RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2010; **24**: 3485–3492
Published online in Wiley Online Library (wileyonlinelibrary.com) DOI: 10.1002/rcm.4783

RCM

A comparison of the fragmentation pathways of $[Cu^{II}(M_a)(M_b)]^{\bullet 2+}$ complexes where M_a and M_b are peptides containing either a tryptophan or a tyrosine residue

Yuyong Ke[1], Houssain El Aribi[2], Chi-Kit Siu[3], K.W. Michael Siu[1] and Alan C. Hopkinson[1]*

[1]Department of Chemistry and Centre for Research in Mass Spectrometry, York University, 4700 Keele Street, Toronto, Ontario, Canada M3J 1P3
[2]Applied Biosystems Deutschland GmbH, Frankfurter Strasse 129 B, 64293 Darmstadt, Germany
[3]Department of Biology and Chemistry, City University of Hong Kong, 83 Tat Chee Avenue, Kowloon Tong, Hong Kong, China

Received 7 July 2010; Revised 6 September 2010; Accepted 12 September 2010

$[Cu^{II}(M_a)(M_b)]^{\bullet 2+}$ complexes, where M_a and M_b are dipeptides or tripeptides each containing either a tryptophan (W) or tyrosine (Y) residue, have been examined by means of electrospray tandem mass spectrometry. Collision-induced dissociations (CIDs) of complexes containing identical peptides having a tryptophan residue generated abundant radical cations of the peptides; by contrast, for complexes containing peptides having a tyrosine residue, the main fragmentation channel is dissociative proton transfer to give $[M_a + H]^+$ and $[Cu^{II}(M_b - H)]^{\bullet +}$. When there are two *different* peptides in the complex, each containing a tryptophan residue, radical cations are again the major products, with their relative abundances depending on the locations of the tryptophan residue in the peptides. In the CIDs of mixed complexes, where one peptide contains a tryptophan residue and the other a tyrosine residue, the main fragmentation channel is formation of the radical cation of the tryptophan-containing peptide and not proton transfer from the tyrosine-containing peptide to give a protonated peptide. Copyright © 2010 John Wiley & Sons, Ltd.

Electron ionization (EI) is the classical ionization technique used in generating molecular radical cations $M^{\bullet +}$ for mass spectrometry (MS).[1,2] Extension of EI to amino acids and peptides has been limited because these biologically important molecules have very low vapor pressures and decompose upon heating, thus making it difficult, if not impossible, to create a sufficiently large population of the neutrals in the gas phase for EI.[3] Laser ablation followed by UV photoionization has been successfully used to generate radical cations of peptides,[4–6] but is only effective for peptides that contain aromatic amino acid residues that act as UV chromophores. The advent of electrospray ionization (ESI) enables a general technique whereby protonated amino acids and peptides, $[M + nH]^{n+}$, can be generated in the gas phase.[7] Subsequent electron capture[8] by, or electron transfer[9] to, $[M + nH]^{n+}$ results in $[M + nH]^{\bullet (n-1)+}$, which are hydrogen-rich radical cations. Alternatively, it has been demonstrated that photodissociation of a protonated peptide that was chemically modified to incorporate a labile radical precursor, or one that was noncovalently bound to a ligand containing the labile radical precursor, can produce a peptide radical cation $M^{\bullet +}$.[10,11]

Metal-ion complexes containing amino acids or peptides as ligands can also be generated by ESI and collision-induced dissociation (CID) of some copper(II) complexes has been found to generate molecular radical cations of oligopeptides $M^{\bullet +}$.[12,13] These are species that would have been produced by EI, had it been possible to generate sufficiently large populations of the neutral peptides in the gas phase. In the initial discovery, the ternary complex $[Cu^{II}(dien)(YGGFLR)]^{\bullet 2+}$, where dien = diethylenetriamine ($H_2NCH_2CH_2NHCH_2CH_2NH_2$) and YGGFLR is a leucine enkephalin derivative, TyrGlyGlyPheLeuArg, was shown to dissociate giving the radical cation, $M^{\bullet +}$, of the peptide and $[Cu(dien)]^+$. Subsequently, complexes with various combinations of the peptide, M, and the auxiliary ligand, L (the latter includes triamines, terpyridines, crown ethers and their analogues, or salens), have been shown to generate peptide radical cations when subjected to CID.[14–23]

Fragmentation pathways, all leading to separation of charge, that have been observed are dissociative electron transfer from the peptide to the metal (reaction 1), dissociative proton transfer from the ligand to the peptide (reaction 2), dissociative proton transfer from the peptide to the ligand (reaction 3),

*Correspondence to: A. C. Hopkinson, Department of Chemistry and Centre for Research in Mass Spectrometry, York University, 4700 Keele Street, Toronto, Ontario, Canada M3J 1P3.
E-mail: ach@yorku.ca

Copyright © 2010 John Wiley & Sons, Ltd.

and peptide dissociation and charge separation (reaction 4).[21]

$$[Cu^{II}(L)(M)]^{\bullet 2+} \rightarrow [Cu^{I}(L)]^{+} + M^{\bullet +} \quad (1)$$

$$[Cu^{II}(L)(M)]^{\bullet 2+} \rightarrow [Cu^{II}(L-H)]^{\bullet +} + [M+H]^{+} \quad (2)$$

$$[Cu^{II}(L)(M)]^{\bullet 2+} \rightarrow [Cu^{II}(M-H)]^{\bullet +} + [L+H]^{+} \quad (3)$$

$$[Cu^{II}(L)(M)]^{\bullet 2+} \rightarrow [Cu^{II}(M-b_2)(L)]^{\bullet +} + b_2^{+} \quad (4)$$

Production of the radical cation, $M^{\bullet +}$, in reaction 1 is a dissociative redox reaction and, in the present context, gives the desired product. Considerable experimental effort has been expended on maximizing the yield of $M^{\bullet +}$.[15–23] This dissociative redox reaction strategy for producing $M^{\bullet +}$ has been elaborated to include the use of triply charge metal ions (Cr^{3+}, Mn^{3+}, Fe^{3+}, and Co^{3+}), a variety of ligands, and constituents of nucleic acids.[24–28]

Much of the work on the radical cations of peptides has been focused on using ever-more complicated ligands to exert control on the fragmentation chemistry. A path that was much less frequently followed, but nonetheless equally interesting, was to examine the fragmentation chemistry of complexes in which the auxiliary ligand was also a peptide. We reported that the dissociation of $[Cu^{II}(W)_2]^{\bullet 2+}$ (W = tryptophan) yielded both $W^{\bullet +}$ (*m/z* 204) and the protonated 3-methyleneindolenine cation (*m/z* 130, the side chain of tryptophan) in high abundance, while the fragmentation of $[Cu^{II}(Y)_2]^{\bullet 2+}$ (Y = tyrosine) gave a mixture of $Y^{\bullet +}$ (*m/z* 181) and $[Y+H]^{+}$ (*m/z* 182 in higher abundance), as well as the *p*-hydroxybenzyl cation (*m/z* 107) and the *p*-cresol radical cation (*m/z* 108) originating from the side chain of tyrosine.[29] These results are comparable to those obtained in the CID of $[Cu^{II}(dien)W]^{\bullet 2+}$ and $[Cu^{II}(dien)Y]^{\bullet 2+}$, respectively.[16]

Herein we report the dissociation chemistries of complexes $[Cu^{II}(M_a)(M_b)]^{\bullet 2+}$, where both M_a and M_b are peptides, each containing either a tryptophan or tyrosine residue. Peptides containing these two residues were selected because tryptophan and tyrosine have the lowest ionization energies of the naturally occurring amino acids and the presence of one of these residues in a peptide is expected to facilitate formation of radical cations. In some cases, M_a and M_b are identical, while in most cases they are different and permit insight into competitive electron transfer from M_a or M_b to $Cu^{\bullet 2+}$ and/or competitive proton transfer between M_a and M_b.

EXPERIMENTAL AND COMPUTATIONAL

Experiments were performed on an ion-trap mass spectrometer (LCQ, Finnigan-MAT LCQ) and a triple quadrupole mass spectrometer prototype (API3000 equivalent, Applied Biosystems/MDS SCIEX) equipped with an electrospray ionization (ESI) source. On the LCQ, the typical electrospray voltage was 4.5 kV, the sheath gas was 0.3 L/min and the capillary temperature was 120–160°C. Two series of dipeptides and tripeptides containing tryptophan and tyrosine residues were examined on the ion trap: these were WG, GW, WGG, GWG and GGW; GY, YG, YGG, GYG, and GGY (G = glycine). The amino acids, tryptophan and tyrosine, were examined on the API3000, where the typical electrospray voltage was 5.5 kV and the declustering potential was 5 V. Laboratory collision energy (E_{lab}) is used and simply defined as DC offset between Q_0 and RO2 on the API3000. Samples were typically 100 μM in $Cu(ClO_4)_2$ and 200 μM in peptides in a 50:50 water/methanol solution. Typical sample flow rates for electrospray were 3 μL/min. Ion lineage was determined by using successive stages of mass spectrometry. All peptides were purchased from Bachem BioSciences, Inc. (King of Prussia, PA, USA). The amino acids, tryptophan and tyrosine, and $Cu(ClO_4)_2$ were purchased from Sigma (St. Louis, MO, USA). All CID experiments were performed on complexes with each Cu isotope at low collision energies with N_2 as collision gas in the API3000 and He in the LCQ; the Cu isotope used in each figure is specified. The choice of isotope selected was based on minimizing isobaric interferences. 3,3-d-Tyrosine (denoted Y-d_2 on Fig. 1(c)) was also used to minimize isobaric interferences.

All density functional theory calculations were performed using the Gaussian 03 program.[30] Structure optimizations were executed at the UB3LYP/6-31++G(d,p) level and the resulting structures were all verified to be at minima by harmonic frequency calculations.[31–33]

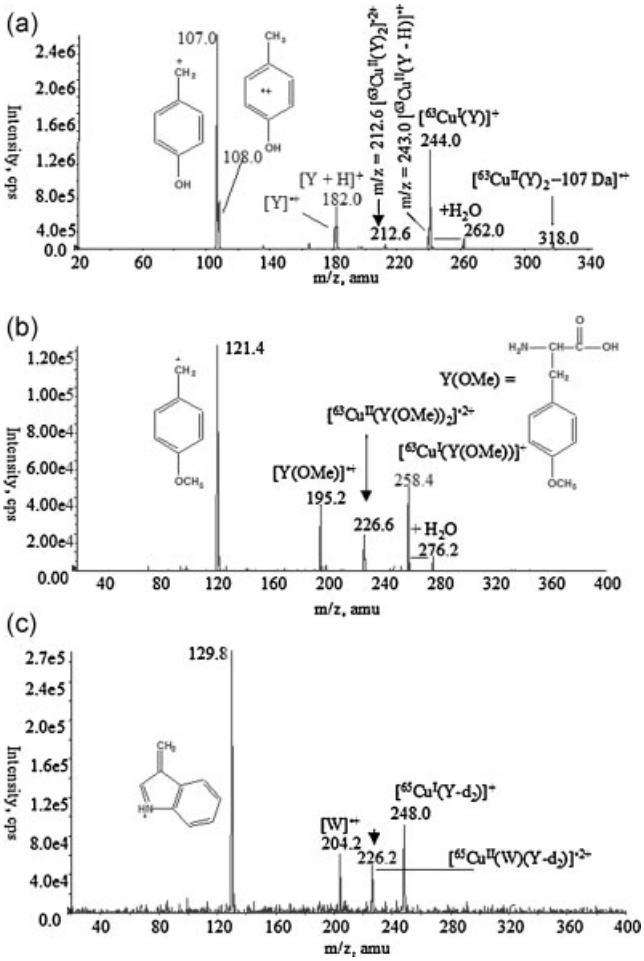

Figure 1. CID spectra of (a) $[^{63}Cu^{II}(Y)_2]^{\bullet 2+}$, (b) $[^{63}Cu^{II}(Y(OMe))_2]^{\bullet 2+}$, and (c) $[^{65}Cu^{II}(W)(Y\text{-}d_2)]^{\bullet 2+}$ all at $E_{lab} = 7$ eV. The use of the ^{65}Cu-containing complex and the d_2 amino acid of Y in (c) was necessitated by isobaric interferences. The bold arrow indicates the precursor ion.

Copyright © 2010 John Wiley & Sons, Ltd.

DOI: 10.1002/rcm

RESULTS AND DISCUSSION

$[Cu^{II}(M_a)(M_b)]^{\bullet 2+}$ complexes in which M_a and M_b were amino acids tryptophan and/or tyrosine

Fragmentation of $[Cu^{II}(W)_2]^{\bullet 2+}$ followed reaction 1, dissociative electron transfer, giving as primary product ions the radical cation $W^{\bullet +}$ and the complementary ion $[Cu^{I}(W)]^{+}$. In comparison, dissociation of $[Cu^{II}(Y)_2]^{\bullet 2+}$ gave two reaction channels: dissociative electron transfer (reaction 1) resulting in $Y^{\bullet +}$ and $[Cu^{I}(Y)]^{+}$, and proton transfer (reaction 2) resulting in $[Y+H]^{+}$ and $[Cu^{II}(Y-H)]^{\bullet +}$) (Fig. 1(a)). Dissociative electron transfer was the more abundant channel as evidenced by the higher abundance of $[Cu^{I}(Y)]^{+}$ relative to $[Cu^{II}(Y-H)]^{\bullet +}$; a significant fraction of nascent $Y^{\bullet +}$ were hot and dissociated to give the *p*-hydroxybenzyl cation (*m*/*z* 107) and the *p*-cresol radical cation (*m*/z 108).[29] Methylation of the phenol on the tyrosine side chain prevented the proton transfer reaction, as shown in the CID spectrum of $[Cu^{II}(Y(OMe))_2]^{\bullet 2+}$ (Fig. 1(b)), where only radical cation formation was observed. Likewise in the CID of the mixed complex $[Cu^{II}(W)(Y)]^{\bullet 2+}$ (Fig. 1(c)), dissociative electron transfer was the only active channel and the only radical cationic product apparent was $W^{\bullet +}$, i.e., the products were reminiscent of those of $[Cu^{II}(W)_2]^{\bullet 2+}$. The exclusivity of $W^{\bullet +}$ over $Y^{\bullet +}$ is probably a reflection of the lower ionization energy of tryptophan relative to that of tyrosine.[34,35]

The phenolic hydrogen of tyrosine apparently plays an essential role in controlling the proton transfer reaction (reaction 2). Several structures of $[Cu^{II}(Y)_2]^{\bullet 2+}$ were optimized using DFT calculations at the B3LYP/6-31++G(d,p) level (Fig. 2(a)). The lowest-energy isomer has a charge-solvation-salt-bridge (CS-SB) structure in which one tyrosine is in the canonical form dicoordinated through the carbonyl oxygen and the nitrogen of the amino group (CS), while the other tyrosine is in the zwitterionic form with the phenolate anion attached to the copper and the amino group protonated (SB) (structure **I**). This structure formally separates the two positive charges while leaving one close to copper; structures V and VI, in which both ligands are zwitterionic and the charges are further apart on protonated amino groups, have higher energy. Dissociation of the zwitterionic tyrosine, followed by proton transfer from the phenol of the canonical tyrosine to the phenolate oxygen, gives the lowest energy combination of products $[Y+H]^{+}$ and $[Cu^{II}(Y-H)]^{\bullet +}$ (**VII** in Fig. 2(b)) and is exothermic by 4.2 kcal mol^{-1}. One unusual aspect of the possible products is that the zwitterionic structure **VII** has lower energy than ions **VIII** and **IX**, both of which have less charge separation. Details of the dissociation mechanism were not studied.

Methylation of the phenol group prevents the formation of structure **I**. A structure of type **1** cannot be formed from methylated tyrosine, but **II** and **III** (at relative enthalpies of 3.0 and 2.6 kcal mol^{-}, respectively, on the $[Cu^{II}(Y)_2]^{\bullet 2+}$ surface) are possible. Dissociative proton transfers of **II** and **III** give **VIII**; these reactions are endothermic by 3.3 and 3.7 kcal mol^{-1}, possibly explaining why proton transfer does not occur in the CID of $[Cu^{II}(Y(OMe))_2]^{\bullet 2+}$.

$[Cu^{II}(M_a)(M_b)]^{\bullet 2+}$ complexes in which both peptides contained a *tryptophan* residue

(a) M_a and M_b were identical

CID of complexes $[Cu^{II}(M)_2]^{\bullet 2+}$, where M = WG, GW, WGG, GWG, and GGW, all resulted in high abundances of radical cations, $M^{\bullet +}$ (Table 1), and their decomposition products,

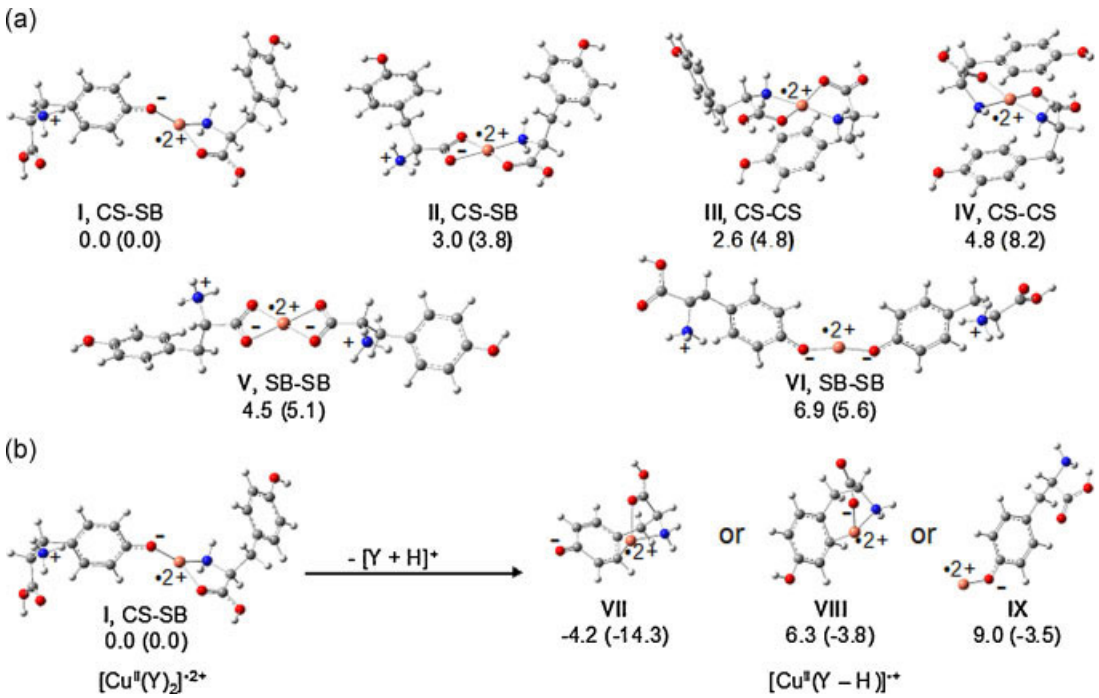

Figure 2. (a) Structures of $[Cu^{II}(Y)_2]^{\bullet 2+}$ as optimized at the B3LYP/6-31++G(d,p) level of theory. Numbers are ΔH°_0 and, in brackets, ΔG°_{298} (kcal mol^{-1}). All energies are relative to that of structure **I**. (b) Possible structures for $[Cu^{II}(Y-H)]^{\bullet +}$. Energies are for the reaction $[Cu(Y)_2]^{\bullet 2+} \rightarrow [Cu^{II}(Y-H)]^{\bullet +} + [Y+H]^{+}$.

Copyright © 2010 John Wiley & Sons, Ltd.

DOI: 10.1002/rcm

Table 1. Relative abundances of fragments from $[Cu^{II}(M)_2]^{\bullet 2+}$ at relative collision energies of 7%. (values in bold have abundances greater than 10%)

Compounds	$[Cu^{I}(M)]^{+}$	[a] $M^{\bullet +}$	[b] $[Cu^{II}(M - H)]^{\bullet +}$	$[M + H]^{+}$
$[Cu^{II}(M)_2]^{\bullet 2+}$	(%)	(%)	(%)	(%)
$[Cu^{II}(WG)_2]^{\bullet 2+}$	**48.7**	**51.2**	0.0	0.0
$[Cu^{II}(GW)_2]^{\bullet 2+}$	**50.2**	**49.8**	0.0	0.0
$[Cu^{II}(WGG)_2]^{\bullet 2+}$	**29.4**	**19.4**	**27.0**	**24.2**
$[Cu^{II}(GWG)_2]^{\bullet 2+}$	**45.1**	**41.2**	6.9	6.8
$[Cu^{II}(GGW)_2]^{\bullet 2+}$	**57.3**	**42.6**	0.0	0.0
$[Cu^{II}(YG)_2]^{\bullet 2+}$	**18.7**	**20.0**	**34.4**	**26.8**
$[Cu^{II}(GY)_2]^{\bullet 2+}$	9.7	3.0	**39.4**	**47.9**
$[Cu^{II}(YGG)_2]^{\bullet 2+}$	2.4	2.2	**53.6**	**41.9**
$[Cu^{II}(GYG)_2]^{\bullet 2+}$	4.4	5.4	**44.9**	**45.4**
$[Cu^{II}(GGY)_2]^{\bullet 2+}$	5.0	3.0	**47.1**	**44.9**

[a] Includes the secondary fragmentation product of $[z_n - H]^{\bullet +}$.
[b] Includes the products due to loss of CO_2 and attachment of H_2O and CH_3OH.

the $(z_n - H)^{\bullet +}$ ions, formed by cleavage of the N–C_α bonds of the tryptophan residues of $WGG^{\bullet +}$ and $WG^{\bullet +}$ (with accompanying neutral losses of ammonia) and of $GWG^{\bullet +}$ (with an accompanying neutral loss of glycinamide).[16] Proton transfer was observed in high abundance with only one of the tryptophan-containing peptides (Fig. 3(a)), WGG, and in low abundance with GWG (Table 1); no proton transfer products were evident in the CID of the $[Cu^{II}(GGW)_2]^{\bullet 2+}$ complex (Table 1). Previously, we had also observed the formation of abundant $[WGG + H]^{+}$ in the CID of $[Cu^{II}(dien)(WGG)]^{\bullet 2+}$.[16] Protonation of the peptide probably occurs at the terminal amino nitrogen and, as trytophan (224.7 kcal mol^{-1}) has a higher proton affinity than glycine (211.9 kcal mol^{-1}),[36] it is probable that WGG has a higher proton affinity than GWG and GGW. This then makes the proton transfer channel more competitive with radical formation in the CID of $[Cu^{II}(WGG)_2]^{\bullet 2+}$ than in those of the other complexes. The binding modes in these $[Cu^{II}(M)_2]^{\bullet 2+}$ complexes are not known, but because of the higher proton affinity of the terminal NH_2 group of WGG, at least one of the two peptides is likely to bind as a zwitterion, coordinated to the copper through the carboxyl anion with the terminal amino group protonated. Such a structure would facilitate proton transfer to the other tryptophan ligand, thereby creating $[Cu^{II}(WGG - H)]^{\bullet +}$ after proton transfer from the carboxylic group; evidence for this latter structure is provided by the abundant CO_2 loss from $[Cu^{II}(WGG - H)]^{\bullet +}$ ($m/z = 380$) (Fig. 3(b)). Proton transfer was not observed in the dissociation of $[Cu^{II}(WG)_2]^{\bullet 2+}$; this is attributed to the lower proton affinity of the smaller dipeptide WG not being competitive with radical formation.

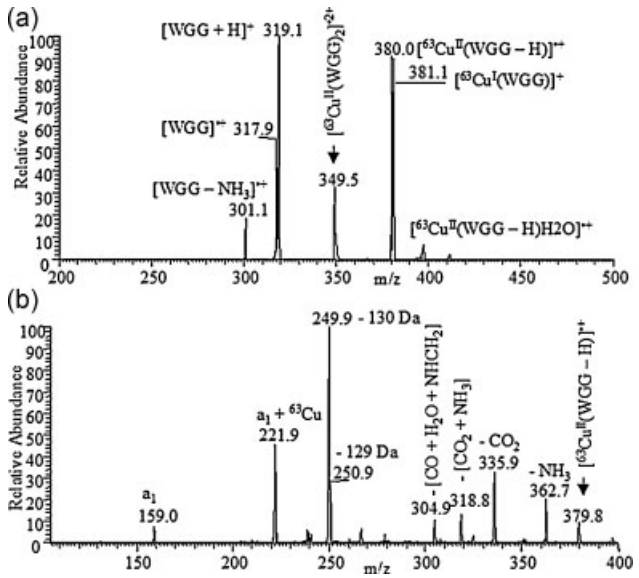

Figure 3. CID spectra of (a) $[^{63}Cu^{II}(WGG)_2]^{\bullet 2+}$ and (b) $[^{63}Cu^{II}(WGG - H)]^{\bullet +}$ at relative collision energies of 8% and 14%. The bold arrow indicates the precursor ion.

(b) M_a and M_b were different peptides

CID of complexes that contain two different peptides can, in principle, yield two different radical cations. Fragmentation of $[Cu^{II}(WG)(WGG)]^{\bullet 2+}$ produced both radical cations in high abundances (Fig. 4(a)), but there was a slightly higher abundance of the larger radical $WGG^{\bullet +}$. From the breakdown curve (Fig. 4(b)) for formation of these two radicals, the ratio $WGG^{\bullet +}/WG^{\bullet +}$ is approximately 2:1 at high collision energies. Proton transfers were very minor.

CID of $[Cu^{II}(WG)(GWG)]^{\bullet 2+}$ resulted mainly in formation of $WG^{\bullet +}$, with a low abundance of $GWG^{\bullet +}$, and a very low abundance of $[WG + H]^{+}$ (Table 2); CID of $[Cu^{II}(WG)(GGW)]^{\bullet 2+}$ yielded only $WG^{\bullet +}$ (Table 2). When the tryptophan residue was at the N-terminus of the tripeptide and the C-terminus of the dipeptide as in $[Cu^{II}(GW)(WGG)]^{\bullet 2+}$, the only major fragmentation pathway produced $WGG^{\bullet +}$. These results indicate that either the peptide radical cation with a tryptophan residue at the N-terminus is the most stable,

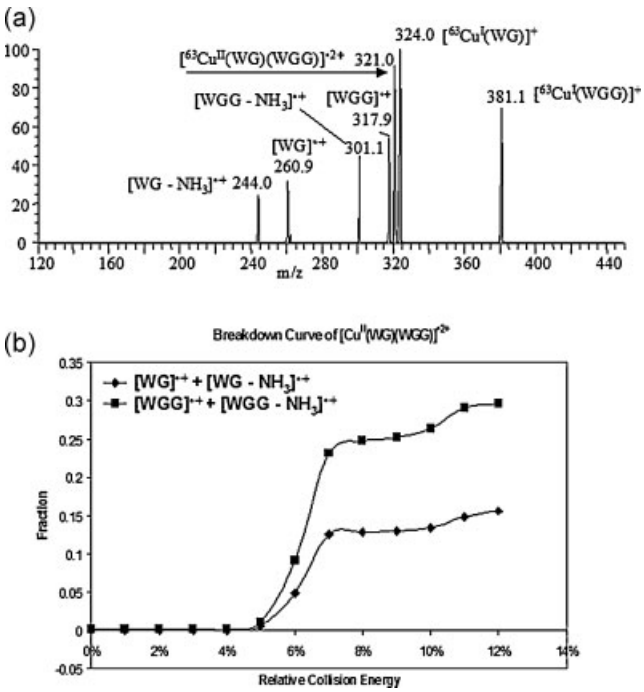

Figure 4. CID spectrum of $[^{63}Cu^{II}(WGG)(WG)]^{\bullet 2+}$ at relative collision energy of 7% and (b) energy-resolved CID of $[^{63}Cu^{II}(WGG)(WG)]^{\bullet 2+}$ for products $WG^{\bullet +}$ and $WGG^{\bullet +}$.

Copyright © 2010 John Wiley & Sons, Ltd.

DOI: 10.1002/rcm

Table 2. Relative abundances of fragments from $[Cu^{II}(M_a)(M_b)]^{\bullet 2+}$, where both M_a and M_b contained tryptophan, at relative collision energies of 7% (values in bold have abundances greater than 10%)

$[Cu^{II}(M_a)(M_b)]^{\bullet 2+}$	$[Cu^{I}(M_a)]^{+}$	$M_b^{\bullet +}$	$[Cu^{I}(M_b)]^{+}$	$M_a^{\bullet +}$	$[M_a + H]^{+}$	$[Cu^{II}(M_b - H)]^{\bullet +}$
M_a M_b	(%)	(%)	(%)	(%)	(%)	(%)
WG, WGG	**27.7**	**31.4**	**19.9**	**17.8**	1.1	2.0
WG, GWG	2.5	3.4	**47.4**	**39.4**	1.6	5.7
WG, GGW	0.0	0.0	**47.6**	**52.4**	0.0	0.0
GW, WGG	**41.0**	**40.9**	1.8	1.1	3.6	4.5
GW, GWG	**26.3**	**50.0**	5.3	5.4	6.5	6.5

or that the binding of the peptide cation to $[Cu^{I}(M)]^{+}$ is the weakest when the tryptophan residue is at the N-terminus. When neither peptide has its tryptophan residue at the N-terminus, as in $[Cu^{II}(GW)(GWG)]^{\bullet 2+}$, the major product is the larger radical cation, $GWG^{\bullet +}$, with ions $GW^{\bullet +}$ and the proton transfer product $[GW + H]^{+}$ in low abundances. When the tryptophan residues are in the same location relative to the N-terminus in two different sized peptides, e.g. in complex $[Cu^{II}(GW)(GWG)]^{\bullet 2+}$, the probable reason for preferential formation of the larger peptide as the radical cation is the greater ability of this peptide to distribute the charge. The larger peptide would also stabilize $[Cu(M)]^{+}$ more effectively, but ligand size is less important here than in the peptide radical cation.

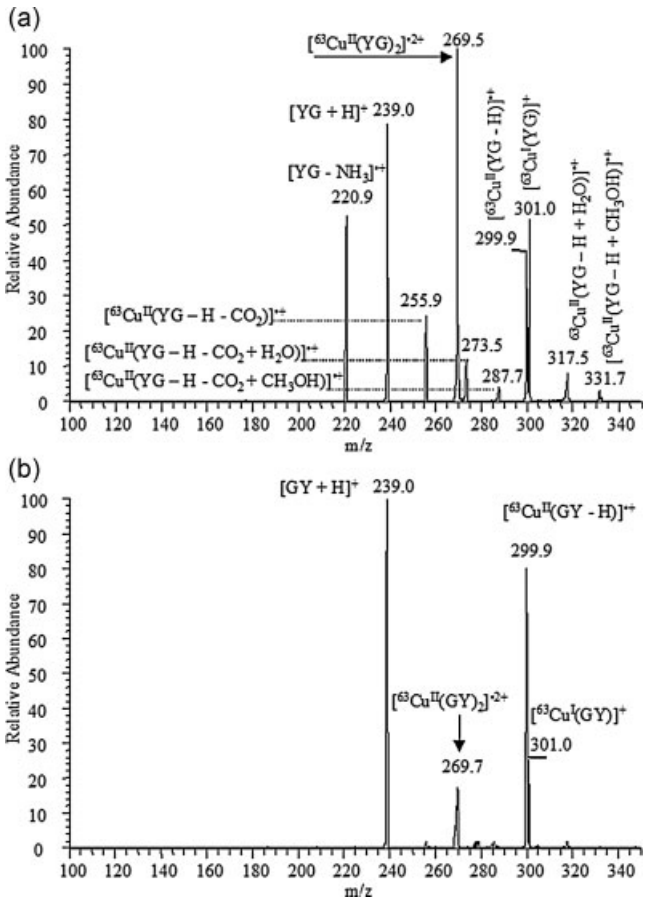

Figure 5. CID spectra of $[^{63}Cu^{II}(M_a)(M_b)]^{\bullet 2+}$: (a) $M_a = M_b = YG$ and (b) $M_a = M_b = GY$ at relative collision energy of 7%. The bold arrow indicates the precursor ion.

$[Cu^{II}(M_a)(M_b)]^{\bullet 2+}$ complexes in which both peptides contained a tyrosine residue

(a) M_a and M_b were identical

The CID spectra of $[Cu^{II}(YG)_2]^{\bullet 2+}$ (Fig. 5(a)) and $[Cu^{II}(GY)_2]^{\bullet 2+}$ (Fig. 5(b)) were the only ones in this study that showed high abundances for *both* radical-cation and proton-transfer products (Table 1). As a result of reaction 1, ions $[Cu^{I}(L)]^{+}$ at *m/z* 301 were formed; the intact radical cations, however, were not observed and this is attributed to facile cleavage of the $N-C_\alpha$ bond. In the case of $[Cu^{II}(YG)_2]^{\bullet 2+}$, the loss of NH_3 from $YG^{\bullet +}$ gave an ion at *m/z* 221; for $[Cu^{II}(GY)_2]^{\bullet 2+}$, the loss of glycinamide, $H_2NCH_2CONH_2$, from $GY^{\bullet +}$ was followed by proton transfer to the glycinamide (*m/z* 75), which lay outside of the mass range of the ion trap for MS^2 for the precursor ion at *m/z* 269.5, but was highly abundant in a triple quadrupole mass spectrometer.[37]

The products of proton transfer reactions were the protonated dipeptides, $[M_a + H]^{+}$ (the fragmentations of which have been extensively studied), and ions $[Cu^{II}(M_b - H)]^{\bullet +}$. Under the conditions required to perform CID on the precursor ions in the ion trap, some of the $[Cu^{II}(YG - H)]^{\bullet +}$ ions (*m/z* 300) became solvated by water or methanol; others lose CO_2 to give a product ion at *m/z* 256 (see Fig. 5(a)). Facile loss of CO_2 has previously been reported[38] from $[Zn^{II}(G - H)]^{+}$ and from $[Cu(M - H)(bpy)]^{\bullet +}$ ions, where M is leucine, isoleucine, or lysine.[39] The attachment of water and methanol to $[Cu^{II}(YG - H)]^{\bullet +}$, but not to $[Cu^{I}(YG)]^{+}$, indicates different binding modes with the former having an additional coordination site available.

Complexes $[Cu^{II}(M)_2]^{\bullet 2+}$, where M is YGG, GYG, or GGY, fragment predominantly by proton transfer (Table 1) and peptide radical cations are in very low abundance (spectra not shown). Unlike the $[Cu^{II}(YG - H)]^{\bullet +}$ ion, $[Cu^{II}(YGG - H)]^{\bullet +}$ does not solvate and does not lose CO_2. The low abundances of radical cation products from complexes containing tripeptides, relative to those containing dipeptides, indicate that lengthening the chain favors proton transfer.

(b) M_a and M_b were different

Complexes $[Cu^{II}(YG)(M_b)]^{\bullet 2+}$, where M_b = YGG, GYG, or GGY, dissociate predominantly by proton transfer to give $[YG + H]^{+}$ and $[Cu^{II}(M_b - H)]^{\bullet +}$ (Table 3). Radical cations $GYG^{\bullet +}$ and $YGG^{\bullet +}$ were also observed, but in very low abundance along with the complementary ion $[Cu^{I}(YG)]^{+}$. No $[M_b + H]^{+}$ was evident; apparently when the two

Copyright © 2010 John Wiley & Sons, Ltd.

DOI: 10.1002/rcm

Table 3. Relative abundances of fragments from $[Cu^{II}(M_a)(M_b)]^{\bullet 2+}$, where both M_a and M_b contained tyrosine, at relative collision energies of 7% (values in bold have abundances greater than 10%)

$[Cu^{II}(M_a)(M_b)]^{\bullet 2+}$	$[Cu^{II}(M_b - H)]^{\bullet +}$	$[M_a + H]^+$	$[Cu^{I}(M_a)]^+$	$M_b^{\bullet +}$
M_a M_b	(%)	(%)	(%)	(%)
YG, YGG	**54.4**	**41.4**	2.2	2.0
YG, GYG	**60.3**	**34.3**	3.2	2.1
YG, GGY	**60.2**	**39.8**	0.0	0.0

peptides are of different lengths, the shorter peptide is the one that is less tightly bound.

If binding in $[Cu^{II}(YG)(M_b)]^{\bullet 2+}$ parallels that in $[Cu^{II}(Y)_2]^{\bullet 2+}$, then the structure of the former that will most effectively delocalize the charge will have the tripeptide in the zwitterionic form and the dipeptide in the canonical form. As a result, the dipeptide will be less tightly bound and will be more easily lost under CID conditions; dissociation of the dipeptide would be accompanied by proton transfer, producing $[YG + H]^+$.

$[Cu^{II}(M_a)(M_b)]^{\bullet 2+}$ complexes with one peptide containing a tyrosine residue and the other a tryptophan residue

From the above, it is apparent that the presence of a tyrosine residue in a peptide favors dissociation products via proton transfer reactions while the presence of a tryptophan residue results in almost exclusive formation of peptide radical cations. As shown above, the CID spectrum of the mixed complex $[Cu^{II}(W)(Y)]^{\bullet 2+}$ showed no evidence of proton transfer, only peptide radical cation formation. It was, therefore, of considerable interest to see whether this pattern would repeat when the amino acids were replaced by peptides, one containing a tyrosine residue and the other a tryptophan residue.

(a) M_a and M_b were both dipeptides

The CID of $[Cu^{II}(WG)(YG)]^{\bullet 2+}$ and $[Cu^{II}(WG)(GY)]^{\bullet 2+}$ both yielded abundant amounts of the peptide radical cation $WG^{\bullet +}$ (Table 4). In the case of $[Cu^{II}(WG)(YG)]^{\bullet 2+}$, low abundances of $YG^{\bullet +}$ and the proton transfer products, $[YG + H]^+$ and $[Cu^{II}(WG - H)]^{\bullet +}$, were also observed. Curiously, the $[Cu^{II}(WG - H)]^{\bullet +}$ ion was not observed in the CID of $[Cu^{II}(WG)_2]^{\bullet 2+}$, but was produced from $[Cu^{II}(WG)(YG)]^{\bullet 2+}$ in very low abundance.

(b) M_a was WGG and M_b a dipeptide containing a tyrosine residue

Radical cation $WGG^{\bullet +}$ was the most abundant product in the CID of both $[Cu^{II}(WGG)(YG)]^{\bullet 2+}$ and $[Cu^{II}(WGG)(GY)]^{\bullet 2+}$. This is in accordance with the results of $[Cu^{II}(WG)(YG)]^{\bullet 2+}$ and $[Cu^{II}(WG)(GY)]^{\bullet 2+}$ in that formation of the radical cation is the dominant dissociation channel in peptides that have an N-terminal tryptophan residue. Low abundances of $[YG + H]^+$ and its complementary ion $[Cu^{II}(WGG - H)]^{\bullet +}$ were also observed in the CID spectrum of $[Cu^{II}(WGG)(YG)]^{\bullet 2+}$, which parallels those in the CID of $[Cu^{II}(WG)(YG)]^{\bullet 2+}$ (Table 4).

(c) M_a was WG and M_b a tripeptide containing a tyrosine residue

The CIDs of all $[Cu^{II}(WG)(M_b)]^{\bullet 2+}$, where M_b is YGG, GYG or GGY, gave only one radical cation, $WG^{\bullet +}$, in high abundance; the proton transfer product $[WG + H]^+$ was also observed, but always in low abundance (Fig. 6). This latter minor channel contrasts with the fragmentation of $[Cu^{II}(WG)(YG)]^{\bullet 2+}$, where the minor channel had proton transfer *away* from the tryptophan-containing peptide, giving $[Cu^{II}(WG - H)]^{\bullet +}$ and $[YG + H]^+$ (Table 4). This result can again be rationalized in terms of the larger, tripeptide being bound to $Cu^{\bullet 2+}$ as a zwitterion, and the loss of WG accompanied by proton transfer, thereby producing $[WG + H]^+$ and $[Cu^{II}(M_b - H)]^{\bullet +}$. In $[Cu^{II}(WG)(YG)]^{\bullet 2+}$, if only one peptide is bound as a zwitterion, it is likely to be the more basic one, WG; dissociation accompanied proton transfer would yield $[YG + H]^+$ and $[Cu^{II}(WG - H)]^{\bullet +}$, as observed.

(d) M_a was WGG and M_b a tripeptide containing a tyrosine residue

The products of three fragmentation pathways were observed in the CIDs of complexes $[Cu^{II}(WGG)(M_b)]^{\bullet 2+}$, where M_b is YGG, GYG, or GGY (Fig. 7). The *only* radical cation formed was $WGG^{\bullet +}$, attributable to the lower ionization energy of tryptophan relative to tyrosine; ion $WGG^{\bullet +}$ had the highest abundance of non-copper-containing ions in all the spectra. Two proton transfer channels, forming $[WGG + H]^+$ and $[M_b + H]^+$, were observed. In the CID spectrum of $[Cu^{II}(WGG)(YGG)]^{\bullet 2+}$, the abundance of $[YGG + H]^+$ is slightly higher than that of $[WGG + H]^+$ (Fig. 7(a)); this is contrary to expectations based on proton affinities. The proton affinity of tryptophan (224.7 kcal mol^{-1}) is slightly higher than that of tyrosine (221.5 kcal mol^{-1})[36] and, assuming that the peptides also protonate on the

Table 4. Relative abundances of fragments from $[Cu^{II}(M_a)(M_b)]^{\bullet 2+}$, where M_a contained tryptophan and M_b contained tyrosine, at relative collision energies of 7% (values in bold have values greater than 10%)

$[Cu^{II}(M_a)(M_b)]^{\bullet 2+}$	$[Cu^{I}(M_a)]^+$	$M_b^{\bullet +}$	$[Cu^{I}(M_b)]^+$	$M_a^{\bullet +}$	$[Cu^{II}(M_a - H)]^{\bullet +}$	$[M_b + H]^+$
M_a M_b	(%)	(%)	(%)	(%)	(%)	(%)
WG, YG	1.6	1.5	**48.4**	**44.8**	2.5	1.2
WG, GY	0.0	0.0	**51.5**	**48.4**	0.0	0.0
WGG, YG	0.0	0.0	**47.4**	**47.4**	3.0	2.1
WGG, GY	0.0	0.0	**49.9**	**49.9**	0.0	0.0

Copyright © 2010 John Wiley & Sons, Ltd.

DOI: 10.1002/rcm

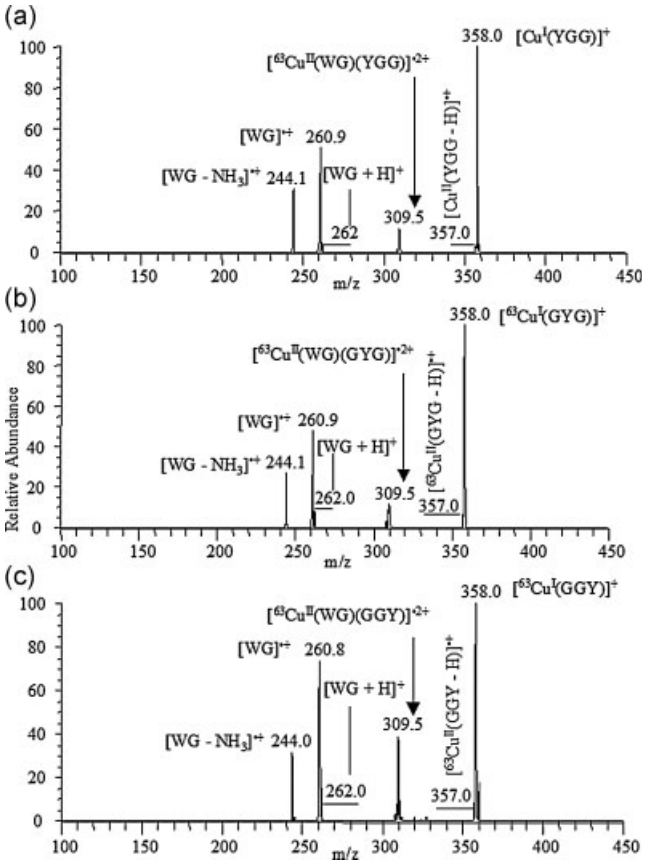

Figure 6. CID spectra of $[^{63}Cu^{II}(M_a)(M_b)]^{\bullet 2+}$ where (a) $M_a = WG$ and $M_b = YGG$; (b) $M_a = WG$ and $M_b = GYG$; and (c) $M_a = WG$ and $M_b = GGY$ at relative collision energy 7%. The bold arrow indicates the precursor ion.

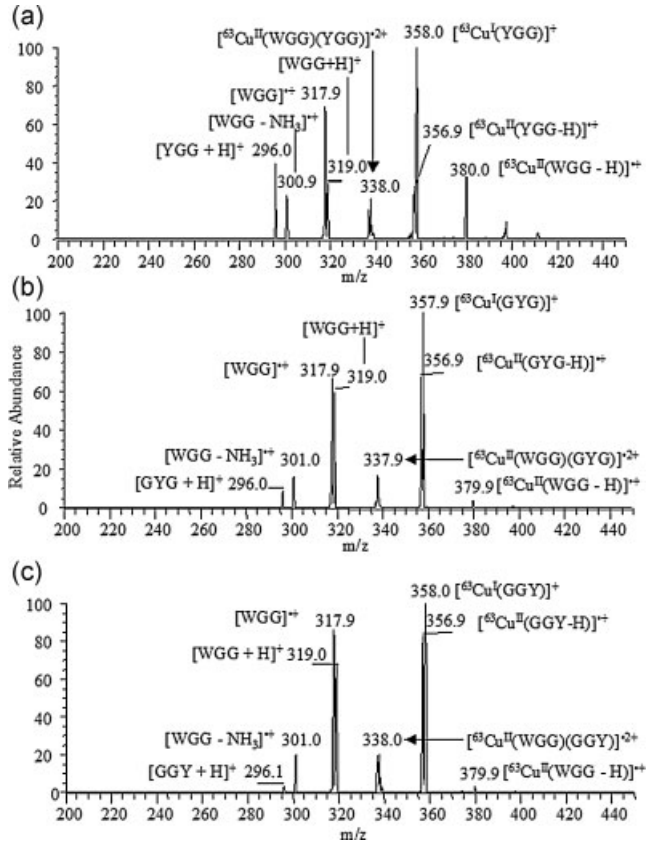

Figure 7. CID spectra of complexes of $[^{63}Cu^{II}(M_a)(M_b)]^{\bullet 2+}$ where (a) $M_a = WGG$ and $M_b = YGG$; (b) $M_a = WGG$ and $M_b = GYG$; and (c) $M_a = WGG$ and $M_b = GGY$ at relative collision energy 7%. The bold arrow indicates the precursor ion.

terminal NH_2 group, then WGG might be expected to have a slightly higher proton affinity than YGG. The other factor determining the relative abundances is the stabilizing effect of the N-terminal residues on the complementary ions, $[Cu^{II}(XGG - H)]^{\bullet +}$. The slight preference for formation of $[YGG + H]^+$ and $[Cu^{II}(WGG - H)]^{\bullet +}$ indicates that the latter factor is dominant.

In the CIDs of complexes $[Cu^{II}(WGG)(M_b)]^{\bullet 2+}$, where the tyrosine residue is not at the N-terminal ($M_b = GYG$ or GGY), ion $[WGG + H]^+$ is the dominant proton transfer product, although $[M_b + H]^+$ ions are present in low abundance (the proton affinity of glycine is 210.5 kcal mol^{-1}, which is considerably lower than that of tryptophan[36] at 224.7 kcal mol^{-1}).

SUMMARY

Collision-induced dissociations of $[Cu^{II}(M_a)(M_b)]^{\bullet 2+}$ complexes, where both M_a and M_b were peptides containing either a tryptophan or a tyrosine residue, led to the formation of radical cations of at least one of the peptides. There is one major difference in how these two classes of complexes fragment. For complexes of peptides containing *only* a tryptophan residue, radical formation was the dominant pathway and the competing proton transfer reaction channel was very minor (or non-existent), except in the CID of $[Cu^{II}(WGG)_2]^{\bullet 2+}$. The ease with which these radical cations were formed is attributed to the low ionization energy of the tryptophan residue, thereby making reduction of the copper complex by electron transfer to Cu(II) a low-energy process. By contrast, the fragmentation of tyrosine-containing complexes was dominated by proton transfer reactions, except in the CID of $[Cu^{II}(YG)_2]^{\bullet 2+}$, where radical formation is almost as abundant as proton transfer. This may be attributed to three factors: when compared with tryptophan, tyrosine has the higher ionization energy; tyrosine attaches to Cu(II) as a zwitterion through the phenolate oxygen; and peptides that contain a tyrosine residue (particularly when it is at the N-terminal) may have slightly lower proton affinities than those with a tryptophan residue.

When the $[Cu^{II}(M_a)(M_b)]^{\bullet 2+}$ complex had one peptide containing a tryptophan residue and the other a tyrosine residue, the products were predominantly radical cations of the tryptophan-containing peptide; proton transfer to the tryptophan-containing peptide was more significant, when both ligands were tripeptides. Only in the CID of $[Cu^{II}(YGG)(WGG)]^{\bullet 2+}$ was there significant extent of proton transfer to the tyrosine-containing peptide.

Within a class of complexes containing different peptides, but with only *one and not both* of the aromatic amino acids, the location of the amino acid residue in the peptide chain dictates which product is formed. In the CIDs of $[Cu^{II}(WG)(M_b)]^{\bullet 2+}$, where M_b is WGG, GWG or GGW,

Copyright © 2010 John Wiley & Sons, Ltd.

DOI: 10.1002/rcm

the radical cation $WGG^{\bullet+}$ forms preferentially to $WG^{\bullet+}$ from the first complex (i.e., $[Cu^{II}(WG)(WGG)]^{\bullet2+}$), but, from the others, $WG^{\bullet+}$ is the major product. This suggests that there is a special stability achieved by having a tryptophan residue at the N-terminus of the radical cation. In the CIDs of complexes $[Cu^{II}(YG)(M_b)]^{\bullet2+}$, where M_b is YGG, GYG or GGY, proton transfer is the dominant reaction and it is always the larger peptide that acts as the proton donor, yielding $[YG + H]^+$. We speculate that a plausible reason for this is that the larger peptide is coordinated as a zwitterion in the complex, thereby most effectively distributing the charge within the complex.

Acknowledgements

This research is supported by the Natural Sciences and Engineering Research Council (NSERC) of Canada and made possible by the facilities of the Shared Hierarchical Academic Research Computing Network (SHARCNET: http://www.sharcnet.ca). Y.K. gratefully acknowledges receipt of an Ontario Graduate Scholarship.

REFERENCES

1. Biemann K. *Mass Spectrometry Organic Chemical Applications*. McGraw-Hill: New York, 1962.
2. Biemann K, McCloskey JA. *J. Am. Chem. Soc.* 1962; **84**: 3192.
3. Jochims H-W, Schwell M, Chotin J-L, Clemino M, Dulieu F, Baumgartel H, Leach S. *Chem. Phys.* 2004; **298**: 279.
4. Grotemeyer J, Schlag EW. *Acc. Chem. Res.* 1989; **22**: 399.
5. Becker CH, Wu KJ. *J. Am. Soc. Mass Spectrom.* 1995; **6**: 883.
6. Cui W, Thompson MS, Reilly JP. *J. Am. Soc. Mass Spectrom.* 2005; **16**: 1384.
7. Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. *Science* 1989; **246**: 64.
8. Zubarev RA, Kelleher NL, McLafferty FW. *J. Am. Chem. Soc.* 1998; **120**: 3265.
9. Syka JEP, Coon JJ, Schroeder MJ, Shabanowitz J, Hunt DF. *Proc. Natl. Acad. Sci. USA* 2004; **101**: 9528.
10. Ly T, Julian RR. *J. Am. Chem. Soc.* 2008; **130**: 351.
11. Sun Q, Yin S, Julian RR. *Anal. Chem.* 2010; **82**: 3826.
12. Chu IK, Rodriquez CF, Lau T-C, Hopkinson AC, Siu KWM. *J. Phys. Chem. B* 2000; **104**: 3393.
13. Chu IK, Rodriquez CF, Hopkinson AC, Siu KWM, Lau T-C. *J. Am. Soc. Mass Spectrom.* 2001; **12**: 1114.
14. Wee S, O'Hair RAJ, McFadyen WD. *Rapid Commun. Mass Spectrom.* 2002; **16**: 884.
15. Chu IK, Siu SO, Lam CNW, Chan JCY, Rodriquez CF. *Rapid Commun. Mass Spectrom.* 2004; **18**: 1798.
16. Bagheri-Majdi E, Ke Y, Orlova G, Chu IK, Hopkinson AC, Siu KWM. *J. Phys. Chem. B* 2004; **108**: 11170.
17. Barlow CK, Wee S, McFadyen WD, O'Hair RAJ. *Dalton Trans.* 2004; 3199.
18. Wee S, O'Hair RAJ, McFadyen WD. *Int. J. Mass Spectrom.* 2004; **234**: 101.
19. Chu IK, Lam CNW, Siu SO. *J. Am. Soc. Mass Spectrom.* 2005; **16**: 763.
20. Ke Y, Zhao J, Verkerk UH, Hopkinson AC, Siu KWM. *J. Phys. Chem. B* 2007; **111**: 14318.
21. Hopkinson AC, Siu KWM. In *Principles of Mass Spectrometry Applied to Biomolecules*, Laskin J, Lifshitz C (eds). Wilson & Sons: New Jersey, 2006; 301.
22. Hopkinson AC. *Mass Spectrom. Rev.* 2009; **28**: 655.
23. Lam CNW, Siu SO, Orlova G, Chu IK. *Rapid Commun. Mass Spectrom.* 2006; **20**: 790.
24. Wee S, O'Hair RAJ, McFadyen WD. *Rapid Commun. Mass Spectrom.* 2005; **19**: 1797.
25. Barlow CK, McFadyen WD, O'Hair RAJ. *J. Am. Chem. Soc.* 2005; **127**: 6109.
26. Barlow CK, Moran D, Radom L, McFadyen WD, O'Hair RAJ. *J. Phys. Chem. A* 2006; **110**: 8304.
27. Cheng P, Bohme DK. *J. Phys. Chem. B* 2007; **111**: 11075.
28. Feketeová L, Yuriev E, Orbell JD, Khairallah GN, O'Hair RAJ. *Int. J. Mass Spectrom.* 2010; in press.
29. Siu C-K, Ke Y, Guo Y, Hopkinson AC, Siu KWM. *Phys. Chem. Chem. Phys.* 2008; **10**: 5908.
30. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Montgomery JA Jr, Vreven T, Kudin KN, Burant JC, Millam JM, Iyengar SS, Tomasi J, Barone V, Mennucci B, Cossi M, Scalmani G, Rega N, Petersson GA, Nakatsuji H, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Klene M, Li X, Knox JE, Hratchian HP, Cross JB, Bakken V, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Ayala PY, Morokuma K, Voth GA, Salvador P, Dannenberg JJ, Zakrzewski VG, Dapprich S, Daniels AD, Strain MC, Farkas O, Malick DK, Rabuck AD, Raghavachari K, Foresman JB, Ortiz JV, Cui Q, Baboul AG, Clifford S, Cioslowski J, Stefanov BB, Liu G, Liashenko A, Piskorz P, Komaromi I, Martin RL, Fox DJ, Keith T, Al-Laham MA, Peng CY, Nanayakkara A, Challacombe M, Gill PMW, Johnson B, Chen W, Wong MW, Gonzalez C, Pople JA. *Gaussian 03*, Gaussian, Inc.: Wallingford CT. 2004.
31. Becke AD. *J. Chem. Phys.* 1993; **98**: 5648.
32. Lee CT, Yang WT, Parr RG. *Phys. Rev. B* 1988; **37**: 785.
33. (a)Hehre WJ, Ditchfield R, Pople JA. *J. Chem. Phys.* 1972; **56**: 2257;; (b)Hariharan PC, Pople JA. *Chem. Phys. Lett.* 1972; **16**: 217;; (c)Chandrasekhar J, Andrade JG, Schleyer PvR. *J. Am. Chem. Soc.* 1981; **103**: 5609.
34. Campbell S, Beauchamp JL, Rempe M, Lichtenberger DL. *Int. J. Mass Spectrom Ion Processes* 1992; **117**: 83.
35. Dehareng D, Dive G. *Int. J. Mol. Sci.* 2004; **5**: 301.
36. Gronert S, Simpson DC, Conner KM. *J. Am. Soc. Mass Spectrom.* 2009; **20**: 2116.
37. Siu C-K, Ke Y, Orlova G, Hopkinson AC, Siu KWM. *J. Am. Soc. Mass Spectrom.* 2008; **19**: 1799.
38. Hoppilliard Y, Rogalewicz F, Ohanessian G. *Int. J. Mass Spectrom.* 2001; **204**: 267.
39. Seymour FL, Turecek F. *J. Mass Spectrom.* 2002; **37**: 533.

Copyright © 2010 John Wiley & Sons, Ltd.

DOI: 10.1002/rcm