Facile Generation of Tripeptide Radical Cations In Vacuo via Intramolecular Electron Transfer in Cu^{II} Tripeptide Complexes Containing Sterically Encumbered Terpyridine Ligands

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Molecular radical cations of tripeptides of the form glycylglycyl(residue X) (GGX^{•+}) are produced by the collision-induced, intramolecular one-electron transfer of [Cu(II)(L)GGX]^{•2+} complexes (L = triamine ligand). We demonstrate, for the first time, the formation of molecular radical cations of all of the aliphatic, basic, aromatic, acidic, and some heteroatom-bearing GGX tripeptides, albeit inefficiently in some cases, by altering the structure of the auxiliary polyamine ligand bound to the copper atom. The design of the ligand allows exquisite control over the nature of the dissociation pathway. Steric hindrance of bulky groups in the ligand affects the binding of the peptide to the copper ion; this interaction is an important factor in determining whether the electron transfer pathway predominates. (J Am Soc Mass Spectrom 2005, 16, 763–771) © 2005 American Society for Mass Spectrometry

Protein radicals are believed to play important roles in various biological processes, such as aging and neurodegenerative diseases [1, 2]. Radical cationic peptides have been examined in the gas phase as models for molecular wires in studies of electrical conduction in biological systems [3, 4]. The diverse range of fragmentations of radical cations provides an analytical means for de novo sequencing, especially for post-translational modifications and for differentiation between the isomeric leucine and isoleucine residues in peptides [5–8]. The reactivities of these radicals remain largely unexplored, however, because of difficulties in generating these species and because of their transient nature. In the gas phase, molecular radical peptides are typically generated using electron impact ionization, but this approach is limited to small molecules (e.g., tripeptides) [9, 10]. Laser desorption using multiphoton ionization circumvents the nonvolatility of peptides and is applicable to the study of peptides that contain aromatic chromophore units [3, 4, 11]. Electron capture dissociation (ECD) has been offered as an alternative approach to the conversion of multiply protonated peptides into radical protonated peptides, but this technique requires Fourier transform ion cyclotron resonance mass spectrometry and is applicable only to relatively long peptides [12]. Recently, a

similar technique that relies on electron transfer has been demonstrated on commercial ion trap instruments [13–15].

An alternative approach for generating radical cationic peptides has appeared recently: it involves the collision-induced, intramolecular one-electron transfer of [Cu^{II}(L)M]^{•2+} (L = dien, terpy) complexes generated in situ from the reaction between [Cu^{II}(L)]^{•2+} and the peptide M [16–22]. This approach is particularly interesting because there is evidence that radicals are generated from the interactions between copper(II) and both the prion protein (involved in mad cow disease) and β -amyloid peptide (involved in Alzheimer's disease) [23, 24]. Despite the importance of radical cationic oligopeptides in chemical and biological systems, our understanding of the factors that govern radical peptide formation extends only to studies of the intrinsic properties of electron transfer processes and to the competing reactions of ligated multiply charged transition metal complexes [25–27]. This approach, however, has been applicable so far only to a limited number of peptides, including those containing tyrosine, tryptophan, lysine, arginine, and histidine residues, when L is either dien or terpy; otherwise, competitive reaction channels predominate [16–20]. Recently, by using crown ether ligands, we extended this approach to generate a number of aliphatic-only radical cationic tripeptides [21].

In this study, we were interested in generating radical tripeptides and search for ligands that would suppress most of the competing reaction channels. The structures of

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Scheme 1. Structure of the ligands.

the ligands are shown in Scheme 1. The generation of radical cations of tripeptides is not an uncommon phenomenon and has been reported by several research groups. O'Hair et al. have recently explored the role of auxiliary ligands in the generation of cationic radicals via low-energy collision induced dissociation (CID) of doubly charged metal polyamine complexes with glycyl(residue X)arginine tripeptides and leucine enkephaline derivatives [22]. The system is, however, still restricted to the generation of M^{*+} species that possess basic amino acid residues. We report herein that using copper(II) complexes containing sterically hindered terpyridine ligands allows us to generate all of the aliphatic only, basic, aromatic, acidic, and some heteroatom-bearing tripeptides from a series of 19 naturally occurring tripeptides of the form GGX that differ only in the identity of their C-terminal residues. The sequences glycylglycyl(residue X) comprise the simplest prototypical tripeptide systems; they are computationally tractable for theoretical calculations that investigate the effect of the amino acid unit on the dissociation pathways because a minimum of two residues is required to generate the oxazolone structure [28, 29] of the $[b_2-H]^+$ ion. Although at present the yields of the radical cations are modest, we demonstrate that the nature of the dissociation pathway can be controlled by ligand design. In particular, steric hindrance of the bulky groups in the ligand affected the binding of the peptide to the copper ion: this interaction is an important factor in determining whether electron transfer pathway predominates. The result of our work should provide valuable information for the design of more efficient molecular radical cationic peptide formation.

Experimental

Mass Spectrometry

All mass spectrometry experiments were conducted using a quadrupole ion trap mass spectrometer (Finni-

gan LCQ, ThermoFinnigan, San Jose, CA), except for that presented in Figure 1c, in which a PE SCIEX API 150EX single quadrupole mass spectrometer (Perkin Elmer-SCIEX, Toronto, Canada) was used. Samples typically comprised 600 mM Cu(II) complex and 50 mM oligopeptide in a water/methanol (50:50) solution. These samples were infused continuously at a typical rate of 5 μ L/min into the pneumatically assisted electrospray probe using air as the nebulizer gas. CID spectra of $[Cu(II)(amine)M]^{*2+}$ complexes were acquired using helium as the collision gas. The injection time and excitation time for CID in the ion trap were 200 and 50 ms, respectively, and the amplitude of the excitation was optimized for each experiment.

Materials

All peptides and chemicals were commercially available (Aldrich and Sigma, St. Louis, MO, Bachem, King of Prussia, PA), except GGD, GGS, GGT, and GGN, which were synthesized according to literature procedures [30]. The 4'-bromo-2,2':6',2''-terpyridine ligand was purchased from HetCat (Basel, Switzerland). The 6-bromo-2,2':6',2''-terpyridine ligand and the copper(II) amine complexes were synthesized according to the experimental procedure described by Newkome et al. [31] and Henke et al. [32], respectively.

Results and Discussion

Figure 1a displays a typical electrospray mass spectrum of a mixture of $[Cu^{II}(\text{dien})](\text{ClO}_4)_2$ and tripeptide GGI. The predominant peaks are the protonated GGI monomer and dimer at 246 and 491 Th, respectively; the doubly charged $[Cu^{II}(\text{dien})\text{GGI}]^{*2+}$ at 206 Th, and the deprotonated complexes $[Cu(\text{dien} - \text{H})]^+$ at 165 Th [We have not assigned the oxidation state of copper in the deprotonated $[Cu(\text{dien} - \text{H})]^+$ since the complex can

Figure 2. CID spectra, with unimass resolution, of $[Cu(\text{amine})-(GGK)]^{2+}$ where (a) amine = dien, (b) amine = terpy. The amplitudes of the resonance excitation RF voltage were 0.39 and 0.76 eV, respectively; helium was used as the collision gas.

be either a singly charged copper(I) and a neutral (dien-H) or an ionic doubly charged copper(II) and a (dien-H)⁻, and $[Cu(\text{dien})(GGI) - H]^+$ at 410 Th, which arose as a result of proton transfer from the acidic proton of the ligand or the tripeptide to the solvent, tripeptide, or counteranion [38]. It is apparent that proton transfer can occur as long as the endoergicity can be overcome by collisional activation. Figure 1b indicates that the relative abundance of the deprotonated complexes can be minimized upon decreasing up-front CID. We speculate that the dissociation products can also be generated by residue gas collision during ion trapping because of the absence of the deprotonated complex $[Cu(\text{dien} - H)]^+$ in the electrospray quadrupole mass spectrum presented in Figure 1c. In general, the relative abundances of the doubly charged $[Cu^{II}(L)GGX]^{2+}$ ions in their respective ESI spectra are similar, but they depend on a number of experimental factors, including the purity of the complexes, the instrument's settings and the nature of the

Figure 1. Electrospray mass spectra of 600 μM $[Cu(L)]^{2+}$ and 50 mM GGI in water/methanol (50:50); (a) L = dien, capillary voltage = 10 V, tube lens offsets = 20 V, using LCQ; (b) L = dien, capillary voltage = 5 V, tube lens offsets = -60 V, using LCQ; (c) L = dien, ion spray voltage = 4500V, declustering potential (DP) = 0V, focusing potential (FP) = 50V, entrance potential (EP) = 2V, prefilter (ST) = -15.5V, using API-150EX.; (d) L = terpy, capillary voltage = 5 V, tube lens offsets = -60 V, using LCQ; and (e) L = 6,6''-Br₂-terpy, capillary voltage = 5 V, tube lens offsets = -60 V, using LCQ.

Table 1. Dissociation pathways^a of [Cu^{II}(amine)GGX]^{•2+} Systems

Ligand/Structures	Eq 1: Electron Transfer [(i)%, (ii)%] ^c	Eq 2: Proton Transfer [(iii)%, (iv)%] ^c	Eq 3: Proton Abstraction [(v)%, (vi)%] ^c	Eq 4: Peptide Fragmentation [(vii)%, (viii)%] ^{c,d,e}	Other Dissociation Pathways: [(Cu ^{II} (L)y ₁) ^{•2+} (%), (Cu ^{II} (L)y ₂) ^{•2+} (%)] ^f
<div style="text-align: center;">dien</div>	Y (22, 28) W (79, 100)	G (36, 99) A (95, 82) L (93, 92) I (91, 91) P (80, 61) V (84, 98) F (46, 56) Y (52, 80) D (98, 84) E (51, 88) H (55, 68) R (80, 90) K (46, 71) M (93, 99) Q (93, 98) S (69, 79) T (76, 44) N (93, 72)	H (31, 23) R (11, 6) K (6, 1) M (1, 3) Q (5, 7) S (14, 20) T (4, 33) N (6, 2)	G (6, b ₂ ⁺ 13) A (4, b ₂ ⁺ 5) L (4, b ₂ ⁺ 7) I (4, b ₂ ⁺ 7) P (11, b ₂ ⁺ 16) V (6, b ₂ ⁺ 8) F (5, b ₂ ⁺ 6) Y (7, b ₂ ⁺ 12) D (6, b ₂ ⁺ 7) E (2, b ₂ ⁺ 4) M (2, b ₂ ⁺ 4) Q (8, b ₂ ⁺ 11) S (4, b ₂ ⁺ 8) T (6, b ₂ ⁺ 17) N (13, b ₂ ⁺ 17)	G (5, 1) A (0, 2) L (0, 1) I (0, 1) P (4, 0) V (0, 2) F (7, 1) D (0, 1) Q (1, 1) S (1, 5) T (1, 2) N (2, 2)
<div style="text-align: center;">Me₅dien</div>	W (53, 49) R (9, 3) K (4, 6)	R (10, 26) K (3, 14)	Y (31, 16) E (90, 64) R (12, 8) K (8, 3) M (4, 6) Q (40, 36) S (23, 48) N (18, 8)	G (68, b ₁ ⁺ 32), (98, b ₂ ⁺ 62) A (68, b ₁ ⁺ 0), (93, b ₂ ⁺ 59) L (97, b ₁ ⁺ 3), (90, b ₂ ⁺ 42) I (100, b ₁ ⁺ 4), (88, b ₂ ⁺ 47) P (70, b ₁ ⁺ 0), (98, b ₂ ⁺ 72) V (96, b ₁ ⁺ 8), (62, b ₂ ⁺ 42) F (20, b ₁ ⁺ 0), (90, b ₂ ⁺ 60) Y (6, b ₁ ⁺ 0), (44, b ₂ ⁺ 23) W (5, b ₁ ⁺ 2) D (22, b ₁ ⁺ 7), (81, b ₂ ⁺ 61) E (18, b ₁ ⁺ 0), (98, b ₂ ⁺ 56) M (7, b ₁ ⁺ 0), (69, b ₂ ⁺ 49) Q (41, b ₁ ⁺ 0), (96, b ₂ ⁺ 78) S (80, b ₁ ⁺ 30), (87, b ₂ ⁺ 58) N (35, b ₁ ⁺ 0), (98, b ₂ ⁺ 46)	G (0, 23) A (0, 29) L (1, 6) I (1, 12) P (25, 2) V (1, 12) F (1, 8) Y (0, 4) D (0, 17) E (0, 32) M (1, 4) Q (19, 12) S (0, 10) N (4, 4)
<div style="text-align: center;">terpy</div>	Y W (68, 63) H (77, 45) R (12, 100) K (80, 71)	T (60, 82)	R (5, 13)	G (3, b ₁ ⁺ 0), (37, b ₂ ⁺ 15) A (4, b ₁ ⁺ 0), (93, b ₂ ⁺ 42) L (1, b ₁ ⁺ 0), (71, b ₂ ⁺ 30) I (4, b ₁ ⁺ 0), (91, b ₂ ⁺ 38) P (79, b ₂ ⁺ 42) V (2, b ₁ ⁺ 0), (66, b ₂ ⁺ 17) F (1, b ₁ ⁺ 0), (96, b ₂ ⁺ 33) Y W (14, b ₂ ⁺ 6) D (92, b ₂ ⁺ 8) E (88, b ₂ ⁺ 27) K (3, b ₂ ⁺ 1) M (78, b ₂ ⁺ 24) Q (33, b ₂ ⁺ 11) N (86, b ₂ ⁺ 23)	G (0, 2) A (22, 68) L (41, 26) I (77, 47) P (54, 1) V (29, 9) F (51, 13) Y D (4, 6) E (21, 23) H (100, 4) K (7, 3) M (60, 31) Q (68, 16) N (59, 4)

tripeptide [27, 38]. As an illustration, Figure 1d and e display the electrospray mass spectra of GGI obtained in the presence of [Cu^{II}(terpy)](ClO₄)₂ and [Cu^{II}(6,6''-Br₂-terpy)](ClO₄)₂, respectively. The use of 6,6''-Br₂-terpy as ligand tends to generate a lower abundance of [Cu^{II}(amine)M]^{•2+} ions.

Figure 2a presents a typical CID spectrum of

[Cu^{II}(dien)(GGK)]^{•2+} ("dien" = diethylenetriamine); no radical cation is observed, but the predominant product ions are protonated GGK and the deprotonated complex of [Cu(dien-H)]⁺. Radical cationic GGX^{•+} is produced through an electron transfer reaction only for [Cu^{II}(dien)(GGX)]^{•2+} complexes in which X is a tyrosine or tryptophan residue, i.e., tripeptides that have

Table 1. Continued

Ligand/Structures	Eq 1: Electron Transfer [(i)%, (ii)%] ^c	Eq 2: Proton Transfer [(iii)%, (iv)%] ^c	Eq 3: Proton Abstraction [(v)%, (vi)%] ^c	Eq 4: Peptide Fragmentation [(vii)%, (viii)%] ^{c,d,e}	Other Dissociation Pathways: [(Cu ^{II} (L)y ₁) ^{•2+} (%), (Cu ^{II} (L)y ₂) ^{•2+} (%)] ^f
6-Br-terpy	Y (64, 27) W (97, 35) H (76, 41) R (99, 48) K (93, 53) P (36, 5)	—	G (0, 1) A (0, 4) L (3, 8) I (7, 14) P (5, 28) V (4, 7) Y (3, 4) E (9, 8) H (66, 73) R (6, 15) K (1, 9) M (5, 4) Q (18, 13) S (1, 31) T (0, 14)	G (80, b ₂ ⁺ 9) A (89, b ₂ ⁺ 26) L (56, b ₂ ⁺ 28) I (85, b ₂ ⁺ 40) P (25, b ₂ ⁺ 26) V (95, b ₂ ⁺ 33) F (90, b ₂ ⁺ 22) Y (8, b ₂ ⁺ 4) D (35, b ₂ ⁺ 9) E (90, b ₂ ⁺ 18) M (91, b ₂ ⁺ 8) Q (19, b ₂ ⁺ 3) N (25, b ₂ ⁺ 5)	G (1, 7) A (19, 21) L (87, 40) I (94, 26) P (82, 3) V (73, 14) F (46, 4) D (1, 1) E (20, 12) M (36, 9) Q (99, 2) N (22, 4)
6,6''-Br ₂ -terpy	G (24, 32) ^b A (96, 26) ^b L (99, 62) I (92, 26) P (82, 20) V (92, 31) F (95, 18) Y (79, 82) W (91, 80) E (86, 22) H (42, 47) R (79, 65) K (85, 54) M (81, 35) Q (34, 8) T (11, 14)	T (21, 30)	G (32, 72) A (25, 63) L (23, 44) I (27, 45) P (74, 91) V (30, 64) F (18, 17) Y (23, 13) D (58, 25) E (40, 58) H (68, 81) R (14, 12) K (12, 12) M (35, 23) Q (99, 59) S (6, 38) T (22, 30) N (12, 16)	G (18, b ₂ ⁺ 14) A (15, b ₂ ⁺ 13) L (7, b ₂ ⁺ 24) I (5, b ₂ ⁺ 20) P (1, b ₂ ⁺ 2) V (12, b ₁ ⁺ 0), (24, b ₂ ⁺ 14) F (18, b ₂ ⁺ 20) D (20, b ₂ ⁺ 14) E (8, b ₂ ⁺ 8) M (23, b ₂ ⁺ 18) S (2, b ₂ ⁺ 1) N (3, b ₂ ⁺ 3)	G (0, 32) A (48, 38) L (56, 21) I (53, 20) P (34, 1) V (85, 34) F (57, 8) D (39, 44) E (10, 6) M (19, 2) Q (40, 0) N (62, 0)

^aNomenclature follows that provided in eqs 1–4.

^bA much lower abundance of M^{•+} ions formed in the GGG and GGA systems. This phenomenon may be due to facile fragmentation of the M^{•+} ions. We attribute the presence of diagnostic fragment ions, such as [a₃ + H]^{•+} and [b₂ – H]^{•+}, to dissociation of the M^{•+} radical cations.

^cRelative abundances as a percentage of product ions are presented (consecutive fragment ions of [a₃ + H]^{•+} and [z₃ – H]^{•+} are included), where (i) = [Cu^I(L)]⁺, (ii) = M^{•+}, (iii) = [Cu^{II}(L – H)]⁺, (iv) = [M + H]⁺, (v) = [Cu^{II}(M – H)]⁺, (vi) = [L + H]⁺, (vii) = [Cu^I(L)(M – b_n)]⁺ and (viii) = b_n⁺ (Scheme 1).

^dTwo dissociation pathways are involved in Eq 4, namely 1) [Cu^{II}(L)(M – b₁)]⁺ and b₁⁺ and (2) [Cu^{II}(L)(M – b₂)]⁺ and b₂⁺.

^eRelative abundances (%) of fragment ions in Eq 4 are reported in the order ([Cu^{II}(L)(M – b₁)]⁺, b₁⁺), ([Cu^{II}(L)(M – b₂)]⁺, b₂⁺).

^fNeutral losses of one glycine unit [Cu^{II}(L)y₂]^{•2+} and two glycine units [Cu^{II}(L)y₁]^{•2+} from the parent ion [Cu^{II}(L)M]^{•2+}, with the charge still located at the C-terminus, are reported.

relatively low ionization energies. The reasons why the competitive reactions overwhelm the electron transfer process for the other [Cu^{II}(dien)(GGX)]^{•2+} ions are not obvious when comparing the second ionization energy of bare copper (IE_{Cu} = 20.3 eV, the highest second ionization energy among the first-row transition metals) [33] with the ionization energies of those amino acids (IE_{amino acids} = 7–11 eV; since reliable ionization energies are known for only a few of the free amino acids, we estimated values for the free amino acids and residues by comparison with the ionization energies of

the side chains provided in NIST-evaluated data) [33]. It seems as though the oxidation of the peptide is always thermodynamically favorable and exoergic. The ionization energy of transition metal complexes, however, are greatly influenced by their ligation, which depends not only on the types of ligands but also the number of them that are chelated, irrespective of whether these ligands are water, ammonia, crown ether, or polyamine moieties. Aliphatic-only radical cationic tripeptides could be generated by substituting the dien ligand with oxygen donor ligands supplied by a crown ether [21].

Figure 3. CID spectra, with open resolution, of $[Cu(\text{amine})(GGP)]^{2+}$ where (a) amine = terpy, (b) amine = 6-Br-terpy. The amplitudes of the resonance excitation RF voltage were 0.50 and 0.64 eV, respectively; helium was used as the collision gas.

As a result, it seems that electron transfer from the peptide to the $[Cu^{II}(\text{amine})]^{2+}$ complex may not always be more favorable than the competitive reactions.

It has been observed that different competitive product ions of $[Cu^{II}(\text{amine})(GGX)]^{2+}$ can be formed upon changing the ligand, the peptide sequence, or the peptide composition [17, 20, 22]. We speculate that the energy barriers of the competitive reactions are comparable to one another. A similar situation has been observed in metal hydrates where electron transfer and proton transfer reactions compete. For these reactions, relative ion abundances are in qualitative agreement with relative barriers of density functional theory calculations [25, 34]. To elucidate the various reaction pathways that occur upon the CID of copper(II) tripeptide complexes, initially we conducted a systematic study of $[Cu^{II}(\text{dien})(GGX)]^{2+}$ complexes. Based on our present results and those of previous studies, there are four known competitive collision-induced dissociation pathways for $[Cu^{II}(L)(GGX)]^{2+}$ ions, including radical peptide formation (eq 1) [16–22, 35, 36], proton transfer to the peptide (eq 2) [16–22, 35–37], proton abstraction from the peptide (eq 3) [16–19, 25], and peptide fragmentation (eq 4) [16–22, 26, 27, 35, 36].

For $[Cu^{II}(\text{dien})(GGX)]^{2+}$, radical peptides $M^{\bullet+}$ are generated through an electron transfer reaction from only two of the 19 tripeptides in which X is a tyrosine or tryptophan residue, i.e., tripeptides that have relatively low ionization energies; for the other tripeptides, the major dissociation pathways are proton transfer and peptide fragmentation (Table 1). Because of the high gas phase basicities of the tripeptides, from the least-basic (GGG) to most-basic (GGR), range from 214 to 243 kcal mol⁻¹ [38], we ascribe the favorable proton transfer reaction of $[Cu^{II}(\text{dien})(GGK)]^{2+}$ to the high proton affinity of the tripeptide GGG (232 kcal mol⁻¹) [38]. The proton transfer may occur not only from one of the exchangeable amino hydrogen atoms of dien [16–20, 22, 37, 39] but also possibly from the acidic α = C–H hydrogen atoms [40].

Proton transfer may be blocked or moderated by

Figure 4. CID spectra, with open resolution, of $[Cu(6,6''\text{-Br}_2\text{-terpy})M]^{2+}$ where (a) M = GGP, (b) M = GGF, (c) M = GGQ. The amplitudes of the resonance excitation RF voltage were 0.54, 0.59 and 0.55 eV, respectively; helium was used as the collision gas.

Figure 5. CID spectra, with unimass resolution, of $[\text{Cu}(\text{amine})(\text{YGGFL})]^{2+}$ where (a) amine = terpy, (b) amine = 6,6''-Br₂-terpy. The amplitudes of the resonance excitation RF voltage were 0.91 and 1.78 eV, respectively; helium was used as the collision gas.

deliberately using ligands, such as *N,N,N',N',N''*-pentamethyldiethylenetriamine (Me₅dien), and 2,2':6',2''-terpyridine (terpy) [17–20], which do not possess NH protons. When L = Me₅dien, however, proton transfer is feasible only for basic tripeptides (X = arginine or lysine); the proton abstraction and peptide fragmentation predominate for most tripeptides, and radicals are formed from three peptides (Table 1). Figure 2b displays the product ion spectrum of $[\text{Cu}^{\text{II}}(\text{terpy})(\text{GGX})]^{2+}$; instead of proton transfer forming protonated GGK, as occurred in the case of the $[\text{Cu}^{\text{II}}(\text{dien})(\text{GGX})]^{2+}$ ion, electron transfer in $[\text{Cu}^{\text{II}}(\text{terpy})(\text{GGX})]^{2+}$ occurred to generate mainly the radical cation $\text{GGK}^{\bullet+}$. Five of the complexes $[\text{Cu}^{\text{II}}(\text{terpy})(\text{GGX})]^{2+}$ exhibit evidence of $\text{M}^{\bullet+}$ formation. The system is restricted to those GGX tripeptides that contain either a basic residue, [16–19] (lysyl, arginyl, or histidyl), or an aromatic residue that has a relatively low ionization energy from 7.5 to 8.8 eV [33], i.e., tyrosyl or tryptophanyl. For the terpy-containing systems, facile peptide cleavage is the prominent reaction pathway that competes with peptide radical formation (Table 1). For example, peptide fragmentation of $[\text{Cu}(\text{terpy})(\text{GGP})]^{2+}$ yields b_2^+ and its complementary fragment $[\text{Cu}(\text{terpy})(\text{GGP} - \text{b}_2)]^+$ through a charge separation. Alternatively, if the charge is retained in the C-terminal fragment, a $[\text{Cu}(\text{terpy})_2]^{2+}$ ion is produced (Figure 3a). Peptide fragmentation, as opposed to peptide cleavage from Cu, may be a result of a strong peptide–Cu bond. Reduction of the strength of the peptide–Cu interaction,

e.g., by introducing bulky groups on the ligand to hinder the binding between the Cu ion and the peptide, may enhance the amount of peptide cleavage and reduce the amount of peptide fragmentation.

Electron transfer from the peptide to Cu is facilitated by strong π -acceptor ligands, such as terpy. Placing electron-withdrawing groups on the terpy ligand should further enhance the electron-transfer pathway. The results we obtained when L = 4'-Cl-terpy or 4'-Br-terpy are, however, essentially the same as those when L = terpy. Thus, electronic effects are insignificant.

To examine steric effects, we investigated terpy ligands that contain Br substituents at the 6- and 6', 6''-positions. For most of the GGX tripeptides, the use of 6-bromo-2,2':6',2''-terpyridine (6-Br-terpy) successfully eliminated the proton transfer, and peptide fragmentation became much less substantial, although it was still observable. The formation of molecular radical peptides occurred, albeit inefficiently, when X = isoleucine, leucine, or proline, but proton abstraction was facile at the same time to generate the protonated ligand $[(6\text{-Br-terpy} + \text{H})]^+$ and its complementary fragment ion of the deprotonated complex of $[\text{Cu}(\text{GGX-H})]^+$ as shown in Figure 3b. On the other hand, when the sterically more encumbered 6,6''-dibromo-2,2':6',2''-terpyridine (6,6''-Br₂-terpy) was used as the ligand, we were able to generate radical cations of all of the aliphatic only, basic, aromatic, and acidic—and some of the heteroatom-bearing—tripeptides GGX except for those in which X = serine, asparagine, or aspartic acid; eleven of these radical cations were not observed when L = dien, Me₅dien, or terpy (Table 1). Figure 4a, b, and c show typical CID spectra of $[\text{Cu}(6,6''\text{-Br}_2\text{-terpy})(\text{GGX})]^{2+}$ yields $\text{GGX}^{\bullet+}$ through a electron transfer and its consecutive fragment ions due to facile fragmentation of the $\text{M}^{\bullet+}$ ions.

Figure 5 compares the results of the use of $[\text{Cu}^{\text{I}}(\text{terpy})(\text{M})]^{2+}$ versus $[\text{Cu}^{\text{II}}(6,6''\text{-Br}_2\text{-terpy})(\text{M})]^{2+}$ when M is a relatively long peptide, YGGFL. For the former system, peptide fragmentation to give $[\text{Cu}^{\text{I}}(\text{terpy})(\text{M} - \text{b}_n)]^+$ and b_n^+ (and their secondary fragments, a_n^+) predominate, while for the latter, peptide fragmentation is absent and electron cleavage to give $\text{YGGFL}^{\bullet+}$ is evident. This observation is in accordance with the expectation of a weaker Cu–YGGFL interaction in $[\text{Cu}^{\text{II}}(6,6''\text{-Br}_2\text{-terpy})(\text{M})]^{2+}$ because of the presence of the bulkier 6,6''-Br₂-terpy ligand.

Conclusions

We have demonstrated the formation of molecular radical cations of GGX tripeptides that lack basic or aromatic residues, albeit inefficiently in some cases, by altering the structure of the auxiliary ligand on the copper atom. The nature of the dissociation pathways can be controlled by ligand design. The steric hindrance of the ligand in the complex is one of the

important factors that govern whether the electron transfer pathway predominates. The 6,6''-Br₂-terpy ligand is a versatile ligand that is capable of facilitating the formation, in the gas phase, of radical cations from a wide variety of tripeptides. We are currently searching for a more efficient system for the formation of peptide radical cations by investigating the effects that various other ligand substituents on the electron transfer process.

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