Dissociation of the N–C_α Bond and Competitive Formation of the $[z_n - H]^{\bullet +}$ and $[c_n + 2H]^+$ Product Ions in Radical Peptide Ions Containing Tyrosine and Tryptophan: The Influence of Proton Affinities on Product Formation

Chi-Kit Siu, Yuyong Ke, Galina Orlova,* Alan C. Hopkinson, and K. W. Michael Siu

Department of Chemistry and Centre for Research in Mass Spectrometry, York University, Toronto, Ontario, Canada

Dissociations at the N–C_α bond of tryptophan and tyrosine residues are the prevalent pathways in the fragmentations of radical cations of tripeptides that contain such as residues. This process involves a proton transfer from the β-carbon of the tryptophan or tyrosine residue to the carbonyl oxygen of the amide group, followed by cleavage of the N–C_α bond, generating low-lying proton-bound dimers that dissociate to give each an ionic and a neutral product. Formation of the $[z_n - H]^{\bullet +}$ or $[c_n + 2H]^+$ ion is a competition between the two incipient fragments for the proton in a dissociating proton-bound dimer. (J Am Soc Mass Spectrom 2008, 19, 1799–1807) © 2008 Published by Elsevier Inc. on behalf of American Society for Mass Spectrometry

Since the initial discovery that selected radical peptide ions can be efficiently formed via collision-induced dissociation (CID) of a copper (II) ternary complex containing an oligopeptide ligand [1–3], there has been a flourish of activity to the extent that the formation and dissociation of radical peptide ions now constitute a growing research area [4–10]. In the ternary complex $[Cu^{II}(L)(M)]^{\bullet 2+}$, where L is an auxiliary ligand and M the oligopeptide, the first auxiliary ligands used were diethylenetriamine (dien) and 2,2′:6′,2″-terpyridine (tpy); most peptides examined initially contained tyrosine or tryptophan as a residue of low ionization energy [1–3]. In a series of articles, Chu and coworkers [4–6] established that judicious choice of the auxiliary ligand can lead to generation of radical peptide ions from virtually any oligopeptides, even those comprising only aliphatic residues; in particular, the use of a sterically encumbered macrocycle, including 1,4,7,10-tetraoxacyclododecane (12-crown-4) and 1,4,7-triazacyclononane (tacn), has a dramatic effect on the yield of $GGX^{\bullet +}$, where X = an aliphatic amino acid residue. A similar effect was also demonstrated using 6,6″-dibromo-2,2′:6′,2″-terpyrine as the auxiliary ligand. O'Hair and coworkers [7] demonstrated the first and only successful non-Cu-based complex system for generating $M^{\bullet +}$; one that involves a trivalent metal ion, Cr^{3+}, Mn^{3+}, Fe^{3+}, and Co^{3+}, the dianionic *N*,*N*′-ethylenebis(salicylideneiminato) as the auxiliary ligand, and M to give a singly charged complex, whose CID under appropriate conditions yields abundant $M^{\bullet +}$. This complex system has been adopted and extensively investigated by Laskin and coworkers [8] using surface-induced dissociation via Fourier-transform ion cyclotron resonance mass spectrometry. Thus, the generation of $M^{\bullet +}$ via redox chemistry has now been established under various time regimes as well as under different instrumental and dissociation conditions.

The dissociations of radical peptide ions are interesting and display rich chemistries, as they differ substantially from those of their protonated counterparts. Under low-energy CID conditions, the dissociations of protonated peptides are largely charge-driven with the mobile proton inducing cleavage principally at the peptide bonds, giving *b*- and *y*-type ions [11, 12]. By contrast, the fragmentations of radical peptide ions are more varied and can be charge- or radical-driven; many of the latter reactions take place at the side chain, e.g., the eliminations of *p*-quinomethide and 3-methylene-3*H*-indole from tyrosine and tryptophan, respectively

Address reprint requests to Dr. K. W. M. Siu, Department of Chemistry and Centre for Research in Mass Spectrometry, York University, 4700 Keele St., Toronto, ON, Canada M3J 1P3. E-mail:kwmsiu@yorku.ca

* Current address: Department of Chemistry, St. Francis Xavier University, Antigonish, Nova Scotia, Canada B2G 2W5.

1044-0305/08/$32.00
doi:10.1016/j.jasms.2008.09.026

Published online October 1, 2008
Received July 24, 2008
Revised September 29, 2008
Accepted September 29, 2008

[1–3, 12], $CH_3^{\bullet}$ and $CH_3CH_2^{\bullet}$ from isoleucine, and $(CH_3)_2CH^{\bullet}$ from leucine [9]. Many of these radical-driven reactions are competitive, probably because the peptides contain the arginine residue which, owing to its high proton affinity (PA), tends to sequester the proton [10]. In a recent study on isomeric radical ions of the simplest tripeptides, $[G^{\bullet}GG]^+$, $[GG^{\bullet}G]^+$, and $[GGG^{\bullet}]^+$, it was found that the barriers against interconversions among these triglycine isomers are ≥44.7 kcal/mol [13], significantly higher than those against tautomerisms of protonated triglycine isomers at <17 kcal/mol [14]. Charge-driven dissociations in both protonated triglycine and triglycine radical cations lead to cleavage of the peptide bonds, producing the b_2^+ and $[b_2 - H]^{\bullet +}$ ion, respectively.

Bagheri-Majdi et al. [3] reported another type of peptide backbone dissociation, one that involves cleavage of the N–C_α bond of the tryptophan residue to produce the $[z_n - H]^{\bullet +}$ ion in oligopeptides that contain glycines as the only other residues. Activation of the N–C_α bonds, giving *c*- and *z*-type ions, is common in other types of dissociation techniques, namely electron-capture dissociation (ECD) [15] and electron-transfer dissociation (ETD) [16], that produce radical cations as a result of, respectively, electron-capture by or electron-transfer to multiply protonated peptide ions [17, 18]. Plausible mechanisms have been examined theoretically; they commonly involve an aminoketyl radical intermediate that can undergo homolytic cleavage of the adjacent N–C_α bond to give an ion-molecule complex between the *c*- and *z*-incipient fragments, with the former being an amide [19–22]. Similarly, in the CID of $[GWG]^{\bullet +}$, the neutral that is lost leading to the observation of abundant $[z_2 - H]^{\bullet +}$ was also postulated to be glycinamide, while in the CID of $[GGW]^{\bullet +}$ the neutral that accompanies the abundant $[z_1 - H]^{\bullet +}$ ion is glycylglycinamide [3, 23]. A mechanism that involves the migration of a β-hydrogen to the N-terminal side followed by a heterolytic cleavage of the N–C_α bond has been proposed [3]. Similar fragmentations have been observed in the cleavage of the N–C_α bond of the tyrosine residue; significantly, the dissociation of $[GYG]^{\bullet +}$ exhibits both the $[z_2 - H]^{\bullet +}$ ion and the $[c_1 + 2H]^+$ ion, while that of $[GGY]^{\bullet +}$ shows only the $[c_2 + 2H]^+$ ion, as the abundant products from cleavage of the tyrosyl N–C_α bond [24]. The most probable structures for the $[c_1 + 2H]^+$ and $[c_2 + 2H]^+$ ions are protonated glycinamide and glycylglycinamide, respectively. Here we compare dissociation reactions of the tryptophan- and tyrosine-containing series of tripeptides, and propose fragmentation mechanisms that account for these observations. For ease of presentation, we will describe these tripeptides as "triglycine derivatives" containing a "heteroresidue" (tyrosine or tryptophan) in different positions. Density functional theory (DFT) at the B3LYP level of theory in conjunction with the 6-311++G(d,p) basis set are used to determine the most plausible fragmentation pathways.

Experimental

Mass Spectrometry

Experiments were performed on a commercially available ion-trap mass spectrometer (Finnigan-MAT LCQ; San Jose, CA) and a prototype triple-quadrupole instrument of the commercially available SCIEX API 3000; Concord, ON, Canada). Both mass spectrometers were equipped with electrospray ionization sources, with 4.5 kV being the optimal electrospray potential on the LCQ and 5.5 kV on the API 3000. Samples were typically 100 μM in $Cu(ClO_4)_2$, 100 μM in the amine ligand—tpy, tacn, or dien—and 100 μM in peptide in 50/50 water/methanol solutions. These were infused for electrospray at 2~3 μL/min. Ion lineage was determined using successive stages of mass spectrometry. On the LCQ, the radical cation was formed by CID of the $[Cu^{II}(L)M]^{\bullet 2+}$ complex in the ion trap as the first stage, while on the API 3000, the same ion was formed via in-source CID. The relative collision energy on the LCQ was typically 4% to 20%, whereas the laboratory collision energy on the API 3000 was 2 to 14 eV.

All chemicals and solvents were available from Sigma-Aldrich (St. Louis, MO). Peptides were purchased from Bachem BioSciences (King of Prussia, PA) or from Sigma-Aldrich. All materials were used as received.

Computational Methods

Calculations were performed using the Gaussian03 quantum chemical program [25]. The total energies of the radical peptide ions were calculated using the unrestricted open-shell formalism, UB3LYP [26, 27]. Preliminary calculations with a Gaussian-type double-ζ 6-31++G(d,p) basis set were used to sketch out potential energy surfaces (PESs) and obtain molecular geometries before optimization with a larger triple-ζ 6-311++G(d,p) basis set; these basis sets include polarization and diffuse functions on all atoms. Local minima and transition structures were optimized and verified by means of harmonic vibrational frequency analyses. Zero-point vibrational energies were evaluated directly using the normal-mode vibrational frequencies without anharmonic scaling. The local minima associated with each transition structure were identified using the intrinsic reaction coordinates method [28]. Natural population analyses were used to determine atomic charges and spin densities [29].

Results and Discussion

Figure 1 shows the CID spectra of (a) $[WGG]^{\bullet +}$, (b) $[YGG]^{\bullet +}$, (c) $[GGW]^{\bullet +}$, (d) $[GGY]^{\bullet +}$, (e) $[GWG]^{\bullet +}$, and (f) $[GYG]^{\bullet +}$; many of the fragment ions are products of N–C_α bond cleavages at the heteroresidues. These include the $[z_3 - H]^{\bullet +}$ ions from $[WGG]^{\bullet +}$ and $[YGG]^{\bullet +}$, the $[z_1 - H]^{\bullet +}$ ion from $[GGW]^{\bullet +}$, the $[c_2 + 2H]^+$ ions from $[GGW]^{\bullet +}$ and $[GGY]^{\bullet +}$, the $[z_2 - H]^{\bullet +}$ ions from

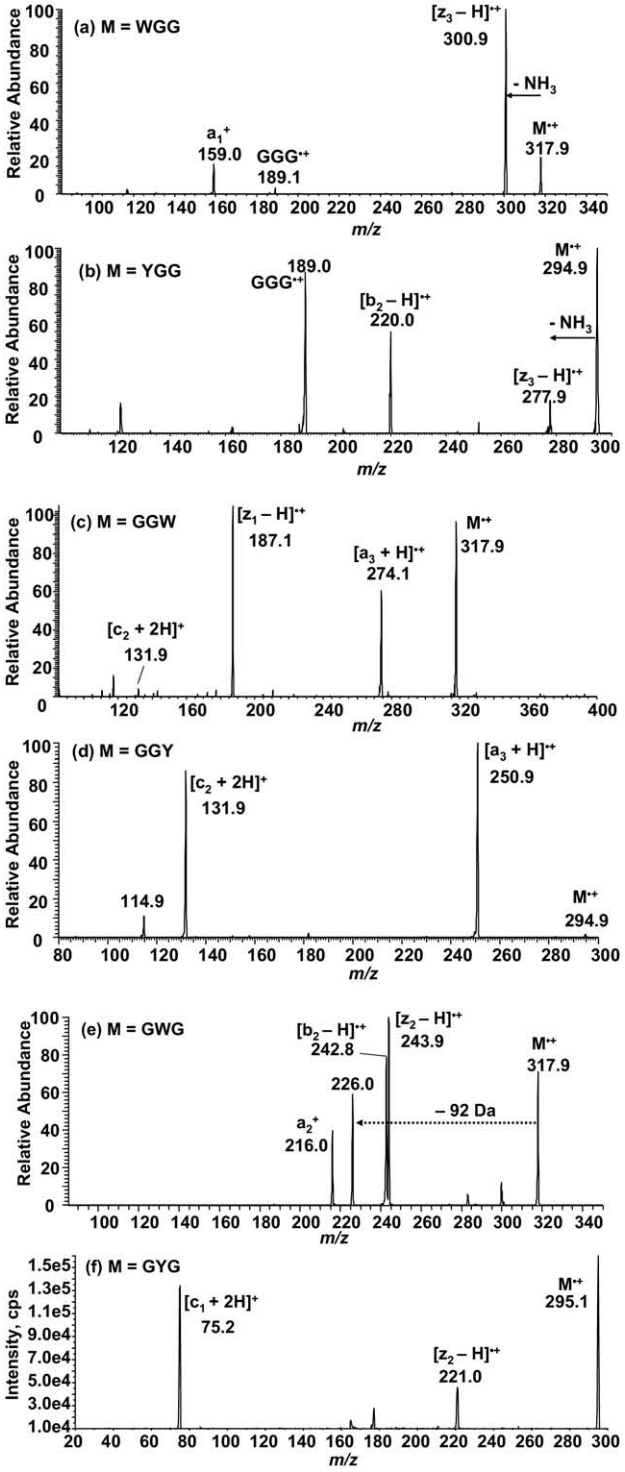


Figure 1. CID spectra of (**a**) $[WGG]^{\bullet+}$ at a relative collision energy of 8%, (**b**) $[YGG]^{\bullet+}$ at 10%, (**c**) $[GGW]^{\bullet+}$ at 8%, (**d**) $[GGY]^{\bullet+}$ at 10%, (**e**) $[GWG]^{\bullet+}$ at 10%, and (**f**) $[GYG]^{\bullet+}$ at a laboratory collision energy of 10 eV.

$[GWG]^{\bullet+}$ and $[GYG]^{\bullet+}$, and the $[c_1 + 2H]^+$ ion from $[GYG]^{\bullet+}$. Other prominent fragment ions include $[GGG]^{\bullet+}$ (more precisely $[G^{\bullet}GG]^+$ [13] formed by C_α–C_β cleavage of the side-chain of $[WGG]^{\bullet+}$ and especially $[YGG]^{\bullet+}$, the $[b_2 – H]^{\bullet+}$ ions from cleavage of the second peptide linkage in $[YGG]^{\bullet+}$ and $[GWG]^{\bullet+}$ plus elimination of the C-terminal glycine residue, and the $[a_3 + H]^{\bullet+}$ ions from cleavage of the C_α–CO_2H bond in $[GGW]^{\bullet+}$ [3, 23] and $[GGY]^{\bullet+}$ plus elimination of CO_2.

Formation of the $[z_n – H]^{\bullet+}$ ion requires nominally transfer of a hydrogen from the C-terminal side to the N-terminal side of the N–C_α bond that cleaves. The origin of this hydrogen is examined here by means of a combination of CID experiments and DFT calculations. Figure 2 shows the CID spectra of derivatives of Trp and Tyr. Loss of CH_3CONH_2 from the radical cations of both *N*-acetyl-tryptophan methyl ester $[AcWOMe]^{\bullet+}$ and *N*-acetyl-tyrosine methyl ester $[AcYOMe]^{\bullet+}$ is in accordance with the expectation that the hydrogen transferred is not the carboxylic hydrogen. Transfer of a hydrogen from the β-carbon or the α-carbon would generate, respectively, a benzylic radical or α-radical, both judged to be quite favorable in energy [23, 30, 31]. The transition structures for these hydrogen transfers are located theoretically employing DFT calculations (Figure 3). The energies of the transition structures for hydrogen migration from the α-carbon to the amide oxygen are $\geq$30 kcal mol^{-1}, which are much higher than those from the β-carbon to the same amide oxygen ($\leq$ 16 kcal/mol). Consequently, the most probable hydrogen transferred is the benzylic-type hydrogen on the β-carbon.

Bagheri-Majdi et al. [3] have suggested a similar mechanism in the dissociation of radical cations of di- and tripeptides, e.g., in the CID of $[WG]^{\bullet+}$, transfer of a benzylic-type hydrogen on the β-carbon as a proton to the NH_2 group leaves the radical center formally on the β-carbon; delocalization of the radical center over the tryptophanyl ring lowers the energy of the resultant structure, which dissociates to give the $[z_2 – H]^{\bullet+}$ ion and NH_3. For peptides where the tryptophan residue is

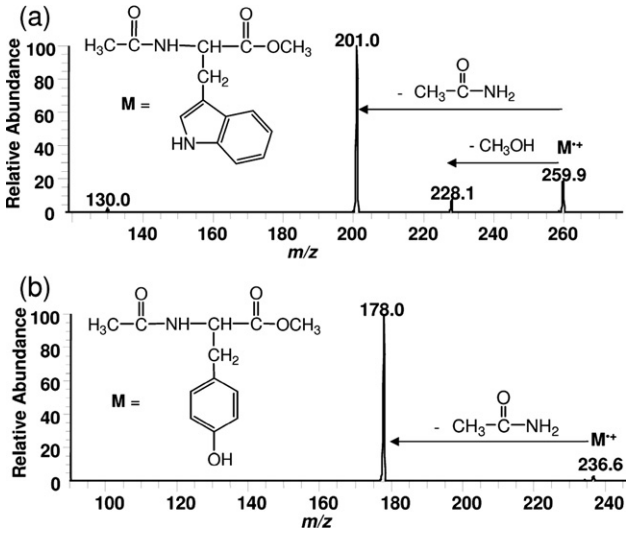


Figure 2. CID spectra of (**a**) $[AcWOMe]^{\bullet+}$ and (**b**) $[AcYOMe]^{\bullet+}$ at a relative collision energy of 8%.

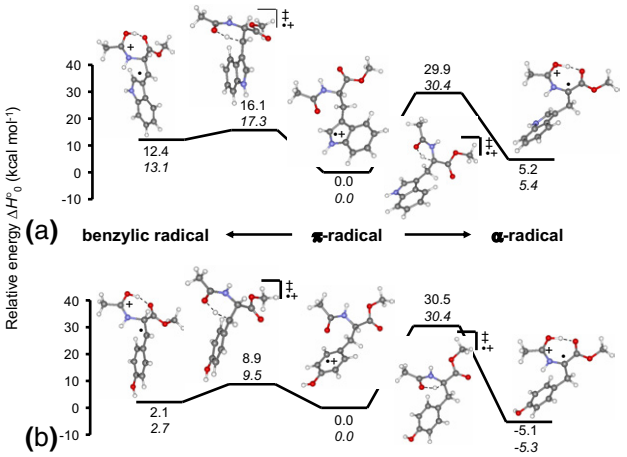


Figure 3. Potential energy surface for the isomerization of (**a**) $[AcWOMe]^{\bullet+}$ and (**b**) $[AcYOMe]^{\bullet+}$. Upper values are ΔH°_0 and lower, italicized values are ΔG°_{298} (kcal mol^{-1}) at B3LYP/6-311++G(d,p).

not in the N-terminal position, e.g., GWG and GGW, the neutral eliminated was postulated to be an amide; thus the neutral eliminated in the former example with formation of the $[z_2 - H]^{\bullet+}$ ion is glycinamide and that in the latter example glycylglycinamide. As the $[z_2 - H]^{\bullet+}$ ion is isobaric with the b_2^+ ion in GWG and GYG, the assignments of $[z_2 - H]^{\bullet+}$ are verified with the CIDs of $[GWA]^{\bullet+}$ and $[AWG]^{\bullet+}$ for the former, and $[GYA]^{\bullet+}$ and $[AYG]^{\bullet+}$ for the latter triglycine derivatives.

Similarly, cleavage of the N–C_α bond to give the $[c_n + 2H]^+$ ion requires nominally transfer of a proton from the C- to the N-terminal fragment. As discussed earlier, the $[c_1 + 2H]^+$ and the $[c_2 + 2H]^+$ ions have been proposed to be protonated glycinamide and protonated glycylglycinamide, respectively [24]. Figure 4 shows the CID spectra of (a) the $[c_2 + 2H]^+$ ion from the fragmentation of $[GGY]^{\bullet+}$ and (b) protonated glycylglycinamide; the two product ion spectra are virtually identical, showing the loss of NH_3 as the only and prominent dissociation reaction. Thus these results are in accordance with our earlier interpretations [24]. A question that should be addressed is why the CID of $[GGW]^{\bullet+}$ gives a prominent $[z_1 - H]^{\bullet+}$ ion, whereas that of $[GGY]^{\bullet+}$ gives an abundant $[c_2 + 2H]^+$ ion, especially as both are products of N–C_α bond cleavage at the heteroresidues.

Formation of the $[z_n - H]^{\bullet+}$ Ion

The dissociation of $[GW]^{\bullet+}$ gives abundant $[z_1 - H]^{\bullet+}$ [3] and will be used here as the prototypical example to illustrate the mechanism that is proposed for formation of $[z_n - H]^{\bullet+}$ in general. Figure 5a shows the PES for the dissociation of $[GW]^{\bullet+}$: the upper values are relative energies at zero temperature (ΔH°_0), while the lower, italicized values are relative free energies at 298 K (ΔG°_{298}), both in kcal mol^{-1}. Ion **1**, a π-radical cation, is the $[GW]^{\bullet+}$ structure at the global minimum. Proton transfer from the β-carbon to the carbonyl oxygen on the peptide linkage gives the distonic ion **2**, in which the radical (as shown on the β-carbon) is delocalized onto the indole π-system [3]. Rotation about the N_2–C_α bond (the italicized subscript hereon refers to the residue number) and formation of the hydrogen-bond $C_1OH^+\cdots OC_2$ gives ion **3**, which is only 7.8 kcal mol^{-1} higher in enthalpy than **1**. Heterolytic cleavage of the N_2–C_α bond and both charge and radical retention on the C-terminal fragment results in a proton-bound dimer of the $[z_1 - H]^{\bullet+}$ ion and glycinamide as its iminol tautomer, $NH_2CH_2C(OH) = NH$, **4A** (Figure 5b). Direct dissociation of **4A** yields product pair **7**; this reaction step is endothermic by 12.5 kcal mol^{-1} (products **7** are 26.9 kcal mol^{-1} above **1**, the structure of $[GW]^{\bullet+}$ at the global minimum). Alternatively, rotation of the indole moiety gives a lower energy ion-neutral **4B**, containing a hydrogen bond (1.677 Å) between indole-NH and the iminol nitrogen of the glycinamide, which is much stronger than that (1.894 Å) in **4A**. A proton transfer from the oxygen to the nitrogen within the iminol moiety generates the more stable canonical glycinamide, which can hydrogen bond to the carboxylic group (**4C**) or the indole-NH group via the amino nitrogen (**4D**) or the amide oxygen (**4E**) of the incipient $[z_1 - H]^{\bullet+}$ ion. Rotations of large groups within ion-neutral complexes are relatively commonplace and are achieved via low barriers [32–34].

The lowest energy structure, **4E**, can dissociate yielding product pair **5**. Internal proton transfer in **4D**, followed by dissociation can give another product pair, **6**, comprising protonated glycinamide, or $[c_1 + 2H]^+$, and the indolyl radical. However, product pair **6** is higher in enthalpy than **5** by 10.2 kcal mol^{-1}, which means the former is noncompetitive versus the latter. The rate-determining step in the dissociation to give

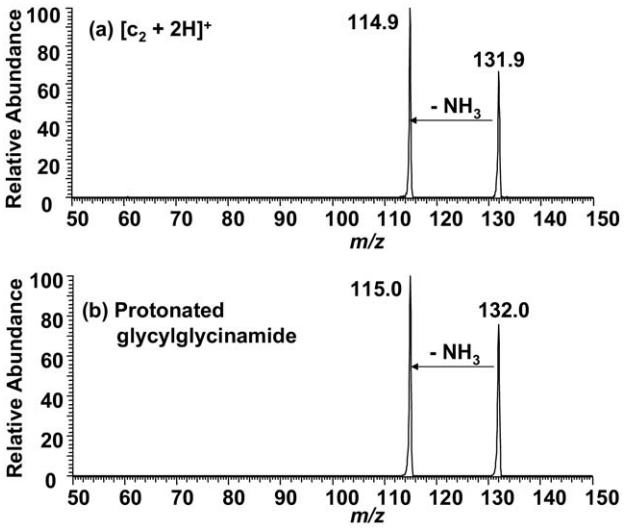


Figure 4. CID spectra of (**a**) the $[c_2 + 2H]^+$ ion from $[GGY]^{\bullet+}$ and (**b**) protonated glycylglycinamide, both at relative collision energies of 6%.

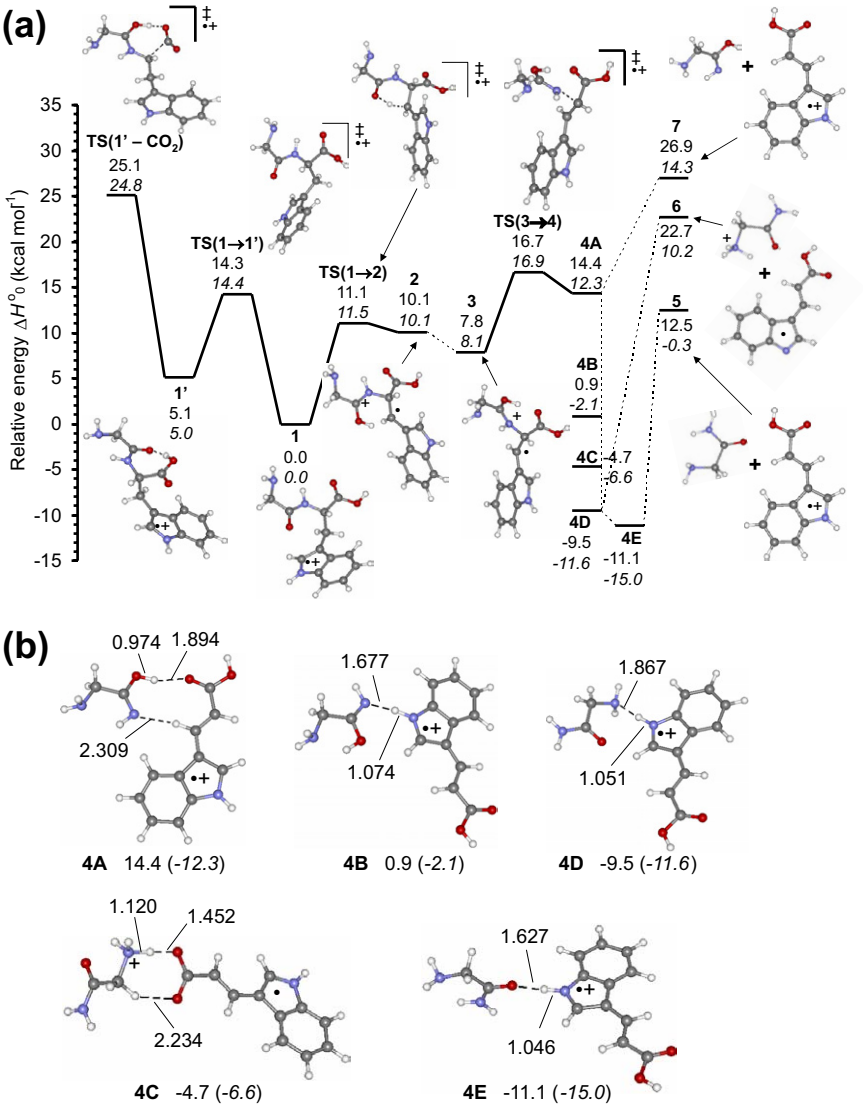


Figure 5. (**a**) Potential energy surface for the dissociation of $[GW]^{\bullet+}$. Upper values are ΔH°_0 and lower, italicized values are ΔG°_{298} (kcal mol^{-1}) at B3LYP/6-311++G(d,p). Energy levels of the minima associated with each transition structure confirmed by IRC calculations are connected by a solid line; otherwise broken lines are used. (**b**) Structures of the proton-bound dimers **4** in (**a**).

products **5** is the cleavage of the N–C_α bond for which the barrier (**TS(3→4)**) is relatively low at 16.7 kcal mol^{-1}. This is to be contrasted with the critical barrier against the loss of CO_2, for which the enthalpy is higher at 25.1 kcal mol^{-1}, via another π-radical intermediate **1'** formed by rotation about bonds of **1**. These DFT predictions are consistent with experimental observations that show the $[z_1 - H]^{\bullet+}$ ion as the predominant product [3, 23].

Formation of the $[c_1 + 2H]^+$ Ion

As in the previous section, we use the dissociation of $[GY]^{\bullet+}$ as the prototypical example that leads to the $[c_n + 2H]^+$ ion (the CID spectrum of $[GY]^{\bullet+}$ is shown in Supporting Information, which can be found in the electronic version of this article). The PES is shown in Figure 6a. Ion **8** is a π-radical cation, the equivalent of **1** for $[GW]^{\bullet+}$. Transfer of a proton from the β-carbon to the glycyl carbonyl oxygen gives the distonic ion **9**. Interestingly, both this ion and the barrier leading to it are considerably lower than their equivalents on the PES of $[GW]^{\bullet+}$, consistent with the fact that the radical cation is accommodated better by the indole side-chain of tryptophan than by the phenol side-chain of tyrosine [30, 31]. Rotation about the C_1^+–OH bond on the glycine residue gives ion **10**; this is followed by rotation about the C_α–C_1 bond to give ion **11**, which is stabilized by a strong hydrogen-bond (1.784 Å) between the amino nitrogen and the proton on the glycyl carbonyl oxygen. Heterolytic cleavage of the N_2–C_α bond gives the ion-neutral complex **12A** (Figure 6b), which is a phenol radical cation solvated by an iminol via two very weak hydrogen bonds between the iminol nitro-

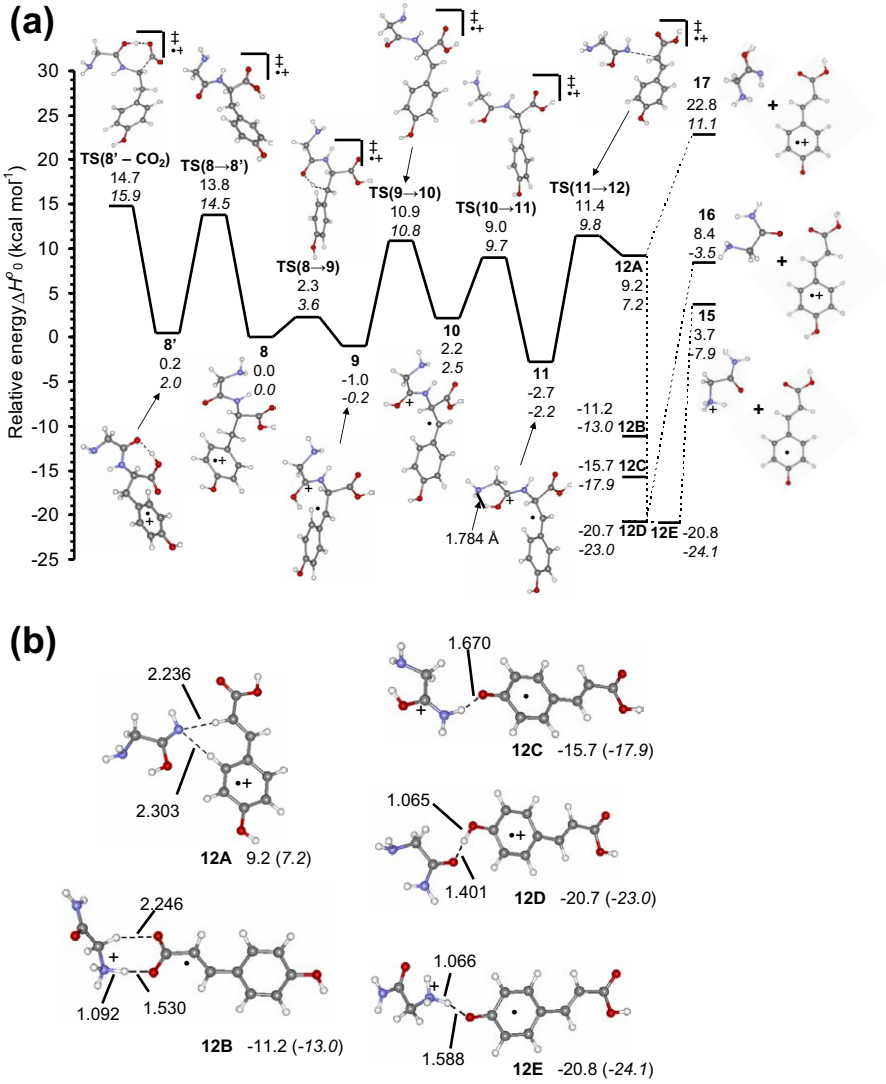


Figure 6. (**a**) Potential energy surface for the dissociation of $[GY]^{\bullet +}$. Upper values are ΔH°_0 and lower, italicized values are ΔG°_{298} (kcal mol^{-1}) at B3LYP/6-311++G(d,p). Energy levels of the minima associated with each transition structure confirmed by IRC calculations are connected by a solid line; otherwise broken lines are used. (**b**) Structures on the proton-bound dimers **12** in (**a**).

gen and the C_{α}-H and C_{ortho}-H hydrogens (2.236 Å and 2.303 Å, respectively). Direct dissociation to products **17** is endothermic by 13.6 kcal mol^{-1} (or 22.8 kcal mol^{-1} relative to **8**). Forming two stronger hydrogen bonds between the carboxylic group and the amide tautomer of glycinamide gives a lower energy complex **12B** (−11.2 kcal mol^{-1} versus **8**). As with the ion-neutral complexes of $[GW]^{\bullet +}$, starting from **12A**, rotation of the phenol moiety and transfer of the phenolic proton to the iminol nitrogen gives a third ion-neutral complex **12C**, a protonated glycinamide solvated by a phenoxy radical in which the hydrogen bond (1.670 Å) between the amide hydrogen and the phenoxy oxygen is strong. Rotation of the protonated glycinamide followed by proton transfer from the carbonyl oxygen to the phenoxy oxygen produces **12D**; this ion-neutral complex is almost isoenergetic to **12E**, the lowest-energy structure on the PES (−20.8 kcal mol^{-1}). Complex **12E** is protonated glycinamide solvated by the phenoxy radical via a short hydrogen bond (1.588 Å); cleavage of the hydrogen bond gives products **15**, protonated glycinamide, or $[c_1 + 2H]^+$, and the phenoxy radical. Alternatively, internal proton transfer and cleaving the N–H bond of **12E** gives the higher enthalpy products **16**, the phenoxy radical cation and neutral glycinamide. These products can also be formed by cleavage of the hydrogen bond in complex **12D**. Products **16** are only 4.7 kcal mol^{-1} above **15** in enthalpy. Figure 7 also shows an alterative, but noncompetitive, route to products **16**. Proton transfer from the carbonyl oxygen to the amino nitrogen in ion **11** gives **13**. Rotation about the $C_{\alpha} - C_1$ bond and proton transfer from the ammonium nitrogen to the amide nitrogen then ensues, resulting in ion **14**. Cleavage of the N_2–C_{α} bond gives product **16**. This mechanism is noncompetitive as the critical transition-state, **TS(13→14)**, is higher than the critical transition-state of

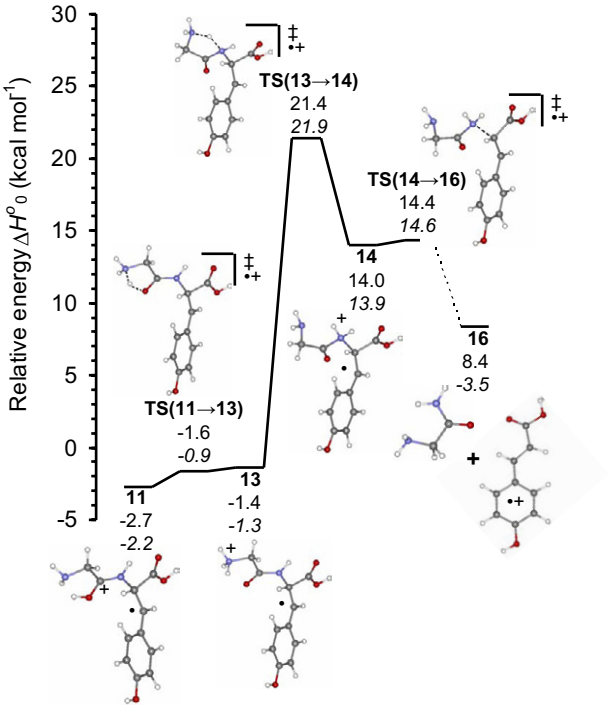


Figure 7. Potential energy surface for the alternative dissociation route of $[GY]^{\bullet+}$. Upper values are $\Delta H°_0$ and lower, italicized values are $\Delta G°_{298}$ (kcal mol^{-1}) at B3LYP/6-311++G(d,p). Energy levels of the minima associated with each transition structure confirmed by IRC calculations are connected by a solid line; otherwise broken lines are used.

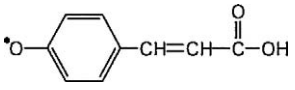
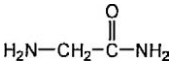
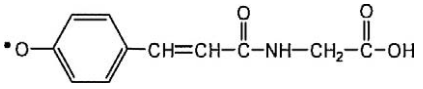
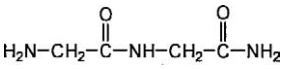
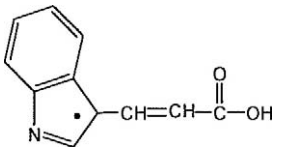
the lower energy pathway, **TS(11→12)**, by 10.0 kcal mol^{-1}.

Proton-Competition Reactions

McLuckey et al. [35] discovered a linear correlation between the logarithm of the product ion abundance ratios, $[\mathbf{A} + H]^+/[\mathbf{B} + H]^+$, in the CID of proton-bound dimers $[\mathbf{A}\cdots H\cdots \mathbf{B}]^+$ and the proton affinities (PAs) of the variable base, **A**. Harrison et al. [36] and Paizs et al. [37] applied a similar relationship to the proton-bound dimer between the N- and C-terminal fragments, formed as an intermediate in the dissociation of a protonated peptide [38] to account for the linear correlation between abundance ratios of product ions, $\log(y_1/b_2)$ and $\log(y_2/b_1)$, and the PAs of the heteroresidue X in protonated tripeptides GGX and XGG, respectively [39–41]. The clue and the means to evaluate potential products of N–C_α bond cleavages are also provided by the proton-bound dimers **4** for $[GW]^{\bullet+}$ and **12** for $[GY]^{\bullet+}$, these intermediates are similar to those formed in the N–C_α bond cleavage of peptide radical cations generated by ECD or ETD [19–22]. The ensuing reaction is a competition between the two incipient fragments for the proton with the preferred channel giving the ion whose neutral has the higher PA, $\Delta H°_{298}$. The PAs of the fragments as determined by B3LYP/6-311++G(d,p) and a known literature experimental value are listed in Table 1. The PA of glycinamide at 217.7 (217.7 [42] kcal mol^{-1} is lower than the PA of the indolyl radical in product **6**, at 227.6 kcal mol^{-1}, resulting in proton attachment to the indolyl radical to give the $[z_1 - H]^{\bullet+}$ ion. Similarly, the PA of glycinamide is higher than the PA of the phenoxy radical in product **15** (phenoxy radical **I** in Table 1) at 212.7 kcal mol^{-1}; thus glycinamide will capture the proton in complexes **12** to give the $[c_1 + 2H]^+$ ion.

Assuming that the fragmentations of triglycine derivatives proceed also via proton-bound dimers, we can now move ahead to evaluate the outcomes of the proton competitions. The PA of the indolyl radical at 227.6 kcal mol^{-1} is slightly higher than the PA of glycylglycinamide at 225.7 kcal mol^{-1}, and is much higher than the PA of the phenoxy radical **I** at 212.7 kcal mol^{-1}

Table 1. Proton Affinities ($\Delta H°_{298}$) of Fragments as a Consequence of N—C_α Bond Cleavage

N-terminal fragment	PA[a]/kcal mol^{-1}	C-terminal fragment	PA[a]/kcal mol^{-1}
		Phenoxy radical I 	212.7
Glycinamide 	217.7 (217.7[b])	Phenoxy radical II 	218.1
Glycylglycinamide 	225.7	Indolyl radical 	227.6

[a]All PAs calculated at the B3LYP/6-311++G(d,p) level.
[b]Reference 42.

(Table 1). This is in accordance with experiments: the $[z_1 - H]^{\bullet +}$ ion is higher in abundance than the $[c_2 + 2H]^+$ ion in the dissociation of $[GGW]^{\bullet +}$ (Figure 1c), while only the $[c_2 + 2H]^+$ ion is evident in the dissociation of $[GGY]^{\bullet +}$ (Figure 1d). The PA of glycinamide at 217.7 kcal mol^{-1} is comparable to that of the phenoxy radical **II** at 218.1 kcal mol^{-1}; expectedly, both the $[c_1 + 2H]^+$ and $[z_2 - H]^{\bullet +}$ ions are observable in the CID of $[GYG]^{\bullet +}$ (Figure 1f). However, the abundance of the $[c_1 + 2H]^+$ ion is higher than that of the $[z_2 - H]^{\bullet +}$ ion. Similar observations have also been made in the dissociation of protonated tripeptides where the heteroresidue is in the central position [40]. In addition, the discrepancy in our case could also have been a result of limited computational accuracy; typical computational errors in DFT calculations at our level of theory relative to the best experimental data are within 2-3 kcal mol^{-1} [43, 44]. Addition of a glycine residue to the indolyl radical increases the PA; the magnitude of this effect can be estimated from the difference in PAs (5.4 kcal mol^{-1}) between the phenoxy radicals **II** and **I**. Thus the indolyl radical from $[GWG]^{\bullet +}$ is expected to have a PA ~233 kcal mol^{-1}, much higher than the PA of glycinamide, and only the $[z_2 - H]^{\bullet +}$ ion is observed as a result of the proton competition (Figure 1e).

The dissociations to give the $[b_2 - H]^{\bullet +}$ and $[a_3 + H]^{\bullet +}$ ions, and the loss of the 92 Da neutral to give the 226 Th ion in the dissociation of $[GWG]^{\bullet +}$, have previously been discussed by Bagheri-Majdi [3] and will not be elaborated here.

Conclusions

The dissociation reactions of radical cations of tryptophan- and tyrosine-containing tripeptides have been compared experimentally using collision-induced dissociation. Many of the fragment ions are products of N–C_α bond cleavages at the heteroresidues, forming either $[z_n - H]^{\bullet +}$ or $[c_n + 2H]^+$ ions. Density functional theory calculations at the B3LYP/6-311++G(d,p) level, using $[GW]^{\bullet +}$ and $[GY]^{\bullet +}$ as prototypical examples, establish that the fragmentation involves a proton transfer from the β-carbon to the carbonyl oxygen of the amide group forming an intermediate in which the charge and unpaired electron are separated and both delocalized in the forms of a protonated amide moiety and a benzylic radical. The energy barrier of this process for $[GW]^{\bullet +}$ is higher than that for $[GY]^{\bullet +}$. Proton-bound dimers are formed in the subsequent N–C_α bond cleavage, giving the critical transition-state. The lowest-energy complexes consist of an indole radical cation solvated by a neutral glycinamide via a hydrogen bond between the carbonyl oxygen and the indole-NH for $[GW]^{\bullet +}$ (**4E**), and a protonated glycinamide solvated by a phenoxy radical via a hydrogen bond between the protonated amino hydrogen and the phenoxy oxygen for $[GY]^{\bullet +}$ (**12E**). The ensuing dissociation is a competition between the two incipient fragments for the proton within the complex with the preferred channel giving the ion whose neutral has the higher proton affinity. Consequently, the $[z_n - H]^{\bullet +}$ ion is formed from all tryptophan-containing tripeptides radical cations, while the $[c_n + 2H]^+$ ion is formed from $[GGY]^{\bullet +}$ and both the $[z_n - H]^{\bullet +}$ and the $[c_n + 2H]^+$ ions are observed from $[GYG]^{\bullet +}$.

Acknowledgments

The authors acknowledge support of this study by the Natural Sciences and Engineering Research Council (NSERC) of Canada and made possible by the facilities of the Shared Hierarchical Academic Research Computing Network (SHARCNET: www.sharcnet.ca). YYK acknowledges receipt of an Ontario Graduate Scholarship.

References

1. Chu, I. K.; Rodriquez, C. F.; Lau, T. C.; Hopkinson, A. C.; Siu, K. W. M. Molecular Radical Cations of Oligopeptides. *J. Phys. Chem. B.* **2000,** *104,* 3393–3397.
2. Chu, I. K.; Rodriquez, C. F.; Hopkinson, A. C.; Siu, K. W. M.; Lau, T. C. Formation of Molecular Radical Cations of Enkephalin Derivatives Via Collision-Induced Dissociation of Electrospray-Generated Copper (II) Complex Ions of Amines and Peptides. *J. Am. Soc. Mass Spectrom.* **2001,** *12,* 1114–1119.
3. Bagheri-Majdi, E.; Ke, Y. Y.; Orlova, G.; Chu, I. K.; Hopkinson, A. C.; Siu, K. W. M. Copper-Mediated Peptide Radical Ions in the Gas Phase. *J. Phys. Chem. B.* **2004,** *108,* 11170–11181.
4. Chu, I. K.; Siu, S. O.; Lam, C. N. W.; Chan, J. C. Y.; Rodriquez, C. F. Formation of Molecular Radical Cations of Aliphatic Tripeptides from their Complexes with Cu-II(12-Crown-4). *Rapid Commun. Mass Spectrom.* **2004,** *18,* 1798–1802.
5. Lam, C. N. W.; Siu, S. O.; Orlova, G.; Chu, I. K. Macrocyclic Effect of Auxiliary Ligand on the Gas-Phase Dissociation of Ternary Copper(II)-GGX Complexes. *Rapid Commun. Mass Spectrom.* **2006,** *20,* 790–796.
6. Chu, I. K.; Lam, C. N. W.; Siu, S. O. Facile Generation of Tripeptide Radical Cations in Vacuo Via Intramolecular Electron Transfer in Cu-II Tripeptide Complexes Containing Sterically Encumbered Terpyridine Ligands. *J. Am. Soc. Mass Spectrom.* **2005,** *16,* 763–771.
7. Barlow, C. K.; McFadyen, W. D.; O'Hair, R. A. J. Formation of Cationic Peptide Radicals by Gas-Phase Redox Reactions with Trivalent Chromium, Manganese, Iron, and Cobalt Complexes. *J. Am. Chem. Soc.* **2005,** *127,* 6109–6115.
8. Laskin, J.; Yang, Z.; Chu, I. K. Energetics and Dynamics of Electron Transfer and Proton Transfer in Dissociation of $Metal^{III}$(Salen)-Peptide Complexes in the Gas Phase. *J. Am. Chem. Soc.* **2008,** *130,* 3218–3230.
9. Wee, S.; O'Hair, R. A. J.; McFadyen, W. D. Side-Chain Radical Losses from Radical Cations Allows Distinction of Leucine and Isoleucine Residues in the Isomeric Peptides Gly-XXX-Arg. *Rapid Commun. Mass Spectrom.* **2002,** *16,* 884–890.
10. Wee, S.; O'Hair, R. A. J.; McFadyen, W. D. Comparing the Gas-Phase Fragmentation Reactions of Protonated and Radical Cations of the Tripeptides GXR. *Int. J. Mass Spectrom.* **2004,** *234,* 101–122.
11. Paizs, B.; Suhai, S. Fragmentation Pathways of Protonated Peptides. *Mass Spectrom. Rev.* **2005,** *24,* 508–548.
12. Wysocki, V. H.; Cheng, G.; Zhang, Q.; Hermann, K. A.; Beardsley, R. L.; Hilderbrand, A. E. Peptide Fragmentation Overview. In *Principles of Mass Spectrometry Applied to Biomolecules*, Laskin, J.; Lifshitz, C., Eds.; Wiley-Interscience: Hoboken, NJ, 2006; pp. 279–300.
13. Chu, I. K.; Zhao, J. F.; Xu, M.; Siu, S. O.; Hopkinson, A. C.; Siu, K. W. M. Are the Radical Centers in Peptide Radical Cations Mobile? The Generation, Tautomerism, and Dissociation of Isomeric α-Carbon-Centered Triglycine Radical Cations in the Gas Phase. *J. Am. Chem. Soc.* **2008,** *130,* 7862–7872.
14. Rodriquez, C. F.; Cunje, A.; Shoeib, T.; Chu, I. K.; Hopkinson, A. C.; Siu, K. W. M. Proton Migration and Tautomerism in Protonated Triglycine. *J. Am. Chem. Soc.* **2001,** *123,* 3006–3012.
15. Zubarev, R. A.; Kelleher, N. L.; McLafferty, F. W. Electron Capture Dissociation of Multiply Charged Protein Cations. A Nonergodic Process. *J. Am. Chem. Soc.* **1998,** *120,* 3265–3266.
16. Syka, J. E. P.; Coon, J. J.; Schroeder, M. J.; Shabanowitz, J.; Hunt, D. F. Peptide and Protein Sequence Analysis by Electron Transfer Dissociation Mass Spectrometry. *Proc. Natl. Acad. Sci. U.S.A.* **2004,** *101,* 9528–9533.
17. Zubarev, R. A. Reactions of Polypeptide Ions with Electrons in the Gas Phase. *Mass Spectrom. Rev.* **2003,** *22,* 57–77.
18. Cooper, H. J.; Hakansson, K.; Marshall, A. G. The Role of Electron Capture Dissociation in Biomolecular Analysis. *Mass Spectrom. Rev.* **2005,** *24,* 201–222.

19. Tureček, F. N–C_α Bond Dissociation Energies and Kinetics in Amide and Peptide Radicals. Is the Dissociation a Nonergodic Process? *J. Am. Chem. Soc.* **2003,** *125*, 5954–5963.
20. Syrstad, E. A.; Tureček, F. Toward a General Mechanism of Electron Capture Dissociation. *J. Am. Soc. Mass Spectrom.* **2005,** *16*, 208–224.
21. Sobczyk, M.; Anusiewicz, W.; Berdys-Kochanska, J.; Sawicka, A.; Skurski, P.; Simons, J. Coulomb-Assisted Dissociative Electron Attachment: Application to a Model Peptide. *J. Phys. Chem. A.* **2005,** *109*, 250–258.
22. Skurski, P.; Sobczyk, M.; Jakowski, J.; Simons, J. Possible Mechanisms for Protecting N–C_α Bonds in Helical Peptides from Electron-Capture (or Transfer) Dissociation. *Int. J. Mass Spectrom.* **2007,** *265*, 197–212.
23. Hopkinson, A. C.; Siu, K. W. M. Peptide Radical Cations. In *Principles of Mass Spectrometry Applied to Biomolecules*, Laskin, J.; Lifshitz, C., Eds.; John Wiley and Sons, Inc.: Hoboken, 2006; pp. 301–335.
24. Siu, K. W. M. Molecular Radical Cations of Peptides: Formation and Dissociation. Gordon Research Conference on Biological Molecules in the Gas Phase. New London, CT, 2003.
25. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A. Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03, 2004 Rev. D.01*; Gaussian Inc.: Wallingford CT, 2004.
26. Becke, A. D. Density-Functional Thermochemistry. 3. The Role of Exact Exchange. *J. Chem. Phys.* **1993,** *98*, 5648–5652.
27. Lee, C. T.; Yang, W. T.; Parr, R. G. Development of the Colle-Salvetti Correlation-Energy Formula into a Functional of the Electron-Density. *Phys. Rev. B* **1988,** *37*, 785–789.
28. Gonzalez, C.; Schlegel, H. B. An Improved Algorithm for Reaction-Path Following. *J. Chem. Phys.* **1989,** *90*, 2154–2161.
29. Reed, A. E.; Weinstock, R. B.; Weinhold, F. Natural-Population Analysis. *J. Chem. Phys.* **1985,** *83*, 735–746.
30. Sutherland, K. N.; Mineau, P. C.; Orlova, G. Radical-Cationic Gaseous Amino Acids: A Theoretical Study. *J. Phys. Chem. A* **2007,** *111*, 7906–7914.
31. Siu, C. K.; Ke, Y. Y.; Guo, Y. Z.; Hopkinson, A. C.; Siu, K. W. M. Dissociations of Copper(II)-Containing Complexes of Aromatic Amino Acids: Radical Cations of Tryptophan, Tyrosine, and Phenylalanine. *Phys. Chem. Chem. Phys.* 2008, DOI: 10.1039/b807692h.
32. McAdoo, D. J. Ion Neutral Complexes in Unimolecular Decompositions. *Mass Spectrom. Rev.* **1988,** *7*, 363–393.
33. Bowen, R. D. Ion-Neutral Complexes. *Acc. Chem. Res.* **1991,** *24*, 364–371.
34. Longevialle, P. Ion-Neutral Complexes in the Unimolecular Reactivity of Organic Cations in the Gas-Phase. *Mass Spectrom. Rev.* **1992,** *11*, 157–192.
35. McLuckey, S. A.; Cameron, D.; Cooks, R. G. Proton Affinities from Dissociations of Proton-Bound Dimers. *J. Am. Chem. Soc.* **1981,** *103*, 1313–1317.
36. Harrison, A. G.; Csizmadia, I. G.; Tang, T. H.; Tu, Y. P. Reaction Competition in the Fragmentation of Protonated Dipeptides. *J. Mass Spectrom.* **2000,** *35*, 683–688.
37. Paizs, B.; Suhai, S. Combined Quantum Chemical and RRKM Modeling of the Main Fragmentation Pathways of Protonated GGG. II. Formation of b_2, y_1, and y_2 Ions. *Rapid Commun. Mass Spectrom.* **2002,** *16*, 375–389.
38. Paizs, B.; Lendvay, G.; Vekey, K.; Suhai, S. Formation of b_2^+ Ions from Protonated Peptides: An Ab Initio Study. *Rapid Commun. Mass Spectrom.* **1999,** *13*, 525–533.
39. Morgan, D. G.; Bursey, M. M. A Linear Free-Energy Correlation in the Low-Energy Tandem Mass-Spectra of Protonated Tripeptides Gly-Gly-Xxx. *Org. Mass Spectrom.* **1994,** *29*, 354–359.
40. Morgan, D. G.; Bursey, M. M. Linear Energy Correlation in the Low-Energy Tandem Mass-Spectra of Protonated Tripeptides Xxx-Gly-Gly but Failure for Gly-Xxx-Gly. *J. Mass Spectrom.* **1995,** *30*, 290–295.
41. Morgan, D. G.; Bursey, M. M. Linear Energy Correlations and Failures in the Low-Energy Tandem Mass-Spectra of Protonated N-Benzoylated Tripeptides—Tools for Probing Mechanisms of Cad Processes. *J. Mass Spectrom.* **1995,** *30*, 595–600.
42. Kinser, R. D.; Ridge, D. P.; Hvistendahl, G.; Rasmussen, B.; Uggerud, E. The Unimolecular Chemistry of Protonated Glycinamide and the Proton Affinity of Glycinamide - Mass Spectrometric Experiments and Theoretical Model. *Chem. Eur. J.* **1996,** *2*, 1143–1149.
43. El Aribi, H.; Shoeib, T.; Ling, Y.; Rodriquez, C. F.; Hopkinson, A. C.; Siu, K. W. M. Binding Energies of the Silver Ion to Small Oxygen-Containing Ligands: Determination by Means of Density Functional Theory and Threshold Collision-Induced Dissociation. *J. Phys. Chem. A* **2002,** *106*, 2908–2914.
44. El Aribi, H.; Rodriquez, C. F.; Shoeib, T.; Ling, Y.; Hopkinson, A. C.; Siu, K. W. M. Threshold Collision-Induced Dissociation Determination and Molecular Orbital Calculations of the Binding Energies of Sodium and Silver Ions to Small Nitrogen-Containing Ligands. *J. Phys. Chem. A* **2002,** *106*, 8798–8805.