Metal-Mediated Formation of Gas-Phase Amino Acid Radical Cations[†,#]

Christopher K. Barlow,[‡] Damian Moran,[§] Leo Radom,*[,§,⊥] W. David McFadyen,*[,‡] and Richard A. J. O'Hair*[,‡,||,⊥]

School of Chemistry, University of Melbourne, Melbourne, VIC 3010, Australia, School of Chemistry, University of Sydney, Sydney, NSW 2006, Australia, Bio21 Institute, University of Melbourne, Melbourne, VIC 3010, Australia, and ARC Centre of Excellence in Free Radical Chemistry and Biotechnology

Received: November 8, 2005; In Final Form: March 5, 2006

The results from an investigation of the collision-induced dissociation (CID) of the ternary complexes $[Cu^{II}(terpy)(AA)]^{2+}$ are presented (terpy = 2,2′:6′,2″-terpyridine; AA = one of the twenty common amino acids). These complexes show a rich gas-phase chemistry, which depends on the identity of the amino acid. For the histidine-, lysine- and tryptophan-containing complexes, oxidative dissociation of the amino acid is observed, yielding the amino acid radical cation. The results of further mass selection and CID of these amino acid radical cations are presented. The CID of the series $[Fe^{III}(salen)(AA)]^{+}$ (where salen = *N*,*N*′-ethylenebis(salicylideneaminato)) is also examined. These complexes undergo loss of the neutral amino acid in all cases, although the radical cation of arginine is also produced and its subsequent fragmentation examined. B3-LYP/6-31G(d) computations were carried out to test aspects of the proposed fragmentation mechanism of the histidine and arginine radical cations.

1. Introduction

There have been several mass spectrometric studies in recent years of gas-phase copper(II) complexes with amino acids or peptides.[1−5] A feature of such copper(II) complexes is their tendency to oxidize the coordinated amino acid or peptide. For example, in a study of the collision-induced dissociation (CID) of histidine-containing peptides coordinated to copper(II), Hu and Loo found that the complexes underwent decarboxylation followed by further radical-driven elimination.[1] Similarly, Tureček and co-workers have shown that the fragmentation of $[Cu^{II}(bipy)(AA - H)]^{+}$ complexes (bipy = 2,2′-bipyridine, AA is an amino acid) typically leads to loss of CO_2 followed by further radical-driven fragmentation of the resulting copper(I) complex of the decarboxylated amino acid radical, $[Cu^{I}(bipy)(AA - H - CO_2)^{\bullet}]^{+}$. Closely related CID experiments involving $[Cu^{II}(bipy)(Arg)]^{2+}$ and $[Cu^{II}(His)_2]^{2+}$ produce cationic radical fragments such as $(Arg - CO_2)^{+\bullet}$ and $(His - CO_2)^{+\bullet}$, respectively, suggesting the transient formation of both the arginine and histidine radical cations.[2,5]

Particularly germane to the present work is the important discovery by Siu and co-workers that CID of the $[Cu^{II}(dien)(YGGFLR)]^{2+}$ complex (dien = diethylenetriamine, Figure 1) leads to the formation of the $YGGFLR^{+\bullet}$ radical cation and the complementary $[Cu^{I}(dien)]^{+}$ complex.[6] This discovery has formed the basis of a method for the formation of peptide radical cations in the gas phase. The method involves the introduction of a neutral peptide into the gas phase as part of a ternary complex in which the peptide is coordinated to a copper(II) ion along with an auxiliary ligand. Upon CID, the peptide is oxidized concomitant with its dissociation from the metal complex, thus forming a cationic peptide radical. Subsequently, several investigations involving this methodology have been undertaken.[6−12] Unfortunately, several reactions compete with the oxidative dissociation pathway, and complexes containing the dien ligand are prone to dissociation by competitive loss of the protonated peptide, protonated ligand, or fragmentation of the peptide. Thus, successful application was restricted to the generation of radical cations of particular peptides containing a tyrosine or a tryptophan residue and were assisted by the presence of a basic residue, specifically arginine, lysine, or histidine.[6,7] Siu and co-workers have also reported the formation of the individual tyrosine and tryptophan radical cations from the corresponding copper(II) dien complexes.[10]

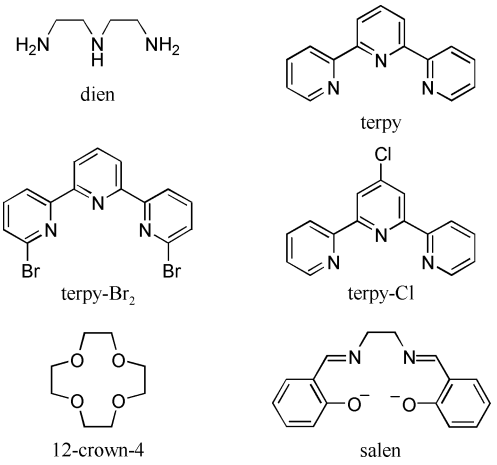


Figure 1. Examples of the auxiliary ligands that have been used in ternary metal complexes to produce cationic peptide radicals.

Much of the proton-transfer chemistry competing with oxidative dissociation is suppressed by the use of 2,2′:6′,2″-

[†] Part of the "Chava Lifshitz Memorial Issue".
[#] This article is part 49 of the series "Gas Phase Ion Chemistry of Biomolecules".
* Email: rohair@unimelb.edu.au; wdmf@unimelb.edu.au; radom@chem.usyd.edu.au.
[‡] School of Chemistry, University of Melbourne.
[§] University of Sydney.
[||] Bio21 Institute, University of Melbourne.
[⊥] ARC Centre of Excellence in Free Radical Chemistry and Biotechnology.

10.1021/jp056471v CCC: $33.50
Published on Web 04/07/2006

terpyridine (terpy, Figure 1) in place of dien. In addition to the formation of radical cations containing aromatic residues, terpy allowed the formation of radical cations containing a basic residue in the absence of a tyrosine or tryptophan.[10,13] This has led to terpy complexes being used in a number of studies by ourselves and others.[12−14] However, even for the terpy complexes, their CID chemistry is complex and often fails to produce the radical cation.[12] Clearly, the dependence of the dissociative chemistry of these metal complexes on the identity of the peptide warrants a more systematic examination. To this end, studies have been undertaken in which a single residue is varied in a tripeptide such as GXR[13] or GGX.[12] However, the position, as well as the identity, of the residues within a peptide has also been shown to be important in directing the CID of the metal complexes.[15]

A number of other ternary metal complexes have also been used to form peptide radical cations. The use of copper(II) complexes with 1,4,7,10-tetraoxacyclododecane (12-crown-4, Figure 1) as the auxiliary ligand allowed the formation of tripeptide radical cations of the form $GGX^{+\bullet}$, where X was an aliphatic residue.[9] This was a significant finding, as aliphatic residues have the highest ionization energies of all the amino acids. More recently, it has been shown that the use of copper(II) complexes incorporating the 6,6″-dibromo-2,2′:6′,2″-terpyridine (terpy-Br_2, Figure 1) auxiliary ligand allows radical cation formation for a much greater range of peptides, including those containing only aliphatic residues.[12] Peptide radical cations have also been formed using complexes that contain metal ions other than copper(II). For example, we recently reported that a number of trivalent metal complexes (Cr^{III}, Mn^{III}, Fe^{III}, and Co^{III}) utilizing dianionic *N*,*N*′-ethylenebis(salicylideneaminato) (salen, Figure 1) as the auxiliary ligand are capable of forming cationic peptide radical ions.[16]

In this paper, we report the CID reactions of a series of complexes of the type $[Cu^{II}(terpy)(AA)]^{2+}$ and $[Fe^{III}(salen)(AA)]^{+}$ (where AA corresponds to one of the 20 common amino acids listed in Table 1). The purpose of these experiments is as follows:

(i) To provide amino acid dependent information relating to the CID of the ternary metal complexes, which may help rationalize the chemistry of the corresponding peptide-containing complexes.

(ii) To form the radical cations of the amino acids in sufficient abundance so as to allow examination of their low-energy CID. Despite the electron impact mass spectrometry (EI-MS) of the 20 common amino acids being known,[17] as well as there being several studies on single photon and multiphoton ionization (MPI) of amino acids,[18] the low-energy CID of the amino acid radical cations has remained in many cases unexplored.[19]

2. Experimental Section

2.1. Mass Spectrometry. Most experiments were conducted on a commercially available quadrupole ion-trap mass spectrometer (Finnigan-MAT model LCQ, San Jose, CA) equipped with electrospray ionization (ESI). In other cases, experiments were performed on a Finnigan LCQ Deca XT Max quadrupole ion-trap mass spectrometer (Finnigan, San Jose, CA) or in the linear ion trap of a Finnigan LTQ FT mass spectrometer (Bremen, Germany), as indicated in the text. In each of the instruments, helium was used as the collision gas. Samples were typically prepared by combining 100 μL of amino acid stock solution (1 mg mL^{-1} in water) with 100 μL of the metal complex stock solution (1 mg mL^{-1} in methanol) and diluting with methanol to a total volume of 1 mL. Sample solutions were sprayed immediately. Experiments involving the deuterium exchange of protons bound to heteroatoms were carried out in a similar fashion, except that water was substituted by deuterium oxide and methanol by methanol-d_1 or methanol-d_4. The $[Fe^{III}(salen)(AA)]^{+}$ ions were abundant under a range of source conditions, while formation of the $[Cu^{II}(terpy)(AA)]^{2+}$ ions in sufficient abundance to allow CID typically required extensive tuning of the electrospray conditions, details of which are included in the Supporting Information. In the case of the methylated histidine derivatives, 1-methylhistidine (1-Me-His), 3-methylhistidine (3-Me-His), and the methyl ester of histidine (His-OMe), the terpy ligand was replaced by 4′-chloro-2,2′:6′,2″-terpyridine (terpy-Cl) (Figure 1). This substitution was necessitated by an unfortunate isobaric overlap: The *m*/*z* of the $[Cu^{II}(terpy)(AA)]^{2+}$ ion is 232.5 and the *m*/*z* of $[Cu^{I}(AA)]^{+}$ is 232, making isolation and mass selection of the former doubly charged complex extremely difficult. We adopt the convention of designating the mass-selected precursor ion in the spectra by an asterisk (*) throughout this paper.

2.2. Materials. All amino acids were purchased from Sigma-Aldrich as the L-isomer (98% purity), except for 3-methyl-L-histidine (Sigma) and 1-methyl-L-histidine (Fluka). The complexes $[Cu^{II}(terpy)(NO_3)]\cdot H_2O$ and $[Fe^{III}(salen)]Cl$ were prepared according to literature procedures.[20,21] The complex $[Cu^{II}(terpy-Cl)(NO_3)]$ was prepared following the procedure used to prepare $[Cu^{II}(terpy)(NO_3)]\cdot H_2O$. Deuterated solvents were purchased from Cambridge Isotope Laboratories, Andover, MA.

2.3. Computational Methodology. All geometries were optimized at the B3-LYP/6-31G(d) level of theory, with frequencies calculated at the same level to confirm that the structures correspond to either true minima or first-order saddle points on the potential energy surface and to provide zero-point vibrational energy (ZPVE) corrections. The ZPVE corrections, scaled by the appropriate factor (0.9806),[22] were applied to all energies. The $\langle S^2 \rangle$ values were checked and showed negligible spin contamination for open-shell systems. Because of the high degree of conformational flexibility of arginine, it was impractical to systematically explore all the conformers of the molecule at the B3-LYP/6-31G(d) level of theory. Therefore, the (Monte Carlo) conformation search function of MacSpartan 02[23] was used at the AM1 level of theory. In addition, the Gaussian 03[24] scan keyword was used to generate conformers followed by optimization (HF/3-21G(d)) and single-point energy computations (B3-LYP/6-31G(d)//HF/3-21G(d)). The Gaussian 03[24] program package was used for all B3-LYP/6-31G(d) calculations. Geometries, energies, ZPVEs, and $\langle S^2 \rangle$ values not reported in the text are included as Supporting Information.

3. Results and Discussion

3.1. CID of the $[Cu^{II}(terpy)(AA)]^{2+}$ Complexes. In discussing the CID of the $[Cu^{II}(terpy)(AA)]^{2+}$ complexes, it is convenient to divide the reactions into four classes (Scheme 1). Reaction 1 is the loss of the neutral amino acid, that is, nonoxidative dissociation. Reaction 2 involves oxidative dissociation of the amino acid to form the corresponding radical cation and the complementary $[Cu^{I}(terpy)]^{+}$ ion. In many cases, the $[Cu^{I}(terpy)]^{+}$ ion is observed in the absence of its complementary $AA^{+\bullet}$ ion, due to the further fragmentation of the amino acid radical cation following dissociation from the complex. Reaction 3 involves elimination of a positively charged fragment of the amino acid, while the remaining negatively charged fragment remains coordinated to the copper ion. This reaction is almost exclusively limited to elimination of the immonium ion (NH_2CHR^{+}) plus CO to give the $[Cu^{II}(terpy)(OH)]^{+}$ ion

TABLE 1: Summary of the CID Chemistry of the $[Cu^{II}(terpy)(AA)]^{2+}$ Complexes

amino acid	ionization energy (eV)	reaction 1	reaction 2		reaction 3		reaction 4			comments and other losses[d]
		$[Cu^{II}(terpy)]^{2+}$	$AA^{+\bullet}$	$[Cu^{I}(terpy)]^{+}$	$[Cu^{II}(terpy)(OH)^{+}$	$[NH_2CHR]^{+}$	$[Cu^{II}(terpy)AA - CH_2O_2)]^{2+}$	$[Cu^{II}(terpy)AA - H_2O)]^{2+}$	$[Cu^{II}(terpy)(AA - NH_3)]^{2+}$	
Gly[a]	8.8[e]	+	−	−	+	−	+	−	−	primarily undergoes non-oxidative dissociation
Ala[a,b]	8.88[e]	+	−	−	+	−	−	−	−	also exhibits loss of $NH_3 + CO$
Val	8.71[e]	+	−	−	+	+	−	−	−	
Leu	8.51[e]	+	−	−	+	+	−	−	+	
Ile	8.66[e]	+	−	−	+	+	−	−	+	
Pro	8.2[e]	−	−	+	+	+	−	−	−	
Ser	8.8[e]	+	−	+	−	−	+	+	−	loss of H_2O is the primary fragmentation pathway
Thr		+	−	+	+	−	+	+	−	loss of H_2O is the primary fragmentation pathway
Cys	8.0[e]	+	−	−	−	−	−	−	+	primarily loses NH_3, loss of NH_4^+ is also apparent
Met	8.0[e]	−	−	−	+	−	−	−	+	primarily undergoes loss of 48 mass units corresponding to CH_3SH
Asp		−	−	+	+	−	+	−	−	primarily undergoes loss of 46 mass units
Glu		−	−	−	−	−	−	+	−	loss of H_2O is the primary fragmentation pathway
Asn		−	−	−	+	−	−	−	−	shows a loss of 45 mass units which may correspond to NH_3 plus CO
Gln		−	−	+	−	−	−	+	+	loss of NH_3 and H_2O prominent, loss of NH_4^+ is also apparent
Phe	8.5,[e] 8.8[f]	+	−	+	+	+	−	−	−	also shows a product ion at $m/z = 104$
Tyr	8.0[e]	+	−	+	+	−	−	+	−	also shows a product ion at $m/z = 120$
Trp	7.2[e]	−	+	+	−	−	−	−	+	also shows a product ion at $m/z = 130$, which corresponds to the further fragmentation of the radical cation
His		−	+	+	−	−	+	−	−	there is also a product ion at $m/z = 82$
Lys[c]	8.6[e]	−	+	+	+	−	−	−	+	predominantly loses NH_3
Arg[b]		−	+	+	+	−	−	−	−	the arginine radical cation appears to undergo further fragmentation

[a] Note that, for glycine and alanine, the mass of the immonium ion is below the low-mass cutoff of the ion trap, making its detection impossible. [b] This experiment was performed on both the LCQ and LCQ Deca XT Max instruments. [c] This experiment was performed on the LCQ Deca XT Max instrument. [d] All the spectra not included as figures in the text are provided as Supporting Information. [e] Lowest adiabatic ionization energy. See ref 25 and references therein. [f] See ref 26 for conformation-dependent ionization energies of L-phenylalanine.

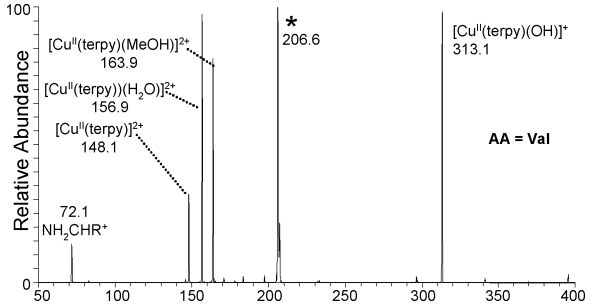
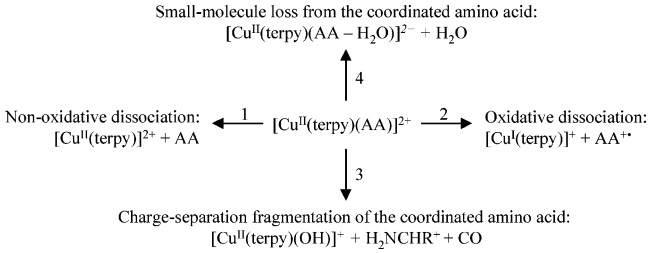


Figure 2. CID of the $[Cu^{II}(terpy)(Val)]^{2+}$ complex. We adopt the convention of designating the mass-selected parent ion by an asterisk (*) throughout this paper.

SCHEME 1: Grouping of the CID Reactions of the $[Cu^{II}(terpy)(AA)]^{2+}$ Complexes into Four Reaction Types



and is most likely driven by the polarizing effect of the copper(II) ion. Reaction 4 involves fragmentation of the coordinated amino acid, typically through the loss of small molecules such as H_2O or NH_3. The most common product ions observed following CID of the $[Cu^{II}(terpy)(AA)]^{2+}$ complexes are given in Table 1, while the fragmentation reactions of the individual complexes are described in more detail below.

3.1.1. The Aliphatic Amino Acids. The CID spectrum of $[Cu^{II}(terpy)(Val)]^{2+}$ illustrates the common types of product ions observed for the aliphatic amino acid containing complexes (Figure 2). The aliphatic amino acids predominantly fragment by non-oxidative dissociation (Scheme 1, reaction 1) or by fragmentation of the amino acid backbone (Scheme 1, reaction 3). All of the aliphatic amino acid complexes, apart from $[Cu^{II}(terpy)(Pro)]^{2+}$, show peaks at $m/z = 148$, 157, and 164 following CID. These ions arise from the loss of the neutral amino acid to give the $[Cu^{II}(terpy)]^{2+}$ complex ($m/z = 148$, reaction 1), which readily undergoes addition of adventitious background water and methanol in the trap to give $[Cu^{II}(terpy)(H_2O)]^{2+}$ ($m/z = 157$) and $[Cu^{II}(terpy)(MeOH)]^{2+}$ ($m/z = 164$), respectively.

The $[Cu^{II}(terpy)(OH)]^{+}$ complex is a prominent product for all the aliphatic amino acids. The complementary b_1 (NH_2CHRCO^+) ions are known to be unstable with respect to decomposition by decarbonylation, forming the corresponding immonium ion (NH_2CHR^+).[27] Consequently, the formation of the $[Cu^{II}(terpy)(OH)]^{+}$ product ion from the aliphatic amino acid complexes (reaction 3) is accompanied by the presence of the immonium ion for all the aliphatic amino acid complexes except glycine and alanine. The absence of the glycine and alanine immonium ions from the CID of their respective complexes is not surprising, as these low-mass ions are not readily captured in the ion trap.

3.1.2. The Aromatic Amino Acids. The CID spectra of the aromatic amino acid complexes are shown in Figure 3. CID of all of the aromatic amino acid complexes yield $[Cu^{I}(terpy)]^{+}$, suggestive of oxidative dissociation of the amino acid. The complementary amino acid radical cations are present for histidine and tryptophan. Despite the presence of the $[Cu^{I}(terpy)]^{+}$ ion in the CID of both $[Cu^{II}(terpy)(Phe)]^{2+}$ and $[Cu^{II}(terpy)(Tyr)]^{2+}$ (Figure 3A,B), the corresponding amino acid radical cations are absent. Both spectra exhibit a product ion 61 mass units lower than the radical cation, which may correspond to the formation of the styrene and 4-hydroxystyrene radical cations, respectively. The mechanism for the formation of these product ions is, however, unclear. CID of the tryptophan complex primarily proceeds by oxidative dissociation to produce both $[Cu^{I}(terpy)]^{+}$ and $Trp^{+\bullet}$ (Figure 3C). In addition, the tryptophan complex yields a minor loss of NH_3 as well as a product ion at $m/z = 130$, which corresponds to the benzylic ion, 3-methylene-3*H*-indole-1-ium.

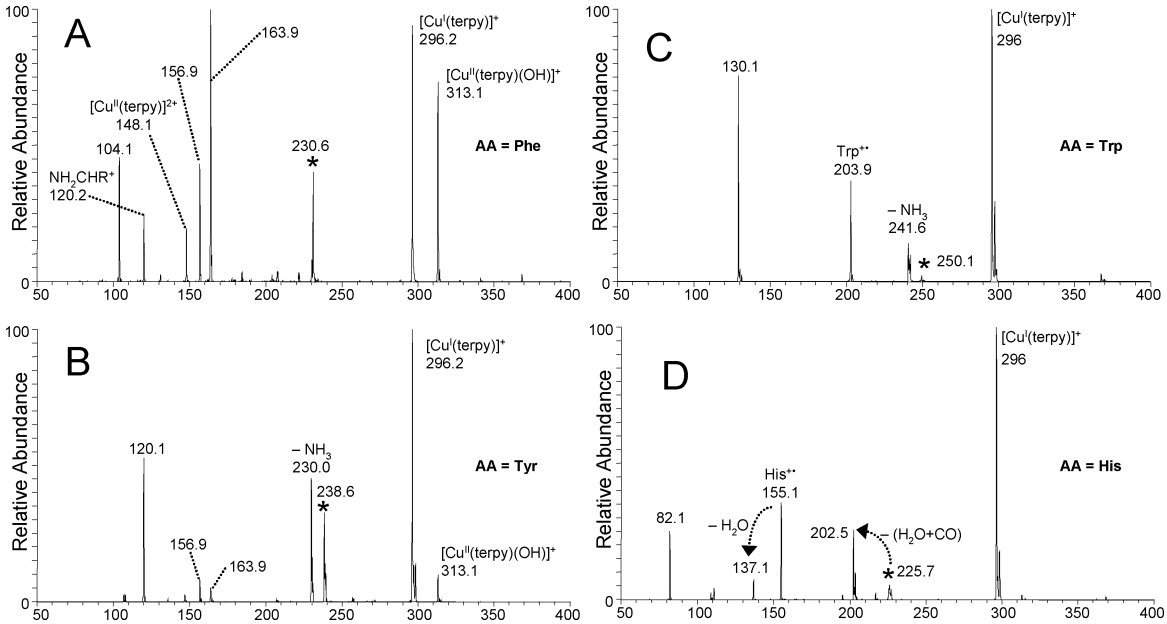


Figure 3. (A) CID spectra of the $[Cu^{II}(terpy)(Phe)]^{2+}$ complex. The peak at $m/z = 120$ corresponds to the immonium ion and $m/z = 104$ would correspond to $C_8H_8^{+\bullet}$. (B) CID of $[Cu^{II}(terpy)(Tyr)]^{2+}$ complex. The peak at $m/z = 120$ in this case corresponds to $C_8H_8O^{+\bullet}$. Peaks in spectra A and B at $m/z = 156.9$ and 163.9 correspond to $[Cu^{II}(terpy)(H_2O)]^{2+}$ and $[Cu^{II}(terpy)(MeOH)]^{2+}$ complexes, respectively. (C) CID of $[Cu^{II}(terpy)(Trp)]^{2+}$ complex. The peak at $m/z = 130$ corresponds to protonated 3-methyleneindolenine radical cation. (D) CID of the $[Cu^{II}(terpy)(His)]^{2+}$ ion.

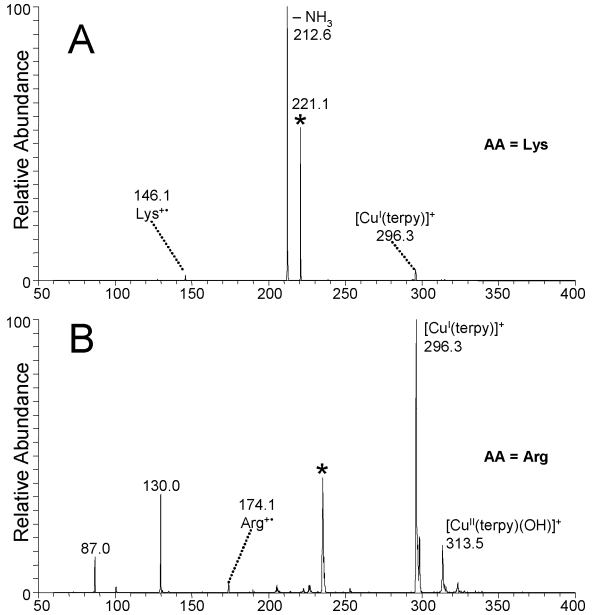


Figure 4. (A) CID of the $[Cu^{II}(terpy)(Lys)]^{2+}$ complex (LTQ FT). (B) CID of the $[Cu^{II}(terpy)(Arg)]^{2+}$ complex.

The CID spectrum of the $[Cu^{II}(terpy)(His)]^{2+}$ complex shows the loss of H_2O and CO, which is in competition with the formation of the histidine radical cation (Figure 3D). In addition, a product ion at $m/z = 82$ is also present, which may be assigned as the loss of iminoacetic acid to provide the 4-methyleneimidazole radical cation (Scheme 3). Interestingly, this ion is observed in the CID of the $[Cu^{II}(His)_2]^{2+}$ complex and the 70 eV EI-MS spectrum.[5,17,28]

3.1.3. Lysine and Arginine. Complexes containing the two most basic amino acids, lysine and arginine, show evidence of redox chemistry (Figure 4). The lysine-containing complex produces the lysine radical cation as a minor product, the fragmentation proceeding primarily by the loss of NH_3 (Figure 4A). Tureček and co-workers previously reported on the CID of $[Cu^{II}(bipy)(Lys)]^{2+}$, which also gives prominent loss of NH_3.[2] CID of the $[Cu^{II}(terpy)(Arg)]^{2+}$ complex provides the $[Cu^{I}(terpy)]^{+}$ ion as the main product ion, but the complementary arginine radical cation is only present in very low abundance, apparently readily undergoing loss of CO_2 to give an ion at $m/z = 130$ (Figure 4B). Further trapping and CID of the ion at $m/z = 130$ leads primarily to the formation of an ion at $m/z = 87$, which we assign as the ethylguanidine radical cation, and a less abundant ion at $m/z = 101$ corresponding to the loss of methanimine. The ethylguanidine radical cation is also present in the MS^2 spectrum. Possible mechanisms for these losses are described in further detail in section 3.6.

3.2. Discussion of the CID of $[Cu^{II}(terpy)(AA)]^{2+}$. To successfully form the amino acid radical cation, there are two requirements. First, dissociation of the amino acid from the metal complex must be competitive with (and ideally favored over) fragmentation of the coordinated amino acid. Second, dissociation must result in oxidation of the amino acid. The aliphatic amino acids tend to satisfy the first criterion but not the second, as demonstrated by the formation of the $[Cu^{II}(terpy)]^{2+}$ and related product ions and the absence of the $[Cu^{I}(terpy)]^{+}$ ion. This may be attributed to the relatively high ionization energies (IEs) of the aliphatic amino acids (Table 1). The aromatic amino acids, which generally have lower ionization energies, satisfy both criteria, as demonstrated by the presence of the $[Cu^{I}(terpy)]^{+}$ and radical-related product ions. Of the remaining amino acids, Gln, Ser, and Thr also show evidence of redox chemistry, as indicated by the presence of small amounts of the $[Cu^{I}(terpy)]^{+}$ product ion, suggesting that the second criterion is satisfied. The first criterion is not met, however, as these complexes primarily undergo dissociation by fragmentation of the coordinated amino acid. Unlike the aliphatic amino acid complexes, fragmentation is not generally driven by loss of a charged immonium ion, but by intramolecular proton transfer, leading to the loss of small molecules such as H_2O, NH_3, or CH_3SH.

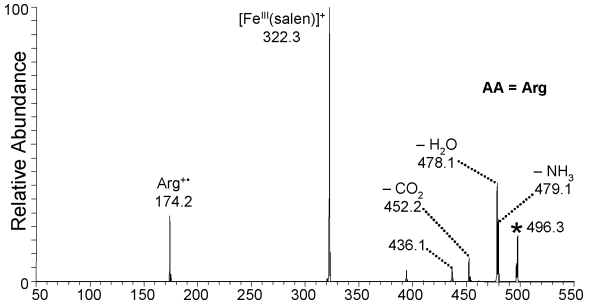


Figure 5. CID of the $[Fe^{III}(salen)(Arg)]^{+}$ complex, clearly showing the formation of the arginine radical cation.

The competitive loss of small molecules is likely to be driven by two factors. The copper(II) ion appears, in these cases, to act as a Lewis acid facilitating proton transfer from the site of binding on the amino acid to a different site, thereby triggering subsequent small-molecule loss. In addition, neighboring group participation is known to be important in the elimination of small molecules for a number of protonated amino acids.[29] Both intramolecular acid−base chemistry between the side chain and the backbone and neighboring group processes should be facilitated by the presence of a flexible functionalized side chain such as those found in Ser, Thr, Met, Glu, Asp, Asn, Gln, and Cys. In contrast, the aromatic amino acids Phe, Trp, and Tyr possess side chains for which both proton transfer between the side chain and the backbone and neighboring group processes are sterically less favorable. Although both $[Cu^{II}(terpy)(Tyr)]^{2+}$ and $[Cu^{II}(terpy)(Trp)]^{2+}$ lose NH_3, small-molecule loss from complexes containing the aromatic amino acids Phe, Trp, and Tyr is not a prevalent fragmentation pathway. The loss of NH_3 may be accounted for by proton transfer occurring across the amino acid backbone from the carboxylic acid.

It appears likely that it is not only the comparatively low IE of the aromatic amino acids that enables formation of the radical cation counterparts, but also the absence of facile pathways for small-molecule loss. Conversely, the failure of complexes containing a flexible functionalized side chain to undergo extensive oxidative dissociation may not be due to their greater IE, but rather to the extremely facile loss of small molecules from these complexes.

3.3. CID of the $[Fe^{III}(salen)(AA)]^{+}$ Complexes. For the 20 common amino acids, CID of the $[Fe^{III}(salen)(AA)]^{+}$ complexes leads essentially to non-oxidative dissociation of the neutral amino acid from the complex to provide the $[Fe^{III}(salen)]^{+}$ ion. However, for the arginine-containing complex, competitive formation of the arginine radical cation also occurs (Figure 5).

The tendency for the $[Fe^{III}(salen)(AA)]^{+}$ complex to undergo non-oxidative dissociation demonstrates the importance of the charge state in directing the dissociation of these complexes. For the doubly charged $[Cu^{II}(terpy)(AA)]^{2+}$ complexes, there is a strong driving force for pathways that result in the separation of charge. This driving force promotes dissociation involving charge transfer, such as oxidative dissociation, at the expense of non-oxidative dissociation. In contrast, the $[Fe^{III}(salen)(AA)]^{+}$

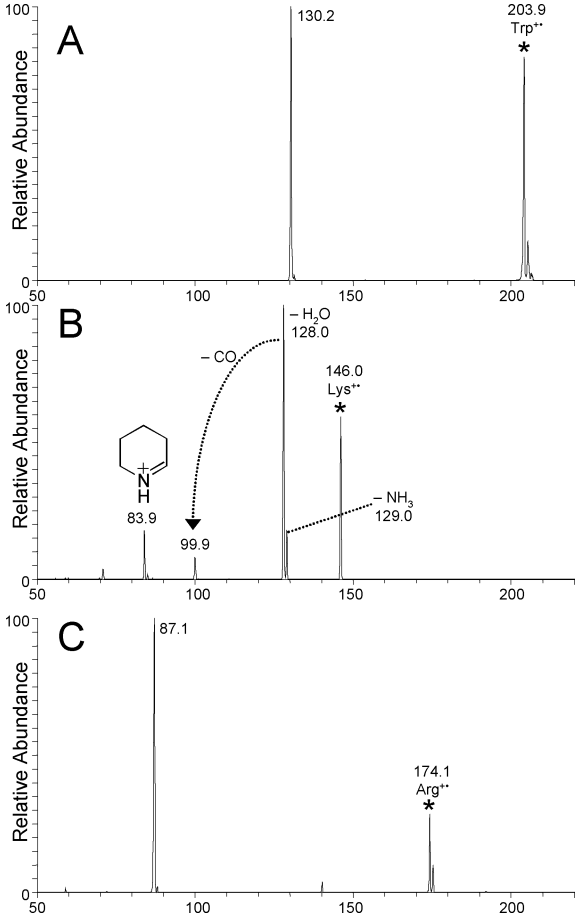


Figure 6. (A) CID of the tryptophan radical cation. (B) CID of the lysine radical cation (LTQ FT). (C) CID of the arginine radical cation formed following dissociation from the $[Fe^{III}(salen)(Arg)]^+$ complex leads to the loss of dehydroalanine to give the ethylguanidine radical cation.

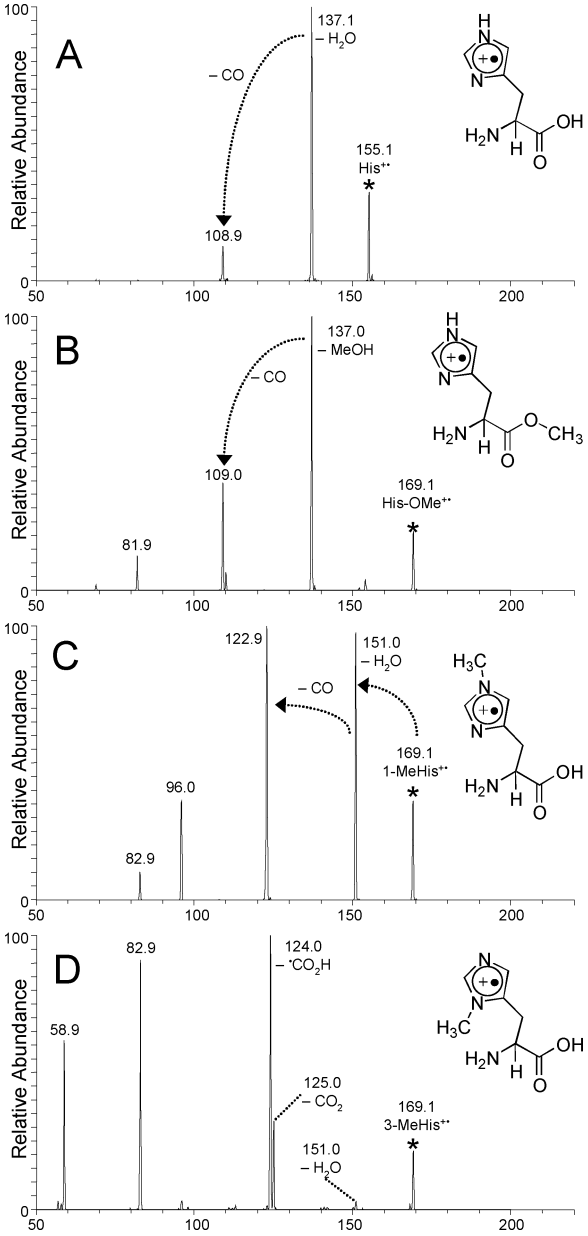


Figure 7. CID of the histidine radical cation (A), the histidine methyl ester radical cation (B), 1-methylhistidine radical cation (C), and 3-methylhistidine radical cation (D). Note that experiments B−D were performed on the Finnigan-LTQ, rather than the LCQ.

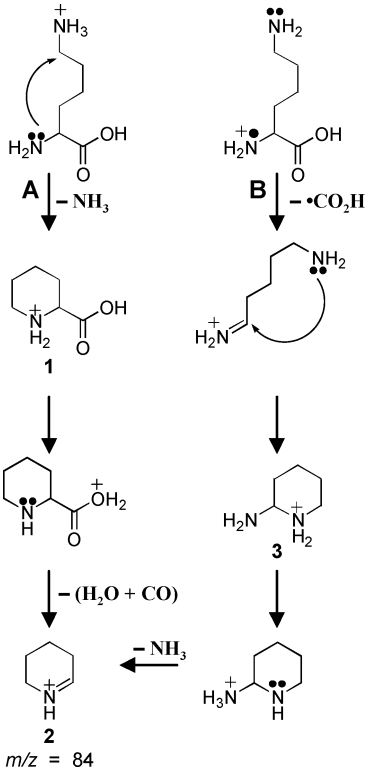
complexes have a net charge of +1, and thus, both oxidative and non-oxidative dissociation lead to the formation of a singly charged ion as well as the neutral product.

3.4. CID of the Amino Acid Radical Cations. A significant outcome of this work is that the tryptophan, histidine, lysine, and arginine radical cations were formed in sufficient abundance to allow their further mass selection for CID studies. The CID spectra of the tryptophan, lysine, and arginine radical cations are shown in Figure 6, while the CID spectrum of $His^{+\bullet}$ is shown in Figure 7A. CID of the tryptophan radical cation leads to the exclusive formation of protonated 3-methylene-3*H*-indole-1-ium (Figure 6A), consistent with a previous report by Siu and co-workers.[10] This product also dominates the 70 eV EI-MS and the MPI spectrum of tryptophan.[17,18] CID of the lysine radical cation (Figure 6B) leads predominantly to the loss of H_2O, with minor loss of H_2O + CO and NH_3. A fragment ion at $m/z = 84$ is also present, the identity of which is not immediately apparent. However, an ion at $m/z = 84$ is also observed in the CID spectra of protonated lysine, which may correspond to the same ion. CID of $[Lys + H]^+$ leads to elimination of side chain NH_3 following nucleophilic attack by the backbone amine to form protonated pipecolic acid (**1**) (Scheme 2), which then eliminates H_2O and CO to give protonated 2,3,4,5-tetrahydropyridine (**2**), $m/z = 84$ (pathway A, Scheme 2).[30] CID of $Lys^{+\bullet}$ may follow a similar mechanism, most likely by elimination of $^{\bullet}CO_2H$ followed by nucleophilic attack by the side chain amine at the α-carbon, leading to the formation of protonated 2-aminopiperidine (**3**), which then eliminates NH_3 to form **2** (pathway B, Scheme 2). CID of the arginine radical cation leads almost exclusively to the loss of dehydroalanine to give the ethylguanidine radical cation (Figure 6C), and these results are discussed below in section 3.5.

3.5. Fragmentation of the Histidine Radical Cation. Mass selection and CID of $His^{+\bullet}$ results in loss of H_2O and H_2O + CO. This is also the dominant fragmentation pathway for protonated histidine,[28] suggesting that the fragmentation of the long-lived radical cation may be charge-directed, rather than radical-directed.

In addition to examining the histidine radical cation, we have also been able to form the radical cation of three related derivatives: 1-methylhistidine (1-MeHis), 3-methylhistidine (3-MeHis), and the methyl ester of histidine (His-OMe).[31] The CID spectra of $His\text{-}OMe^{+\bullet}$, $1\text{-}MeHis^{+\bullet}$, and $3\text{-}MeHis^{+\bullet}$ (Figure 7B,C,D) provide insights into the mechanism of H_2O loss from $His^{+\bullet}$. Neither $His\text{-}OMe^{+\bullet}$ nor $1\text{-}MeHis^{+\bullet}$ differ significantly from $His^{+\bullet}$ in their CID chemistry. The methyl ester, His-

SCHEME 2: Proposed Mechanisms for the Formation of Protonated 2,3,4,5-Tetrahydropyridine (2) from Protonated Lysine (A) (ref 30) and Lysine Radical Cation (B)

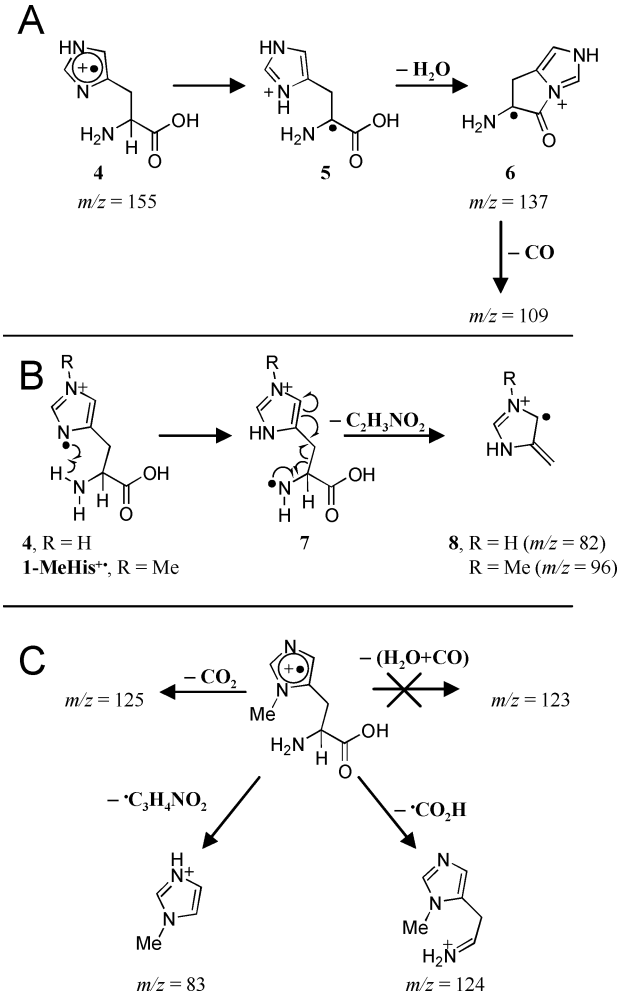


OMe$^{+\bullet}$, undergoes loss of MeOH and CO in close analogy with His$^{+\bullet}$ (Figure 7B). Similarly, CID of 1-MeHis$^{+\bullet}$ predominantly proceeds by loss of H_2O and H_2O + CO (Figure 7C).

A possible rationale for these observations is provided in Scheme 3A and involves nitrogen in the 3-position of the imidazole ring acting as a proton shuttle, triggered by the abstraction of the hydrogen atom from the α-carbon. Subsequent H_2O and H_2O + CO losses are equivalent to those in protonated histidine, which proceed by proton transfer from the 3-position on the imidazole ring.[32] This mechanism apparently specifically involves the nitrogen in the 3-position, as methylation at this position dramatically alters the CID spectrum (Figure 7D), with loss of H_2O being negligible and new pathways involving CO_2 and $^{\bullet}CO_2H$ loss becoming apparent.

Scheme 3B provides a possible mechanism for the formation of the 4-methyleneimidazole radical cation (**8**) (m/z = 82), via a McLafferty-type rearrangement. Hydrogen-atom abstraction from the amine by the nitrogen in the 3-position is followed by homolytic cleavage of the $C_\alpha-C_\beta$ bond to eliminate iminoacetic acid. Although the m/z = 82 product ion is not observed in the His$^{+\bullet}$ spectrum, it is present in both the [Cu^{II}(terpy)(His)]$^{2+}$ and His-OMe$^{+\bullet}$ spectra. It is also the main product ion in the 70 eV EI-MS.[17] Furthermore, CID of 1-MeHis$^{+\bullet}$ provides a product ion at m/z = 96, corresponding to the 1-methyl derivative. Notable in the context of the mechanism proposed in Scheme 3B is the absence of a product ion at either m/z = 82 or 96 in the spectrum of 3-MeHis$^{+\bullet}$, which implicates the specific involvement of the nitrogen at the 3-position rather than the 1-position. Both these mechanisms (Scheme 3A and 3B) appear to specifically involve hydrogen-atom abstraction by the N3 rather than the N1 nitrogen. We attribute this observation to the restricted capacity of the nitrogen in the 1-position to become sufficiently close to the amino acid backbone.

SCHEME 3: Fragmentation of the Histidine Radical Cation. (A) Proposed Mechanism for the Loss of Water from the Histidine Radical Cation. (B) Proposed Mechanism for the Formation of the 4-Methylene Imidazole Radical Cation. (C) Fragmentation of 3-MeHis$^{+\bullet}$



A preliminary examination of aspects of the potential energy surface (Figure 8) using B3-LYP/6-31G(d) yields results that are consistent with the mechanisms proposed in Scheme 3A,B.[33] The calculations show in the first place that, while the enthalpy for the elimination of water (**4** → **6**, Scheme 3A) is exothermic by 26 kJ mol^{-1}, the McLafferty rearrangement (**4** → **8**, Scheme 3B) is endothermic by 47 kJ mol^{-1}. In addition, the calculations predict barriers associated with the rate-limiting steps that are quite similar for the two pathways, the loss of water being slightly lower (by 12 kJ mol^{-1}). For comparison, our experimental results suggest that the rapid fragmentation observed immediately following oxidative dissociation (MS^2 spectrum) proceeds predominantly by the higher-energy McLafferty rearrangement (Scheme 3B), whereas in the low-energy CID of the isolated histidine radical cation, the lower-energy loss of water (Scheme 3A) takes place. The internal energy of the histidine radical cation immediately following oxidative dissociation, in the MS^2 experiment, is undoubtedly above the activation energy for both processes. The transition structure associated with the McLafferty rearrangement is likely to be favored entropically (because it is less tight) relative to the transition structure associated with the highly concerted loss of water, consistent with its prevalence in the MS^2 experiment. This contrasts with the behavior observed in the low-energy CID of the isolated

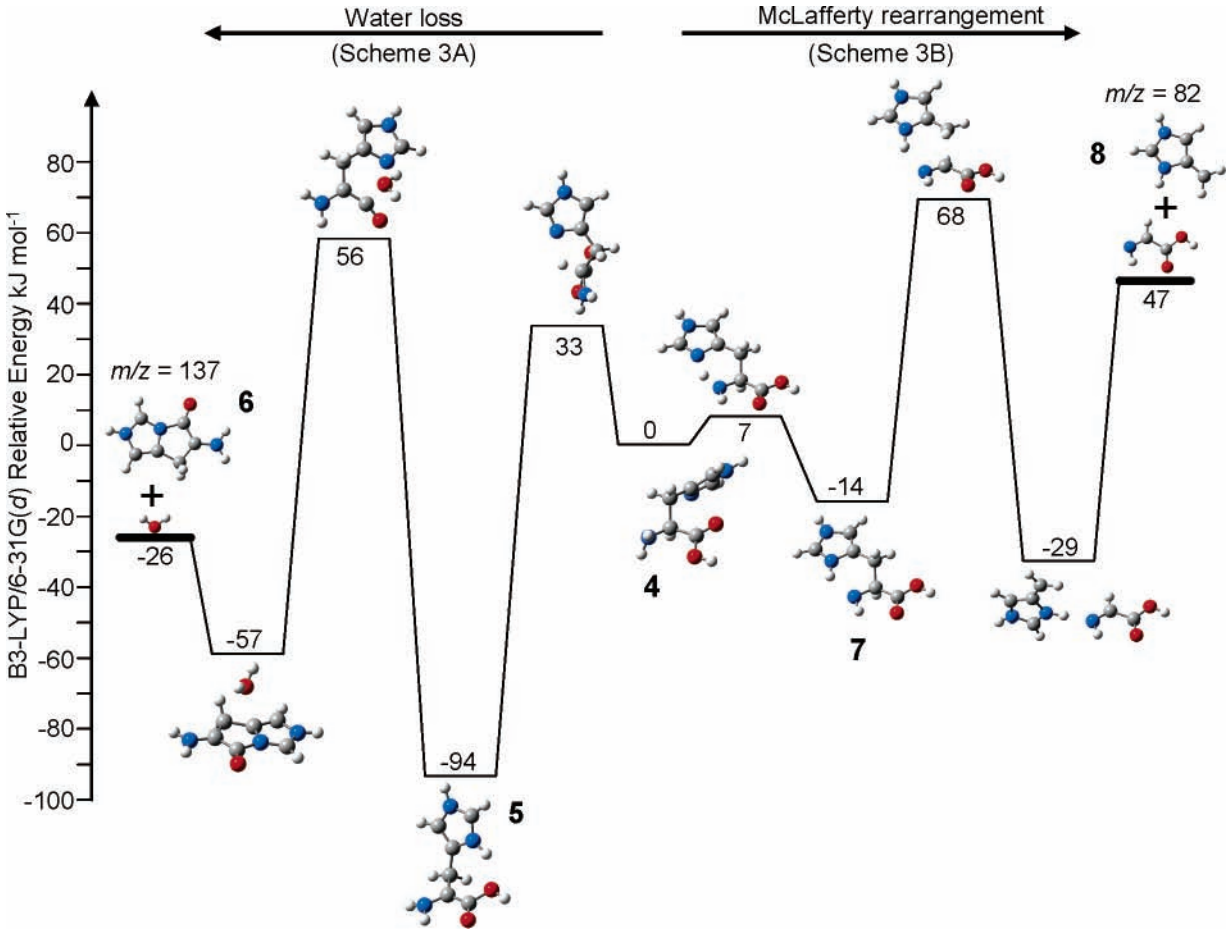


Figure 8. The B3-LYP/6-31G(d) potential energy surface for the histidine radical cation (kJ mol^{-1}). Bold lines represent dissociation channels. Barriers associated with bond rotations are omitted for clarity.

histidine radical cation (MS^3). In this case, we have a slow-heating process involving numerous low-energy collisions, thereby favoring the lower-energy loss of water.

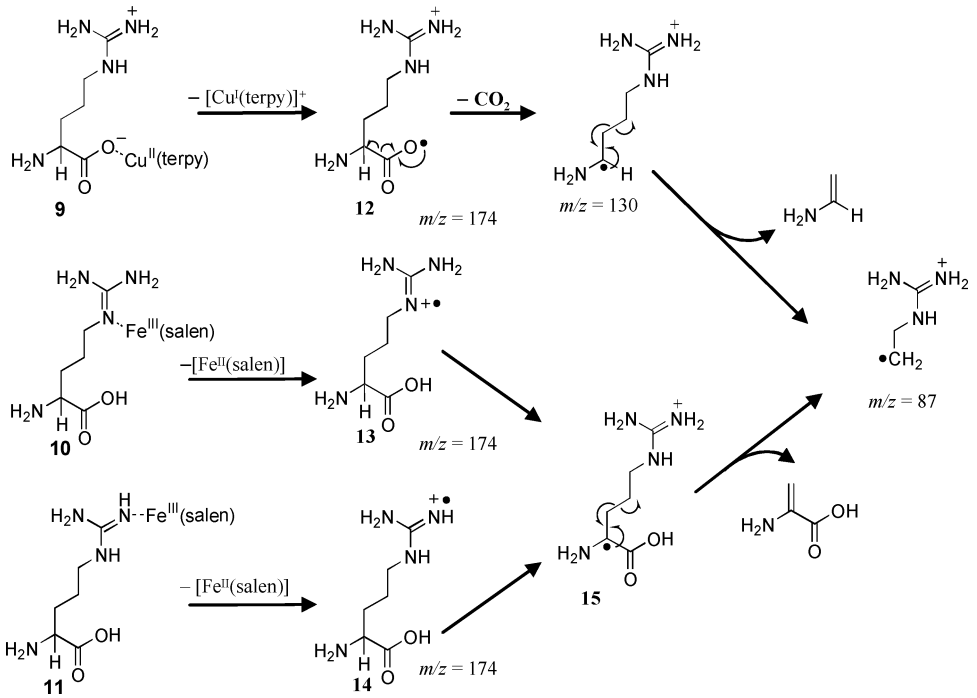
3.6. Comparison of $[Fe^{III}(salen)(Arg)]^+$ and $[Cu^{II}(terpy)(Arg)]^{2+}$. In the current investigation, we observe that both the $[Cu^{II}(terpy)(Arg)]^{2+}$ and $[Fe^{III}(salen)(Arg)]^+$ complexes undergo oxidative dissociation to form an arginine radical cation. The arginine radical cation formed from $[Cu^{II}(terpy)(Arg)]^{2+}$ rapidly undergoes (in the MS^2 experiment) further fragmentation by the loss of CO_2, to give an ion at $m/z = 130$. This may undergo still further fragmentation by loss of vinylamine to give the ethylguanidine radical cation ($m/z = 87$). Tureček and co-workers have previously reported the same product ions following CID of the related $[Cu^{II}(bipy)(Arg)]^{2+}$ complex.[2] They proposed that the arginine radical cation is formed as a zwitterionic-type structure in which the carboxylate is deprotonated, and we suggest that the arginine adopts an equivalent structure in the $[Cu^{II}(terpy)(Arg)]^{2+}$ complex, structure **9** (Scheme 4). Arginine is a good candidate to adopt a salt-bridge-type structure, due to the high basicity of the guanidine group. Indeed there have been a number of studies concerned with the question of whether arginine exists as a gas-phase zwitterion,[34−39] with the most recent of these showing that the non-zwitterionic form of arginine is more stable by 13.6 kJ mol^{-1}.[38,39] However, it has been demonstrated both theoretically[40] and experimentally[41] that coordination of an alkali metal ion may induce a salt-bridge-type structure.

CID of the arginine radical cation formed following oxidative dissociation from $[Fe^{III}(salen)(Arg)]^+$ contrasts with both its 70 eV EI-MS[17e] and charge-directed fragmentation obtained following MPI.[18] In addition, it does not show loss of CO_2 in the MS^2 experiment, as is observed for the $[Cu^{II}(terpy)(Arg)]^{2+}$ case. Indeed, further CID of the $Arg^{+\bullet}$ formed from the iron complex does not lead to CO_2 loss at all, but rather the elimination of dehydroalanine, which implies that dissociation proceeds through a distonic radical cation in which the charge is located on the fully protonated guanidinium side chain and the radical on the α-carbon (**15**, Scheme 4). Such a tautomer is unlikely to be formed directly following dissociation from the complex, as it would involve initial coordination of the arginine to the iron as a carbanion. More probable is coordination of arginine as a non-zwitterionic tautomer, as in complexes **10** or **11** in Scheme 4. We find that the radical cation of both the N-acetylated and methyl ester derivatives of arginine may be formed using iron(III) salen, which is consistent with the proposed complexes **10** and **11** (Scheme 4).

We propose that, following oxidative dissociation of arginine, the radical cation (either **13** or **14**, Scheme 4) tautomerizes to **15**, prior to fragmentation. Direct evidence for this rearrangement comes from exchange of the heterobound protons for deuteriums. CID of the deuterium-enriched radical cation leads to product ions at $m/z = 91$ and $m/z = 92$ (Figure 9). The lower-mass product ion is consistent with our proposed rearrangement. We attribute formation of the second product ion to intramolecular exchange between the heterobound proton/deuteriums.

Our experimental results are suggestive of the presence of several tautomeric forms of arginine. However, because these forms are not directly observable, it is useful to employ computational techniques to examine the viability of the proposed mechanistic scheme. Table 2 lists the B3-LYP/6-31G(d)

SCHEME 4: Proposed Mechanism for the Formation and Fragmentation of the Arginine Radical Cation from Copper(II) (A) and Iron(III) (B and C) Complexes



relative energies of the lowest-energy conformers of the tautomers discussed above, along with a fifth tautomer (**16**) identified during the conformational search process.

It is clear that the most stable tautomer is **15**, the α-centered radical, which lies considerably lower in energy (by more than 100 kJ mol^{-1}) than the second most stable tautomer, **16**, followed by **12**, **13**, and **14**, which are of comparable energy. The low energy of isomer **15** can be readily understood. In the first place, **15** contains a radical site that is stabilized by the NH_2 donor and COOH acceptor groups, i.e., it is strongly captodatively stabilized.[42] In addition, the charge is highly delocalized within the guanidinium moiety.

We have proposed in Scheme 4 that tautomers **13** and **14** might readily rearrange to **15**. The significantly lower energy of **15** relative to **13** or **14** demonstrates that there is a strong enthalpic driving force for the tautomerization. In addition, we have located a transition structure (TS) for the interconversion of **13** and **15**, as shown in Figure 10 (**TS1**), that lies only 28 kJ mol^{-1} above the most stable conformer of **13**. Although there are likely to be several TSs for each possible tautomerization as a result of the flexible side chain of arginine, making it impractical to comprehensively investigate the entire potential energy surface, the energy obtained for **TS1** places a very modest upper limit on the barrier for conversion of **13** to **15**. This provides further support for the mechanism proposed in Scheme 4. Our preliminary calculations of the pathway linking **14** and **15** indicate that the details are sensitive to the level of theory employed. However, our best calculations suggest a low barrier separating **14** from **15**. We have also found that the TS for loss of CO_2 from **12** (Figure 10, **TS2**) lies only 4 kJ mol^{-1} above the most stable conformer of **12**, demonstrating that this fragmentation process is virtually barrierless. This again is consistent with the mechanism for the formation of $m/z = 130$ proposed in Scheme 4.

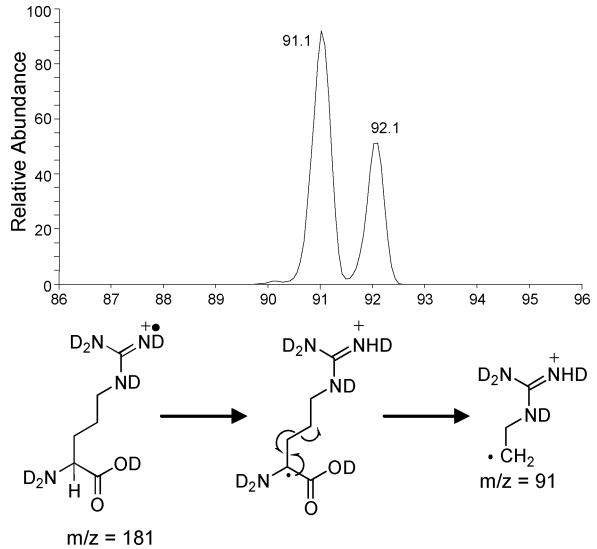
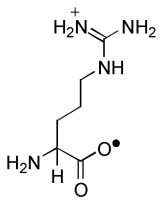
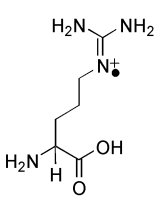
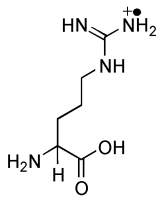
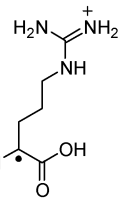
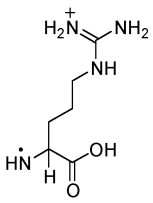


Figure 9. CID of the arginine radical cation in which the heteroatom protons have been exchanged for deuterium. Only the product ions are shown. Observation of the $m/z = 91$ ion is consistent with the proposed tautomerization to tautomer **15** prior to fragmentation. We attribute the product ion at $m/z = 92$ to intramolecular H/D scrambling between the heterobound proton/deuteriums.

What implications does the chemistry observed for the arginine radical cation have for that involving larger peptides? The unstable tautomer formed following dissociation of the copper complex leads to rapid fragmentation by a single pathway. In contrast, we observe that, for the arginine radical cation produced following dissociation from the iron complex, the initially formed tautomer may rearrange to one that is much more stable, specifically a tautomer in which the charge is located on the basic side chain and the radical is located at the α-carbon. Arginine possesses only one α-carbon. In peptides, on the other hand, the radical site may be located on any of the α-carbons, leading to a diverse range of tautomers and presumably a richer fragmentation chemistry. If, however, a radical cation is produced that undergoes rapid fragmentation immediately following its dissociation from the complex, it is likely to do so by a single pathway, and much of the structural information regarding the peptide is lost. In support of this concept, we have demonstrated previously that [Cu^{II}(terpy)-

TABLE 2: Relative B3-LYP/6-31G(d) Energies (kJ mol^{-1}) of the Lowest-Energy Conformers (A−E) of the Five Tautomers Examined[a]

	 **12**	 **13**	 **14**	 **15**	 **16**
A	128	132	138	0	107
B	132	135	145	3	109
C	134	136	155	6	113
D	135	140	158	8	114
E	135	141	160	24	114

[a] There are several resonance forms for these radical cations involving the delocalization of both the charge and the radical site; so, the assignment of charge and radical site is primarily provided for ease of discussion.

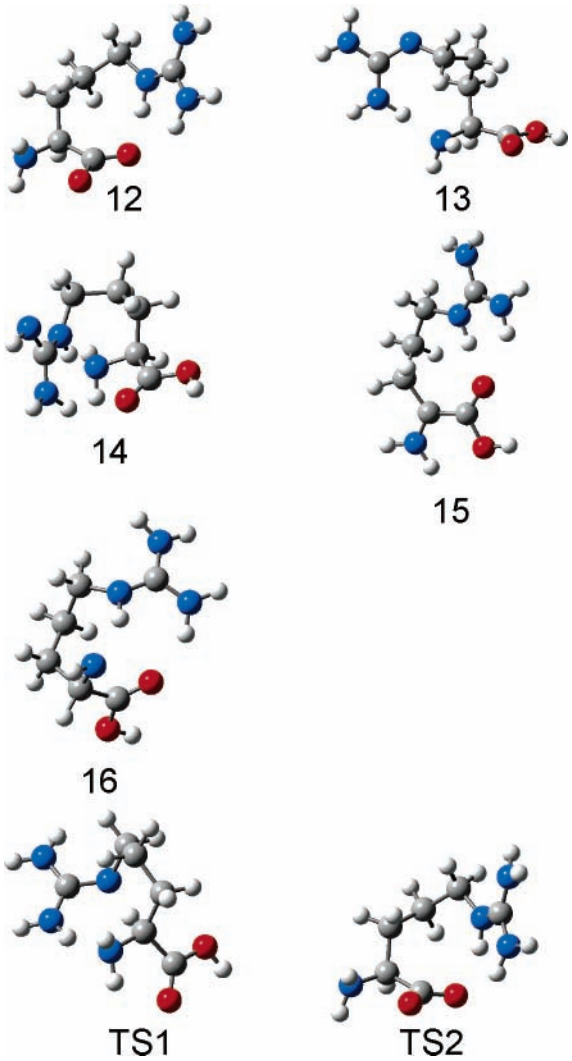


Figure 10. The lowest-energy conformers (**A**, Table 2) of the various tautomers of the arginine radical cation (**12**−**16**) and transition structures for the rearrangement of tautomer **13** to **15** (**TS1**) and for the loss of CO_2 from tautomer **12** (**TS2**).

(WGGFLR)]$^{2+}$ and [Fe^{III}(salen)(WGGFLR)]$^{+}$ both undergo oxidative dissociation to produce the WGGFLR$^{+\bullet}$ radical cation.[16] The peptide radical produced from the copper complex predominantly undergoes further fragmentation by loss of 3-methyleneindolenine from the tryptophan side chain, thus making mass selection of WGGFLR$^{+\bullet}$ impossible. In contrast, the WGGFLR$^{+\bullet}$ radical cation produced following dissociation from the iron complex is the main product ion, undergoing only very minor loss of 3-methyleneindolenine. It is significant that loss of 3-methyleneindolenine following CID of the WGGFLR$^{+\bullet}$ radical cation is only a minor process. The major fragmentation processes correspond to several competing radical-driven pathways that must necessarily proceed from different tautomeric forms of the peptide radical cation.

4. Conclusions

CID of the series of [Cu^{II}(terpy)(AA)]$^{2+}$ complexes shows a strong dependence on the nature of the amino acid. When the amino acid is aliphatic, the complexes tend to undergo fragmentation while coordinated to the copper, or non-oxidative dissociation, in line with their relatively high ionization energies. Complexes containing aromatic amino acids, on the other hand, tend to undergo redox chemistry forming abundant [Cu^{I}(terpy)]$^{+}$ ions. The successful formation of radical cations of the aromatic amino acids (His and Trp) can be attributed not only to their comparatively low ionization energies, but also to their stability with respect to intramolecular proton-driven small-molecule loss. The current study indicates that the redox potential of the [Cu^{II}(terpy)]$^{2+}$ system may also be capable of oxidizing many *non*-aromatic amino acids. However, the corresponding amino acid radical cation is not formed in many cases because of the propensity of these amino acids to undergo small-molecule loss. In these instances, there appear to be analogies with the fragmentation of protonated amino acids, and thus, the copper ion appears to mainly play the role of a Lewis acid. In contrast to the [Cu^{II}(terpy)(AA)]$^{2+}$ complexes, the [Fe^{III}(salen)(AA)]$^{+}$ complexes predominantly undergo non-oxidative dissociation, indicating that the redox potential is insufficient to oxidize many of the amino acids.

The low-energy CID spectra of the tryptophan, lysine, histidine, and arginine radical cations show interesting rearrangement and fragmentation behavior, which can be rationalized with the aid of theoretical calculations. For example, B3-LYP/6-31G(d) computations support the idea that the histidine radical cation rearranges to an α-centered radical before undergoing water loss, which is facilitated by the imidazole nitrogen in the 3-position acting as a proton shuttle. Similarly, B3-LYP/6-31G(d) computations support the proposal that the

arginine radical cation formed from the iron complex undergoes rearrangement to an α-centered radical cation before the radical-driven elimination of dehydroalanine. In contrast, the arginine radical cation formed from the corresponding copper complex is unstable and rapidly undergoes decarboxylation. Arginine is postulated to bind in a non-zwitterionic form in the $[Fe^{III}(salen)(Arg)]^+$ complex, but in a zwitterionic-type structure in the $[Cu^{II}(terpy)(Arg)]^{2+}$ complex.

Note Added in Proof: A paper has just appeared on the gas phase ionization and fragmentation of histidine using VUV radiation. The ionization energy was determined to be 8.3 eV: Wilson, K. R.; Belau, L.; Nicolas, C.; Jimenez-Cruz, M.; Leone, S. R.; Ahmed, M. *Int. J. Mass Spectrom.* **2006**, *155*, 249−250.

Acknowledgment. We gratefully acknowledge the award of an Australian Postgraduate Research Scholarship (to C.K.B.), a University of Sydney Sesqui Postdoctoral Fellowship (to D.M.), and funding from the Australian Research Council (to R.A.J.O., W.D.M., and L.R.). We also gratefully acknowledge generous allocations of computing time from the Australian Partnership for Advanced Computing, the Australian National University Supercomputing Facility, the Australian Centre for Advanced Computing and Communication, and the Victorian Institute for Chemical Sciences (VICS) High Performance Computing Facility. We thank the trustees of the A.E. Rowden White foundation for funds to purchase the Thermo Finnegan LCQ DECA mass spectrometer and the ARC and VICS for partial funding to purchase the LTQ-FTMS.

Supporting Information Available: CID spectra of $[Cu^{II}(terpy)(AA)]^{2+}$ for those AA not included in the text are included in Figure S1. Also included are the CID spectra of $[Fe^{III}(salen)(NAcArg)]^+$ and $[Fe^{III}(salen)(ArgOMe)]^+$ (Figure S3) and the high-resolution CID spectra of $[Cu^{II}(terpy)(Ala)]^{2+}$ (Figure S2). B3-LYP/6-31G(d) Cartesian coordinates, energies, ZPVEs and $\langle S^2 \rangle$ for the arginine conformers **A**−**E** of isomers **12**−**16** and for **TS1** and **TS2** are given in Tables S1−S6, and similarly, the histidine structures **4**−**8**, shown in Figure 8, are given in Table S7. The electrospray conditions used for the formation of the $[Cu^{II}(terpy)(AA)]^{2+}$ complexes are given in Table S8. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Hu, P.; Loo, J. A. *J. Am. Chem. Soc.* **1995**, *117*, 11314.

(2) Gatlin, C. L.; Tureček, F.; Vaisar, T. *J. Mass Spectrom.* **1995**, *30*, 1617.

(3) Gatlin, C. L.; Tureček, F.; Vaisar, T. *J. Mass Spectrom.* **1995**, *30*, 1605.

(4) Gatlin, C. L.; Tureček, F.; Vaisar, T. *J. Am. Chem. Soc.* **1995**, *117*, 3637.

(5) Lavanant, H.; Hecquet, E.; Hoppilliard, Y. *Int. J. Mass Spectrom.* **1999**, *185/186/187*, 11.

(6) Chu, I. K.; Rodriquez, C. F.; Lau, T. C.; Hopkinson, A. C.; Siu, K. W. M. *J. Phys. Chem. B* **2000**, *104*, 3393.

(7) Chu, I. K.; Rodriguez, C. F.; Hopkinson, A. C.; Siu, K. W. M. *J. Am. Soc. Mass Spectrom.* **2001**, *12*, 1114.

(8) Wee, S.; O'Hair, R. A. J.; McFadyen, W. D. *Rapid Commun. Mass Spectrom.* **2002**, *16*, 884.

(9) Chu, I. K.; Siu, S. O.; Lam, C. N. W.; Chan, J. C. Y.; Rodriquez, C. F. *Rapid Commun. Mass Spectrom.* **2004**, *18*, 1798.

(10) Bagheri-Majdi, E.; Ke, Y.; Orlova, G.; Chu, I. K.; Hopkinson, A. C.; Siu, K. W. M. *J. Phys. Chem. B* **2004**, *108*, 11170.

(11) Barlow, C. K.; Wee, S.; McFadyen, W. D.; O'Hair, R. A. J. *Dalton Trans.* **2004**, 3199.

(12) Chu, I. K.; Lam, C. N. W.; Siu, S. O. *J. Am. Soc. Mass Spectrom.* **2005**, *16*, 763.

(13) Wee, S.; O'Hair, R. A. J.; McFadyen, W. D. *Int. J. Mass Spectrom.* **2004**, *234*, 101.

(14) Wee, S.; White, J. M.; McFadyen, W. D.; O'Hair, R. A. J. *Aust. J. Chem.* **2003**, *56*, 1201.

(15) Wee, S.; O'Hair, R. A. J.; McFadyen, W. D. *Int. J. Mass Spectrom.* **2006**, *249*−*250*, 171.

(16) Barlow, C. K.; McFadyen, W. D.; O'Hair, R. A. J. *J. Am. Chem. Soc.* **2005**, *127*, 6109.

(17) (a) Junk, G.; Svec, H. *J. Am. Chem. Soc.* **1963**, *85*, 839. (b) Biemann, K.; Seibl, J.; Gapp, F. *J. Am. Chem. Soc.* **1961**, *83*, 3795. (c) Biemann, K.; McCloskey, J. A. *J. Am. Chem. Soc.* **1962**, *84*, 3192. (d) Heyns, K.; Gruetzmacher, H. F. *Liebigs Ann. Chem.* **1963**, *667*, 194. (e) For evaluated EI-MS data, see the *National Institute of Standards and Technology Chemistry WebBook* at http://webbook.nist.gov/chemistry/.

(18) (a) Grotemeyer, J.; Walter, K.; Boesl, U.; Schlag, E. W. *Int. J. Mass Spectrom. Ion Processes* **1987**, *78*, 69. (b) Koester, C.; Grotemeyer, J. *Org. Mass Spectrom.* **1992**, *27*, 463. (c) Nagra, D. S.; Zhang, J. Y.; Li, L. *Anal. Chem.* **1991**, *63*, 2188. (d) Vorsa, V.; Kono, T.; Willey, K. F.; Winograd, N. *J. Phys. Chem. B* **1999**, *103*, 7889.

(19) Detailed comparisons of the fragmentation reactions of radical cations of amino acids formed from metal complexes with those formed via EI or photoionization are difficult, due to the quite different energy and time regimes for the various ionization techniques. The ideal study would be to experimentally determine the kinetics of fragmentation upon excitation of long-lived, thermalized radical cations and to then model the kinetics using RRKM calculations. Professor Lifshitz recently pioneered such studies (for example, see: Hu, Y.; Hadas, B.; Davidovitz, M.; Balta, B.; Lifshitz, C. *J. Phys. Chem. A* **2003**, *107*, 6507), but these were curtailed by her untimely death.

(20) Castro, I.; Faus, J.; Julve, M.; Gleizes, A. *J. Chem. Soc., Dalton Trans.* **1991**, 1937.

(21) Gerloch, M.; Lewis, J.; Mabbs, F. E.; Richards, A. *J. Chem. Soc. A* **1968**, 112.

(22) Scott, A. P.; Radom, L. *J. Phys. Chem.* **1996**, *100*, 16502.

(23) *Spartan 02*; Wavefunction, Inc., Irvine, CA. Except for molecular mechanics and semiempirical models, the calculation methods used in *Spartan 02* have been documented in Kong, J.; White, C. A.; Krylov, A. I.; Sherrill, D.; Adamson, R. D.; Furlani, T. R.; Lee, M. S.; Lee, A. M.; Gwaltney, S. R.; Adams, T. R.; Ochsenfeld, C.; Gilbert, A. T. B.; Kedziora, G. S.; Rassolov, V. A.; Maurice, D. R.; Nair, N.; Shao, Y.; Besley, N. A.; Maslen, P. E.; Dombroski, J. P.; Daschel, H.; Zhang, W.; Korambath, P. P.; Baker, J.; Byrd, E. F. C.; Van Voorhis, T.; Oumi, M.; Hirata, S.; Hsu, C.-P.; Ishikawa, N.; Florian, J.; Warshel, A.; Johnson, B. G.; Gill, P. M. W.; Head-Gordon, M.; Pople, J. A. *J. Comput. Chem.* **2000**, *21*, 1532. A discussion and assessment of commonly used calculation methods may be found in Hehre, W. J. *A Guide to Molecular Mechanics and Quantum Chemical Calculations*; Wavefunction: Irvine, 2002.

(24) 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.; and Pople, J. A. *Gaussian 03*, revision C.02; Gaussian, Inc.: Wallingford, CT, 2004.

(25) Campbell, S.; Beauchamp, J. L.; Rempe, M.; Lichtenberger, D. L. *Int. J. Mass Spectrom. Ion Processes* **1992**, *117*, 83.

(26) Lee, K. T.; Sung, J.; Lee, K. J.; Park, Y. D.; Kim, S. K. *Angew. Chem., Int. Ed.* **2002**, *41*, 4114.

(27) O'Hair, R. A. J.; Reid, G. E. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 1220.

(28) Dookeran, N. N.; Yalcin, T.; Harrison, A. G. *J. Mass Spectrom.* **1996**, *31*, 500.

(29) O'Hair, R. A. J. *J. Mass Spectrom.* **2000**, *35*, 1377.

(30) Rogalewicz, F.; Hoppilliard, Y.; Ohanessian, G. *Int. J. Mass Spectrom.* **2000**, *195/196*, 565.

(31) Note that these radical cations were formed following dissociation from the corresponding $[Cu^{II}(terpy\text{-}Cl)(AA)]^{2+}$ complexes, as discussed in the Experimental Section.

(32) Tureček, F.; Kerwin, J. L.; Xu, R.; Kramer, K. J. *J. Mass Spectrom.* **1998**, *33*, 392.

(33) Further details will be published separately. We note that a complete theoretical description of all possible fragmentation reactions of the radical cations of the larger amino acids would be a computationally demanding

proposition due to the large conformational space and the existence of multiple tautomeric structures. Indeed, the theoretical treatment of even the simplest amino acid, glycine, is complicated by the existence of multiple conformations and tautomers. See: Lu, H.-F.; Li, F.-Y.; Lin, S. H. *J. Phys. Chem. A* **2004**, *108*, 9233.

(34) Price, W. D.; Jockusch, R. A.; Williams, E. R. *J. Am. Chem. Soc.* **1997**, *119*, 11988.

(35) Chapo, C. J.; Paul, J. B.; Provencal, R. A.; Roth, K.; Saykally, R. J. *J. Am. Chem. Soc.* **1998**, *120*, 12956.

(36) Maksic, Z. B.; Kovacevic, B. *J. Chem. Soc., Perkin Trans 2* **1999**, 2623.

(37) Skurski, P.; Gutowski, M.; Barrios, R.; Simons, J. *Chem. Phys. Lett.* **2001**, *337*, 143.

(38) Rak, J.; Skurski, P.; Simons, J.; Gutowski, M. *J. Am. Chem. Soc.* **2001**, *123*, 11695.

(39) Gdanitz, R. J.; Cardoen, W.; Windus, T. L.; Simons, J. *J. Phys. Chem. A* **2004**, *108*, 515.

(40) Jockusch, R. A.; Price, W. D.; Williams, E. R. *J. Phys. Chem. A* **1999**, *103*, 9266.

(41) Cerda, B. A.; Wesdemiotis, C. *Analyst* **2000**, *125*, 657.

(42) Viehe, H. G.; Janousek, Z.; Merenyi, R.; Stella, L. *Acc. Chem. Res.* **1985**, *18*, 148.