According to Shaik and co-workers, as the ability of the substrate to delocalize charge away from the reaction center increases, structure **3b** becomes more important relative to **3a**, and consequently the **3** → **2** curve becomes flat in the initial stages of the reaction. Under these circumstances f is relatively large. In contrast, if there is an initial localization of charge in the substrate, **3a** predominates over **3b**, and a relatively small value of f results. In summary, in the VBCM model constructed by Shaik et al., the magnitude of the activation barrier for an S_N2 reaction depends on the interplay between the donor–acceptor energy gap and delocalization of the excess charge transferred to the reaction center.

In the ion–molecule complexes of [$ClCH_2CN{\cdot}Cl^-$] and [$CH_3Cl{\cdot}Cl^-$], nearly all of the stabilization relative to separated reactants arises from electrostatics alone and can be accounted for by considering a point charge in the full multipole field of the neutral (see Section II). In brief, formation of the ion–molecule complexes does not involve substantial changes in electronic structure. This conclusion is likely to be valid for most substrates, at least those reacting with Cl^-. Therefore, in applying the VBCM model, the reactants and products in Scheme IV are those species that exist immediately before and after the substitution step, these being the two chemically equivalent ion–molecule complexes. The activation energy referred to in the model is thus the energy difference between the ion–molecule complex and the S_N2 transition state, which is consistently denoted as ΔE^* here. Otherwise, the effects of long-range electrostatic interactions spoil the analysis, and the qualitative reaction profiles in Figure 8 and Scheme IV do not match.

According to Shaik,[149] the two effects which govern the value of ΔE^* are in competition for many systems and often very nearly cancel one another. In substrates with π systems α to the reaction center, enhanced electron affinities arise because low-lying, unoccupied π^* orbitals are present, but at the same time resonance delocalization of excess charge transferred to the reaction center is facilitated. In comparing eqs 1 and 2 according to the VBCM model, Shaik[149] has attempted to quantify the competition between gap and slope, or delocalization, factors by calculating relative barrier heights within the formalism. For the CH_3Cl substrate, $E_g \approx 113$ kcal mol^{-1} and $f \approx 0.25$, whereas for $ClCH_2CN$ these values are about 88 kcal mol^{-1} and 0.32, respectively. The E_g and f factors balance one another to the extent that the ΔE^* values differ by only 0.2 kcal mol^{-1}, CH_3Cl having the lower barrier. These predictions cannot be regarded as quantitative, although there is good agreement with the final ab initio value for the ΔE^* difference between the two systems, i.e., 1 kcal mol^{-1}, but with $ClCH_2CN$ having the lower barrier. Whether such agreement between a qualitative bonding model and rigorous ab initio quantum mechanical computations is fortuitous or fundamental is by no means clear. Shi and Boyd[154] have criticized the form of the VBCM wave function, but their objections pertain primarily to S_N2 cross reactions, not identity exchange processes. Perhaps a combination of qualitative VBCM models for identity S_N2 processes and Marcus theory for S_N2 cross reactions will prove useful as further investigations proceed.

Acknowledgment. We are grateful to the National Science Foundation and to the Stanford Data Center for support of this research. We thank Drs. R. G. Gilbert and S. C. Smith for giving us a copy of their RRKM program and for helpful discussion regarding the details of the theory. We also thank J. L. Wilbur for help with the experimental thermodynamic measurements and Dr. A. G. Császár for helpful discussion concerning the computational details.

Registry No. Cl^-, 16887-00-6; $ClCH_2CN$, 107-14-2; CH_3Cl, 74-87-3; Cl_2, 7782-50-5; $^{37}Cl^-$, 42150-36-7.

Supplementary Material Available: Tables of total electronic energies for all ab initio calculations along with the complete SQM force field for $ClCH_2CN$, the C_s- and C_1-symmetry chloride complexes, and the S_N2 transition state (10 pages). Ordering information is given on any current masthead page.

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Strong Interactions of Anionic Peptides and Alkaline Earth Metal Ions: Metal-Ion-Bound Peptides in the Gas Phase

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Abstract: Tripeptides comprising amino acids with neutral side chains interact with alkaline earth metal ions to form gas-phase anionic complexes of the composition [tripept + Met^{2+} − $3H^+$]$^-$ under fast atom bombardment. The metal ion binds to the deprotonated C-terminal carboxylate group and to the two amide nitrogens. Because the C-terminal and the central amino acid are tightly bound by the metal ion, they are not vulnerable to collisionally activated decompositions in a tandem mass spectrometer. Instead, the significant fragmentations occur at the N-terminal amino acid site, which is the least tightly bonded. Ions are formed by elimination of an imine and the imine plus CO from the N-terminus (product ions are assigned as x_2 + H and y_2, respectively). Other major fragmentations of this complex include dehydrogenation and loss of an ammonia molecule. Peptides with functionalized side chains, such as those of serine, threonine, and phenylalanine, lose the side chains readily when they are bound to metal ions and submitted to collisional activation. Other fragmentation channels are largely suppressed, indicating direct metal ion–side chain interaction. Fragmentation mechanisms are proposed on the basis of results with isotopically labeled peptides and from MS/MS/MS experiments.

Introduction

Tandem mass spectrometric (MS/MS) studies of peptides have gained momentum recently owing principally to the capacity for peptide sequencing. N-Blocked and other modified peptides can be readily sequenced by applying tandem mass spectrometry to desorbed $(M + H)^+$ ions.[1] Metal ion/peptide adducts have been

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studied less frequently by tandem mass spectrometry. Nevertheless, MS/MS offers the opportunity for understanding the intrinsic interactions of metal ions and peptides as well as for determining peptide sequence.[2]

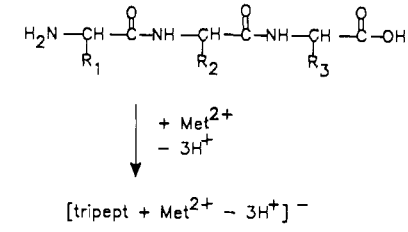
The nature of adducts formed from alkali metal ions and peptides has been the main subject thus far.[2] Controversies have arisen over whether the adducts have an unique structure in which the alkali metal ion binds preferentially to the peptide chain or to the C-terminus.[2a-d,f-j] The preferred binding site should be directly related to relative metal ion affinities of the various ligand sites of a peptide. The consequences of the binding, whatever its nature, are that most Li^+ and Na^+ adducts fragment by expelling CO and an imine from the C-terminus to give a new adduct in which the peptide size is reduced by one amino acid.[2d,h,j] The reaction dominates both collisionally activated and metastable ion fragmentation processes. A similar reaction also occurs for certain protonated peptides, but it is not as general as that promoted by Li^+ or Na^+.[2e] The C-terminal amino acid can be readily identified on the basis of these rearrangements.

Gas-phase alkaline earth metal ion/peptide interactions have also been studied,[2k] and adduct fragmentations give extensive sequence information. Alkaline earth metal ions are thought to bind preferentially to deprotonated amide groups to give complexes that are positively charged.

Deprotonated peptides were studied recently by negative ion FAB.[3] The usefulness of this method for sequencing di-/tripeptides and the influence of the side chains on the fragmentation of deprotonated di-/tripeptides were investigated.[3a-d]

Recently, Operti et al.[4] reported a study of gas-phase reactions between Cu^{2+} and α-amino acids. The metal ion forms $[AA + Cu^+]^+$, $[2AA + Cu]^+$, $[2AA + Cu - H]^+$, and $[AA + G + Cu]^+$ under fast atom bombardment (FAB) ionization conditions, where AA is leucine or valine and G is glycerol. Gas-phase interactions between Pd^{2+} and angiotensin II were studied by Macfarlane's group[5] using PDMS. The Pd^{2+} binding sites were elucidated by comparing the spectrum of protonated angiotensin II and that of the Pd–angiotensin II adduct.

Scheme I



Transition metal/peptide complexes, in which the amide hydrogens are deprotonated to form anionic complexes in solution,[6] require for deprotonation a "primary ligating site" or "anchor". The metal ion binds to the anchor site to chelate the amide nitrogen by substituting for a proton. The N-terminal amine group and histidine side chain are effective metal anchors. One line of the research[6b-f] has been aimed at the nature of the metal/peptide interactions. Studies include the kinetics of both the protonation processes at the amide nitrogen and the replacement of the peptide by other ligands. The incentive for the study, however, is to develop an understanding of biologically important metal/protein interactions. For example, doubly charged metal ions chelate tetraglycine at the N-terminal amine group and the three deprotonated amide nitrogens,[7a-c] and indeed it was experimentally shown that Cu^{2+} and Ni^{2+} interact with the amino terminus of the proteins.[7d,e]

Alkaline earth metal ions, however, do not form complexes in solutions in which the amide NH groups are deprotonated.[6a] Although there have been numerous attempts to observe the interactions between peptides and alkali and alkaline earth metal ions, no complexes have been reported thus far. The ligating ability of alkaline earth metal ions is thought to be too weak to deprotonate amide groups.[6a]

Recently Adams and co-workers[8] and we independently discovered that anionic complexes between alkaline earth metal ions and peptides of the form $[pept + Met^{2+} - 3H^+]^-$ can be produced in the gas phase. Furthermore, the existence of gas-phase metal–bis(peptide) complexes of the form $[2pept + Met^{2+} - 3H^+]^-$ was also established in our laboratory. Doubly charged transition metal ions also form metal-bound peptides and metal–bis(peptide) complexes in the gas phase. The existence of these metal/peptide complexes not only provides a chance to compare the interactions of transition metal ions and peptides in solution with those in the gas phase but also offers the only opportunity so far available to study peptide/alkali and alkaline earth metal ion complexes, a class of complexes that are not detectable in solution.

The purpose of the research reported in this paper is to examine the novel metal-bound peptides $[pept + Met^{2+} - 3H^+]^-$ by using tandem mass spectrometry. The peptides for this study contain only neutral amino acids (e.g., Gly, Ala, Val, Pro, Leu, Ser, and Thr), and the metal ions are Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+}. The foci of the study are the structure of the complexes and the mechanisms of their fragmentation. In the accompanying paper we report on the metal–bis(peptide) complex $[2pept + Met^{2+} - 3H^+]^-$ and in a future paper on transition metal ion complexes.

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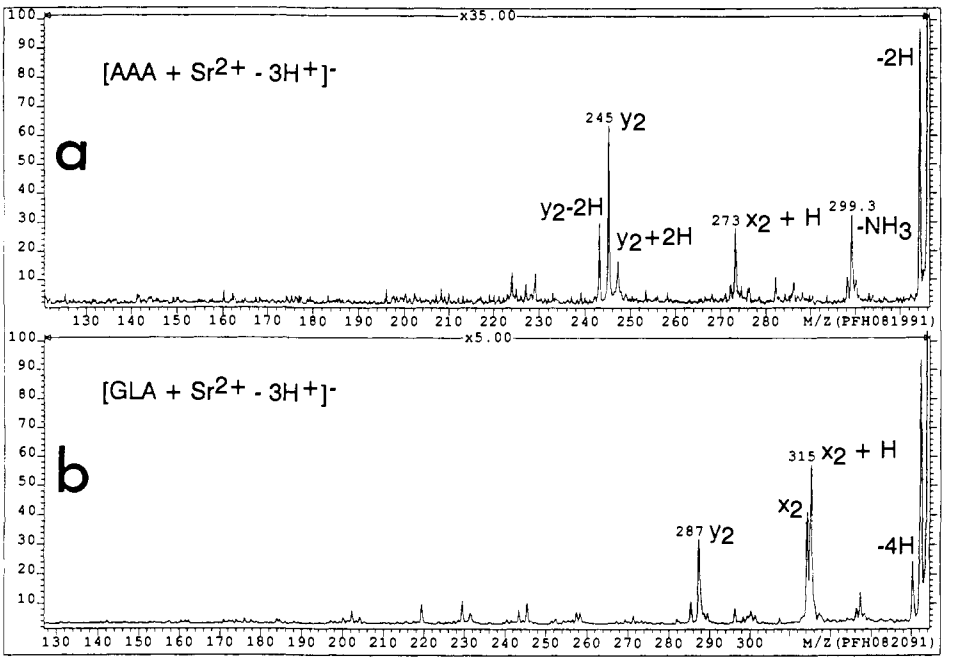


Figure 1. CAD spectra of Sr^{2+}-bound tripeptides: (a) $[AAA + Sr^{2+} - 3H^+]^-$ m/z 316; (b) $[GLA + Sr^{2+} - 3H^+]^-$ m/z 344.

The results for these simple systems are important reference points for investigations of more complex peptide systems.

Results and Discussion

Formation of [pept + Met^{2+} – $3H^+$]$^-$. In order that the anionic complex [pept + Met^{2+} – $3H^+$]$^-$ can be formed, three protons must be removed from the peptide. The availability of acidic protons, therefore, is an important factor for determining whether the complex can be desorbed into the gas phase (see Scheme I). Tripeptides and larger peptides readily form this anion because there are at least three acidic sites in the peptide (two or more amide sites and the C-terminal COOH group). A few dipeptides also form this anion, but the anionic complex is usually of low abundance. An explanation is that the hydrogen atoms on the N-terminal amino group are not sufficiently acidic to allow the necessary third deprotonation to occur. An acetylated dipeptide (e.g., Ac-GL), however, is readily desorbed as [Ac-GL + Ca^{2+} – $3H^+$]$^-$. The introduction of the acetyl group enhances the acidity of the terminal NH group and permits formation of the necessary triply negative peptide.

When basic alkaline earth metal ion salts, such as hydroxides, acetates, and oxides, are used as the metal ion source in the FAB matrix, higher ion currents than those from neutral alkaline earth metal ion salts are produced. The basicity of these salts may assist the deprotonation of the peptides. Supporting evidence is that if acid (HBr) is added to either a $Ba(OH)_2$- or $Ca(OH)_2$-saturated glycerol/thioglycerol (1:1) matrix, the metal-ion-bound peptide can no longer be detected in the gas phase. Deprotonation processes to give a peptide bearing a 3– charge are apparently crucial for desorption of the complex.

Small peptides with basic side chains, such as those of histidine and arginine, do not form significant gas-phase [pept + Met^{2+} – $3H^+$]$^-$ ions. One possible reason is that the triple deprotonation accompanying the complex formation is more difficult for these peptides containing basic amino acids. Another reason is that the basic site may interact with the metal ion, leading to the formation of other structures that compete with the formation of the anionic complex.

Both the nature of the metal salt and its concentration in the matrix affect the formation of metal-ion-bound peptides. The hydroxides of Ca^{2+}, Sr^{2+}, and Ba^{2+} are the best metal salts for this purpose probably because they are highly basic. The $Mg(OH)_2$-saturated matrices, however, are not effective for generating significant metal-ion-bound peptides, although the hydroxide is the most basic among the Mg^{2+} salts. The small solubility of $Mg(OH)_2$ in glycerol may be the explanation. $Mg(Ac)_2$ is basic, dissolves in glycerol, and can be used for the desorption of relatively abundant Mg^{2+}-bound peptides. On the other hand, a high concentration of metal ion in the matrix depresses the formation of metal-ion-bound peptides. An experiment with $Sr(Ac)_2$ shows that too low a concentration of metal ion (ca. 0.01 F) in the matrix militates against the formation of metal ion/peptide complexes; only the deprotonated peptide $[M - H]^-$ is observed if the metal ion concentration is lower than 0.01 F. On the other hand, the formation of metal ion/peptide complexes can be completely suppressed if high concentrations of Sr^{2+} (ca. 1 F) are used, although the workable metal ion concentration range is fairly wide, from 0.01 to 1 F of $Sr(Ac)_2$.

Collisionally Activated Decompositions (CAD) of Metal-Bound Peptides. Peptides with Aliphatic Side Chains. The CAD spectra of [pept + Met^{2+} – $3H^+$]$^-$ formed from tripeptides that contain only neutral amino acids are generally simple with respect to the CAD spectra of peptides that are desorbed as positive (either deprotonated or metal ion cationized) ions (Figure 1 and Table I). There are three common abundant fragment ions, y_2, x_2 + H (these ions are formally [y_2 + Met^{2+} – $2H^+$]$^-$ and [x_2 + Met^{2+} – $2H^+$]$^-$, but we will refer to them simply as x_2 + H and y_2, which is in accord with the generally accepted nomenclature[1a]), and that formed by loss of dihydrogen. The losses that accompany the formation of the y_2 and x_2 + H ions involve groups of the N-terminal amino acid; the expelled neutrals are likely to be an imine and carbon monoxide ($HN{=}CHR_1$ + CO) or only the imine ($HN{=}CHR_1$), respectively (see later discussions on their formation mechanisms).

The [pept + Met^{2+} – $3H^+$]$^-$ ions of peptides that have a glycine terminus generate abundant x_2 radical anions (Figure 1b and Table I) by loss of $^{\bullet}CH_2NH_2$. For these metal-ion-bound peptides that have a nonglycine terminus, the x_2 ion is only weakly produced.

Other ions formed by the decomposition of the N-terminal amino acid include that formed by loss of NH_3; this ion is abundant when the peptides have a phenylalanine terminus (Table I). The two ions that adjoin y_2 in mass are 2 mass units higher than and 2 mass units lower than y_2 (Figure 1) and are abundant for some peptide complexes.

Another feature of the CAD of the alkaline-earth-metal-bound peptides is the elimination of dihydrogen. This elimination is highly favorable even though the peptides in these complexes are already triply deprotonated (Figure 1 and Table I). For most peptides studied here, the loss of dihydrogen is the most facile process in the fragmentation of the [pept + Met^{2+} – $3H^+$]$^-$ ion.

Table I. Relative Abundances of Major Fragment Ions of Sr^{2+}-Bound Tripeptides

peptides	$(x_2 + H)/x_2$	$(y_2 - 2H)/y_2/(y_2 + 2H)$	loss of CO_2	loss of H_2	loss of NH_3	others
AAA	26/nd[a]	30/65/13	7	100	31	
ALA	28/nd	11/37/8	nd	100	36	
GFA	nd/nd	nd/nd/nd	nd	15	nd	M - 92 (100)
GGG	20/21	nd/11/nd	10	100	nd	
GGF	nd/nd	nd/nd/nd	nd	nd	nd	M - 92 (100)
GGL	55/55	13/48/nd	25	100	nd	M - 4H (8)
GGV	66/75	32/66/14	43	100	18	M - 84 (78)
GLA	59/41	7/30/nd	nd	100	11	
MAS	nd/nd	nd/nd/nd	4	3	5	M - 30
PGG	2/nd	nd/5/nd	nd	100	nd	
TVL	nd/nd	nd/nd/nd	nd	nd	nd	M - 44 (100) M - H_2O (25)
VGG	19/nd	14/100/35	nd	100	nd	

[a] Not detected.

The $[pept + Sr^{2+} - 3H^+]^-$ ion of Gly-Leu-Ala even undergoes a loss of 4 H ($2H_2$).

One product ion that may involve the C-terminus is that formed by a loss of 44 mass units, a process that is only competitive for some peptides (Table I). The 44 mass units losses for GGV and GGL complexes may include those of C_3H_8 from the side chains. Side chains of the C-terminal amino acid residue may be particularly vulnerable to collisional activation.

Peptides with Functionalized Side Chains. Metal-ion-bound peptides that contain a phenylalanine, serine, or threonine behave differently upon collisional activation. The loss of a side chain dominates, and the otherwise prominent $x_2 + H$ and y_2 ions are largely suppressed (see Table I). The $[TVL + Ca^{2+} - 3H^+]^-$ complex decomposes to an abundant ion by loss of 44 mass units, whereas the complexes of GFA and MAS fragment extensively by a loss of 92 and 30 mass units, respectively. When the peptide contains more than one functionalized side chain, the loss of one is usually prevalent. An example is $[MAS + Ca^{2+} - 3H^+]^-$ (see Table I); the only side chain loss involves that of serine.

It was reported by others[9] that for transition-metal-ion-bound peptides, the peptide side chains interact directly with the metal ion. The complex in solution favors a conformation in which the side chains, even aliphatic ones such as that of leucine, position above the plane containing the metal ion and peptide ligands. This direct metal ion–side chain interaction is not solvent-related and may account for the facile side chain loss from gas-phase metal-ion-bound peptides.

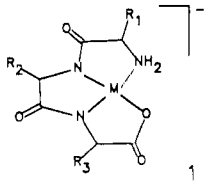
The side chain losses from threonine and serine also occur upon FAB desorption. In the formation of Ca^{2+}-bound TVL, the threonine side chain is lost to generate a putative Ca^{2+}-bound GVL (see later discussion for evidence). This product ion is more abundant than the precursor ion (ca. 5:1). Apparently the threonine side chain does not survive well the FAB ionization. Similar metal ion influence over the serine residue in MAS is also observed.

The facile losses of peptide side chains upon FAB are considered to require metal ion–side chain interaction because these losses do not occur for $(M + H)^+$ ions or metal cationized peptides. The ready losses of serine and threonine side chains, however, were observed in the CAD of deprotonated dipeptides. The side chain loss was proposed to be initiated by the negative charge located at the C-terminus.[3b]

The overall fragmentation chemistry is interpreted to indicate that the C-terminal amino acid and the central amino acid of the tripeptides are tightly bound to the metal ion. The part of the peptide chain that is most accessible to fragmentation is the N-terminal amino acid. The dominance of such processes as elimination of dihydrogen and losses of side chains of the constituent amino acids is consistent with the idea that metal ion bonding in the complex is strong.

Structure of $[pept + Met^{2+} - 3H^+]^-$. For simple neutral peptides, the three most acidic hydrogens are at the C-terminal carboxylate group and at the two amide nitrogens. An amino group is generally not considered to be acidic with respect to carboxylic and amide groups, and the deprotonation of an amino group in the interaction of peptides with doubly charged metal ions has not been observed in solution.[6] It, however, was observed that deprotonated amino groups are found in Cu^{3+} and Au^{3+} complexes at high pH.[10] Therefore, the two amide nitrogen atoms and the C-terminal carboxylate group are expected to be deprotonated and to form the strongest bonds with the metal ion in the structure $[pept + Met^{2+} - 3H^+]^-$. The N-terminal amino group may also be involved in the bonding, but the interaction is likely to be weaker. This is demonstrated in the formation of $[Ac\text{-}GL + Ca^{2+} - 3H^+]^-$ ion. Although Ac-GL has three acidic sites, it lacks a fourth coordination group. The ability to desorb a metal-bound peptide apparently is not affected significantly if the fourth coordination is not available.

The fragmentation behavior discussed above is entirely consistent with the structure we propose for the complex **1**. The



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multiring structure has the C-terminal and the central amino acid residues deprotonated, and these sites are thus available for strong coordinate covalent bonding. The neutral N-terminal amino group probably bonds, but we have no direct evidence. There are, however, solution analogies in which complexes of alkaline earth metals and various ligands have coordination numbers greater than three.[11] The nature of the bonding is in accord with the $x_2 + H$ and y_2 ion formation. The processes that lead to the losses of H_2, NH_3, and side chains of the peptide are other means of dissipating the energy gained upon collision without disrupting the strong metal bonding. Therefore, the fragmentation of the peptide chain (except at the N-terminus) is unfavorable. For positive metal ion/peptide adducts, reactions of a charge-remote[12] nature occur at various parts of the molecule even if the metal ion binds to a specific site.[13]

In addition to the information provided by the CAD of metal-ion-bound peptides, results from deuterium-labeled peptides

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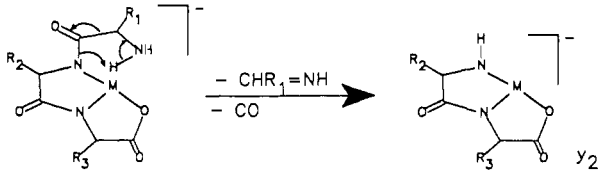
Table II. Neutral Losses for the Major Fragment Ions of Alkaline-Earth-Metal-Ion-Bound Peptides[a]

peptide	m/z[b]	$y_2 - 2H$[c]	y_2	$y_2 + 2H$[c]	$x_2 + H$[c]	$M - 2H$[c]
VGG (Ca)	268	101	99	97	71	2
VGG-d_5 (Ca)	270	103	100	97	73	2 & 3
GGV (Sr)	316	59	57	55	43	2
GGV-d_5 (Sr)	318	61	58	55	44	2 & 3
PGG (Ca)	266	99	97	n/a[d]	69	2
PGG-d_5 (Ca)	268	100	98	n/a	70	3
AAA (Sr)	316	73	71	69	43	2
AAA-d_5 (Sr)	318	75	72	69	45	2 & 3

[a] The data were acquired with the three sector tandem mass spectrometer. For isotopically labeled peptides, the peak widths are too broad (ca. 1.5 u) to assess accurately the extent, if any, of H/D scrambling. Peak shape analysis, however, reveals that if any H/D interchange occurs, it is less than 10%. The metal ions used in the experiments are given in parentheses. [b] The masses of the metal-ion-bound peptides. [c] Hydrogen or deuterium. [d] Not applicable.

Scheme II



are key evidence that supports this structure assignment. Two active hydrogens are left in the tripeptide $[pept + Met^{2+} - 3H^+]^-$ complex because three of a total five are lost in its formation. The complex formed from a deuterium-labeled tripeptide (all five active hydrogens replaced by deuteriums) is 2 mass units heavier than that of the unlabeled peptide. The mass loss accompanying the formation of $x_2 + H$ ion from Val-Gly-Gly-d_5 is 73 mass units, whereas the mass loss for the same process of the unlabeled peptide is 71 mass units (see Table II and the detailed discussion on $x_2 + H$ ion formation mechanism). The 2 mass units difference indicates that both of the two remaining active hydrogens are lost. Therefore, they are located at the N-terminus because formation of $x_2 + H$ involves one hydrogen transfer from the N-terminal side to the remaining C-terminal side. The formation of $M - NH_3$ ions from some peptide complexes supports the contention that the N-terminal NH_2 is not deprotonated.

Two other lines of evidence are consistent with the structure assignment. First, tripeptides without a free acidic C-terminus do not produce any detectable $[tripept + Met^{2+} - 3H^+]^-$. This indicates that a C-terminal free acid is essential to the formation of this anionic complex. Second, tripeptides with proline as the central amino acid do not form any detectable complex, pointing to the need of a middle amide hydrogen that can be lost as a proton.

Fragmentation Mechanisms. y_2 Ions. The masses of the y_2 ions from tripeptides reveal that losses of N-terminal amino acid residues are accompanied by hydrogen rearrangement. The mass loss for the formation of y_2 ion from Val-Gly-Gly-d_5 complex is 100 mass units, which is 1 mass unit higher than that for the unlabeled peptide (Table II). This confirms that one of the hydrogen atoms on the N-terminal amino group is transferred. A mechanism for the formation of the y_2 ion (see Scheme II) shows that one of the amino group hydrogens is transferred as a proton to the second deprotonated amide nitrogen atom, and that triggers bond cleavages resulting in losses of carbon monoxide and an imine. The metal binding in the product ion is not significantly affected by the fragmentation.

Although the mechanism we propose for the formation of y_2 ions does not directly involve the metal ion, most peptides show metal ion dependence for this process. With respect to the abundance of undecomposed metal complexes, the abundance of y_2 increases as the metal is changed from Ca^{2+} to Sr^{2+} to Ba^{2+} (see Figure 2). The y_2 ions are gas-phase dipeptide complexes, which desorb by FAB with the greatest of difficulty, indicating some instability (there are only two sites that are readily deprotonated in a dipeptide and yet three are required to form the complex). It is likely that Ba^{2+}–dipeptide complexes are more stable than Sr^{2+} complexes and these in turn are more stable than those involving Ca^{2+}. This trend in product stability impacts transition structure energy and geometry because the reactions are endothermic.

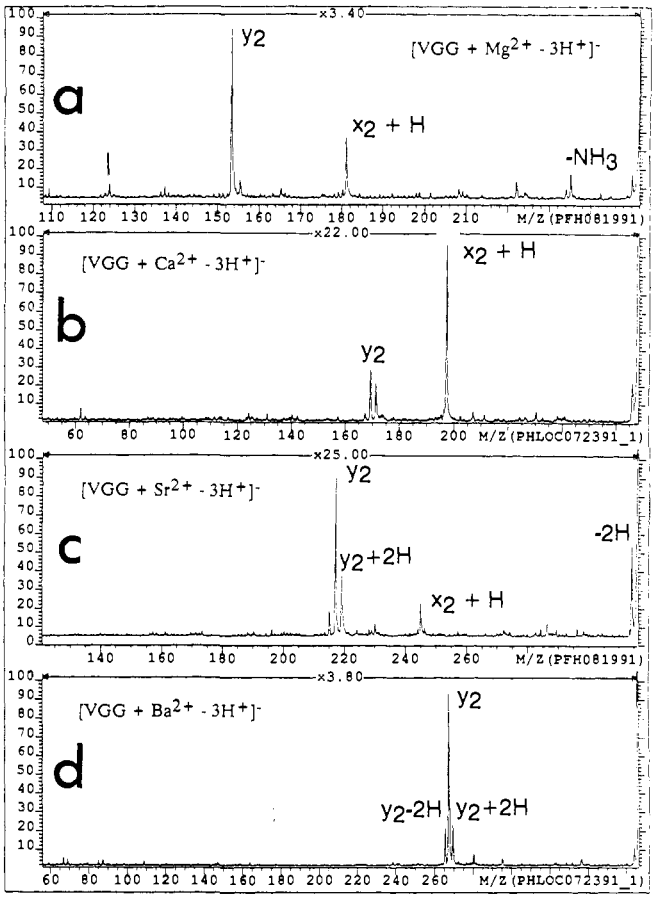
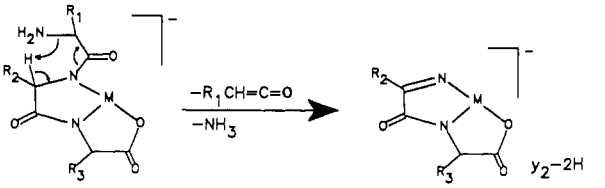


Figure 2. CAD spectra of alkaline-earth-metal-bound VGG monomers: (a) $[VGG + Mg^{2+} - 3H^+]^-$ m/z 252; (b) $[VGG + Ca^{2+} - 3H^+]^-$ m/z 268; (c) $[VGG + Sr^{2+} - 3H^+]^-$ m/z 316; (d) $[VGG + Ba^{2+} - 3H^+]^-$ m/z 366.

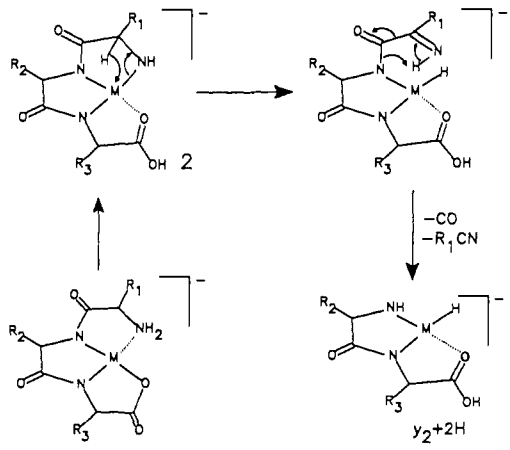
Scheme III



$y_2 - 2H$ Ions. As mentioned earlier, two adjoining mass ions of the compositions $y_2 - 2H$ and $y_2 + 2H$ are also formed along with the y_2 ion. In order that the $y_2 - 2H$ ion be formed, one hydrogen atom must be transferred from the portion of the peptide that binds the metal ion to the N-terminal leaving group. For peptides in which the active hydrogens are replaced by deuteriums, the mass of this ion is 3 mass units less than that of y_2 ion. This shows that the product ion does not contain any deuterium. The α-hydrogen of the central amino acid is proposed to be transferred to the N-terminal amino group, leading to scission of the amide bond (see Scheme III). The product ion retains similar metal ion binding to that of the original complex.

$y_2 + 2H$ Ions. The transfer of three hydrogens from the N-terminal amino acid residue to the portion bound by the metal ion must accompany the formation of $y_2 + 2H$ ions. For peptides in which active hydrogens are replaced by deuterium, the mass of this ion is 3 mass units greater than that of the y_2 ion, indicating that both of the two deuterium atoms are retained in $y_2 + 2H$ ion. The neutral lost is likely a nitrile (R_1CN) except for when

Scheme IV



the N-terminal amino acid is glycine, and then it is hydrogen cyanide (HCN).

As proposed in Scheme IV, the formation of y_2 + 2H ions proceeds through an intermediate in which the N-terminal amino group is deprotonated, leaving one of the three original binding sites in the neutral form and less strongly bound to the metal ion (structure **2**, a recent theoretical study[14] using glycine as a model, showed that the carbonyl oxygen is a better binding site for Na^+ than the hydroxyl oxygen of a carboxylic acid group). The y_2 + 2H ion begins to form as the α-hydrogen of the N-terminal amino acid is transferred to the metal ion as a hydride. Successive hydrogen transfers from the N-terminal amino group to the second amide nitrogen initiate the process of losing neutral carbon monoxide and a nitrile. The product ion is a doubly deprotonated dipeptide bound to a metal hydride. If this mechanism is correct, tripeptides with proline as the N-terminal amino acid should not produce the y_2 + 2H ion, and this is consistent with the experimental results for PGG.

Although the y_2 + 2H ion is usually produced less abundantly than the y_2 ion in CAD, it is formed more abundantly in metastable decompositions. The overall energetics for the y_2 + 2H ion formation must be lower than those for the y_2 ion even though an intermediate structure is involved. The energy barriers for the reactions in Scheme IV must be smaller than the activation barrier for y_2 ion formation. This is likely the result of the greater stability of the y_2 + 2H ion compared to the y_2 ion. For the low energy metastable decompositions, the energetically favored y_2 + 2H ion has a higher probability to form. When the internal energy of the precursor ions is raised by collisional activation, the one-step y_2 ion formation becomes kinetically favored.

Structure **2** is also the likely intermediate for fragmentations in which 44 and 46 mass units (CO_2 and HCO_2H, respectively) are lost. Although these losses are not facile for alkaline-earth-metal-ion-bound peptides, they are major fragmentation channels for transition-metal-ion-bound tripeptides. A detailed discussion will be presented in a future paper.

x_2 + H Ions. The formation of x_2 + H ions occurs by two different mechanisms as indicated by the experimental results for labeled peptides Val-Gly-Gly-d_5, Gly-Gly-Val-d_5, Ala-Ala-Ala-d_5, and Pro-Gly-Gly-d_4 (Table II). The mass loss for the formation of x_2 + H ion from Val-Gly-Gly-d_5, Pro-Gly-Gly-d_4, and Ala-Ala-Ala-d_5 is 2 mass units greater than that for the corresponding unlabeled peptides. Both of the deuterium atoms on the terminal amino group are lost as part of the neutral, and the hydrogen rearranged to the C-terminal side is not from the N-terminal amino group. The mass loss for the same process from Gly-Gly-Val-d_5, however, is 1 mass unit greater than that for the unlabeled peptide, which indicates that one of the hydrogen atoms of the N-terminal amino group is transferred to the C-terminal side and the other one is lost along with elements of an imine.

The resulting structures of the x_2 + H ions from both types of peptides, however, are identical as indicated by consecutive activation (MS/MS/MS) experiments.[15] Gly-Leu-Ala and Ala-Leu-Ala have different N-terminal amino acids, yet the x_2 + H ions produced from the metal complexes of these peptides have the same nominal mass. The mass spectra produced by collisionally activating these two x_2 + H ions should reflect the differences in their structures. The two spectra are nearly identical. Mechanisms for the formation of x_2 + H ions are proposed in Scheme V on the basis of the above evidence.

The formation of x_2 + H ion from metal-ion-bound peptides that have a glycine N-terminus also proceeds through a 1,4-hydrogen transfer (Scheme V) to eliminate an imine. When the N-terminal amino acid residue has a β-hydrogen, the process starts by a transfer of a hydride from the β-position to the metal ion (Scheme V). Both types of peptides then undergo consecutive electron transfers that result in a loss of vinylamine or an imine and the formation of a isocyanate group at the new N-terminus. This intermediate (**3**) may then isomerize to **4**. The driving force for this mechanism is probably the positive charge on the metal ion. We also proposed for y_2 + 2H ion formation a mechanism in which a hydride transfer to the metal ion is also the first step of the process (Scheme IV).

These mechanisms that involve hydride transfer to the metal ion should show that the abundances of product ions depend on the nature of the metal. That is clear for the process giving y_2 + 2H with respect to that for y_2. For x_2 + H ion formation in peptide complexes having glycine at the N-terminus, there is little if any metal ion dependence, whereas weak metal dependence pertains to those peptides having other amino acids at the N-terminus (Figure 2). Given that the latter peptides transfer hydride from carbon rather than from nitrogen, even though both transfers are possible, it is likely that hydride transfer from nitrogen requires higher energy, washing out subtleties of metal ion dependence. This is also in accord with the fact that the x_2 + H ion formation favors the β-hydrogen over the amino group hydrogen transfer.

The high activation energy of the amino group hydrogen transfer mechanism is also reflected by the production of an x_2 ion in the CAD of metal-bound peptides that have a glycine N-terminus. The x_2 ion results from a homolytic cleavage process that produces a radical anion and a radical neutral. Because x_2 ions are not produced to any significant extent in the CAD of metal-ion-bound peptides that form x_2 + H ions by the β-hydrogen transfer mechanism, the amino group hydrogen transfer mechanism must have a relatively high energy requirement so that the radical anion formation can compete.

Loss of Dihydrogen. The CAD of metal-bound labeled peptides including Val-Gly-Gly-d_5, Ala-Ala-Ala-d_5, Gly-Gly-Val-d_5, and Pro-Gly-Gly-d_4 also shed light on the mechanism for loss of dihydrogen. In addition to loss of H_2, expulsion of HD also occurs from $[pept\text{-}d_5 + Met^{2+} - 3D^+]^-$. The expulsion of HD is consistent with a product ion that is formed by loss of one of the hydrogens on the N-terminal NH_2 group and one of the α-hydrogens of the N-terminal amino acid. The product ion contains an extended π-system and is, therefore, stable. The loss of dihydrogen from the metal-bound labeled peptides indicates that other mechanisms are also possible. The 1,2-elimination of an α-hydrogen and a β-hydrogen is one alternative. The product ion formed by this mechanism also has extended conjugation. The loss of 4 H from some of the metal-ion-bound peptides, such as those of PGG, GLA, and GGL, may point to the serial occurrence of both mechanisms.

Thermal dehydrogenation normally requires a catalyst. The 1,2-elimination is symmetry-forbidden.[16] Nevertheless, 1,2-eliminations were observed in metastable ion decompositions of small ions.[17] Accompanying the event is a large kinetic energy

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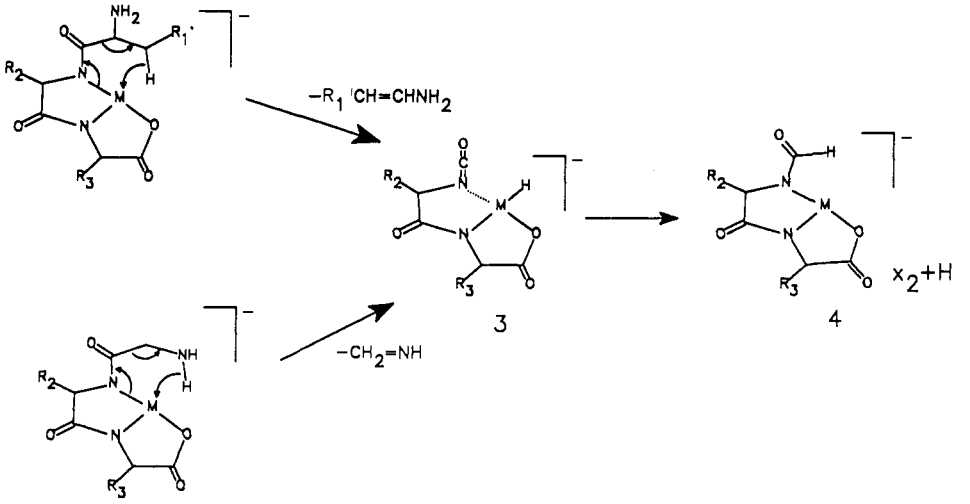
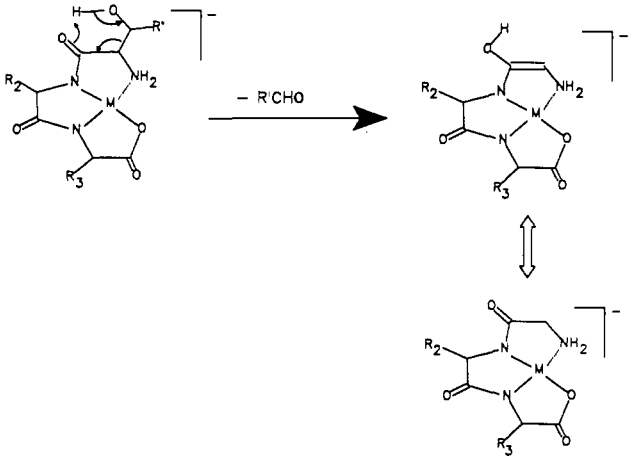
(17) Williams, D. H.; Hvistendahl, G. *J. Am. Chem. Soc.* **1974**, *96*, 6753–6755.

Scheme V



Scheme VI



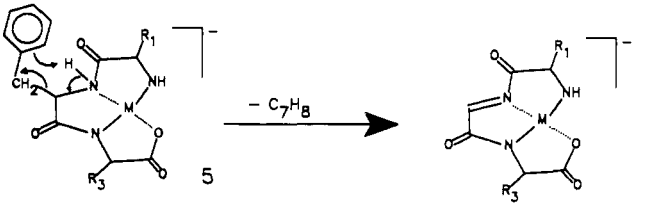
release. The relatively facile loss of dihydrogen in the CAD of metal-bound peptides may be a result of metal participation in the reaction transition structure.

Losses from Side Chains of Serine, Threonine, and Phenylalanine. The threonine, serine, and phenylalanine side chains are seen as the most vulnerable toward CA among those of neutral amino acids, and they may be lost as CH_3CHO, CH_2O, and $PhCH_3$, respectively. As mentioned earlier, the facile losses of these side chains may be an effect of direct metal ion–side chain interactions. These side chains, owing to their electron donating abilities, are all capable of interacting with the metal ion. That interaction weakens the O–H bond for serine and threonine side chains, lowering the activation energy for the process.

The mechanisms for serine and threonine side chain losses are likely to be similar because both of the side chains contain a β-OH group (Scheme VI). The product ions have structures that are identical to those of the precursor ions except that the serine and threonine residues are converted to glycine residues. This is supported by an MS/MS/MS experiment. For example, Ca^{2+}-bound TVL, upon CA, forms a predominate product ion by losing 44 mass units. If indeed the threonine residue is converted to glycine in the process, then the CAD spectrum of this product ion should be similar to that of GVL. That is, losses of 29 and 57 mass units (corresponding to $x_2 + H$ and y_2 ion formation from N-terminal glycine) should occur, and indeed they are the major fragmentations. Like the fragmentation of other metal-bound peptides with N-terminal glycine, ions formed by losing 44 and 17 mass units are also observed.

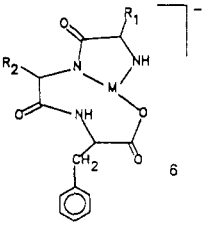
The losses of 92 mass units from metal-bound phenylalanine-containing peptides do not have positional dependence. Metal-bound FGG, GFA, and GGF all undergo predominent loss of 92

Scheme VII



mass units. Results from deuterium labeled peptides (all active hydrogens are replaced by deuterium atoms) show that an active hydrogen is lost along with the benzyl group. It is likely that the benzene ring participates in the elimination process (Scheme VII).

For tripeptides that have a central phenylalanine residue, the reaction probably proceeds through an intermediate structure in which the N-terminal amino group is deprotonated and the amide group of the phenylalanine residue has a proton (see structure **5**). The metal-bound peptides that have a C-terminal phenylalanine residue require an intermediate for C_7H_8 loss in which the first amide group is not deprotonated (**6**). For peptides with



phenylalanine at the N-terminus, the hydrogen removed may originate from the N-terminal amino group.

Future Prospects. The limited availability of acidic sites in the tripeptides studied here assures us that metal-bound tripeptides have one favored structure, which we depict as structure **1**. Larger peptides have more than three potential deprotonation sites and may assume more than one structure. Tetrapeptides have four sites that have nearly equal acidity[6a,18] (i.e., the C-terminal COOH and the three amide groups), and thus there can be more than one structure: structure **7** has a C-terminal carboxylate and two adjoining deprotonated amide groups, whereas structure **8** has three deprotonated amide groups.

Collisional activation of metal-bound tetrapeptides should produce product ions that are characteristic of both structures if they exist. Both the N-terminal ions formed by losses of H_2O and CO_2 from structure **8** and the C-terminal ions $x_2 + H$ and y_2 from structure **7** as well as $x_3 + H$ and y_3 from structure **8** are observed for Ca^{2+}-bound VAAF. This provides an opportunity to study the relative affinity of the metal ion toward the C-terminal

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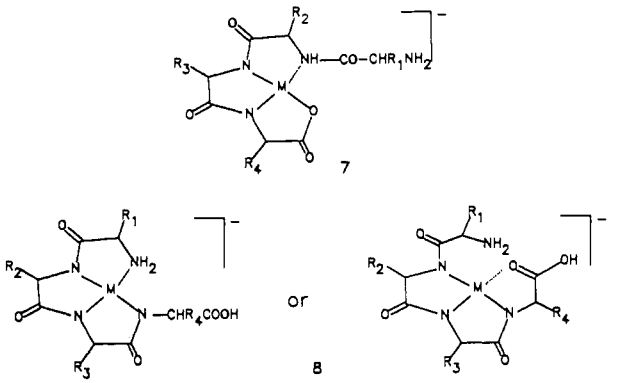

carboxylate and the amide groups. The results of this study will be presented in a future paper.

Conclusion

Tripeptides interact with alkaline earth metal ions to form anionic complexes of the composition [tripept + Met^{2+} - $3H^+$]$^-$. The structure involves coordinate covalent bonding of the metal ion to three sites: the deprotonated C-terminal carboxylate group and the two deprotonated amide nitrogens. The N-terminal amino group is more loosely bound to the metal ion. Deprotonation of amide hydrogens to give an alkaline earth metal complex bearing a negative charge is unique in the gas phase. In solution, solvation must weaken the interactions between peptides and alkaline earth metal ions to an extent that deprotonation of amide NH cannot occur.

The strong bonding in the gas-phase anionic complex is underscored by the fragmentations to lose 2 H, 4 H, and NH_3 as well as those to expel side chains of phenylalanine, serine, and threonine. The facile reactions giving x_2 + H and y_2 ions are also consistent with the proposed structure. The key step in the formation of both x_2 + H and y_2 + 2H ion involves a novel hydride transfer to the metal ion. Thus, the relative abundances of these two ions are metal dependent and increase in the order Ca^{2+} > Sr^{2+} > Ba^{2+}.

Tandem mass spectrometry of these anionic metal-bound tripeptides also provides some sequence information. The N-terminal amino acid is readily determined on the basis of two dominant ions, y_2 and x_2 + H.

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 on which the mass spectra and tandem mass spectra were acquired were a Kratos MS-50 and VG four-sector ZAB-T. The Kratos MS-50 is a triple-sector mass spectrometer of EBE geometry, which was previously described.[19] It is equipped with a commercially available 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. Both field-free regions 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.[15]

The ZAB-T four-sector tandem mass spectrometer consisted of two high-mass, double-focusing mass spectrometers.[20] 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 were 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).

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 the 25-keV Cs^+ atom beam for the desorption of the [pept + Met^{2+} - $3H^+$]$^-$ ion.

For FAB-MS-MS-MS experiments,[15] the Kratos MS-50 was used. The source-produced ions were activated in the first field-free region, and the collision cell pressure was adjusted so that the product signal was maximized. The product ion of interest was selected by setting the first ESA and the magnet at appropriate values. This selected product ion was then collisionally activated in the third field-free region with 50% of main beam suppression, and the product ions were recorded by scanning the second ESA.

For experiments with deuterium labeled peptides, the peptide and the matrix were mixed on the tip of an FAB probe. D_2O (4–5 μL) was then added to the mixture. After the solution was mixed, the probe was inserted into the prevacuum system of the mass spectrometer and the volatile water (H_2O, HDO, and D_2O) was pumped away for ca. 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-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 No. CHE9017250).

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