A New Copper Containing MALDI Matrix That Yields High Abundances of [Peptide + Cu]⁺ Ions

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The dinuclear copper complex (α-cyano-4-hydroxycinnamic acid (CHCA) copper salt $(CHCA)_4Cu_2$), synthesized by reacting CHCA with copper oxide (CuO), yields increased abundances of $[M + xCu - (x-1)H]^+$ (x = 1–6) ions when used as a matrix for matrix-assisted laser desorption ionization (355 nm Nd:YAG laser). The yield of $[M + xCu - (x-1)H]^+$ (x = 1~6) ion is much greater than that obtained by mixing peptides with copper salts or directly depositing peptides onto oxidized copper surfaces. The increased ion yields for $[M + xCu - (x-1)H]^+$ facilitate studies of biologically important copper binding peptides. For example, using this matrix we have investigated site-specific copper binding of several peptides using fragmentation chemistry of $[M + Cu]^+$ and $[M + 2Cu - H]^+$ ions. The fragmentation studies reveal interesting insight on Cu binding preferences for basic amino acids. Most notable is the fact that the binding of a single Cu^+ ion and two Cu^+ ions are quite different, and these differences are explained in terms of intramolecular interactions of the peptide-Cu ionic complex. (J Am Soc Mass Spectrom 2009, 20, 1263–1271) © 2009 Published by Elsevier Inc. on behalf of American Society for Mass Spectrometry

Copper ions (Cu^+, Cu^{2+}, and the multimeric form Cu_x^{n+}) play important roles in many chemical and biochemical processes, such as oxidation, dioxygen transport, and electron-transfer; many of the functions in these processes result from copper ions interacting with proteins and peptides [1]. In plants, copper is a cofactor for plastocyanin, copper/zinc superoxide dismutase (Cu/ZnSOD), ethylene receptors for the apoplastic oxidases, and plays important roles in photosynthesis, respiration, and antioxidant activity [2–4]. Copper has also been implicated in human neurodegenerative diseases, such as Alzheimer's disease; presumably such affects involve the interaction of copper ions with peptides and/or proteins [5–7]. Most of our knowledge concerning copper in biological systems is derived from solution and solid-state studies [8]; however, studies of gas-phase species can be used to probe intrinsic Cu-peptide and Cu-protein interactions in low dielectric environments, which are more closely related to biological membrane systems [9]. Studies performed in the absence of solvent also simplify the chemistry and eliminates solvent stabilization of metal ion-ligand interactions [9–11]. Although comparison of gas-phase and solution-phase data could potentially yield important information regarding solvent dependent Cu-biomolecule interactions, the ligand interactions are typically very different. For example, in solution the basic amino acid side chains are protonated, which weakens the binding energy 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].

In previous studies, matrix-assisted laser desorption ionization (MALDI) mass spectrometry has been used to study the energetics of copper ion (Cu^+ and Cu^{2+}) binding sites in model peptides [12–16]. Results from these MALDI studies suggest that peptide–metal ion complexes are formed by a reductive process which yields primarily $[M + Cu(I)]^+$ ions, whereas, the formation of peptide–metal ion complexes by electrospray ionization (ESI) yields almost exclusively $[M + Cu(II) - H]^+$ ions. More recently, Prudent and Girault showed that both $[M + Cu(I)]^+$ and $[M + Cu(II) - H]^+$ ions can be formed by ESI; however, formation of $[M + Cu]^+$ ions is only observed if the ESI emitters are composed of solvable copper anodes [17]. Peptides that do not possess basic amino acids do not strongly bind to copper ions and the $[M + Cu]^+$ ions are either not observed by mass spectrometry or are formed at very low abundance. Bluhm and coworkers used a combination of electronic structure calculations and experimental data to determine copper binding sites for modeled systems based on monodentate and bidentate interactions [18], and the measured and calculated Cu^+ ion

affinity order were in agreement with previous data reported by Wesdemiotis [19]; the monodentate Cu$^+$ binding energies for amino acids follow the order Arg > His > Lys > Cys > Ser, and bidentate Cu$^+$ relative binding energies are Arg > Lys > His > Gln > Asn > Glu > Asp. In both cases, arginine is the most favorable binding site of Cu$^+$ for gas-phase peptides. Based on fragmentation studies, Shields and coworkers proposed that the primary Cu$^+$ binding site in peptides with an N-terminal arginine is the guanidine group of arginine and the N-terminal amine [13]. That is, the principal fragment ions of [M + Cu]$^+$ peptide ions containing an N-terminal arginine are [a$_n$ + Cu − H]$^+$ and [b$_n$ + Cu − H]$^+$ fragment ions. They also described the fragmentation reactions of [M + Cu]$^+$ in terms of a "mobile proton" model, by which the fragmentation occurs remote from the Cu$^+$ ion attachment site, and involves metal ion-promoted deprotonation to generate a new site of protonation. For peptides with more than one basic residue, the interaction between the Cu$^+$ and the basic residues is described in terms of competitive binding; however, few studies have been performed to determine Cu$^+$ binding sites for multiple basic residue containing peptides in terms of the binding preference. Also, we have found no reports on the fragmentation of multiple copper-binding peptides, such as [M + 2Cu − H]$^+$ ions. Addressing these issues will shed light on the Cu$^+$ binding chemistry for peptides containing multiple basic residues and the interactions of Cu$^+$ ions with peptides and proteins in biological systems.

There are two commonly used methods to generate gas-phase peptide-Cu ions using MALDI: (1) co-mixing a metal salt such as CuCl$_2$, CuO, or CuSO$_4$ with the peptide and organic matrix [20–32], and (2) the method first reported by Shields et al. where [M + Cu]$^+$ is generated by desorbing the sample from a CHCA matrix deposited onto an oxidized copper plate [33]. Presumably the latter method involves dissolution of CuO from the metal surface followed by reaction of CuO with the peptide and/or matrix to yield peptide-Cu complexes. Both methods produce abundant peptide [M + Cu]$^+$ ions; however, the dominant ions observed in the mass spectrum usually correspond to the [M + H]$^+$ ion with a lower abundance of the [M + Cu]$^+$ ions. Here, we introduce a new copper matrix, which simplifies the sample preparation and greatly enhances the yield of copper adducted peptide ions, especially peptide-Cu ions containing multiple Cu. The improved yields of these peptide-Cu ions result in higher quality tandem MS signals, which enable studies of the binding and fragmentation chemistry of peptide-Cu complexes.

Experimental

Chemicals and Materials

Copper oxide (CuO) and α-cyano-4-hydroxycinnamic acid (CHCA) were obtained from Sigma (St. Louis, MO). The CHCA was recrystallized before use. The

organic solvents used for mass spectrometry were HPLC grade, all the other chemicals were reagent grade, and the water is in high purity (18MΩ; Barnstead International, Dubuque, IA). The peptides Ac-(AAKAA)$_n$-NH$_2$ (n = 2–4) were purchased from Genscript Corporation (Piscataway, NJ). Other peptides used in the experiments were purchased from Sigma (St. Louis, MO) and used without further purification. Ac-(AAKAA)$_2$-OH and Ac-(AAKAA)$_2$-OCH$_3$ peptides were synthesized from Ac-(AAKAA)$_2$-NH$_2$ following standard protocols [34].

Synthesis of the Copper Complex

The copper α-cyano-4-hydroxycinnamic acid complex (Cu–CHCA) was synthesized by reacting CHCA with copper oxide (CuO). The reaction was carried out by dissolving 200 mg of α-cyano-4-hydroxycinnamic acid (CHCA) and 170 mg of copper oxide (CuO) powder in 7:3 (vol:vol) distilled deionized H$_2$O:acetonitrile (ACN) solution, followed by 15 min of sonication to form a suspension. The suspension was then incubated in a water bath at 65 °C for 8 h and cooled to room temperature. Following several steps of filtration and recrystallization, needle-like crystals were obtained (see supplemental material, which can be found in the electronic version of this article).

Characterization of the Copper Complex

The Cu–CHCA complex was characterized by single crystal X-ray diffraction, MALDI-TOF mass spectrometry, and UV/VIS spectroscopy. The single crystal X-ray diffraction data (see supplemental material) indicated that four α-cyano-4-hydroxycinnamic acid ligands complexed to a dinuclear copper center in a paddlewheel-like structure [35]. The distance between the two copper atoms of ~2.6 Å is typical of the noncovalent binding of Cu–Cu in other paddlewheel complexes. Each copper atom is five-coordinated with four oxygen atoms from the two carboxylate groups and one oxygen atom from the solvent molecule (tetrahydrofuran), thus the chemical formula of the complex is (CHCA)$_4$Cu$_2$. The MALDI mass spectrum (see supplemental material) of the Cu–CHCA complex contains a dominant peak at m/z 441.0, which corresponds to the protonated (CHCA)$_2$Cu species. The UV/VIS spectrum (see supplemental material) shows that the Cu–CHCA complex has a strong absorption in the UV wavelength range, which is similar to that of CHCA. Also, the Cu–CHCA complex has a weak absorption in the visible light wavelength range, which is an indication of the existence of Cu^{2+} ions.

Mass Spectrometry

The MALDI MS experiments described herein were performed on a tandem time-of-flight mass spectrometer (4700 Proteomics Analyzer; Applied Biosystems, Framingham, MA), and all tandem MS experiments

were performed using a collision energy setting of 1 kV and air as the collision gas.

Theoretical Calculations

Theoretical calculations using density functional theory (DFT) have been performed to gain a better understanding of peptide-Cu ion structure. The B3LYP functional was used [36–38]. The triple-ξ quality basis set with a small-core effective core potential (SDD) was used for the Cu atoms [39], while all the other atoms were treated with the triple-ξ quality Pople style basis set with diffuse and polarization functions (6-311++G(d,p)) [40, 41]. This combination of basis sets will be referred to as TZBS. No symmetry restriction of any kind was imposed in the process of geometry optimization. All calculations were performed using the Gaussian 03 program suite [42].

Results and Discussion

Here, we describe the utility of the Cu–CHCA complex as a MALDI matrix. LDI from crystals of Cu–CHCA yield higher abundances of $[M + xCu - (x-1)H]^+$ ions ($x = 1–6$). To illustrate this point, LDI mass spectra obtained by using copper sulfate, a copper plate (CuO) and the Cu–CHCA complex are shown in Figure 1. Note that the yield of $[M + Cu]^+$ ions using Cu–CHCA is considerably higher than that obtained using copper salt or a copper plate and that the ratio of $[M + Cu]^+$ to $[M + H]^+$ ions has also increased (Figure 1). For the peptide ACTH (1-17) (SYSMEHFRWGKPVGKKR), which contains six basic amino acids, we observe $[M + xCu - (x-1)H]^+$ where x ranges from 1 to 6, and the ratio of $[M + Cu]^+$ ions to $[M + H]^+$ is ~1.3:1. Conversely, when using copper sulfate or CHCA/copper plate, the most abundant peaks in the spectra

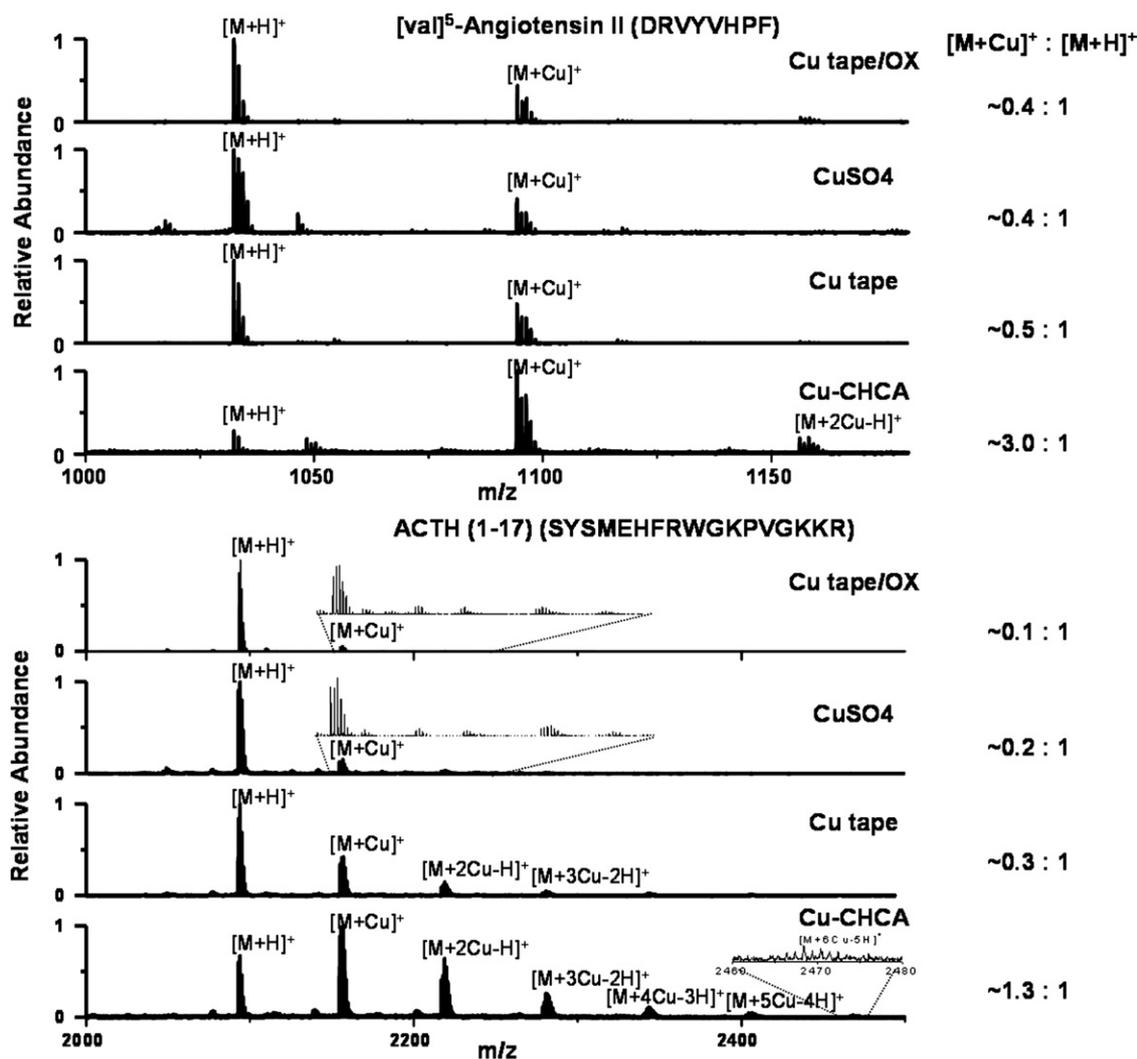**Figure 1.** Partial mass spectra for [Val]^5-Angiotensin II and ACTH (1-17) obtained by using four different copper sources: copper tape with an oxidized surface; CuSO$_4$ solution; copper tape with a nonoxidized surface; copper matrix Cu–CHCA. The peptides amounts deposited on the MALDI plate were kept constant at 1 pmol for all the experiments. The molar ratio of CuSO$_4$/peptide and Cu–CHCA/peptide was kept constant at 5.

correspond to $[M + H]^+$ ions while a very low abundance of $[M + xCu - (x-1)H]^+$ ions are observed.

The increased abundances for $[M + Cu]^+$ produced by Cu–CHCA reveal rich MS/MS spectra containing Cu^+, which improves our ability to investigate Cu-peptide and Cu–protein complexes. In addition, Cu–CHCA affords opportunities to study the competitive binding of Cu^+ in $[M + xCu - (x-1)H]^+$ ions, i.e., Cu uptake by biologically important peptides and proteins. That is, if a peptide contains multiple Cu^+ binding sites, are there other factors that determine Cu^+ binding; does the addition of Cu^+ to a peptide influence the binding site of subsequent Cu^+ ions? How does accumulation of Cu^+ by specific amino acids influence Cu^+ binding?

Figure 2 contains tandem mass spectra for $[M + Cu]^+$ ions of [Val]^5-angiotensin II and ACTH (1-17). Both spectra contain abundant b_i and y_i ions, and all fragment ions contain Cu^+, i.e., the Cu^+ binding affinity of the peptide is higher than the energy required to fragment the molecule. On closer inspection of the fragment ions, we can determine the Cu^+ binding site, and the relative abundance of b and y-type ions indicates whether Cu^+ prefers to bind to the basic residues close to the N-terminus or the C-terminus. For example, on the basis of relative binding energies (BE) we would predict that Cu^+ preferentially binds to Arg^2 and His^6; BE for Arg^2 (~73 kcal/mol) is greater than that for His^6 (~64 kcal/mol) [18]. We would also expect to observe b_2, b_3, b_4, and b_5 fragment ions for $[M + Cu]^+$ ions if Cu^+ is

bound to Arg^2. Similarly, signature fragment ions for Cu^+ binding to His^6 would be y_3, y_4, y_5, and y_6. The relative abundances of these signature b and y-type ions is about 2:1, thus apparently ~67% of the Cu^+ ions bind to Arg^2 and ~33% of the Cu^+ ions bind to His^6. On the basis of our previous studies we would argue that Cu^+ prefers to bind to Arg^2, owing to the stabilization afforded by bidentate complexation to the N-terminal NH_2 group [18].

Figure 3 contains the fragment ion spectra of Ac-(AAKAA)$_2$Y $[M + Cu]^+$ ions with three different C-terminal groups: an amide group, a free acid and a methyl ester. This peptide contains two basic lysine residues; thus, Cu^+ ions can bind to either Lys^3 or Lys^8. In addition, our previous studies suggest a preference for binding to Lys^3 because of its ability to form a bidentate interaction with the N-terminus [18]. The fragmentation spectra of the $[M + Cu]^+$ ions of the peptide ion with different C-termini are dominated by both b- and y-type fragment ions, all of which contain Cu^+. The b_3 ion is the lowest m/z b-type fragment ion observed and the lowest m/z y-type fragment ions correspond to y_4 ions, which supports our assignment of Cu^+ binding to either Lys^3 or Lys^8. Note also that the b-type fragment ions are more dominant than the y-type fragment ions; the ratio of b- to y-type ions is ~70% to 30% for all the three spectra, respectively (Table 1). Presumably, the higher abundance of b-type fragment ions indicates that Lys^3, which is close to the N-terminus, has a higher binding affinity for Cu^+ than the
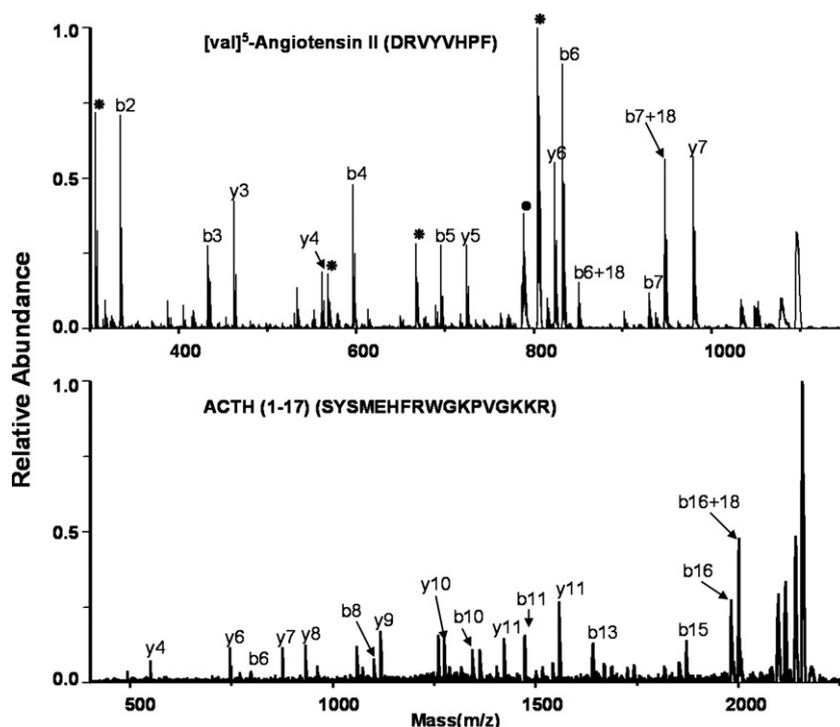

Figure 2. Tandem mass spectra of $[M + Cu]^+$ of two peptides: [Val]^5-Angiotensin II and ACTH (1-17). All fragment ions in the spectra have bound Cu^+. (Filled circle) denotes internal fragment ions with Cu^+ attached; (asterisk) denotes a-type fragment ions with Cu^+ attached; b- and y- type fragment ions with Cu^+ attached are the dominant ions for both peptides.
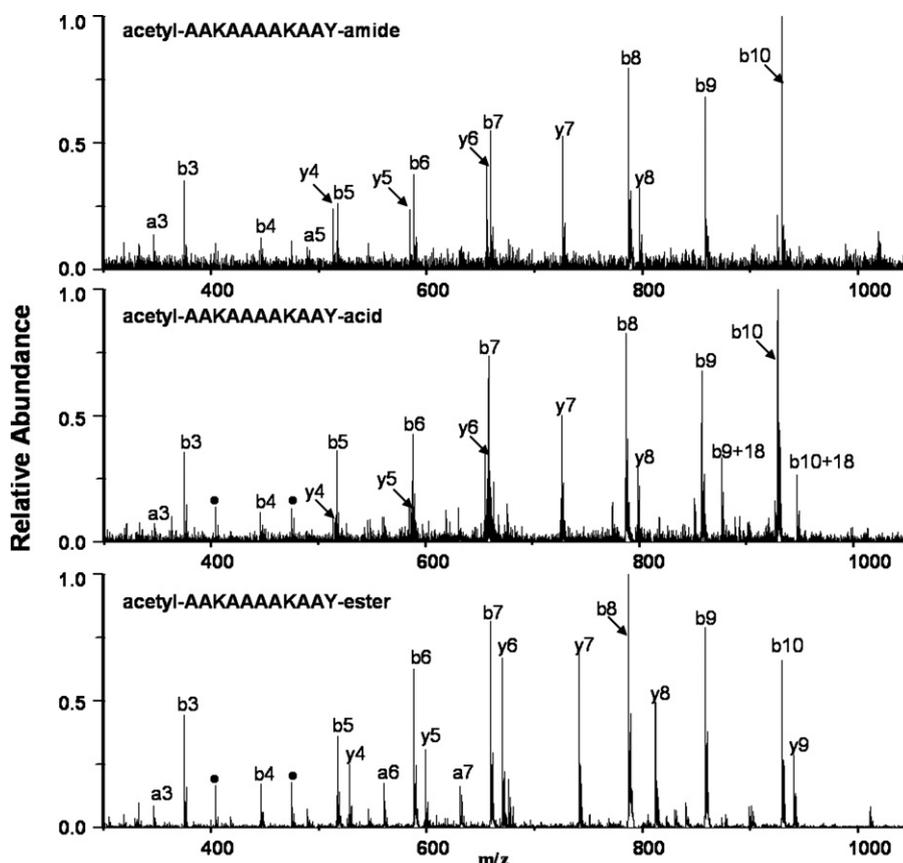
Figure 3. Tandem mass spectra of $[M + Cu]^+$ of Ac-$(AAKAA)_2$Y with three different C-terminal groups: amide, free acid, and ester. All fragment ions in the spectra have bound Cu^+. (Filled circle) denotes internal fragment ions with Cu^+ attached. The fragment ion spectra of the $[M + Cu]^+$ ions of the peptide ion with different C-termini are dominated by both *b*- and *y*-type fragment ions, all of which contain Cu^+, and the abundance ratio of *b*- to *y*-type ions is ~70% to 30% for all the three spectra, respectively.

Lys[8]; thus, the position of the lysine residues plays an important role in the binding preference of Cu^+ to peptides. Also, this data suggests that the C-terminus has less influence on the fragmentation of $[M + Cu]^+$ ions compared to the position of the lysine residue.

We also examined the fragmentation chemistry for model peptide ions containing multiple Cu ions. For these ions, the C-terminus strongly affects the fragmentation reactions. For example, the fragment ion spectra of the $[M + 2Cu - H]^+$ ions of the peptide Ac-

$(AAKAA)_2$-Y with three different C-terminal groups (Figure 4)) are quite different from those of the $[M + Cu]^+$ ions (Figure 3). Specifically, inspection of the $[M + 2Cu - H]^+$ fragment ion spectra shows that all the observed fragment ions contain two coppers while fragment ions containing a single Cu^+ are not observed, which suggests that both copper ions are bound to the same site or in close proximity. The $[M + 2Cu - H]^+$ fragment ion spectra are dominated by *b*- and *y*-type fragment ions, and the ratio of *b*- to *y*-type ions varies

Table 1. Relative binding preference of Cu^+ to peptides with multiple basic residues. For example, K^3 is the percentage of peptide with Cu^+ bound to the third lysine. For multiple lysine containing peptides Ac-$(AAKAA)_n$ Y-NH_2 (n = 3,4), the Cu^+ ions preferentially bind to the lysine near the termini; however, for multiple histidine containing peptide WGGHDGPHAPGDH, the most favorable binding sites for Cu^+ ions are His[8] and His[13]

| Peptide	$[M + Cu]^+$	$[M + 2Cu–H]^+$
R^1PGFSPFR^8	R^1: 57%; R^8: 43%	R^1: 58%; R^8: 42%
R^1PPGFSPFR^9	R^1: 73%; R^9: 27%	R^1: 74%; R^9: 26%
acetyl-AAK^3AAAAK^8AAY-amide	K^3: 70%; K^8: 30%	K^3: 47%; K^8: 53%
acetyl-AAK^3AAAAK^8AAY-free acid	K^3: 68%; K^8: 32%	K^3: 5%; K^8: 95%
acetyl-AAK^3AAAAK^8AAY-ester	K^3: 70%; K^8: 30%	K^3: 45%; K^8: 55%
acetyl-AAK^3AAAAK^8AAAAK^{13}AAY-amide	K^3: 69%; K^{13}: 31%	K^3: 49%; K^{13}: 51%
acetyl-AAK^3AAAAK^8AAAAK^{13}AAAAK^{18}AAY-amide	K^3: 68%; K^{18}: 32%	K^3: 46%; K^{18}: 54%
WGGH^4DGPH^8APGDH^{13}	H^8: 46%; H^{13}: 54%	H^8: 42%; H^{13}: 58%
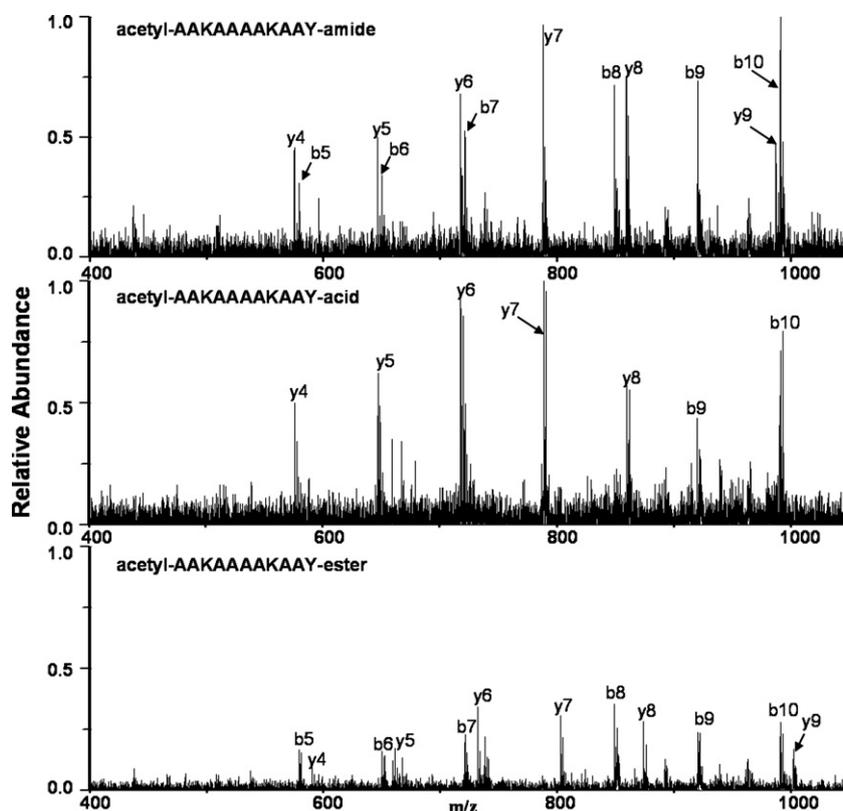
Figure 4. Tandem mass spectra of the $[M + 2Cu - H]^+$ of Ac-(AAKAA)$_2$Y with three different C-terminal groups: amide, free acid, and ester. All fragment ions in the spectra contain two Cu$^+$. Both copper ions are bound to the same site or in close proximity. For peptide ions with an amide or ester C-terminus, the abundance ratio of b- to y-type ions is ~45% to 55%, whereas peptide ions with a free acid C-terminus yield very different ratio of b- to y-type ions, viz. 5% to 95%.

depending on the nature of the C-terminal group. For peptide ions with an amide or ester C-terminus, the ratio of b- to y- type ions is ~45% to 55% (Table 1), which suggests that the copper ions bind to the two lysine residues with roughly equal probability. On the other hand, peptide ions with a free acid C-terminus yield a very different ratio of b- to y-type ions, viz. 5% to 95% (Table 1), which suggest that the copper ions strongly prefer Lys^8. Our interpretation of this experimental result is that the free acid C-terminus is a better Cu$^+$ ligand than the amide or methyl ester groups; this is probably an indication that the −COOH proton is lost in forming $[M + 2Cu - H]^+$ ions.

A plausible way to describe the $[M + 2Cu - H]^+$ ions is in terms of individual Cu$^+$ ions attached to the €-amino group of Lys^3 and Lys^8 and the deprotonated C-terminus because it is unlikely that the Cu$^+$ ions are bound as a Cu$_2^{2+}$ [43]. These ideas were explored using theoretical studies (at the DFT/B3LYP/TZBS level) on several model systems, viz. various structures of deprotonated Lys-Ala containing two Cu$^+$ ions $[M + 2Cu - H]^+$ (Figure 5a), the dinuclear Cu$^+$ complex of deprotonated Ala-Ala (AA, label A) and n-propylamine (PA, label B) $[M + 2 Cu - H]^+$ (Figure 5b), and the y$_4$ Ac-(AAKAA)$_2$-Y $[M + 2Cu - H]^+$ fragment ion (Figure 6). The lowest energy structure obtained for the Lys–Ala dinuclear

complex has both Cu$^+$ ions interacting with the carboxylate group and an amide oxygen. The lowest energy structure obtained for the AA/PA-2Cu $[M + 2 Cu - H]^+$ complex (Figure 5b) is similar to the Lys–Ala dinuclear complex in that both Cu$^+$ ions interact with the carboxylate group and an amide oxygen. The large binding energies (49.83 and 38.81 kcal/mol) observed in the AA/PA-2Cu $[M + 2 Cu - H]^+$ complex suggest that the Cu ions can be better coordinated by the carboxylate and amide groups, and this information was further used to developed a series of candidate structures for the y$_4$ Ac-(AAKAA)$_2$-Y $[M + 2Cu - H]^+$ ions (Figure 6). The lowest energy structures (labeled A, B, and C) are all quite similar, having the two Cu$^+$ ions interacting with the carboxylate group and backbone amide groups of Lys^8. Higher energy conformations, i.e., Cu$^+$ ions located at the Tyr^{11} and the carboxylate group (structure F, 87.13 kcal/mol) and at the Lys^8 and Tyr^{11} (structure G, ~169.89 kcal/mol), are also shown for comparison in Figure 6. In the case of the high-energy structure G, it should be pointed out that the loss of the desired conformation was avoided by terminating the optimization process after ten geometry optimization cycles. Using the model structures contained in Figures 5 and 6 the preference for forming y-type CID product ions, which retain both Cu$^+$ ions
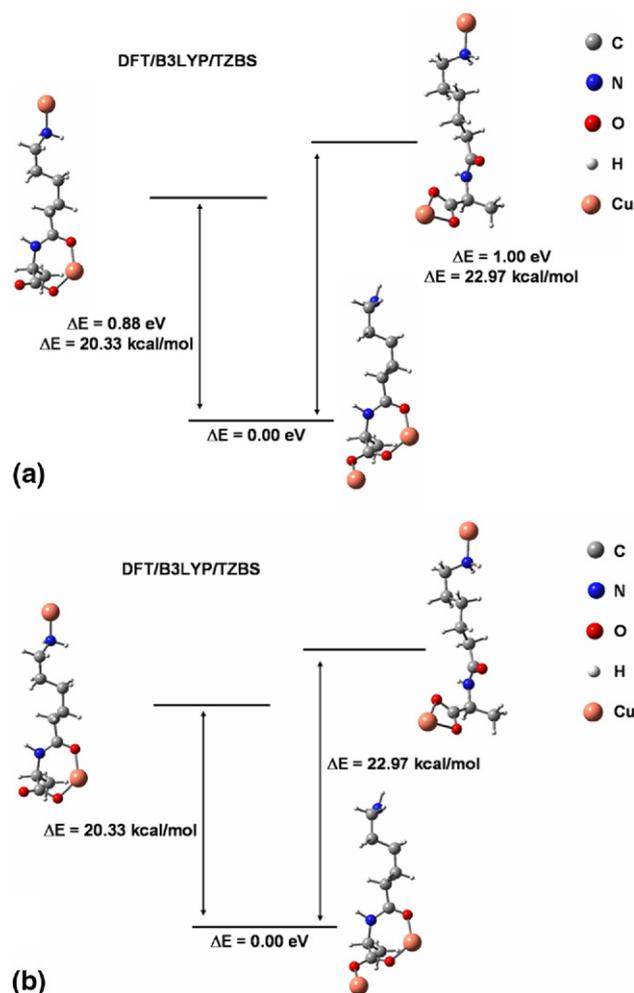
Figure 5. Model structures used to study the copper binding site and energy for: (**a**) deprotonated Lys-Ala containing two Cu^+ ions $[M + 2Cu - H]^+$, and (**b**) the dinuclear Cu^+ complex of deprotonated Ala-Ala (AA, label A) and n-propylamine (PA, label B) $[M + 2Cu - H]^+$. All calculations were performed at the DFT/B3LYP/TZBS level.

bind to the lysine near the termini. The ratio of b- to y-type ions of $[M + Cu]^+$ is ~70% to 30%, which suggests that lysine residues near the N-terminus has a higher Cu^+ affinity than lysine residues that are located near the C-terminus. We also found that the competitive binding of Cu^+ to basic residues close to N-terminus versus C-terminus depends on the amino acid. For example, for a multiple histidine containing peptide such as WGGHDGPHAPGDH, we do not observe signature fragment ions for $[M + Cu]^+$ and $[M + 2Cu - H]^+$ in which Cu^+ ions bind at His^4 (data not shown). Thus, the most favorable binding sites for Cu^+ ions are His^8 and His^{13} probably because of the peptide second-
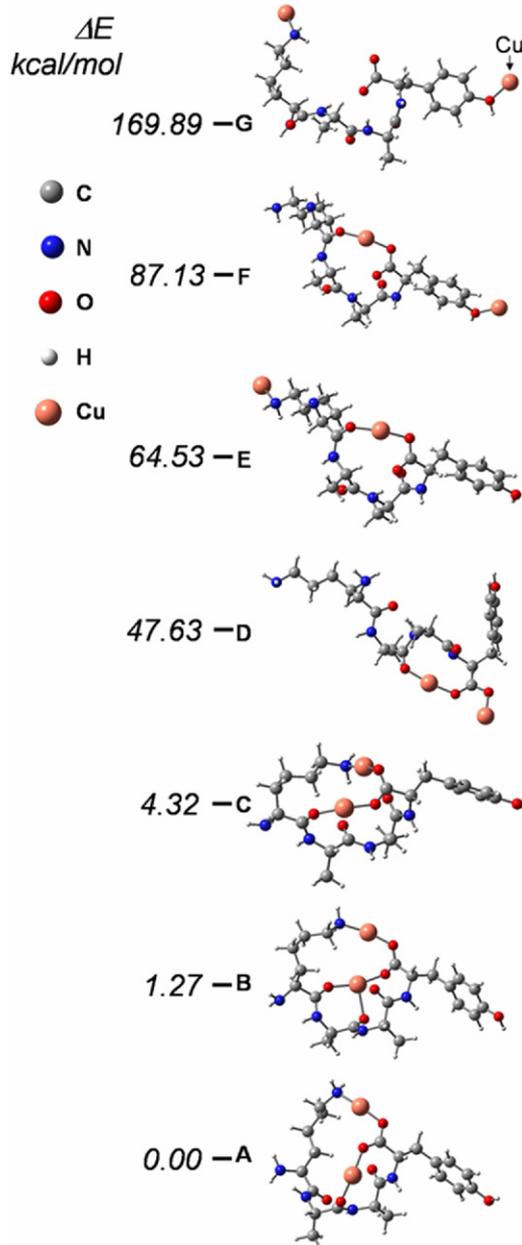

Figure 6. Candidate structures obtained at the DFT/B3LYP/TZBS level for the y_4 fragment ion of the $[Ac-(AAKAA)_2-Y + 2Cu - H]^+$ peptide ion.

can be rationalized. For example, the collisional activation of the $Ac-(AAKAA)_2-Y$ $[M + 2Cu - H]^+$ ion weakens the interaction between the €-amino group of Lys^3 and subsequent backbone cleavage yields fragment ions with the charge carried by the C-terminus. The lowest energy C-terminal charge carrying ions are the y-type ions [44].

We also examined fragmentation reactions of $[M + Cu]^+$ and $[M + 2Cu - H]^+$ of histidine and arginine containing peptide ions (Table 1). The arginine containing peptides RPGFSPFR and RPPGFSPFR (bradykinin) contain abundant b-type ions which we interpret as evidence for binding preferences to the N-terminal arginine. Note also that this trend is observed for both $[M + Cu]^+$ and $[M + 2Cu - H]^+$ ions. The peptides $Ac-(AAKAA)_nY-NH_2$ (n = 3, 4) yield fragment ions that suggest the Cu^+ ion is bound to Lys^3 and Lys^{13} (n = 3) and Lys^3 and Lys^{18} (n = 4), i.e., the N- and C-terminal residues, thus apparently the Cu^+ ions preferentially

ary structure, i.e., His[4] may be sterically hindered by other amino acid residues, thereby reducing the Cu^{+} ion affinity of His[4]. Residues such as tryptophan and aspartic acid may also play roles in copper binding. For example, we have found that acidic groups such as oxidized cysteine strongly influence Cu^{+} ion binding. Specifically, for the peptide laminin (CDPGYIGSR), we found that the oxidized cysteine (via sulfonic acid) will partially shift the Cu^{+} binding from the arginine to the C-terminal cysteine, owing to the bidentate binding of Cu^{+} by the acidic sulfonic acid, similar to that for the carboxylic acid group discussed above.

Conclusions

We have synthesized a novel copper matrix and demonstrated the utility of the copper matrix for studies of copper binding peptides. The yields of copper adducted peptides in the gas-phase are increased significantly using this copper matrix as compared with other methods previously used to generate peptide-Cu^{+} ions. We investigated the fragmentation chemistry of $[M + Cu]^+$ and $[M + 2Cu - H]^+$ of model peptides, and explored the influence of modifying the C-terminal group on the fragmentation pattern of the peptide-Cu^{+} ions. We have also explored the influence of the amino acid on the copper binding site and the fragmentation pattern. This work provides a new approach towards investigating the binding of copper ions to peptides and this new method will likely help understand the interactions of copper ions with peptides and proteins in the gas phase. Future work will focus on studying the gas-phase structure of copper-binding peptides which will provide a detailed understanding of copper adduction in the gas-phase. The idea of the copper matrix can also be extended to other transition metals such as nickel and iron to investigate the binding of metal ions to functionalized organic molecules, which are also important in many biological systems.

Acknowledgments

Appendix A
Supplementary Material

Supplementary material associated with this article may be found in the online version at doi:10.1016/ j.jasms.2009.02.025.

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