Amino Acid Influence on Copper Binding to Peptides: Cysteine Versus Arginine

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Matrix assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry (MS) and theoretical calculations [density functional theory (DFT)] were utilized to investigate the influence of cysteine side chain on Cu^+ binding to peptides and how Cu^+ ions competitively interact with cysteine ($-SH/SO_3H$) versus arginine. Results from theoretical and experimental (fragmentation reactions) studies on $[M + Cu]^+$ and $[M + 2Cu - H]^+$ ions suggest that cysteine side chains ($-SH$) and cysteic acid ($-SO_3H$) are important Cu^+ ligands. For example, we show that Cu^+ ions are competitively coordinated to the $-SH$ or SO_3H groups; however, we also present evidence that the proton of the SH/SO_3H group is mobile and can be transferred to the arginine guanidine group. For $[M + 2Cu - H]^+$ ions, deprotonation of the $-SH/SO_3H$ group is energetically more favorable than that of the carboxyl group, and the resulting thiolate/sulfonate group plays an important role in the coordination structure of $[M + 2Cu - H]^+$ ions, as well as the fragmentation patterns. (J Am Soc Mass Spectrom 2010, 21, 522–533) © 2010 American Society for Mass Spectrometry

Copper ions (Cu^+, Cu^{2+}) play important roles in many chemical and biochemical processes, including oxidation, dioxygen transport, and electron transfer and, in many cases, the functions in these processes result from copper ions either as mono-metal or multi-metal complexes of peptides or proteins [1–5]. Redox chemistry ($Cu^{2+} \leftrightarrow Cu^+$) is involved in many enzymatic processes, but there are numerous examples, such as copper chaperone proteins and copper transport proteins, where the biological processes are limited to a single oxidation state, i.e., Cu^{2+} or Cu^+ [6]. Most of our knowledge concerning copper in biological systems is derived from solution and solid-state studies [7]; however, studies of gas-phase Cu-peptide and Cu-protein interactions may provide new understanding of peptide-metal ion interactions in low dielectric environments [8–11]. Although comparisons of gas-phase and solution-phase data could potentially yield important information regarding solvent dependent Cu-biomolecule interactions, the ligand interactions in gas phase and solution phase are typically very different. For example, in solution, the side chains of basic amino acids (arginine, lysine, and histidine) are protonated, which reduces the Cu^+ and Cu^{2+} binding energies of N-donor ligands, whereas in the gas phase, Cu^+ and Cu^{2+} have strong preferences for binding to arginine, lysine, and histidine [12, 13].

Much of the previous work on gas-phase peptide–Cu^+ and peptide–Cu^{2+} complexes was directed toward the relative binding energies of Cu^+ and Cu^{2+} to specific amino acids and how the relative Cu ion binding energies affect the fragmentation reactions of the complex [12–18]. More recently, Lim and Vachet developed a methodology based on metal-catalyzed oxidation (MCO) reactions and mass spectrometry (MS) to determine the Cu^{2+} binding sites in copper metalloproteins [19]. They utilize MCO reactions to oxidize the amino acids in the Cu^{2+} binding sites and MS to identify the amino acids that have been oxidized [20, 21]. We recently reported the synthesis of a "paddle-wheel" dinuclear copper matrix that afforded new capabilities for studies of both mono-metal and multi-metal containing peptide complexes [22]. These studies clearly illustrated that the C-terminal carboxyl group is an important Cu^+ ligand, especially for multi-copper peptide ions such as $[M + 2Cu - H]^+$, whereas the C-terminal amide or the methyl ester group has less influence on the Cu^+ coordination. The differences in the binding sites for the mono-metal and di-metal species arise as a result of deprotonation of the acidic C-terminal carboxyl and, as a consequence, the Cu^+ ions are charge-solvated by the resulting carboxylate, the lysine €-amino group, and the backbone amide groups [22].

In biological systems, cysteine is an important ligand in terms of the functions of copper proteins [23–26], and 35% of the copper (Cu^+, Cu^{2+}) ligands are cysteine residues [27–29]. For example, copper-zinc superoxide dismutase (Cu/Zn SOD), a copper containing protein, which catalyzes the conversion of superoxide anion (O_2^-) into O_2 and H_2O_2, protects cells against oxidative stress [30]. It has been suggested that cysteine (position 111) in Cu/Zn SOD is a potential copper ligand that is responsible for the function of Cu/Zn SOD [26]. Cys111

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is also a primary target for oxidative modification, and the so-called "copper hypothesis" suggests that copper bound to or released by mutant SOD generates free radicals that cause oxidative damage to motor neurons [25]. These studies suggest that understanding how copper ions (Cu^+, Cu^{2+}) interact with cysteine residues is essential to understanding the biological functions of copper proteins.

In this paper, we investigate the fragmentation chemistry of $[M + xCu - (x–1)H]^+$ (x = 1,2) ions in an effort to determine the influence of the cysteine residue on the Cu^+ binding, specifically competition for Cu^+ between cysteine and the guanidine group of arginine. Theoretical calculations are also used to generate the candidate structures of the peptide–metal ion complex, which provide theoretical evidences to support our experimental observations. In related work, Vachet and coworkers compared the effects of ligand donor group on dissociation of Cu(II) complexes, and their results suggest that Cu^{2+} ions prefer to remain coordinated to thio group rather than other functional groups such as amino and imidazole [31, 32]. Kass and coworkers utilized H/D exchange experiments and high level calculations to compare the gas-phase acidities of the cysteine thiol group and carboxyl group [33]. They reported that the side-chain thiol in cysteine is more acidic than the carboxyl group in gas phase, and deprotonated cysteine is thus predicted to be a thiolate ion [33]. If this is the case, the deprotonation of the thiol group would be highly competitive with that at the carboxyl group. For peptides containing both cysteine and arginine residues, it appears likely that the thiolate group could function as an important Cu^+ ion ligand. This study focuses on the competitive binding of Cu^+ ions to peptides that contain both cysteine and arginine.

Experimental

Copper oxide (CuO) and α-cyano-4-hydroxycinnamic acid (CHCA) were obtained from Sigma (St. Louis, MO, USA). CHCA was recrystallized in ethanol before use. The α-cyano-4-hydroxycinnamic acid (CHCA) copper salt $(CHCA)_4Cu_2$ was synthesized as previous described [22]. The organic solvents (CH_3OH, CH_3CN) used for mass spectrometry were HPLC grade, and the water was in high purity (18MΩ; Barnstead International, Dubuque, IA, USA). Laminin (925-933) (CDPGYIGSR) and Cys-Kemptide (CLRRASLG) were purchased from Sigma (St. Louis, MO, USA) and used without further purification. Cysteine residues were alkylated [34] or oxidized [35] following standard protocols.

Mass Spectrometry

The MALDI experiments described herein were performed on a tandem time-of-flight mass spectrometer (4700 Proteomics Analyzer, Applied Biosystems, Framingham, MA, USA). Tandem mass experiments were performed using a collision energy setting of 1 kV and air as the collision gas. $(CHCA)_4Cu_2$ (10 mg/mL in 60% acetonitrile containing 0.1% trifluoacetic acid) was used as the MALDI matrix to generate $[peptide + xCu - (x–1)H]^+$ ions [22]. Peptide samples (0.5 nmol) were mixed with matrix solution at a ratio of 1:3 (vol:vol) and a 1 μL aliquot was deposited to a stainless steel target. Samples were dried in air at room temperature.

Theoretical Calculations

Theoretical calculations using density functional theory (DFT) were performed to gain a better understanding of the stability of the peptide-Cu ion structures. The B3LYP LACV3P basis set (DFT/B3LYP/LACV3P**)

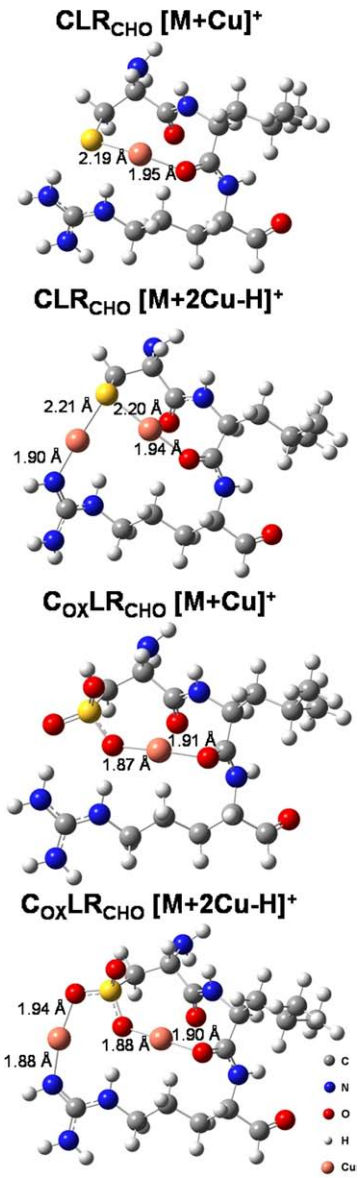


Figure 1. Lowest energy candidate structures obtained at the DFT/B3LYP/LACV3P** level for the $[M + Cu]^+$ and $[M + 2Cu - H]^+$ ions of peptides CLR_{CHO} and $C_{ox}LR_{CHO}$. The "$_{CHO}$" denotes an aldehyde C-terminus. For comparison purposes, other candidate structures are included in the Supplemental Information.

was used for these studies [36–38]. The LACV3P basis set is a triple-ζ contraction of the LACVP basis set [39] developed and tested at Schrödinger, Inc. [40]. In particular, Cu ions were treated using effective core potentials, while all the other atoms were treated with the 6-311G** basis set. No symmetry restriction of any kind was imposed in the process of geometry optimization. A vibration frequency analysis was performed for all the optimized structures at the level of calculation employed. For the reported structures, all frequencies are observed to be real, indicating that they correspond to the true minima in the respective potential energy hypersurfaces. All calculations were performed with the Jaguar 6.0 software using the pseudospectral method to minimize the computational time [40].

Candidate structures were constructed to study the copper ion(s) attachment to a small, model peptide sequence CLR. Initial calculations of copper ion(s) interacting with the oxidized and non-oxidized form of the Cys side chain were performed to minimize the computational time. As a result, the backbone orientations and docking sites of the copper ion(s) for both forms of the Cys side chain were obtained. Analogous, backbone orientations and docking sites for the Arg side chain were explored. These configurations were used as starting geometries for the peptide sequence

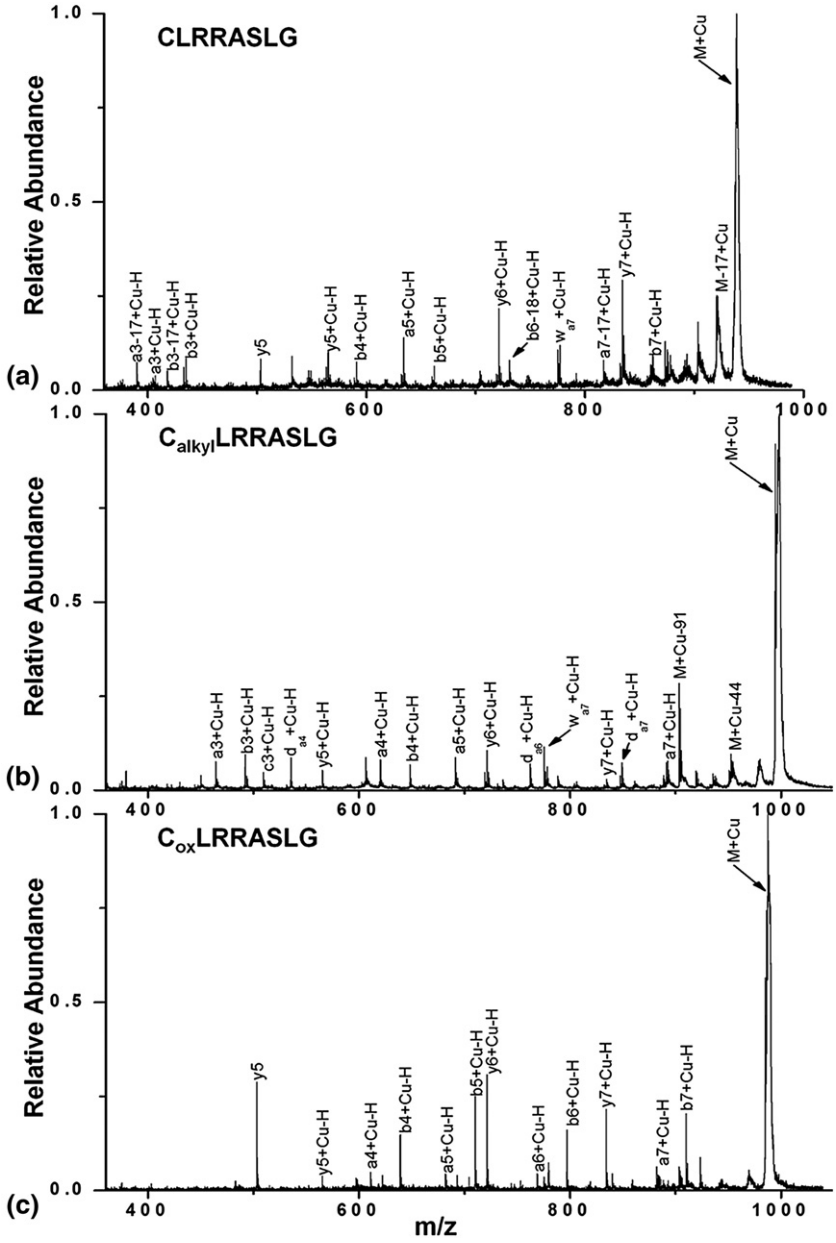
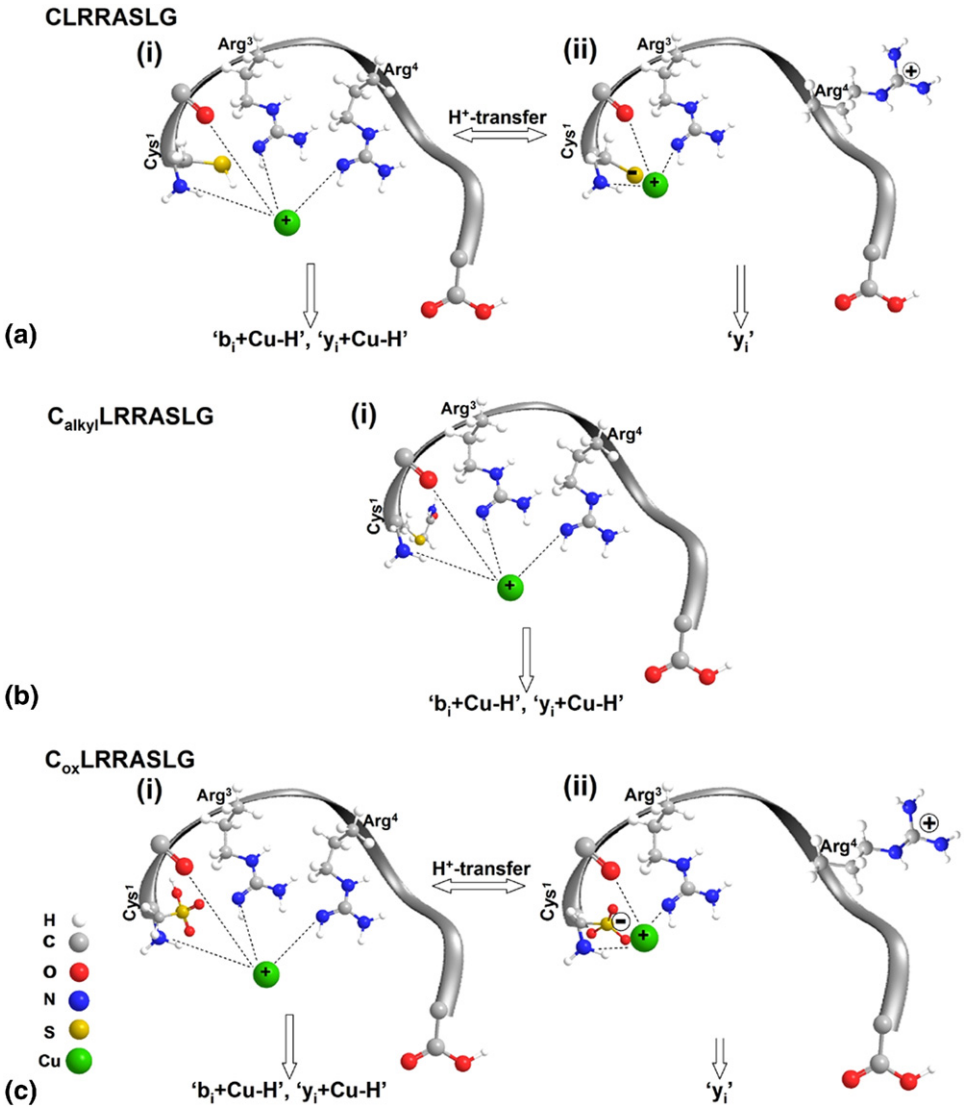


Figure 2. Fragment ion spectra of the $[M + Cu]^+$ ions of (**a**) CLRRASLG, (**b**) C_{alkyl}LRRASLG, (**c**) C_{ox}LRRASLG. The 91 mass shift (i.e., $[M + Cu - 91]^+$ in (**b**) is due to the loss of the alkylated cysteine side chain ($SHCH_2CONH_2$).

CLR. Copper attachments involving the C-terminus were explored in a previous paper [22] and were not considered here. Candidate structures and geometry files of the peptide sequence CLR for the oxidized and non-oxidized form of the Cys side chain are included in the Supplemental Information, which can be found in the electronic version of this article.

Results and Discussion

Previous work has shown that Cu^+ ions preferentially bind to electron-rich systems in gas phase (i.e., N-terminal amino group, the side chain of lysine, histidine, and arginine, and the deprotonated C-terminal carboxyl group) [17, 22]. Kass and coworkers reported that in gas phase, the cysteine side-chain thiol is more acidic than the carboxyl group, and deprotonated cysteine is thus predicted to be a thiolate ion, making it a potential electron rich group [33]. Therefore, it would be interesting to see how the Cu^+ ions competitively bind to the cysteine thiol versus other electron-rich system such as the guanidino group of arginine. The potential for interactions of cysteine thiol with Cu^+ ions were first examined by using theoretical calculations (at the DFT/B3LYP/LACV3P** level) on a small, model peptide sequenced CLR. The C-terminus was blocked by addition of an aldehyde group to eliminate the possible interaction of a carboxyl group with Cu^+ ions, which was described previously [22]. For a comparison, the interactions of Cu^+ ions with cysteine thiol versus cysteine sulfonic acid were also examined. Figure 1 contains the lowest energy structures obtained at the DFT/B3LYP/LACV3P** level for the $[M + Cu]^+$ and $[M + 2Cu - H]^+$ ions of CLR_{CHO} and $C_{ox}LR_{CHO}$ ("$_{CHO}$" denotes an aldehyde C-terminus). These simulated structures reveal that the guanidino group of arginine is an important ligand for Cu^+ ions; however, the cysteine side-chain also participates in the coordination of Cu^+



Scheme 1

ions. For example, a plausible configuration for $[M + Cu]^+$ ions is that the proton from the initial thiol or the sulfonic acid group is transferred to the basic guanidine, resulting in a protonated arginine side chain, and the Cu^+ ion is charge-solvated by the resulting thiolate or sulfonate group and the backbone carbonyls (see Figure 1). In addition, deprotonation of the thiol or sulfonic acid is energetically favorable for $[M + 2Cu - H]^+$ ions and, as a consequence, the interaction of the resulting thiolate or sulfonate group with Cu^+ ions contributes to the gas-phase structure of the $[M + 2Cu - H]^+$ ions. These results raise several questions regarding the binding of Cu^+ ions to thiol/sulfonic acid versus guanidine in larger peptide ions. How does the competitive Cu^+ binding affect the fragmentation of the peptides?

To address these questions, we examined the fragmentation reactions of the $[M + Cu]^+$ and $[M + 2Cu - H]^+$ ions of cys-kemptide (CLRRASLG). We also examined the modified Cys-kemptide where the N-terminal cysteine was modified by iodoacetamide [34] or performic acid [35], denoted as C_{alkyl} or $C_{ox,}$ respectively. Figure 2 contains the fragment ion spectra of the $[M + Cu]^+$ ions for peptides XLRRASLG (X = C, C_{alkyl} and C_{ox}). In the case of CLRRASLG, the fragment ion spectrum of $[M + Cu]^+$ ions contains both C- and

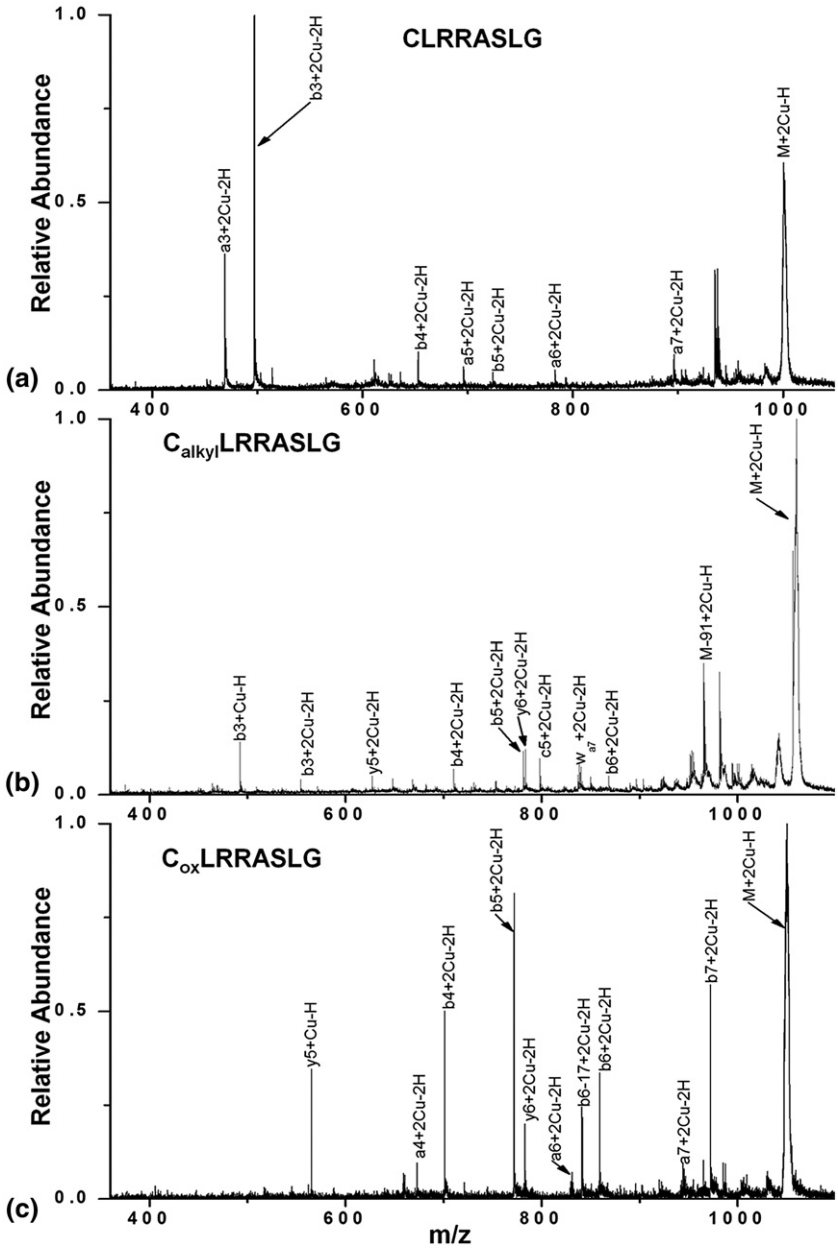
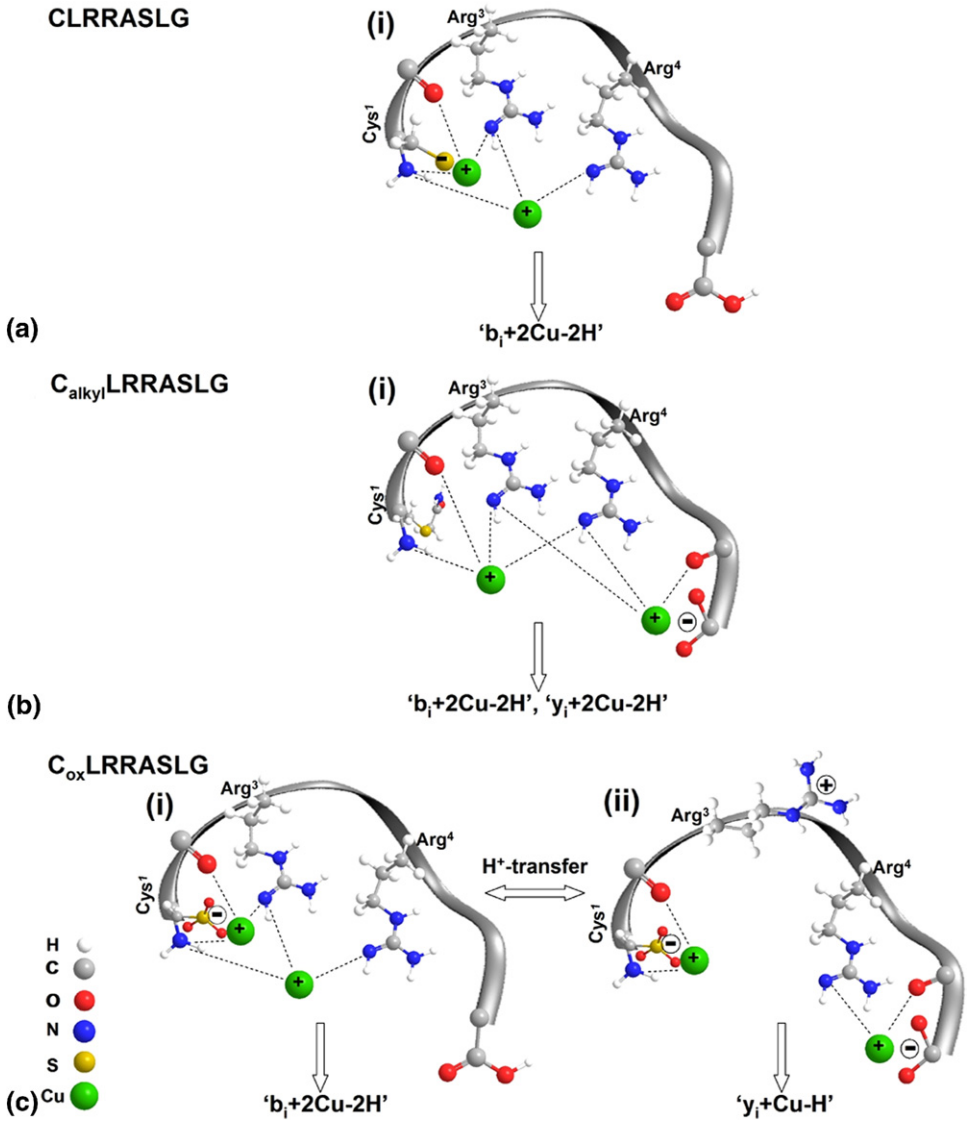


Figure 3. Fragment ion spectra of the $[M + 2Cu - H]^+$ ions of (**a**) CLRRASLG, (**b**) C_{alkyl}LRRASLG, (**c**) C_{ox}LRRASLG. The 91 mass shift, i.e., $[M - 91 + 2Cu - H]^+$ in (**b**) is due to the loss of the alkylated cysteine side chain $SHCH_2CONH_2$.

N-terminal fragment ions, including $[a_i + Cu - H]^+$, $[b_i + Cu - H]^+$, and $[y_i + Cu - H]^+$ ions. The relative abundance ratios of C- and N-terminal fragment ions are 52% and 48%, respectively. We interpret this observation as evidence that Cu^+ can bind to either Arg^3 or Arg^4, and there is no strong preference for binding of Cu^+ to the N-terminal cysteine. Scheme **1Ai** contains a proposed configuration showing that Cu^+ interacts with the guanidine group(s) of arginine(s), the backbone carbonyls, and the N-terminal amine group. Note, however, that a small peak corresponding to the y_5 ion without Cu^+ is observed. An explanation for the formation of the y_i ions, which is consistent with the theoretical results for CLR, is that Cu^+ promotes intramolecular proton transfer from the cysteine thiol to the arginine guanidine. That is, the Cu^+ ion is charge-solvated by the resulting thiolate, and the arginine side chain is protonated (Scheme **1Aii.** Note that the protonated guanidine group is projected away from the Cu^+ charge solvating center because this group is not a good Cu^+ ligand, i.e., the group acts as an isolated charge site). This explanation is further supported by the absence of y_i fragment ions for the peptide C_{alkyl}LRRASLG (Figure 2b). That is, the $-CH_2CONH_2$ group blocks the pathway for the intramolecular proton transfer thus all fragment ions are formed with Cu^+ attached (Scheme **1Bi**). Interestingly, when the cysteine thiol is oxidized to sulfonic acid, the abundance of the y_5 is increased (Figure 2c). Thus, apparently, oxidation of the cysteine increases the extent of the intramolecular proton transfer, generating larger population of the conformation shown in Scheme **1Cii** than that shown in Scheme **1Aii**.

We also examined the fragmentation chemistry of the $[M + 2Cu - H]^+$ ions for XLRRASLG (X = C, C_{alkyl}, and C_{ox}). Fragment ion spectra of the $[M + 2Cu - H]^+$ ions are very different from that of the $[M + Cu]^+$ ions, and the cysteine modifications significantly affect the fragmentation results. For example, we observe predom-



Scheme 2

inantly $[a_3 + 2Cu - 2H]^+$ and $[b_3 + 2Cu - 2H]^+$ fragment ions for the $[M + 2Cu - H]^+$ ions of CLRRASLG (Figure 3a); however, very different fragment ions are observed if the cysteine is alkylated (Figure 3b). A plausible explanation for the high abundance of the N-terminal fragment ions is that the deprotonation of the cysteine side chain occurs rather than the C-terminal carboxyl group, owing to its higher gas-phase acidity over that of the carboxyl [33]. Consequence, the two Cu^+ ions are strongly coordinated to N-terminal CLR residues. That is, the two Cu^+ ions are charge-solvated by the resulting thiolate, the arginine guanidine(s), the backbone carbonyl(s), and the N-terminal amine (Scheme **2Ai**). This interpretation is further evidenced by the fragmentation reactions of the peptide C_{alkyl}LRRASLG (Figure 3b). That is, no significant preference of the N-terminal fragment ions for $[M + 2Cu - H]^+$ are observed, considering that the abundance ratio of N-/C-fragment ions is ~58/42 for C_{alkyl}LRRASLG (Figure 3b) versus ~100/0 for CLRRASLG (Figure 3a). These results are in good agreement with our theoretical calculations, where deprotonation of the cysteine thiol is energetically favorable for $[M + 2Cu - H]^+$ ions, and blocking the thiol results in a different deprotonation site along the peptide backbone, which significantly changes the Cu^+ coordination environment for $[M + 2Cu - H]^+$ ions (Scheme **2Bi**).

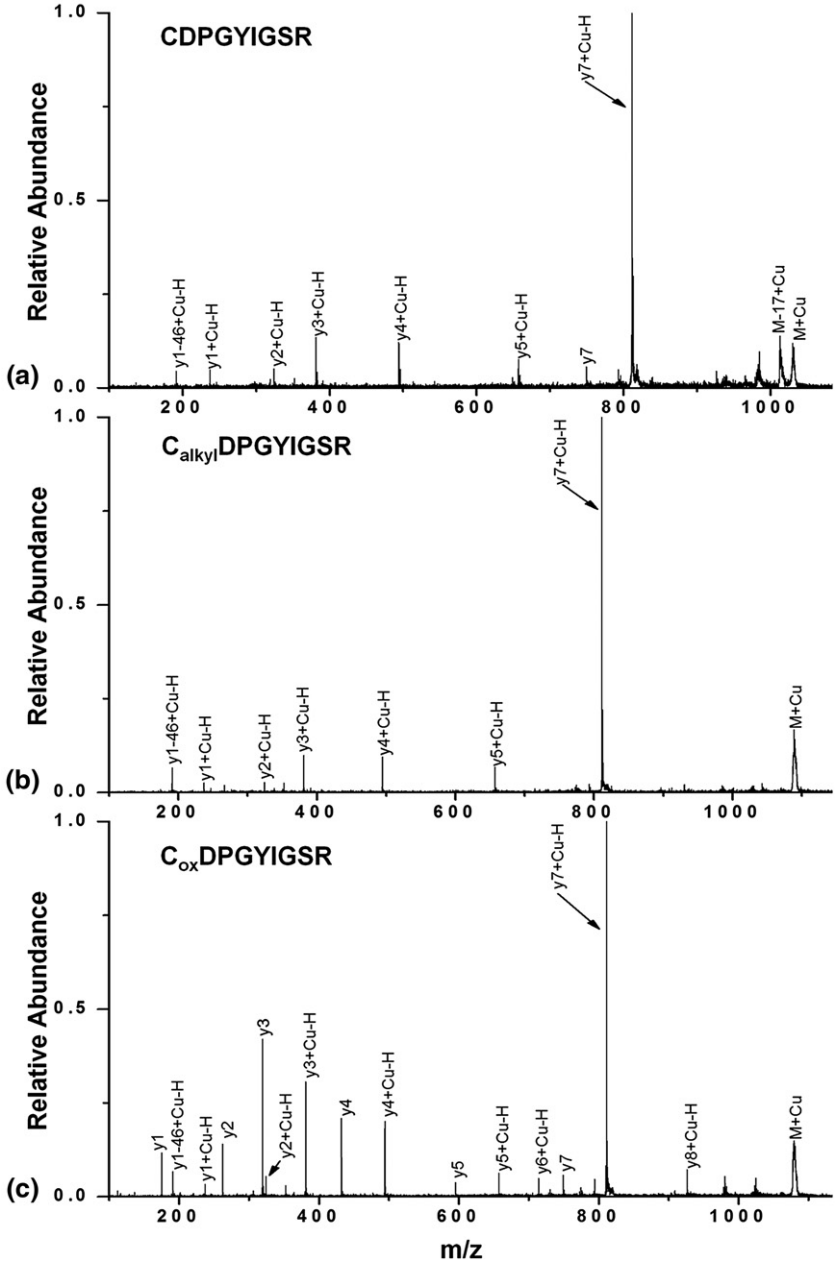
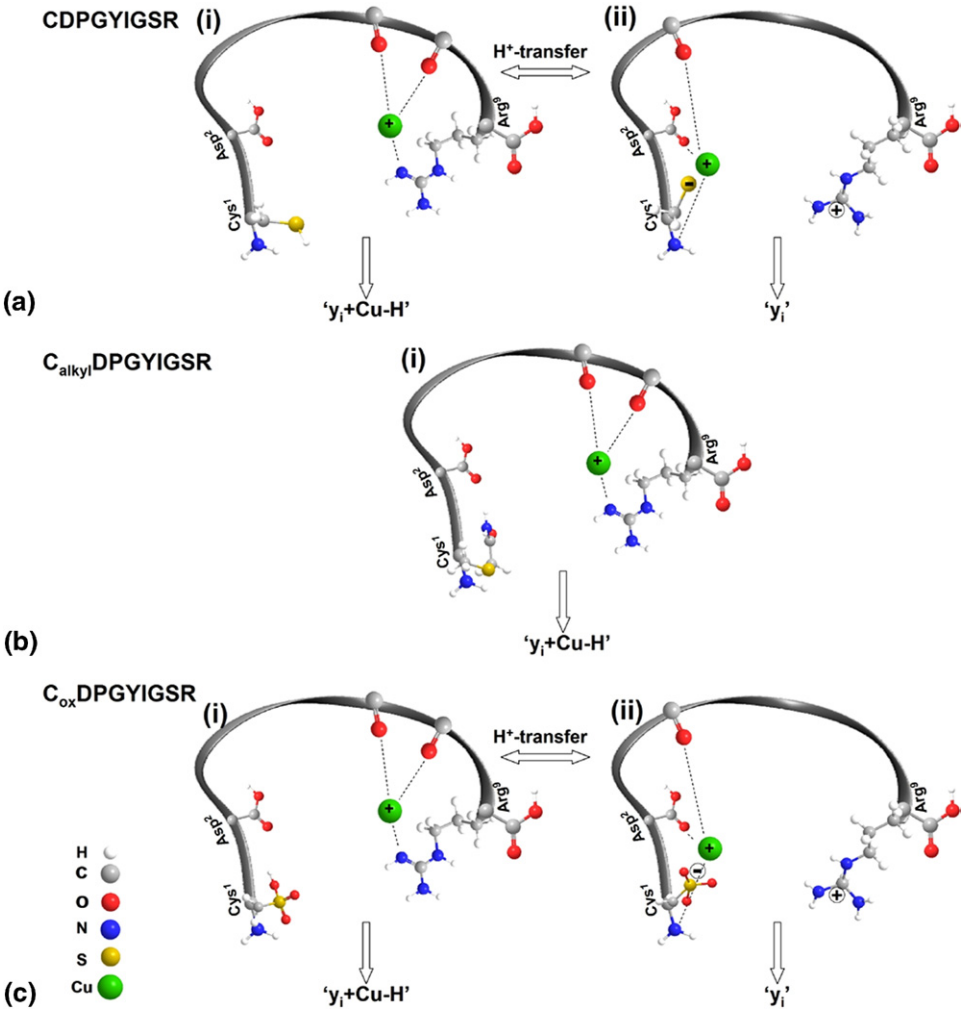


Figure 4. Fragment ion spectra of the $[M + Cu]^+$ ions of (**a**) CDPGYIGSR, (**b**) C_{alkyl}DPGYIGSR, (**c**) C_{ox}DPGYIGSR.

Oxidizing the cysteine also influences the Cu^+ coordination. For example, the fragment ion spectrum of the C_{ox}LRRASLG $[M + 2Cu - H]^+$ ion contains predominantly $[b_i + 2Cu - 2H]^+$ ($i \geq 4$) fragment ions; note that the $[b_3 + 2Cu - 2H]^+$ fragment ions are absent. Thus, apparently the position of arginine also plays a role in the Cu^+ coordination, i.e., Cu^+ ions prefer to bind to Arg^4 rather than Arg^3 for C_{ox}LRRASLG. Two additional observations are consistent with our proposed Cu^+ binding preference of Arg^4: (1) the smallest N-terminal fragment ions of $[M + 2Cu - H]^+$ ions for C_{ox}LRRASLG are $[b_4 + 2Cu - 2H]^+$ and $[a_4 + 2Cu - 2H]^+$ (Figure 3c), indicating that at least one Cu^+ ion is attached to either Arg^3 or Arg^4; and (2) the peak at *m/z* 565.1, which is assigned as $[y_5 + Cu - H]^+$, suggests that Arg^4 is the preferred Cu^+ binding site; otherwise we would expect $[y_6 + Cu - H]^+$ to also be observed (Scheme **2Cii**).

For comparison, we also examined the peptide Laminin (CDPGYIGSR) aimed at addressing how the Cu^+ ion interacts with cysteine versus arginine if the two side chains are located at the two termini. For example, is the intramolecular proton transfer process noted above the result of close proximity of the two groups? The $[M + Cu]^+$ ion of CDPGYIGSR yields exclusively C-terminal fragment ions (Figure 4a). The $[y_7 + Cu - H]^+$ ion is the most abundant, which indicates an enhanced backbone cleavage between the aspartic acid and proline residue with the Cu^+ remaining on the C-terminus. We interpret the preference for $[y_i + Cu - H]^+$ ions, especially $[y_1 + Cu - H]^+$, as evidence that Cu^+ ion is bound to the arginine side-chain (Scheme **3Ai**). Interestingly, we also observed a small peak corresponding to the y_7 ion that does not contain Cu^+; however, the y_7 peak is absent for $[M + Cu]^+$ ions of C_{alkyl}DPGYIGSR (Figure 4b), a result which suggests that the formation of y_7 ion for $[M + Cu]^+$ ions of CDPGYIGSR is also owing to an intramolecular proton transfer. That is, the proton from the thiol is transferred to the arginine guanidine, and Cu^+ is charge-solvated by the resulting thiolate group (Scheme **3Aii**). More interestingly, however, when the cysteine thiol group is oxidized to sulfonic acid, the intramolecular proton transfer appears to occur to a larger extent. For example, in Figure 4c, we observe a significantly high abundance of y_i ions that do not contain Cu^+. The abundance ratio of the $[y_i + Cu - H]^+$ to y_i is ~70% to 30%. We



Scheme 3

interpret this result as evidence that the coordination structure of the $[M + Cu]^+$ ions is dependent on the position of the cysteine and arginine, and oxidizing the cysteine residue favors coordinate Cu^+ ions (Scheme **3Cii**).

We also investigated the fragmentation chemistry of the $[M + 2Cu - H]^+$ ions of CDPGYIGSR (Figure 5). Results from these experiments suggest that the fragmentation of $[M + 2Cu - H]^+$ ions is sensitive to the nature of the cysteine side chain, and different fragment ion spectra are observed when the cysteine side chain is modified (C_{alkyl} and C_{ox}). For example, the fragment ion spectrum of $[M + 2Cu - H]^+$ ions of CDPGYIGSR contains three types of fragment ions, viz. $[y_i + 2Cu - 2H]^+$, $[y_i + Cu - H]^+$, and y_i, and the relative abundance ratios of these ions are 42%, 48%, and 10%, respectively (Table 1). We propose that $[y_i + 2Cu - 2H]^+$ and $[y_i + Cu - H]^+$ are formed from the coordination conformations such as **4Ai** and **4Aii** shown in Scheme **4**, respectively; note that the differences in these two conformations depend on where the deprotonation site is, i.e., cysteine thiol or C-terminal carboxyl. We would expect that the conformation (Scheme **4Aiii**) to yield predominantly y_i ions with no Cu^+ attached. That is, the two Cu^+ ions are charge-solvated by the cysteine thiolate and the carboxylate of the aspartic acid, respec-

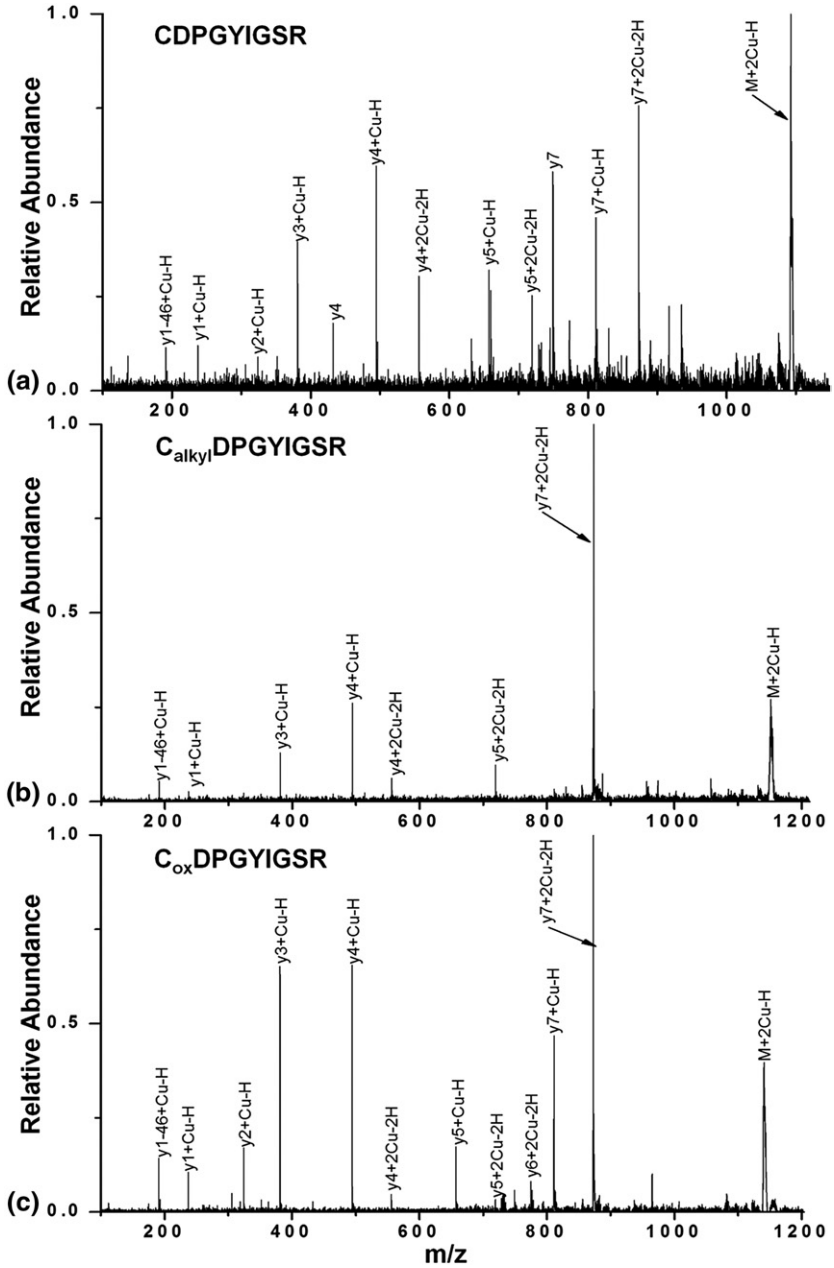


Figure 5. Fragment ion spectra of the $[M + 2Cu - H]^+$ ions of (**a**) CDPGYIGSR, (**b**) C_{alkyl}DPGYIGSR, (**c**) C_{ox}DPGYIGSR.

Table 1. Relative abundance ratio of the fragment ions for $[M + Cu]^+$ and $[M + 2Cu - H]^+$

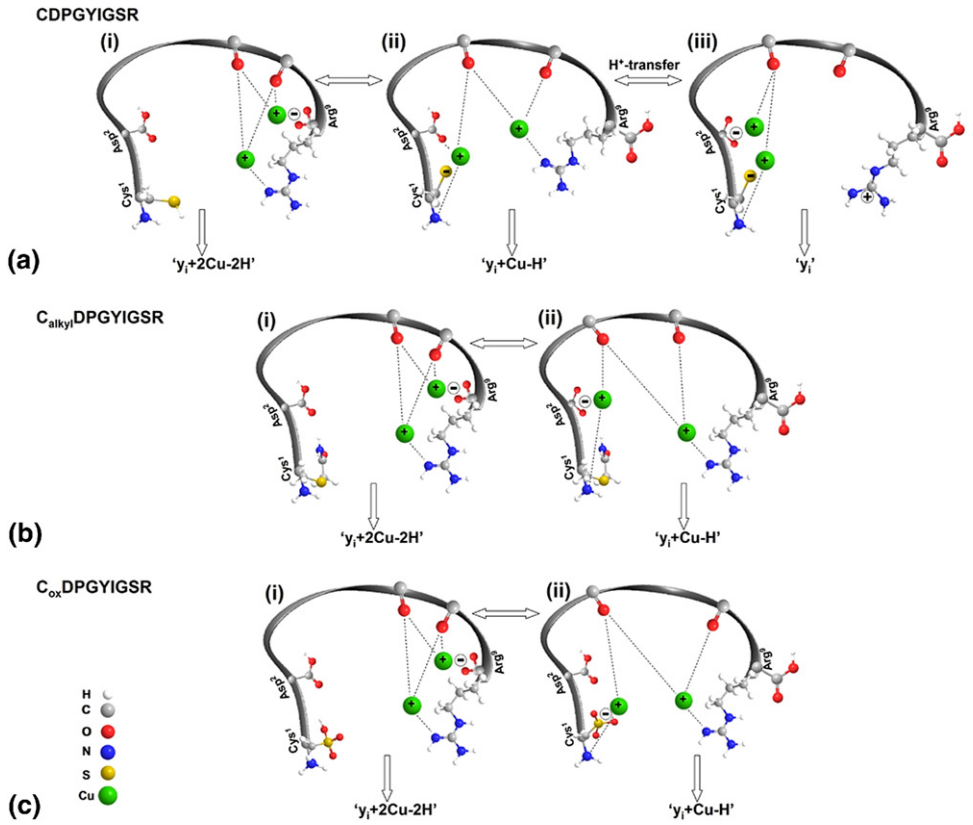
	$[M + Cu]^+$		$[M + 2Cu - H]^+$		
	$\Sigma(y_i + Cu - H)$	Σy_i	$\Sigma(y_i + 2Cu - 2H)$	$\Sigma(y_i + Cu - H)$	Σy_i
CDPGYIGDR	>99%	<1%	42%	48%	10%
C_{alkyl}DPGYIGDR	100%	N/A	70%	30%	N/A
C_{ox}DPGYIGDR	65%	35%	44%	56%	N/A

For example, for $[M + Cu]^+$ ions, the relative abundance ratio of "$y_i + Cu - H$" ions = $\Sigma(y_i + Cu - H)/(\Sigma(y_i + Cu - H) + \Sigma y_i)$.

tively, and the arginine side chain is protonated via an intramolecular proton transfer. This assumption is supported by the fragment ion spectrum of C_{alkyl}DPGYIGSR. The fragment ion spectrum of the modified peptide C_{alkyl}DPGYIGSR contains only two types of fragment ions, viz. $[y_i + 2Cu - 2H]^+$ and $[y_i + Cu - H]^+$, and y_i ions are not observed. The absence of y_i ions for C_{alkyl}DPGYIGSR provides evidence that blocking the cysteine thiol group reduces the interaction with Cu^+ ion. In addition, these data suggest that at least one Cu^+ must be located at the C-terminus (charge-solvated by the C-terminal carboxyl and/or the arginine guanidine). For the $[M + 2Cu - H]^+$ ion of C_{ox}DPGYIGSR, we observed exclusively $[y_i + 2Cu - 2H]^+$ and $[y_i + Cu - H]^+$ fragment ions with relative abundance ratios of 44% and 56%, respectively (Table 1). The higher abundance of the $[y_i + Cu - H]^+$ ions suggests a larger population of the conformation (Scheme 4**Cii**), where the sulfonic acid is the primary protonation site, and each Cu^+ ion is individually charge-solvated by the sulfonate and the arginine guanidine group, respectively. As a consequence, only one Cu^+ ion remains on the C-terminal fragment ions (Scheme 4**Cii**). A possible explanation for the formation of higher abundance of $[y_i + Cu - H]^+$ ions is the fact that the deprotonation energy for sulfonic acid is less than that for carboxylic acid [41]. Thus, deprotonation of the sulfonic acid is more energetically favorable over that of the C-terminal carboxyl group, and the lower deprotonation energy of sulfonic acid results in a more stable coordination structure (Scheme 4**Cii**).

Conclusions

These studies clearly show that cysteine and cysteic acid side chains ($-SH/SO_3H$) are important Cu^+ ion ligands, and these metal ion-ligand interactions have a strong influence on the fragmentation chemistry of



Scheme 4

$[M + Cu]^+$ and $[M + 2Cu - H]^+$ ions. In addition, we demonstrate that Cu^+ ions are competitively coordinated to the $-SH/SO_3H$ groups versus guanidine group via an intramolecular proton transfer. This is probably not surprising, considering the Cu^+ binding energy for the two groups differs by ~5 kcal/mol [17, 42]. It is particularly interesting to note that upon binding of Cu^+ ions to the cysteine or cysteic acid side chains, the proton (on SH or SO_3H) is transferred to the arginine guanidine group, resulting in a thiolate-Cu^+ or sulfonate-Cu^+ group and a protonated arginine side chain. On the basis of the data reported herein, we cannot determine whether this proton migration occurs as a result of Cu^+ attachment to SH or SO_3H (i.e., a process similar to that was proposed previously [13]), or whether the proton is mobilized as a result of the collisional activation process. We are currently investigating these issues further by comparing ion abundances observed by using 193 nm photodissociation with those observed by CID and by ion mobility-mass spectrometry (IM-MS) and molecular dynamics simulations. These studies underscore the role of peptide-metal ion interactions on the fragmentation chemistry of gas-phase peptide ions as well as the level of information that can be extracted regarding the specificity of metal ion binding.

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Appendix A Supplementary Material

Supplementary material associated with this article may be found in the online version at doi:10.1016/j.jasms.2009.12.020.

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