

Formation of molecular radical cations of aliphatic tripeptides from their complexes with Cu^{II}(12-crown-4)

Ivan K. Chu^{1*}, S. O. Siu¹, Corey N. W. Lam¹, Jenny C. Y. Chan¹
and Christopher F. Rodriguez²

¹Department of Chemistry, The University of Hong Kong, SAR China

²Department of Chemistry, McNeese State University, 4205 Ryan Street, P.O. Box 90455, Lake Charles, LA 70609, USA

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Molecular radical cations have proven to be difficult to generate from aliphatic peptides under electrospray ionization mass spectrometry (ESI-MS) conditions. For a family of small aliphatic peptides GGX, where X=G, A, P, I, L and V, these cations have been generated by electrospraying a mixture of Cu²⁺, 12-crown-4 and GGX in methanol/water. GGX⁺ is readily formed from the collision-induced dissociation (CID) of [Cu^{II}(12-crown-4)(GGX)]²⁺. The formation of these aliphatic peptide radical ions from these complexes, in cases where it is not possible from the corresponding complexes involving a series of amine ligands instead of 12-crown-4, is likely due to the second ionization energy of the [Cu^I(12-crown-4)(GGX)]⁺ complex being higher than that of the corresponding [Cu^I(amine)(GGX)]⁺ complex. Using these 12-crown-4 complexes, GGI can be differentiated from the isomeric GGL by comparing the CID spectra of their [a₃+H]⁺ ions. Copyright © 2004 John Wiley & Sons, Ltd.

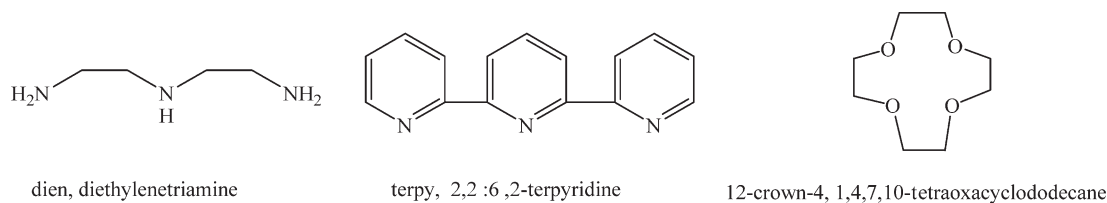
The fragmentation chemistry of protonated peptides has yielded valuable information for the identification of peptides. This is in contrast to the chemistry of radical cations of oligopeptides which, although fascinating, is still in its infancy. Peptide radicals are believed to play important and diverse roles in various biological processes such as aging and Parkinson's disease.^{1–4} They have been used as models for molecular wires in studies of electrical conduction in biological systems.⁵ They have also been used in investigations of intramolecular vibrational redistribution in unimolecular fragmentations.⁶ Despite the importance of oligopeptide radical cations in chemical and biological systems, generating these transient species has proven difficult and therefore very little is known about their chemistry.

Siu and coworkers have recently reported the generation of molecular radical cations, formed by electrospraying a mixture of [Cu^{II}(dien)]²⁺ (where dien = diethylenetriamine, Scheme 1) and the oligopeptide (M), of a number of peptides via low-energy collision induced dissociation (CID) of [Cu^{II}(dien)M]²⁺. These peptide radical cations undergo diverse fragmentations which may provide an alternative method for peptide sequencing. However, with dien as the ligand, this particular methodology is only applicable to peptides that contain tyrosyl or tryptophanyl residues.^{7–9} Several studies have extended this approach to the use of [Cu^{II}(terpy)]²⁺ (where terpy = 2,2':6',2''-terpyridine, Scheme 1) for generating peptide radical cations. In addition to peptides with tyrosyl and tryptophanyl residues, the [Cu^{II}(terpy)]²⁺ complex is also capable of generating radical cations of

oligopeptides containing basic amino acid residues such as arginine, lysine and histidine.^{9–12} A study conducted by O'Hair and coworkers showed that leucine and isoleucine residues can be differentiated using peptide radical ions; these residues are isomeric and their fragmentation chemistries in protonated peptides are virtually identical, certainly under conditions of low collision energy.¹⁰ However, [Cu^{II}(terpy)M]²⁺ tends to undergo *in situ* competitive fragmentations on the peptide backbone to yield b_n⁺ ions plus its complementary fragment ions [Cu^{II}(terpy)(M-b_n)]⁺ or proton abstraction from the peptide to the auxiliary ligand, terpy.^{11,12} Moreover, this system is not capable of generating radical cations containing only aliphatic amino acid residues, which is a very challenging problem due to the relatively high ionization energies (IEs) of aliphatic amino acids. While reliable IEs of all the free amino acids are not available, they can be estimated from the IEs of molecules corresponding to the side chains, which are obtained from NIST evaluated data. For example, the IE of leucine is 10.68 eV, while those of tyrosine, tryptophan, arginine, lysine and histidine are 8.34, 7.51, 9.10, 8.73 and 8.81 eV, respectively.¹³ Currently, no molecular radical cation of an aliphatic tripeptide has been produced successfully using this general approach.

Glycylglycylglycine (GlyGlyGly) is the simplest model system that is computationally tractable for theoretical calculations on the fragmentation of the peptide backbone; GGG⁺ is the simplest model for fragmentations of radical cations of peptides because a minimum of two residues is required to generate the oxazolone structure of [b₂-H]⁺ ion from the dissociation of the peptide.^{9,14,15} Herein we report a remarkable system, based on [Cu^{II}(12-crown-4)]²⁺ (see Scheme 1 for the structure and IUPAC name of 12-crown-4), that is capable of generating molecular radical cations of a series of aliphatic

*Correspondence to: I. K. Chu, Department of Chemistry, The University of Hong Kong, SAR China.
E-mail: ivankchu@hku.hk



Scheme 1. Structures of ligands.

tripeptides GGX, with X=G, A, P, I, L and V. This work should greatly expand the scope of investigations of molecular radical cations of peptides.

EXPERIMENTAL

All peptides and chemicals were commercially available (Aldrich and Sigma, St. Louis, MO, USA, and Bachem, King of Prussia, PA, USA). Copper(II) amine complexes were synthesized according to the experimental procedure described by Henke *et al.*¹⁶ Copper(II) crown ether complexes were prepared *in situ* by dissolving 600 mM of copper(II) nitrate with 600 mM of crown ether in a 50:50 water/methanol solution. Experiments were conducted using a quadrupole ion trap mass spectrometer (Finnigan LCQ, ThermoFinnigan, San Jose, CA, USA). Samples were typically 600 μ M in Cu^{II}-complex and 50 μ M in oligopeptide in a 50:50 water/methanol solution. These were continuously infused at a typical rate of 5 μ L/min into the pneumatically assisted electrospray probe with air as the nebulizer gas. CID spectra of [Cu^{II}-(amine)M]²⁺ and [Cu^{II}(12-crown-4)M]²⁺ were acquired using helium as the collision gas. The excitation time for CID in the ion trap was 50 ms, and the amplitude of the excitation AC was optimized in each experiment.

RESULTS AND DISCUSSION

Proton transfer from the amine groups of dien to the peptide is one of the competitive reactions for [Cu^{II}(dien)M]²⁺ complexes, especially for highly basic tripeptides M. 12-Crown-4 is a cyclic polyether ligand that has no exchangeable protons, and therefore competitive proton transfer can be avoided during the formation of radical cations of M.^{17,18} Figure 1 shows the CID spectrum of [Cu^{II}(12-crown-4)(GGW)]²⁺, with GGW⁺ as the most abundant fragment. A comparison of the product ion spectra of GGW⁺, formed by CID of (a) [Cu^{II}(terpy)(GGW)]²⁺ and (b) [Cu^{II}(12-crown-4)(GGW)]²⁺, is shown in Fig. 2; the fragmentation patterns are virtually identical, implying that the precursor ions are identical.

More significantly [Cu^{II}(12-crown-4)]²⁺ is able to produce radical cations from peptides containing only aliphatic amino acid residues. The generation of radical cations of small organic molecules is not uncommon and has been reported by several research groups.^{19,20} For example, Brodbelt and coworkers have shown the generation of aromatic radical cations via low-energy CID of doubly charged metal polyether complexes.¹⁹ Here we report what is, to the best of our knowledge, the first example of radical cations of tripeptides containing only aliphatic amino acid residues. The reasons for the enhanced capabilities of [Cu^{II}(12-crown-4)]²⁺ in the formation of peptide radicals are not obvious, considering

that the second ionization energy of [Cu^I(12-crown-4)]⁺ is higher than that of [Cu^I(terpy)]⁺. Clearly, the electron transfer reaction can become thermodynamically favorable relative to the competing reactions of proton addition to the peptide, proton abstraction from the peptide, neutral loss of ligand or peptide, and peptide fragmentation. Unfortunately, there are only sparse experimental thermochemical data available related to doubly charged transition-metal ions. Density function theory (DFT) calculations have suggested that the second ionization energy of a metal complex depends not only on the type, but also the number of coordinated ligands.¹⁸ Charge stripping experiments on ligated copper(I) complexes provide values of 18.6 and 17.0 eV for the second ionization energy of [Cu(H₂O)]⁺ and [Cu(NH₃)]⁺, respectively, in agreement with the proposed higher redox potentials of copper–oxygen than copper–nitrogen complexes.^{18,21–23} Figure 3 shows a comparison of the CID spectra of (a) [Cu^{II}(terpy)(GGP)]²⁺ and (b) [Cu^{II}(12-crown-4)(GGP)]²⁺. No radical cations are observed in the dissociation of [Cu^{II}-(terpy)(GGP)]²⁺, for which the predominant reaction channel is peptide backbone cleavage. On the other hand, the CID of [Cu^{II}(12-crown-4)(GGP)]²⁺ reveals the abundant formation of GG⁺ at *m/z* 229, together with peptide fragment ions. The presence of [z₂-H]⁺ and [a₃+H]⁺ ions in this spectrum further supports the existence of the peptide radical cations, as these fragment ions are rarely observed in low energy (<100 eV) CID spectra of protonated peptides. (In this case [a₃+H]⁺ ions can also be represented as [M-CO₂]⁺.)

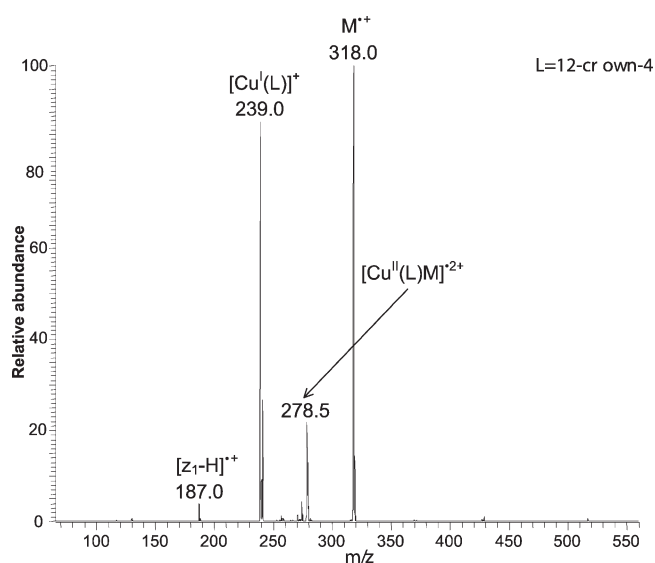


Figure 1. CID spectrum of [Cu(12-crown-4)(GGW)]²⁺ showing GGW⁺ as the most abundant fragment ion. The amplitude of the resonance excitation RF voltage was 0.35 eV.

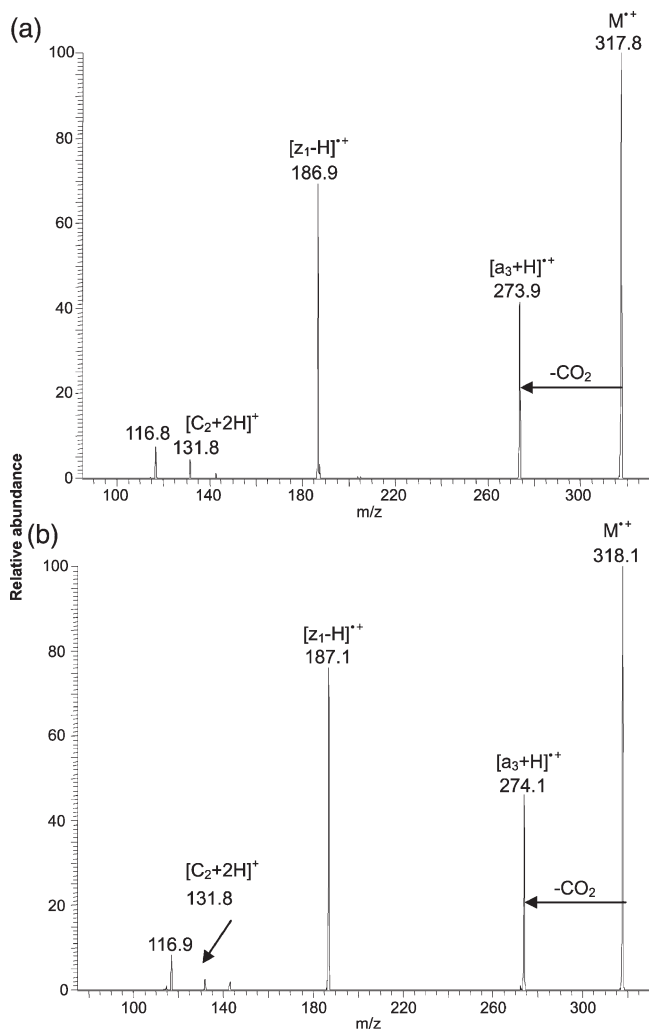


Figure 2. CID spectra of the GGW⁺ ions derived from (a) [Cu(terpy)(GGW)]²⁺ and (b) [Cu(12-crown-4)(GGW)]²⁺. The amplitudes of the resonance excitation RF voltage were 0.64 and 0.48 eV, respectively.

Peptide radical cations are also readily generated in the same manner for other aliphatic tripeptides, including GGG, GGA, GGI, GGL and GGV, although the relative abundances of the radical cations are different. As an illustration, Fig. 4 shows the CID spectrum of [Cu^{II}(12-crown-4)(GGI)]²⁺. The reduced complex [Cu^{II}(12-crown-4)]⁺ is formed at *m/z* 239, together with its complementary radical cation GGI⁺ at *m/z* 245. It is encouraging that GGI, which has a relatively high IE, can yield observable GGI⁺ although at low abundance due to its facile fragmentation. It is noteworthy that CID of the [a₃+H]⁺ ions from (a) [Cu^{II}(12-crown-4)(GGL)]²⁺ and (b) [Cu^{II}(12-crown-4)(GGI)]²⁺ yields fragmentation patterns that are significantly different, and this approach can therefore be used to differentiate between leucine and isoleucine residues (Figs. 5(a) and 5(b)). In the [a₃+H]⁺ ion derived from GGL, C₃H₇ loss from the leucine side chain is the major fragment ion, whereas, in the case of GGI, C₂H₅ loss from the isoleucine side chain predominates. This behavior is similar to that observed in the fragmentation of doubly and singly charged (deprotonated) complexes of [Cu^{II}(bpy)]²⁺ with GGI and GGL (where bpy = 2,2'-bipyridine).^{10,24,25}

Figure 6 shows the CID spectrum of GGI⁺. An interesting observation is that the [a₃+H]⁺ ion is not generated in the

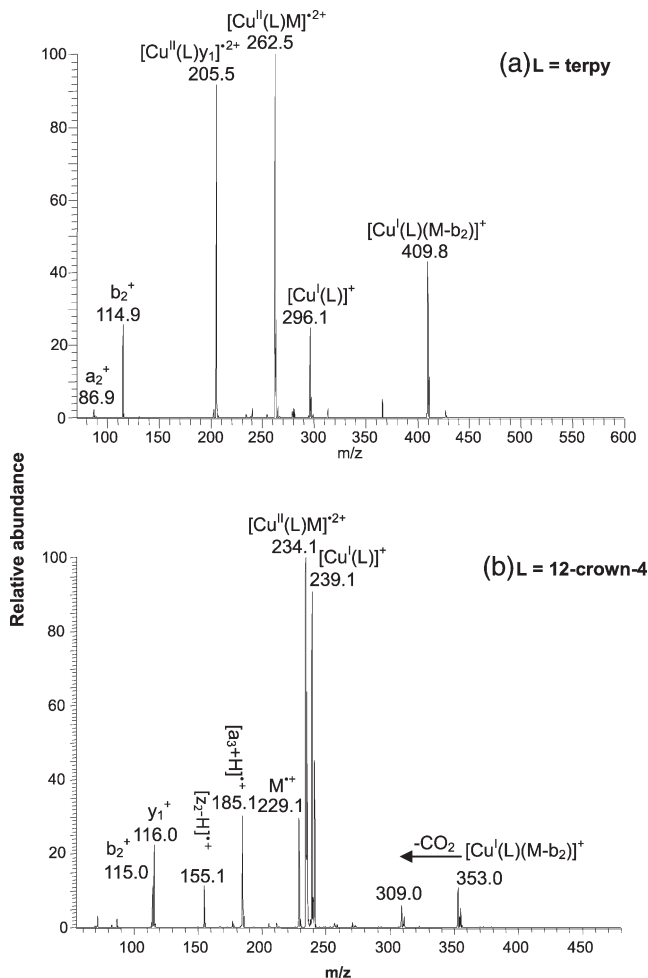


Figure 3. CID spectra of (a) [Cu(terpy)(GGP)]²⁺ and (b) [Cu(12-crown-4)(GGP)]²⁺. The amplitudes of the resonance excitation RF voltage were 0.45 and 0.52 eV, respectively.

CID of GGI⁺ at different collision energies; instead the [b₂-H]⁺ becomes the predominant fragment ion at *m/z* 114. However, in most cases, the [a₃+H]⁺ ion is the most abundant ion in the CID of [Cu^{II}(12-crown-4)(GGX)]²⁺ for all

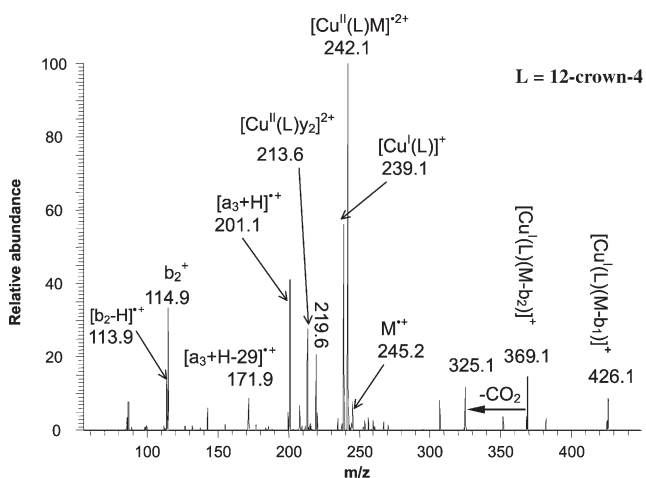


Figure 4. CID spectrum of [Cu(12-crown-4)(GGI)]²⁺. The amplitude of the resonance excitation RF voltage was 0.88 eV.

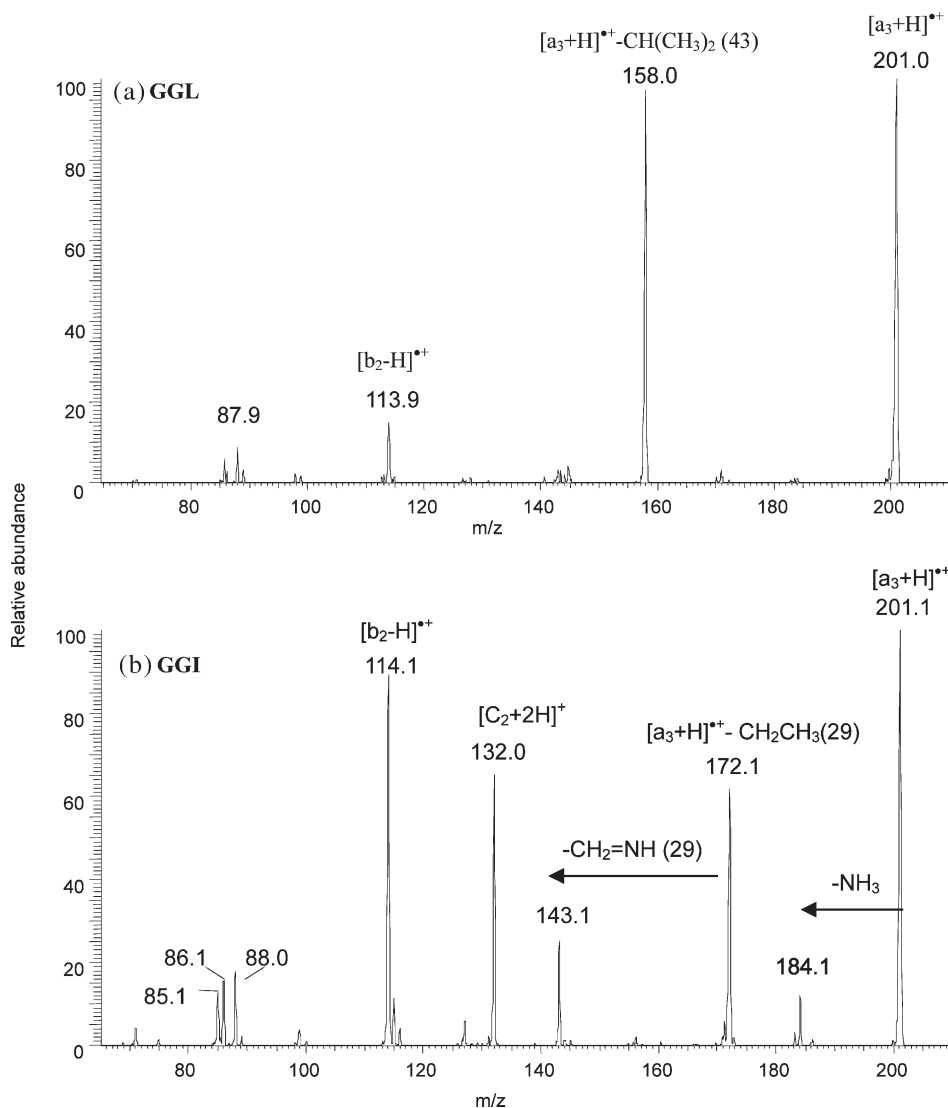


Figure 5. CID spectra of the $[a_3+H]^+$ ions derived from (a) $[\text{Cu}(12\text{-crown-4})(\text{GGL})]^{2+}$ and (b) $[\text{Cu}(12\text{-crown-4})(\text{GGI})]^{2+}$. The amplitudes of the resonance excitation RF voltage were 0.67 and 0.48 V, respectively.

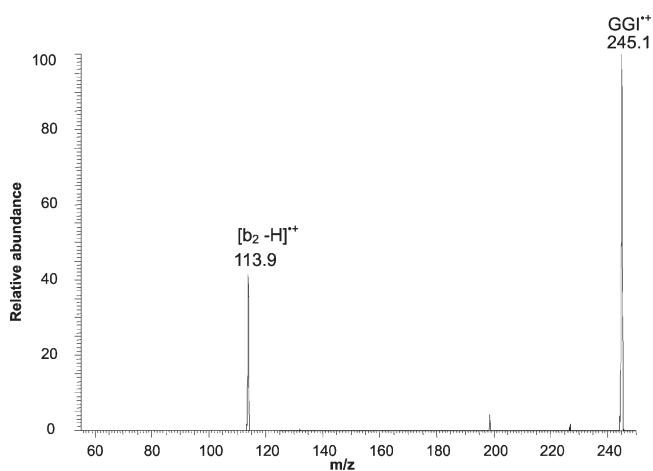


Figure 6. CID spectrum of the GGI^+ ion derived from $[\text{Cu}(12\text{-crown-4})(\text{GGI})]^{2+}$. The amplitude of the resonance excitation RF voltage is 0.53 eV.

aliphatic tripeptides, although the M^+ ion is always observed as such. Our current speculation is that two or more isomers of GGX^+ are generated initially as a result of competitive electron removal at different sites of the peptide, and one of these isomers is relatively stable while the other fragments rapidly to give the $[a_3+H]^+$ ion. The $\text{N}-\text{C}_\alpha$ bond cleavage is common in electron capture dissociation of oligopeptides.^{27–30} Similar observations of facile CO_2 loss via $\text{N}-\text{C}_\alpha$ bond cleavage have been reported in molecular cationic tripeptides with a C-terminal arginine residue,¹¹ and recent DFT calculations have given an energy requirement of only about 27.7 kcal/mol for CO_2 loss from the radical cation GW^+ .⁹ Alternatively, whether the radical fragment ion $[a_3+H]^+$ originates directly from the complexes itself, or via other fragmentation channels, requires further investigation.

CONCLUSIONS

In conclusion, we have described a new method for producing molecular radical cations of tripeptides from the

collision induced dissociation of $[\text{Cu}^{\text{II}}(12\text{-crown-4})(\text{GGX})]^{2+}$. The reagent $[\text{Cu}^{\text{II}}(12\text{-crown-4})]^{2+}$ is a very versatile complex that is capable of generating molecular radical cations of aliphatic tripeptides GGX in the gas phase. The isomeric GGL and GGI can be differentiated by significant differences in the product ion spectra of their $[\text{a}_3+\text{H}]^+$ ions. The potential of the $[\text{Cu}^{\text{II}}(12\text{-crown-4})]^{2+}$ system in the generation of radical cations of other oligopeptides or amino acids is currently under investigation.

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REFERENCES

1. Stubbe J, van der Donk WA. *Chem. Rev.* 1998; **98**: 705.
2. Fu S, Davies MJ, Stocker R, Dean RT. *Biochem. J.* 1998; **333**: 519.
3. Beckman KB, Ames BN. *Physiol. Rev.* 1998; **78**: 547.
4. Linton S, Davies MJ, Dean RT. *Exp. Gerontol.* 2001; **36**: 1503.
5. Weinkauff R, Schanen P, Metsala A, Schlag EW, Burgle M, Kessler H. *J. Phys. Chem.* 1996; **100**: 18567.
6. Cui W, Hu Y, Lifshitz C. *Eur. Phys. J. D* 2002; **20**: 565.
7. Chu IK, Rodriguez CF, Lau TC, Hopkinson AC, Siu KWM. *J. Am. Soc. Mass Spectrom.* 2001; **12**: 1114.
8. Chu IK, Rodriguez CF, Lau TC, Hopkinson AC, Siu KWM. *J. Phys. Chem. B* 2000; **104**: 3393.
9. Bagheri-Majidi E, Ke Y, Orlova G, Hopkinson AC, Siu KWM. *J. Phys. Chem. B* 2004; in press.
10. Wee S, O'Hair RAJ, McFadyen WD. *Rapid Commun. Mass Spectrom.* 2002; **16**: 884.
11. Wee S, O'Hair RAJ, McFadyen WD. *Int. J. Mass Spectrom.* 2004; **234**: 101.
12. Lam CNW, Chan JCY, Rodriguez CF, Chu IK. *Proc. 51st ASMS Conf. Mass Spectrometry and Allied Topics*, Montreal, QC, Canada, June, 2003, TPJ175.
13. (a) Hunter EP, Lias SG. In *NIST Chemistry WebBook*. Available: <http://webbook.nist.gov/chemistry/>; (b) Hunter EP, Lias SG. *J. Phys. Chem. Ref. Data* 1998; **27**: 413.
14. Yalcin T, Khouw C, Csizmadia IG, Peterson MR, Harrison AG. *J. Am. Soc. Mass Spectrom.* 1995; **6**: 1165.
15. Yalcin T, Csizmadia IG, Peterson MR, Harrison AG. *J. Am. Soc. Mass Spectrom.* 1996; **7**: 233.
16. Henke W, Kremer S, Reinen D. *Inorg. Chem.* 1983; **22**: 2858.
17. Chu IK, Lau T-C, Siu KWM. *J. Mass Spectrom.* 1998; **33**: 811.
18. (a) Tsierkezos NG, Schroder D, Schwarz H. *Int. J. Mass Spectrom.* 2004; **235**: 33; (b) Schwarz H. *Angew. Chem. Int. Edn Engl.* 2003; **42**: 4442; (c) Schröder H, Schwarz J, Wu C, Wesdemiotis C. *Chem. Phys. Lett.* 2001; **343**: 258; (d) Shi T, Orlova G, Guo J, Bohme DK, Hopkinson AC, Siu KWM. *J. Am. Chem. Soc.* 2004; Web release on 05-Jun-2004.
19. Shen J, Brodbelt JS. *J. Am. Soc. Mass Spectrom.* 1999; **10**: 126.
20. Kohler M, Leary JA. *J. Am. Soc. Mass Spectrom.* 1997; **8**: 1124.
21. Dai P, McCullough-Catalano S, Boulton M, Jones AD, Lebrilla CB. *Int. J. Mass Spectrom. Ion Processes* 1995; **144**: 67.
22. Schroder D, Barsch S, Schwarz H. *Int. J. Mass Spectrom.* 1999; **192**: 125.
23. Martinez A, Salcedo R, Sansores LE, Medina G, Gasque L. *Inorg. Chem.* 2001; **40**: 301.
24. Turecek F. In *Electrospray Ionization Mass Spectrometry: Fundamentals, Instrumentation, and Applications*, Cole RB (ed). John Wiley: New York, 1997.
25. Vaisar T, Gatlin CL, Rao RD, Seymour JL, Turecek F. *J. Mass Spectrom.* 2001; **36**: 306.
26. Seymour JL, Turecek F. *J. Mass Spectrom.* 2002; **37**: 533–U4.
27. Zubarev RA. *Mass Spectrom Rev.* 2003; **22**: 57.
28. Kjeldsen F, Haselmann KF, Sorensen ES, Zubarev RA. *Anal. Chem.* 2003; **75**: 1267.
29. Schröder D, Soldi-Lose H, Schwarz H. *Aust. J. Chem.* 2003; **56**: 443.
30. Kruger NA, Zubarev RA, Horn DM, McLafferty FW. *Int. J. Mass Spectrom.* 1999; **185/186/187**: 787.