Gas-Phase Interactions of Transition-Metal Ions and Di- and Tripeptides: A Comparison with Alkaline-Earth-Metal-Ion Interactions

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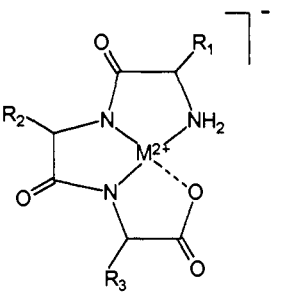
Abstract: Di- and tripeptides containing amino acids with nonacidic or nonbasic side chains bond with transition metals to give [tripept + Met^{2+} – $3H^+$]$^-$ that exist in various structural forms in the gas phase. This is in sharp contrast to the nearly homogeneous structure population of the corresponding alkaline-earth-metal-bound tripeptides in which the C-terminal COOH group and the two amide NH groups are deprotonated and bond to the metal ion. The structure-determining factor is the difference in ligand affinity of the metal ions. Alkaline-earth-metal ions are oxyphilic, whereas transition-metal ions favor nitrogen ligands in solution. That property is carried over to the gas phase and may be regarded as an intrinsic property. Because transition metals favor nitrogen ligands, dipeptides form abundant metal-bound peptides of these metals. Collisionally activated decompositions (CAD) measured on a tandem mass spectrometer show that transition-metal-bound tripeptides fragment both at the C-terminus by losing CO_2 and H_2CO_2 and at the N-terminus, where alkaline-earth-metal-bound tripeptides decompose. Transition-metal complexes containing serine, threonine, phenylalanine, and methionine lose elements of the side chain. For peptides containing several amino acids with functionalized side chains, fragments from each are observed, in contrast with the predominant loss of a single side chain from corresponding alkaline-earth complexes. Another contrast is bis(peptide) complexes which are formed less readily with transition metals than with alkaline-earth-metal ions.

Introduction

Dipositively charged transition-metal ions, including Ni(II),[1] Cu(II),[2] ^{195}Pt(II),[3] and Pd(II),[4] interact with tripeptides in solution to form [pept + Met^{2+} – $3H^+$]$^-$ complexes that have a square planar structure (**1**). The C-terminal carboxylate group and the two amide NH groups are deprotonated. For tetrapeptides, however, a [pept + Met^{2+} – $4H^+$)$^{2-}$ is formed in which the C-terminal carboxylic acid and the three amide NH groups are deprotonated. The C-terminal COO^- group, however, is not involved in the metal/tetrapeptide coordination of the planar square complex either in solution[1b] or in the crystal.[5] The nature of the metal–peptide interactions has been revealed by extensive CD and, more recently, NMR studies of the kinetics of protonation and of ligand replacement.[1-4]

Interactions of metal ions and deprotonated peptides in solution are only established for transition-metal ions. The failure to observe any interactions of alkali and alkaline-earth-metal ions in solution has led to the hypothesis that these metal ions are unable to deprotonate amide hydrogens.[6] In our previous papers,[7,8] however, we reported the intrinsic ability of alkaline-earth-metal ions to deprotonate amide nitrogens and form anionic metal-bound peptides and metal–bis(peptide) complexes. We characterized the structures of these gas-phase complexes by investigating their collisionally activated decompositions (CAD) as a function of amino acid composition and sequence. Adams and co-workers[9a] showed even earlier that alkaline-earth-metal ions can deprotonate amide nitrogens and form cationic complexes. More recently,[9b] they also reported negatively charged, metal-bound peptide complexes.



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The gas-phase, alkaline-earth-metal-bound tripeptides have a structure that is similar to that of **1**.[7] The binding between the metal ion and the N-terminal amino group of simple tripeptides (i.e., those that contain amino acids with no acidic or basic side chains) is weaker than that with the deprotonated C-terminal carboxyl group and the deprotonated amides. The fragmentation of this complex occurs mostly at the N-terminal amino acid residue, forming two abundant peptide chain fragments y_2 and

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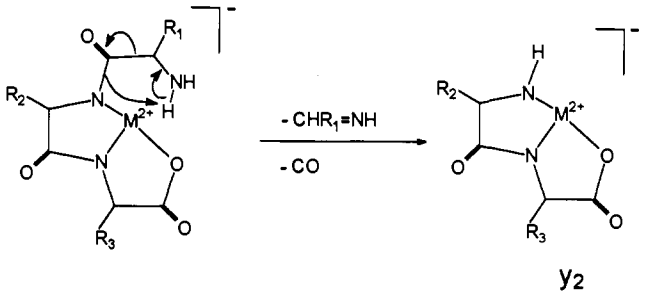
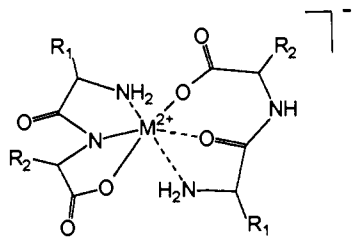
Scheme I



y_2

$(x_2 + H)$ (see Schemes I and II).[7,10] The $(x_2 + H)$ ion is formed by two different mechanisms whose relative importance is determined by the nature of the N-terminal amino acid (Scheme II).

Alkaline-earth-metal–bis(peptide) complexes have a structure in which the metal ion interacts with the two peptides via at least six-coordinate bonding. The three deprotonated groups include the C-terminal carboxylate groups of both peptides and one amide group (**2**).[8] A feature in the fragmentation of this bis(peptide) complex is the formation of (z – H), w, and v ions by high-energy processes. Sequence ions are also formed through scission of the peptide chains.



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We report here that transition-metal-ion-bound peptides and bis(peptide) complexes can also be introduced by FAB into the gas phase. The fragmentations of these anionic complexes enjoy some common features with those of alkaline-earth-metal complexes, but also show unique features. Moreover, because transition-metal complexes also exist in the condensed phase, there is an opportunity to contrast gas-phase and solution-phase interactions as well as to assess the differences in interactions within the complexes of different metal ions.

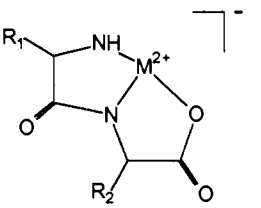
Results and Discussion

Transition-Metal-Bound Di- and Tripeptides. Desorption of Metal-Bound Peptides and Bis(peptides). Tripeptides can be desorbed into the gas phase as metal-bound complexes, but not bis(peptide) complexes, with transition-metal ions (Figure 1A), whereas dipeptides desorb to give both complexes (Figure 1B). The metal-bound dipeptides are of higher abundance than are the metal–bis(dipeptide) complexes. This property of transition-metal ions is in contrast with that of alkaline-earth-metal ions, for which metal–bis(peptide) complexes desorb more abundantly than do metal-bound di-/tripeptides.[8] In solution, divalent transition-metal ions do not induce deprotonation of the N-terminal amine group of a dipeptide, and instead Ni^{2+} [11] and Co^{2+} [12] produce hexacoordinate bis(dipeptide) complexes. Larger peptides form square planar complexes because the deprotonation of the second peptide nitrogen is easier than that of the first.[1c] The metal ion bonding to the first deprotonated amide nitrogen promotes deprotonation of the second amide nitrogen, producing the planar complex in which spin pairing gives the complex both kinetic and thermodynamic stability.[13]

In solution, a primary ligating site or anchor is needed so that deprotonation and chelation of amide groups can occur.[6] The N-terminal NH_2 group serves as an effective anchor for transition-metal ions, whereas the C-terminal carboxyl group does not. The opposite is true for the gas-phase deprotonation of peptides by alkaline-earth-metal ions.[7,8] The formation of transition-metal–peptide complexes in the gas phase, like those of alkaline-earth complexes, requires a free C-terminal COO^- for the peptides studied here. Evidence comes from peptides with a modified N-terminus such as Ac-AAA and *N*-formyl-MLF, which readily form gas-phase, metal-bound peptides and bis(peptide) complexes. If the peptide is further derivatized (e.g., esterified or amidated) at the C-terminus, a metal-bound peptide cannot be desorbed. Examples are Ac-AAA-OCH_3 and PLG-NH_2.

Differences in the properties of metal ions in the gas phase and in solution are also manifest. In solution, the solvent interacts with both peptides and metal ions, possibly modifying their interactions. As mentioned earlier, alkaline-earth-metal ions do not induce deprotonation of peptides in solution, but desorption of such species into the gas phase is readily accomplished.[7,8] A $[tripept + Co^{2+} - 3H^+]^-$ ion is not stable in solution,[14] but the complex is generated readily in the gas phase (Figure 1A). Stable Cu^{2+} complexes were extensively studied in solution,[2] but they are difficult to desorb by FAB into the gas phase.

CAD and Structure of Transition-Metal-Bound Dipeptides. The three deprotonation sites for the type of dipeptides studied here can only be the C-terminal COOH group, the single amide NH group, and the N-terminal NH_2 group (see structure **3**). As a result, transition-metal complexes do not form in solution, but they can be produced in the gas phase. Upon CA, the complexes of Ni^{2+}, Mn^{2+}, and Fe^{2+} principally lose imine molecules ($R_2CH{=}NH$) from the N-terminus to form x_1 ions, which then expel CO_2 (Figure 2). The formation of $(x_1 - CO_2)$ may occur via expulsion of first CO_2 and then the imine, or vice versa.



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For Co^{2+} complexes, however, a different product ion $(x_1 + 2H)$ is generated by loss of a nitrile molecule. A proposed mechanism involves metal–hydride bond formation and two hydrogen rearrangements (Scheme III). An Fe^{2+} induces competitive losses of both a nitrile and an imine. The dominance of a particular process depends on the nature of the metal ion; there is, however, no correlation with the ionization energies of the metals. The subject of nitrile loss is for a later investigation.

(10) The convention used here to denote fragment ions is the same as that proposed by Roepstorff and Fohlman (Roepstorff, P.; Fohlman, J. *Biomed. Mass Spectrom.* **1984**, *11*, 601) and modified by Biemann and Martin (Biemann, K.; Martin, S. A. *Mass Spectrom. Rev.* **1987**, *6*, 1). Small letters are used to denote the fragmentation sites, and the subscript number specifies the number of amino acid residues in the fragment. The number and the sign that follow a letter indicate the net number and the direction of hydrogen transfer, respectively. If the number of required H transfers is the same as for the fragmentation of a peptide $(M + H)^+$, the product ion is denoted in the same manner as if it were formed from an $(M + H)^+$.

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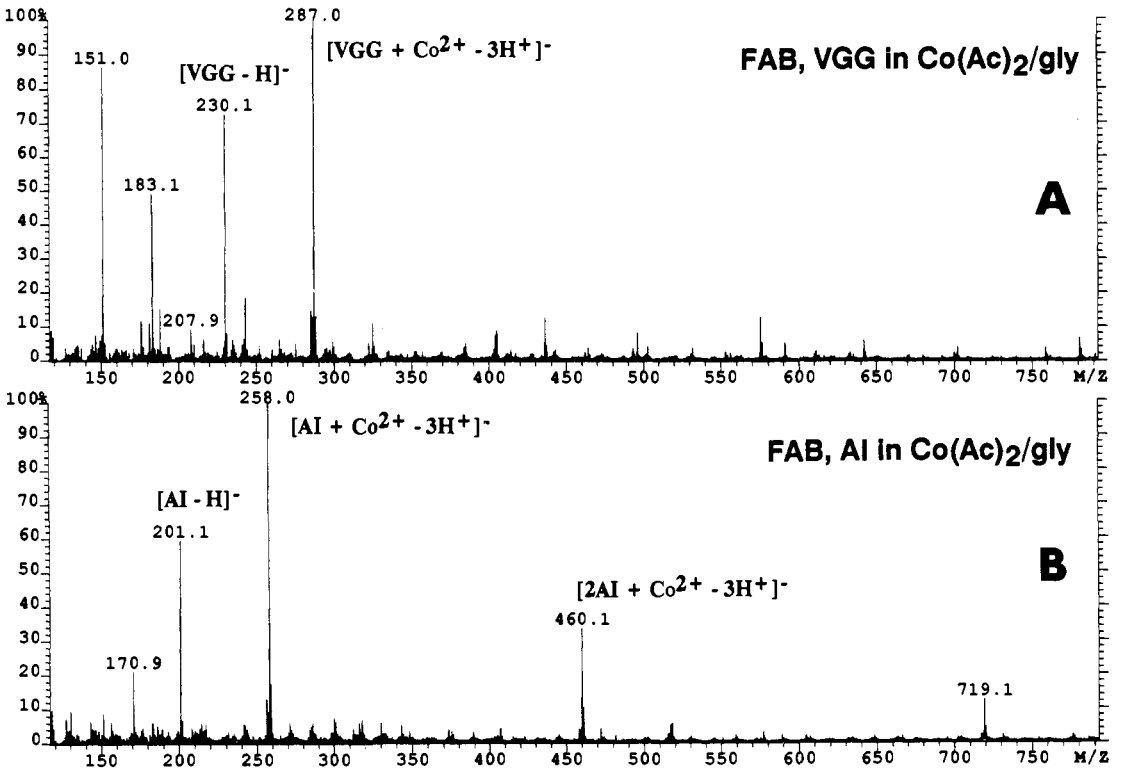
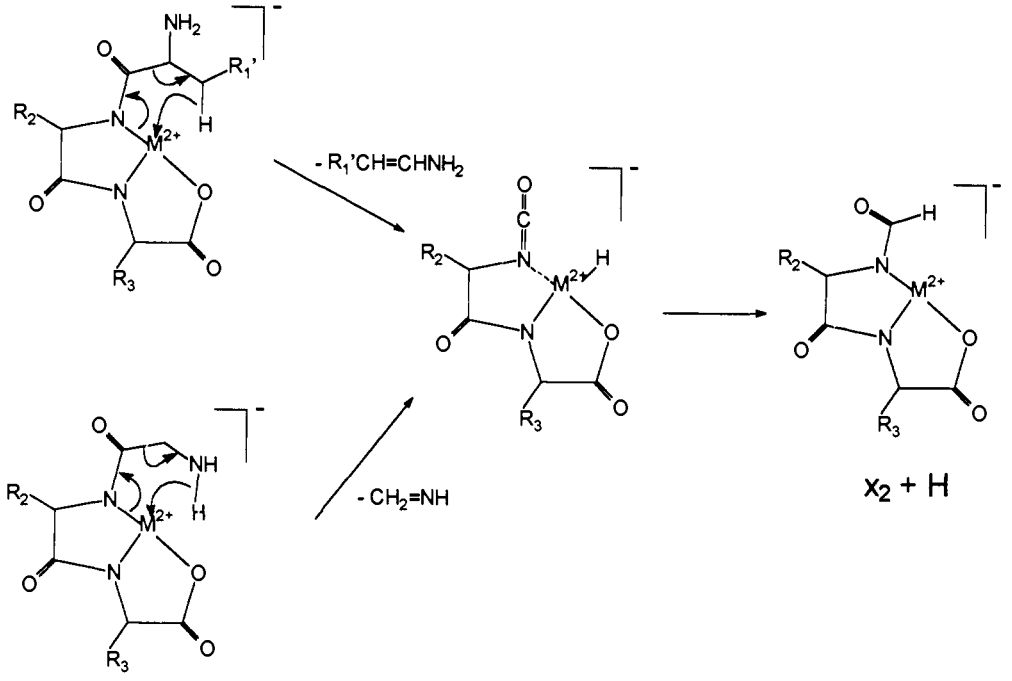


Figure 1. FAB spectra of (A) VGG and (B) AI in $Co(Ac)_2$/glycerol (0.5 F). The ion at m/z 719 may be $[3AI + 2Co^{2+} - 5H^+]^-$.

Scheme II



Doubly charged metal ions usually remain as part of the charged species in the CAD of metal-bound peptides and bis(peptide) complexes. Loss of the metal ion as a part of the neutral species however, is observed for transition-metal-bound dipeptides [labeled as $(x_1 - M)^-$ in Figure 2]. The metal ion is lost along with the doubly negative N-terminal species R_1CHNH.

CAD of Transition-Metal-Bound Tripeptides. Upon CA, transition-metal-bound tripeptides show some fragmentations that are similar to those of alkaline-earth-metal-bound tripeptides.[7] The dehydrogenation process is general. The y_2 and $(x_2 + H)$ ions, which are the only abundant product ions in the CAD of alkaline-earth-metal-bound simple peptides, are also readily formed here (Figure 3). The $(x_2 + H)$ and y_2 ion formation mechanisms that were proposed for complexes of alkaline-earth-metal ions[7] are likely to apply to the transition-metal complexes (Schemes I and II).

Two abundant ions that are characteristic of transition-metal-bound tripeptides, however, are formed by losing 44 and 46 u. In metastable ion decompositions, the loss of 44 u overwhelmingly dominates. The loss of CO_2 requires that the C-terminal COO^- be weakly bonded to the metal (Scheme IV), in accord with the weaker ligand field strength of COO^- compared to that of NH_2. The loss of H_2CO_2 (Scheme V) is proposed to require a nonionized C-terminal COOH group. Supporting evidence is the CAD of transition-metal complexes of APG, GPA, and dipeptides, which do not fragment via losses of CO_2 and H_2CO_2. To form a metal-

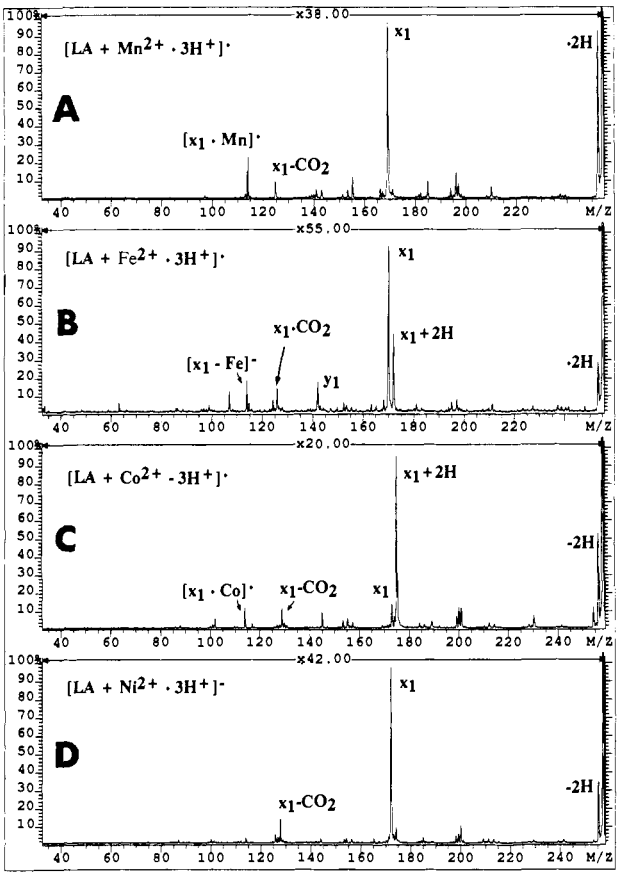


Figure 2. CAD spectra of transition-metal-bound LA complexes: (A) Mn^{2+}, m/z 254; (B) Fe^{2+}, m/z 255; (C) Co^{2+}, m/z 258; and (D) Ni^{2+}, m/z 257.

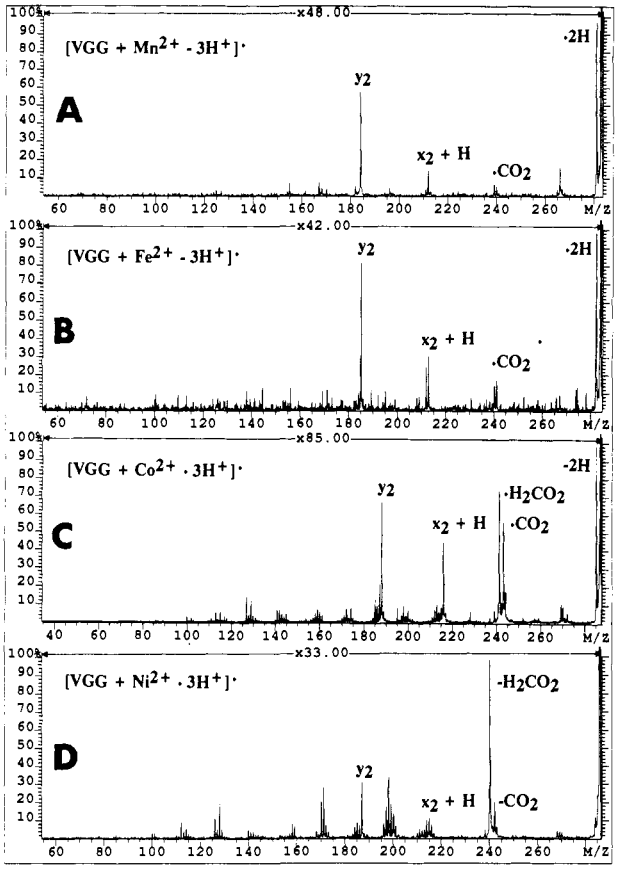
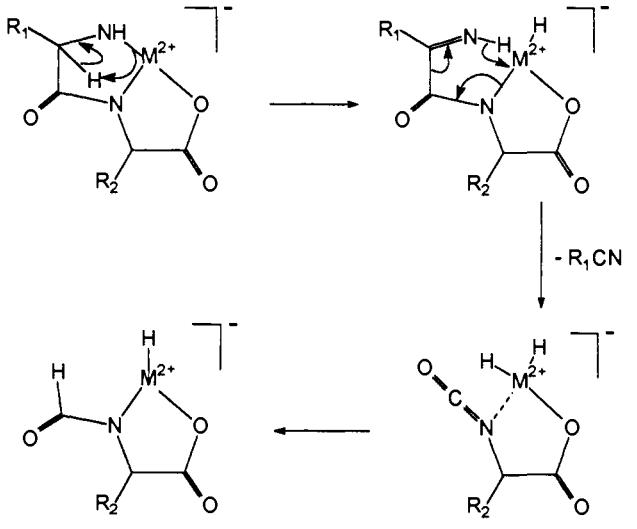


Figure 3. CAD spectra of transition-metal-bound VGG complexes: (A) Mn^{2+}, m/z 283; (B) Fe^{2+}, m/z 284; (C) Co^{2+}, m/z 287; and (D) Ni^{2+}, m/z 286.

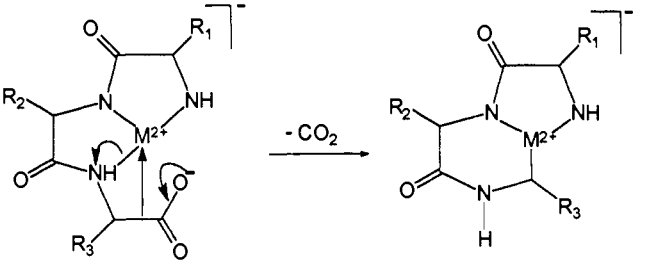
Scheme III



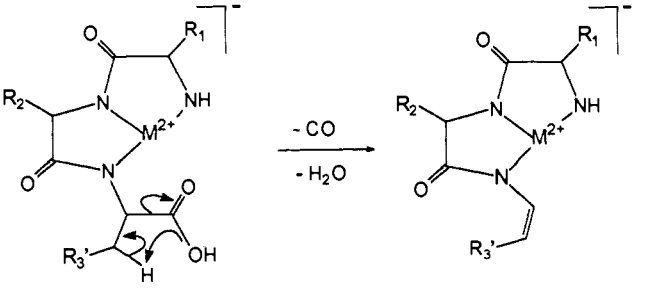
bound peptide from the proline-containing peptides, the C-terminal carboxylate group must be deprotonated to provide the necessary three negative charge sites. Similarly, a structure in which the C-terminus remains as COOH is not possible for metal-bound dipeptides.

To expel H_2CO_2, two hydrogens in addition to CO_2 must be removed from the precursor. There are at least two mechanisms. One involves a loss of CO_2, presumably as shown in Scheme IV, and then dehydrogenation of a side chain. The other involves a loss of H_2O and CO and requires a side chain on the C-terminal amino acid (Scheme V). According to the latter mechanism, an active hydrogen and a β-hydrogen of the C-terminal amino acid residue are lost. Two lines of evidence support the second

Scheme IV



Scheme V

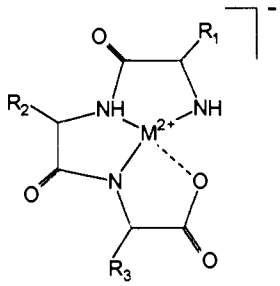


mechanism. Complexes of GGG do not undergo loss of 46 u. The $[GFA\text{-}d_5 + Co - 3D^+]^-$ ion undergoes a primary loss of 47 u and a minor loss of 46 u, the latter pointing to some involvement of the first mechanism.

The competitiveness of the two mechanisms varies with the amino acid composition and location. The Co^{2+}-bound GFA undergoes 46-u loss primarily by the second mechanism (Scheme V), whereas Co^{2+}-bound GGV-d_5 loses 46 u competitively via both mechanisms, as indicated by the comparable abundances of the ions formed by losses of 46 and 47 u.

For the transition-metal-ion complexes studied here, the relative abundances of C-terminal and N-terminal fragments are metal-ion-dependent. The abundances of the N-terminal ions (formed by losses of 44 and 46 u) increase in the order Mn < Fe < Co < Ni. The CAD spectra of Mn- and Fe-containing complexes (Figure 3A,B) resemble those of alkaline-earth-metal-ion complexes (i.e., the C-terminal product ions dominate the CAD).

Structure of Transition-Metal-Bound Tripeptides. We propose that the gas-phase transition-metal-bound tripeptides have not only the same structure (**1**) as their solution counterparts but also other structures. The existence of structure **1** is supported by the experiments with derivatized peptides described above and by the CAD spectra of these gas-phase complexes. Other structural forms are indicated by experiments with proline-containing tripeptides. Like other simple tripeptides, APG and GPA form gas-phase transition-metal-bound tripeptides. The N-terminal NH_2 group is apparently deprotonated by transition-metal ions if a third, more acidic nitrogen site is not available (deprotonation of CH groups to form enolates is also possible, but chelate structures involving bonding with the negatively charged ligands cannot form). Therefore, in addition to structure **1**, structures **4–6**, in which the N-terminal NH_2 group is deprotonated, are also feasible. The facile desorption of transition-metal-bound dipeptides provides direct support for this interpretation. On the basis of comparable abundances of the C-terminal and N-terminal ions, structures **1** and **6** should coexist in a composite structure population.



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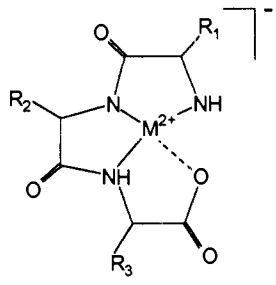

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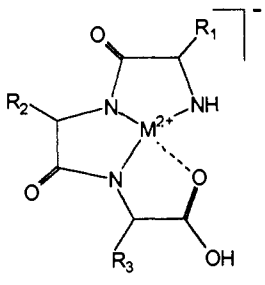

6

The deprotonation of an amine group by trivalent metal ions such as Cu^{3+} or Au^{3+} at high pH in solution was previously reported.[15] Although divalent metal ions such as Cu^{2+} and Ni^{2+} in similar complexes are not capable of deprotonating amine groups in solution,[15] these divalent metal ions (the metal ions studied here include Ni^{2+}, Co^{2+}, Fe^{2+}, and Mn^{2+}) readily form gas-phase $[pept + Met^{2+} - 3H^+]^-$ ions, suggesting that the NH_2–terminus is deprotonated.

If the N-terminal NH_2 group is deprotonated in transition-metal complexes, why is AAA-OCH_3 not desorbed as an anionic transition-metal-bound peptide? One explanation may be that a COO^- group serves as a metal anchor. In solution, the COOH group has a much higher acidity than that of both amide NH and amine groups, although the COOH and amide NH groups have similar intrinsic acidity.[6,16] The C-terminal carboxylate may bind to the metal ion, and then the metal ion assists the deprotonation of the amide and/or the amine groups. Another and more likely explanation recognizes that the peptide must be negatively charged to form a detectable complex. Those peptides with an ionized COOH undergo more readily triple deprotonation than do those with a nonionizable C-terminus. In this case, the principal bonding to the metal is via nitrogen ligands, some negative and some neutral; this bonding scheme is consistent with the various ligand field strengths.

The factors that determine the relative stabilities of the various complexes (**1**, **4–6**) are the energies required for deprotonation and those released in metal–ligand bond formation. We know from a previous study[7] that alkaline-earth-metal-bound tripeptides assume structure **1**, which is consistent with the oxyphilic nature of these metal ions.[17] Transition-metal ions, however, induce deprotonation of the less acidic amine groups because their nitrogen ligand affinity[15] is intrinsically high (i.e., there is a greater ligand field for nitrogen than oxygen). In other words, the energy released in forming the metal–nitrogen bond compensates for the high energy required for the deprotonation of the amine group. Therefore, the stabilities of structures **1** and **6** are more comparable for transition-metal complexes than they are for alkaline-earth-metal complexes, and this accounts for the greater propensity of the former to fragment via losses of CO_2 and H_2CO_2 from the C-terminus. In addition to the three deprotonated amide nitrogens, the fourth binding ligand is the neutral N-terminal amine group, as mentioned above. The amine group is expected to coordinate more strongly to Ni^{2+} than does the C-terminal carboxylate, as is the case in solution and in the crystal structure.

Tripeptides Containing Phenylalanine. Phenylalanine residues influence the fragmentation of metal-bound tripeptides. In addition to the loss of a side chain, an N-terminal phenylalanine induces abundant loss of ammonia;[8] a central phenylalanine induces abundant v_2[18] and $[y_2 - C_7H_7]$ ions, which do not occur metastably (Figure 4). The complexes of peptides containing C-terminal phenylalanine undergo a dominant side chain loss. This fragmentation pattern is in sharp contrast to that of alkaline-earth-metal-bound tripeptides that contain phenylalanine; for these the side chain loss is, in most cases, the only significant fragmentation.[7]

In metastable ion fragmentations, metal(II)-bound tripeptides containing phenylalanine readily lose the side chain as either $C_7H_7^\bullet$ or C_7H_8; the ratio of the losses depends on the nature of the metal ion in the complex and the position of the phenylalanine residue in the peptide. For tripeptides that have a central phenylalanine residue (e.g., GFA), a C_7H_7 radical is expelled from the metal-bound peptides when the complexing metal ion is Ni^{2+} or Co^{2+}. All complexes of alkaline-earth-metal ions (Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+}) undergo a loss of C_7H_8, whereas manganese and iron complexes give losses of both C_7H_8 and $C_7H_7^\bullet$ (Figure 5).

The collision gas pressure also influences the relative abundance of the ions formed in the losses of 91 and 92 u. Generally, the abundance of the less-favored ion in metastable ion fragmentation increases with increases in collision cell pressure. For example, 92-u losses from complexes of Fe and Co are ca. 25 and 5% (relative to 91-u loss), respectively, in metastable ion decompositions (Figure 5). The percentages increase to 60 and 35% in the CAD when the collision cell pressure is sufficient to reduce

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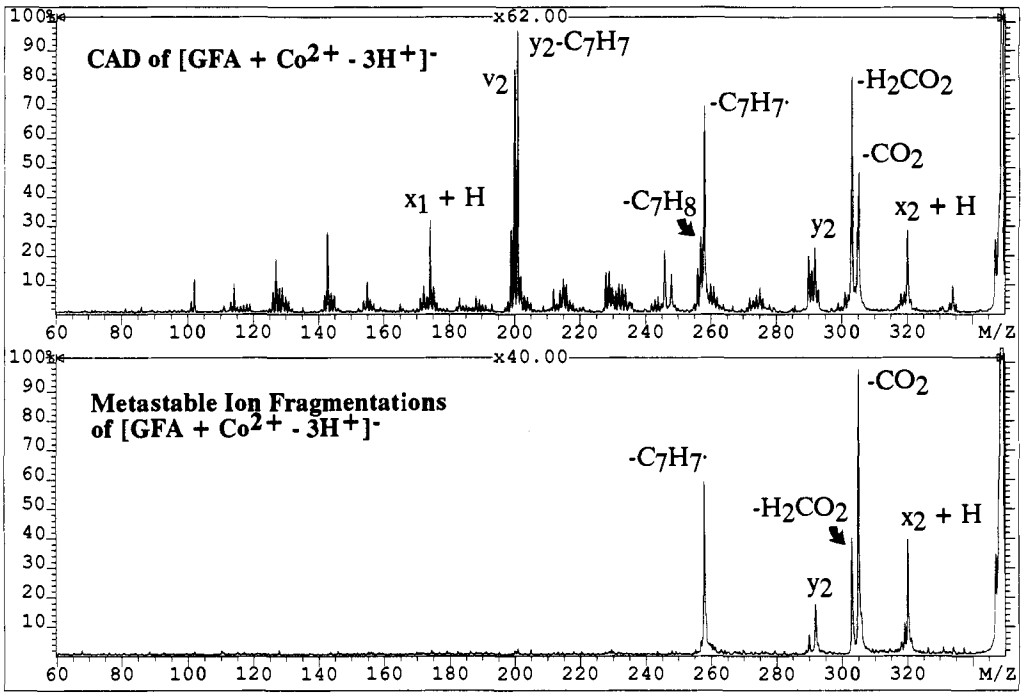


Figure 4. CAD (A, top) and metastable (B, bottom) spectra of Co^{2+}-bound GFA, m/z 349. The CAD spectrum was acquired with main beam suppression of approximately 75%.

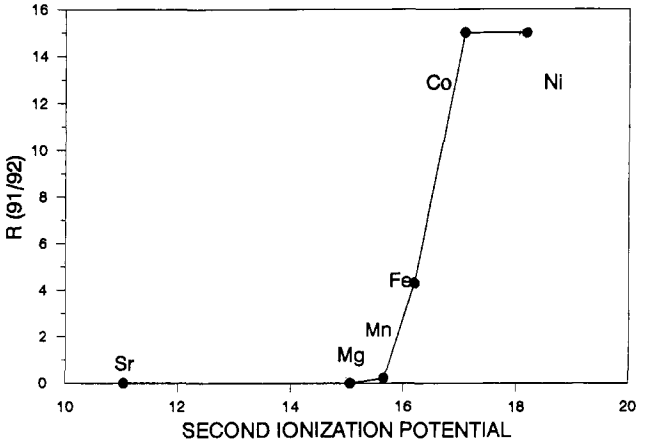


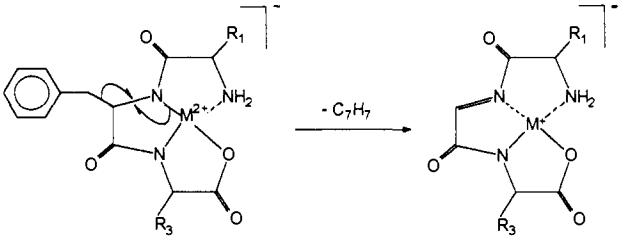
Figure 5. Metastable ion phenylalanine side chain loss from [GFA + Met^{2+} – $3H^+$]$^-$ as a function of the second ionization energies. The maximum $R(91/92)$ is chosen as 15 because the fluctuation of the base line allows a peak of approximately 5% relative intensity to be precisely defined.

the parent ion beam by approximately 75%. The 91-u loss does not occur metastably from Mg^{2+} complexes, whereas it is significant (35%) upon CA.

We described in a previous study[7] the loss of C_7H_8 from alkaline-earth-metal-bound tripeptides containing phenylalanine. The departing $C_6H_5CH_2$ couples with an amide or amine hydrogen of the phenylalanine residue. Different precursor structures should be involved when the location of phenylalanine is changed. For the GFA complex, the precursor has a neutral amide NH that adjoins the N-terminus, and for FGG and GGF the precursors have a neutral N-terminal NH_2 and an amide group adjoining the C-terminus, respectively. Because the structures of alkaline-earth-metal-bound tripeptides and those of transition-metal counterparts are similar, the propensity to expel 92 u from these complexes should be similar.[7]

In the mechanism proposed here for the loss of 91 u from phenylalanine (see Scheme VI), the radical generated by the homolytic cleavage expelling C_7H_7· induces the homolytic cleavage of the metal–amide nitrogen bond. The result is that the metal

Scheme VI



ion undergoes one-electron reduction to become singly charged. If the mechanism is correct, the ionization energies of the metal should affect the relative competitiveness of reactions. As shown in Figure 5, a distinction can be made for complexes containing Mn or Fe. For metals that have a second ionization energy lower than that of Mn (15.64 eV),[19] metastable C_7H_8 loss is dominant for GFA complexes; for metals having a second ionization energy higher than that of Fe (16.18 eV), metastable C_7H_7· loss is dominant.

Ions of metals that have high second-ionization energies can readily undergo reduction to the +1 state, facilitating loss of $C_6H_5CH_2$· (Figure 5, Ni^{2+}, Co^{2+}, Fe^{2+}, and Mn^{2+}). For metals that have lower second-ionization energies (e.g., the alkaline-earth-metal ions), the loss of C_7H_8 dominates. In metastable ion decompositions, the 91-u loss is observed for the GFA–Mn complex but not for the GFA–Mg complex. The second-ionization potentials of Mn and Mg differ by only 0.6 eV (Figure 5). The second-ionization energy of Mn appears to be the lowest that allows the metastable 91-u loss process.

If the mechanism is correct, then 91-u loss is also expected from metal-bound peptides that have a C-terminal phenylalanine because the two major structures (**1** and **6**) of the precursor readily undergo 91-u loss, and this is observed for GGF. Metal-bound peptides that have an N-terminal phenylalanine residue, however, experience different constraints. The reaction that leads to 91-u loss readily occurs for structure **6**, but not for **1** of FGG complexes. On the other hand, structure **1** does accommodate 92-u loss. It

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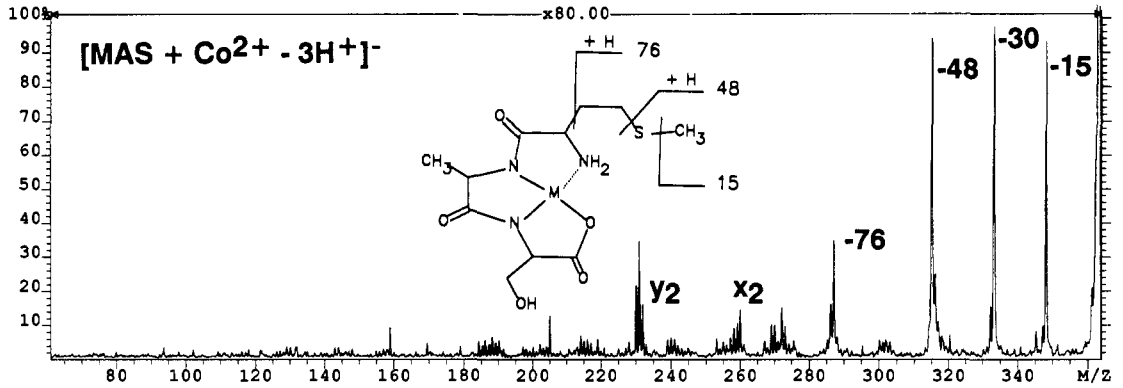
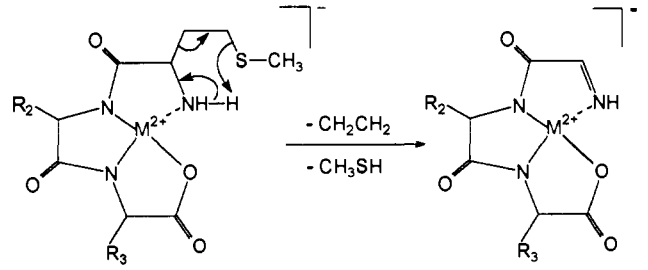
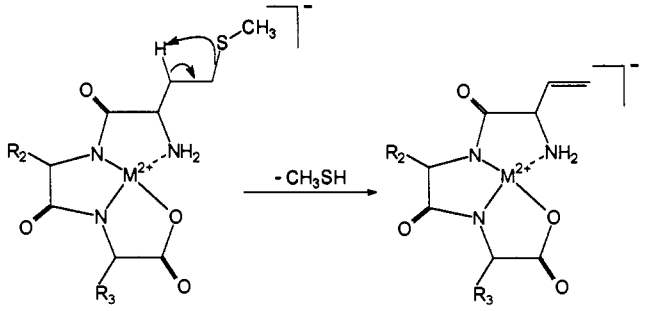


Figure 6. CAD spectrum of Co^{2+}-bound MAS, m/z 363.

Scheme VII



Scheme VIII



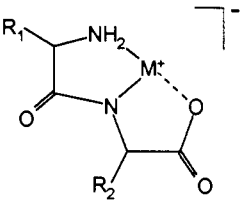
is not surprising that Ni^{2+}-bound GGF gives both 92- and 91-u losses (5:2), in contrast to the exclusive loss of 91 u from peptides having central and C-terminal phenylalanines.

Peptides Containing Serine, Threonine, and Methionine. Side chains like those of serine, threonine, and methionine fragment extensively upon CA when the peptides containing these side chains are involved in metal bonding. Even peptide backbone fragmentation can be minor (see Figure 6). Serine and threonine side chains fragment by losing CH_2O and CH_3CHO, respectively.[7] From the methionine side chain are losses of CH_3, CH_3SH (48 u), and CH_2CH_2 + CH_3SH (76 u). If more than one side chain is present in the peptide, fragments of each are observed. For example, the serine side chain loss of 30 u and the methionine side chain losses of 15, 48, and 76 u occur for Co^{2+}-bound MAS (Figure 6).

A mechanism for serine and threonine side chain losses was proposed in a previous study[7] and supported by sequential activation (MS/MS/MS) studies of alkaline-earth-metal-bound tripeptides. The hydroxyl hydrogen is rearranged to the carbonyl oxygen via a six-membered-ring transition structure, triggering the Cα–Cβ bond cleavage. This mechanism should also supply here. The losses of CH_3SH and C_2H_4 coupled with that of CH_3-SH from the methionine side chain may follow mechanisms proposed in Schemes VII and VIII. Both processes involve losses of neutral species, and the metal bonding remains unaffected.

Although transition-metal-ion complexes undergo fragmentation of all functionalized side chains, alkaline-earth complexes that contain serine, methionine, and threonine show loss of the most vulnerable side chain. Peptide chain fragmentations and the decompositions of other side chain(s) are often totally suppressed; for example, the loss of CH_2O from serine generates the only fragment ion of Sr^{2+}-bound MAS. One explanation for the more extended influence of transition metals is the multiplicity of structures for the transition-metal-bound peptides. The smaller ionic radii (relative to Ca^{2+}, Sr^{2+}, and Ba^{2+}) may be another because Mg^{2+}-bound GFA does show more fragmentations than the complexes of other alkaline-earth metals.

Transition-Metal–Bis(dipeptide) Complexes. Transition-metal–bis(dipeptide) complexes decompose overwhelmingly by losing a dipeptide to form a metal(II)-bound dipeptide (**3**). Metal reduction also occurs in the loss of a peptide radical (peptide – H•), leading to the formation of metal(I)-bound dipeptides (**7**).



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For most of the complexes studied here, the metal(I)-bound dipeptides are second most abundant. In addition, a deprotonated dipeptide is observed at low abundance (approximately 10%) (see Figure 7). Other ions of low abundance include y_1, x_0, and w_1 ions (for nomenclature explanation, see ref 10). For bis-(peptide) complexes of Ni^{2+}, the abundances of the deprotonated peptide and metal(I)-bound dipeptide are significantly enhanced when compared to those of other metal-ion complexes.

In general, the fragmentations of transition-metal–bis(dipeptide) complexes are similar to those of alkaline-earth–bis-(dipeptide) complexes, except that those leading to the formation of metal(I or II)-bound peptides are more dominating. This suggests that bis(dipeptide) complexes of the two metal groups have similar structures (structure **2**), in which the metal binds to one doubly deprotonated and another singly negative peptide deprotonated at the C-terminus. The C-terminal COOH group and the only amide NH group are likely to be deprotonated in the doubly negative peptide. The metal ion coordinates with at least six sites of the two peptides, each contributing three binding sites.

A prismatic or distorted primatic geometry was previously proposed for alkaline-earth-metal–bis(peptide) complexes.[8] Transition-metal ions, however, usually adopt an octahedral geometry when they form six-coordinate complexes. In the crystal structure of the $Na_2Ni^{2+}(GG)_2 \cdot nH_2O$ (n = 8 or 10) complex,[12] Ni^{2+} coordinates two doubly deprotonated GGs via six coordination sites. Each peptide contributes three sites, and the spatial arrangement of the six ligands is octahedral. Transition-metal–

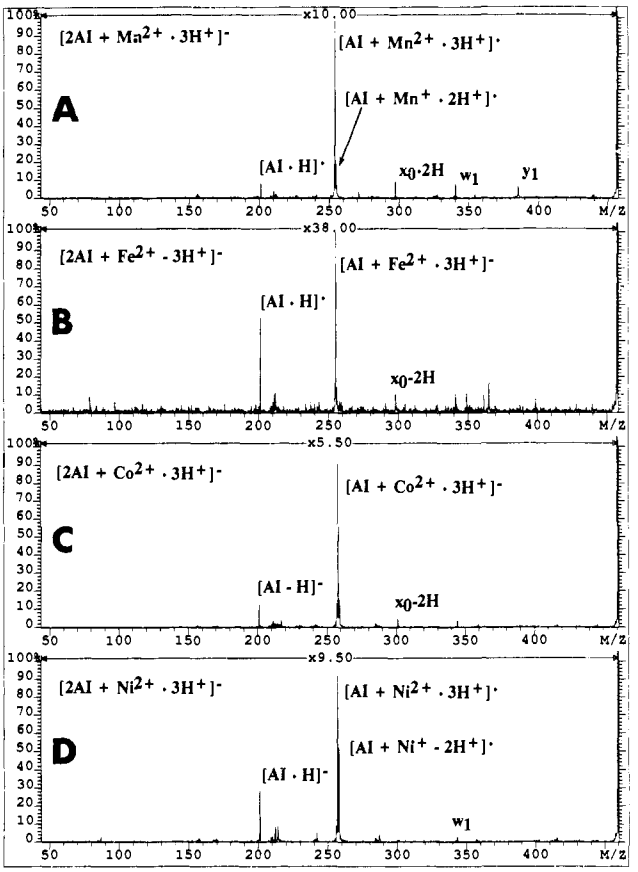

Figure 7. CAD spectra of transition-metal–bis(AI) complexes: (A) Mn^{2+}, m/z 456; (B) Fe^{2+}, m/z 457; (C) Co^{2+}, m/z 460; and (D) Ni^{2+}, m/z 459.

bis(peptide) complexes in the gas phase similarly have two peptides bound to the metal ion, except that one of the peptides is singly deprotonated. This, however, may preclude octahedral geometry. According to Freeman,[20] if a metal is bound to three donor groups in a single peptide chain and the central group is nitrogen, the three donor atoms lie in the same coordination plane. Therefore, a tetrahedral configuration is unfavorable both energetically and geometrically when a neutral amide nitrogen is the central binding group. An alternative is a prismatic or distorted geometry if six coordination is involved. Other options would have the metal ion coordinated to fewer than six sites.

Conclusion

The formation and CAD of transition-metal–dipeptide and –tripeptide complexes differ significantly from those of alkaline-earth complexes. Fundamental properties of the metal ions offer explanations for the differences. Alkaline-earth metals are oxyphilic,[17] and transition metals favor nitrogen ligands. The high nitrogen affinity of transition metals and consequently the strong metal–nitrogen bond[15] enable the deprotonation of amine groups, which are only deprotonated by trivalent transition-metal ions in solution. Thus, we can understand why transition-metal-bound dipeptides can be desorbed, but not their alkaline-earth counterparts, and why transition-metal-bound tripeptides exist as composite structures, whereas alkaline-earth complexes favor a single structure. There is a similar explanation for the disparate metal–peptide bonding in bis(peptide) complexes of the two metal classes. Two of the three negative sites of the two peptides are oxygen ligands. The oxyphilic alkaline-earth metals, therefore, interact strongly with these peptides. On the other hand, the interactions of transition metals with the same ligands are expected to be less strong. Upon CA, the complexes fragment primarily by losing a peptide to form metal-bound peptides in which two of the three negative charges are at nitrogen sites.

The strong coordinating ability of transition metals contributes to a cooperative deprotonation of peptide nitrogens.[6] The deprotonation does not stop with the first peptide nitrogen but continues to furnish a hexacoordinate metal–bis(peptide) complex for peptides larger than dipeptides. This reduces considerably the driving force for transition-metal–bis(peptide) complexes to form. Because alkaline-earth metals form more abundant bis-(peptide) complexes than metal-bound peptides, the deprotonation of a peptide nitrogen by alkaline-earth metals must be less effective.

Experimental Section

Reagents. The peptides used in this work were commercially available from Sigma Chemical Company (St. Louis, MO). Alkaline-earth-metal-ion hydroxides were from Fisher Scientific Company (Fair Lawn, NJ). Glycerol and thioglycerol were purchased from Aldrich Chemical Company (Milwaukee, WI).

Instrumentation. The mass spectrometers used for this work were a Kratos MS-50 and a VG four-sector ZAB-T. The Kratos MS-50 is a triple-sector mass spectrometer of EBE geometry, which was previously described.[21] It was equipped with a commercially availabe FAB source and an Ion Tech saddle-field atom gun (Ion Tech, Middlesex, England), which produced a ca. 6-keV Ar atom beam for FAB desorption. Field-free regions both between ESA-1 and the magnet and between the magnet and ESA-2 were equipped with standard collision cells. When an MS/MS experiment was performed, MS-1 (ESA-1 and the magnet) was used to select the precursor ion, and a MIKES scan was conducted by scanning the field of ESA-2 to obtain the product ion spectrum. This experiment was described earlier by us.[22]

The ZAB-T four-sector tandem mass spectrometer consisted of two high-mass, double-focusing mass spectrometers.[23] The design of MS-2 is a reverse geometry Mattauch-Herzog type (BE). The instrument was equipped with a Cs gun that provided a 17-keV Cs^+ beam (the overall energy for desorption was 25 keV). When mass spectra were acquired, only MS-1 and the intermediate detector was used. When MS/MS experiments were conducted, MS-1 was used to select the precursor ion at a mass resolution of ca. 1500, and a B/E scan was taken with MS-2 to record the product ions produced by collisional activation in the collision cell located between MS-1 and MS-2. The object slit of MS-2 was closed so that the peak of the selected ion went from flat to round top (slit fully illuminated), so that the resolution of the product ions was ca. 1000 (FWHH).

Although all the spectra published here were taken with the four-sector tandem instrument, those from the three-sector were in good agreement except that the mass resolution for the analysis of product ions was poorer.

Procedures. For FAB-MS/MS experiments, a few micrograms of the peptide was mixed on a stainless steel tip of a FAB probe with glycerol/thioglycerol (1:1) that was saturated with alkaline-earth-metal-ion hydroxide or had ca. 0.5 F metal acetate in it. The tip was then exposed to a 25-keV Cs^+ atom beam for the desorption of the $[pept + Met^{2+} - 3H^+]^-$ ion.

For experiments with deuterium-labeled peptides, the peptide and the matrix were mixed on the tip of a FAB probe; then D_2O (4–6 μL) was added to the mixture. After mixing, the probe was inserted into the prevacuum system of the mass spectrometer, and the water (H_2O, HDO, and D_2O) was pumped away for approximately 4 min. The procedure was repeated four times. The probe was then introduced into the FAB source. On the basis of the full scan FAB spectrum of $[pept\text{-}d_5 + D]^+$, at least 90% of the active hydrogens of the peptide were replaced.

Acknowledgment. This work was supported by the National Science Foundation (Grant CHE9017250).

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