LIAD, see: Dow, A., Wittrig, A., Kenttämaa, H.I.: Laser-induced acoustic desorption (LIAD) mass spectrometry. Eur. J. Mass Spectrom. 18, 77–92 (2012).
10. Perez, J., Ramirez-Arizmendi, L.E., Petzold, C.J., Guler, L.P., Nelson, E.D., Kenttämaa, H.I.: Laser-induced acoustic desorption/chemical ionization in Fourier transform ion cyclotron resonance mass spectrometry. Int. J. Mass Spectrom. **198**, 173–188 (2000)
11. Shea, R.C., Habicht, S.C., Vaughn, W.E., Kenttämaa, H.I.: Design and characterization of a high-power laser-induced acoustic desorption probe coupled with a Fourier transform ion cyclotron resonance mass spectrometer. Anal. Chem. **79**, 2688–2694 (2007)
12. Shea, R.C., Petzold, C.J., Campbell, J.L., Li, S., Aaserud, D.J., Kenttämaa, H.I.: Characterization of laser-induced acoustic desorption coupled with a Fourier transform ion cyclotron resonance mass spectrometer. Anal. Chem. **78**, 6133–6139 (2006)
13. Shea, R.C., Petzold, C.J., Liu, J.A., Kenttämaa, H.I.: Experimental investigations of the internal energy of molecules evaporated via laser-induced acoustic desorption into a Fourier transform ion cyclotron resonance mass spectrometer. Anal. Chem. **79**, 1825–1832 (2007)
14. Crawford, K., Campbell, J., Fiddler, M., Duan, P., Qian, K., Gorbaty, M., Kenttämaa, H.I.: Laser-induced acoustic desorption/Fourier transform ion cyclotron resonance mass spectrometry for petroleum distillate analysis. Anal. Chem. **77**, 7916–7923 (2005)
15. Pinkston, D., Duan, P., Gallardo, V., Habicht, S., Tan, X., Qian, K., Gray, M., Müllen, K., Kenttämaa, H.I.: Analysis of asphaltenes and asphaltene model compounds by laser-induced acoustic desorption/Fourier transform ion cyclotron resonance mass spectrometry. Energy Fuel **23**, 5564–5570 (2009)
16. Fenn, J.B., Mann, M., Meng, C.K., Wong, S.F., Whitehouse, C.M.: Electrospray ionization for mass-spectrometry of large biomolecules. Science **246**, 64–71 (1989)
17. Karas, M., Hillenkamp, F.: Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. Anal. Chem. **60**, 2299–2301 (1988)
18. Nordhoff, E., Ingendoh, A., Cramer, R., Overberg, A., Stahl, B., Karas, M., Hillenkamp, F., Crain, P.F.: Matrix-assisted laser desorption ionization mass-spectrometry of nucleic-acids with wavelengths in the ultraviolet and infrared. Rapid Commun. Mass Spectrom. **6**, 771–776 (1992)
19. Wells, J., McLuckey, S.: Collision-induced dissociation (CID) of peptides and proteins. Methods Enzymol. **402**, 148–185 (2005)
20. Zubarev, R.A., Kelleher, N.L., McLafferty, F.W.: Electron capture dissociation of multiply charged protein cations. A nonergodic process. J. Am. Chem. Soc. **120**, 3265–3266 (1998)
21. Zubarev, R.A., Kruger, N.A., Fridriksson, E.K., Lewis, M.A., Horn, D.M., Carpenter, B.K., McLafferty, F.W.: Electron capture dissociation of gaseous multiply-charged proteins is favored at disulfide bonds and other sites of high hydrogen atom affinity. J. Am. Chem. Soc. **121**, 2857–2862 (1999)
22. Zubarev, R.: Reactions of polypeptide ions with electrons in the gas phase. Mass Spectrom. Rev. **22**, 57–77 (2003)
23. Wang, B.-H., McLafferty, F.W.: Electron impact excitation of ions from larger organic molecules. Org. Mass Spectrom. **25**, 554–556 (1990)
24. Lioe, H., O'Hair, R.A.: Comparison of collision-induced dissociation and electron-induced dissociation of singly protonated aromatic amino acids, cystine, and related simple peptides using a hybrid linear ion trap-FT-ICR mass spectrometer. Anal. Bioanal. Chem. **389**, 1429–1437 (2007)
25. Duan, P., Qian, K., Habicht, S.C., Pinkston, D.S., Fu, M.K., Kenttämaa, H.I.: Analysis of base oil fractions by ClMn(H2O)(+) chemical ionization combined with laser-induced acoustic desorption/Fourier transform ion cyclotron resonance mass spectrometry. Anal. Chem. **80**, 1847–1853 (2008)
26. Petzold, C.J., Ramirez-Arizmendi, L.E., Heidbrink, J.L., Perez, J., Kenttämaa, H.I.: Gas-phase reactions of charged phenyl radicals with neutral biomolecules evaporated by laser-induced acoustic desorption. J. Am. Soc. Mass Spectrom. **13**, 192–194 (2002)
27. Stein, S.E.: In NIST Chemistry WebBook, NIST Standard Reference Database Number 69; Linstrom, P.J., Mallard, W.G. (Eds.) NIST Mass Spec Data Center: Gaithersburg, MD (2014).
28. Johnstone, R.A.W.: Mass spectrometry for chemists and biochemists, 2nd edn. Cambridge University Press, New York (1996)
29. Wang, X.-B., Woo, H.-K., Wang, L.-S., Minofar, B., Jungwirth, P.: Determination of the electron affinity of the acetyloxyl radical (CH3COO) by low-temperature anion photoelectron spectroscopy and ab initio calculations. J. Phys. Chem. A **110**, 5047–5050 (2006)
30. Price, W.D., Jockusch, R.A., Williams, E.R.: Is arginine a zwitterion in the gas phase? J. Am. Chem. Soc. **119**, 11988–11989 (1997)
31. Rak, J., Skurski, P., Simons, J., Gutowski, M.: Low-energy tautomers and conformers of neutral and protonated arginine. J. Am. Chem. Soc. **123**, 11695–11707 (2001)
32. Ke, Y., Zhao, J., Verkerk, U.H., Hopkinson, A.C., Siu, M.K.W.M.: Histidine, lysine, and arginine radical cations: isomer control via the choice of auxiliary ligand (L) in the dissociation of $[Cu^{II}(L)(\text{amino acid})]^{\bullet 2+}$ complexes. J. Phys. Chem. B **111**, 14318–14328 (2007)

33. McLafferty, F.W., Tureček, F.: Interpretation of mass spectra. University Science Books, Sausalito (1993)
34. Budnik, B.A., Haselmann, K.F., Zubarev, R.A.: Electron detachment dissociation of peptide di-anions: an electron–hole recombination phenomenon. Chem. Phys. Lett. **342**, 299 (2001)
35. Fung, Y., Adams, C., Zubarev, R.: Electron ionization dissociation of singly and multiply charged peptides. J. Am. Chem. Soc. **131**, 9977–9985 (2009)
36. Zubarev, R., Haselmann, K., Budnik, B., Kjeldsen, F., Jensen, F.: Account: towards an understanding of the mechanism of electron-capture dissociation: a historical perspective and modern ideas. Eur. J. Mass Spectrom. **8**, 337–349 (2002)