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Comparing the gas-phase fragmentation reactions of protonated and radical cations of the tripeptides GXR☆

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Dedicated to Professor Alan Marshall on the occasion of his 60th birthday and in recognition of his many important contributions to fundamental and analytical mass spectrometry. One of us (RAJO) thoroughly enjoyed a sabbatical with Alan at the National High Magnetic Field Laboratory ICR Program.

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Abstract

Electrospray ionization (ESI) mass spectrometry of methanolic solutions of mixtures of the copper salt (2,2′:6′,2″-terpyridine)copper(II) nitrate monohydrate ([Cu(II)(tpy)$(NO_3)_2$]·H_2O) and a tripeptide GXR (where X = 1 of the 20 naturally occurring amino acids) yielded [Cu(II)(tpy)(GXR)]$^{\bullet 2+}$ ions, which were then subjected to collision induced dissociation (CID). In all but one case (GRR), these [Cu(II)(tpy)(GXR)]$^{\bullet 2+}$ ions fragment to form odd electron GXR$^{\bullet +}$ radical cations with sufficient abundance to examine their gas-phase fragmentation reactions. The GXR$^{\bullet +}$ radical cations undergo a diverse range of fragmentation reactions which depend on the nature of the side chain of X. Many of these reactions can be rationalized as arising from the intermediacy of isomeric distonic ions in which the charge (i.e. proton) is sequestered by the highly basic arginine side chain and the radical site is located at various positions on the tripeptide including the peptide back bone and side chains. The radical sites in these distonic ions often direct the fragmentation reactions via the expulsion of small radicals (to yield even electron ions) or small neutrals (to form radical cations). Both classes of reaction can yield useful structural information, allowing for example, distinction between leucine and isoleucine residues. The gas-phase fragmentation reactions of the GXR$^{\bullet +}$ radical cations are also compared to their even electron $[GXR + H]^+$ and $[GXR + 2H]^{2+}$ counterparts. The $[GXR + H]^+$ ions give fewer sequence ions and more small molecule losses while the $[GXR + 2H]^{2+}$ ions yield more sequence information, consistent with the 'mobile proton model' described in previous studies. In general, all three classes of ions give complementary structural information, but the GXR$^{\bullet +}$ radical cations exhibit a more diverse loss of small species (radicals and neutrals). Finally, links between these gas-phase results and key radical species derived from amino acids, peptides and proteins described in the literature are made.

Keywords: Electrospray ionization; Multistage mass spectrometry; Protonated peptides; Radical cations of peptides; Cu(II) complexes; Leucine-isoleucine distinction; Mobile proton; Mobile radical; Radical directed fragmentation

1. Introduction

A vast literature and continued research efforts are testament to the key roles that radicals play in biological processes [1]. Indeed, one of the simplest radicals, NO$^{\bullet}$, was chosen as Science magazine's "*Molecule of the Year*" [2] and has been shown to play a wide range of beneficial (e.g. assisting the immune system to fight viral, bacterial and parasitic infections; stroke prevention, vascular smooth muscle relaxation; inhibition of blood clotting; as a treatment in male impotence) and deleterious roles in the body (e.g. cell destruction in cancer and other diseases; progression of neurological diseases such as multiple sclerosis) [3]. Radicals derived from biological molecules can also play beneficial as well as deleterious roles. Examples of the former range from fruit ripening [1] to their involvement in the chemistry of enzymes [4] and drugs which target DNA [5], while their deleterious roles are highlighted by damage to biomolecules [6] such as DNA [7–9] and proteins [10]. Radical damage to DNA can lead to strand breaks, while protein damage can lead to: oxidation of side chains; formation of reactive groups (e.g. HOOR); fragmentation; cross-linking; changes in hydrophobicity and conformation; and changes in susceptibility to proteolysis.

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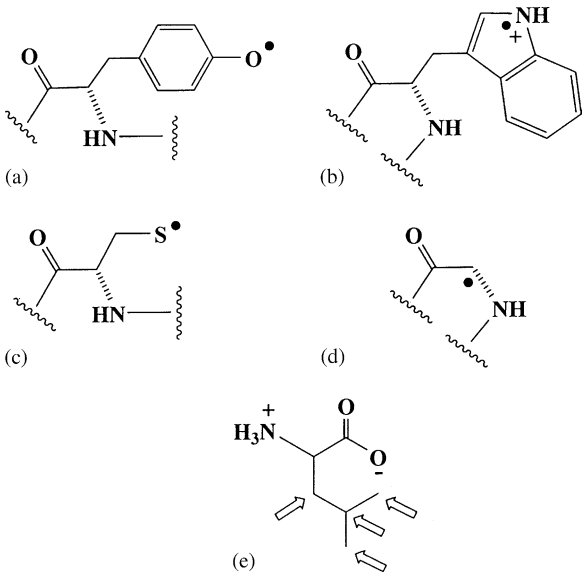


Fig. 1. Some key radicals involved in enzymes and radical damage [11,12]: (a) tyrosyl radical involved in class I ribonucleotide reductase [11]; (b) tryptophyl radical cation found in cytochrome oxidase [12]; (c) cysteinyl radical involved in pyruvate formate lyase; (d) α-glycyl radical found in class III ribonucleotide reductase [12] and (e) arrows indicate sites of H• abstraction of leucine by HO• as probed by H/D exchange reactions [12].

A significant effort has been invested in understanding the types of radicals formed in amino acids, peptides and proteins and the types of reactions that they undergo. Some of the key radical species which play a role in enzymes are shown in Fig. 1 and include: the tyrosyl radical involved in class I ribonucleotide reductase (Fig. 1a, [11]); tryptophyl radical cation found in cytochrome oxidase (Fig. 1b, [11]); cysteinyl radical involved in pyruvate formate lyase (Fig. 1c, [11]); α-glycyl radical found in class III ribonucleotide reductase (Fig. 1d, [11]). In addition, recent studies have used H/D exchange reactions to probe the sites of H• abstraction in amino acids and peptides by the highly reactive hydroxyl radical [12]. Fig. 1e uses arrows to indicate the sites of attack by HO• onto leucine. Note that these reactivity studies utilize intermolecular H• transfer reactions. Other reactivity studies using different radicals reveal that abstraction of a H• from amino acids, peptides and proteins can be quite selective to yield regiospecific products. The selectivity of attack at different sites becomes more pronounced for less reactive attacking radicals [9]. Radical stabilizing factors and polar effects [13] which influence the transition state energies for H• abstraction may also play a role. Once a radical is formed on a peptide or a protein, it can also undergo intramolecular H• transfer reactions to translocate the radical site to a different position. A number of intramolecular H• transfer reactions have been reported [8,9] and recent DFT calculations provide support for the transfer of H• from the C_α–H bonds of glutathione to the thiyl radical [14].

There has been considerable recent interest in the study of radicals derived from biomolecules in the gas phase. One of the attractive features of the gas phase is that intrinsic properties can be examined experimentally unperturbed by solvent and counterion effects. These experiments can also act as benchmarks for theoretical studies. This approach has become possible in the past decade due to exciting advances in mass spectrometry such as electrospray ionization (ESI) and the application of novel methods to generate radicals and charged radicals from biomolecules. A key reason for the interest in odd electron species of biomolecules is that they provide complementary information to their even electron counterparts, which can prove useful in analysis via tandem mass spectrometry [15]. For example, side chain losses from radical cations of peptides can provide useful structural information [16] and can be used to distinguish leucine and isoleucine residues [17,18].

While a review of the exciting and emerging area of gas-phase biomolecule radicals is beyond the scope of this introduction, we note that these species can be formed via a diverse range of processes including: (i) various types of laser ionization methods to produce radical cations [19]; (ii) neutralization-reionization [20]; (iii) various electron-ion interactions to generate radical cations [21] and radical anions [22], of which electron capture dissociation (ECD) has been the most studied [23,24]; (iv) ion-ion reactions to generate radical anions [25]; (v) charge stripping of neutrals or ions [26]; (vi) radical losses from even electron ions under high energy collision induced dissociation (CID) conditions [27]; (vii) via redox processes involving metal ions [17,28–31].

From a chemical perspective, method (vii) is attractive since in principle a wide range of metals and biomolecules can be explored and the different redox properties of various metal species can be exploited. While Amster and co-workers showed that bare metal ions can undergo redox processes with amino acids in the gas phase [28], a major breakthrough came when Siu et al. established that CID of ESI generated $[Cu(II)(L_3)M]^{\bullet 2+}$ complexes (where L_3 = a tridentate ligand) can be used to generate radical cations of peptides, $M^{\bullet +}$ (Eq. (1)) [29,30]. An important aspect of Siu's approach is that it can be carried out on a wide range of tandem mass spectrometers equipped with electrospray ionization and CID capabilities. To date, three studies have appeared on the gas-phase chemistry of radical cations of peptides using copper complexes [17,29,30] and recently we have shown that silver-adenine complexes can form radical cations under CID conditions [31].

$$[Cu(II)(dien)(M)]^{\bullet 2+} \xrightarrow{CID} [Cu(I)(dien)]^{+} + M^{\bullet +} \quad (1)$$

Considerable efforts have been directed towards understanding the fragmentation mechanisms of protonated peptides [32] through the use of experimental and theoretical studies on model systems [33,34] and database searching [35,36]. Some factors which are important for the formation of sequence and non-sequence ions include: (i) proton mobility [37]; (ii) neighbouring group reactions [33]; and (iii) the influence of salt bridges, which can play a role in fragmentation reactions including rearrangements [38]. Typically, a

'non-mobile proton' situation is encountered for peptides in which the number of ionizing proton(s) ≤the number of arginine residue(s). This is due to the high proton affinity of arginine, which allows each arginine residue to 'sequester' a proton. A recent statistical analysis of a MS/MS database has not only extended the idea of 'mobile versus non-mobile proton' to include 'partially mobile proton', but also revealed the role of individual residues and pairwise effects in influencing the fragmentation of peptide bonds [35].

Under low energy collision conditions for which the 'mobile proton' requirement is met, protonated peptides tend to fragment via charge directed cleavage reactions to give both sequence ions and non-sequence ions. In contrast, under 'non-mobile' conditions, peptides fragment via charge remote processes [39]. These charge remote processes can occur in systems in which the proton is sequestered by an arginine (the most basic) side chain or which have a specific fixed charge [40] such as the phosphonium salts developed by Watson [41]. It is important to recognize that while charge remote processes can still yield structurally useful sequence and non-sequence ions, the mechanisms by which they form can be quite different to those formed by charge directed processes [41].

In contrast, the gas-phase chemistry of radical cations of peptides is not so well understood and in this report, we address this situation. We compare the gas-phase fragmentation reactions of the even electron $[M+H]^+$ and $[M+2H]^{2+}$ ions with their odd electron $M^{\bullet+}$ counterparts for a library of 20 tripeptides GXR (where X = 1 of the 20 naturally occurring amino acids). Given the presence of the highly basic side chain of arginine in these GXR peptides, we are particularly interested in evaluating whether the fragmentation reactions of the odd electron $M^{\bullet+}$ ions of these peptides provide indirect evidence for the formation of distonic ions in which the charge (i.e. proton) is sequestered by the arginine side chain, while the radical is located at other sites, such as those previously described for amino acids, peptides and proteins (Fig. 1).

2. Experimental methods

2.1. Materials

The 20 tripeptides, GXR were purchased from SynPep (Dublin, CA, U.S.A.) with a stated minimum purity of 95% and used without further purification. (2,2′:6′,2″-Terpyridine)copper(II) nitrate monohydrate (hereafter designated as $[Cu(II)(tpy)(NO_3)_2]\cdot H_2O$) was synthesized according to a literature procedure [42].

2.2. Mass spectrometry

All experiments were performed using a commercially available quadrupole ion trap mass spectrometer (Finnigan-MAT model LCQ, San Jose, CA) equipped with electrospray ionization (ESI). Samples were prepared by dissolving 0.1 mg of the peptide (GXR) and 0.1 mg of the $[Cu(II)(tpy)(NO_3)_2]\cdot H_2O$ complex in 1.0 ml of CH_3OH and were then immediately introduced to the mass spectrometer at 3.0 μl/min via the electrospray ionization source. Each sample required careful tuning to maximize the signal of the metal complex $[Cu(II)(tpy)(GXR)]^{\bullet 2+}$ ions. The typical source conditions were: spray voltage, 4.0–5.5 kV; capillary temperature, 100–250 °C; nitrogen sheath pressure, 0 psi; capillary voltage, −135 to +135 V; tube lens offset voltage, −200 to +200 V. CID experiments were performed utilizing the advanced scan functions of the LCQ instrument. The isolation windows used were 0.7–3 Th for the doubly charged ions and 0.8–1.3 Th for the singly charged ions.

Several deuterium labeling experiments were performed in which acidic protons on heteroatoms were allowed to exchange for deuteriums. Thus, D_9-GXR (X = A, F, L and I) peptides were prepared in situ by stirring GXR (1 mg) in D_2O (1 ml) for 48 h. A 0.1 ml amount of this solution and $[Cu(II)(tpy)(NO_3)_2]\cdot H_2O$ (0.1 mg) were then added to CD_3OD (0.9 ml) before being analyzed by mass spectrometry.

Since multistage MS^3 experiments were performed, we have used the symbolism of Cooks and co-workers [43] to define each stage of mass spectrometry for all of the figures. Filled circles represent mass selected ions (with the *m/z* value of the selected ion placed next to the circle) and open circles represent mass scans.

3. Results and discussion

Previous studies on ternary complexes $[Metal(L)(M)]^{2+}$ have shown that the nature of both the metal and its ancillary ligand (L) can influence the formation of a radical cation of a biomolecule [29,44]. Thus, ligands which have acidic protons (such as the NH protons on dien) can undergo proton transfer (Eq. (2)) to the biomolecule rather than the desired electron transfer reaction (Eq. (1)) [29,44]. To overcome this problem we have used the terpyridine ligand in all of our attempts to form the radical cations of GXR (Eq. (3)). This method has been successful and radical cations have been formed for all the peptides except GRR.

$$[Cu(II)(dien)(M)]^{\bullet 2+} \xrightarrow{CID} [Cu(II)(dien)\text{-}H]^+ + [M+H]^+ \quad (2)$$

$$[Cu(II)(tpy)(M)]^{\bullet 2+} \xrightarrow{CID} [Cu(I)(tpy)]^+ + M^{\bullet +} \quad (3)$$

Before discussing the fragmentation reactions of the $[GXR+H]^+$, $[GXR+2H]^{2+}$ and $GXR^{\bullet+}$ ions in detail individually, we briefly examine the spectra of the four systems (X = S, C, Y and E) shown in Figs. 2–5. These data reveal that each of these ions provides complementary structural information. All of the $[GXR+H]^+$ ions provide little sequence ion information (apart from the formation

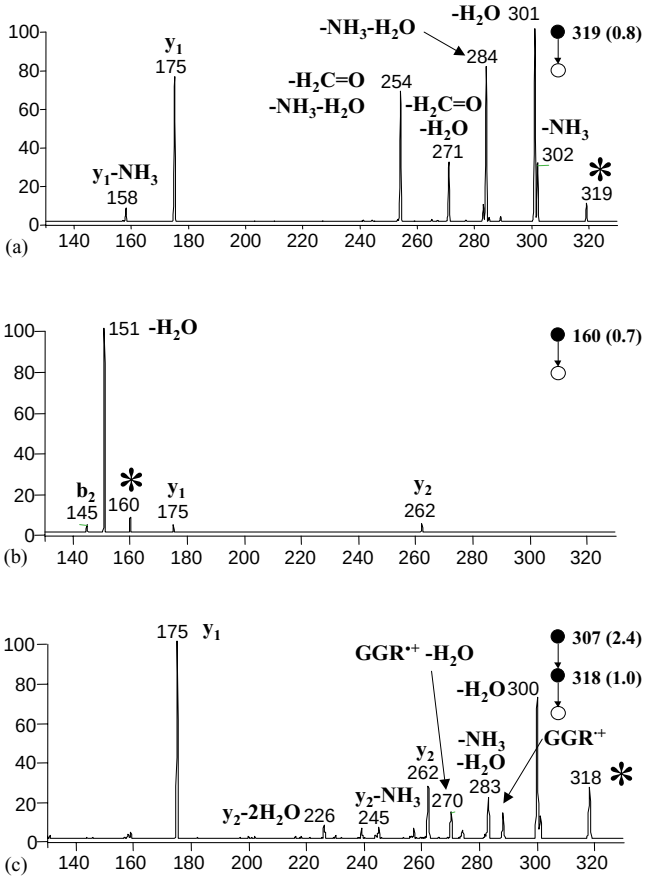


Fig. 2. (a) CID MS/MS reactions of the $[GSR + H]^+$ ion; (b) CID MS/MS reactions of the $[GSR + 2H]^{2+}$ ion and (c) CID MS^3 reactions of the $GSR^{\bullet+}$ ion. A (✻) denotes the precursor ion. The number in bracket denotes the width of the isolation window used (in Th) in the mass-selection process. No significant ions are observed below *m/z* 130.

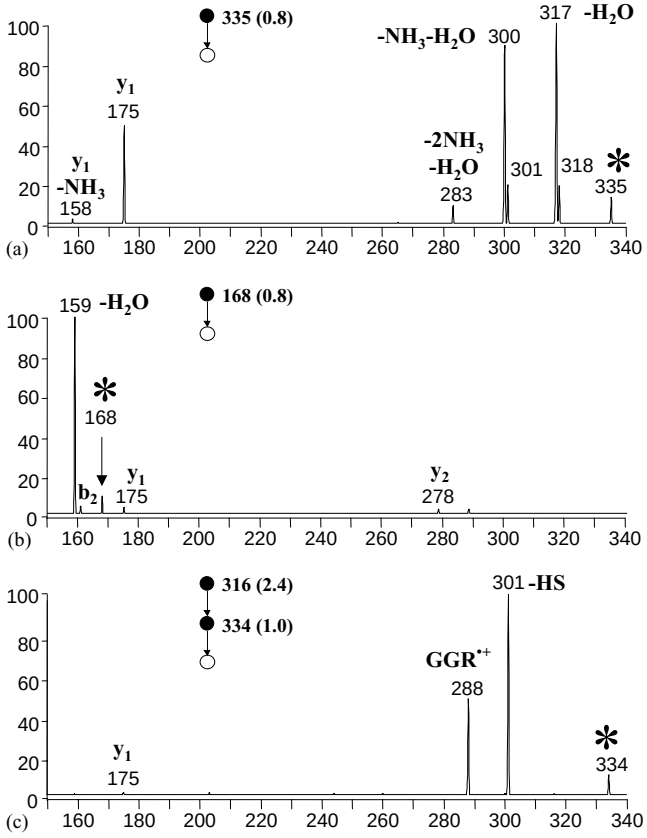


Fig. 3. (a) CID MS/MS reactions of the $[GCR + H]^+$ ion; (b) CID MS/MS reactions of the $[GCR + 2H]^{2+}$ ion and (c) CID MS^3 reactions of the $GCR^{\bullet+}$ ion. A (✻) denotes the precursor ion. The number in bracket denotes the width of the isolation window used (in Th) in the mass-selection process. No significant ions are observed below *m/z* 150.

of the y_1 sequence ion), often undergoing the loss of small molecules such as NH_3 and H_2O individually and in combination (Figs. 2a, 3a, 4a and 5a). These competing sequence and non-sequence ion forming reactions of the $[GXR+H]^+$ ions are discussed in further detail in Section 3.1. The $[GXR + 2H]^{2+}$ ions provide more sequence ion information, with b_2 and y_2 ions often being observed, although in some instances the yield of these sequence ions is small relative to the formation of non-sequence ions via small molecule losses (see for example Figs. 2b, 3b and 5b). The fragmentation reactions of the $[GXR + 2H]^{2+}$ ions are discussed in further detail in Section 3.2. A comparison of Figs. 2c, 3c, 4c and 5c reveals that across the range of systems studied, the radical cations exhibit diverse and rich chemistry. In some instances a complete y sequence ion series is observed in competition with small molecule losses (Figs. 2c and 5c) while in others the small molecule losses dominate (Figs. 3c and 4c). The types of small molecules lost from the $GXR^{\bullet+}$ ions are more diverse than their even electron counterparts, with structurally informative side chains losses often occurring (e.g. Figs. 3c, 4c and 5c). The fragmentation reactions of these $GXR^{\bullet+}$ ions are discussed in more detail in Section 3.4. All but one of the 20 $GXR^{\bullet+}$ ions were produced by reaction (3). Possible reasons why this reaction fails for $GRR^{\bullet+}$ are discussed in Section 3.3, which examines the gas-phase fragmentation of the precursor $[Cu(II)(tpy)(GXR)]^{\bullet2+}$ ions in detail. Section 3.5 provides an overview which compares the fragmentation reactions of even and odd electron ions of these tripeptides.

3.1. MS/MS on the $[GXR+H]^+$

Scheme 1 illustrates the bonds that need to be cleaved to form sequence ions, as well as the generally accepted structures of these sequence ions. Formation of the complementary y_1 and b_2 sequence ions involves the cleavage of amide bond B. Since the precursor ions, $[GXR+H]^+$ possess only one proton, both of the y_1 and b_2 fragments are in competition for this proton within an ion-molecule complex. Which of these two fragments is protonated and hence detected in the MS/MS spectra depends on the relative proton affinity of the y_1 versus b_2 fragments [45]. Since arginine is the most basic residue, the proton is largely retained on the y_1 fragment in the fragmentation of $[GXR + H]^+$. As a result, the

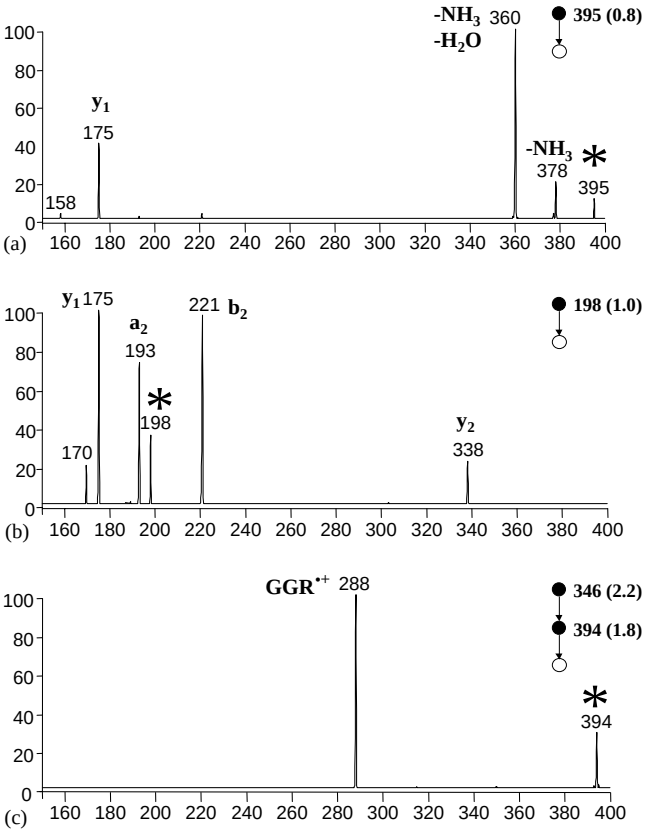


Fig. 4. (a) CID MS/MS reactions of the $[GYR + H]^+$ ion; (b) CID MS/MS reactions of the $[GYR + 2H]^{2+}$ ion and (c) CID MS^3 reactions of the $GYR^{\bullet+}$ ion. A (✻) denotes the precursor ion. The number in bracket denotes the width of the isolation window used (in Th) in the mass-selection process. No significant ions are observed below *m/z* 150.

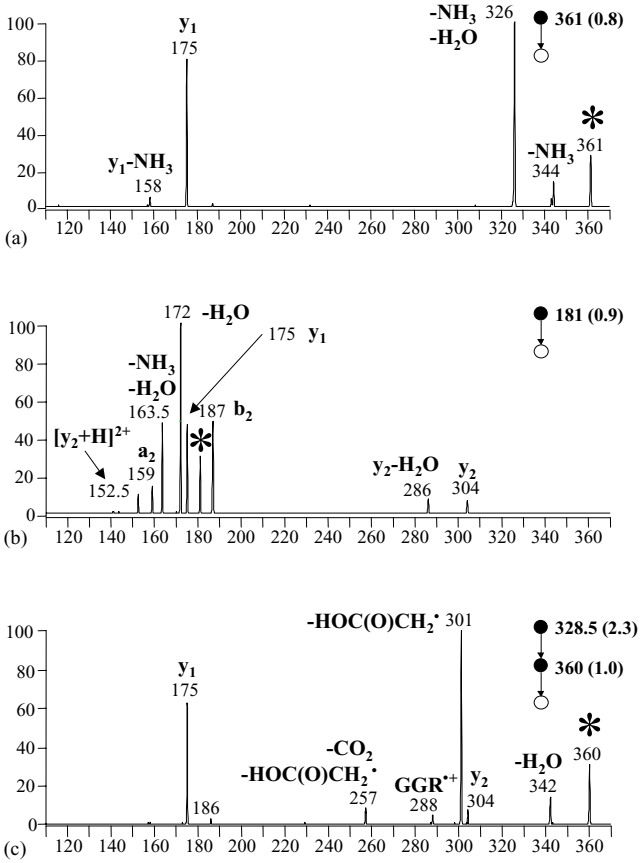
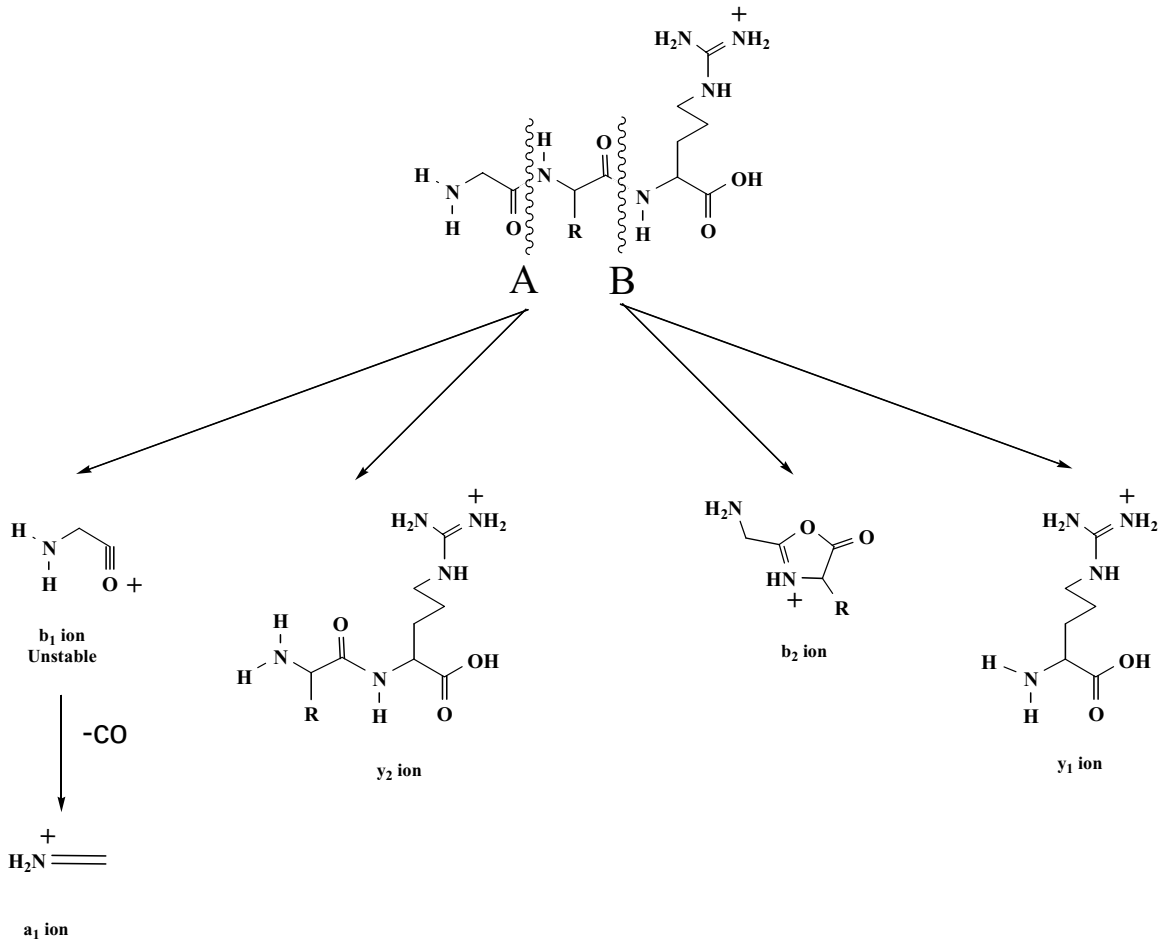


Fig. 5. (a) CID MS/MS reactions of the $[GER+H]^+$ ion; (b) CID MS/MS reactions of the $[GER+2H]^{2+}$ ion and (c) CID MS^3 reactions of the $GER^{\bullet+}$ ion. A (✻) denotes the precursor ion. The number in parenthesis denotes the width of the isolation window used (in Th) in the mass-selection process. No significant ions are observed below *m/z* 110.

y_1 ion is much more abundant than b_2 ions in the fragmentation reactions of the $[GXR + H]^+$ ions.

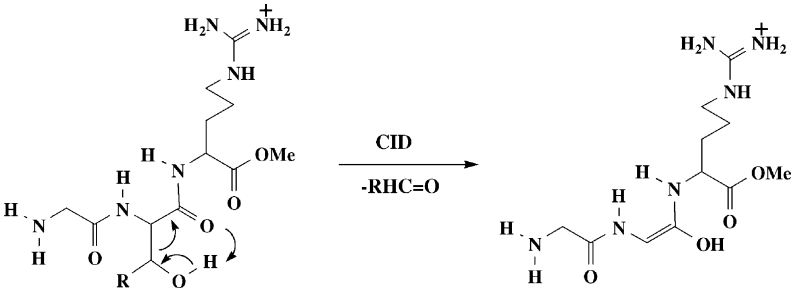
The major CID products of $[GXR + H]^+$ are given in (Table 1). Typically only the y_1 ion is observed, while the complementary b_2 ions are only observed in a few instances. In contrast, y_2 ions are absent. In many cases, non-sequence ions arising from the loss of small molecules such as H_2O and NH_3 dominate the MS/MS spectra. These results are consistent with the work of Kapp et al. [35] who have noted that a protonated peptide which has the number of ionizing proton(s) $\leq$the number of arginine residue(s) gives less sequence information. This is due to the sequestering of the 'mobile proton' by arginine residue in $[GXR + H]^+$ ions, which leads to a 'non-mobile proton' condition that yields less sequence information and produces non-sequence ions.

One of the unusual fragmentation results of the $[GXR + H]^+$ that we observed involves the behaviour of the $[GSR + H]^+$ and $[GTR + H]^+$ ions, which lose $[RCH{=}O + H_2O/NH_3]$ (R = H when X = S; R = Me when X = T). Such losses are not observed in the fragmentation of the $[GSR + 2H]^{2+}$ and $[GTR + 2H]^{2+}$ ions, which mainly lose H_2O instead. While the loss of H_2O is a common fragmentation behaviour of protonated serine/threonine containing peptides and involves loss of H_2O from the side chain [33], the loss of RCH=O from serine or threonine is not normally observed in the fragmentation of protonated serine/threonine containing peptides or protonated serine/threonine amino acids. However, related losses have been observed in the fragmentation of deprotonated serine/threonine containing peptides [46] and in the fragmentation of Cu(II) complexes of deprotonated serine/threonine amino acids [47] as well as in the fragmentation of calcium complexes of deprotonated serine/threonine containing peptides [48]. In order to probe whether the loss of aldehydes occurred from a salt bridge structure involving a deprotonated negatively charged C-terminus, CID was also carried out on the esters, $[GSR\text{-}OMe + H]^+$ and $[GTR\text{-}OMe + H]^+$, which cannot adopt a salt bridge structure. However, both these methylated peptides also lose $[RCH{=}O + H_2O/NH_3]$ (data not shown). This indicates that the loss of $[RCH{=}O + H_2O/NH_3]$ from the singly protonated peptides, $[GSR + H]^+$ and $[GTR + H]^+$, is not due to the presence of a deprotonated negatively charged C-terminus. It is possible that the arginine residue sequesters the proton of the singly protonated peptide and promotes the loss of an aldehyde as illustrated



Scheme 1.

in Eq. (4). A related mechanism has been proposed for the loss of RCH=O from [Cu(II)(bpy)(serine/threonine-H)]$^+$ [47].



(4)

[GKR + H]$^+$ also yields an unusual fragmentation product, which most likely corresponds to a combination of the following losses: (i) the guanidinium group from the arginine side chain; (ii) H_2O and CO from the C-terminus and (iii) the N-terminal glycine residue. The loss of the guanidinium group from arginine side chain may be a result of the attack of the protonated guanidinium group by the nucleophilic lysine side chain. Unfortunately, the sequence of these losses cannot be determined from the CID spectrum of [GKR + H]$^+$ since the individual losses are not observed to any significant extent.

3.2. MS/MS on the [GXR+2H]$^{2+}$

All of the [GXR + 2H]$^{2+}$ ions have either one 'mobile proton', one 'partially mobile proton' ([GHR + 2H]$^{2+}$ and [GKR + 2H]$^{2+}$) or no 'mobile proton' ([GRR + 2H]$^{2+}$). Hence, most [GXR + 2H]$^{2+}$ ions provide more sequence information than their [GXR + H]$^+$ counterparts (Table 2). The competitive fragmentation pathways for the formation

Table 1
Abundance of CID products of $[GXR + H]^+$ relative to the most intense peak in the spectrum (%)

GXR	y_1	y_1-NH_3	b_2	$-NH_3$	$-2NH_3$	$-NH_3-H_2O$	$-H_2O$	$-2H_2O$	Other products
X = aliphatic residue									
GGR	100	33	5	70	12	97	18	8	$-3NH_3$ (6%)
GAR	85	10		30		100	13	7	
GVR	75	10		23		100	8		
GIR[a]	60	10	5	25		100	10		
GLR[a]	80	15	5	25		100	7	5	
GPR	100	5		45		35	63	63	$-NH_3$, $-2H_2O$ (30%), $m/z = 250$ (8%)
X = aromatic residue									
GFR	45			22		100	7		
GYR	40			20		100			
GWR	40		20	22		100	10		
X = acidic residue									
GDR	100			10		40	13		$[M + H - NH_3 - H_2O - CO_2]^+$ (50%)
GER	80	7		15		100	6		
X = basic residue									
GHR	75		10	20		100	19	7	$m/z = 322$ (20%), $m/z = 290$ (8%), $m/z = 309$ (5%), $m/z = 317$ (5%)
GKR	100			17		15	10	13	$-2NH_3$, $-H_2O$ (5%), $-NH_3$, $-2H_2O$ (6%), $-HN=C(NH_2)_2$, $-H_2O$, $-CO$, $-HN=CH_2-CO$ (40%)
GRR	40					10	100		$b_2 + H_2O$ (20%)
X = heteroatom residue (non-aromatic)									
GCR	50			20	20 (or $-H_2S$)	90	100		$-2NH_3$, $-H_2O$ (10%)
GMR	55	8		25		100	5	5	
GSR	75	8		35		85	100	10	$-H_2C=O$, $-H_2O$ (35%), $-H_2C=O$, $-H_2O$, $-NH_3$ (70%)
GTR	47			18		15	33		$-CH_3CH=O$ (5%), $-CH_3CH=O$, $-H_2O$ (45%), $-CH_3CH=O$, $-H_2O$, $-NH_3$ (100%)
GNR	30			48	8	100	27	7	
GQR	55		5	27	17	100	10		

Products of relative abundance less than 5% are not listed.
[a] These results were previously reported [17].

of sequence ions from $[GXR + 2H]^{2+}$ may be understood by a slight modification to Scheme 1. Unlike $[GXR + H]^+$ which have only one proton (for which both the y_1 and b_2 fragments compete), $[GXR + 2H]^{2+}$ have two protons, allowing both y_1 and b_2 fragments to be protonated and thus, in the fragmentation of $[GXR + 2H]^{2+}$, the y_1 and its complementary b_2 and/or a_2 ions are observed with almost equal abundances. The 'mobile proton' also induces the formation of y_2 or $[y_2 + H]^{2+}$ ions via cleavage of amide bond A. For the singly charged y_2 ions, the complementary b_1 ion is not observed as it is not stable [49] and readily loses CO to form the a_1 ion, which is not detected due to the low mass cut-off of the ion trap.

Despite the presence of a 'mobile proton', some of the $[GXR+2H]^{2+}$ ions only form low abundance sequence ions. They are (Table 2): (i) $[GNR + 2H]^{2+}$ and $[GQR + 2H]^{2+}$, which lose mainly NH_3; (ii) $[GCR + 2H]^{2+}$, $[GTR + 2H]^{2+}$ and $[GSR + 2H]^{2+}$, which lose mainly H_2O; (iii) $[GVR + 2H]^{2+}$, $[GLR + 2H]^{2+}$ and $[GIR + 2H]^{2+}$ which form minor amounts of the y_2 ions.

How do our results compare with previous studies on the gas-phase fragmentation reactions of peptides? Abundant NH_3 loss from $[GNR + 2H]^{2+}$ and $[GQR + 2H]^{2+}$ is not surprising since significant loss of NH_3 from the side chain amides is common in the fragmentation of asparagine and glutamine containing peptides [45,50]. Abundant loss of H_2O from $[GCR + 2H]^{2+}$, $[GTR + 2H]^{2+}$ and $[GSR + 2H]^{2+}$ is entirely consistent with the known behaviour of cysteine, threonine and serine residues which often undergo abundant water loss in low energy peptide fragmentation reactions [33]. $[GER + 2H]^{2+}$ also loses H_2O abundantly, but this is less consistent with the fragmentation reactions of other protonated peptides containing glutamic acid, which generally give less water loss [50]. Despite these common non-sequence ion products due to NH_3 and H_2O losses, it is interesting to note the lack of sequence ion formation in the fragmentation of $[GNR + 2H]^{2+}$, $[GQR + 2H]^{2+}$, $[GCR + 2H]^{2+}$, $[GTR + 2H]^{2+}$ and $[GSR + 2H]^{2+}$. In contrast, the previously published related systems, $[GQG + H]^+$ and $[GCG\text{-}OMe + H]^+$, containing glutamine and cysteine as interior residues, have been found to give >5% sequence ions [50,51]. Presumably, the arginine residue is playing a role in the present $[GXR + 2H]^{2+}$ system.

It is interesting to note that peptides containing aliphatic residues $[GVR + 2H]^{2+}$, $[GLR + 2H]^{2+}$ and $[GIR + 2H]^{2+}$ also produce y_2 ions in low abundance. This observation is consistent with the work of Kapp et al. [35], who showed that valine, isoleucine and leucine display a C-terminal positive cleavage effect, which would suggest that more y_1/b_2 ions should be formed than y_2/b_1 ions for simple tripeptides.

Table 2
Abundance of CID products of $[GXR + 2H]^{2+}$ relative to the most intense peak in the spectrum (%)

GXR	y_1	y_2	$[y_2 + H]^{2+}$	b_2	a_2	$-NH_3$	$-H_2O$	Other products
X = aliphatic residue								
GGR	30	100			18			
GAR	100	33	7	70	15			
GVR	100			75	17			
GIR[a]	100			15	60			
GLR[a]	100			28	61			
GPR	80	25	100	45	17			
X = aromatic residue								
GFR	100	20	25	85	65			
GYR	100	20		97	73			$m/z = 169.5$ (20%)
GWR	75	15		100	25		55	$m/z = 181$ (55%), $m/z = 172.5$ (7%)
X = acidic residue								
GDR	95	100			80		75	$y_2 - H_2O$ (10%), $a_2 - H_2O$ (30%)
GER	50	10	10	50	15		100	$-H_2O$, $-NH_3$ (50%), $y_2 - H_2O$ (10%)
X = basic residue								
GHR	20		35	25			10	$-H_2O$, $-NH_3$ (100%), $b_2 + H_2O$ (5%)
GKR	100	12	45	22		70	10	$-H_2O$, $-NH_3$ (71%), $-H_2O$, $-2NH_3$ (22%), $[y_2 + H]^{2+}-NH_3$ (20%), related/immonium ion ($m/z = 129$) (55%), $b_2 - NH_3$ (12%), $a_2 - NH_3$ (10%), $b_2 + H_2O$ (10%)
GRR	63			45		50	8	$-H_2O$, $-NH_3$ (100%), $b_2 - NH_3$ (35%), $b_2 + H_2O$ (27%), $m/z = 272$ (10%), $m/z = 173.5$ (8%), related/immonium ion ($m/z = 100$) (8%), related/immonium ion ($m/z = 112$) (8%)
X = heteroatom residue (non-aromatic)								
GCR							100	
GMR	100	45	45	67	45			$-CH_3SH$ (35%), $m/z = 113$ (15%)
GSR		5					100	
GTR							100	
GNR	10	7	5			100		$-H_2O$, $-NH_3$ (15%), $GXR + H^+ - NH_3$ (20%), $y_2 - NH_3$ (7%)
GQR						100		

Products of relative abundance less than 5% are not listed.
[a] These results were previously reported [17].

$[GRR + 2H]^{2+}$ fragments mainly via NH_3 and H_2O loss. Although $[GRR + 2H]^{2+}$ also generates some y_1 and b_2 sequence ions, it does not yield a significant abundance of the y_2 ion. This is not surprising since $[GRR + 2H]^{2+}$ has no 'mobile proton' and is expected to yield less sequence ions.

3.3. MS/MS on $[Cu(II)(tpy)(GXR)]^{\bullet 2+}$

As noted in Section 2, mixtures of $[Cu(II)(tpy)(NO_3)_2]\cdot H_2O$ and the peptide were dissolved in CH_3OH and immediately introduced into the mass spectrometer via the electrospray ionization source. After careful tuning to maximize the signal of the metal complex $[Cu(II)(tpy)(GXR)]^{\bullet 2+}$ ions, reasonable abundances of these $[Cu(II)(tpy)(GXR)]^{\bullet 2+}$ ions were generally observed.

There are 2 major competing fragmentation pathways in the CID of $[Cu(II)(tpy)(GXR)]^{\bullet 2+}$: (i) the desired redox reaction involving the oxidation of GXR to $GXR^{\bullet +}$ (Eq. (3)), and (ii) loss of protonated tpy (Eq. (5)). When $X \neq R$, formation of $GXR^{\bullet +}$ (Eq. (3)) is the dominant pathway. When X = R, the pathway of Eq. (5) dominates and radical formation is largely "switched off". The lack of the formation of $GRR^{\bullet +}$ upon CID of $[Cu(II)(tpy)(GRR)]^{\bullet 2+}$ may indicate a stronger binding affinity of GRR to Cu(II). The relative abundance of $[Cu(II)(tpy)(GRR)]^{\bullet 2+}$ is significantly lower than the relative abundances of other $[Cu(II)(tpy)(GXR)]^{\bullet 2+}$ which may be a result of the interior arginine. In most cases, trace loss of NH_3 from $[Cu(II)(tpy)(GXR)]^{\bullet 2+}$ is also observed (Eq. (6)).

$$[Cu(II)(tpy)(M)]^{\bullet 2+} \xrightarrow{CID} [Cu(II)(M - H)]^{\bullet +} + [tpy + H]^{+} \quad (5)$$

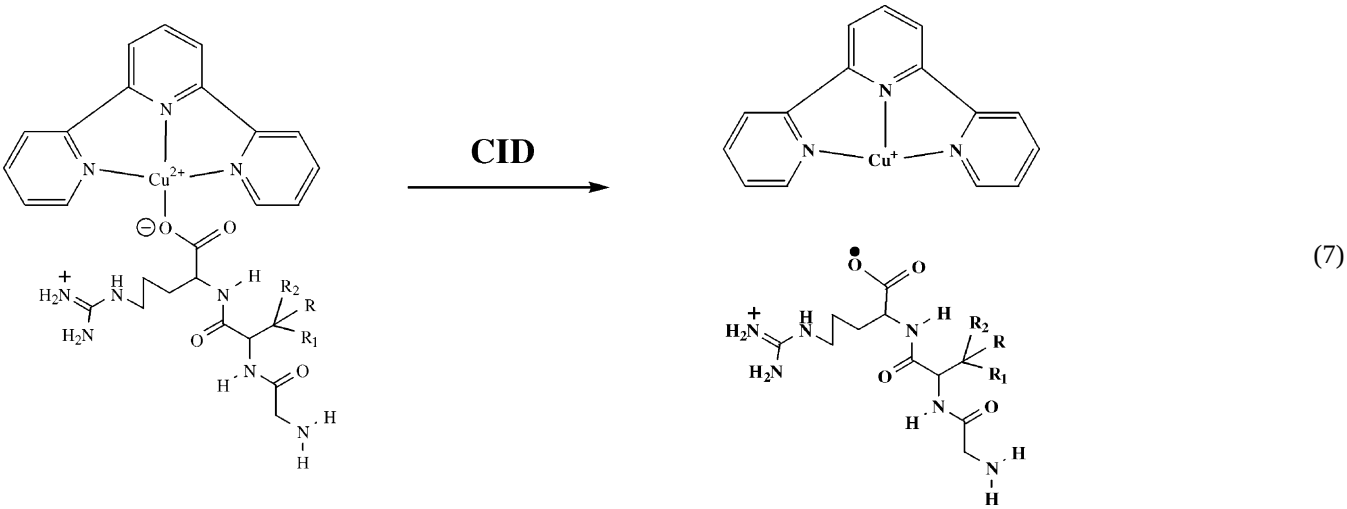
$$[Cu(II)(tpy)(M)]^{\bullet 2+} \xrightarrow{CID} [Cu(II)(tpy)(M - NH_3)]^{\bullet 2+} + NH_3 \quad (6)$$

Some key questions regarding the formation and subsequent reactivity of the radical cations of the tripeptides GXR are as follows:

(i) How does the initial binding of the peptide to the copper in the ternary complex $[Cu(II)(tpy)(GXR)]^{\bullet 2+}$ influence the formation of radical cations?
(ii) Does the initially formed radical cation undergo prompt fragmentation induced by the radical site?
(iii) Can the initially formed radical cation undergo intramolecular $H^{\bullet}$ transfer to yield a new radical site which undergoes different fragmentation processes?

While we cannot directly answer question (i), we note that copper(II) complexes not only exhibit a range of coordination numbers (typically from 4 to 6), but can adopt distortions from ideal geometries [52]. Cu(II) is a borderline hard acid, but the hard base functions of ligands can be complicated by their ability to reduce Cu(II) to Cu(I). Thus there are potentially a number of binding motifs for the tripeptides GXR. Nonetheless, the presence of the highly basic arginine side chain means that the GXR peptides have an opportunity to bind to the Cu(II)(tpy) moiety as zwitterions via the negatively charged carboxylate group. The binding of carboxylate ions to Cu(II) is well documented. Indeed, not only has Turecek shown that a free carboxylate is a requirement for amino acids to bind to related copper bipyridine complexes under ESI/MS condition [53], but there are crystal structures of related ternary complexes of amino acids which clearly reveal the binding of the carboxylate to copper. Many of these systems are further complicated by secondary interactions such as H-bonding or π–π stacking [54].

Thus we speculate that upon CID, GXR are oxidized by electron transfer from the carboxylate oxygen (to [Cu(II)(tpy)]) to form a distonic $GXR^{\bullet+}$ ion [55] with the radical site at the carboxylate oxygen and the charge on the arginine side chain (Eq. (7)). We have found that the initially formed $GXR^{\bullet+}$ ions often undergo further fragmentation during MS/MS of the $[Cu(II)(tpy)(GXR)]^{\bullet 2+}$ complexes, such as side chain fragmentation and the loss of CO_2 (Table 3). Based upon recent work by Schröder et al. [56] and Bossio et al. [57], who have demonstrated that carboxylate radicals are relatively unstable in the gas phase, we suggest that the loss of CO_2 is a diagnostic fragmentation reaction of the initially formed distonic ion in which the radical site is located at the carboxylate oxygen. It is interesting to note that carboxylate radicals are implicated in the solid state chemistry of irradiated *N*-acetyl amino acids, di- and tripeptides and that carboxylate radicals of peptides and proteins also undergo decarboxylation reactions in the solid state [8].



In most cases, CO_2 loss occurs to a lesser extent upon CID of $GXR^{\bullet+}$ at the MS^3 stage (see Section 3.4 for a detailed discussion of the fragmentation reactions of $GXR^{\bullet+}$ under MS^3 conditions). The reason may be that upon CID of $[Cu(II)(tpy)(GXR)]^{\bullet 2+}$ complexes at the MS^2 stage, one of the resultant populations of the $GXR^{\bullet+}$ ions rapidly undergoes decomposition via CO_2 loss, while the remaining populations undergoes isomerization via $H^{\bullet}$ rearrangements leading to more stable $GXR^{\bullet+}$ ions. The possibility of radical site migration via intramolecular $H^{\bullet}$ transfer is consistent with both the known behaviour of peptides and proteins in the condensed phase [8,9,14] as well as recent work by Schröder et al., who showed that carboxyl radicals can undergo intramolecular $H^{\bullet}$ transfer under NRMS conditions [56]. Section 3.4 discusses the other fragmentation reactions of $GXR^{\bullet+}$ ions under MS^3 conditions.

3.4. MS^3 on $GXR^{\bullet+}$

The major fragmentation products of $GXR^{\bullet+}$ under MS^3 conditions are listed in Table 4 according to the type of side chain present in X. We suggest that many of these fragmentation reactions are driven by the radical site in isomeric distonic $GXR^{\bullet+}$ ions in which the radical site is located at different sites (Scheme 2). The isomeric distonic $GXR^{\bullet+}$ precursor ions may arise from processes involving intramolecular $H^{\bullet}$ transfer, a 'mobile radical' process. Furthermore, the different radical sites produce different fragmentation products (Scheme 2). Generally, the major fragmentation products of $GXR^{\bullet+}$ are $[GXR\text{-}CO_2]^{\bullet+}$ (A) (Eq. (8)), $G(G^{\bullet})R^{+}$ (B) (Eq. (9)), (C) (Eq. (10)) and the y_1 and y_2 ions (Eqs. (11) and (12)). Note that in our discussion, we use $(G^{\bullet})XR^{+}$ and $G(X^{\bullet})R^{+}$ to designate an α-radical on the first and second residue, respectively.

Formation of (B) is promoted by abstraction of a side chain $H^{\bullet}$ (not at the β position) followed by the loss of even-electron unsaturated molecule(s) to form an α-centered radical. The formation of (B) likely requires the following conditions: (i) an X side chain which is longer than the alanine side chain, (ii) a low bond dissociation energy of the R–H bond (R=C, N, S, O) from which the $H^{\bullet}$ can be

Table 3
Abundance of CID products of $[Cu(II)(tpy)(GXR)]^{\bullet 2+}$ relative to the most intense peak in the spectrum (%)

GXR	$GXR^{\bullet +}$	$[Cu^{I}(tpy)]^{+}$	$tpy + H^{+}$	$[Cu(II)(GXR - H)]^{+}$	$-NH_3$	$GXR^{\bullet +}$-CO_2 (A)	$G(G^{\bullet})R^{+}$ (B)	(C)	(C) $- CO_2$	y_1	$[Cu(II)(GXR - y_1)]^{+}$	Other products
X = aliphatic residue												
GGR	40	100	5		12	6						
GAR	28	100	5			17						*m/z* = 303 (5%), *m/z* = 355 (7%)
GVR	25	100				5						
GIR[a]	42	100			6	5						$y_2 - NH_3$ (6%)
GLR[a]	55	100			20				7			$GGR^{\bullet +}$-CO_2 (10%)
GPR	75	100				13						*m/z* = 215 (8%)
X = aromatic residue												
GFR	55	100	6		15	10						
GYR	100	80					15					
GWR	30	100				10						
X = acidic residue												
GDR	20	100	6		20					10	27	$GXR^{\bullet +} - NH_3$ (7%), $GXR^{\bullet +}$ – (relative ion-H^{+}) (40%)
GER	25	100			22					10	25	*m/z* = 235 (10%), $GXR^{\bullet +}$-H_2O (6%)
X = basic residue												
GHR	6	100	17	10								
GKR	40	100	15	10	10	7						$[Cu(II)(GXR - H)\text{-}CO_2]^{+}$ (5%)
GRR		10	43	55								$-H_2N^{\bullet}$ (15%), *m/z* = 267 (8%)
X = heteroatom residue (non-aromatic)												
GCR	75	95										
GMR	30	100			12			13	8			*m/z* = 281 (7%)
GSR	52	100	12	6		7						
GTR	13	100										*m/z* = 328 (6%)
GNR	45	100	13	7	10			11	5			
GQR	50	100	12	13	13	8			8			$GXR^{\bullet +}$–H_2O (8%)

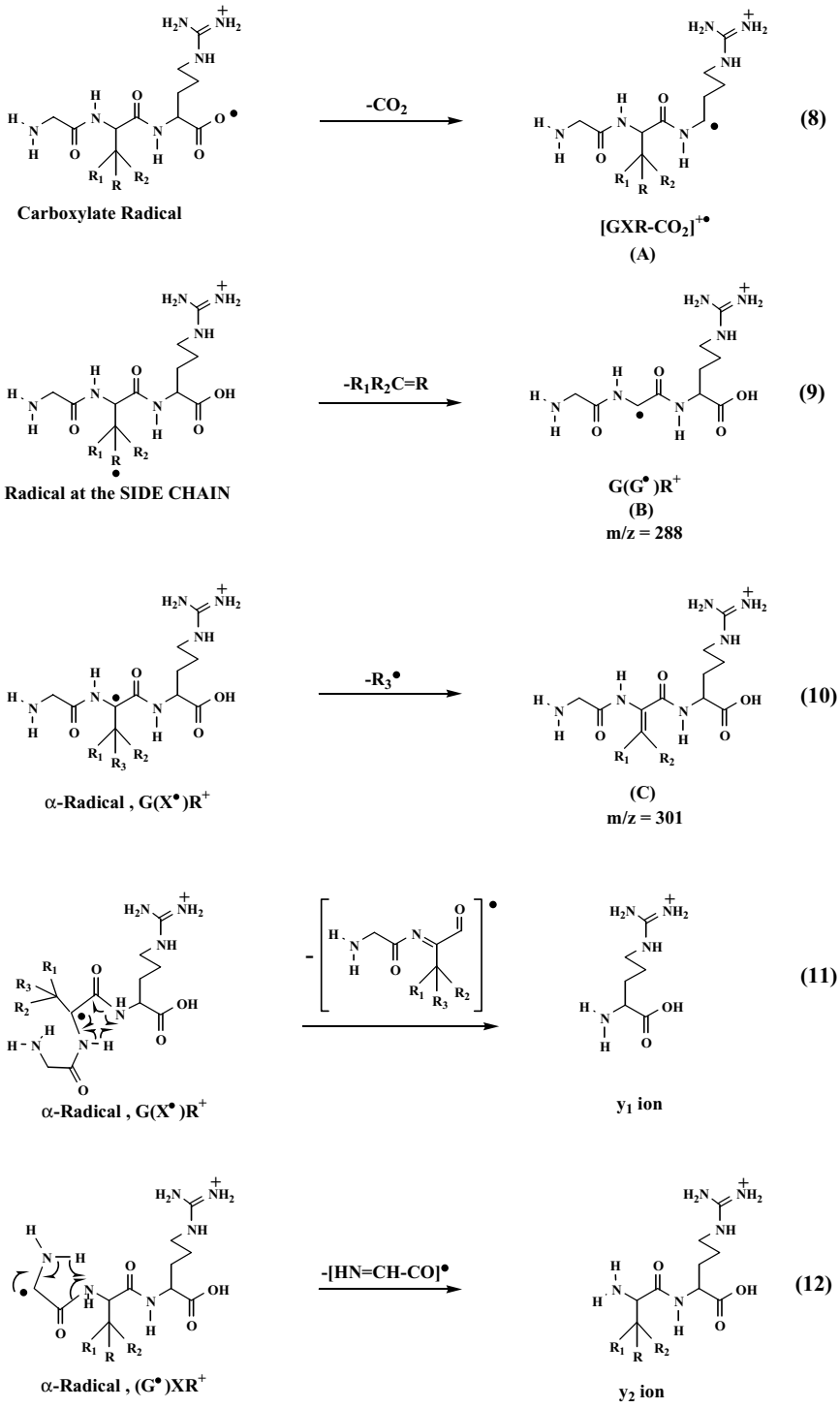
Products of relative abundance less than 5% are not listed.

[a] These results were previously reported [17].

Table 4
Abundance of CID products of $GXR^{\bullet+}$ relative to the most intense peak in the spectrum

$GXR^{\bullet+}$	y_1 (%)	y_2 (%)	$GXR^{\bullet+}$-CO_2 (A) (%)	$G(G^{\bullet})R^+$ (B) (%)	(C) (%)	(C) − CO_2 (%)	$-NH_3$ (%)	$-H_2O$ (%)	Other products
X = aliphatic residue									
$GGR^{\bullet+}$ (100%)	35	12	5						
$GAR^{\bullet+}$ (28%)	100	21	16						*m/z* = 200 (5%)
$GVR^{\bullet+}$ (17%)	100	38	7		–$Me^{\bullet}$ (20%)	$-CO_2$, –$Me^{\bullet}$ (10%)		5	$z_1^{\bullet+}$ (8%), *m/z* = 225 (5%)
D_9-$GIR^{\bullet+}$ (13%)	70	30		18	–$Et^{\bullet}$ (100%), –$Me^{\bullet}$ (8%)	$-CO_2$, –$Et^{\bullet}$ (17%)			
D_9-$GLR^{\bullet+}$ (35%)	70	20		32	100	8			
$GPR^{\bullet+}$ (20%)	100	18							*m/z* = 270 (8%)
X = aromatic residue									
$GFR^{\bullet+}$ (27%)	100	40	45					6	*m/z* = 225 (10%), $z_1^{\bullet+}$ (20%), *m/z* = 276 (12%), $[b_2 - H^{\bullet}]^{\bullet+}$ (9%)
$GYR^{\bullet+}$ (10%)				100					
$GWR^{\bullet+}$ (35%)	100	80	60					8	–[3-$^{\bullet}CH_2$-1H-indole] (7%), –[3-$^{\bullet}CH_2$-1H-indole]-H_2O (9%), –[3-$^{\bullet}CH_2$-1H-indole]-CO_2 (12%), *m/z* = 225 (10%), $y_1 - NH_3$ (5%), $z_1^{\bullet+}$ (13%), *m/z* = 315 (10%), $y_2 - NH_3$ (20%)
X = acidic residue									
$GDR^{\bullet+}$ (22%)	100	8	10					5	$-2CO_2$ (5%)
$GER^{\bullet+}$ (35%)	65	10		5	100	10		17	
X = basic residue									
$GHR^{\bullet+}$ (100%)	32	8	20				5	10	$z_1^{\bullet+}$ (12%), *m/z*=225 (5%)
$GKR^{\bullet+}$ (25%)	100	15	15	50	22	5	6	15	$GGR^{\bullet+}$-CO_2 (20%), $-2H_2O$ (8%), −62 Da (5%), $-H_2NCH_2^{\bullet}$ (6%), $-H_2N^{\bullet}$ (5%), *m/z* = 316 (9%)
$GRR^{\bullet+}$									
X = heteroatom residue (non-aromatic)									
$GCR^{\bullet+}$ (12%)				50	100				
$GMR^{\bullet+}$ (10%)	33	10		20	100				$-CH_3S^{\bullet}$ (8%)
$GSR^{\bullet+}$ (27%)	100	25		15	13	5		75	$y_2 - 2H_2O$ (6%), $y_2 - NH_3$ (5%), $GGR^{\bullet+}-H_2O$ (13%), $-H_2O$, $-NH_3$ (23%)
$GTR^{\bullet+}$ (100%)	40	12		12	6		6	23	$y_2 - 2H_2O$ (5%), $y_2 - NH_3$ (15%), (C) − NH_3 (8%)
$GNR^{\bullet+}$ (13%)	20	13			100	15	18	7	(C) − NH_3 (5%)
$GQR^{\bullet+}$ (15%)	40	15		7	100	15	25	6	$y_2 - H_2O$ (5%)

Products of relative abundance less than 5% are not listed.



Scheme 2.

abstracted, (iii) the absence of unfavorable polar effects that may influence the transition state energy of H• abstraction [13], (iv) energetically favorable conformations of $GXR^{\bullet+}$ to facilitate H• abstraction, and (v) accessible side chain fragmentation pathways leading to a stable α-centered radical. Note that in some cases (e.g. $GSR^{\bullet+}$ and $GTR^{\bullet+}$), isomer(s) of (B) can be formed via even-electron pathways (cf. Eq. (4)).

The even-electron species, (C), is formed via side chain radical loss from an α-radical of an X residue. An examination of Table 4 reveals that side chain radical loss can vary from being non-existent through to a dominant pathway. For example, HS• is readily lost from $GCR^{\bullet+}$ while HO• loss is only minor for $GSR^{\bullet+}$. The abundance of (C) is likely to depend on the stability of the side chain radical lost. Interestingly, as we noted previously for $GIR^{\bullet+}$ [17], two

different radicals are lost: $Me^{\bullet}$ and $Et^{\bullet}$. The latter radical is lost in higher abundance, consistent with the 'largest alkyl loss rule' observed in the 'α-cleavage' fragmentation reactions of many radical cations of small organic systems [58].

Since y_2 ions are not formed from the fragmentation of $[GXR + H]^+$, formation of y_2 ions from $GXR^{\bullet+}$ must occur via an odd-electron pathway. We propose the mechanism shown in Eq. (12), Scheme 2, based on: (i) analogies with the mechanism of H_2O loss from an α-centered glycyl radical proposed by Polce and Wesdemiotis [59] and the mechanism of CH_3NH_2 loss from the N-radical of 2-glycyl N-methylamide proposed by Turecek and Carpenter [60]; (ii) deuterium labeling studies carried out using peptides in which all the protons on heteroatoms were exchanged for deuteriums. One such experiment is illustrated for D_9-$GAR^{\bullet+}$ in Fig. 6. The results of these experiments are summarized in Scheme 3 and reveal that the majority of y_2 ions formed from D_9-$GXR^{\bullet+}$ (X = A and F) possess eight deuteriums (data not shown for D_9-$GFR^{\bullet+}$). Note that the carboxylate proton of these D_8-y_2 ions is unlabelled since it is formed from the original carboxylate radical, which abstracts a $H^{\bullet}$ from a carbon center (either from an α-carbon or the side chain).

Since the y_1 ion is formed upon CID of $[GXR + H]^+$, it can be generated via either an even electron and/or an odd electron pathway (Eq. (11)) from $GXR^{\bullet+}$. Given the deuterium labeling experiments described in Scheme 3, radical processes still play a role in the formation of this y_1 ion.

We now consider in detail the competition between various fragmentation reactions of different classes of individual amino acid residues (Table 4). In order to provide a mechanistic rationale for these fragmentation reactions, we have used deuterium labeling in selected cases and also considered thermochemical aspects for $H^{\bullet}$ transfer reactions using the bond dissociation energies for model systems listed in Table 5.

3.4.1. X = aliphatic residue

3.4.1.1. $GGR^{\bullet+}$. Once formed, $GGR^{\bullet+}$ undergoes rapid radical site rearrangements to yield various isomeric $GGR^{\bullet+}$ in which the radical is located at different sites. $G(G^{\bullet})R^+$ (B) is very likely to be one of the radical site rearrangement products of $GGR^{\bullet+}$ and the only fragmentation products observed are the y_1 ion, y_2 ion and (A) (Table 4).

3.4.1.2. $GAR^{\bullet+}$. Formation of (B) from $GXR^{\bullet+}$ is preceded by the abstraction of a side chain hydrogen atom. This abstraction cannot occur from the β position to lead to the formation of (B). Hence, $GAR^{\bullet+}$ does not fragment to yield (B). $GAR^{\bullet+}$ also does not fragment to yield (C) since this would require the loss of the least stable $H^{\bullet}$ radical from the alanine side chain. As a result, fragmentation of $GAR^{\bullet+}$ yields the y_1 and y_2 ions and (A) only (Table 4). It is interesting to note that in the condensed phase, $HO^{\bullet}$ attacks glycine and alanine residues almost exclusively at the C_{α}-H position along the peptide main chain [8]. In order to gain further insights into the fragmentation mechanism of $GAR^{\bullet+}$, labeling studies were carried out. The CID spectrum of D_9-$GAR^{\bullet+}$ (Fig. 6) reveals that the majority (87–90%) of the y_1 and y_2 ions formed from the fragmentation of D_9-$GAR^{\bullet+}$ are the D_7-y_1 and D_8-y_2 ions. If all acidic

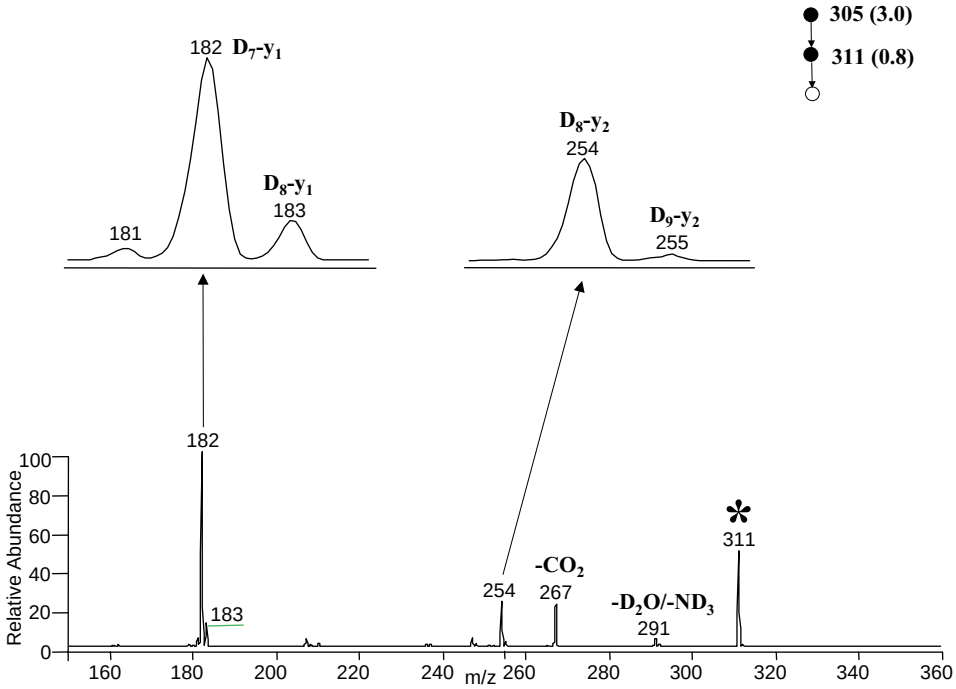
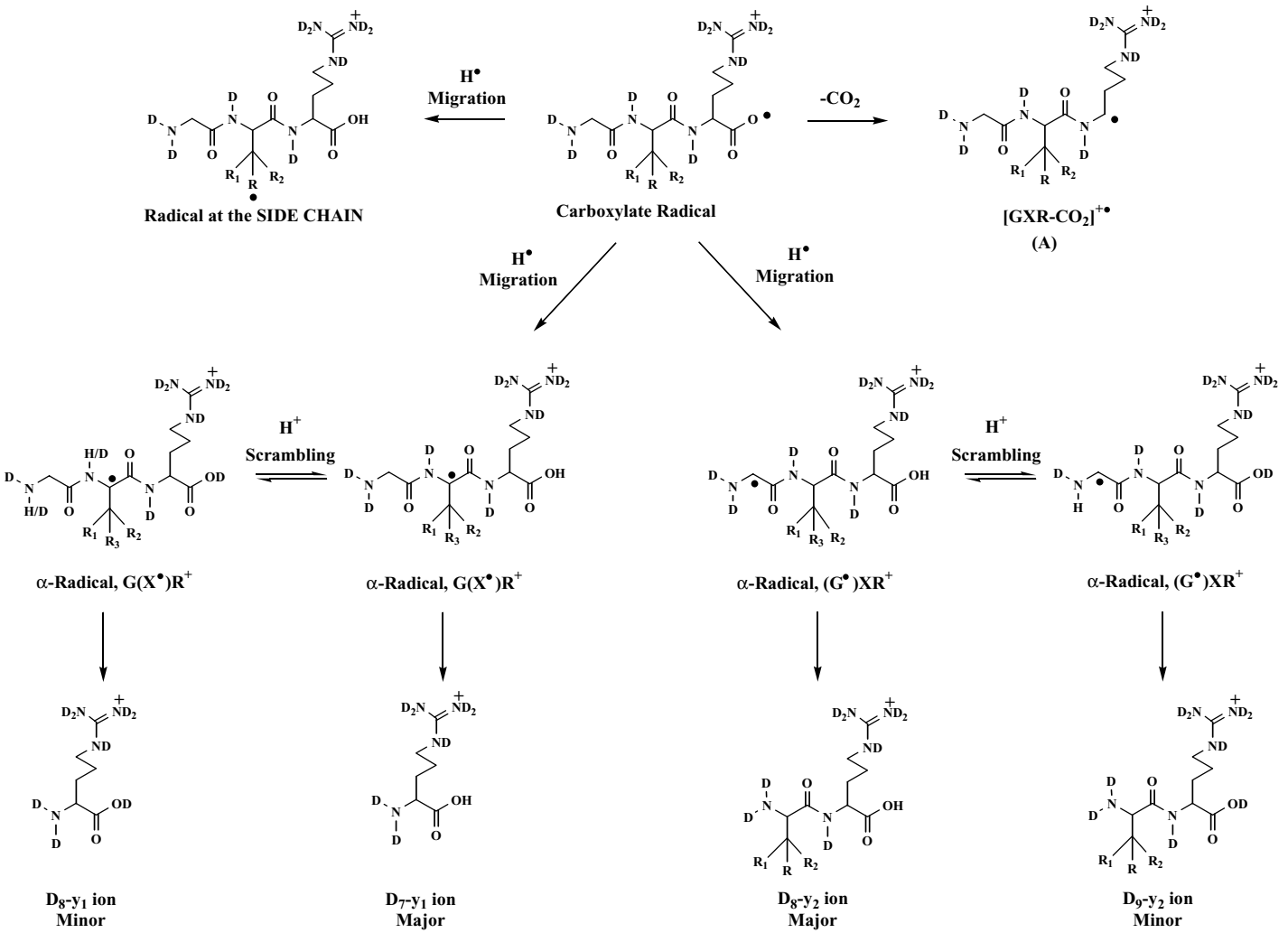


Fig. 6. CID MS^3 reactions of the D_9-$GAR^{\bullet+}$ ion. A (✻) denotes the precursor ion. The number in bracket denotes the width of the isolation window used (in Th) in the mass-selection process.


Radical at the SIDE CHAIN
H• Migration
Carboxylate Radical
-CO2
[GXR-CO2]+•
(A)
H• Migration
H• Migration
H+ Scrambling
H+ Scrambling
α-Radical, G(X•)R+
α-Radical, G(X•)R+
α-Radical, (G•)XR+
α-Radical, (G•)XR+
D8-y1 ion Minor
D7-y1 ion Major
D8-y2 ion Major
D9-y2 ion Minor

Scheme 3.

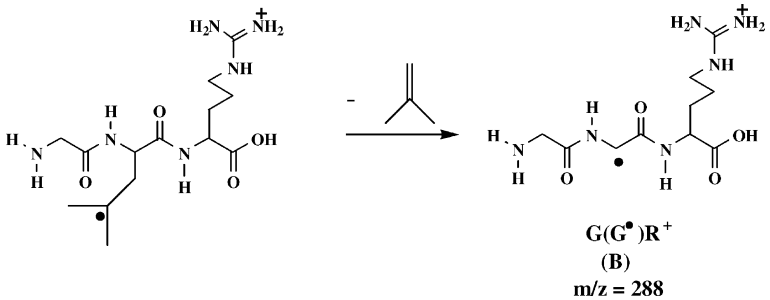
Table 5
Useful bond dissociation energy (BDE) of relevant simple organic compounds as models of amino acid side chains for the interpretation of the fragmentation behaviour of $GXR^{\bullet+}$

$GXR^{\bullet+}$	Equivalent R–H bond in organic compound	BDE ($kJ\,mol^{-1}$)	Reference
X = aliphatic residue			
$GGR^{\bullet+}$	–	–	
$GAR^{\bullet+}$	–	–	
$GVR^{\bullet+}$	$HC(\mathbf{CH_3})_3$	425.2 ± 2.1	[61]
$GLR^{\bullet+}$	$CH_3CH_2\mathbf{C}(CH_3)_2\mathbf{H}$	404.0 ± 6.3	[62]
$GIR^{\bullet+}$	$CH_3\mathbf{CH_2}C(CH_3)_2H$	408.6	[63]
	$CH_3CH_2C(\mathbf{CH_3})_2H$	421.3	[63]
$GPR^{\bullet+}$	–	–	
X = aromatic residue			
$GFR^{\bullet+}$	–	–	
$GHR^{\bullet+}$	–	–	
$GYR^{\bullet+}$	$C_6H_5\mathbf{O–H}$	361.9 ± 8	[62]
$GWR^{\bullet+}$	**N–H** of indole ring	368.2	[64]
X = heteroatom residue (non-aromatic)			
$GCR^{\bullet+}$	$CH_3\mathbf{S–H}$	365.3 ± 2.5	[62]
	$\mathbf{H_2S}$	381.6 ± 2.9	[62]
$GMR^{\bullet+}$	$CH_3\mathbf{SCH_3}$	384.9 ± 5.9	[62]
$GSR^{\bullet+}$	$CH_3CH_2\mathbf{O–H}$	437.7 ± 3.4	[62]
	$\mathbf{H_2O}$	498 ± 4	[62]
$GTR^{\bullet+}$	$(CH_3)_3\mathbf{CO–H}$	439.7 ± 4	[62]
	$\mathbf{H_2O}$	498 ± 4	[62]
$GDR^{\bullet+}$	**H–C**OOH	392.7	[65]
$GNR^{\bullet+}$	**H–C**ONH_2	Not available	
$GER^{\bullet+}$	$CH_3CH_2CH_2CO\mathbf{O–H}$	443.1 ± 8	[62]
	$CH_3CH_2\mathbf{CH_2}COO–H$	397.7	[65]
$GQR^{\bullet+}$	$CH_3CH_2CH_2CO\mathbf{NH_2}$	Not available	
	$CH_3CH_2\mathbf{CH_2}CONH_2$	Not available	
$GKR^{\bullet+}$	$CH_3\mathbf{NH_2}$	418.4 ± 10	[66]
	$\mathbf{CH_3}NH_2$	390.4 ± 8	[66]

The bonds of interest are in bold.

protons are fully exchanged, the y_1 and y_2 ions should contain 8 and 9 deuteriums, respectively. The fact that not all the acidic protons of the majority of y_1 and y_2 ions are deuterated may be accounted for by the mechanism proposed in Scheme 3, in which the radical site is formed initially at a heteroatom and then migrates to other carbon center(s) via $H^{\bullet}$ abstraction. Given that CO_2 loss is also observed, we suggest that the initially formed radical is the carboxylate radical. About 10% of the y_1 and y_2 ions formed have all acidic protons deuterated. This may be due to proton scrambling involving the newly formed carboxylic acid moiety (CO_2H) (Scheme 3).

3.4.1.3. $GLR^{\bullet}$, $^{+}GIR^{\bullet+}$ and $GVR^{\bullet+}$. We have previously described the fragmentation chemistry of $GLR^{\bullet+}$ and $GIR^{\bullet+}$ [17]. In the CID spectra of $GLR^{\bullet+}$ and $GIR^{\bullet+}$ [17], the y_2 ion is isobaric with $G(G^{\bullet})R^{+}$ (B). However, the possibility of the formation of (B) was not considered in our previous report [17] because we did not expect $H^{\bullet}$ to be abstracted from the aliphatic carbon center at the side chain (due to the relatively high bond dissociation energy compared to the C_α–H bond). Nevertheless, our recent observation in a related project of the loss of $H_2C{=}C(CH_3)_2$ from the leucine side chain to form α-centered radicals in the fragmentation of XGGFLR= $^{\bullet+}$ (where X = Y, W and G) has prompted us to reexamine the fragmentation of $GLR^{\bullet+}$ and $GIR^{\bullet+}$ via deuterium labeling studies. The CID spectra of D_9-$GLR^{\bullet+}$ and D_9-$GIR^{\bullet+}$ (Fig. 7) reveal the fragmentation channel that was hidden for the unlabelled $GLR^{\bullet+}$ and $GIR^{\bullet+}$ ions [17]. The CID spectra of D_9-$GLR^{\bullet+}$ and D_9-$GIR^{\bullet+}$ (Fig. 7) show an ion of significant abundance at *m/z* 297, which corresponds to the formation of the D_9-$G(G^{\bullet})R^{+}$ ion. It is likely that *m/z* 297 also corresponds to the D_9-y_2 ion, but the trace amount of the D_9-y_2 ion formed in the CID of D_9-$GAR^{\bullet+}$ (Fig. 6) and D_9-$GFR^{\bullet+}$ (data not shown) implies that the peaks corresponding to *m/z* 297 in the CID spectra of D_9-$GLR^{\bullet+}$ and D_9-$GIR^{\bullet+}$ are likely to be mainly due to the formation of D_9-$G(G^{\bullet})R^{+}$. Eqs. (13) and (14) illustrate the proposed mechanisms for the formation of $G(G^{\bullet})R^{+}$ from $GLR^{+\bullet}$ and $GIR^{\bullet+}$. Since <5% of (B) is formed in the fragmentation of $GVR^{\bullet+}$, the formation of (B) from $GIR^{\bullet+}$ most likely arises from the secondary γ radical instead of the primary γ radical at the side chain of $GIR^{\bullet+}$. The relative abundance of $G(G^{\bullet})R^{+}$ formed from $GLR^{\bullet+}$, $GIR^{\bullet+}$ and $GVR^{\bullet+}$ can be explained by the relative bond strength of the C–H bonds being broken. The bond strengths are in the order of tertiary C–H (leucine side chain) < secondary C–H (isoleucine side chain) < primary C–H (valine side chain) (Table 5). It is interesting to note, however, that in the condensed phase, $HO^{\bullet}$ can abstract $H_\gamma^{\bullet}$ from valine and $H_\gamma^{\bullet}$ as well as $H_\delta^{\bullet}$ from leucine [8] despite the relatively high bond strength of primary C_γ–H and C_δ–H bonds. This is probably due to the high reactivity of $HO^{\bullet}$ which results in it being less selective in the abstraction of $H^{\bullet}$.



(13)

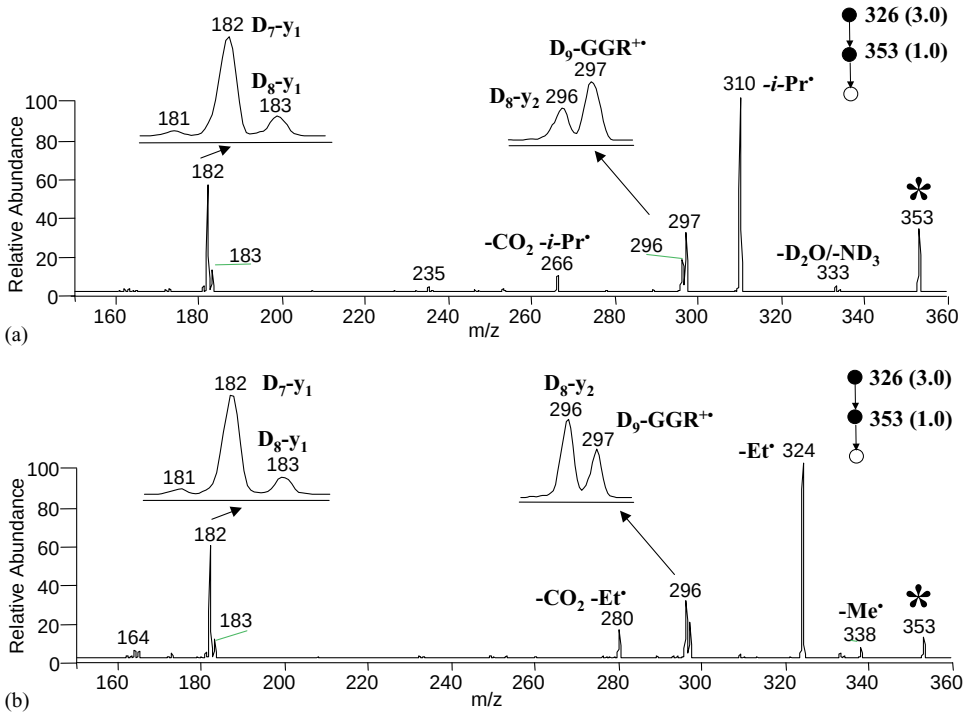
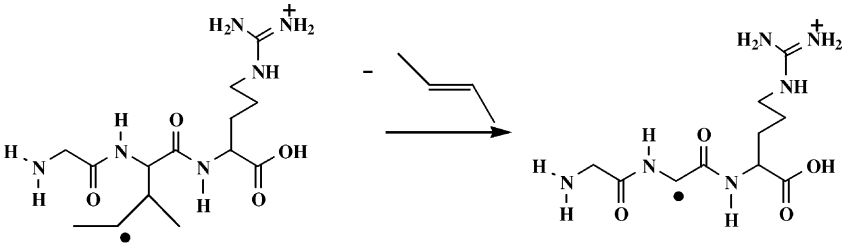


Fig. 7. (a) CID MS^3 reactions of the D_9-GLR$^{\bullet+}$ ion and (b) CID MS^3 reactions of the D_9-GIR$^{\bullet+}$ ion. A (✻) denotes the precursor ion. The number in parenthesis denotes the width of the isolation window used (in Th) in the mass-selection process.

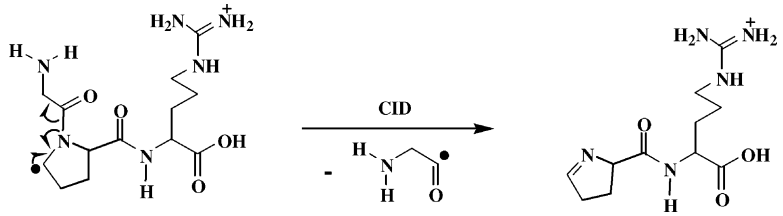


G(G$^{\bullet}$)R^{+}
(B)
m/z = 288

(14)

Unlike GLR$^{\bullet+}$ and GIR$^{\bullet+}$ which exhibit abundant side chain radical (*i*-Pr$^{\bullet}$ and Et$^{\bullet}$, respectively) losses to form (C), loss of Me$^{\bullet}$ from GVR$^{\bullet+}$ is not a major process. This may be due to the relative instability of Me$^{\bullet}$ compared to *i*-Pr$^{\bullet}$ and Et$^{\bullet}$ since the loss of Me$^{\bullet}$ from GIR$^{\bullet+}$ is also a minor process.

3.4.1.4. GPR$^{\bullet+}$. The major fragmentation products of GPR$^{\bullet+}$ are the y_1 and y_2 ions. There is also a peak at *m/z* 270 (8%) in the CID spectrum of GPR$^{\bullet+}$ (Table 4), which may correspond to the loss of $H_2NCH_2C(O)^{\bullet}$. We propose that the δ-radical, instead of the α-radical (of the proline residue), is the precursor for the loss of $H_2NCH_2C(O)^{\bullet}$ (Eq. (15)). This is because unlike other aliphatic residues in which the α-radical is the most stable radical, the most stable radical of the proline residue is the δ-radical [67]. The instability of α-radicals of proline residues is due to severe steric interactions between the carbonyl groups of proline and glycine residues which prevent the proline α-radical from adopting a planar conformation that is essential for the stabilization of the α-radical via the captodative effect [67].

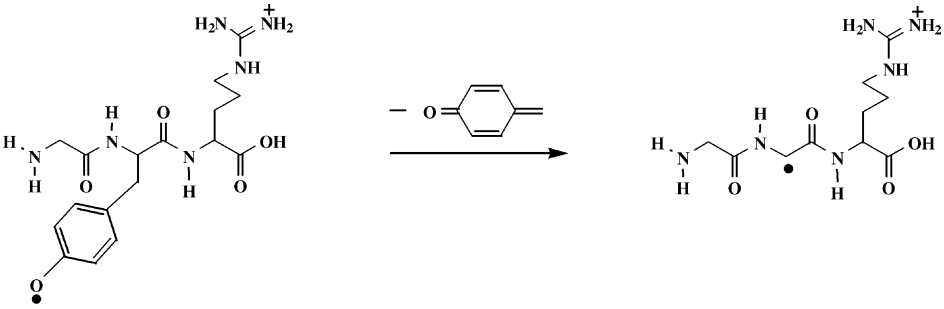


(15)

3.4.2. X = aromatic residue

With the exception of $GYR^{\bullet+}$, the $GXR^{\bullet+}$ which contain aromatic residues (X = F, H and W) essentially all produce the same major fragmentation ions: y_1 ion, y_2 ion and $[GXR\text{-}CO_2]^{\bullet+}$ (A). None of the $GXR^{\bullet+}$ (X = F, H, W and Y) ions undergo radical losses from their side chains to form (C) (Eq. (15) of Scheme 2). This may be due to the instability of the radicals that must be lost to generate (C).

The formation of $G(G^{\bullet})R^{+}$ (B) from $GYR^{\bullet+}$ (Eq. (9) of Scheme 2) is interesting, as there is a stark contrast in the behaviour of the various aromatic residues. Thus, fragmentation of $GYR^{\bullet+}$ yields one major product ion, $G(G^{\bullet})R^{+}$ (B), via the loss of *p*-quinomethide from the tyrosine side chain with the formation of other fragment ions, such as $[GYR\text{-}CO_2]^{\bullet+}$, occurring at less than 5% relative abundance (Fig. 4c). Note that the loss of *p*-quinomethide has been observed before for radical cations of tyrosine containing peptides, but only when the tyrosine is contained at the N-terminus. This loss appears to be one of the most significant side chain losses occurring for tyrosine containing peptide radical cations generated from copper(II) complexes [29,30].



(D) $G(G^{\bullet})R^{+}$ (B) m/z = 288 (16)

Despite the importance of this loss, the exact mechanism for the formation of $G(G^{\bullet})R^{+}$ (B) from $GYR^{\bullet+}$, is not clear. Siu and co-workers [29,30] have suggested that the tyrosine side chain binds to the Cu(II) center in its deprotonated form (i.e. as a phenolate anion) which upon CID, yields a phenol radical via electron transfer. Another possibility involves $H^{\bullet}$ migration from the O–H bond of phenol to other radical sites, which is driven by the low O–H bond strength of phenol ($361.9 \pm 8\,kJ\,mol^{-1}$) [62]. Regardless of the precise mechanism for the formation of the phenol radical, (D) seems likely to be the precursor to the loss of *p*-quinomethide (Eq. (16)). Note that related radicals play a role in enzyme chemistry (compare (D) with Fig. 1a).

Although a major loss of 3-methylene indolenine from the tryptophan side chain has been observed in the fragmentation of tryptophan containing peptide radical cations in which the tryptophan residue is at the N-terminus [30] and the formation of neutral indolyl radical (the precursor for the loss of 3-methylene indolenine from tryptophan) has been detected in the condensed phase [8,9], CID of $GWR^{\bullet+}$ shows hardly any loss of 3-methylene indolenine (<5%). The lack of formation of $G(G^{\bullet})R^{+}$ from $GWR^{\bullet+}$ versus $GYR^{\bullet+}$ may be attributed to the absence of a conformation that facilitates $H^{\bullet}$ transfer from indole to carboxylate radical to yield neutral indolyl radical.

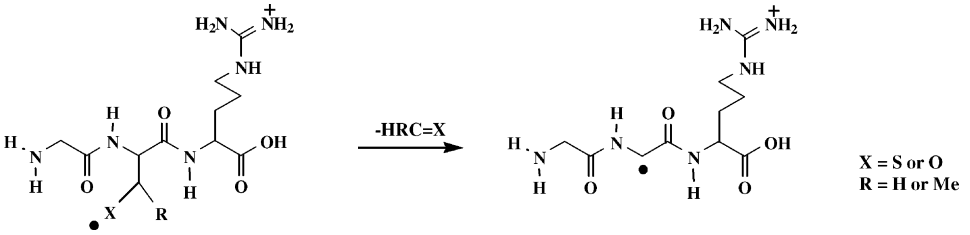
The formation of $G(G^{\bullet})R^{+}$, as illustrated in Eq (9) of Scheme 2, requires that the side chain not only be a good $H^{\bullet}$ donor (i.e. have a low R–H bond dissociation energy) but that the resultant side chain radical be able to initiate the loss of unsaturated molecule(s) with concomitant formation of the stable α-centered radical. The unlikely $H^{\bullet}$ loss from the phenylalanine side chain and the inability of the imidazole radical (formed via $H^{\bullet}$ abstraction from the histidine side chain) to trigger the loss of unsaturated molecules may explain the absence of the formation of $G(G^{\bullet})R^{+}$ in the fragmentation reactions of $GFR^{+\bullet}$ and $GHR^{\bullet+}$.

3.4.3. X = heteroatom residue (non-aromatic)

3.4.3.1. $GCR^{\bullet+}$, $GMR^{\bullet+}$, $GSR^{\bullet+}$ and $GTR^{\bullet+}$. The radical cations $GCR^{\bullet+}$, $GMR^{\bullet+}$, $GSR^{\bullet+}$ and $GTR^{\bullet+}$ all yield both (B) and (C). We note that the peak at *m/z* 288 in the CID spectrum of $GTR^{\bullet+}$ may correspond to both (A) and (B). Since $GSR^{\bullet+}$ loses $H_2C{=}O$ to form (B) but forms little of (A) via CO_2 loss, we suggest that the loss of 44 Da from $GTR^{\bullet+}$ corresponds mainly to the loss of $CH_3CH{=}O$ instead of the loss of CO_2. Note that Gatlin et al. also see a preferred loss of $CH_3CH{=}O$ from deprotonated threonine bound to Cu(II) [47]. In some instances, related precursors are likely to yield (B), as illustrated in Eq. (17). Note that a mechanism similar to the one shown in Eq. (17) has been proposed for the loss of formaldehyde from alkoxy radicals formed at the β-carbon on peptides and proteins (β-scission reaction) in the condensed phase [9]. The relative abundance of (B) formed from $GCR^{\bullet+}$ and $GMR^{\bullet+}$ is greater than that obtained from $GSR^{\bullet+}$. This is probably due to the lower S–H bond strength in $GCR^{\bullet+}$ as well as the lower $C_{\gamma/\varepsilon}$–H bond strengths in $GMR^{\bullet+}$ (note that different radical precursors are involved in the formation of (B) from $GMR^{\bullet+}$) compared to the O–H bond strengths in $GSR^{\bullet+}$ (Table 5). Note that for $GCR^{\bullet+}$, the precursor in Eq. (17)

(where X = S and R = H) corresponds to a sulfhydryl radical, which is known to play a role in enzyme chemistry (cf. Fig. 1c). We note that the loss of $H_2C{=}O$ from $GSR^{\bullet+}$ can also occur via an even-electron process (cf. Eq. (4)) instead of an odd-electron process (Eq. (17)). However, the product(s) formed via the even-electron pathway will not be (B) but rather the isomer(s) of (B). The peak at *m/z* 288 in the CID spectra of $GSR^{\bullet+}$ is of higher intensity than that observed for $GVR^{\bullet+}$ even though the primary C_γ-H bond strength of the valine side chain is less than the O–H bond strength of serine (Table 5). This observation may be accounted for by the formation of the isomers of (B) in addition to (B) itself from $GSR^{\bullet+}$.

The relative abundance of (C) formed from $GCR^{\bullet+}$ and $GMR^{\bullet+}$ is also greater than that obtained from $GSR^{\bullet+}$. This observation can again be explained by the relative stability of the radical lost in forming (C), viz, $HO^\bullet$ is less stable than $HS^\bullet$ and $CH_3SCH_2^\bullet$ (Table 5). Note that (C) formed via the loss of $HO^\bullet$ from $GSR^{+\bullet}$ is isobaric with the loss of NH_3. Deuterium labeling studies cannot differentiate between these two losses since hydrogen scrambling can occur. In either case, the relative abundance of (C) formed from $GCR^{+\bullet}$ and $GMR^{+\bullet}$ is greater than that from $GSR^{\bullet+}$.



G(G•)R+
(B)
m/z = 288

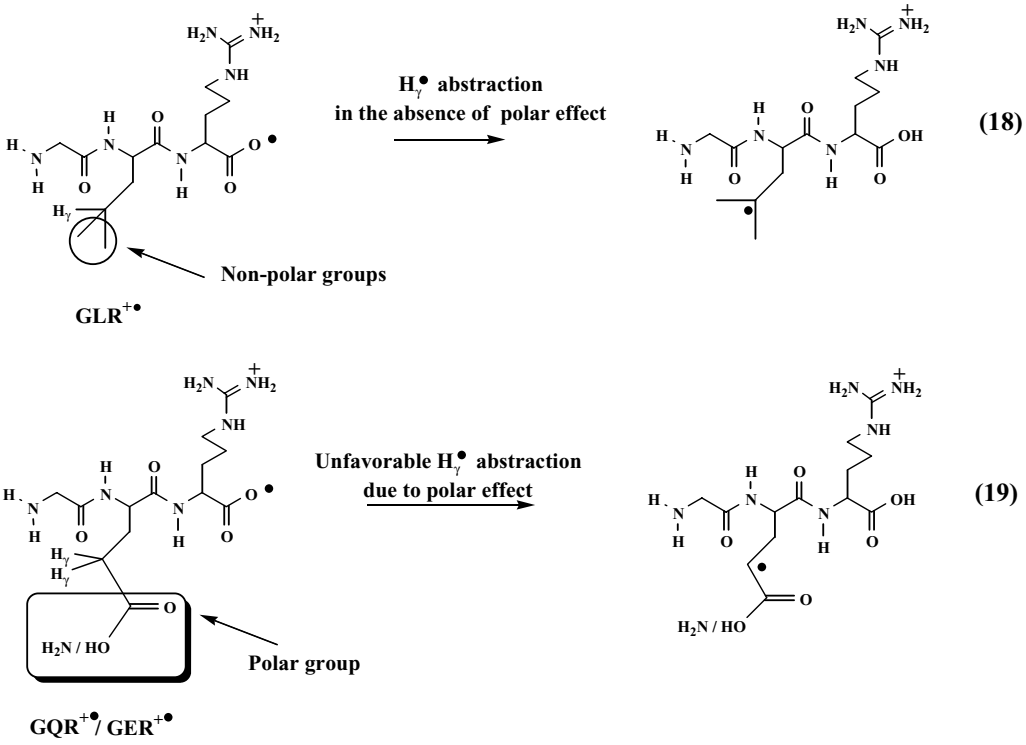
(17)

3.4.3.2. GDR•+, GER•+, GNR•+ and GQR•+. Both $GDR^{\bullet+}$ and $GNR^{\bullet+}$ do not yield (B) since abstraction of $H_\beta^\bullet$ and $H_\delta^\bullet$ will not yield this species. Although both $GER^{\bullet+}$ and $GQR^{\bullet+}$ have abstractable hydrogen atoms ($H_\gamma^\bullet$ and the carboxylate $H^\bullet$ of the glutamic acid residue as well as $H_\gamma^\bullet$ and the amide $H^\bullet$ of glutamine residue) which will allow formation of (B), little of (B) is formed. Note that for $GER^{\bullet+}$ the peak at *m/z* 288 could also correspond to $z_2^{\bullet+}$. In either case, however, little of this ion is formed from $GER^{\bullet+}$.

Due to the similarity in the C_α-H bond strength of butanoic acid (which serves as a model for the glutamic acid side chain) with the tertiary C–H bond strength of isopentane (which serves as a model of leucine side chain) (Table 5), it is interesting to compare the behaviour of $GER^{\bullet+}$ which yields little of (B) with that of $GLR^{\bullet+}$ which forms (B) in significant amounts as discussed above. The O–H bond strength of butanoic acid, ~443 kJ mol^{-1} [62] is higher than the C_α–H bond strength of butanoic acid and the tertiary C–H bond strength of isopentane, both about 400 kJ mol^{-1} [62]. A significant amount (32%) of (B) is formed from $GLR^{\bullet+}$ but not from $GER^{\bullet+}$, despite the similarity in these C–H bond strengths. This suggests that polar effects may play a role in the H_γ abstraction efficiency from the side chain of glutamic acid compared to leucine as illustrated in Scheme 4 [13]. Thus, abstraction of $H_\gamma^\bullet$ from leucine is not hindered by a polar effect (Eq. (18)). In contrast, unfavourable polar effects may raise the transition state energy associated with abstraction of $H_\gamma^\bullet$ from glutamic acid (Eq. (19)). Data on the N-H and the C_α–H bond strengths of butanamide (which can serve as a model of glutamine side chain) are not available. However, the same explanation may apply to account for the lack of formation of (B) from $GQR^{\bullet+}$ since the H_γ's of the side chain of glutamine are also beside the polar and electron-withdrawing amide group (Eq. (19) of Scheme 4). It is interesting to note, however, that in the condensed phase, $HO^\bullet$ abstracts H_γ's from both glutamic acid and glutamine [8]. Once again, the high reactivity of $HO^\bullet$ may be the reason for the lack of selectivity of $HO^\bullet$ in $H^\bullet$ abstraction since polar effects do not play significant roles in highly exothermic reaction [13].

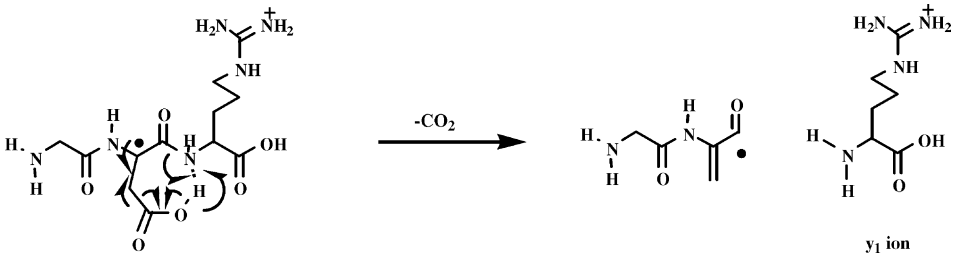
$GER^{\bullet+}$, $GNR^{\bullet+}$ and $GQR^{\bullet+}$ yield (C) as the major fragmentation product although in the case of $GDR^{\bullet+}$, (C) was formed in low abundance. Note that for $GNR^{\bullet+}$, (C) (*m/z* 301) is isobaric with (A). In order to identify this peak at *m/z* 301, we compare the CID (MS^4) spectrum of this peak with the CID (MS^4) spectra of (C) formed from other $GXR^{\bullet+}$. Among the other $GXR^{\bullet+}$ ions that yield (C), only $GMR^{\bullet+}$, $GER^{\bullet+}$ and $GQR^{\bullet+}$ yield (C) with sufficient abundance for MS^4 studies to be carried out. The CID spectrum (an MS^4 experiment) of the peak at *m/z* 301 formed from $GNR^{\bullet+}$ is identical with the CID spectra (MS^4 experiments) of (C) formed from $GQR^{\bullet+}$, $GER^{\bullet+}$ and $GMR^{\bullet+}$ (data not shown). This indicates that the *m/z* 301 peak formed from the CID of $GNR^{\bullet+}$ corresponds mainly to (C).

The failure of $GDR^{\bullet+}$ to form significant amounts of (C) cannot be due to the instability of the $HOC(O)^\bullet$ lost, since the stability of the isoelectronic $H_2NC(O)^\bullet$ formed from $GNR^{\bullet+}$ appears to permit the formation of (C). Instead, we propose that $GDR^{\bullet+}$ adopts a conformation in which the acidic proton of the aspartic acid side chain is hydrogen bonded to the amide nitrogen C-terminus to the aspartic acid residue (Eq. (20)). This conformation leads to the formation of the y_1 ion (via the mechanism proposed in Eq. (20)) instead of the formation of (C). Hence, in the fragmentation of



Scheme 4.

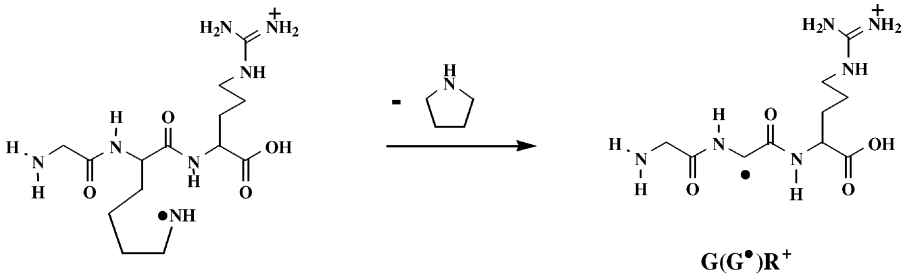
$GDR^{\bullet+}$, the α-radical which would normally lead to abundant formation of (C), fragments to form the y_1 ion instead.



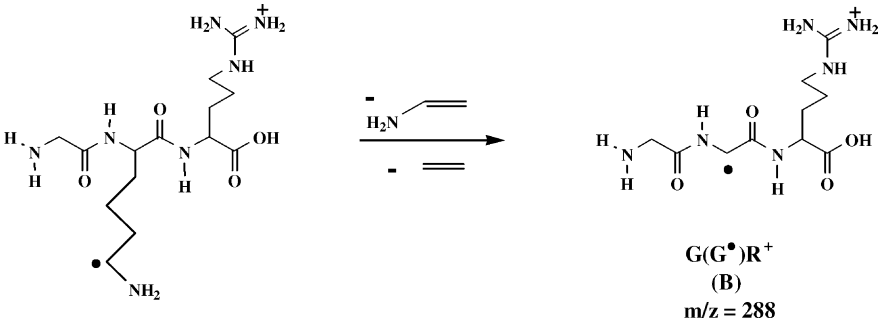
(20)

3.4.3.3. GKR•+. In the lysine side chain, radical formation occurs preferentially at the ϵ position or at the $–NH_2$ terminus [68]. The formation of (B) from these radical precursors can occur via two possible pathways as shown in Eqs. (21) and (22). The extent to which abstraction occurs at either site of the lysine side chain depends upon the nature of the radical that abstracts the H• [68]. Although the C_α–H bond is weaker than the N-H bond in an alkyl amine (Table 5), H• abstraction from C_α–H versus N-H in alkylamines cannot be predicted on this basis [68,69].

In order to probe which H• (C_ε–H or N–H) is abstracted from the lysine side chain in the formation of (B), deuterium labeling studies, in which all the protons on the heteroatoms were exchanged for deuteriums, were carried out. These studies (data not shown) revealed that most of the H• abstracted from the lysine side chain (in the formation of (B)) was $H_\varepsilon^\bullet$ instead of the H• from the $–NH_2$ terminus (Eq. (22)).



(21)



$G(G^{\bullet})R^{+}$
(B)
m/z = 288 (22)

$GKR^{\bullet+}$ yields both (B) and (C). Most of the non-aromatic $GXR^{\bullet+}$ that yield both (B) and (C) yield (C) in preference to (B). $GKR^{\bullet+}$ is the only radical that yields (C) in significantly less amounts than (B). The formation of (B) from $GKR^{\bullet+}$ depends on the bond dissociation energy of C_{ε}–H at the lysine side chain, which is influenced by the stability of $H_2NCH^{\bullet}CH_2CH_2CH_2R$. The abundance of (C) is affected by the stability of $H_2NCH_2CH_2CH_2^{\bullet}$, the radical lost from lysine side chain. The greater stability of the resonance stabilized carbon radical $H_2NCH^{\bullet}CH_2CH_2CH_2R$ compared to the primary radical $H_2NCH_2CH_2CH_2^{\bullet}$ which is remote from the NH_2 group, may be the reason for the more abundant formation of (B) over (C).

3.4.3.4. $GRR^{\bullet+}$. As $GRR^{\bullet+}$ forms in low abundance, presumably due to the strong binding affinity of GRR to Cu(II), MS^3 study of $GRR^{\bullet+}$ was not possible.

3.5. An overview of the complementary fragmentation reactions of the $[GXR+H]^+$, $[GXR+2H]^{2+}$, and $GXR^{\bullet+}$ ions

The classes of fragmentation reactions observed for $[GXR+H]^+$, $[GXR+2H]^{2+}$, and $GXR^{\bullet+}$ ions are summarized in Table 6. Differences in the abundances and types of sequence ions formed in the MS/MS spectra of the $[GXR+2H]^{2+}$ ions relative to their $[GXR+H]^+$ counterparts can readily be rationalized by the 'mobile proton' model. Thus, the $[GXR+H]^+$ ions in which the proton is sequestered by the arginine side chain tend to yield limited sequence information (only y_1 and b_2 ions are formed) while abundant small molecule loss is observed. $[GXR+2H]^{2+}$ ions yield additional sequence ion information since y_2 ions are often observed. CID of the $GXR^{\bullet+}$ ions yields structurally useful sequence and non-sequence ions. Not only are the sequence ions formed in different mechanisms to those formed from even electron precursor ions, but the non-sequence ion losses are more diverse for the $GXR^{\bullet+}$ ions. It appears that radical cations of arginine containing peptides do not possess a conventional structure in which the radical and charge are located at the same site, but rather a distonic ion structure in which the charge is located at the arginine via sequestering of a proton while the radical is located at a different site. Under collision induced dissociation conditions, other radical sites can be accessed via a 'mobile radical' condition. Having the radical sites at different positions such as the peptide backbone and the side chains opens up a diverse range of fragmentation pathways that can be regarded as 'charge remote' in the sense that they are directed by the radical site rather than the charge site. Since these reactions are directed by the radical site, many of the side chain losses have no precedence in the low

Table 6
Summary of main classes of reactions of even and odd electron ions of GXR

Type of Side Chain of X	$[GXR+H]^+$		$[GXR+2H]^{2+}$		$GXR^{+\bullet}$		
	sequence ions	small molecule loss	sequence ions	small molecule loss	sequence ions	small molecule loss	side chain radical loss
Aliphatic (X=G,A,V,I,L,P)	y_1 b_2 (X=G,I,L)	H_2O, NH_3	y_1, b_2/a_2 $y_2/[y_2+H]^{2+}$ (X=G,A,P)		y_1, y_2	CO_2 (X=G,A,V,I,L) $H_2C=C(CH_3)_2$ (X=L) $CH_3CH=CHCH_3$ (X=I)	$Me^{\bullet}$ (X=V) *i*-$Pr^{\bullet}$ (X=L) $Et^{\bullet}$, $Me^{\bullet}$ (X=I)
Aromatic (X=F,W,Y)	y_1 b_2 (X=W)	H_2O, NH_3	y_1, b_2/a_2, $y_2/[y_2+H]^{2+}$	H_2O (X=W)	y_1, y_2 (X=F, W) $[b_2\text{-}H^{\bullet}]^{+\bullet}$ (X=F)	CO_2, H_2O (X=F,W) *p*-quinomethide (X=Y)	[3-$^{\bullet}CH_2$-1H-indole] (X=W)
Basic (X=H,K,R)	y_1 b_2 (X=H,R)	H_2O, NH_3	y_1, b_2/a_2 $y_2/[y_2+H]^{2+}$ (X=H,K)	NH_3, H_2O	y_1, y_2 (X=H,K)	CO_2, H_2O, NH_3 (X=H,K) $H_2C=CH_2+H_2C=CHNH_2$ (X=K)	$H_2NCH_2^{\bullet}$, (X=K) $H_2NCH_2CH_2CH_2^{\bullet}$ (X=K)
Acidic (X=D,E)	y_1	H_2O, NH_3 CO_2 (X=D)	y_1, b_2/a_2, $y_2/[y_2+H]^{2+}$	H_2O NH_3 (X=E)	y_1, y_2	CO_2, H_2O $H_2C=CHCOOH$ (X=E)	$HOC(O)CH_2^{\bullet}$ (X=E)
$CONH_2$ (X=N,Q)	y_1 b_2 (X=Q)	H_2O, NH_3	y_1, $y_2/[y_2+H]^{2+}$ (X=N)	NH_3 H_2O (X=N)	y_1, y_2	CO_2, H_2O, NH_3 $H_2C=CHCONH_2$ (X=Q)	$H_2NC(O)^{\bullet}$ (X=N) $H_2NC(O)CH_2^{\bullet}$ (X=Q)
SH (X=C,M)	y_1	H_2O, NH_3 May be H_2S (X=C)	y_1, b_2/a_2, $y_2/[y_2+H]^{2+}$ (X=M)	CH_3SH (X=M) H_2O (X=C)	y_1, y_2 (X=M)	$H_2C=S+H_2C=CH_2$ or $H_3CSCH=CH_2$ (X=M) $H_2C=S$ (X=C)	$CH_3SCH_2^{\bullet}$, $CH_3S^{\bullet}$ (X=M) $HS^{\bullet}$ (X=C)
OH (X=S,T)	y_1	H_2O, NH_3 $H_2C=O$ (X=S) $CH_3CH=O$ (X=T)		H_2O	y_1, y_2	H_2O, NH_3 $H_2C=O$ (X=S) $CH_3CH=O$ (X=T)	$HO^{\bullet}$

Parentheses represent the residues which yield specific sequence ions or losses. The absence of parentheses indicates that all X undergo this fragmentation.

energy gas-phase fragmentation reactions of even electron protonated peptides.

4. Conclusions

The current study expands on our previous comment that "an obvious way of accessing different gas-phase fragmentation pathways of peptides is to change the nature of the charge (singly versus multiply charged; positive versus negative ion)" [33] to show that another way of inducing new fragmentation reactions is to open up peptide radical chemistry. The new radical directed fragmentation reactions discussed in this paper are caused by $H^{\bullet}$ deficient processes which unmask reactive radical sites on the peptide backbone and side chains due to the sequestering of the charge by the arginine side chain. As such, they are quite different to the radical fragmentation reactions observed for ECD [23,24], which can be regarded as being driven by $H^{\bullet}$ rich processes.

We are currently examining the fragmentation reactions of other metal complexes $[Metal(L)(M)]^{n+}$ to evaluate the roles of the metal, ancillary ligand (L) and the peptide (M) in: (i) generating radical cations of M; and (ii) controlling the subsequent fragmentation reactions of $M^{\bullet+}$. In addition, we are investigating different ways to generate radical cations of peptides in order to help define the role of the charge and radical sites in controlling their fragmentation reactions. In particular, we are examining derivatives: (i) in which the charge is fixed; and (ii) which possess a weak bond which can be broken in a homolytic fashion to allow a radical site to be generated at a specific amino acid residue within a peptide [70].

Note added in proof

Since this manuscript was accepted, a paper has appeared on the generation of lysine side chain radicals via mass spectrometry: D.S. Masterson, H. Yin, A. Chacon, D.L. Hachey, J.L. Norris, N.A. Porter, J. Am. Chem. Soc. 126 (2004) 720.

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