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The role of the position of the basic residue in the generation and fragmentation of peptide radical cations☆

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Dedicated to the memory of Professor Charva Lifshitz and her many important contributions to gas-phase ion chemistry.

Abstract

Using simple di- and tripeptides GX, GGX, GXG, XG and XGG, the influence of the position of the basic residue, X (X = R, K and H), on the formation of peptide radical cations ($M^{\bullet+}$) from $[Cu^{II}(tpy)M]^{2+}$ complexes (where tpy = 2,2′:6′,2″-terpyridine) was probed. It was found that $M^{\bullet+}$ is formed with greatest abundance when the basic residue is at the C-terminus. For arginine containing peptides, this may be due to further fragmentation of $GRG^{\bullet+}$, $RG^{\bullet+}$ and $RGG^{\bullet+}$ at the MS^2 stage. For lysine and histidine containing peptides, when the basic residue is not located at the C-terminus, competing fragmentation pathways that lead to peptide backbone cleavage are more facile than $M^{\bullet+}$ formation. In order to gain some insights into the binding modes of these peptides to $[Cu^{II}(tpy)]^{2+}$, the formation and fragmentation of copper(II) complexes of tripeptides protected as their carboxy methyl/ethyl esters (M–OR′, R′ = Me/Et) were also probed. The products of the competing fragmentation pathways of $[Cu^{II}(tpy)M]^{2+}$, as well as the formation and fragmentation of $[Cu^{II}(tpy)(M–OR')]^{2+}$, suggest that the unprotected peptides, M, mainly bind as zwitterions to $[Cu^{II}(tpy)]^{2+}$.

The fragmentation reactions of the radical cations ($M^{\bullet+}$) were also studied. Radical driven side chain fragmentation reactions of $M^{\bullet+}$ are dependent on both the position of the residue as well as the identity of other residues present in the peptide radical cations. GR and RG, which undergo rearrangement to form a mixed anhydride in their protonated forms, do not undergo the same rearrangement in their radical cation forms.

Keywords: Electrospray ionization; Multistage mass spectrometry; Protonated peptides; Radical cations of peptides; Cu(II) complexes

1. Introduction

Considerable attention has focussed on understanding the fundamental gas-phase chemistry of charged radicals derived from biomolecules. This is due to the fact that a number of experimental techniques have been developed to study radical species, as well as the promise of radical species to provide complementary structural information to their even-electron counterparts. Inspired by Siu and coworkers' pioneering work on the use of ternary transition metal complexes to generate peptide radical cations [1], several studies have addressed the influence of the three components of these complexes, viz. the auxiliary ligand, the metal and the peptide, in the generation of peptide radical cations [2–8]. Early work by Siu and coworkers suggested the presence of both a basic residue as well as a tyrosine/tryptophan residue in the peptide was a requirement for the generation of peptide radical cations ($M^{\bullet+}$) from ternary Cu(II) complexes, $[Cu^{II}(L^3)M]^{2+}$ (L^3 = dien (diethylenetriamine), Me_5dien (*N,N,N′,N″,N″*-pentamethyldiethylenetriamine) or tpy; M = peptide) [1]. Since then, however, the situation has been clarified and recently it has been shown that the presence of either a tyrosine or a tryptophan residue is sufficient for peptide radical cation generation from $[Cu^{II}(dien)M]^{2+}$ [3]. Likewise, we have shown that the presence of a basic residue alone also allows the formation of the tripeptide radical cations, $GXR^{\bullet+}$, using $[Cu^{II}(tpy)GXR]^{2+}$ as the starting ternary complex [4]. Moreover, the subsequent fragmentation of the peptide radical cations was shown to yield complementary structural information to that derived from their protonated analogues [4].

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Several studies have clearly demonstrated that the position of a residue within a peptide can influence its fundamental gas phase properties and reactivity. For example, the position of a histidine residue in a tripeptide not only influences its gas phase proton affinity [9], but also the preferred site of cleavage in the CID spectrum of the protonated tripeptide methyl ester [10]. In the emerging field of gas phase peptide radical cations, an important question is "How does the site of a residue within a peptide influence the generation and subsequent fragmentation reactions of its radical cation?". The only study to have systematically addressed this issue is that of Siu and coworkers [3], who showed that the formation of peptide radical cations of di- and tripeptides from $[Cu^{II}(dien)M]^{2+}$ is influenced by the position of the tryptophan residue [3]. For example, the GGW complex almost exclusively yields the radical cation, while the complexes of GWG and WGG form significant amounts of the protonated peptide. More importantly, this study showed that these peptides exhibit unique gas phase chemistry involving facile fragmentation around the α-carbon of the tryptophan residue to give $[z_n - H]^{\bullet+}$ ions. Given that our previous study has shown that it is possible to use $[Cu^{II}(tpy)]^{2+}$ to generate radical cations of peptides that do not contain a tryptophan/tyrosine residue but contain an arginine residue [4], here we probe the influence of the position of the basic residue (arginine, lysine and histidine) on the formation of peptide radical cations from $[Cu^{II}(tpy)M]^{2+}$ complexes, as well as the subsequent fragmentation of $M^{\bullet+}$.

2. Experimental methods

2.1. Materials

GH, GGH, GHG, HG, HGG, diketopiperazine, GR and RG were purchased from Bachem (Bubendorf, Switzerland) and GGR, GRG, RGG, GK, GGK, GKG, KG and KGG were obtained from SynPep (Dublin, CA, U.S.A.) with a stated minimum purity of 95%. These peptides were used without further purification.

The methyl esters of the peptides (M–OMe) were prepared following a literature procedure [11]. The ethyl esters of the peptides containing arginine (M–OEt) were prepared by adding an ethanolic solution of HCl (2 M, 0.3 mL) to M–OMe (1 mg). The reaction was allowed to proceed for 3 h at room temperature and then the solvent was removed.

$[Cu^{II}(tpy)(NO_3)_2]\cdot H_2O$ was synthesized according to a literature procedure [12].

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). For the analysis of $[Cu^{II}(tpy)(M)]^{2+}$ and $[Cu^{II}(tpy)(M–OR')]^{2+}$, stock solutions of peptides (M or M–OR′) and a stock solution of $[Cu^{II}(tpy)(NO_3)_2]\cdot H_2O$ were first prepared. The stock solutions of the peptides were prepared by dissolving 1 mg of peptide in 1 mL of MeOH or H_2O, whereas the stock solution of $[Cu^{II}(tpy)(NO_3)_2]\cdot H_2O$ was prepared by dissolving 1 mg of $[Cu^{II}(tpy)(NO_3)_2]\cdot H_2O$ in 1 mL of MeOH. 100 μL of the peptide stock solution was then mixed with 100 μL of $[Cu^{II}(tpy)(NO_3)_2]$ stock solution and incubated for 2 h before addition of 0.8 mL of MeOH. Samples were introduced into the mass spectrometer at 3.0 μL/min via the electrospray ionization source. In each case the instrument required careful tuning to maximize the signal of the metal complex $[Cu^{II}(tpy)(M/M–OR')]^{\bullet 2+}$ ions. The typical source conditions were: spray voltage, 4.0–5.5 kV; capillary temperature, 100–250 °C; nitrogen sheath pressure, 20–80 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. For $[Cu^{II}(tpy)(M/M–OR')]^{\bullet 2+}$ ions, an envelope of two isotopes (arising from ^{63}Cu, and ^{65}Cu) was mass selected. When it was possible, a single isotope (arising from ^{63}Cu) was mass selected.

Since multistage MS^3 experiments were performed, we have used the symbolism of Cooks et al. [13] to define each stage of mass spectrometry for all of the figures.

3. Results and discussion

In several instances, similar types of fragment ions are observed in the CID spectra of the $[Cu^{II}(tpy)M]^{2+}$ complexes and the corresponding protonated peptides. For this reason, we first discuss the fragmentation reactions of the protonated peptides, before examining the influence of the basic residue position in the generation of peptide radical cations from $[Cu^{II}(tpy)]^{2+}$ complexes. Finally, for those complexes which yield "long-lived" radical cations (i.e. those that do not completely dissociate in the MS/MS spectrum), their CID fragmentation are described.

3.1. MS/MS on $[M + H]^+$ and $[M–OR' + H]^+$

The fragmentation products of all the protonated peptides $[M + H]^+$ and protonated tripeptides protected as their methyl/ethyl esters $[M–OR' + H]^+$ are given in Table 1. Note that fragmentation reactions of some of these molecules have previously been studied by our group [10,14] and by Yalcin et al. [16a]. We have reported our results on the dissociation of arginine containing dipeptides [14] and the fragmentation of cyclo(GH) as well as methyl esters of histidine containing peptides [10] while Yalcin et al. have described the fragmentation of lysine containing peptides which were mostly achieved via metastable ion dissociation [16a]. Since CID is the activation method employed in the current work, the fragmentation of protonated lysine containing peptides was re-examined so that their fragmentation reactions could be directly compared with the other peptides under similar experimental conditions.

Generally, NH_3 loss and H_2O loss is particularly abundant in arginine containing peptides and histidine containing peptides, respectively. The arginine containing peptides also yield b_n–NH_3 ions. While we have not probed the mechanisms for

Table 1
Abundance of CID products of $[M + H]^+$ and $[M–OR' + H]^+$ relative to the most intense peak in the spectrum (%)

M	y_2	b_1	a_1	y_1	b_2	$–H_2O$	$–NH_3$	Other products	
X at C-terminus									
GR					17		100	$-2NH_3$	(8)
								$b_2 - NH_3/-H_2O\ -NH_3$	(8)
								$y_1 - NH_3$	(13)
								$y_1 - H_2O$	(17)
								−60 Da	(12)
								−59 Da	(15)
GGR				100		20	70	$-2NH_3$	(12)
								$b_3 - NH_3/-H_2O\ -NH_3$	(93)
								$b_3 - H_2O/-2H_2O$	(12)
								$y_1 - NH_3$	(30)
GGR–OEt							100	a_3	(8)
								−EtOH	(12)
								$-2NH_3$	(40)
								$-H_2O\ -NH_3$	(80)
								$b_3 - NH_3$	(6)
								$y_1 - NH_3$	(55)
								y_1 − 59 Da	(6)
								−42 Da	(10)
								$-3NH_3$	(25)
GK					100		20	$y_1 - H_2O$ (m/z = 129 Th)	(50)
								Related ion (m/z = 84 Th)	(5)
GGK	5			100				$-H_2O$	(17)
								$b_3 - H_2O$	(10)
								$-H_2O\ -NH_3$	(32)
								$y_1 - NH_3$	(22)
								$y_1 - H_2O$ (m/z = 129 Th)	(90)
								Related/immonium ion (m/z = 84 Th)	(20)
GGK–OMe	17			100				$-H_2O$	(25)
								$b_3 - H_2O$	(6)
								$-H_2O\ -NH_3$	(50)
								$y_1 - NH_3$	(30)
								y_1 − MeOH	(55)
								Related/immonium ion (m/z = 84 Th)	(8)
GH					100			Related/immonium ion (m/z = 110 Th)	(5)
								$y_1 - H_2O$(m/z = 138 Th)	(5)
GGH				100				$-H_2O$	(58)
								$-H_2O\ -NH_3$	(14)
								Related/immonium ion (m/z = 110 Th)	(5)
GGH–OMe				100				−MeOH	(6)
								$-H_2O$	(53)
								$-H_2O\ -NH_3$	(27)
X as Interior Residue									
GRG	15				75 or $y_2 - H_2O$	5	35	$-2NH_3$	(5)
								$-H_2O\ -NH_3$	(100)
								$b_2 - NH_3$	(55)
GRG–OEt					100 or y_2 − EtOH		27	$-2NH_3$	(7)
								$-H_2O\ -NH_3$	(80)
								$b_2 - NH_3$	(72)
GKG					100 or $y_2 - H_2O$			$-H_2O$	(15)
								$-H_2O\ -NH_3$	(70)
								$y_2 - H_2O$ (m/z = 129 Th)	(10)
GKG–OMe					100 or y_2 − MeOH			$-H_2O$	(10)
								$-H_2O\ -NH_3$	(47)
								$y_2 - H_2O$ (m/z = 129 Th)	(7)
GHG					70 or $y_2 - H_2O$			$-H_2O$	(7%)
								$-H_2O\ -NH_3$	(100)

Table 1 (*Continued*)

M	y_2	b_1	a_1	y_1	b_2	$-H_2O$	$-NH_3$	Other products	
GHG–OMe					55 or y_2-MeOH			$-H_2O$	(100)
								$-H_2O$ $-NH_3$	(52)
X at N-terminus									
RG	Spectrum same as $[GR+H]^+$								
RGG		47			15	8	100	−59 Da	(8)
								$b_2 - NH_3$	(10)
								$b_1 - NH$	(5)
RGG–OEt		40					100	−59 Da	(8)
								$b_2 - NH_3$	(20)
								$a_2 - NH_3$	(6)
								$b_1 - NH_3$	(6)
KG		100			15		35	Related/immonium ion (m/z = 84 Th)	(8)
KGG		100			5		10	$-H_2O$	(8)
								Related/immonium ion (m/z = 84 Th)	(10)
KGG–OMe		100			5		7	$-H_2O$	(8)
								Related/immonium ion (m/z = 84 Th)	(10)
HG		35	30		100			$-H_2O$	(8)
								Related/immonium ion (m/z = 84 Th)	(10)
HG		35	30		100				
HGG		45	52		100			$-H_2O$	(100)
								$b_3 - H_2O$	(20)
HGG–OMe		28	28		90			$-H_2O$	(100)
								$b_3 - H_2O$	(7)
Cyclo(GH)/Diketopiperazine							67	–CO	(100)
								$-CH_2$=NH	(5)
								$-CO-NH_3$	(43)
								$-2CO-NH_3$	(5)

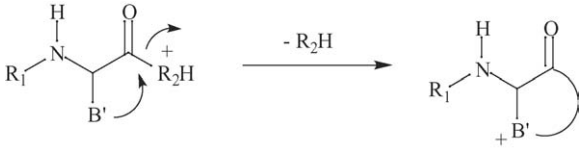
Products of relative abundance less than 5% are not listed.

these losses, it is interesting to note that the b_n–NH_3 ions may arise from the initial loss of NH_3 from the arginine side chain, as recently demonstrated by Laskin et al. for larger arginine containing peptides [15].

As noted previously, $[GR+H]^+$ and $[RG+H]^+$ yield identical CID spectra since they undergo rearrangement to form a mixed anhydride [14]. For $[GX+H]^+$ and $[XG+H]^+$, where X = K or H, identical fragmentation products were generated, but their relative abundances are different (Table 1), suggesting incomplete isomerization. Note that in Table 1, the fragmentation products y_1–H_2O formed from $[GX+H]^+$ are the same as the b_1 ions of $[XG+H]^+$. The immonium ion with nominal m/z = 110 Th formed from $[GH+H]^+$ is the same as the a_1 ion of $[HG+H]^+$.

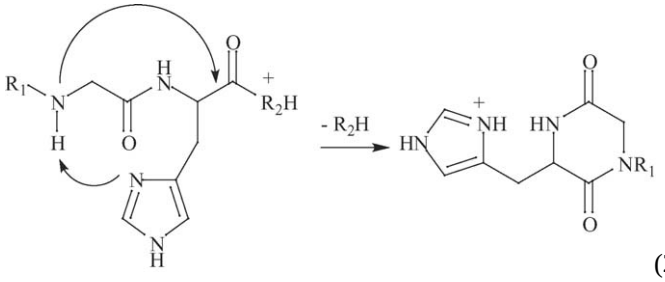
3.1.1. MS/MS on $[GX+H]^+$ and $[GGX+H]^+$

With the exception of $[GR+H]^+$, which undergoes rearrangement to form a mixed anhydride [14], the common major fragmentation products of $[GX+H]^+$ and $[GGX+H]^+$ are the loss of H_2O and /or formation of y_1 ions (as well as the further fragmentation products of these primary fragmentation products). The loss of H_2O may be a side-chain driven process and can involve either the OH of the C terminal carboxylic acid (eq. (1) for X = R/K [16,17] or eq. (2) for X = H [18]) or the carbonyl oxygen atom of a peptide bond [10,17]. As noted previously, histidine appears to be unique in its ability to form b_2 ions with a diketopiperazine structure (eqs. (2) and (3)) [10].



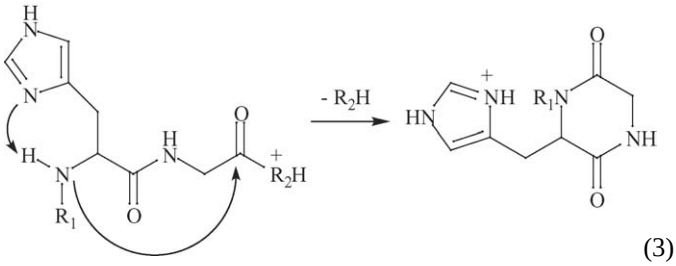
(1)

where B′ is side chain of basic residue R_1 H or nitrogen atom of peptide bond and R_2 is OH or nitrogen atom of peptide bond.



(2)

where R_1 is H or nitrogen atom of peptide bond and R_2 is OH or nitrogen atom of peptide bond.



(3)

where R_1 is H or nitrogen atom of peptide bond and R_2 is OH or nitrogen atom of peptide bond.

3.1.2. MS/MS on $[GXG + H]^+$

The common major fragmentation products of $[GXG + H]^+$ are: (i) the formation of b_2 ions; and (ii) the loss of H_2O and/or NH_3. While we have not attempted to determine the structure of these b_2 ions and their mechanisms of formation, it is worth recalling that studies on related systems suggest that cyclic structures involving the side chain (cf. eqs. (1) and (2)) are more stable than oxazolone structures which are proposed as structures of b_n ions of aliphatic peptides [10,17]. Thus, the mechanisms for the formation of b_2 ions from $[GRG + H]^+$ and $[GKG + H]^+$ are likely to be analogous to the formation of b_3 and b_2 ions from $[GGR + H]^+$ and $[GK + H]^+$ respectively (eq. (1)) while the mechanisms for the formation of b_2 ions from $[GHG + H]^+$ may be analogous to the formation of b_2 ions from $[GH + H]^+$ (eq. (2)). Finally, abundant H_2O loss from $[GHG + H]^+$ is likely a side-chain driven process [10].

3.1.3. MS/MS on $[XG + H]^+$ and $[XGG + H]^+$

Apart from $[RG + H]^+$, which undergoes rearrangement to form a mixed anhydride [14], the common major fragmentation products of $[XG + H]^+$ and $[XGG + H]^+$ are b_1 ions. The b_1 ions of arginine, lysine and histidine are likely to adopt cyclic structures as a result of side chain mediated peptide bond cleavage, as proposed in eq. (1) [16].

3.1.4. MS/MS on $[M–OR' + H]^+$ and $[Cyclo(GH)+H]^+$

The fragmentation patterns of protonated cyclo(GH) (Table 1) and the methyl esters of the histidine containing tripeptides ($[M–OMe + H]^+$) (Table 1) are in accord with previous reports [10]. Generally, the CID of $[M–OR' + H]^+$ is analogous to the fragmentation of the protonated unprotected peptides, $[M + H]^+$ as shown in Table 1. The only exception is $[GGR–OEt + H]^+$, in which the formation of the y_1 ion and the loss of $2H_2O$ are suppressed while the losses of 42 and 51 Dalton (which may correspond to losses of HN=C=NH and $3NH_3$ respectively) are enhanced. Significant suppression of y_1 ion formation in the fragmentation of $[GGR–OEt + H]^+$ may imply that formation of the y_1 ion from $[GGR + H]^+$ largely involves a salt bridge intermediate which results from mobilization of H^+ from the C-terminal carboxylic acid to the C-terminal amide bond. Formation of such an intermediate is prevented in $[GGR–OEt + H]^+$.

3.2. MS/MS on $[Cu^{II}(tpy)(M)]^{2+}$ and $[Cu^{II}(tpy)(M–OR')]^{2+}$

3.2.1. MS/MS on $[Cu^{II}(tpy)(M)]^{2+}$

The abundance of the radical cations of XG, XGG, GXG, GX and GGX (X = R, K, or H) generated from $[Cu^{II}(tpy)M]^{2+}$ along with all the fragmentation products of $[Cu^{II}(tpy)M]^{2+}$ are listed in Table 2. Note that our results for the GGX peptides are in good agreement with a very recent paper by Chu et al. [6]. Examination of Table 2 reveals that the yield of $M^{\bullet+}$ formed from $[Cu^{II}(tpy)M]^{2+}$ depends on the position of the basic residue, X, within the peptide, with the most abundant $M^{\bullet+}$ being formed when X is at the C-terminus.

A closer examination of the fragmentation reactions of the $[Cu^{II}(tpy)M]^{2+}$ complexes (Table 2) reveals that in addition to radical cation formation (eq. (4)), other fragmentation pathways involve cleavage of the peptides, with a fragment of the peptide remaining bound to $[Cu^{II}(tpy)]^{2+}$ (for example eq. (5)). These other fragmentation pathways are analogous to the fragmentation of the protonated peptides themselves (Table 1), although with different relative abundances. These other fragmentation pathways compete with the formation of radical cations from $[Cu^{II}(tpy)M]^{2+}$. In the rest of this work, these other fragmentation pathways will be referred to as 'competing fragmentation pathways'.

$$[Cu^{II}(tpy)M]^{2+} \rightarrow [Cu^{I}(tpy)^{+}] + M^{\bullet+} \quad (4)$$

$$[Cu^{II}(tpy)M]^{2+} \rightarrow [Cu^{II}(tpy)(M)–(b_n)]^{+} + b_n{}^{+} \quad (5a)$$

$$[Cu^{II}(tpy)M]^{2+} \rightarrow [Cu^{II}(tpy)(M)–(b_n–H)]^{2+} + (b_n–H) \quad (5b)$$

For lysine and histidine containing peptides which have the basic residue at the C-terminus, viz. GX and GGX (where X = K and H), the dissociative redox reaction depicted in equation (4) predominates and the competing fragmentation pathways are largely suppressed with the exception of GGH. Besides yielding abundant $GGH^{\bullet+}$, fragmentation of $[Cu^{II}(tpy)(GGH)]^{2+}$ also affords significant amounts of $[Cu^{II}(tpy)(GGH)–(b_2–H)]^{2+}$ (which is synonymous with the designation $[Cu^{II}(tpy)(y_1–H)]^{2+}$). The formation of this complex is analogous to the formation of the y_1 ion in the fragmentation of $[GGH + H]^+$.

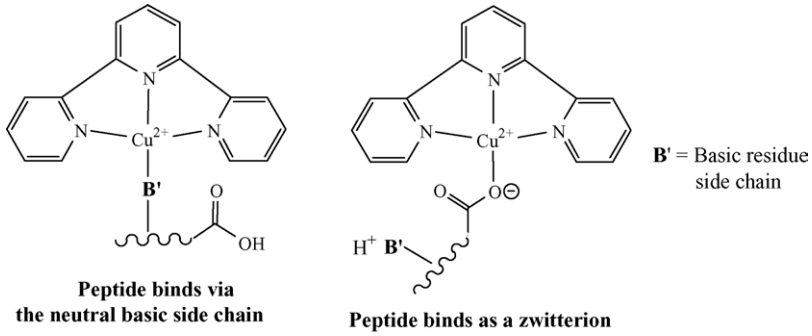
In contrast, when the basic lysine or histidine residue is not at the C-terminus, the side-chain assisted competing fragmentation pathways predominate in the fragmentation of $[Cu^{II}(tpy)M]^{2+}$ while the dissociative redox reaction depicted in equation (4) is largely suppressed, rendering low yields of $M^{\bullet+}$. The side-chain assisted competing fragmentation pathways in this case are mainly: (i) NH_3 loss from $[Cu^{II}(tpy)(KG)]^{2+}$ and $[Cu^{II}(tpy)(KGG)]^{2+}$ (presumably caused by the attack of the N-terminal lysine side-chain on the backbone) [19]; (ii) side-chain assisted amide bond cleavage.

For the peptides containing arginine, the competing fragmentation pathways of $[Cu^{II}(tpy)M]^{2+}$ are also more pronounced in the case where the arginine residue is not at the C-terminus (M = RG, RGG and GRG) than the case where the arginine residue is at the C-terminus (M = GR and GGR). However,

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

M	$M^{\bullet+}$	$[Cu^{I}(tpy)]^{+}$	$[Cu^{II}(tpy)(M)–(b_1 - H)]^{2+}$	$[Cu^{II}(tpy)(M)–(b_1)]^{+}$	b_1	a_1	$[Cu^{II}(tpy)(M)–(b_2 - H)]^{2+}$	$[Cu^{II}(tpy)(M) - (b_2)]^{+}$	b_2	a_2	Other products	
X at C-terminus												
GR	95	100										
GGR	65	100									$[Cu^{II}(tpy)M - NH_3]^{2+}$	(20)
											$[tpy + H]^{+}$	(8)
											$M^{\bullet+} - CO_2$	(10)
GK	100	90									$[Cu^{II}(tpy)M - NH_3]^{2+}$	(8)
GGK	100	75									$M^{\bullet+} - H_2O$	(8)
GH	87	100	5								$[tpy + H]^{+}$	(5)
											$[Cu^{II}(tpy)(M) - (\text{related ion})]^{+}$	(9)
GGH	60	100	5				73	6			$[tpy + H]^{+}$	(6)
											$[Cu^{II}(tpy)M - H]^{+}$	(6)
											$[Cu^{II}(tpy)(M) - (\text{related ion})]^{+}$	(5)
											$M^{\bullet+} - H_2O$	(12)
X as interior residue												
GRG	20	100						25	10	15	$[Cu^{II}(tpy)M - NH_3]^{2+}$	(23)
											$[Cu^{II}(tpy)M - H_2O - NH_3]^{2+}$	(8)
											$[tpy + H]^{+}$	(17)
											$[Cu^{II}(tpy)M - H]^{+}$	(15)
											$M^{\bullet+} - CO_2$	(6)
											immonium ion (m/z = 100 Th)	(6)
											$b_2 - NH_3$	(10)
GKG	7	25						100	30		Related/immonium ion (m/z = 129 Th)	(23)
											$[Cu^{II}(tpy)M - H_2O - NH_3]^{2+}$	(8)
											$[Cu^{II}(tpy)M - NH_3]^{2+}$	(8)
											$[Cu^{II}(tpy)M - H_2O]^{2+}$	(5)
GHG	5	30						100	51		b_2 ion $- NH_3$	(5)
											$[Cu^{II}(tpy)M - H_2O]^{2+}$	(15)
											$[Cu^{II}(tpy)M - H_2O - NH_3]^{2+}$	(50)
X at N-terminus												
RG	8	100		6							$[Cu^{II}(tpy)M - NH_3]^{2+}$	(25)
											$[tpy + H]^{+}$	(15)
											$[Cu^{II}(tpy)M - 60\ Da]^{+}$	(15)
											m/z = 87 Th	(5)
RGG	5	100		15							$[Cu^{II}(tpy)M - NH_3]^{2+}$	(25)
											$[Cu^{II}(tpy)M - 59/2]^{2+}$	(12)
											$[Cu^{II}(tpy)M - 60\ Da]^{+}$	(12)
											$M^{\bullet+}–CO_2$	(15)
KG	1	45		45	12						$[Cu^{II}(tpy)M–NH_3]^{2+}$	(100)
											Related/immonium ion (m/z = 84 Th)	(5)
KGG	2	27		90	40						$[Cu^{II}(tpy)M–NH_3]^{2+}$	(100)
											Related/immonium ion (m/z = 84 Th)	(5)
HG	3	43		100	5	45						
HGG	1	25		100	8	50		12	12			

Products of relative abundance less than 5% (except $M^{\bullet+}$) are not listed.



Scheme 1. Possible binding modes of peptide (M) in $[Cu^{II}(tpy)M]^{2+}$.

unlike the case of lysine or histidine containing peptides, these competing fragmentation pathways are minor processes. In the fragmentation of $[Cu^{II}(tpy)M]^{2+}$ where M = an arginine containing peptide, the dissociative redox reaction always predominates regardless of the position of the arginine residue. This is exhibited by the formation of $[Cu^{I}(tpy)]^{+}$ as the most abundant fragmentation product in all cases. However, it is puzzling why the complementary products $M^{\bullet+}$ are not always in high abundance, especially when the arginine residue is not at the C-terminus. One possible rationale for the low abundance of $M^{\bullet+}$ when the arginine residue is not at the C-terminus is that the $M^{\bullet+}$ undergoes further fragmentation to yield low-mass product ions that are not readily detected in the LCQ mass spectrometer.

In summary, for arginine containing peptides, the dissociative redox reaction always predominates regardless of the position of the arginine residue although the abundance of $M^{\bullet+}$ is low when the arginine residue is not at the C-terminus. In contrast, for lysine and histidine containing peptides, the dissociative redox reaction only predominates when the basic residue is at the C-terminus, whereas competing fragmentation pathways predominate when the basic residue is not at the C-terminus.

The observation that the position and the type of the basic residue dictate the dominant fragmentation pathway of $[Cu^{II}(tpy)M]^{2+}$ is intriguing. This may be a result of the binding modes of these peptides to $[Cu^{II}(tpy)]^{2+}$. There are two possible binding modes of these peptides to $[Cu^{II}(tpy)]^{2+}$: (i) via the basic side chain or (ii) as a zwitterion via the C-terminal carboxylate group (Scheme 1). The loss of b_1 and b_2 ions from $[Cu^{II}(tpy)M]^{2+}$ for M = XG/XGG and GXG respectively suggests that M binds to $[Cu^{II}(tpy)]^{2+}$ via the carboxylate group at the C-terminus and not at the side chain of the basic residue. This is consistent with the findings of Hu et al. who suggest that Cu^{+} associates with the peptide C-terminus [20]. However, they also found that the naked Cu^{2+} ion binds to the histidine residue when the C-terminus is an amide rather than a carboxylic acid [20].

In order to probe which of these binding modes is adopted by the peptides in $[Cu^{II}(tpy)M]^{2+}$ complexes, the formation and fragmentation of $[Cu^{II}(tpy)(M–OR')]^{2+}$ complexes of the nine tripeptides protected as methyl/ethyl esters listed in Table 1 were examined, and these results are discussed below.

3.2.2. MS/MS on $[Cu^{II}(tpy)(M–OR')]^{2+}$ and $[Cu^{II}(tpy)(Cyclo\{GH\})]^{2+}$

The presence of methyl/ethyl ester protecting groups will prevent peptide binding in the zwitterionic form to $[Cu^{II}(tpy)]^{2+}$. Hence, if the binding mode of unprotected peptides in $[Cu^{II}(tpy)M]^{2+}$ is zwitterionic, the $[Cu^{II}(tpy)(M–OR')]^{2+}$ complexes would either not be formed, or if formed, would exhibit different fragmentation patterns to their $[Cu^{II}(tpy)M]^{2+}$ counterparts. The M–OR′ listed in Table 1 were employed to examine the influence of the type and position of the basic residue on the binding modes of tripeptides to $[Cu^{II}(tpy)]^{2+}$.

For M–OR′ = arginine and lysine containing peptides, $[Cu^{II}(tpy)(M–OEt)]^{2}$ and $[Cu^{II}(tpy)(M–OMe)]^{2+}$ respectively were not detected. This is consistent with the observation of Gatlin et al. [21] that the ternary complex $[Cu^{II}(bpy)(M–OMe)]^{2+}$ was not detected in the ESI/MS of the methyl ester of arginine. This implies that arginine and lysine containing peptides bind to $[Cu^{II}(tpy)]^{2+}$ as zwitterions, consistent with discussions above.

For M = histidine containing tripeptides, the $[Cu^{II}(tpy)(M–OMe)]^{2+}$ complexes were detected, but they exhibit different fragmentation patterns from $[Cu^{II}(tpy)M]^{2+}$ (Figs. 1–3).

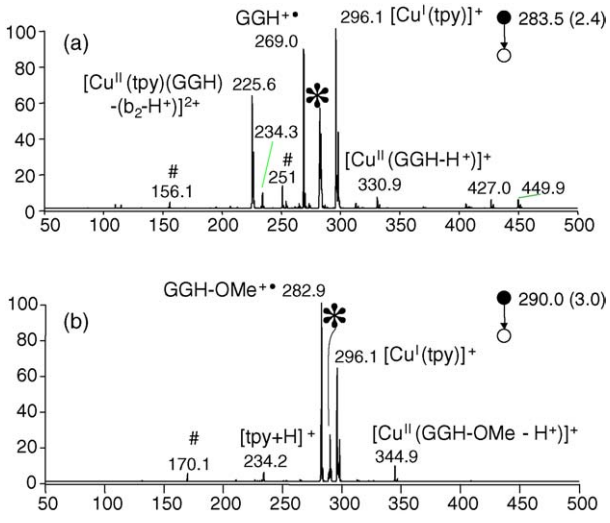


Fig. 1. (a) MS/MS spectra of $[Cu^{II}(tpy)(GGH)]^{2+}$; and (b) MS/MS spectra of $[Cu^{II}(tpy)(GGH–OMe)]^{2+}$. *The precursor ion. The number in brackets denotes the width of the isolation window (in Th) used in the mass-selection process. #The further fragmentation product of the peptide radical cation, $M^{\bullet+}$ or $M–OMe^{\bullet+}$.

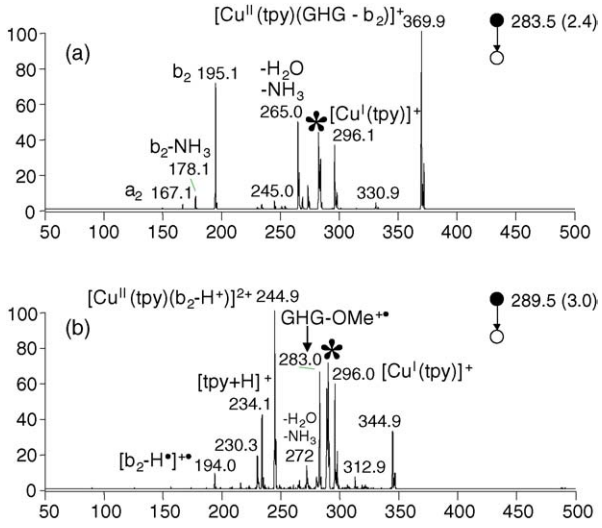


Fig. 2. (a) MS/MS spectra of $[Cu^{II}(tpy)(GHG)]^{2+}$; and (b) MS/MS spectra of $[Cu^{II}(tpy)(GHG–OMe)]^{2+}$. *The precursor ion. The number in brackets denotes the width of the isolation window (in Th) used in the mass-selection process. #The further fragmentation product of the peptide radical cation, $M^{\bullet+}$ or $M–OMe^{\bullet+}$.

In the fragmentation of $[Cu^{II}(tpy)(M–OMe)]^{2+}$, the competing fragmentation pathways that are present in the fragmentation of $[Cu^{II}(tpy)M]^{2+}$ are largely suppressed while the dissociative redox reaction dominates, particularly for GGH–OMe and HGG–OMe. For $[Cu^{II}(tpy)(GHG–OMe)]^{2+}$, although the dissociative redox reaction is not the dominant fragmentation pathway, it is greatly enhanced compared to the fragmentation of $[Cu^{II}(tpy)(GHG)]^{2+}$. The dominant fragmentation pathway of $[Cu^{II}(tpy)(GHG–OMe)]^{2+}$ is the formation of $[Cu^{II}(tpy)(b_2–H)]^{2+}$, which is absent in the fragmentation of $[Cu^{II}(tpy)(GHG)]^{2+}$. A possible explanation for the differences in the fragmentation pathways of the $[Cu^{II}(tpy)(M–OMe)]^{2+}$ and

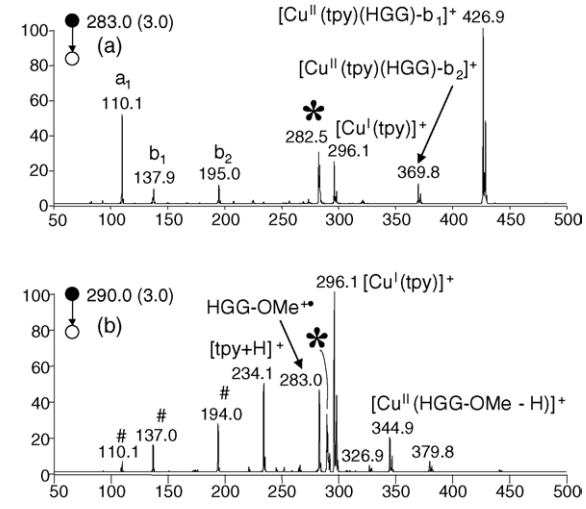


Fig. 3. (a) MS/MS spectrum of $[Cu^{II}(tpy)(HGG)]^{2+}$; and (b) MS/MS spectrum of $[Cu^{II}(tpy)(HGG–OMe)]^{2+}$. *The precursor ion. The number in brackets denotes the width of the isolation window (in Th) used in the mass-selection process. #The further fragmentation product of the peptide radical cation, $M^{\bullet+}$ or $M–OMe^{\bullet+}$.

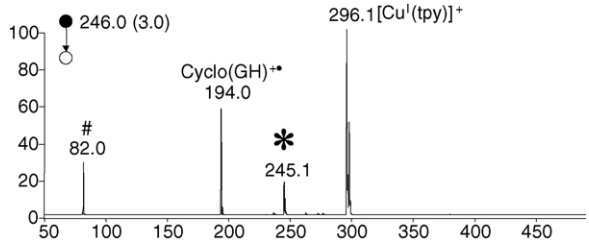


Fig. 4. MS/MS spectrum of $[Cu^{II}(tpy)(cyclo(GH))]^{2+}$. *The precursor ion. The number in bracket denotes the width of the isolation window (in Th) used in the mass-selection process. #The further fragmentation product of the peptide radical cation, $M^{\bullet+}$.

$[Cu^{II}(tpy)M]^{2+}$ complexes may be due to differences in binding modes, with the peptide esters unable to bind as zwitterions.

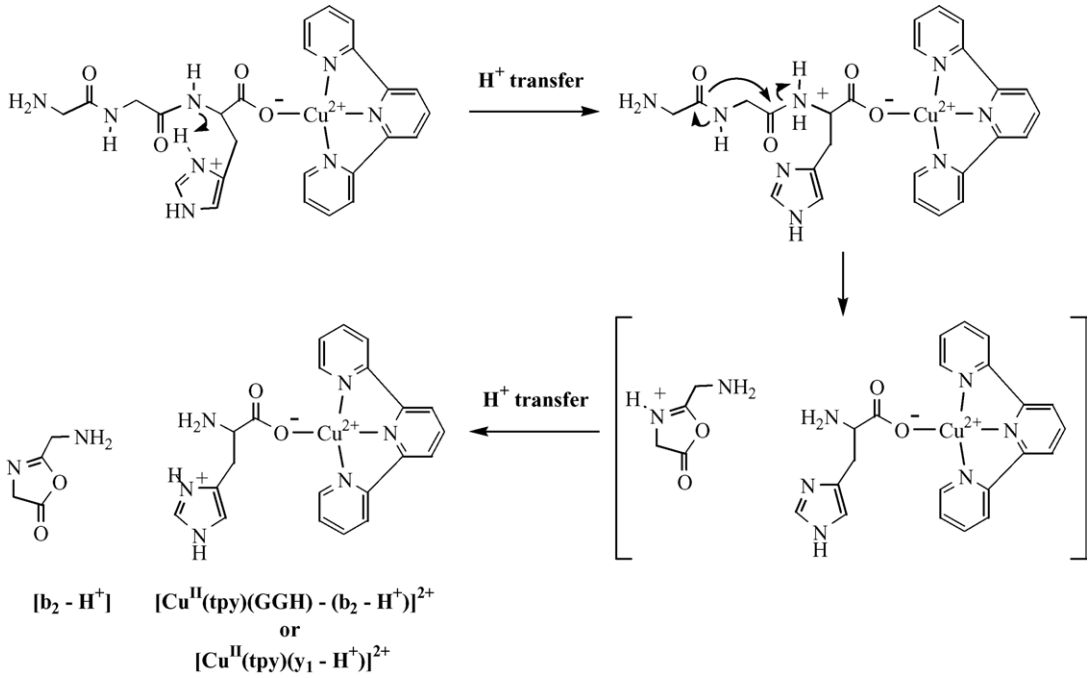
For $[Cu^{II}(tpy)(GHG)]^{2+}$ and $[Cu^{II}(tpy)(HGG)]^{2+}$, the competing fragmentation pathways depicted in eq. (5) dominate and peptide radical cation formation is largely suppressed while the reverse is true for $[Cu^{II}(tpy)(GHG–OMe)]^{2+}$ and $[Cu^{II}(tpy)(HGG–OMe)]^{2+}$. It is therefore very likely that the binding mode of GHG and HGG in the majority (if not all) of $[Cu^{II}(tpy)(GHG)]^{2+}$ and $[Cu^{II}(tpy)(HGG)]^{2+}$ respectively is zwitterionic. To further confirm that the histidine side chain can bind to $Cu^{II}(tpy)$, the formation of the $Cu^{II}(tpy)$ complex of the cyclic dipeptide cyclo(GH) was probed. $[Cu^{II}(tpy)(cyclo(GH))]^{2+}$ was observed in the ESI mass spectrum and its CID spectrum (Fig. 4) is dominated by the dissociative redox fragmentation pathway (eq. (4)).

Taken together, our observations involving the formation and the fragmentation of $[Cu^{II}(tpy)M]^{2+}$, $[Cu^{II}(tpy)(M–OR')]^{2+}$ and $[Cu^{II}(tpy)(cyclo(GH))]^{2+}$ suggest that the unprotected peptides M most likely bind to $[Cu^{II}(tpy)]^{2+}$ as zwitterions. The zwitterionic binding mode of M to $[Cu^{II}(tpy)]^{2+}$ allows us to explain the dominant fragmentation pathways of the $[Cu^{II}(tpy)M]^{2+}$ complexes. As mentioned above, for peptides with a basic residue at the C-terminus, peptide radical cation formation dominates, while competing fragmentation pathways are suppressed. The exception is $[Cu^{II}(tpy)(GGH)]^{2+}$, which forms both $GGH^{\bullet+}$ and the $[Cu^{II}(tpy)(GGH)–(b_2–H)]^{2+}$ ion. The facile formation of this latter ion is unique to GGH and may be due to the ability of the histidine side chain to act as a proton shuttle, thereby facilitating proton transfer to the peptide backbone as shown in Scheme 2 [10].

3.3. MS^3 on $M^{\bullet+}$ and $M–OR'^{\bullet+}$

$M^{\bullet+}$ formed from $[Cu^{II}(tpy)M]^{2+}$ generally have low absolute abundance, especially when X is not at the C-terminus. The abundances of $RGG^{\bullet+}$ and $KG^{\bullet+}$ generated from $[Cu^{II}(tpy)M]^{2+}$ are too low to allow MS^3 experiments to be carried out. The fragmentation products of the rest of the peptide radical cations are listed in Table 3.

One of the most dramatic differences in the fragmentation behaviour of $M^{\bullet+}$ versus $[M+H]^+$ involves the dipeptides GR and RG. We have previously noted that $[GR + H]^+$ and $[RG + H]^+$ yield identical CID spectra since they undergo rearrangement to form a mixed anhydride via a mechanism which involves salt



Scheme 2. Proposed mechanism for the formation of $[Cu^{II}(tpy)(GGH)–(b_2–H)]^{2+}$.

bridge formation [14]. In contrast, $GR^{\bullet+}$ and $RG^{\bullet+}$ fragment differently (Fig. 5). Even if the initial structures of $GR^{\bullet+}$ and $RG^{\bullet+}$ formed upon CID of $[Cu^{II}(tpy)M]^{2+}$ are carboxylate radicals, salt bridge formation is still possible in $GR^{\bullet+}$ and $RG^{\bullet+}$ once the carboxylate radical abstracts a $H^{\bullet}$ from another part of the peptide. The absence of rearrangement of $GR^{\bullet+}$ and $RG^{\bullet+}$ to form a mixed anhydride implies that the barrier(s) for rearrangement to form an anhydride is higher than the barrier for fragmentation.

It is interesting to note that while the fragmentation of arginine containing dipeptide radical cations is dominated by radical driven processes, the major fragmentation pathways of $GGR^{\bullet+}$ and $GRG^{\bullet+}$ are the formation of y_1 and $[b_2 - H^{\bullet}]^{\bullet+}$ respectively.[1] These species are most likely formed via even-electron processes since y_1 and b_2 ions are formed abundantly from $[GGR + H]^+$ and $[GRG + H]^+$ respectively.

Generally, for radical cations of lysine containing peptides (with the exception of $KG^{\bullet+}$, the abundance of which is too low to allow MS^3 fragmentation experiments to be carried out), the fragmentation reactions are dominated by $[b_n - H^{\bullet}]^{\bullet+}$ formation. The formation of $[b_n - H^{\bullet}]^{\bullet+}$ may be an even-electron process proceeding via side-chain assisted amide bond cleavage (cf. eq. (1)). Among these lysine containing peptide radical cations, fragmentation of $GGK^{\bullet+}$ exhibits the most extensive radical driven processes involving side chain cleavage.

As for histidine containing peptide radical cations, when the histidine residue is at the C-terminus, the major fragmentation product is the y_1 ion. When the histidine residue is not at the C-terminus, $[b_n - H^{\bullet}]^{\bullet+}$ formation is the dominant fragmentation product, and may proceed via side-chain assisted amide bond cleavage (cf. eqs. (1)–(3)).

The fragmentation mechanisms of peptide radical cations derived from a library of tripeptides GXR (where X = all of the naturally occurring amino acids except R) have been discussed in detail previously [4]. Some of the mechanisms proposed in that work are reproduced to illustrate analogous fragmen-

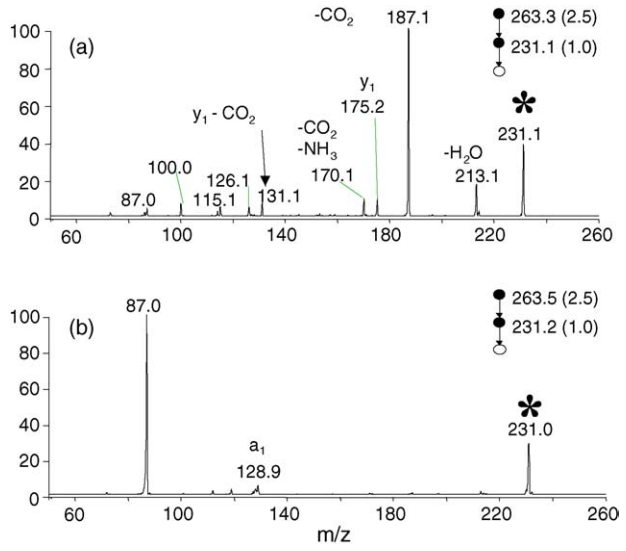


Fig. 5. (a) CID MS^3 reactions of the $GR^{\bullet+}$ ion; and (b) CID MS^3 reactions of the $RG^{\bullet+}$ ion. *The precursor ion. The number in brackets denotes the width of the isolation window (in Th) used in the mass-selection process.

[1] A referee has highlighted the challenges in understanding the gas phase fragmentation chemistry of peptide radical cations, which can either be initiated by the charge site or the radical site, and which can also involve H^+ (mobile proton) or H atom migrations. While terms such as "α cleavage" and "homolytic cleavage" are widely used to describe the fragmentation reactions of conventional radical cations in EI mass spectrometry, we use the term radical driven process to highlight that this reaction involves the radical site.

Table 3
Abundance of CID products of $M^{\bullet+}$ relative to the most intense peak in the spectrum (%)

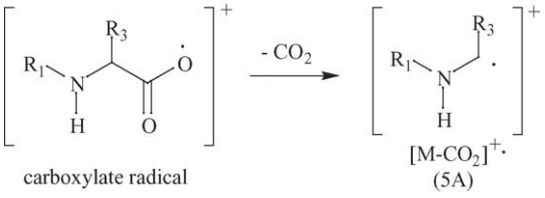
M	y_2	$[b_1 - H^{\bullet}]$	y_1	$[b_2 - H^{\bullet}]^{\bullet+}$	$[a_2 - H^{\bullet}]^{\bullet+}$	$[b_3 - H^{\bullet}]^{\bullet+}$	$[a_3 - H^{\bullet}]^{\bullet+}$	$-CO_2$	$-NH_3$	Other products	
X at C-terminus											
GR			10	17 ($-H_2O$)				100		$-NH_3$, $-CO_2$	(10)
						Not applicable				$y_1 - CO_2$	(8)
										Related/immonium ion (m/z = 100 Th)	(7)
GGR	40		100			8 ($-H_2O$)		8	8	Related/immonium ion (m/z = 100 Th)	(10)
GK				100 ($-H_2O$)					15	$-2NH_3$	(8)
						Not applicable				$[b_2 - H^{\bullet}]^{\bullet+} - NH_3/-H_2O-NH_3$	(15)
										$-NH_2CH_2^{\bullet}$	(10)
										m/z = 128Th	(25)
GGK			15	8		100 ($-H_2O$)			20	$[b_3 - H^{\bullet}]^{\bullet+} - H_2O/-2H_2O$	(45)
										$-NH_2CH_2CH_2CH_2^{\bullet}$	(20)
										$-NH_2CH_2CH_2CH_3$[b]	(55)
										$GGG^{\bullet+}$	(10)
										$y_1 - H_2O$(m/z = 129)	(10)
										m/z = 128Th	(50)
GH			100	42 ($-H_2O$)	8 ($-H_2O$ –CO)				28	$-NH_3$,$-CO_2$	(18)
						Not applicable				$y_1 - CO_2$	(12)
										Related ion (m/z = 13 8 Th)/$y_1 - H_2O$	(20)
										Immonium ion (m/z = 110 Th)	(12)
GGH	15		100			25 ($-H_2O$)	15 ($-H_2O$ –CO)	25		$[b_3 - H^{\bullet}]^{\bullet+} - H_2O/-2H_2O$	(5)
										$-CO_2-H_2O$	(12)
										$y_2 - CO_2$	(5)
										$y_1 - CO_2$	(5)
										$-$[Related ion $- H^{\bullet}]^{\bullet+}$	(16)
										Immonium ion (m/z = 110 Th)	(8)
X as Interior Residue											
GRG	30			100		37($-H_2O$)		35	25	$-NH_2CH_2^{\bullet}$	(10)
										m/z = 230 Th	(22)
										$-$60 Da	(30)
										$-$62 Da	(10)
										$[b_2 - H^{\bullet}]^{\bullet+} - NH_3$	(6)
										a_2	(45)
										$-$104 Da	(10)
										Related/immonium ion (m/z = 100)	(12)
										m/z = 87 or related/immonium ion	(13)
GKG				100		30($-H_2O$)				$[b_3 - H^{\bullet}]^{\bullet+} - NH_3/-H_2O-NH_3$	(15)
										a_2	(5)
GHG				100							
X at N-terminus											
RG							Not applicable			m/z = 87 Th or rel/immonium ion	(100)
RGG[a]											
KG[a]											
KGG		100		50						$-CO$	(8)
										$[b_2 - H^{\bullet}]^{\bullet+} - 2NH_3$[b]	(10)
										$[b_2 - H^{\bullet}]^{\bullet+} - 2NH_3 - CO$[b]	(10)
HG		100					Not applicable				
HGG		45		35		5($-H_2O$)			10	Immonium ion (m/z = 110 Th)/a_2	(5)

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

[a] The abundance of $M^{\bullet+}$ is not sufficient for analysis by CID.

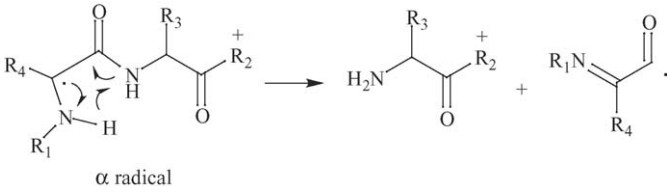
[b] A possible assignment.

tation mechanisms of the peptide radical cations discussed here. Radical driven reactions that are commonly observed in the fragmentation of the peptide radical cations discussed in this paper (Table 3) are CO_2 loss (eq. (6)) [22] as well as y_1 and y_2 ion formation (eq. (7)). Note that the y_1 ions of tripeptide radical cations can also be formed via even-electron processes.



(6)

where R_1 is nitrogen atom of peptide bond.



(7)

where R_1 is H or nitrogen atom of peptide bond and R_2 is OH or nitrogen atom of peptide bond.

3.3.1. MS^3 on $GX^{\bullet+}$ and $GGX^{\bullet+}$

3.3.1.1. $GR^{\bullet+}$. As shown in Fig. 5a, the major fragmentation product of $GR^{\bullet+}$ is $[GR–CO_2]^{\bullet+}$ (A) which is formed from a carboxylate radical (eq. (6), [4,22]). This also suggests that unlike its protonated counterpart, $GR^{\bullet+}$ does not undergo rearrangement to form a mixed anhydride.

3.3.1.2. $GK^{\bullet+}$. Most of the fragmentation products of $GK^{\bullet+}$ are analogous to the fragmentation products of its protonated counterpart, implying that radical driven processes do not play an important role in the fragmentation of $GK^{\bullet+}$.

3.3.1.3. $GH^{\bullet+}$. $GH^{\bullet+}$ yields more backbone cleavage than its protonated analogue. $GH^{\bullet+}$ yields the y_1 ion which is absent in the fragmentation of $[GH + H]^+$. Since the y_1 ion is formed only from $GH^{\bullet+}$ and not from $[GH + H]^+$, it is likely that this y_1 ion is formed via an odd-electron process (eq. (7)).

3.3.1.4. $GGR^{\bullet+}$ and $GGH^{\bullet+}$. Fragmentation reactions of $GGR^{\bullet+}$ and $GGH^{\bullet+}$ also yield more sequence ions than their protonated counterparts. Both the protonated species and the radical cations of GGR and GGH yield y_1 ions. However, only the radical cations yield y_2 ions. Hence, these y_2 ions may also be formed via an odd-electron process (eq. (7)).

3.3.1.5. $GGK^{\bullet+}$. As well as small molecule losses and amide bond cleavages, $NH_2CH_2CH_2CH_2^{\bullet}$ is lost from the lysine side chain. A related loss has been observed in the fragmentation of $GKR^{\bullet+}$ [4] and was proposed to originate from the α-radical of the lysine residue. $GGK^{\bullet+}$ also loses $NH_2CH_2CH_2CH_3$ which was not observed in the fragmentation of $GKR^{\bullet+}$.

3.3.2. MS^3 on $GXG^{\bullet+}$

The most abundant fragmentation products of $GXG^{\bullet+}$ are $[b_2 - H^{\bullet}]^{\bullet+}$ ions. The analogous even-electron b_2 ions are also the major fragmentation products of $[GXG + H]^+$. This implies that formation of $[b_2 - H^{\bullet}]^{\bullet+}$ ions from $GXG^{\bullet+}$ mainly occurs through even-electron processes. These even-electron processes most likely occur via intermediates in which the protonated basic side chains transfer the ionizing proton to the amide nitrogen of the C-terminus prior to fragmentation via mechanisms related to those shown in eqs (1) and (2) [10,17]. Note that formation of $[b_2 - H^{\bullet}]^{\bullet+}$ requires the radical site to be on either the N-terminal residue or the interior residue.

The loss of $NH_3 + H_2O$ and/or H_2O is not significant in the fragmentation of $GXG^{\bullet+}$, unlike the case of protonated GXG. Apart from that, fragmentation of $GKG^{\bullet+}$ and $GHG^{\bullet+}$ is analogous to the fragmentation of their protonated counterparts, and presumably occurs mainly via even-electron processes. As for $GRG^{\bullet+}$, there is significant CO_2 loss and more products are produced from the fragmentation of the arginine side chain when compared to $[GRG + H]^+$. $GRG^{\bullet+}$ also yields significant amounts of the a_2 ion and a product with $m/z = 230$ Th (which may arise from loss of the guanidinium side chain), both of which are absent in the fragmentation of $[GRG + H]^+$.

The fragmentation of similar systems, $GXR^{\bullet+}$ (X = K and H) has been discussed previously [4]. It is interesting to compare the fragmentation of $GXG^{\bullet+}$ with the fragmentation of $GXR^{\bullet+}$ (X = K and H). As noted above, $GXG^{\bullet+}$ (X = K and H) mainly forms $[b_2 - H^{\bullet}]^{\bullet+}$, presumably via even-electron processes which require transfer of H^+ from the protonated X side chains to the peptide backbone; that is, radical driven processes are not significant in the fragmentation of $GXG^{\bullet+}$. In contrast, for $GXR^{\bullet+}$ apart from major y_1 ion formation (the product complementary to $[b_2 - H^{\bullet}]^{\bullet+}$ formed in the fragmentation of $GXG^{\bullet+}$), fragmentation yields significant amounts of other products formed via radical driven processes, such as y_2 ion formation and side chain losses. The reason for enhanced radical driven processes in the fragmentation of $GXR^{\bullet+}$, compared to $GXG^{\bullet+}$, is most likely due to the presence of the basic arginine residue in $GXR^{\bullet+}$ which sequesters the ionizing proton, and hence suppresses even-electron processes (cf. eqs (1) and (2)). This may also be the reason that $GRG^{\bullet+}$ exhibits more radical driven processes than $GKG^{\bullet+}$ and $GHG^{\bullet+}$.

3.3.3. MS^3 on $XG^{\bullet+}$ and $XGG^{\bullet+}$

As mentioned earlier, among the radical cations which have the basic residue at the N-terminus ($XG^{\bullet+}$ and $XGG^{\bullet+}$), the abundances of $RGG^{\bullet+}$ and $KG^{\bullet+}$ are too low to allow MS^3 experiments to be carried out. As for the fragmentation of the other $XG^{\bullet+}$ and $XGG^{\bullet+}$, small molecule (NH_3 and H_2O) losses are generally suppressed compared to their protonated analogues.

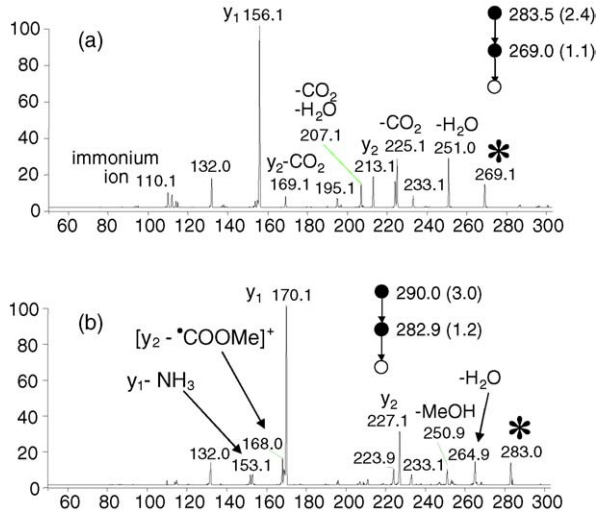


Fig. 6. (a) CID MS^3 reactions of the $GGH^{\bullet+}$ ion; and (b) CID MS^3 reactions of the $GGH–OMe^{\bullet+}$ ion. *The precursor ion. The number in brackets denotes the width of the isolation window (in Th) used in the mass-selection process.

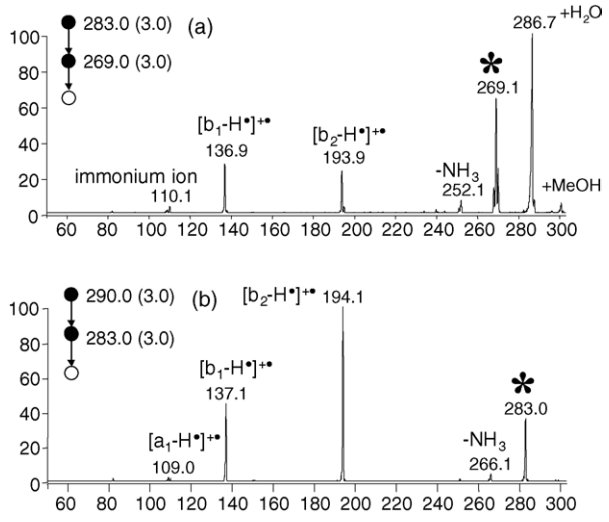


Fig. 7. (a) CID MS^3 reactions of the $HGG^{\bullet+}$ ion; (b) CID MS^3 reactions of the $HGG–OMe^{\bullet+}$ ion. *The precursor ion. The number in brackets denotes the width of the isolation window (in Th) used in the mass-selection process.

The $[b_1 - H^{\bullet}]^{\bullet+}$ fragment is the most abundant product arising from $HG^{\bullet+}$ $KGG^{\bullet+}$ and $HGG^{\bullet+}$. The fragmentation of $RG^{\bullet+}$, however, does not yield $[b_1 - H^{\bullet}]^{\bullet+}$ consistent with the suggestion that formation of $[b_n - H^{\bullet}]^{\bullet+}$ occurs via an even-electron process which requires the transfer of the H^+ from the protonated side chains of the basic residue to the peptide backbone. Since arginine is the most basic residue, more energy is required to transfer the proton from the arginine side chain compared to that of lysine and histidine.

3.3.4. MS^3 on $M–OR'^{\bullet+}$ and $[Cyclo(GH)]^{\bullet+}$

The abundance of $GHG–OMe^{\bullet+}$ is too low to allow MS^3 experiments to be carried out. The CID spectra of $GGH–OMe^{\bullet+}$ and $HGG–OMe^{\bullet+}$ are broadly similar to their unprotected analogues and are shown in Figs. 6 and 7 respectively. One of the main differences between the fragmentation of $GGH^{\bullet+}$ and $GGH–OMe^{\bullet+}$ (Fig. 6) is the formation of products due to CO_2 loss in the fragmentation of $GGH^{\bullet+}$ but not in the fragmentation of $GGH–OMe^{\bullet+}$. This is not surprising since the presence of the methyl ester protecting group at the C-terminus of $GGH–OMe^{\bullet+}$ makes CO_2 loss unlikely. The other difference between the fragmentation of $GGH^{\bullet+}$ and $GGH–OMe^{\bullet+}$ is the formation of $[y_2 - {}^{\bullet}COOMe]^+$ in the fragmentation of $GGH–OMe^{\bullet+}$ but not in the fragmentation of $GGH^{\bullet+}$.

As shown in Fig. 7, the similarities are most pronounced in fragmentation products of $HGG^{\bullet+}$ and $HGG–OMe^{\bullet+}$. Examination of the data shown in this figure also indicates that $HGG^{\bullet+}$ readily forms adducts with adventitious H_2O and MeOH while this is not the case for $HGG–OMe^{\bullet+}$. The reasons for such differences in behaviour are not clear.

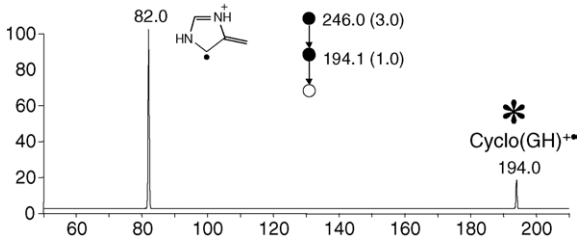
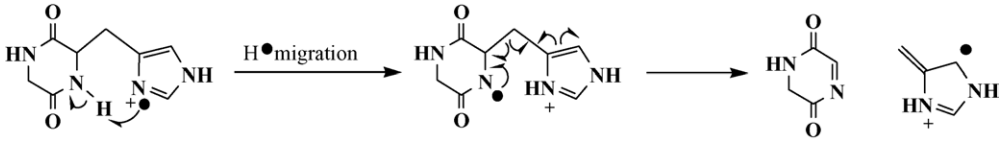


Fig. 8. CID MS^3 reactions of the $[cyclo(GH)]^{\bullet+}$ ion. *The precursor ion. The number in brackets denotes the width of the isolation window (in Th) used in the mass-selection process.

To account for the observations that the CID spectra of $GGH–OMe^{\bullet+}$ and $HGG–OMe^{\bullet+}$ are broadly similar to that of their respective unprotected analogues, we propose that in the esterified systems GGH–OMe and HGG–OMe, the initial radical is formed at the histidine side chain whereas for the unprotected peptides GGH and HGG, the initial radical may form at either the carboxylate or histidine side chain. Regardless of the site of radical formation, rearrangement then occurs to form common precursors for fragmentation. The CID spectrum of $[cyclo(GH)]^{\bullet+}$ (Fig. 8) shows only one fragmentation product at $m/z = 82$ Th. We propose that the formation of this product likely involves $H^{\bullet}$ abstraction by the histidine side chain as shown in Scheme 3.



Scheme 3. Proposed mechanism for the fragmentation of $[Cyclo(GH)]^{\bullet+}$.

4. Conclusions

GX, GGX, GXG, XG and XGG (where X = R, K and H) may adopt a zwitterionic binding mode in $[Cu^{II}(tpy)M]^{2+}$ although histidine containing peptides may also bind to $[Cu^{II}(tpy)]^{2+}$ via the histidine side chain. The dissociative redox reaction that leads to $M^{\bullet+}$ formation from $[Cu^{II}(tpy)M]^{2+}$ only dominates when competing fragmentation pathways which lead to peptide backbone cleavage are suppressed. These competing fragmentation pathways require the transfer of an ionizing proton from the basic side chain to the peptide backbone to promote peptide backbone cleavage driven by even-electron processes. The high basicity of the arginine side chain may contribute to less facile peptide backbone cleavage compared to the dissociative redox reaction channel of $[Cu^{II}(tpy)M]^{2+}$ regardless of the position of the arginine residue. However, when the arginine residue is not at the C-terminus, the abundance of $M^{\bullet+}$ is low, presumably due to prompt further fragmentation of these $M^{\bullet+}$ at the MS^2 stage. As for lysine and histidine containing peptides, the dissociative redox reaction that leads to $M^{\bullet+}$ formation only predominates when the basic residue is at the C-terminus, whereas competing fragmentation pathways predominate when the basic residue is not at the C-terminus, rendering low yields of $M^{\bullet+}$. Taken together, our results indicate that the abundance of $M^{\bullet+}$ formed from $[Cu^{II}(tpy)M]^{2+}$ is at its greatest only when the basic residue is at the C-terminus.

Fragmentation reactions of $M^{\bullet+}$ examined in this work are driven by both even-electron and odd-electron processes. The main fragmentation reactions of $M^{\bullet+}$ which differ from their protonated counterparts are those which are radical driven, including side-chain fragmentation, CO_2 loss and formation of y_{m-1} ion (where m is the number of residues in the peptides). Side chain fragmentation reactions driven by radical processes are dependent on both the position of the residue as well as the type of other residues present in the peptide radical cation. Some of the $M^{\bullet+}$ yield more sequence information than their protonated analogues. Arginine containing dipeptides, which undergo rearrangement to form a mixed anhydride in their protonated forms, do not undergo the same rearrangement in their radical cation forms. Unlike tryptophan containing peptide radical cations [3], $[z_n - H]^{\bullet+}$ ions are not important fragment ions for the simple arginine, histidine or lysine peptides studied here. These studies highlight a rich chemistry of peptide radical cations which depends on both the nature of the residues as well as their positions within the peptide chain.

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