Gas-Phase Coordination Properties of Zn^{2+}, Cu^{2+}, Ni^{2+}, and Co^{2+} with Histidine-Containing Peptides

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Received December 14, 1994[⊗]

Abstract: Transition metal ion−neutral peptide complexes produced by electrospray ionization (ESI) were studied by tandem mass spectrometry with regard to their metal binding structure. Zn^{2+}, Co^{2+}, and Ni^{2+}−histidine containing peptide complexes dissociate at histidine sites upon collisional activation, indicating metal ion binding primarily at the histidine site. The Cu^{2+}−neutral peptide complexes, however, dissociate to produce an ion by losing CO_2 from the C-terminus, and other product ions by reactions at the C-terminus, suggesting the association of Cu^{2+} with the peptide C-terminus.

Introduction

The combination of amino acids in the metal binding domains of metalloenzymes at least partially defines the type of metal cofactor(s) required for their function. For example, Ca^{2+}-binding ligands at Ca^{2+}-binding sites of proteins are exclusively oxygen-based groups, therefore Asp/Asn and Glu/Gln are the most frequent residues in these sites.[1] The most frequently found amino acid residue in the catalytic Zn^{2+} sites of Zn^{2+}−metalloenzymes is histidine followed by glutamic acid, aspartic acid, and cysteine.[2,3] It is of interest to know the undermining factors in the natural selection processes, and whether there are correlations between the frequency for a residue in the metal binding sites and the intrinsic affinity of the residues toward the metal ion.

The interactions between metal ions and peptides in solution have been studied for decades.[4] Transition metal ions weakly interact with peptide amide oxygens in solution at pH values where the peptide bonds are not deprotonated. This interaction, however, is strengthened if groups capable of anchoring metal ions, e.g., the N-terminal NH_2 group, histidine, and cysteine, are present. The metal ion chelates to the anchoring group and an amide oxygen to achieve higher stability. Thus, transition metal ions interact with peptides either at the N-terminus or at these residue sites.

Mass spectrometric reactions usually are unimolecular, which presents a unique opportunity for investigating intrinsic interacting properties between two or more species. Mass spectrometry (MS) has been used to study the interactions between peptides and metal ions via structure elucidation.[5−14] For MS to be a useful method, however, the system in question must meet some basic requirements. For example, the chemical system intended for the study of metal ion/peptide interaction should involve interacting species that resemble those in solution, and the metal binding structure should also be reflected in their mass spectrometric reactions (metastable or collisionally induced).

In our previous study[5] we reported the gas-phase interactions between Zn^{2+} and angiotensin peptides which contain one or two histidines. The study was possible due to the abundant formation of a complex involving Zn^{2+} and a neutral peptide (i.e., the peptide does not carry a net charge) by electrospray ionization. Histidine was found to be the primary Zn^{2+} site of the peptides. In this paper we extend the research to include other histidine-containing peptides and transition metal Zn^{2+}, Ni^{2+}, Cu^{2+}, and Co^{2+} ions and to explore the fragmentation chemistry of these complexes and the differences in coordination chemistry between these metal ions.

Experimental Section

Mass Spectrometer. ESI-MS analyses were performed with a Finnigan MAT 900Q forward geometry hybrid mass spectrometer (Bremen, Germany) equipped with a position-and-time-resolved-ion-counting array detector (PATRIC) after the magnet and before quadrupole analyzer.[15,16] Analyses were performed at full accelerating potential (5 kV), except for linked scan at constant B/E experiments of multiply-charged parent ions. Sample solutions were infused through the ESI source at a flow rate of 1 μL min^{-1}. Mass spectra were typically acquired at 20−30 s decade^{-1}.

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[⊗] Abstract published in *Advance ACS Abstracts*, November 1, 1995.

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The electrospray ionization interface on the double focusing instrument is based on a heated glass capillary inlet (0.5 mm i.d. × 6.2 mm o.d. × 218 mm length).[17] Droplet desolvation was accomplished by a countercurrent stream of warm nitrogen (~60 °C) and by energetic collisions in the electrospray interface. The energy of the collisions is controlled by the voltage difference between the tube lens surrounding the metallized exit of the glass capillary and the first skimmer element of the interface (ΔV_{TS}). The typical range of ΔV_{TS} used for this work was +90 to 120 V. Sulfur hexafluoride coaxial to the spray was also used to suppress corona discharges.

The tandem mass spectrometry (MS/MS) data were acquired by scanning the magnet and the electrical analyzer simultaneously at a constant B/E ratio. Collisionally activated dissociation (CAD) was performed in the first field free region by introducing helium gas sufficient to reduce the precursor ion abundance to 50%. For operation with the PATRIC array detector, a 2% mass-to-charge (*m/z*) range of the *m/z* centered on the detector was used. Due to the use of an array detector on a two-sector instrument any precursor selectivity of a B/E linked scan was lost. (In practice, the *m/z* range that is passed tends to be larger than 2%; however, the transmission for ions outside the window declines exponentially.) The precursor beam is actually a sum of ions whose masses fall within the 2% window of the intended precursor mass. All product ions of all ions in the precursor beam were also recorded simultaneously. This inherent lack of precursor ion selectivity sometimes causes small artifacts in the MS/MS spectrum if there are any other potential precursor ions, including precursors containing heavy isotopes, whose masses fall in this mass window. For example, the doubly protonated peptides are observed in the collisionally activated dissociation of some metal–peptide complexes because the protonated species are much more abundant than the metal–peptide complexes in the ionization. The abundance of the doubly-protonated peptide species can be minimized relative to the (M + metal)$^{2+}$ ion by increasing ΔV_{TS} (the 2+ protonated peptide is more susceptible to collisional dissociation than the metal-bound peptide). The inclusion of the entire isotropic distribution of the precursor ion in the precursor beam, on the other hand, yields product ions that possess a similar isotopic distribution pattern. This feature is an aid for identifying metal-containing product ions with metals that have unique isotope distribution patterns. In addition, the use of the array detector may be less discriminative to singly charged product ions with large kinetic energy release. Because of the 2% *m/z* window simultaneously detected, the singly charged ions with less or more energy due to the large kinetic energy release will be less discriminated during a constant B/E scan than with a conventional point detector.

Materials. The angiotensin peptides angiotensin I (DRVYIHPFHL), Ac-angiotensin I, des-Asp^1-angiotensin I, bullfrog angiotensin I (DRVYVHPFNL), goosefish angiotensin I (NRVYVHPFHL), angiotensin II (DRVYIHPF), des-Asp^1-angiotensin II, Sar^1-angiotensin II, angiotensin III (RVYIHPF), and Ile^7-angiotensin III were purchased from Sigma Chemical (St. Louis, MO). Peptides EGVYVHPV, PHPFHLFVY, AAAYAA, and LAHMYVGK were purchased from BACHEM Bioscience (King of Prussia, PA). Ala^6-angiotensin II was provided by Dr. Wayne Cody (Parke-Davis Pharmaceutical), and Ac-EGVYVHPV-NH_2 was synthesized by Dr. Dan Holsworth (Parke-Davis Pharmaceutical).[18]

Results and Discussion

Complex formation between a neutral peptide and a doubly charged metal ion involves metal ion coordination to neutral ligands. In the absence of residues that anchor metal ions, the metal ion chelates to amide oxygens. Such interactions are usually weak. Residues such as histidine and cysteine are considered to be better metal binding ligands than amides in a neutral peptide.[4] A structure that involves a metal ion chelating to these groups and the neighboring amide(s) is more favorable than those involving only amide groups. Thus, the presence of metal-anchoring residues should promote the formation of metal ion–neutral peptide complexes.

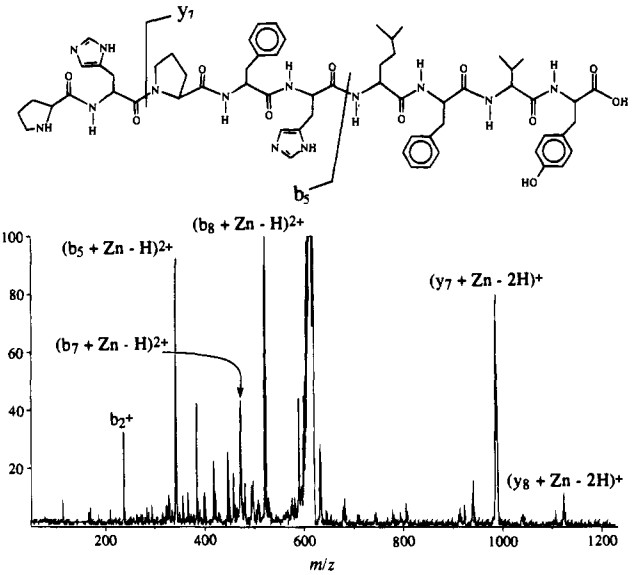

Figure 1. The CAD spectrum of the (PHPFHLFVY + Zn)$^{2+}$ ion.

Our assumption throughout this report is that the major structural form for the initial complex is a neutral peptide coordinated to a doubly charged metal ion. However, it is conceivable that a minor population of the peptide species may be zwitterionic, which we believe is much less stable than a structure involving a neutral peptide. Charge separation reactions (reactions that yield more than one charged species from a multiply charged precursor) may be very important for revealing the possible existence of zwitterionic peptides. However, we do not believe that the main dissociation process involves a zwitterionic peptide. The fragmentation chemistry and possible structures of these complexes described in this paper are based on the major structural form. Whether some of the dissociation processes involve zwitterionic species does not compromise the main point of this paper, to explore the differences between zinc metal ion and the other three transition metal ions (Cu^{2+}, Ni^{2+}, and Co^{2+}) upon interacting with histidine-containing peptides.

In our previous paper[5] we discussed the interactions between *angiotensin* peptides and zinc metal ion. For a complex involving a neutral peptide and a Zn^{2+} ion, the histidine residue is the favored metal binding site. As a result, for the peptides studied, upon collisional activation or under metastable conditions, the complex dissociates nearly exclusively to form (a_n + Zn^{2+} − H)$^{2+}$ and (b_n + Zn^{2+} − H)$^{2+}$ ions by reactions occurring at the histidine sites [compared to the dissociation behavior for the (M + 2H)$^{2+}$ peptide]. We extend this investigation by including other histidine-containing peptides and other transition metals. Again, the main theme of this study is that the charge of the metal ion induces fragmentation at the metal binding site. However, reactions that occur away from the metal-binding site were also found. The premise that the metal ion induces fragmentation at the metal sites does not exclude fragmentation from occurring at other sites of the peptides. For the peptides studied that contain histidine but are not directly related to angiotensin-type sequences, the histidine-containing fragment was a prominent fragment ion.

Zn^{2+}, Co^{2+}, and Ni^{2+} Complexes. The CAD spectrum of the Zn^{2+}–PHPFHLFVY complex is shown in Figure 1. {The nomenclature used to label the product ions is based on a conventional notation.[5,19] A superscript is added to indicate charge state. The small letters should be understood to have

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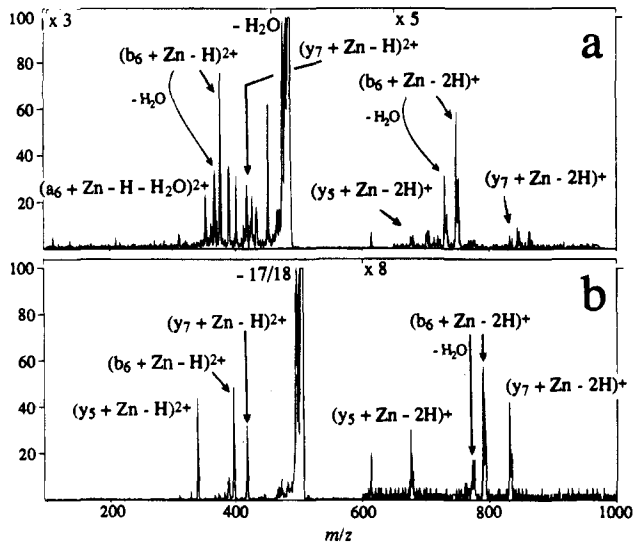

Figure 2. The metastable ion spectra of (a) the (EGVYVHPV + $Zn)^{2+}$ ion and (b) the (Ac-EGVYVHPV + $Zn)^{2+}$ ion. The measured and theoretical *m/z* values for the b- and y-type ions in (b) are as follows: $(y_5 + Zn - H)^{2+}$, (338.1/338.1); $(b_6 + Zn - H)^{2+}$, (395.0/395.1); $(y_7 + Zn - H)^{2+}$, (416.2/416.0); $(y_5 + Zn - 2H)^{+}$, (675.2/675.3); $(b_6 + Zn - 2H)^{+}$, (789.2/789.3); and $(y_7 + Zn - 2H)^{+}$, (831.2/831.4).

the same compositions as they are formed from $(M + H)^+$ precursor ions. Metal attachment and hydrogen movements are labeled explicitly [e.g., $(b_6 + Zn - H)^{2+}$ denotes a Zn^{2+}-deprotonated b_6^+ ion]}. Consistent with the angiotensin peptides studied, an abundant $(b_5 + Zn - H)^{2+}$ ion originating from reactions at the His^5 site indicates His^5 is a primary Zn^{2+} coordination site. The formation of $(b_7 + Zn - H)^{2+}$ and $(b_8 + Zn - H)^{2+}$ ions can be explained by a structure where the Zn^{2+} binds to one of the two histidine sites. The abundant $(y_7 + Zn - H)^{2+}$ and weakly-formed $(y_8 + Zn - H)^{2+}$ ions suggest His^2 is not as good a Zn^{2+} anchor as His^5, most likely due to the constraints imposed by the two neighboring proline residues. The formation of a non-zinc-containing b_2^+ ion may be further support for a structure in which the preferred metal binding site is His^5 (i.e., not His^2).

We showed in our previous study[5] that the N-terminal NH_2 group is a weaker Zn^{2+} site than a histidine residue. Further evidence is presented here in the comparative study of the EGVYVHPV and Ac-EGVYVHPV-NH_2 peptides. Upon collisional activation, the Zn^{2+} complexes of both EGVYVHPV and Ac-EGVYVHPV-NH_2 (Figure 2) produce ions from reactions at histidine sites [$(a_6 + Zn - H)^{2+}$, $(b_6 + Zn - H)^{2+}$, and $(y_{5-7} + Zn - H)^{2+}$ ions], pointing to the metal anchoring role of the histidine residue.

A loss of a water molecule from the precursor or the metal-containing a or b product ions (abbreviated as a- or b-type ions in the following discussion) is a facile process in the metastable fragmentation of both Zn^{2+}–EGVYVHPV and Zn^{2+}–Ac-EGVYVHPV-NH_2 complexes (Figure 2). The loss of water from the a- or b-type ions suggests that either the process that gives rise to a b-type ion or the water loss process is charge remote in nature. It is less likely that the reactions leading to the formation of a- or b-type ions are thermal decomposition due to the high dependence of these reactions on the location of histidine. Thus, the observed facile loss of water is not entirely caused by metal binding, although the slightly smaller

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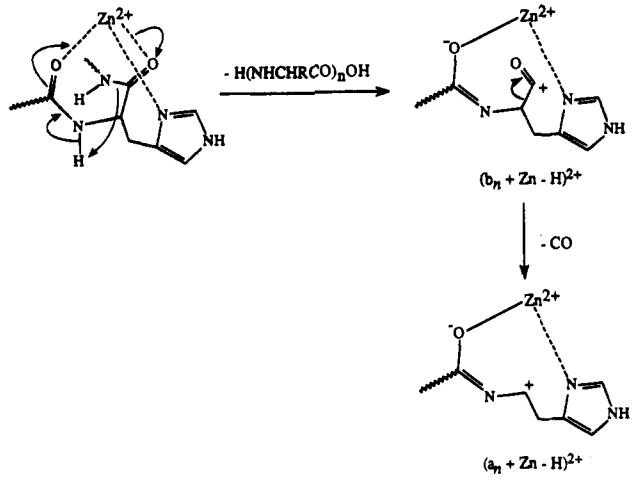
Scheme 1



$(b_6 - H_2O)/b_6$ intensity ratio for the Ac-EGVYVHPV-NH_2–zinc complex than that for the EGVYVHPV–zinc complex may indicate some N-terminal metal binding that promotes water loss (Figure 2). Blocking of the termini of a peptide can change the chemical properties of a peptide. It is therefore not surprising that this affects the overall metal-binding characteristics to some unknown degree.

In addition to CAD spectra, metastable ion spectra were used for elucidation of the dissociation mechanisms (e.g., Figure 2). Low-energy-reacting configurations of the precursor complexes are most likely to be probed by metastable ion decompositions.[6] As Reiter *et al.* states, "The most structurally revealing reactions are low-energy charge-induced cleavages that occur in immediate proximity to the metal-ion binding site."[6]

Dissociation of the amide X–Pro bond has been observed to be a favored process for multiply charged ions under CAD conditions, but Loo *et al.*[20] provided evidence from low-energy CAD experiments that the process may not be as favorable when a positively-charged basic residue (i.e., Arg, Lys, His) precedes the proline residue. It is unlikely that proline plays a major role in the formation of the $(b_n$ + metal) product ions for histidine- and proline-containing peptides. As shown previously,[5] the CAD spectrum for (Ala^7-angiotensin II + $Zn)^{2+}$ (where Pro^7 has been replaced by alanine) is virtually the same as that of the (angiotensin II + $Zn)^{2+}$ complex; the $(b_6 + Zn)^{2+}$ product is the dominant fragment species. Furthermore, CAD of Ala^6-angiotensin II, which contains the Ala-Pro unit, produces a metal-attached b_6 ion with comparable abundance to the b_5 and b_7 metal-containing product ions.

Although the precursor ions involve a neutral peptide, collisional activation of the complex induces deprotonation of the peptide. Because metal ion coordination is necessary for the a-/b-type ion formation reactions to occur, the charge on the metal ion must play a role in the process. Considering that the reactions occur *primarily* at the metal binding site and that the deprotonation process should lead to a stable metal binding structure for the product ion, we propose that the hydrogen removed with the C-terminal part of the peptide in the formation of the $(b_n + Zn^{2+} - H)^{2+}$ ion is from the histidine amide nitrogen (Scheme 1). The resulting product ion involves metal binding to a net negatively charged oxygen and the positive charges are separated, which should also be a stabilizing factor for the product ion. The formation of $(a_n + Zn^{2+} - H)^{2+}$ is merely a one-step elimination of the CO group from the corresponding metal-containing b-type ion.

Co^{2+}– and Ni^{2+}–angiotensin II complexes yielded very similar fragmentations as the Zn^{2+} complexes (compare the

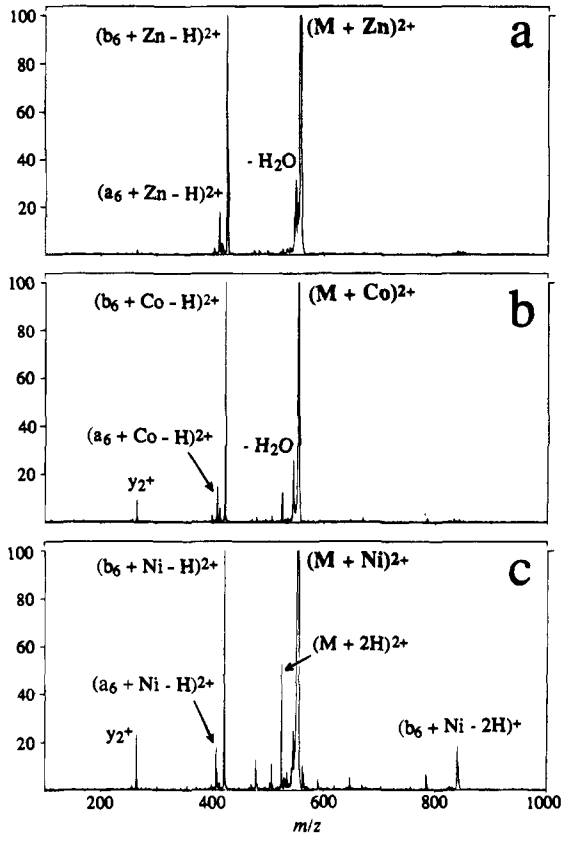


Figure 3. The metastable ion spectra of (a) the $(DRVYIHPF + Zn)^{2+}$ ion, (b) the $(DRVYIHPF + Co)^{2+}$ ion, and (c) the $(DRVYIHPF + Ni)^{2+}$ ion.

metastable spectrum in Figure 3a with Figures 3b and 3c).[5] Namely, $(a_6 + Zn^{2+} - H)^{2+}$ and $(b_6 + Zn^{2+} - H)^{2+}$ ions produced by the fragmentation at the histidine site are the dominant species. This apparently is also a result of metal ion binding to the histidine residue site. One unique fragmentation from the Ni^{2+}–angiotensin II complex is the formation of a $(b_6 + Ni - 2H)^+$ ion, in which the Ni^{2+} ion substitutes for *two* protons. Complexes of Zn^{2+} and Co^{2+} with these peptides do not produce this product ion substantially (Figures 3a and 3b, respectively). It is a common practice to substitute Co^{2+} for Zn^{2+} or Cu^{2+} in metalloproteins due to their desirable physical properties for NMR studies.[21] The enzymes typically maintain most of their activity upon metal substitution. Ni^{2+} substitution has been reported less frequently because the enzymes, in some cases, lose activity. The unique behavior of Ni^{2+} may stem from the same combination of redox and coordination properties that leads to the selection of Ni^{2+} for the biological hydrogenation, desulfurization, and carbonylation reactions.[22]

A non-metal-containing product ion formed with moderate abundance is the y_2^+ ion. The abundance of this ion in the CAD spectrum of angiotensin II $(M + 2H)^{2+}$ ion is about 35% relative to the most abundant product ion b_6^+, as shown previously.[5] Although the peptide $(M + 2H)^{2+}$ ion is present due to the poor precursor selection resolution in our metal-binding experiment, the b_6^+ ion is much less abundant than the y_2^+ ion. Therefore, the y_2^+ ion is at least partially produced from the metal–peptide complexes. Given the number of hydrogen transfers required to form a y_2^+ ion, the peptide most likely has a neutral C-terminal COOH group.

The y_2^+ ion, nominally, is a complementary ion to the $(b_6 + Ni - 2H)^+$ ion (Figure 3c), which in sum (mass and charge) account for the intact molecule.[20] Another pair of complementary ions, b_2^+ and $(y_7 + Zn - 2H)^+$, were observed for the PHPFHLFVY–zinc peptide complex (Figure 1). There appears to be some correlation between the abundances of these complementary ions, i.e., they are simultaneously abundant. They, therefore, may be produced from the same precursor as products of the same reaction. We, however, do not have evidence to support that a charge separation mechanism was involved in the reaction.

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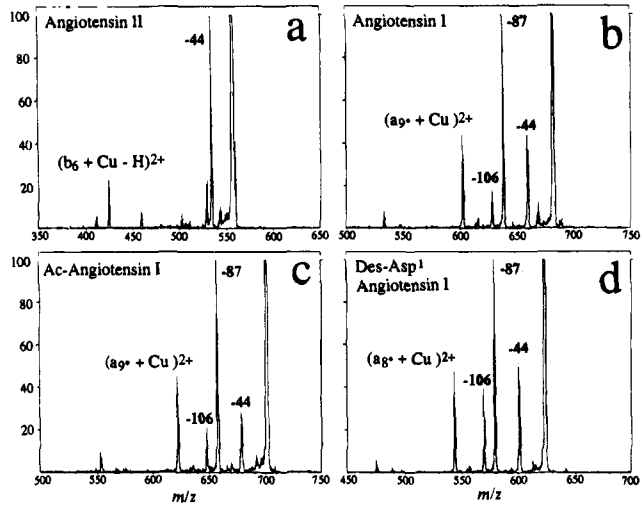

Figure 4. The ESI-CAD spectra of (a) the $(DRVYIHPF + Cu)^{2+}$ ion, (b) $DRVYIHPFHL + Cu)^{2+}$ ion, (c) the $(Ac\text{-}DRVYIHPFHL + Cu)^{2+}$ ion, and (d) the $(RVYIHPFHL + Cu)^{2+}$ ion. There are two ions not covered in the mass range for (d), *m/z* 1156 $[(M + Cu - 87),^{\bullet+}$ 5% relative to $(M - 87)^{2+}$ ion], and *m/z* 1051 [2% relative to $(M - 87)^{2+}$ ion]. The *m/z* values measured for the $(M + Cu)^{2+}$ ion of des-Asp^1-angiotensin I and the ions formed by losing CO_2 (−44), $CO_2 + C_2H_5^{\bullet}$ (−87), $OC_6H_4CH_2$ (−106), and the $(a_8^{\bullet} + Cu)^{2+}$ ion are 622.0, 600.0, 578.5, 569.1, and 543.0, respectively.

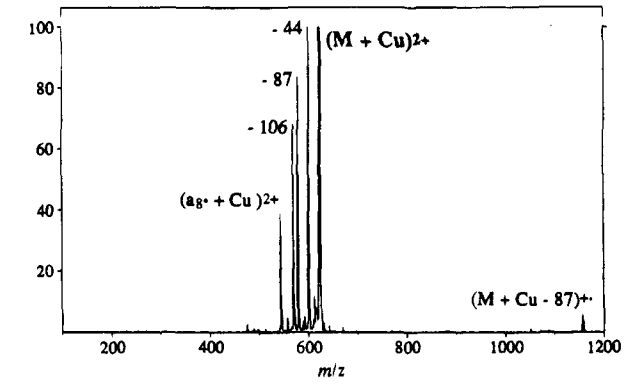

Figure 5. The metastable ion spectra of the $(RVYIHPFHL + Cu)^{2+}$ ion.

Cu^{2+} Complexes. In solution, histidine acts as a Cu^{2+} anchor as well as for other transition metal ions.[4] The gas-phase interaction of Cu^{2+} with the peptides studied, however, was observed to show different characteristics to those of Zn^{2+}, Ni^{2+}, and Co^{2+} ions. A loss of 44 mass units typically is the most facile reaction in the CAD of Cu^{2+}–angiotensin peptide complexes (Figure 4). The loss of 44 u yields the most abundant product ion in the metastable ion spectra of the angiotensin I peptides as shown in Figure 5 (compare Figure 4d and Figure 5). Modification of the N-terminus, such as acetylation (Figure 4c) or the removal of the N-terminal aspartic acid from angiotensin I (Figure 4d) and angiotensin II peptides, does not mitigate the process of losing 44 u. Amidation of the C-terminus, however, completely removes this fragmentation channel (Figure 6). In fact, Cu^{2+} complexes of peptides with

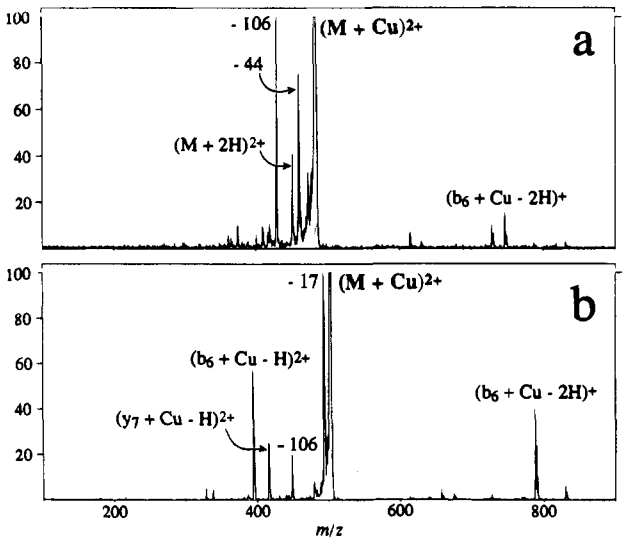

Figure 6. The metastable ion spectra of the (a) (EGVYVHPV + Cu)$^{2+}$ ion and the (b) (Ac-EGVYVHPV-NH_2 + Cu)$^{2+}$ ion.

a C-terminal amide decompose in a similar fashion as their Zn^{2+}-complex counterparts (compare Figures 2b and 6b). When the C-terminal residue of the peptide is a leucine, a reaction that leads to the loss of 87 u also occurs (Figure 4b–d). The product of this reaction may sometimes be more abundant than that of the loss of 44 u. An MS/MS experiment on the ESI source-produced $(M - 44)^{2+}$ ion (with a high ΔV_{TS} voltage difference) shows that the $(M - 44)^{2+}$ ion can be the precursor of the $(M - 87)^{2+}$ ion. Substitution of the C-terminal leucine by isoleucine (Des-Asp^1, Ile^8-angiotensin II) results in the formation of the $(M - 73)^{2+}$ ion [i.e., (44 + 29)]. Furthermore, the presence of a histidine residue in the peptide is not necessary for this reaction to occur; the $(M - 44)^{2+}$ ion is the most abundant product in the CAD spectrum of the Cu^{2+}–Ala^6-angiotensin II complex.

This evidence indicates that the 44 u loss from Cu^{2+}–peptide complexes originates from the removal of CO_2 from the C-terminal carboxylate group, and major fragmentations also occur in the vicinity of the C-terminus. If the argument that the metal ion induces fragmentation at the metal ion site is used to justify the fragmentation of Cu^{2+} complexes, then the Cu^{2+} ion is most likely coordinated with the C-terminus of the peptide. An isolated C-terminus does not give rise to an abundant 44 u loss as for the cases of protonated peptides or Zn^{2+}/Ni^{2+}/Co^{2+} complexes. For peptides with an amide C-terminus, Cu^{2+} interacts with the peptide in the same fashion as Zn^{2+}, Co^{2+}, and Ni^{2+} ions because Cu^{2+} binding to the C-terminus is not likely.

It is interesting to note that an alkali metal ion cationized peptide with a C-terminal leucine decomposes to give a loss of 88 u.[23,24] Because changes, such as amidation and methylation, incurred at the C-terminus do not mitigate against this reaction, a pyrolysis mechanism was proposed for the process. This reaction was used as an indication that alkali metal ions are not associated with the C-terminus. For Cu^{2+}–peptide complexes, amidation at the C-terminus discourages the reactions that lead to the losses of 44 and 87 u. Thus, the Cu^{2+} ion must be associated with the C-terminal carboxylate group and be involved in the reaction.

Cu^+ along with some other singly charged transition metal ions (e.g., Co^+, Ni^+, and Fe^+) has been shown to have the ability

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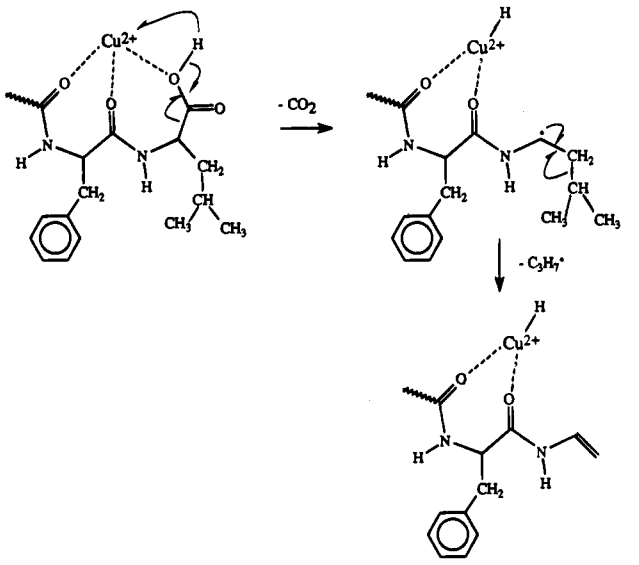
Scheme 2



to insert into a C–H, O–H, or an allylic or propargylic C–C bond (a so called oxidative addition reaction) when anchored by a double or triple bond or a functional group such as nitrile.[25–27] The result of a C–H or O–H bond insertion is that the metal ion gains a hydrogen atom, and the free electron so produced on the atom from which the hydrogen atom is subtracted induces α-cleavage, leading to the elimination of part of the molecule. We propose that Cu^{2+} association with a peptide C-terminus promotes a similar process of subtracting hydrogen atom from the carboxylate group leading to the loss of CO_2 (Scheme 2). The subsequent loss of 43 u from leucine and 29 u from isoleucine are products of an additional α-cleavage reaction. Cu^+ is generally presumed unable to participate in oxidative addition reactions due to its d^{10} electron configuration.[25] The hydrogen subtraction by Cu^{2+} ion may be more foreseeable.

A recent study[28] by ESI-MS on Cu(II) amino acid complexes in the gas phase showed that the $(Cu^{\bullet 2+}RCOO^{\bullet -}bpy)^+$ ions of leucine and isoleucine also decompose by losing CO_2 followed by losing $C_3H_7^\bullet$ and $C_2H_5^\bullet$, respectively. It is therefore also possible that a deprotonated C-terminal carboxylate group is involved in Cu^{2+} binding and the reactions proceed in a mechanism proposed by Gatlin et al. (Scheme 3). The first step of the reaction involves the reduction of the Cu^{2+} metal ion to Cu^+. The resulting radical on the carboxylate oxygen induces loss of CO_2 and subsequent loss of $C_3H_7^\bullet$ from leucine and $C_2H_5^\bullet$ from isoleucine.

It is also observed that an $(M - 106)^{2+}$ ion is produced weakly from Cu^{2+}–angiotensin I peptides (Figures 4 and 5) and most abundantly from the EGVYVHPV–Cu^{2+} complex (Figure 6). The common residue among those peptides which is likely to lose 106 u ($OC_6H_4CH_2$) is tyrosine. The Ac-EGVYVHPV–amide–Cu^{2+} complex, which has the metal binding at His^6, does not undergo this fragmentation. It appears that the tyrosine residue needs to be placed a few residues away from the Cu^{2+} ion for the reactions to occur. Again, these metal-containing fragments can be explained by reactions that occur away from the metal-binding site. Thus, the nature of the process may be a similar reaction to that producing a loss of

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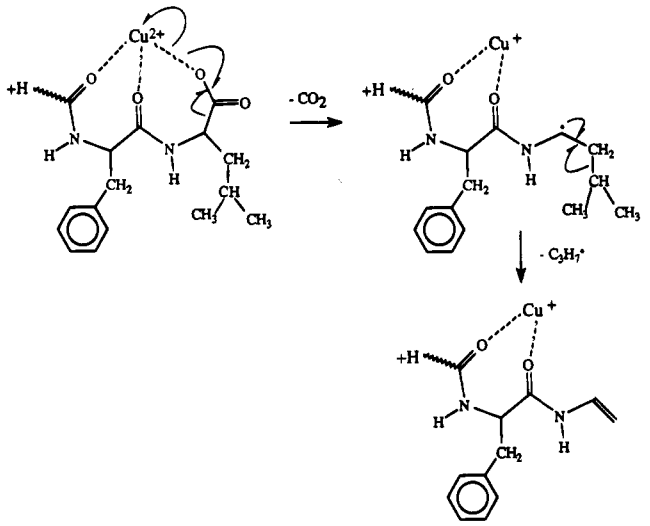
Scheme 3



44 u, but may also be thermal decomposition. The distance requirement is likely due to the necessary space to fold the peptide and to orientate the tyrosine OH group toward the metal ion.

The versatile reactivity of Cu^{2+} is also shown in the formation of a weak $(M + Cu - 87)^{\bullet+}$ product ion, observed in the CAD and metastable fragmentation of $(angiotensin\ I + Cu)^{2+}$ (Figures 4d and 5, respectively). It may, however, be explained by a mechanism similar to that depicted in Scheme 2, except that the hydrogen rearranged is a hydride. The sequence should lead to loss of CO_2 and $C_3H_7^+$ and the formation of the $(M + Cu - 87)^{\bullet+}$ ion. Its detailed mechanism, however, is beyond the scope of this study.

Another unique feature of Cu^{2+} reactivity is the production of a radical $(a_{n-1}^{\bullet} + Cu)^{2+}$ ion from Cu^{2+}–angiotensin I peptide complexes (Figures 5 and 6). Sometimes it is accompanied by the weakly formed $(a_{n-2}^{\bullet} + Cu)^{2+}$ ion. The formation of these ions does not involve the deprotonation of the remaining part of the peptide, unlike the formation of $(a + Zn - H)^{2+}$ ions. The formation of these ions, however, may point to the possibility that some population of the Cu^{2+} complex may have Cu^{2+} binding at the histidine site or the N-terminus. Further study, possibly involving the use of extended tandem mass spectrometry experiments (i.e., MS/MS/MS), is necessary to fully elucidate the nature of these dissociation pathways.

Conclusion

Electrospray ionization and tandem mass spectrometry are shown to be a useful method for studying intrinsic peptide–metal ion interactions. In the gas phase, histidine is the preferred anchor for Zn^{2+}, Co^{2+}, and Ni^{2+} ions. The Cu^{2+} ion, however, appears to have some affinity to the peptide C-terminal carboxylate group. For histidine-containing peptides, the histidine residue is also a Cu^{2+}-binding site. When the peptide C-terminus is not a carboxylate group the histidine residue is the primary Cu^{2+}-binding site.

Many researchers have presented evidence to suggest that data from gas-phase ESI-MS measurements are useful in describing biochemical structures found in the solution phase. Several noncovalent complexes[29] and protein folding experiments[30–32] have been carried out with MS. The importance of water in the solution structure of a macromolecule has not escaped these workers. Wolynes has reflected the seemingly inconsistent notion that biomolecular folding can occur in the vacuum.[33] It is difficult to assess the biological relevance of the data we present. Directly relating the chemistry deduced from a gas-phase system to solution chemistry may be inappropriate at this time. It is unknown how solvation alters the intrinsic properties of a system. Finding the answer to this question will be the final goal achieved by a collective study of many different systems. Our work can only be a building block toward this goal.

JA9440359

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