Methionine, α-methylmethionine and S-methylcysteine radical cations: generations and dissociations in the gas phase†

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Methionine, α-methylmethionine and S-methylcysteine radical cations have been formed by oxidative dissociations of [CuII(M)(CH3CN)]2+ complexes. The radical cations M•+ were trapped, and CID spectra (MS3) of these ions are presented. Fragmentations of the methionine and S-methylcysteine radical cations, initiated by migration of the α-carbon hydrogen atom to the sulfur, trigger the losses of water and thiomethanol from methionine and thiomethanol from S-methylcysteine. Deuterium labeling experiments show that considerable H–D scrambling and rearrangements involving N–H and S–H hydrogens occur in the methionine radical cation prior to fragmentation. An additional channel for S-methylcysteine is the loss of ammonia following β-hydrogen migration. Methylation at the α-carbon of methionine results in a radical cation that fragments differently. Two neutral losses from α-methylmethionine, NH3 and methyl vinyl sulfide, CH2=CH–S–CH3, are initiated by γ-hydrogen migration; a third channel is the loss of COOH. DFT computations at the B3LYP/6-311++G(d,p) level have been used to test aspects of the proposed fragmentation mechanisms of the radical cations.

Introduction

Protein-based radicals play critical roles in some of the most elaborate biosyntheses in nature, including photosynthesis and substrate oxidation. Radical centers are located on aromatic- and sulfur-containing amino acid residues, as well as on the glycine residue. Generation of many of these charged or neutral radicals involves oxidation of an amino acid residue by a neighboring metal cofactor. Until recently radical cations of peptides have proved to be difficult to generate in the gas phase. The low vapor pressures of peptides made the traditional method for producing organic radical cations, electron ionization, not viable. Recently, copper(II)-containing ternary complexes [CuII(L)(M)]2+, where L is typically an amine and M is a peptide or amino acid, have been employed as a source for peptide and amino acid radical cations in the gas phase. Collision-induced dissociation (CID) of the complex leads to dissociative electron transfer, resulting in the formation of M•+. Radical cation formation occurs most efficiently when the peptide contains an amino acid residue that has a low ionization energy. One shortcoming of this method is the existence of competitive channels, mainly proton transfer in which a proton migrates either from the auxiliary ligand to the peptide to create [M + H]+ ions, or vice versa giving [Cu(M – H)]+ and [L + H]+. By judicious choice of auxiliary ligands that are devoid of acidic hydrogens: terpyridines; macrocyclics 1,4,7-triazacyclononane, 1,4,7,10-tetraoxacyclododecane and 2,5,8,11-tetraoxadecane; or large bidentate ligands, this competing proton-transfer reaction is largely suppressed.

Peptide radical cations M•+ have much richer and more varied chemistries than those of protonated peptides, [M + H]+. Under low-energy CID conditions, the dissociations of protonated peptides are primarily charge-driven with the mobile proton inducing cleavage principally at the peptide bonds, giving b- or y-ions. By contrast, the fragmentation of radical peptide ions can either be charge- or radical-driven, and this can to some extent be controlled by, for example, sequestering the proton on the side chain of a basic amino acid to suppress charge-induced reactions. Laskin et al. showed that the gas-phase fragmentation of odd-electron M•+, [M + H]2+, and [M – 2H]•+ ions, where M was angiotensin III (HVYIHFP), share many similarities, thus suggesting strongly that the fragmentation pathways are radical-driven. Furthermore, radical-driven bond cleavages often occur at locations distant from the α-radical center in the initially formed radical cation, thereby indicating that mobility of the radical is an important factor. In a very recent study on isomeric radical ions of the simplest tripeptides, [GG•]2+, [GG•G]2+ and [GGG•]2+, it was found that the barriers against interconversion among these triglycine isomers are ≥ 44.7 kcal mol−1, significantly higher than those against tautomerism of protonated triglycine isomers (< 17 kcal mol−1). Charge-driven dissociations of both protonated triglycine and triglycine radical cations lead to cleavage of the peptide bonds, producing the b2 and [b2 – H]+ ions, respectively.
Amino acid radical cations are much more fragile than protonated amino acids, and only a few have been reported. Radical cations of aromatic amino acids, tryptophan, tyrosine and histidine, have been observed in the CID spectra of [CuII(M)]2+ and [CuII(L)(M)]2+.[8] The basic amino acid radical cations Arg+ and Lys+ were present in the CID spectra of complexes [CuII(L)(M)]2+, where the auxiliary ligand was 1,3,5-triazacyclononane (tacn) or 2,2′:6′,2″-terpyridine (terpy).[9] Arg+ was also formed in the oxidative dissociation of [FeIII(salen)(Arg)]2+, where salen is the N,N'-ethylenebis(salicyldenediaminato) dianion.9 Basic amino acids facilitate the formation of ζ-radicals that have captodative structures in which the charge and spin are formally separated; delocalization of the charge onto the carboxyl group adjacent to the radical center through hydrogen bonding enriches the electron-withdrawing properties, and is highly stabilizing. Very recently, infrared multiple photon dissociation (IRMPD) spectroscopy established unambiguously that the observable His+ has the captodative structure.10

The auxiliary ligand, L, appears to play a significant role in determining the structure of the amino acid radical cation that is formed in oxidative dissociation. Barlow et al. found that the Arg+ ion formed in high abundance in the fragmentation of [FeIII(salen)(Arg)]+ dissociated solely by the loss of dehydroalanine (H2C═C(NH2)(COOH)) to give an ion at m/z 87, whereas dissociation of [CuII(terpy)(Arg)]2+ resulted in Arg+ (in low abundance), [Arg–CO2]+ and the ion at m/z 87. The difference in fragmentation was attributed to different types of binding of arginine to the metal. It was proposed that, in [FeIII(salen)(Arg)]+, arginine in the canonical form is bound in a monodentate fashion to the iron through a nitrogen of the guanidine side chain; dissociation of this complex then gives a radical cation in which both the charge and spin are localized on the guanidine group. By contrast, in [CuII(terpy)(Arg)]2+, it was proposed that arginine binds in the zwitterionic form to copper, and oxidative dissociation results in a zwitterionic ion in which the radical resides on the carboxy group and the charge on the protonated guanidine group. Carboxy radicals are known to easily lose CO2,11 hence the fragmentation pathway where the auxiliary ligand L is acetone.

Here we report generations of the radical cations of methionine, ζ-methylmethionine and ζ-methylcysteine, in the CID of [CuII(M)(L)]2+,[8] where L = acetonirole. The binding modes of the amino acids to copper and the fragmentation behaviors of the radical cations have been investigated theoretically using density functional theory (DFT).

**Experimental section**

1. **Chemicals**

All amino acids, their derivatives, and methionine-2-d4 (minimum 98 atom% deuterium) where the ζ-hydrogen was replaced by deuterium were available from Sigma-Aldrich (St. Louis, MO). Copper(II)-containing ternary complexes were prepared in 1 mL 1:1 water–acetonitrile solutions, by mixing copper(II) perchlorate hexahydrate with the amino acid or its derivatives to a final concentration of 200 μM [CuII(M)(CH3CN)]2+. The collision gas used for both instruments was nitrogen. Samples for both MS 2 and MS 3 spectra were recorded typically using 1% of a parent ion and a collision energy of 20 eV.

2. **Mass spectrometry**

The mass spectrometers used were prototype versions of the API 2000 linear ion trap instrument and the API 3000 triple-quadrupole mass spectrometer (both MDS SCIEX). The collision gas used for both instruments was nitrogen. Samples were introduced by means of pneumatically assisted electrospray at flow rates of 50–60 μL h⁻¹. For the ion trap mass spectrometer, SM2 and SM3 spectra were recorded typically with a fill time of 50 to 100 ms and a scan speed of 1000 Th s⁻¹. We adopt the convention of labeling the mass-selected precursor ion in the spectra with an asterisk (*). Throughout this work, unspecified Cu isotopes are always 63Cu; the
3. Computational methods

All calculations were performed using the Gaussian03 quantum chemical program. The total energies of Cu(II) complexes and radical cations were calculated by the unrestricted open-shell formalism within the framework of Becke’s three-parameter DFT hybrid functional, B3LYP, which is based on a mixture of Hartree–Fock exchange and Becke and Lee–Yang–Parr exchange–correlation functional. Standard Pople Gaussian-type basis sets, 6-31++G(d,p), and 6-311++G(d,p), were employed. Local minima and transition structures were optimized and characterized by harmonic frequency analyses. Zero-point vibration energies were evaluated directly using normal-mode frequencies without anharmonic scaling. The local minima associated with all transition states were identified using the intrinsic reaction coordinates method. Atomic charges and spin densities were evaluated using natural population analysis.

Results and discussion

1. Dissociations of the \([\text{Cu}^{II}(M) \text{CH}_3\text{CN})_2]^{2+}\) ions

(a) Methionine. The CID spectrum of \([\text{Cu}^{II}(\text{Met}) \text{CH}_3\text{CN})_2]^{2+}\) \((m/z\ 147)\) in Fig. 1a is dominated by singly charged ions arising from dissociative electron transfer: \(\text{Met}^{+} (m/z\ 149)\), its fragmentation products \((m/z\ 131, 116\) and 101, vide infra), and the complementary ion in the complex dissociation, \([\text{Cu}^{I}(\text{CH}_3\text{CN})]^{+}\) \((m/z\ 145)\). Two dipositive fragments corresponding to the loss of one or two acetonitriles, \([\text{Cu}^{II}(\text{Met})(\text{CH}_3\text{CN})]^{2+}\) \((m/z\ 126.5)\) and \([\text{Cu}^{II}(\text{Met})]^{2+}\) \((m/z\ 106)\), are also observed. Ion \([\text{Cu}^{II}(\text{Met})(\text{CH}_3\text{CN})]^{2+}\) also dissociated efficiently to give \(\text{Met}^{+}\) and its complementary ion \([\text{Cu}^{I}(\text{CH}_3\text{CN})]^{+}\) at \(m/z\ 104\). It is noteworthy that there was no dissociative proton-transfer reaction or the loss of CH\(_3\)SH from the complex, as these were the dominant dissociation channels when the auxiliary ligand was dien or terpy.

Copper(II) has a d\(^9\) electronic configuration and forms four strong metal–ligand dative bonds in a square-planar arrangement at the equatorial positions; however, five-coordinate complexes of Cu(II) are known although the fifth interaction is typically weaker. Sulfur atoms from thiolate, thioether, disulfide or inorganic sulfur have been shown to act as a donor ligand in a variety of copper complexes. DFT performed at the UB3LYP/6-31++G(d,p) level of theory showed the preferred binding mode for methionine in \([\text{Cu}^{II}(\text{Met})(\text{CH}_3\text{CN})]^{2+}\) to be canonical (Fig. 2), where the Cu is di-coordinated by the amino nitrogen and the sulfur atom; there is also a weaker third interaction between Cu and the carbonyl oxygen with a relatively long bond distance (2.302 Å). Binding of the two acetonitriles to Cu completes the penta-coordination geometry. Elimination of one acetonitrile from \([\text{Cu}^{II}(\text{Met})(\text{CH}_3\text{CN})]^{2+}\) (CS) is endothermic by 33.0 kcal mol\(^{-1}\), while that of two acetonitriles is endothermic by 93.9 kcal mol\(^{-1}\). By contrast, the dissociative electron-transfer reaction to yield Met\(^+\) is exothermic by 33.9 kcal mol\(^{-1}\), albeit there is an activation barrier due to charge separation.

(b) S-Methylcysteine. The dissociation of \([^{65}\text{Cu}^{II}(\text{S-methyl-Cys})(\text{CH}_3\text{CN})]^{2+}\) \((m/z\ 141)\) (Fig. 3) displays a pattern similar to that of \([\text{Cu}^{II}(\text{Met})(\text{CH}_3\text{CN})]^{2+}\): dissociative electron transfer to give \((\text{S-methyl-Cys})^{+}\) \((m/z\ 135)\), and losses of CH\(_3\)CN to give \([^{65}\text{Cu}^{II}(\text{S-methyl-Cys})(\text{CH}_3\text{CN})]^{2+}\) \((m/z\ 120.5)\) and \([^{65}\text{Cu}^{II}(\text{S-methyl-Cys})^{+}\) \((m/z\ 100)\) in low...
abundances. Ions at m/z 117.7 and 87.1 were attributed to the losses of NH₃ and CH₃SH from (S-methyl-Cys)/C₁₅⁺.

(c) α-Methylmethionine. The [CuII(α-methyl-Met)-(CH₃CN)₂]²⁺ ion (m/z 154) readily loses one acetonitrile, and the second one with more difficulty, giving ions at m/z 133.6 and 113.0 (Fig. 4). Dissociative electron transfer to give the α-methylmethionine radical cation (m/z 163), as well as its dissociation products (vide infra) and [CuI(CH₃CN)₂]⁺ (m/z 145) is the dominant fragmentation pathway.

2. Dissociations of the radical cations

(a) Methionine. The radical cation, Met⁺, was mass-selected and subjected to a third stage of CID (Fig. 5a). The most abundant fragment ion was at m/z 131, the [b₁ - H]⁺ ion, produced via the loss of water. Subsequent dissociation of this ion involved the loss of CH₃ from the sulfur atom to give the cation at m/z 116, a reaction previously observed for CH₃SCH₂⁺. There was also a minor dissociation channel from Met⁺: the loss of thiomethanol to give the radical cation at m/z 101. Examination of the CID spectrum of (Met-2-d₁)⁺ in which the α-hydrogen of methionine was replaced by deuterium (Fig. 5b) revealed the loss of H₂O or HOD to give ions at m/z 132 and 131 in high abundance; CID of the ions at m/z 132 resulted in the losses of *CH₂D and *CH₃ (product ions at m/z 116 and 117 in Fig. 5c–1); the ion at m/z 131, in lower abundance in Fig. 5b, lost only *CH₃ (product ion at m/z 116, Fig. 5c–2), as this precursor should not contain any deuterium. The minor pathway in the CID of (Met-2-d₁)⁺ produced ions at m/z 102 and 101, corresponding to the loss of CH₃SH and CH₃SD or CH₂DSH (Fig. 5b). These results show that the deuterium from the α-carbon is efficiently scrambled onto the carboxyl and methyl groups and presumably onto the NH₂ group.

These experimental observations can be rationalized in terms of Scheme 1. The initially formed Met⁺⁻⁻¹ (Met-2-d₁)
radical cation probably has a canonical structure in which both the positive charge and the radical are formally localized on the sulfur atom. Collisional activation can induce a 1,4-deuterium atom shift from the α-carbon to the sulfur to give a distonic ion $\text{Met}^{+}-2a$ which dissociates via several channels. One pathway, initiated by $\text{D}^+$ transfer from the sulfur to the hydroxyl group followed by nucleophilic attack by the sulfur atom on the carbonyl carbon, results in the loss of HDO to give the product ion at $m/z$ 131; this in turn can lose the methyl radical to give the ion at $m/z$ 116. A more minor pathway, initiated by nucleophilic attack by the carbonyl oxygen on the γ-carbon, results in displacement of CH$_3$SD to give the product ion at $m/z$ 101.

The deuteron on the sulfur atom in $\text{Met}^{+}-2a$ can transfer to the amino group by 1,5-D shift to yield a distonic $\text{Met}^{+}-5a$ in which the charge is formally localized on the NH$_2$D group and the spin on the α-carbon. This process is virtually barrierless and $\text{Met}^{+}-5a$ is of similar energy to $\text{Met}^{+}-2a$ (*vide infra*, section 3). Ion $\text{Met}^{+}-2a$ can be reformed from $\text{Met}^{+}-5a$, and in the process an H$^+$ from the amino group can be transferred back to the sulfur atom, resulting in H–D scrambling of N–H and S–D prior to the elimination of water.

Migration of a proton from the S-CH$_3$ group of $\text{Met}^{+}-1$, made acidic by the adjacent positively charged sulfur, via a 1,6-proton shift gives $\text{Met}^{+}-6$. This is followed by a 1,5-D shift from the α-carbon to S-CH$_2$, giving $\text{Met}^{+}-5b$ that contains the SCH$_2$D group. This can subsequently result in the elimination of H$_2$O (product ion $m/z$ 132) followed by CH$_3$D loss (product ion $m/z$ 116), or the elimination of CH$_2$DSH (product ion $m/z$ 101). DFT investigations on the energetics and details of these reactions are discussed below in section 3.
The fragmentation of Met is the loss of ammonia (not observed in cysteine). This can be achieved by migration of a proton from the sulfur to the carbonyl oxygen as a catalyst. The destination of this proton formed from the elimination of NH$_3$. Loss of NH$_3$ is the dominant pathway for fragmentation of Met$^+$, as shown in Fig. 7. The CID of Met$^+$ with the radical on the side chain eliminates NH$_3$, probably by nucleophilic attack by either the sulfur or the carbonyl oxygen. Loss of methyl vinyl sulfide or a cyclic thioether leads to the formation of the captodative iminium ion, and another ion at m/z 89, assigned as Ala$^+$, generated by the loss of the side chain as the neutral methyl vinyl sulfide. A minor product at m/z 128 corresponds to the loss of water from the ion at m/z 146. Each of these three major fragmentation pathways is the result of cleavage of a different bond at the crowded x-carbon.

Both major products in the fragmentation of Met$^+$ are formed via formation of the x-radical. In the fragmentation of (x-methyl-Met)$^+$, there is no evidence for migration of the x-CH$_3$. Consequently, the fragmentation pathways are completely different from those of Met$^+$. Steric crowding at the x-carbon and the acidity of protons adjacent to the sulfur carrying the positive charge appear to be the determining factors. Loss of COOH is a common reaction in the CID of peptide/amino acid radical cations and can be achieved by transferring the charge and unpaired electron onto the carboxyl group from the sulfur. Loss of COOH then reduces the strain around the x-carbon and produces an iminium ion, the a$_1$ ion of x-methylmethionine. The other pathways involve migration of a proton from the x-carbon to the carbonyl oxygen, or from the S-methyl group to the same two receiving groups (Scheme 2). N-protonated ions with the radical on the side chain eliminate NH$_3$, probably by nucleophilic attack by either the sulfur or the carbonyl oxygen. Loss of methyl vinyl sulfide or a cyclic thioether leads to the formation of the captodative x-radical cation of alanine, H$_2$NCH$_3$(CH$_3$)COOH$^+$. DFT investigations are discussed below in section 3.

3. Computational investigations

(a) Methionine. DFT calculations at the UB3LYP/6-311++G(d,p) level of theory were employed in a systematic examination of the structures and fragmentation mechanisms. Fig. 8a–c show the potential energy surfaces (PESs) for the reactions shown in Scheme 1. The most stable canonical structure, Met$^+$-1, formed by dissociative electron transfer from [Cu$_2$(Met)(CH$_3$CN)$_2$]$^+$ (CS), has the majority of its charge and spin on the sulfur atom. The fairly long and flexible side chain permits the sulfur radical to make an intramolecular, two-center-three electron hemibond to the N atom, thereby forming a five-membered ring. The calculated S–N distance is 2.574 Å; extensive spin delocalization is evident with 64% of the spin on the sulfur and 33% of the spin on the nitrogen atom. Migration of the x-carbon to the sulfur via TS-I produces Met$^+$-2a, a distonic radical cation with the radical formally located on the x-carbon and charge on the sulfur. Met$^+$-2a is higher in enthalpy ($\Delta H_0$) than Met$^+$-1 by 10.0 kcal mol$^{-1}$, and the barrier ($\Delta H_0^*$) against the 1,4-H atom transfer via TS-I is 13.1 kcal mol$^{-1}$. One possible ensuing step, migration of a proton from the sulfur to the OH group, followed by the loss of a H$_2$O molecule via TS-2 to give the [b$_1$ – H]$^+$ ion, has a barrier of 20.2 kcal mol$^{-1}$. The overall dissociation reaction from Met$^+$-1 to [b$_1$ – H]$^+$ and water is endothermic by 8.4 kcal mol$^{-1}$. The loss of CH$_3$ from [b$_1$ – H]$^+$ to give the iminium ion at m/z 116 is 30.5 kcal mol$^{-1}$ above [b$_1$ – H]$^+$ (Fig. 8a).

Rotation about the C$_x$–C bond in Met$^+$-2a plus proton transfer from the sulfur to the carbonyl oxygen gives the ion at...
the global minimum, Met$^+$$-3$, a captodative structure in which the radical is stabilized by the adjacent electron-donating NH$_2$ group and electron-withdrawing C(OH)$_2^+$ group. The barrier against formation of Met$^+$$-3$ is 27.0 kcal mol$^{-1}$, relative to Met$^+$$-1$. Transfer of the proton back to the S atom, followed by nucleophilic attack by the carbonyl oxygen on the \(\gamma\)-carbon and with concomitant elimination of CH$_3$SH has an activation enthalpy of 30.9 kcal mol$^{-1}$. Sampling and mass-isolating ions for CID in our mass spectrometers involve a large number of collisions and conditions under which isomerism with low-energy barriers can readily take place. These conditions favor formation of the global minimum structure, especially one that is surrounded by relatively high reaction barriers. Met$^+$$-3$ meets this requirement. Upon collisional activation, Met$^+$$-3$ can isomerize to give Met$^+$$-2a$, which can then dissociate to give [b$_1$ – H]$^+$ and water; the barrier against this reaction is 32.0 kcal mol$^{-1}$ (relative to Met$^+$$-3$). Alternatively, collisionally activated Met$^+$$-3$ can also dissociate to give the ion at m/z 101 and thiomethanol. This latter reaction has a higher barrier of 35.9 kcal mol$^{-1}$. These results are consistent with the experiment on the \(\alpha\)-deuterium labeled ion where eliminations of HDO (product ion m/z 131) followed by the loss of CH$_3$H (product ion m/z 116) and CH$_3$SD (product ion m/z 101) (Fig. 5b and 5c–2) were observed.

The mechanism of the scrambling reaction proposed in Scheme 1 is shown in Fig. 8b. For Met$^+$$-2a$, rotation about the C$_\gamma$–S bond leads to rotamer Met$^+$$-4a$ and the activation barrier against this interconversion tautomeric reaction is 14.9 kcal mol$^{-1}$, only 1.8 kcal mol$^{-1}$ higher than that for the 1,4 \(\alpha\)-hydrogen migration (vide supra). The proton (or deuteron for Met-2-d$_1$) transfer then occurs from the sulfur to the amino group, giving the distonic structure Met$^+$$-5a$ which is 1.6 kcal mol$^{-1}$ lower in enthalpy than Met$^+$$-2a$. This process is barrierless on the enthalpy surface. Note that Met$^+$$-2a$ can be reformed from Met$^+$$-5a$ using a different proton on the amino group, thereby leading to scrambling. The barriers against the proton-switching reactions are relatively low (6.5 kcal mol$^{-1}$ relative to Met$^+$$-5a$) compared with those leading to dissociations in Fig. 8a; hence, there is a large amount of scrambling.

Fig. 8c shows additional scrambling reactions, which involve a methyl hydrogen on the sulfur. Starting with Met$^+$$-1$, a 1,6-H$^+$ shift from the CH$_3$ group leads to the distonic Met$^+$$-6$, via a barrier of 15.6 kcal mol$^{-1}$. In the second step, the \(\alpha\)-deuterium atom undergoes a 1,5-H shift to the carbon radical center S–CH$_2$ via TS-8 to give Met$^+$$-5b$, whose subsequent isomerization to Met$^+$$-2c$ is facile. Ion Met$^+$$-2c$ then eliminates H$_2$O followed by the losses of CH$_3$D$^+$ and CH$_2$DSH in the CID of (Met-2-d$_1$)$^+$ (Fig. 5b and 5c–1).

From the fragmentation mechanisms discussed above, there are significant rearrangements prior to dissociation of Met$^+$. Canonical Met$^+$$-1$ formed in the CID of [Cu$^{11}$,(Met-2-d$_1$)-\(\text{CH}_3\text{CN}_2\)]$^{2+}$ isomerizes to Met$^+$$-2a–c$, ions that have the deuterium in three different locations. It is of note that the
abundance of the product ion at \( m/z \) 132 (via the loss of \( H_2O \)) is higher than that at \( m/z \) 131 (via the loss of \( HDO \)), in accordance with the interpretation of extensive scrambling (Fig. 5b). In addition, the higher barrier against the loss of \( CH_3SH \) is consistent with the experimental observation that the loss of \( H_2O \) is more prevalent than that of \( CH_3SH \) (Fig. 5a).

(b) \( S \)-Methylcysteine. As shown in Fig. 6, (\( S \)-methyl-Cys)*+ fragments prominently to give the dehydroalanine radical.
cation, \( \text{NH}_2^+ \text{C}(\text{CH}_2)\text{COOH} \) (\( m/z \) 87 Th), by the loss of \( \text{CH}_3\text{SH} \). The calculated reaction profile is shown in Fig. 9a. Starting with the lowest-energy canonical structure, \((\text{S-methyl-Cys})^+ \cdot 1\), migration of the \( \alpha \)-hydrogen to the sulfur creates a distonic ion \((\text{S-methyl-Cys})^+ \cdot 2\), in which the charge is formally located on the sulfur and the radical center on \( \text{C}_\alpha \); this structure has a long \( \text{C}_\beta - \text{S} \) distance (2.534 Å) and is essentially \( \text{H}_2\text{N}^+ \text{C}(\text{CH}_2)\text{COOH} \) solvated by \( \text{CH}_3\text{SH} \). The barrier against the 1,3-hydrogen shift is 19.1 kcal mol\(^{-1}\), slightly higher than that of the 1,4-hydrogen shift in Met\(^+ \cdot 1\) (13.1 kcal mol\(^{-1}\), Fig. 8a). Dissociation of the \((\text{S-methyl-Cys})^+ \cdot 2\) complex to give \( \text{H}_2\text{N}^+ \text{C}(\text{CH}_2)\text{COOH} \) and \( \text{CH}_3\text{SH} \) is endothermic by 11.3 kcal mol\(^{-1}\). Collisionally activated \((\text{S-methyl-Cys})^+ \cdot 1\) will give the products in one step, as the barrier (19.1 kcal mol\(^{-1}\)) is at a higher energy than the products.

The energy profile leading to the loss of \( \text{NH}_3 \) from \((\text{S-methyl-Cys})^+ \cdot 1\) is given in Fig. 9b. The proton source is the \( \beta - \text{CH}_2 \) group, made acidic by the adjacent positively charged sulfur. The migration takes place via two 1,4-proton shifts, involving the carbonyl oxygen as a catalyst, and with the nitrogen being the destination; heterolytic cleavage of the \( \text{N} - \text{C}_\alpha \) bond then ensues, giving \( \text{CH}_3\text{S}^+ \cdot \text{CH} = \text{CH} - \text{COOH} \) at \( m/z \) 118 and \( \text{NH}_3 \). The critical barrier on this pathway is at 22.7 kcal mol\(^{-1}\). In comparison, the critical barrier against a direct 1,3-H\(^+\) shift from the \( \beta \)-carbon to the amino nitrogen is 27.4 kcal mol\(^{-1}\) (see ESI†, Fig. S1). We also investigated the reaction channel for migration of a proton from the methyl group to the \( \text{NH}_2 \) group. A higher energy barrier, 32.1 kcal mol\(^{-1}\), was found, making this pathway energetically uncompetitive (see ESI†, Fig. S1).

(c) \( \alpha \)-Methionine. The reaction profile for \( \alpha \)-methylmethionine is shown in Fig. 10a. Starting from the lowest-energy structure, canonical \((\alpha \text{-methyl-Met})^+ \cdot 1\), migration of a proton from the \( \gamma \)-carbon to the \( \text{NH}_2 \) group via TS-13 produces distonic \((\alpha \text{-methyl-Met})^+ \cdot 2\), in which the charge resides formally on the nitrogen and the radical on the \( \gamma \)-carbon. The barrier against this 1,4-proton shift is 15.4 kcal mol\(^{-1}\). Extension of the \( \text{N} - \text{C}_\alpha \) bond results in elimination of \( \text{NH}_3 \) with a barrier of 29.1 kcal mol\(^{-1}\) (relative to \((\alpha \text{-methyl-Met})^+ \cdot 1\)). The barrier against a subsequent loss of water via 1,4-proton migration from the \( \beta \)-carbon to the \( \text{OH} \) group is much higher at 52.9 kcal mol\(^{-1}\), consistent with the observation of low abundance for the resulting ion at \( m/z \) 128 (Fig. 7). We also investigated the viability of

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Fig. 9 Reaction profile for dissociation of the S-methylcysteine radical cation at the UB3LYP/6-311 + G(d,p) level of theory: (a) the loss of \( \text{CH}_3\text{SH} \) and (b) the loss of \( \text{NH}_3 \) (energies are \( \Delta H_0 \) in kcal mol\(^{-1}\)).
transferring an S-methyl proton to the amino group and losing NH₃ (see ESI†, Fig. S2). The barrier against abstracting a proton from the methyl group to give (α-methyl-Met)⁺⁻⁵ is low at 15.6 kcal mol⁻¹, i.e., comparable to that against the 1,4-proton shift described above. However, the barrier against the elimination of NH₃ from (α-methyl-Met)⁺⁻⁵ is 42.6 kcal mol⁻¹, making this pathway uncompetitive. (α-methyl-Met)⁺⁻¹ can also fragment by losing COOH to give an iminium ion at m/z 118. The barrier against that reaction is 26.1 kcal mol⁻¹, slightly lower than that for NH₃ loss; this is contrary to the observed abundance for the two losses, but it is well within the accuracy in the DFT calculations.

There are two possible reaction pathways that can lead to the product ion Ala⁺⁺ at m/z 89 (Fig. 10b); both involve losing the α-methylmethionine side chain as CH₂–CH–S–CH₃. Starting from (α-methyl-Met)⁺⁻², cleavage of the Cα–Cβ bond yields a distonic Ala⁺⁺ in which the charge resides on the NH₃ group and the radical on the α-carbon. The critical energy is the endothermicity of this dissociation reaction, at 34.8 kcal mol⁻¹; this is 5.7 kcal mol⁻¹ higher than the barrier against the elimination of NH₃ (see Fig. 10a). An alternative and more competitive pathway is initiated by migration of a proton on the γ-carbon of (α-methyl-Met)⁺⁻¹ to the carbonyl oxygen to create isomer (α-methyl-Met)⁺⁻³, in which the charge is on the COH₂ group and the radical on the γ-carbon. The barrier against this 1,5-proton shift is the endothermicity at 26.9 kcal mol⁻¹. Subsequent loss of CH₂–CH–S–CH₃ has a barrier of 28.4 kcal mol⁻¹ and gives the lowest-energy isomer of Ala⁺⁺, which is stabilized captodatively. Parenthetically, it is also of note that the isomerization from distonic-Ala⁺⁺ to captodative-Ala⁺⁺ has a barrier of only 5.2 kcal mol⁻¹ (details not shown).

We have also examined the reaction profile for migration of a proton from the S-methyl group to the carbonyl oxygen, followed by the loss of the side chain as trimethylene sulfide. The barrier against this 1,7-proton shift is 32.8 kcal mol⁻¹, 4.4 kcal mol⁻¹ higher than that against the aforementioned 1,5-proton shift that eliminates the side chain as
CH$_2$=CH–S–CH$_3$, which strongly suggests that this new pathway is uncompetitive.

Conclusions

The collision-induced dissociations of [Cu(M)(CH$_3$CN)$_2$]$^{2+}$ complexes (M = methionine, $\alpha$-methylmethionine, and $\beta$-methylcysteine) produce abundant M$^+$ radical cations. The low-energy CID spectra of the radical cations show interesting rearrangement and fragmentation reactions. DFT shows that the methionine radical cation isomerizes to an $\alpha$-centered radical before undergoing water loss. By contrast, in the $\beta$-methylcysteine radical cation, the $\alpha$-hydrogen migrates to the sulfur, leading to the facile loss of CH$_3$SH. The fragmentation of ($\alpha$-methyl-Met)$^+$ is entirely different from that of Met$^+$, resulting in the loss of NH$_3$, H$_2$O, *$^+$COOH, and the side chain, the last of which gives the alanine radical cation. DFT shows that abstracting a proton from the $\gamma$-carbon by either the amino group followed by NH$_3$ loss, or by the carboxyl group followed by CH$_3$=CH–S–CH$_3$ loss, as well as the loss of *$^+$COOH are energetically favorable processes.

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References