Gas-Phase Fragmentations of Anionic Complexes between Peptides and Alkaline Earth Metal Ions: Structure-Specific Side-Chain Interactions

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Abstract: The first study of gas-phase anionic $(M + Cat^{2+} - 3\ H)^-$ complexes between 59 peptides and Mg^{2+}, Ca^{2+}, and Ba^{2+} is reported. Formation of the complexes requires deprotonation of three sites. Peptide complexes that contain aprotic (hydrocarbon) amino acid side chains decompose primarily to give $(x_{n-m} + Cat^{2+} - 2\ H)^-$, $(y_{n-m} + Cat^{2+} - 2\ H)^-$, and other ions that contain the C-terminus. Molecular mechanical calculations reveal that these product ions arise from complexes that contain Cat^{2+} nonspecifically bound to deprotonated amides and/or the C-terminus. In sharp contrast, peptide complexes that contain the protic amino acids Asp, Glu, Tyr, His, or Trp decompose to give unique N-terminal $(c_{n-m} + Cat^{2+} - 2\ H)^-$ ions that instead contain the alkaline earth metal ion bound to the deprotonated side chain and to two deprotonated amides. The experimental data and molecular mechanical calculations support formation of structurally specific precursor ion complexes that involve specific side-chain binding interactions. Other evidence for specific side-chain binding interactions is provided in a comparison of the chemistry of the alkaline earth metal ions to that of some transition metal(II) ions.

Introduction

Alkaline earth metal ions such as Mg^{2+} and Ca^{2+} are essential for a variety of biological processes. Calcium is required for blood clotting,[1] for contraction of muscles,[1] and for secretion of hormones such as insulin.[1b-d] The activation or deactivation of enzymes is also metal ion dependent. For example, Mg^{2+}-Ca^{2+}-ATPase catalyzes the hydrolysis of ATP and requires both metal ions for activation.[1a,d] Phospholipase A_2, which catalyzes the hydrolysis of fatty acid esters of phosphoglycerides, requires Ca^{2+} but will bind with either Ba^{2+} or Sr^{2+}, which will inhibit its enzymatic activity.[1d] Metal ion interactions also affect the stability of a peptide or protein. Thermolysin requires four Ca^{2+} ions to protect its thermal stability,[1b-d,2] and trypsin requires one Ca^{2+} ion per mole to protect the enzyme from autolysis and denaturation.[1d]

Metal ion binding sites in proteins are specific for particular metal ions. The metal ion induces conformational changes to maximize the metal ion–ligand interactions,[1b,2c,3] and each metal ion prefers certain types of ligand interactions. The larger Ca^{2+} prefers to form looser complexes that can have variable bond lengths.[1b,3,4] The smaller Mg^{2+} instead prefers to form tight complexes with precise bond lengths. The differences in preferred binding interactions may explain why a protein can be inhibited by chelation of Mg^{2+}, but activated by binding with Ca^{2+} or vice versa.[5]

Solvated interactions between peptides and metal ions have been well characterized by solution-phase techniques.[1-5] The intrinsic or gas-phase interactions, however, have just begun to be understood. Recently, the gas-phase chemistry of cationic complexes between peptides and alkali metal ions has been investigated.[6-11] One of the main objectives has been to determine the location of the metal ion in the gas-phase complexes via interpretation of metastable ion and collision-induced decomposition (CID) mass spectra. The exact location and coordination of the alkali metal ion, however, are difficult to determine unequivocally.

In our earlier investigation of cationic gas-phase $(M + Cat^{2+} - H)^+$ complexes between peptides and alkaline earth metal ions, one objective was similarly to determine the nature of the metal ion complexation.[12] Mechanisms for formation of the most abundant sequence (product) ions were proposed, and it was concluded that the alkaline earth metal ion is bonded nonspecifically to a deprotonated amide. It was also suggested that intramolecular, multisite binding interactions occurred between the metal ion and peptide functional groups. We have now extended this research to anionic $(M + Cat^{2+} - 3\ H)^-$ complexes between Mg^{2+}, Ca^{2+}, and Ba^{+2} and 59 tri- through hexapeptides,

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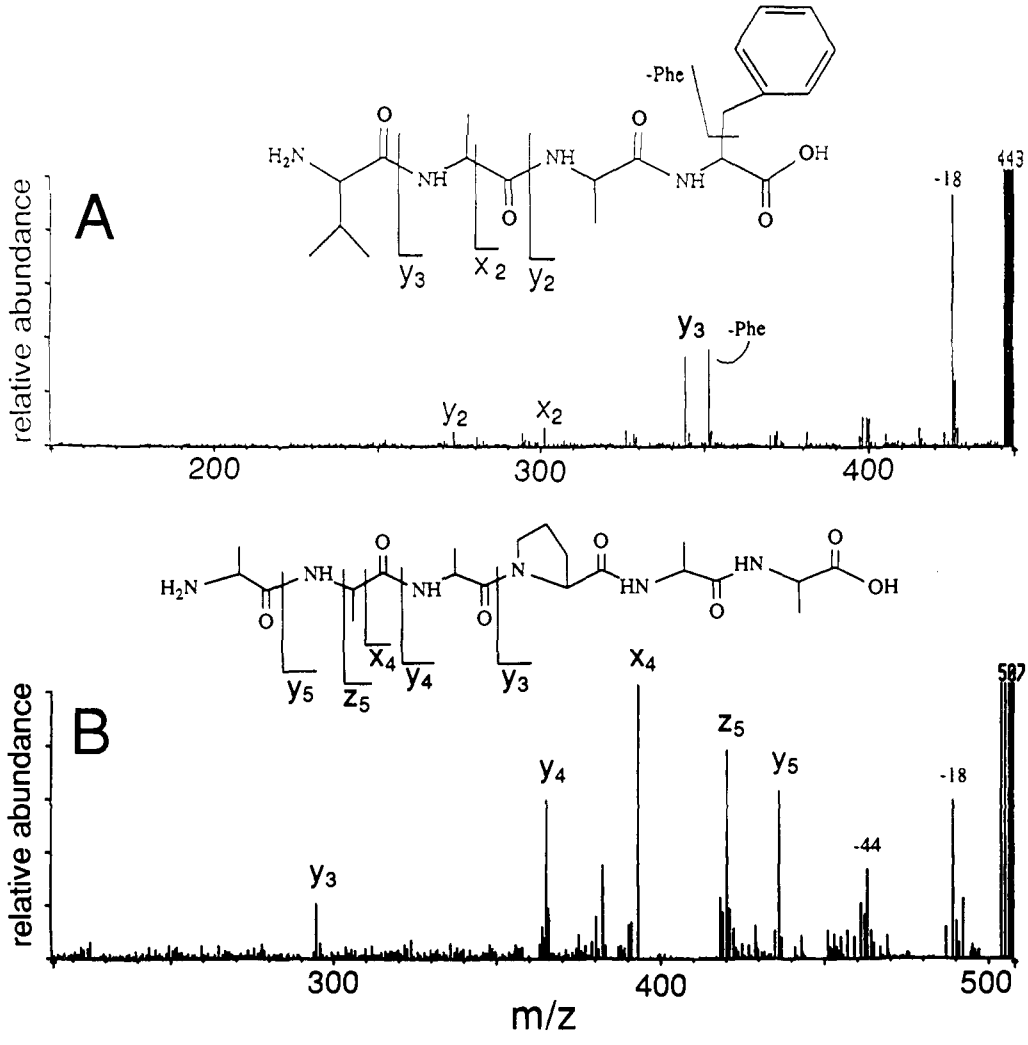

Figure 1. CID spectra of (M + Ca – 3 H)$^-$ complexes of (A) Val-Ala-Ala-Phe and (B) Ala-Ala-Ala-Pro-Ala-Ala. Ions labeled x_{n-m}, y_{n-m}, and z_{n-m} are $(x_{n-m}$ + Ca – 2 H)$^-$, $(y_{n-m}$ + Ca – 2 H)$^-$, and $(z_{n-m}$ + Ca – 3 H)$^{\bullet-}$ ions, respectively. Loss of the Phe side chain as $-C_7H_8$ is labeled as -Phe. FAB-desorbed ions were produced by bombarding the samples with Ar.

most of which contain the protic side chains His, Tyr, Glu, Asp, Trp, Ser, or Thr. Hu and Gross[13] recently published data for tripeptides, none of which contain protic side chains except for Ser or Thr. We also have incorporated molecular mechanical calculations to address structures of the precursor ion complexes. Camilleri and co-workers[14] similarly used molecular modeling to address protein conformations related to electrospray ionization mass spectra. Leary and co-workers[10b] instead used semiempirical calculations to address binding between Li^+ and Gly-Gly-Gly, as related to mass spectral fragmentations. Results presented here provide evidence for the formation of structurally specific intramolecular gas-phase chelation complexes between the peptides and the alkaline earth metal ions.

Results and Discussion

The gas-phase (M + Cat^{2+} – 3 H)$^-$ complexes were prepared in the same general manner as before[12] (also see Experimental Section). Here, however, initial formation of the highly abundant anionic complexes by fast atom bombardment (FAB) requires deprotonation of three peptide functional groups. Sites for deprotonation are the C-terminal carboxylic acid (or amide), the amide groups, and some amino acid side chains. If the complex involved a peptide that contained only aprotic (hydrocarbon) amino acid side chains, the most likely sites for deprotonation would be the C-terminus and amide groups. For peptides that contained protic amino acids, however, additional sites for deprotonation would be the amino acid side chains.

Results for Ca^{2+}. The CID spectra of (VAAF + Ca – 3 H)$^-$ and (AAAPAA + Ca – 3 H)$^-$ complexes in Figure 1 demonstrate the types of decompositions that are typical for peptide–alkaline earth metal ion complexes that contain only aprotic (hydrocarbon) amino acids. The C-terminal $(x_{n-m}$ + Cat^{2+} – 2 H)$^-$, $(y_{n-m}$ + Cat^{2+} – 2 H)$^-$, and $(z_{n-m}$ + Cat^{2+} – 3 H)$^{\bullet-}$ ions (labeled x_{n-m}, y_{n-m}, and z_{n-m} in Figure 1[15]) are the most prevalent sequence (product) ions. Fragmentations in Figure 1 and in six other spectra are consistent with formation of C-terminal sequence ions from precursor complexes that contain the metal ion bonded in chelated structures that either involve the deprotonated C-terminus and two deprotonated amides or involve three deprotonated amides.

For example, complexes of VAAF that decompose to give $(y_2$ + Ca – 2 H)$^-$ ions must have the metal ion bonded to the deprotonated C-terminus and the two adjacent deprotonated

(13) Hu, P.; Gross, M. L. *J. Am. Chem. Soc.*, **1991**, *114*, 9153–9160. They also present data for bis(peptide) complexes (*J. Am. Chem. Soc.* **1991**, *114*, 9161–9169).

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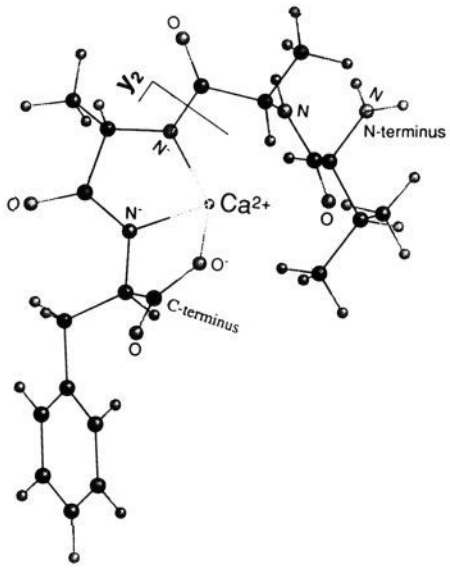
(15) The terminology used here for the sequence ions generally follows that of Biemann (Biemann, K. *Biomed. Environ. Mass Spectrom.* **1988**, *16*, 99–111) and is consistent with terminology used in our previous papers. It should be noted that the terminology describes the masses of the fragment ions, not their mechanism for formation. Furthermore, the alphabetical designation indicates the location of the cleavage reaction. For example, the $(x_{n\ m}$ + Ca – 2 H) closed shell ion arises via cleavage between RC–CO bonds and the product ion actually would be better described chemically as $(x_{n\ m}$ – 3 H^+ + Ca^{2+} + H$^\bullet$). We have chosen, however, to use the shortened terminology for the sake of brevity.

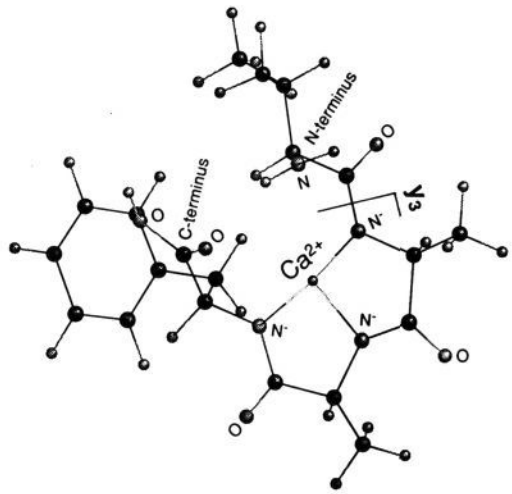
Chart I

VAAF



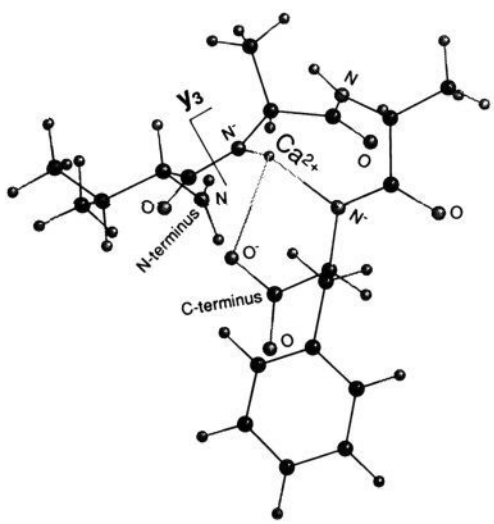
Structure 1a Strain Energy = 19.8 kcal mol^{-1}

VAAF



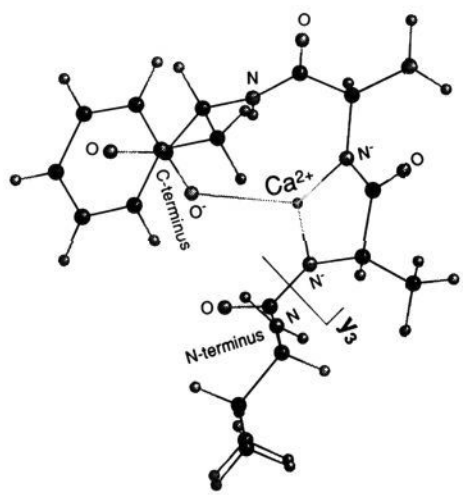
Structure 1b Strain Energy = 16.2 kcal mol^{-1}

VAAF



Structure 1c Strain Energy = 16.5 kcal mol^{-1}

VAAF



Structure 1d Strain Energy = 27.0 kcal mol^{-1}

amides (structure **1a**: all structures and minimized energies are the results of molecular mechanical calculations, see Experimental Section for details). The more highly abundant $(y_3 + Ca - 2H)^-$ ions in the spectrum, on the other hand, can be formed from three different precursor complexes of three different relative energies (structures **1b**, **1c**, and **1d**). The lowest energy structure (structure **1b**) that can give the $(y_3 + Ca - 2H)^-$ ions has the metal ion coordinated to three deprotonated amides. Structure **1c**, which is slightly higher in energy, involves binding to the deprotonated C-terminus, to the adjacent amide, and to the distal amide; and structure **1d** involves binding to the deprotonated C-terminus and to the two distal amides. All four isomeric precursor complexes, however, may give rise to the product ions that are formed by losses of the Phe side chain (–92 u or $-C_7H_8$). Product ions that are formed by losses of water are also abundant in CID spectra of complexes of tetrapeptides or larger peptides, but (M

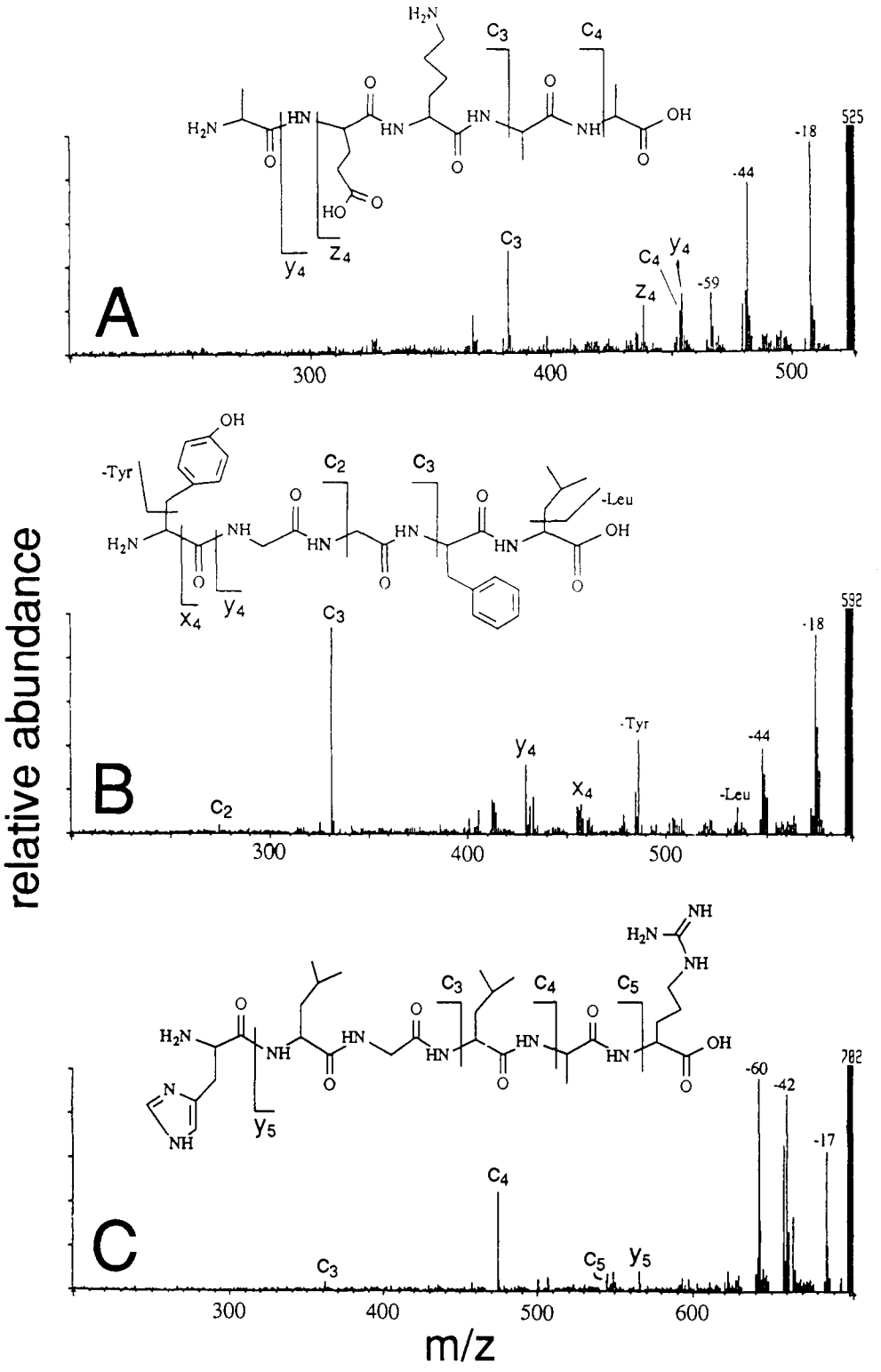


Figure 2. CID spectra of (M + Ca − 3 H) complexes of (A) Ala-Glu-Lys-Ala-Ala, (B) Tyr-Gly-Gly-Phe-Leu, and (C) His-Leu-Gly-Leu-Ala-Arg. Ions labeled c_{n-m} and z_{n-m} are $(c_{n-m} + Ca - 2\ H)^-$ and $(z_{n-m} + Ca - 3\ H)^{\bullet -}$ ions, respectively. FAB-desorbed ions were produced by bombarding the sample with Ar.

+ Ca − 3 H)$^-$ complexes of tripeptides do not decompose to give such ions.[13] This implies that loss of water requires a free C-terminal neutral carboxylic acid, which is present only in the lowest energy structure **1b**. Thus, the most energetically favored precursor isomer, structure **1b**, which would be of high abundance in the precursor ion beam, can fragment competitively to give loss of H_2O, loss of the Phe side chain, and the $(y_3 + Ca - 2\ H)^-$ ions. In contrast, the less energetically favored structure **1a**, which would be of lower abundance in the precursor ion beam, gives rise to the $(y_2 + Ca - 2\ H)^-$ ions. Fragmentations of this less abundant isomer undoubtedly contribute to the lower abundances of the $(y_2 + Ca - 2\ H)^-$ product ions relative to the higher abundances of $(y_3 + Ca - 2\ H)^-$ ions in the spectrum (Figure 1A).

The chemistry of larger peptides, such as the hexapeptide AAAPAA shown in Figure 1B, is similar to that for VAAF in that no N-terminal sequence ions are formed. For example, theoretical calculations reveal that complexation between Ca^{2+} and AAAPAA can occur at several sites along the peptide backbone and the presence of Pro does not constrain the binding

to occur only at the C-terminus. Thus, the $(M + Ca - 3\ H)^-$ complex that contains Ca^{2+} bonded to the deprotonated C-terminus and to two adjacent deprotonated amides is of virtually the same energy (25.4 kcal mol^{-1}) as the isomer that contains Ca^{2+} instead bound to the three deprotonated amides closest to the N-terminus (24.9 kcal mol^{-1}). The former isomer could give rise to the $(y_3 + Ca - 2\ H)^-$ and larger fragment ions, whereas the latter isomer could only give rise to the $(y_5 + Ca - 2\ H)^-$ ions.

The fragmentation pattern changes significantly for peptide $(M + Cat^{2+} - 3\ H)^-$ complexes that contain at least one protic amino acid (Figure 2): There now arises one or more $(c_{n-m} + Cat^{2+} - 2\ H)^-$ sequence ions that instead contain the N-terminus and the protic amino acid. Specifically, complexes that contain either Glu (E) (Figure 2A), Asp (D), Tyr (Y) (Figure 2B), His (H) (Figure 2C), or Trp (W) decompose to give N-terminal $(c_{n-m} + Cat^{2+} - 2\ H)^-$ ions, unlike complexes that contain only aprotic (hydrocarbon) amino acid side chains.[16] Complexes that contain Ser or Thr as the protic amino acid give no $(c_{n-m} + Cat^{2+} - 2\ H)^-$ ions but primarily losses of the side chains.[17] Complexes that contain Arg also give abundant product ions that are formed by losses of different parts of the side chain (see the product ions labeled -17, -42, and -60 in Figure 2C). Complexes that contain more than one protic amino acid decompose to give both N-terminal and C-terminal sequence ions and also product ions that arise from internal cleavages.

The protic side chain clearly must play an important role in binding the metal ion to form the precursor complexes and to form the resultant $(c_{n-m} + Cat^{2+} - 2\ H)^-$ ions. Decompositions of 31 complexes that contain only one protic amino acid reveal certain structural requirements for formation of the product ions (Table I). The first obvious requirement, as discussed above, is that either His, Trp, Tyr, Asp, or Glu must be present to provide one site for deprotonation of the precursor complex. Another is that two amides must be available for deprotonation. Experimentally, we find that the two deprotonated amides in the precursor complexes of $(c_{n-m} + Cat^{2+} - 2\ H)^-$ ions can be adjacent to each other and adjacent to the protic amino acid, can be adjacent to each other but not adjacent to the protic amino acid, or can be separated by another amide group in the peptide. Molecular mechanical calculations provide more insight into these experimental data. For example, precursor complexes of AEKAA that contain Ca^{2+} intramolecularly bonded to the deprotonated Glu (E) side chain and to two adjacent deprotonated amides (structure **2**) have the lowest strain energy among the different isomers that can give rise to $(c_3 + Ca - 2\ H)^-$ ions (Table I and Figure 2A). These isomers thus would be abundant in the precursor ion beam. Consequently, most of the $(c_3 + Ca - 2\ H)^-$ product ions would be formed from such complexes. In contrast, $(c_3 + Ca - 2\ H)^-$ ions of HLGLAR (Table I and Figure 2C) are primarily formed from precursor isomers in which the metal ion is bonded to the deprotonated His amino acid side chain and two adjacent distal deprotonated amides (structure **3**). The $(c_4 + Ca - 2\ H)^-$ ions of AEKAA (Figure 2A), however, are primarily formed from complexes that contain the metal ion bonded to the deprotonated Glu (E) side chain, an adjacent deprotonated amide, and a distal deprotonated amide (structure **4**).

The structural requirements for formation of $(c_{n-m} + Cat^{2+} - 2\ H)^-$ product ions shown experimentally in Table I and the calculated structures shown in structures **2–4** indicate that the cleavage reactions reveal the specific location of the metal ion binding site in the peptides: *The cleavage reactions occur in immediate proximity to the metal ion binding site.* Further experimental data, however, can be obtained from evaluations of

Table I. Relative Abundances (%) of $(c_{n-m} + Ca - 2\ H)$ Product Ions from $(M + Ca - 3\ H)^-$ Complexes that Contain Only One of Either Asp, Glu, Tyr, His, or Trp[a]

peptide	c_2	c_3	c_4	c_5
VIHN[b]		1.5		
N-benzoyl-GHL	0.28			
p-OH-*N*-benzoyl-GHL	0.17			
VHLGP[c,d]	0.031	0.10		
VHLGP	0.10	0.13		
HLGLAR		0.15	0.96	0.083
LHGLAR		0.090	1.00	0.11
LGHLAR		0.12	0.85	0.16
KWK[d,e]	1.4			
YGGFL	0.14	1.7		
YGGFL-NH_2	0.15	0.98		
YGGLFK	0.085	0.54	0.67	0.12
YGGFM	0.063	1.1	0.93	
GYGFM	0.038	0.74	0.10	
GGYFM		0.32	0.088	
YPFPG				
YPFAG		0.80		
YAFPG	0.025		0.097	
RRPYIL			0.074	
AAYGPA[c]		0.071		
AYGPAA[c,d]			0.30	
AYGPAA			0.61	
AADVPA		0.083		
ADVPAA			0.91	
ADVAAA[d]		0.21	1.0	0.24
VPDPR				
VEGGK[c,d]		0.49	0.24	
VEGGK	0.054	0.52	0.091	
AEKAA	0.071	0.94	0.29	
KKGE				
VGSE				

[a] The relative abundances are expressed as % relative to the main beam and include the contributions from metastable ion fragmentations. The protic amino acid is shown in boldface. The designation c_{n-m} is shorthand for $(c_{n-m} + Cat^{2+} - 2\ H)^-$. The precursor ions were formed by bombarding the samples with 8-keV Xe atoms at an atom gun current of 2 mA. [b] The mass of the $(c_3 + Ca - 2\ H)^-$ ion is the same as that of the $(z_3 + Ca - 3\ H)^{\bullet-}$ ion. [c] The peptides originally contained Thr or Ser but lost the side chains during FAB desorption to give $(M + Cat^{2+} - 3\ H - 44)^-$ or $(M + Cat^{2+} - 3\ H - 30)^-$ ions, respectively. The structures of these species are the same as those of peptides that contain Gly instead of Thr or Ser, as indicated in the Table.[17] [d] The precursor ions were produced by bombarding the samples with 7-keV Ar atoms at an atom gun current of 2 mA. [e] The mass of the $(c_2 + Ca - 2\ H)^-$ ion is the same as the mass of a product ion from loss of the Trp side chain.

possible mechanisms for formation of the product ions.[11,12] The $(c_{n-m} + Cat^{2+} - 2\ H)^-$ ions arise from cleavages of N$^-$–CHR bonds via transfer of hydrogen from the C-terminal neutral leaving group to the N-terminal ionic product. A hydrogen could be transferred either from (1) a nearby amino acid side chain, (2) a –CH– group of an amino acid residue, (3) a C-terminal carboxylic acid or amide, or (4) an amide. Fragmentations of structurally diverse peptides (Table I) provide some information regarding the source of hydrogen that is transferred in the reactions.

The first possibility, transfer of hydrogen from a proximal side chain, can be eliminated by considering fragmentations of complexes that contain Gly (G, side chain R = H). For example, complexes of VEGGK decompose to give $(c_3 + Cat^{2+} - 2\ H)^-$ ions via cleavage between the two G residues (Table I). This indicates that hydrogen is not transferred from an amino acid side chain directly adjacent to the site of cleavage in the reaction. Furthermore, formation of $(c_2 + Cat^{2+} - 2\ H)^-$ ions from complexes of both VEGGK and VHLGP (cleavage between E and G, and H and L, respectively) indicates that hydrogen is likewise not transferred from an amino acid side chain that is two residues away from the site of cleavage. Thus, hydrogen is transferred from other sources.

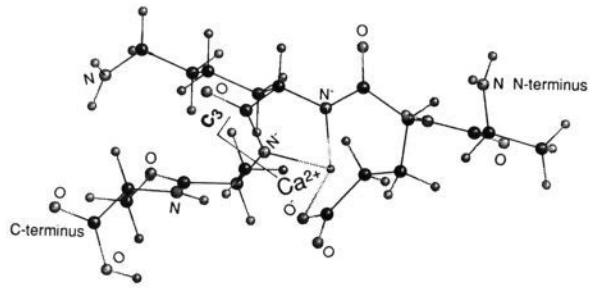
If hydrogen were transferred from the –CH– group of an amino acid residue distant from the site of cleavage toward the

(16) Facile deprotonation of the His side chain is not surprising because $\Delta H°_{(acid)}$ for imidazole is 352.5 kcal mol^{-1}, which is less than that for *N*-methylacetamide (362.3 kcal mol^{-1}) and other amides (Meot-Ner, M. *J. Am. Chem. Soc.* **1988**, *110*, 3071–3075).

(17) Zhao, H.; Reiter, A.; Teesch, L. M.; Adams, J. *Int. J. Mass Spectrom. Ion Processes*, in press.

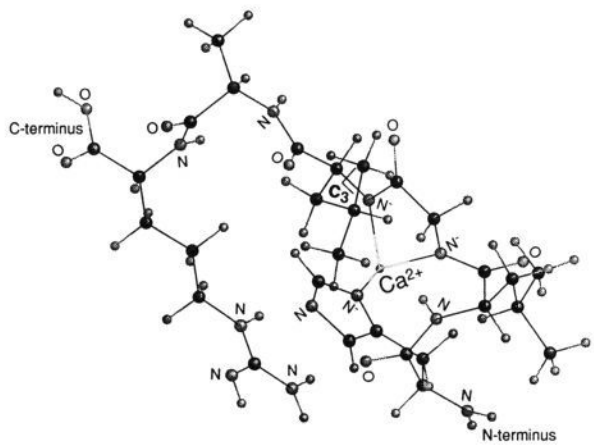
Chart II

AEKAA



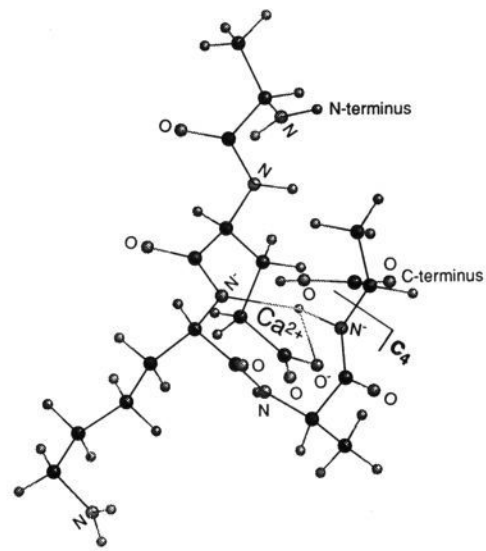
Structure 2 Strain Energy = 13.7 kcal mol^{-1}

HLGLAR



Structure 3 Strain Energy = 37.2 kcal mol^{-1}

AEKAA

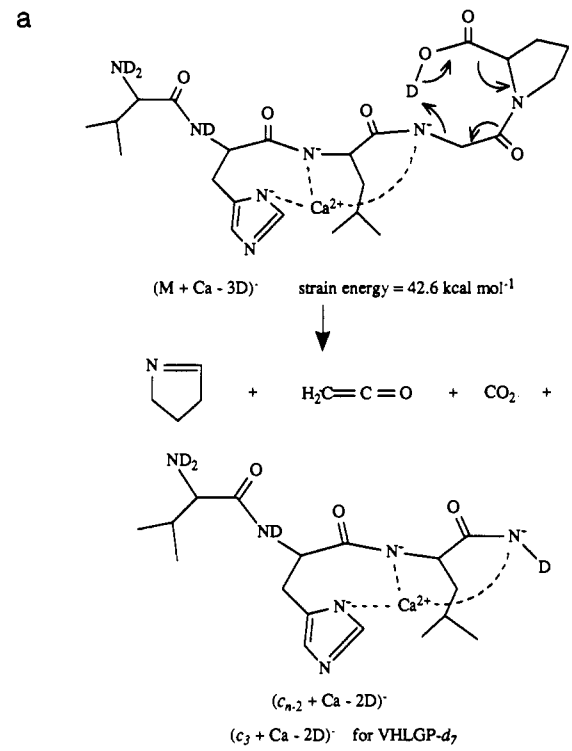


Structure 4 Strain Energy = 11.5 kcal mol^{-1}

C-terminus (the second possibility), formation of product ions would be via a mechanism analogous to that previously proposed[12] for formation of cationic $(c_{n-m} + Cat^{2+})^+$ product ions from $(M + Cat^{2+} - H)^+$ precursor complexes. To test this hypothesis, two peptides YPFAG(d_2)-NH_2 and VEGG(d_2)K were synthesized with deuterium on the Gly residue ($-CD_2-$) at the C-terminus in the former and adjacent to Lys (K) in the latter. Theoretically, complexes of YPFAG(d_2)-NH_2 should decompose to give $(c_3 + Cat^{2+} - 2\ H)^-$ ions that contain one deuteron and are up-shifted in mass by 1 u. The CID spectrum, however, reveals no mass shift for the $(c_3 + Cat^{2+} - 2\ H)^-$ ions. Thus, hydrogen is not transferred from the α carbon of the Gly residue. Furthermore, VEGG(d_2)K complexes give neither $(c_3 + Cat^{2+} - 2\ H)^-$ nor $(c_2 + Cat^{2+} - 2\ H)^-$ ions that contain deuterium. Therefore, $(c_{n-m} + Cat^{2+} - 2\ H)^-$ ions are not formed via a mechanism analogous to that previously proposed.[12] Interestingly, the cationic $(M + Cat^{2+} - H)^-$ complexes of YPFAG(d_2)-NH_2 do decompose as expected and as previously predicted.[12]

The third possibility, hydrogen transfer from a C-terminal –OH or $-NH_2$, can be addressed by using deuterium-labeled peptides formed by deuterium exchange. The peptide VHLGP was chosen because it decomposes to give abundant $(c_3 + Cat^{2+} - 2\ H)^-$ ions, because it contains Pro at the C-terminus, which eliminates the amide NH as a source of hydrogen, and because it does not contain extraneous side chains that might be involved in the exchange reaction. The $(M + Ca - 3\ D)^-$ complexes between VHLGP-d_7 and Ca^{2+} decompose to yield $(c_3 + Ca - 2\ D)^-$ ions that are 4 u above the mass of the nonlabeled ions. Thus, the source of hydrogen that is transferred appears to be the C-terminus. One possible mechanism to account for the data would involve an eight-membered transition state, in which the resulting neutral products are CO_2, ketene, and an imine (Scheme Ia—*the complexes shown in this and all mechanisms are the lowest energy structures from which the product ions can arise*). Another possible mechanism would involve a different eight-membered transition state, but the leaving group would instead

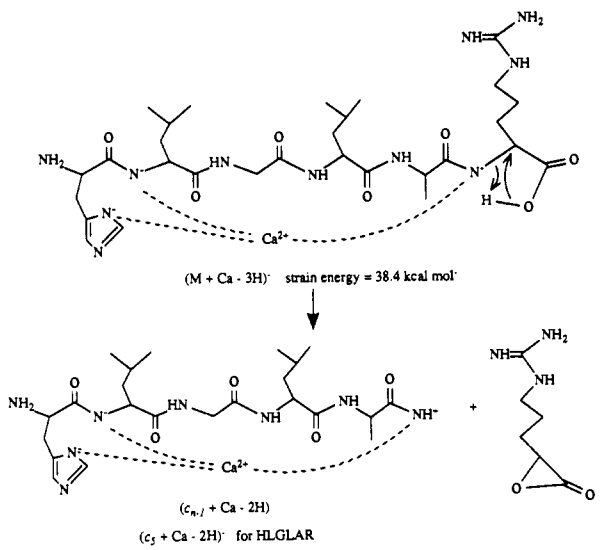
Scheme I



involve a six-membered ring (Scheme Ib). It should be noted that if the C-terminal carboxylate were deprotonated and the deuterium atom were instead located on the second nitrogen from the C-terminus, the carboxylate anion would be involved in metal ion chelation. It is highly unlikely that the $(c_{n-2} + Cat^{2+} - 2\ H)^-$ ions would arise from such a structure because the reaction then would require cleavage of the Ca–O⁻ chelate bond.

Schemes Ia and Ib, however, would only apply to formation of $(c_{n-2} + Cat^{2+} - 2\ H)^-$ ions (cleavage of the N⁻–CH bond of the second amino acid from the C-terminus). Formation of higher mass $(c_{n-1} + Cat^{2+} - 2\ H)^-$ ions could occur via a mechanism similar to that shown in Scheme Ib, differing in that the cyclic leaving group would be a three-membered ring (Scheme II). Unfavorable formation of the relatively unstable neutral product via a five-membered transition state, which would result in low rates of the reactions in the CID time frame, could account for

Scheme II



the low abundances of, or no detectable, $(c_{n-1} + Cat^{2+} - 2\ H)^-$ ions in the spectra of all the peptides studied, except for those in which Pro blocks the more favorable $n - 2$ (Scheme I) cleavage reaction (AAYTPA, AADVPA, and YAFPG in Table I). The unfavorable kinetics would control relative product ion abundances despite possible favored, lower energy precursor ion formation. For example, the precursor isomers that give rise to the $(c_{n-1} + Cat^{2+} - 2\ H)^-$ or $(c_4 + Ca - 2\ H)^-$ ions of AEKAA (Figure 2A and structure **4**) have a strain energy of 11.5 kcal mol^{-1}, which is lower than the 13.7 kcal mol^{-1} energy of the precursor isomers that instead give the $(c_{n-2} + Cat^{2+} - 3\ H)^-$ or $(c_3 + Ca - 2\ H)^-$ ions (Figure 2A and structure **2**). Thus, the precursors of the $(c_4 + Ca - 2\ H)^-$ ions would be formed in higher abundances than the precursors of the $(c_3 + Ca - 2\ H)^-$ ions. We propose, however, that the $(c_4 + Cat^{2+} - 2\ H)^-$ product ions are less abundant than the $(c_3 + Cat^{2+} - 2\ H)^-$ ions (Figure 2A) because the reaction kinetics are less favorable. It is again highly unlikely that the $(c_{n-1} + Cat^{2+} - 2\ H)^-$ ions arise from a structure in which the metal ion is instead bonded to the deprotonated C-terminus because the transition state would then require breaking the chelate Ca–O⁻ bond.

It is possible that the lower mass $(c_{n-m} + Cat^{2+} - 2\ H)^-$ ions, in which $m \geq 3$, are formed via mechanisms analogous to that shown in Scheme I. This is less likely, however, because such mechanisms would involve relatively unfavorable 11-membered or larger transition states. The fact that such ions are formed, and furthermore that such ions are abundant in CID spectra of some of the larger peptides studied, such as the $(c_3 + Ca - 2\ H)^-$ ions ($n = 6$ and $m = 3$) of YGGFLK (Figure 3B), suggests that the mechanism for their formation should have a kinetically favorable transition state. In these cases, hydrogen may be transferred from an amide group (the fourth possibility) that is one (Scheme IIIa) or two (Scheme IIIb) amino acid residues away from the reaction site, as shown for the pentapeptide VHLGP (Scheme IIIa) and the hexapeptide HLGLAR (Scheme IIIb), respectively. It should be noted that the neutral product in Scheme IIIa also involves a three membered ring that would lower the rate of the reaction. This factor may account for the lower relative abundances of the lower mass $(c_{n-m} + Cat^{2+} - 2\ H)^-$ product ions compared to the higher mass ions.

Comparison to Mg^{2+} and Ba^{2+}. The gas-phase ion chemistry of $(M + Cat^{2+} - 3\ H)^-$ complexes that contain Mg^{2+} and Ba^{2+} is similar to that of complexes that contain Ca^{2+} (Figure 3). Previous studies of cationic complexes between peptides and alkaline earth[12] and alkali[11a] metal ions reveal significant changes in fragmentation patterns with changing size of the metal ion. It was hypothesized[11a,12] that the trends reflect specific intramo-

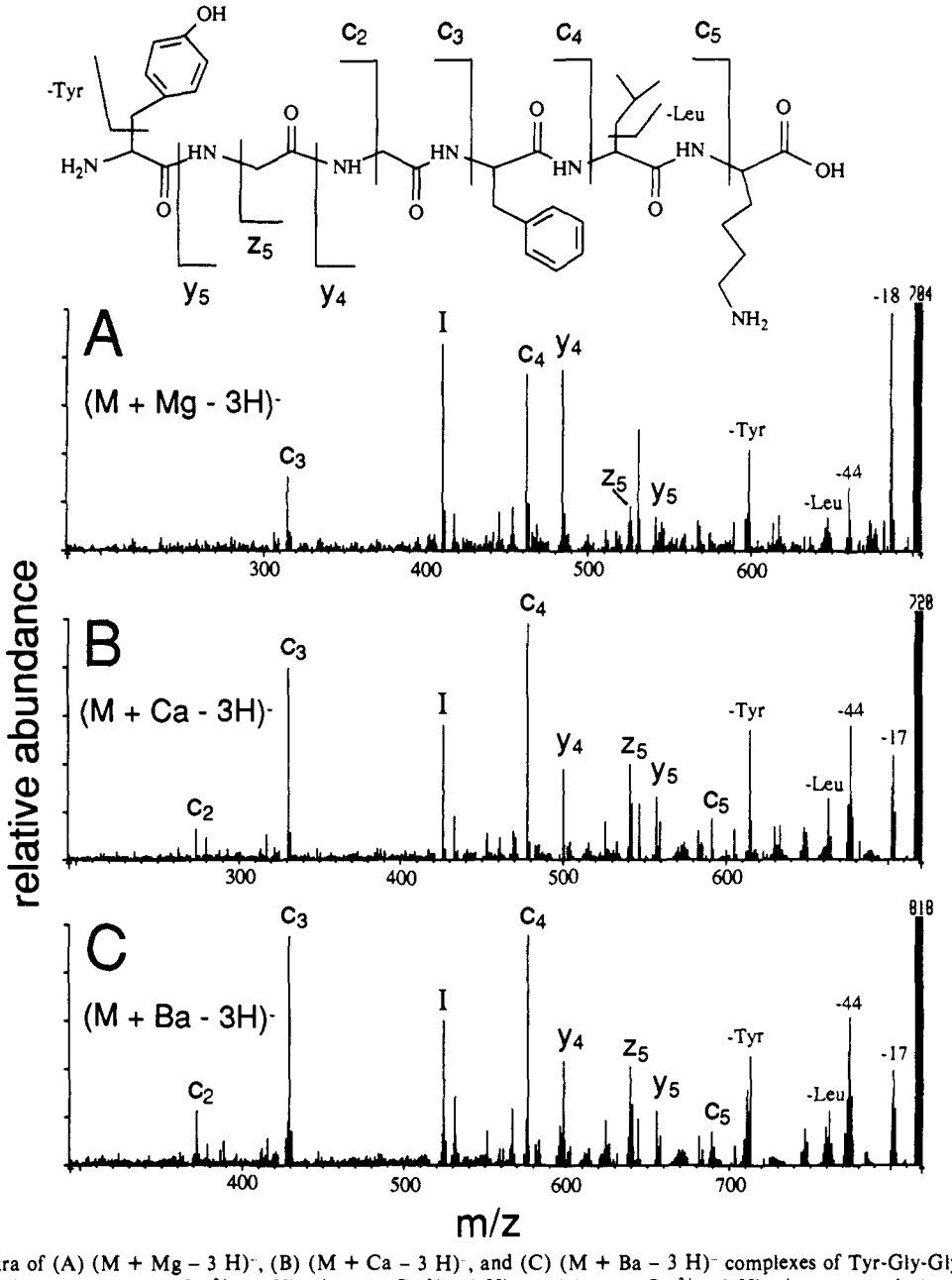


Figure 3. CID spectra of (A) (M + Mg - 3 H)⁻, (B) (M + Ca - 3 H)⁻, and (C) (M + Ba - 3 H)⁻ complexes of Tyr-Gly-Gly-Phe-Leu-Lys. Ions labeled x_{n-m}, y_{n-m}, and z_{n-m} are $(x_{n-m} + Cat^{2+} - 2\ H)^-$, $(y_{n-m} + Cat^{2+} - 2\ H)^-$, and $(z_{n-m} + Cat^{2+} - 3\ H)^{\bullet-}$ ions, respectively. Ions labeled c_{n-m} are $(c_{n\ m} + Cat^{2+} - 2\ H)^-$ ions. Ions labeled -Tyr or -Leu are ions that are formed by loss of the Tyr ($-C_7H_6O$) or the Leu ($-C_4H_9$) side chain, respectively. I represents a fragment ion that appears to be formed by internal cleavages that give losses of both the C- and N-termini. FAB-desorbed ions were produced by bombarding the samples with Xe.

lecular interactions between the metal ion and several sites in the peptide. Unfortunately, CID spectra of cationic complexes cannot provide unequivocal evidence in support of this idea.

Here, changes in the fragmentation pattern also occur with increasing size of the metal ion. Except for some peptides that contain binding sites that are specific for Ca^{2+}, relative abundances of $(c_{n-m} + Cat^{2+} - 2\ H)^-$ ions generally increase with increasing size of the metal ion. Furthermore, the highest mass C-terminal $(y_{n-1} + Cat^{2+} - 2\ H)^-$ ions also generally increase in relative abundance, while lower mass $(y_{n-m} + Cat^{2+} - 2\ H)^-$ ions decrease with increasing size of the metal ion. These trends are shown in Figure 3 and in the relative product ion abundances that are reported in Table II.

At this point, we have insufficient data to draw definitive conclusions regarding the most influential reasons for the changes in relative ion abundances exemplified in Figure 3 and Table II. Preliminary molecular mechanical calculations indicate that the abundances are affected by changes in the relative populations of different fragmenting precursor isomers whose relative energies change with changing size of the metal ion. In addition, however, relative reaction kinetics and competitive shifts should also affect product ion abundances. We do have experimental and theoretical data for complexes that contain Ca-specific side chains, and results for Mg^{2+}, Ca^{2+}, and Ba^{2+} support fragmentations of the most energetically favorable structurally-specific isomers. Discussion of these results will be presented in detail elsewhere.

Comparison to Transition Metal Ions. Further evidence regarding the involvement of structurally-specific isomers in the fragmentation chemistry can be obtained by comparing the chemistry of the alkaline earth metal ion complexes to that of some first transition metal series ions. For example, CID spectra of $(M + Ca^{2+} - 3\ H)^-$, $(M + Co^{2+} - 3\ H)^-$, and $(M + Ni^{2+} - 3\ H)^-$ complexes are significantly different (compare Figure 2B to Figure 4). Full details of the transition metal ion chemistry will be presented later, but the sharp differences in the spectra and results from preliminary molecular mechanical calculations

Scheme III

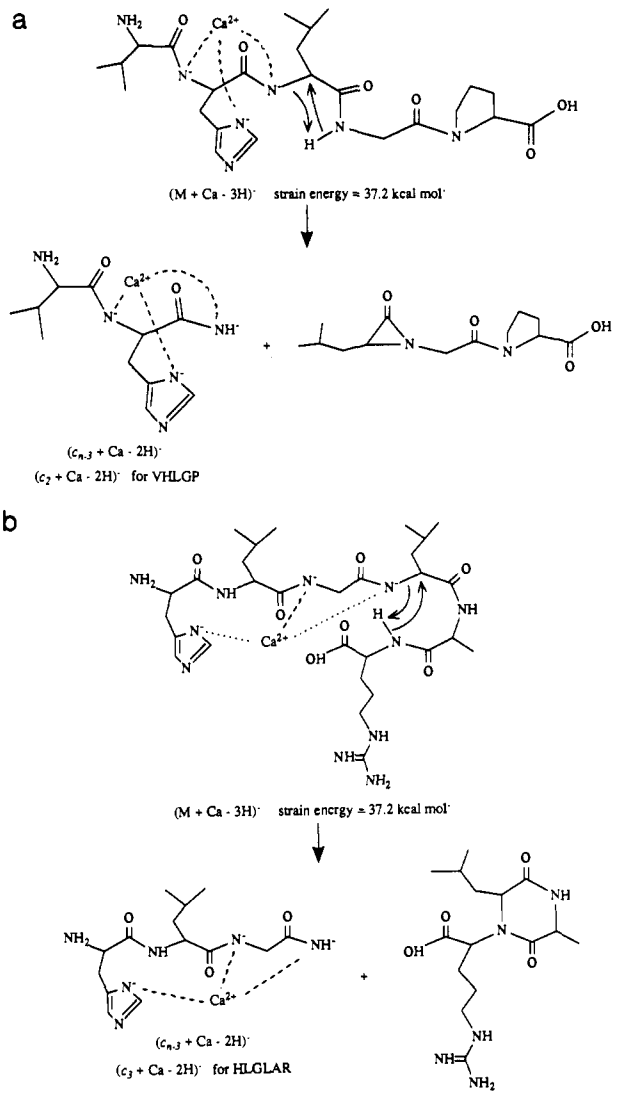


Table II. Relative Abundances (%)[a] of $(c_{n-m} + Cat^{2+} - 2\ H)^-$ and $(y_{n\ m} + Cat^{2+} - 2\ H)^-$ Product Ions in CID Spectra[b] of YGGFLK Complexes

metal ion	c_2[c]	c_3[c]	c_4[c]	c_5[c]	y_4[c]	y^{5}[c]
Mg	0.028	0.19	0.46	ND[d]	0.47	0.091
Ca	0.085	0.54	0.67	0.12	0.26	0.18
Ba	0.20	0.86	0.86	0.12	0.39	0.20

[a] The relative abundances are expressed as % relative to the main beam. [b] The precursor ions were produced by bombarding the samples with 8-keV Xe atoms at an atom gun current of 2 mA. [c] The designation $c_{n\ m}$ is shorthand for $(c_{n\ m} + Cat^{2+} - 2\ H)^-$ and y_{n-m} is shorthand for $(y_{n-m} + Cat^{2+} - 2\ H)^-$. [d] ND = none detected.

indicate clear changes in the binding chemistry. Simple kinetic arguments cannot account for the changes in relative ion abundances of the N-terminal $(c_{n-m} + Cat^{2+} - 2\ H)^-$ ions vs other N-terminal and C-terminal sequence ions. The changes can only arise from significant differences in precursor ion structures and metal ion binding interactions.

Conclusions

Anionic $(M + Cat^{2+} - 3\ H)^-$ complexes between alkaline earth metal ions and peptides that contain aprotic (hydrocarbon) amino acids fragment to give C-terminal sequence ions. Molecular mechanical calculations indicate that the fragmenting precursor isomers preferentially contain the metal ion nonspecifically bound to three deprotonated amides or to two deprotonated amides and the deprotonated C-terminal carboxylate.

In sharp contrast, complexes that contain one protic amino acid (either Asp, Glu, Tyr, His, or Trp) fragment to give abundant N-terminal $(c_{n-m} + Cat^{2+} - 2\ H)^-$ sequence ions. Results from mass spectrometric experiments and from molecular mechanical calculations reveal that formation of these sequence ions requires metal ion binding to a specific deprotonated amino acid side chain and to two amides. Furthermore, the cleavage reactions occur in immediate proximity to the metal ion binding site. This means that formation of the anionic product ions gives precise information about the location of the metal ion in the structurally-specific precursor complexes. The mechanisms for formation of the anionic product ions are not the same as the mechanism previously proposed for formation of analogous cationic $(c_{n-m} + Cat^{2+})^+$ ions in which the metal ion is instead bound nonspecifically to any deprotonated amide in the precursor complex.[12]

The ultimate implication, and application, of our research with peptides and metal ions is to be able eventually to use mass spectrometry for studying some types of binding interactions that occur between metal ions and peptide functional groups in less hydrophilic (more self-solvated) interiors of proteins. Our results with side-chain interactions are of particular relevance because these are the types of interactions expected in the interiors of proteins. Furthermore, the correlation between the molecular mechanical calculations and the gas-phase fragmentation data indicates that the binding interactions observed here are intrinsic, independent of solvation.

Experimental Section

Peptides were obtained either from Sigma or from the Emory University Microchemical Facility (EUMF) and included RYLGYLE and RVYVHPF; HLGLAR, LHGLAR, LGHLAR, ADTHAA, DTHAAA, AAYTPA, AYTPAA, AYGPAA, AADVPA, ADVPAA, ADVAAA, YTGFLT, RYLGYL, RRPYIL, *p*-Glu-LYENK, KEEAE, AAAPAA, and YGGFLK; RYLPT, VHLTP, VHLGP, YPFPG, YPFAG, YAFPG, YGGFH, YGGFM, GYGFM, GGYFM, YGGFL, YGGFL-NH_2, VESSK, VEGGK, VPDPR, AEKAA, RKDVY, RKEVY, FLEEV, FLEEI, FLEEL, FLLEE, and FEELL; VIHN, GPRP, VAAF, KKGE, VGSE, WMDF-NH_2, GGFM, and GGFM-NH_2; GGV, IPI, *N*-benzoyl-GHL, *p*-OH-*N*-benzoyl-GHL, KWK, and MLF. The deuterium-labeled peptides VHLG(d_2)P and VEGG(d_2)K were synthesized by the EUMF.

The matrix used for fast atom bombardment (FAB) was a 2:1 mixture of thioglycerol/glycerol (T/G) saturated with an alkaline earth metal ion hydroxide. The matrix used to obtain the transition metal ion complexes was either T/G or 3-nitrobenzyl alcohol saturated with either $Co(NO_3)_2$ or $Ni(NO_3)_2$. All these materials were obtained from Aldrich.

For the deuterium-exchange experiments, deuterium-labeled VHLGP, T/G, and calcium hydroxide were prepared by dissolving each separately in D_2O and then evaporating the solvent. The procedure was repeated three times. The peptide was then redissolved in D_2O, and 1 μL of this solution was added to deuterium-exchanged T/G saturated with Ca$(OD)_2$ on the FAB probe tip. The cationic and anionic complexes between Ca^{2+} and VHLGP-d_6 and VHLGP-d_7 were the most abundant precursor ions on the FAB full-scan spectra. Both the -d_6 and -d_7 complexes were collisionally activated and their spectra evaluated.

Mass spectrometric experiments were performed by using a VG 70-S, forward-geometry mass spectrometer. Precursor ions were produced by bombarding the sample with 7-keV Ar or 8-keV Xe atoms at an atom gun current of 2 mA. Product ions that were formed by CID in the first field-free region between the ion source and the ESA were observed by using linked scans at a constant ratio of B/E. Helium was used as collision gas (~50% beam reduction). Experiments were performed at a product ion resolution of approximately 1000 (10% valley), and magnet calibration was performed from a mixture of glycerol and CsI. All spectra were acquired by using VG software, and CID spectra are the results of averaging 10–20 scans. Background spectra were acquired for all experiments in order to eliminate artifact ions that might arise from chemical noise.[11] Other experimental details can be found in refs 11 and 12.

Molecular mechanical calculations were performed by usng the program SYBYL, version 5.4 from Tripos Associates, Inc.[18] The strain

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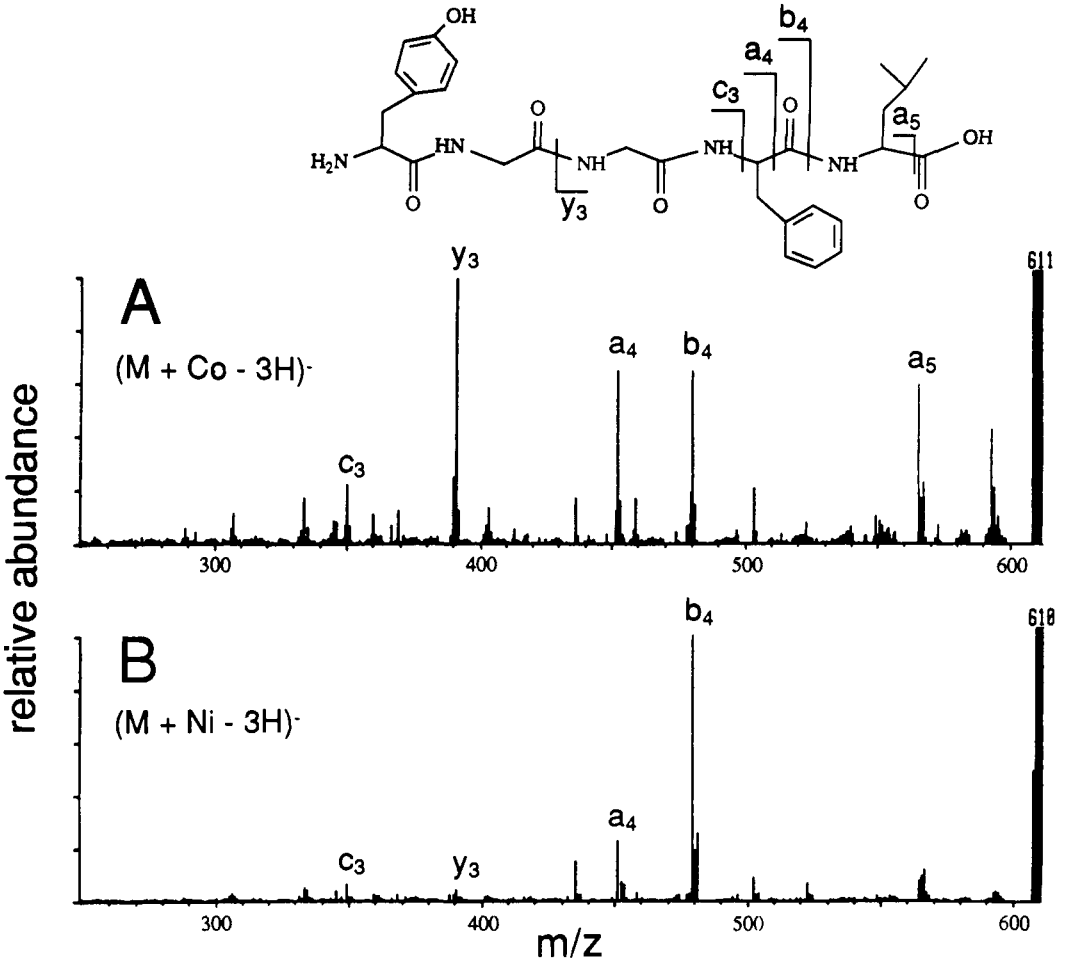

Figure 4. CID spectra of (A) (M + Co − 3 H)$^-$ and (B) (M + Ni − 3 H) complexes of Tyr-Gly-Gly-Phe-Leu. Ions labeled y_{n-m}, c_{n-m}, b_{n-m}, and a_{n-m} are $(y_{n-m} + Me^{2+} - 2\,H)^-$, $(c_{n-m} + Me^{2+} - 2\,H)^-$, $(b_{n-m} + Me^{2+} - 4\,H)^-$, and $(a_{n-m} + Me^{2+} - 4\,H)^-$ ions. FAB-desorbed ions were produced by bombarding the samples with Xe.

energy is described as the summation of the bond length deformation (E_{str}), valence angle deformation (E_{bend}), torsion angle deformation (E_{tors}), out-of-plane deformation (E_{oop}), nonbonded interaction (E_{vdw}), and electron static interaction (E_{ele}), according to eq 1. The force field used

$$E_{total} = \sum E_{strain} + \sum E_{bend} + \sum E_{tors} + \sum E_{oop} + \sum E_{vdr} + \sum E_{ele} \quad (1)$$

to calculate the strain energy was a combination of the parameters described by Raos, Niketic, and Simeon[19] for copper(II) chelation by amino acids and parameters of Tripos. The parameters for the bond length deformation, valence angle deformation, and torsion angle deformation have basis in those described by Simeon and co-workers, whereas the remaining parameters are Tripos parameters. The bond length used for Ca–O is 2.55 Å, an average of the bond lengths reported by Lifson and co-workers[20] for calcium–oxygen interactions. The bond lengths for the rest of the metal-to-oxygen and metal-to-nitrogen bonds were those described by Tripos. This combination of the Simeon and Tripos parameters was used because it gave better agreement with the X-ray crystal structures of the nickel(II) and copper(II) tetraglycine[21] complexes than the parameters of Tripos alone.

The strain energies of the metal ion–peptide complexes were minimized by using the Powell method.[22] To ensure that a global minimum was obtained, the complexes were varied until the strain energies agreed to within 0.5 kcal mol^{-1} after an entire cycle through the complexes. The torsion angles between atoms of the complex backbone and bulky side chains, which are not included in the chelate rings, were varied from 0° to 360° in 60-deg increments, whereas the torsion angles between atoms in the chelate rings were varied from −15° to +15° in 6-deg increments. The variations of the torsion angles were performed sequentially beginning with the C-terminus and ending with the N-terminus, followed by the side chains of the amino acids in the peptides. The structure with the lowest strain energy from the previous set of conformations that were created by varying the torsion angles, as described above, was used as the starting structure for evaluating the next set of conformations.

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