Copper-Mediated Peptide Radical Ions in the Gas Phase

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Molecular radical cations, M\(^{+}\), of amino acids and oligopeptides are produced by collision-induced dissociation of mixed complex ions, [Cu\(^{\text{II}}\)(amine)M]\(^{2+}\), that contain Cu\(^{\text{II}}\), an amine, typically diethylenetriamine (dien), and the oligopeptide, M. With dien as the amine ligand, abundant M\(^{+}\) formation is observed only for the amino acids tryptophan and tyrosine, and oligopeptides that contain either the tryptophanyl or tyrosyl residue. Dissociation of the M\(^{+}\) ion is rich and differs considerably from that of protonated amino acids and peptides. Facile fragmentation occurs around the \(\alpha\)-carbon of the tryptophanyl residue. Cleavage of the N–C\(_{\alpha}\) bond and proton transfer from the exocyclic methylene group in the side chain to the N-terminal residue results in formation of the \([z_n – H]\)^{+} ion and elimination of the N-terminal fragment as ammonia or an amide, depending on the position of the tryptophyl residue. Cleavage of the C\(_{\alpha}–C\) bond of an oligopeptide containing a C-terminal tryptophyl residue and proton transfer from the carboxylic group to the N-terminal fragment (a carbonyl oxygen atom) results in formation of the \([a_2 + H]\)^{+} ion and elimination of carbon dioxide. Both types of fragmentation have no analogous reactions in protonated peptides. For the M\(^{+}\) of tryptophan-glycyl-glycyl-glycine, WGG, elimination of the tryptophanyl side chain results in GGG\(^{+}\)\(\alpha\)-iminium radical ion.

1. Introduction

Protein radicals exhibit fascinating chemistry and are participants in enzyme catalysis in biological systems. They are involved in a number of highly important processes, the most notable of which is the oxidation of water to oxygen for use in a photosynthetic system in plants and algae. Bio-synthesis of protein radicals takes place posttranslationally and requires a metallo cofactor located adjacent to the amino acid residue being oxidized on a subunit or on an activating enzyme involved in the oxidation reaction. Radical sites are commonly located on the glycoprol, tyrosyl, and tryptophyl residues.

Cationic peptide radicals have attracted considerable recent interest as they serve as convenient models for molecular wires in electrical conduction in biological systems. Cationic radicals of amino acids and dipeptides are typically produced after thermal desorption of the neutral species and electron ionization or laser desorption followed by resonant UV two-photon ionization at an aromatic chromophore. Recently, we reported an unprecedented method for producing molecular radical cations, M\(^{+}\), of oligopeptides via collision-induced dissociation (CID) of electrosprayed complexes of Cu(II), a tridentate amine, and an oligopeptide, the \([\text{Cu}^{\text{II}}(\text{amine})\text{M}]^{2+}\) ion. Amines that have been successfully employed include diethylenetriamine (dien), \(N,N,N',N'-\text{pentamethyl-diethylenetriamine (Me}_5\text{-dien)}\), and 2,2',6',2'-terpyridine (terpy). A number of competitive channels in the fragmentation of the \([\text{Cu}^{\text{II}}(\text{amine})\text{M}]^{2+}\) ion have been identified. They are, with respect to the peptide: (1) radical peptide formation, resulting in M\(^{+}\) and \([\text{Cu}(\text{amine}) ]^{+}\); (2) proton addition, producing \([M + H]\)^{+} and \([\text{Cu}^{\text{II}}(\text{amine} – H)]^{+}\); and (3) proton abstraction, producing \([\text{Cu}^{\text{II}}(M – H)]^{+}\) and \([\text{amine} + H]\)^{+}. Oligopeptides that produce abundant M\(^{+}\) upon CID have all contained either a tyrosyl or a tryptophyl residue plus a basic residue, arginyl, lysyl, or histidyl. Furthermore, the \([M – 106]^{+}\) and \([M – 129]^{+}\) ions, corresponding to the loss of p-quinomethide and 3-methylene indolenine, or their isomers, are prominent product ions from tyrosyl- and tryptophanyl-containing peptides, respectively.

Here we report a systematic study that shows abundant M\(^{+}\) ions can be produced from tyrosyl- and tryptophanyl-bearing di- and tripeptides that contain only glycine as the remaining residue(s), i.e., it is not essential to have a basic residue present. It will be shown that the propensity for M\(^{+}\) production is dependent not only on the presence of the critical residues, tyrosyl and tryptophyl, but also on the position of the critical residue in the peptide. The dissociation of selected M\(^{+}\) will be presented. Density functional theory (DFT) examination of key structures and reaction mechanisms will be reported.

2. Experimental Section

Experiments were conducted on a triple quadrupole, a quadrupole/time-of-flight (QqTOF) hybrid, and an ion trap mass...
spectrometer. The first two were prototypes of AB-SCIEX mass spectrometers (Concord, ON) that are commercialized as the API 3000 and the QStar, while the last was the ThermoFinnigan LCQ. The collision gas in the triple quadrupole and the QqTOF instruments was nitrogen, while that in the ion trap mass spectrometer was helium. Samples were typically 600 μM Cu(II)-amine perchlorate and 50 μM in oligopeptide in 50/50 water/methanol. The sample solution was electrosprayed under optimal conditions; nanospray (MDS Protana, Odense, Denmark) was also employed on the QqTOF instrument.

The Cu(II)-amine perchlorate salts were synthesized according to published procedures but with perchlorate replacing nitrate as the counterion. All peptides and chemicals were commercially available (Aldrich and Sigma, St. Louis, MO; and Bachem, King of Prussia, PA).

3. Computational Section

Density functional theory (DFT) with Becke’s hybrid exchange functional and the correlation functional of Lee, Yang, and Parr, B3LYP, was employed. Doubly split-valence basis sets augmented with diffuse and polarization functions on heavy atoms, 6-31+G(d), and with additional diffuse and polarization functions on hydrogen atoms, 6-31++G(d,p), were used. All computations were performed with the Gaussian 98 program suite. Harmonic vibrational frequencies were computed to verify minima and transition-state structures. The connections between transition-state structures and minima on the energy surface were established using the intrinsic reaction coordinates technique as implemented in Gaussian 98.

For indole-containing radical cations, the 6-31++G(d,p) basis set exhibits linear dependence that results in a severe self-consistent field convergence problem. Thus the smaller 6-31+G(d) basis set was employed for indole-containing structures and for the related dissociation of GGG⁺ to smaller fragments (vide infra). Single-point calculations were performed for key structures in the GGG⁺ dissociation using the 6-31++G(d,p) basis set to permit direct comparisons with published results for protonated triglycine. Natural charges and electron densities were obtained using the natural population analysis (NPA) as implemented in Gaussian 98. NPA could not be performed using even the 6-31+G(d) basis set which is nearly linear dependent, the less flexible 6-31G basis set was used in this case.

4. Results and Discussion

4.1. Amino Acids. The 20 α-amino acids were electrosprayed individually with Cu(II)(dien)(ClO₄)₂; the [Cu(II)(dien)M]⁺ ions were mass-selected and subjected to CID. Of the 20, only

Figure 1. CID mass spectra of the [63Cu(II)(dien)M]²⁺ ions of (a) tryptophan, (b) tyrosine, and (c) methionine at relative collision energies of 5, 7, and 6% of 10 eV, respectively. Experiments were performed on a ThermoFinnigan LCQ ion trap mass spectrometer.
tryptophan and tyrosine yielded unambiguous M$^+$ ions. Some amino acids, e.g., lysine and arginine, yielded no observable M$^+$ ions, but there were minor product ions that could have originated from them. Figure 1 shows the product ion spectra of three [63Cu II (dien)M]$^{2+}$ complex ions. For tryptophan (Figure 1a), the prominent product ion at 130 Th, assigned as protonated 3-methylene indolenine, confirms the existence of W$^+$; the 130 Th ion is the most prominent fragment ion observed in the electron ionization mass spectrum of W. The low abundance of the 130 Th fragment in Figure 1a is due to low collision energy and the fact that the precursor ion is [Cu II (dien)M]$^{2+}$. Mass-selecting the W$^+$ and fragmenting it at a higher collision energy resulted in the 130 Th ion being the most abundant ion. Similarly, observation of the product ions at 107 and 108 Th, assigned as protonated p-hydroxybenzyl cation, [p-CH$_2$(C$_6$H$_4$)OH]$^+$, and p-cresol ion, [p-CH$_3$(C$_6$H$_4$)OH]$^+$, in Figure 1b confirms the existence of Y$^+$; these ions are the most abundant fragment ions in the electron ionization mass spectrum of Y. Figure 1c shows the result for methionine as an example of amino acids that do not yield M$^+$.

The observation of significant M$^+$ ions for W and Y among the 20 $\alpha$-amino acids is not surprising in view of the fact that the ionization energies of W and Y are the two lowest. As the ionization energies of only a few amino acids have been evaluated, an estimate of the relative ionization energies of all the amino acids could only be based on a comparison using the ionization energies of the analogous compounds of their side chains. The compounds that have the lowest ionization energies (the corresponding amino acids) and their values$^{18}$ are the following: 3-methyl indole (tryptophan), 7.514 $\pm$ 0.001 eV; p-cresol (tyrosine), 8.34 $\pm$ 0.03 eV; ethyl methyl sulfide (methionine), 8.55 $\pm$ 0.01 eV; butylamine (lysine), 8.73 $\pm$ 0.04 eV; and imidazole (histidine), 8.81 $\pm$ 0.01 eV. With dien as the amine ligand, tryptophan and tyrosine are the only two amino acids that exhibit evidence of M$^+$. Parenthetically, M$^+$ from amino acids such as histidine and lysine are observed with terpy, or a derivative of it, as the ligand (spectra not shown). Terpy and its derivatives lack N-H hydrogens and hence suppress proton transfer from the ligand to the amino acid (vide infra), a pathway that competes with radical peptide formation. However, the use of terpy, relative to that of dien, tends to result in less abundant [Cu II (amine)M]$^{2+}$ ions. Of all the $\alpha$-amino acids, tryptophan is the only one that does not yield any significant quantity of [M $\pm$ H]$^+$ ions; all others form them in abundance during CID of the [Cu II (dien)M]$^{2+}$ complex ions. Complexes containing some amino acids (phen-
4.2. Oligopeptides. The fragmentation spectra of the [63Cu II - (dien)M]²⁺ ions, where M = WGG, GWG, and GGW are shown in Figure 2. It is immediately apparent that the spectra are strikingly different, and it will be shown in the following paragraphs that the differences are due to the placement of the tryptophanyl residue, which dictates the predominant dissociation pathways. In terms of relative yields of M⁺ to [M + H]⁺ in the dissociation of [Cu II(dien)M]²⁺, GGW yields almost exclusively M⁺, GWG yields mostly M⁺ but also some [M + H]⁺, and WGG yields both. (The apparently much lower abundance of M⁺ than that of [M + H]⁺ for the last peptide is due to more facile fragmentation of the M⁺, vide infra.) The ratio between M⁺ and [M + H]⁺ changes with collision energy and presumably reflects the combined effect of the change in branching ratio for the dissociation of the [Cu II(dien)M]²⁺ ion with collision energy plus the relative stability of the M⁺ and [M + H]⁺ ions under collision. Virtually all fragment ions seen in Figure 2 are attributable to the dissociation of M⁺ (vide infra). The ratio of [Cu II(dien)]⁺ to [Cu II(dien − H)]⁺ is a better indicator for the branching ratio of the radical peptide formation to the proton addition channel, as these are relatively stable ions under the experimental conditions.

We shall use two series of tryptophan- and glycine-containing peptides to illustrate the rich and diverse chemistry of oligopeptide radical cations. In addition to the tripeptide series of WGG, GWG, and GGW, we shall also use the dipeptide series of WG and GW.

4.2.1. WG and GW Radical Cations. The CID spectrum of the M⁺ of WG is shown in Figure 3a. The two prominent ions seen are 244 Th, [z 2 - H]⁺, and 159 Th, a₁⁺. Because hydrogens are gained and lost, and ions can contain odd and even numbers of electrons, all details regarding the ions are, therefore, explicitly given in this study. Formation of the [z 2 - H]⁺ ion requires transfer of a hydrogen from the C-terminal fragment to the N-terminal amino group, followed by cleavage of the N−C bond of the tryptophanyl residue and elimination of ammonia. Transfer of the carboxylic hydrogen was ruled out by performing the CID of the M⁺ of the O-methyl ester of WG, which features an equally abundant [z 2 − H]⁺ ion at 258 Th (Figure 3b). Figure 4a shows the optimized structure, ion I, of the [z 2 − H]⁺ ion of WG⁺; the hydrogen that has been transferred to the N-terminal amino group, prior to the loss of ammonia, is from the exocyclic carbon in the tryptophan side chain. The hydrogen involved is a “benzylic-type” hydrogen.

Figure 3. CID mass spectra of the M⁺ ions of (a) WG and (b) the O-methyl ester of WG. Relative collision energy = 10% of 5 eV. Experiments were performed on a ThermoFinnigan LCQ ion trap mass spectrometer.
which, given that the radical center and charge most likely reside on the fused ring system of WG\(^{+}\), is acidic; transfer of it as a proton to the NH\(_2\) group leaves the radical center formally on the exocyclic carbon, but it can delocalize over the tryptophanyl ring.\(^{19}\) Natural population analysis on ion I reveals that the positive charge is uniformly distributed among the ring hydrogen atoms, while the unpaired electron resides mainly on what was the \(\text{R}\)-carbon atom (0.4 e) of the tryptophanyl residue and on the ring carbon atom (0.3 e) adjacent to the exocyclic carbon atom, with the remainder being fairly evenly scattered on the other ring carbon atoms. The two best resonance structures for the \([z_2 - H]^\dagger\) ion are also shown (Figure 4a). Figure 4b shows the CID spectrum of the \([z_2 - H]^\dagger\) ion. The two abundant fragment ions are likely formed via elimination of carbon dioxide from \([z_2 - H]^\dagger\) to give the 200 Th ion, which then eliminates methanimine to give the 171 Th ion.\(^{20}\) Both the 200 and the 171 Th ions are “internal” ions as they have lost both N-terminal and C-terminal components of WG.

The CID spectrum of GW\(^{+}\) is shown in Figure 5. The two abundant fragment ions are 217 Th, \([a_2 + H]^\dagger\), and 187 Th, \([z_1 - H]^\dagger\). Similar to formation of the \([z_2 - H]^\dagger\) ion in WG\(^{+}\), formation of the \([z_1 - H]^\dagger\) ion in GW\(^{+}\) requires also transfer of a hydrogen from the C-terminal to the N-terminal fragment, cleavage of the N-C\(_\alpha\) bond of the tryptophanyl residue, and elimination of the N-terminal fragment as a neutral. The proposed neutral eliminated here, however, is glycinamide.

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**Figure 4.** The \([z_2 - H]^\dagger\) ion of WG\(^{+}\): (a) B3LYP/6-31+G(d)-optimized structure, blue = N, red = O, gray = C; (b) CID mass spectrum of the ion at a relative collision energy of 10% of 5 eV. Experiment performed on a ThermoFinnigan LCQ ion trap mass spectrometer.
results show that the \([z_1 - H]^+\) ion (structure not shown) exhibits all characteristic structural features of the \([z_2 - H]^+\) ion. Formation of the \([a_2 + H]^+\) ion requires transfer of the carboxylic hydrogen to the N-terminal fragment and cleavage of the Cα–C bond of the tryptophan residue, thereby eliminating carbon dioxide. The loss of carbon dioxide is common in radical cations of peptides, whereas it is rare in protonated peptides. Scheme 1 shows the reaction profile at the B3LYP/6-31+G(d)

Figure 5. CID mass spectrom of the M\(^{+}\) ion of GW. Relative collision energy = 10% of 5 eV. Experiment performed on a ThermoFinnigan LCQ ion trap mass spectrometer.
level of a proposed mechanism leading to the \([a_2 + H]^{++}\) ion and CO\(_2\), upper values are \(\Delta H^\circ\) and lower values (in italics) are \(\Delta G^\circ_{298}\) (all energies in kcal/mol). The reaction involves hydrogen bonding of the carboxylic proton to the carbonyl oxygen of the glycyl residue (structure II). Transfer of the hydrogen atom to the carbonyl oxygen and the C\(_\alpha\)–C bond cleavage proceed in one step and result in structure III that dissociates into a tautomer (IV) of the \([a_2 + H]^{++}\) ion and CO\(_2\). 1,4-H transfer from the carbonyl oxygen to the \(\alpha\)-carbon then ensues to result in the \([a_2 + H]^{++}\) ion (V), a structure in which

**Figure 6.** CID mass spectra of (a) the M\(^{++}\) ion of WGG and (b) the 189 Th fragment, GGG\(^{**}\). Relative collision energies: (a) 10% of 5 eV; (b) 8% of 5 eV. Experiments were performed on a ThermoFinnigan LCQ ion trap mass spectrometer.
the charge and the unpaired electron are on the tryptophanyl ring. The rate-determining step of the reaction is the formation of intermediate III, which proceeds via TS(II → III) with a moderate free-energy barrier of 27.7 kcal/mol at 298 K, in keeping with the relative ease in eliminating CO2.

4.2.2. WGG, GWG, and GGW Radical Cations. The fragmentation chemistries of the tripeptide radical cations share many similarities with those of the dipeptide radical cations. The CID spectrum of WGG+ is shown in Figure 6a. As in WG+, WGG+ loses ammonia readily to give the C-terminal ion, the [z3 − H]+ ion. It also forms the a1+ ion. The minor fragment ion at 189 Th, GGG+ formed by eliminating the tryptophanyl side chain is interesting and serves as an example of charge-induced fragmentation in a peptide that is both charged and a radical (vide infra). A proposed mechanism is shown in Scheme 2. A 1,4-proton transfer of the benzylic-type hydrogen to the carbonyl oxygen of the first peptide linkage formally places the unpaired electron on the exocyclic carbon. Cleavage of the C−Cα bond produces GGG+ and the tryptophan side chain as a singlet carbene stabilized by conjugation with the π-system of the tryptophan ring. The CID spectrum of GGG+ is shown in Figure 6b; the abundant fragment ions at 114 and 86 Th are, respectively, the [b2 − H]+ and the [a2 − H]+ ions. Figure 7 shows the optimized structures of the three radical cations. Ion VI is the lowest energy structure of GGG+, structure VII is the lowest energy structure of the [b2 − H]+ ion, structure VIII is the lowest energy structure of the b2+ ion as reported in ref 21, and structure IX is the lowest energy structure of the [a2 − H]+ ion.

Figure 7. Structures of GGG+ and its fragment ions: structure VI was optimized at the B3LYP/6-31+G(d) level; all other structures were optimized at the B3LYP/6-31++G(d,p) level. Structure VI is the lowest energy structure of GGG+, structure VII is the lowest energy structure of the [b2 − H]+ ion, structure VIII is the lowest energy structure of the b2+ ion as reported in ref 21, and structure IX is the lowest energy structure of the [a2 − H]+ ion.
a captodative radical with both a strongly electron-withdrawing group \([-\text{C(OH)}\text{NH}^+\] and a powerful donor (\(\text{NH}_2^-\)) attached to the radical center. However, NPA of the DFT-optimized structures reveals that the positive charge is effectively delocalized on the amide group (\(\text{OCN}\)), while the unpaired electron is shared almost equally between the amino nitrogen and the \(\text{R}-\text{carbon}\).

The \([b_2-H]^+\) ion, structure VII (Figure 7), is a protonated oxazolone and shares many structural features with the \(b_2^+\) ion derived from a protonated peptide. In structure VII, the oxazolone ring and the exocyclic chain are planar; the \(\text{C}^-\text{C}\) and \(\text{N}^-\text{C}\) bonds in the exocyclic chain are short and have considerable double-bond character. Unlike the nitrogens in the \(b_2^+\) ion,\(^22,23\) those in VII are trans rather than cis. In the latter structure, planarity at the radical center results in delocalization of the charge onto the N-terminal amino group; there is electrostatic repulsion between the two \(\text{NH}_2^+\) groups, and this is minimized by adoption of the trans configuration. As a result, the cis isomer is 3.9 kcal/mol higher in free energy than the trans isomer. Structure IX is the lowest energy structure of the \([a_2-H]'^+\) ion; here, the unpaired electron is effectively delocalized. This open structure is 26.4 kcal/mol lower in energy (\(\Delta G_{298}^\circ\) at the B3LYP/6-31++G(d,p) level of theory) than its cyclic isomer, X, formed by nucleophilic attack by the amino nitrogen on the imino carbon.\(^23\) Cyclization localizes the unpaired electron on the \(\alpha\)-carbon of the first residue and localizes the formal positive charge on the \(\text{NH}_2\) group; the localized unpaired electron does not participate in binding and results in a higher ion energy. By contrast, for the \(a_2^+\) ion, the cyclic isomer, protonated 4-imidazolidone, is lower in free energy than the open iminium ion by 8.9 kcal/mol in free energy.\(^23\)

Formation of the \([b_2-H]^+\) ion from GGG\(^++\) exhibits a strong parallel with that of the \(b_2^+\) ion from protonated triglycine.\(^24\) Figure 8 shows the critical intermediate in which the proton is attached to the amide nitrogen and the rate-determining transition state on the reaction profile of the fragmentation of protonated triglycine to the \(b_2^+\) ion (Figure 2 of ref 24) and the corresponding structures for the fragmentation of GGG\(^++\) to the \([b_2-H]^+\) ion. Structure XI is the critical intermediate for the radical peptide; the proton has been transferred from the carbonyl oxygen of the first peptide bond to the amide nitrogen.
of the second amide bond. The corresponding structure for protonated triglycine is structure \textit{6} in ref 24. The radical cation structure \textit{XI} and protonated structure \textit{6} are 19.9 and 18.8 kcal/mol higher in free energy than their corresponding lowest energy structures, structure \textit{VI} for GGG$^+$ and structure \textit{2} for protonated triglycine.\textsuperscript{24} In structure \textit{2}, the proton is residing on the carbonyl oxygen of the first peptide linkage and is hydrogen-bonded to the amino nitrogen. From structure \textit{XI}, the carbonyl oxygen of the first peptide linkage then attacks the carbonyl carbon of the second peptide linkage and the C–N bond lengthens to give transition state TS(XI → \textit{products}), which is similar to TS (6 → \textit{7}) in ref 24. The barrier height is 29.4 kcal/mol in free energy.

Figure 9. CID mass spectrum of the M$^+$ ion of GWG. Relative collision energy = 10\% of 5 eV. The inset shows the CID mass spectrum of the [z_2 − H]$^+$ ion, which is virtually identical to the [z_2 − H]$^+$ ion from WG$^+$ (see Figure 4b).

Figure 10. CID mass spectra of (a) the M$^+$ ion of GGW and (b) the [a_3 + H]$^+$ ion. Relative collision energy: (a) 10\% of 5 eV; (b) 12\% of 5 eV. The proposed structure of the 143 Th ion is shown.
energy for the fragmentation of GGG$^{+}$, while it is 32.5 kcal/mol for that of protonated triglycine (both at the B3LYP/6-31+G(d,p) level). The lower barrier for the former is in keeping with our general observation of the relative fragility of radical peptide ions versus their protonated counterparts. For the radical peptide ion, separation of the products results in the $[b_2 - H]^{+}$ ion and neutral glycine. Structure XII shows the singly occupied molecular orbital (SOMO) of TS(XI → products). It is evident that the unpaired electron resides on the N-terminal fragment and is remote from the C–N bond that is about to break. Thus, even for GGG$^{+}$, the unpaired electron is a mere spectator in the cleavage of the C–N bond to form the $[b_2 - H]^{+}$ ion and neutral glycine. For both GGG$^{+}$ and protonated triglycine, fragmentation is induced by proton transfer to the nitrogen of the second peptide linkage, which weakens the C–N bond.$^{25,26}$

Similar to GGG$^{+}$, the $[z_1 - H]^{+}$ ion of WGG$^{+}$ can also lose its C-terminal glycine to give an internal ion at 226 Th, which upon further collisional activation can lose CO directly to give an ion at 198 Th. Alternatively, the 226 Th ion can first lose a hydrogen atom to give a 225 Th ion, which then loses CO to give a product ion at 197 Th. The ease of CO loss suggests that the 226 Th ion may have an oxazolone structure.

The CID spectrum of GWG$^{+}$ is shown in Figure 9. As before, fragmentation around the α-carbon of the tryptophanyl residue is observed. Cleavage of the N–C$_n$ bond and hydrogen transfer to the N-terminal fragment results in the $[z_2 - H]^{+}$ ion at 244 Th, the CID spectrum of which is shown in the inset. This CID spectrum is virtually identical to the CID spectrum of the $[z_2 - H]^{+}$ ion formed from WG$^{+}$ (Figure 4b), a strong indication that the two ions have an identical structure and similar mechanisms of formation. However, the N-terminal neutral fragment that is formed in the fragmentation of GW$^{+}$ is likely to be glycaminide rather than ammonia, which is formed in the fragmentation of WG$^{+}$. The fragment ion at 243 Th is the $[b_2 - H]^{+}$ ion formed after elimination of the C-terminal glycine. Elimination of the C-terminal residue was confirmed using GWA$^{+}$, which gives an identical $[b_2 - H]^{+}$ ion at 243 Th. This $[b_2 - H]^{+}$ ion is also likely to be an oxazolone formed in a mechanism similar to the one discussed above for GGG$^{+}$. Cleavage of the C$_n$–C bond with charge retention on the N-terminal fragment results in the $[a_2 - H]^{+}$ ion. The fragment ion at 226 Th is isobaric with the 226 Th ion discussed above for WGG$^{+}$, but is probably not an oxazolone, as it does not fragment to lose CO easily.

Figure 10a shows the CID spectrum of the GGW$^{+}$ ion. As in the fragmentation of GW$^{+}$ (Figure 5), there are two major products resulting from either loss of CO$_2$ or loss of an amide from the N-terminal, as a consequence of cleavage at the N–C$_n$ bond of the tryptophanyl residue. Fragmentation of the C$_n$–C bond and proton transfer to the N-terminal fragment results in the $[a_2 + H]^{+}$ ion, probably in a mechanism corresponding to that shown in Scheme 1. The CID spectrum of the $[a_2 + H]^{+}$ ion is shown in Figure 10b. Following the trend of fragmenting the N–C$_n$ bond of the tryptophanyl residue, the most facile fragmentation of the $[a_2 + H]^{+}$ ion is the elimination of glycglycynamide to give the 143 Th ion; cleavage of the other two N–C$_n$ bonds is also observed albeit with much lower abundance. Fragmentation of the N–C$_n$ bond of the GGW$^{+}$ ion gives the $[z_1 - H]^{+}$ ion at 187 Th (Figure 10a). The CID spectrum of this $[z_1 - H]^{+}$ ion is identical to that of the $[z_2 - H]^{+}$ ion formed from GW$^{+}$, suggesting strongly that the ions have identical structures.

5. Conclusion

Tryptophan or tyrosine, present in the form of an amino acid or as a residue in an oligopeptide, is mandatory for abundant formation of the molecular radical cation from fragmentation of the [Cu$_{11}$]-complex ion. The M$^{+}$ ion’s fragmentation chemistries are rich, fascinating, and different from those of protonated peptides. The most facile fragmentations are around the α-carbon of the tryptophanyl residue. Fragmentation of the N–C$_n$ bond and proton transfer to the N-terminal fragment leads to formation of the $[z_2 - H]^{+}$ ions and elimination of a neutral amide or ammonia. Fragmentation of the C$_n$–C bond and hydrogen transfer to the N-terminal fragment leads to formation of the $[a_2 + H]^{+}$ ions and elimination of carbon dioxide. $[z_2 - H]^{+}$ ions of the same length (the same n) have identical CID spectra and have the same structure, irrespective of the N-terminal fragments eliminated.

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Supporting Information Available: Total energies and Cartesian coordinates for all structures reported. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes


(19) The mechanism of the transfer of the benzylic-type proton to the amino nitrogen will be the subject of a future report.

(20) These products are likely to arise from $I_b$ via a 1,6-H transfer of the carboxylic hydrogen to the carbonyl oxygen of the peptide linkage and cleavage of $\text{CO}_2$ (see Scheme 1); an $\alpha$-cleavage then eliminates methanimine.


