# Strong Interactions of Anionic Peptides and Alkaline Earth Metal Ions: Bis(peptide) Complexes in the Gas Phase

**Peifeng Hu and Michael L. Gross***

*Contributions from the Midwest Center For Mass Spectrometry, Department of Chemistry, University of Nebraska—Lincoln, Lincoln, Nebraska 68588-0304.* *Received March 18, 1992*

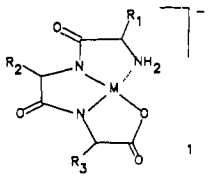
**Abstract:** Small peptides interact with alkaline earth metal ions under conditions of fast atom bombardment to form abundant metal-bis(peptide) complexes of the composition $[2pept + Met^{2+} - 3H^+]^-$. The structure of these gas-phase bis(peptide) complexes, as elucidated by their collisionally activated decompositions (CAD), involves metal ion binding to the deprotonated C-terminal carboxylate groups of both peptides and to a deprotonated amide nitrogen of one of the constituent peptides. Because the C-termini of both peptides in the metal-bis(peptide) complexes are strongly bound to the metal ion, the major product ions from the complex contain a metal ion bound to a doubly deprotonated peptide and to a C-terminal part of the second peptide. These features of the CAD may make these fragmentations useful in sequencing small peptides because there is little complication of fragment ions containing the N-terminus. Other decompositions involve side chain losses to give ions such as z - H, w, and v ions. This chemistry reflects the inherent strong metal bonding in the complex. The z - H ions are observed in the decomposition of metal-bound peptides that contain phenylalanine and alanine. They are not formed in the CAD of any other systems such as those of protonated peptides and metal-cationized peptides.

## Introduction

Protonated peptides[1] and metal-ion-cationized peptides[2] have been the subject of tandem mass spectrometric studies because their spectra are useful for determining peptide sequence and for elucidating intrinsic interactions between the metal ion and the peptide. For peptide sequencing, collisionally activated decompositions (CAD) of protonated peptides have been the first choice because rich sequence information is produced. Alkali-metal-ion-cationized peptides, on the other hand, readily reveal the C-terminal amino acid upon collisional activation (CA) by means of a dominant fragmentation to lose the C-terminal amino acid residue. Similar results pertain to the metastable ion decompositions of certain protonated peptides.[3] Alkali-metal-ion-cationized peptides are also of interest because the precise nature of the structure and bonding in the gas phase is a challenging question and should reflect intrinsic binding. Interpretations on the interactions are thus far divergent.[2c-i]

Alkaline-earth-metal-ion-cationized peptides were recently studied by Adams et al.[4] For these species, the peptide bears one negative charge and the metal ion is doubly positive, giving the complex a net charge of 1+. A wealth of sequence information, like that obtainable from protonated peptides,[1a-c] is produced when these species are submitted to CA. The fragmentation was justified in terms of the hypothesis that alkaline earth metal ions bind preferentially to deprotonated amide functional groups.

In the accompanying study of the gas-phase interaction of alkaline earth metal ions and peptides,[5] we described the formation and fragmentation of an *anionic* complex, $[tripept + Met^{2+} - 3H^+]^-$, in which the constituent amino acids contain neutral side chains and the peptide bears three negative charges. The fragmentation of this anion upon collisional activation can be used to characterize the N-terminus. The structure is one in which the metal ion binds to the tripeptide at the C-terminal carboxylate group and to the two deprotonated amide nitrogens (**1**). The



1

N-terminal amino group is less strongly bound to the metal ion, and consequently the fragmentation is centered near it.

Along with the anionic monomeric complexes of the composition $[pept + Met^{2+} - 3H^+]^-$, alkaline earth metal ions, upon FAB ionization, also form bis(peptide) complexes of the composition $[2pept + Met^{2+} - 3H^+]^-$. We report here on the formation, collisionally activated decompositions, and structure of these gas-phase anionic metal-bis(peptide) complexes in which the peptides are comprised of amino acids having neutral side chains.

## Results and Discussion

**Formation of Metal-Bis(peptide) Complexes.** In addition to the metal-ion-bound peptide,[5] metal-bis(peptide) complexes $[2pept + Met^{2+} - 3H^+]^-$ can also be introduced to the gas phase by fast atom bombardment (FAB) or liquid secondary ion mass spectrometry (LSIMS) (Figure 1). Dipeptides, as we described in the accompanying paper,[5] cannot form abundant metal-bound peptides because the N-terminal amino group does not deprotonate as readily as the amide groups. Metal-bis(dipeptide) complexes, however, are desorbed abundantly into the gas phase upon FAB (e.g., note the $m/z$ 489 ion in FAB ionization of the dipeptide AI in Figure 1A). For tripeptides, the abundance of the metal-bis(peptide) complexes is usually greater than that of the metal-bound peptide $[pept + Met^{2+} - 3H^+]^-$. Compare, for example, the abundance of the $m/z$ 631 [bis(peptide)] and that of the $m/z$ 358 ions (metal-bound peptide), for ALA (Figure 1B). Metal-bis(peptide) complexes are also formed from tetrapeptides (Figure 1C); however, their abundances are significantly lower than those of the metal-bound peptides.

Experiments with $Sr(Ac)_2$ and VGG indicate that metal-bound-peptides and metal-bis(peptide) and tris(peptide) complexes start to desorb from a matrix having concentrations of both $Sr(Ac)_2$ and VGG higher than 0.01 F. The relative abundances of metal-bound peptides and metal-bis(peptide) and metal-tris(peptide) complexes stay relatively constant for a matrix in which the peptide concentration ranges from 0.02 to 1 F and the metal

(1) (a) Biemann, K.; Martin, S. A. *Mass Spectrom. Rev.* **1987**, *6*, 1-76. (b) Biemann, K.; Scoble, H. A. *Science* **1987**, *237*, 992-998. (c) Biemann, K. *Biomed. Environ. Mass Spectrom.* **1988**, *16*, 99-111. (d) Johnson, R. S.; Martin, S. A.; Biemann, K. *Int. J. Mass Spectrom. Ion Proc.* **1988**, *86*, 137-153. (e) Johnson, R. A.; Martin, S. A.; Biemann, K. *Anal. Chem.* **1987**, *59*, 2621-2625. (f) Hunt, D. F.; Yates, J. R., III; Schabanowitz, J.; Winston, S.; Hauer, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 6233-6237.

(2) (a) Russell, D. H.; McGlohon, E. S.; Mallis, L. M. *Anal. Chem.* **1988**, *60*, 1818-1824. (b) Mallis, L. M.; Russell, D. H. *Anal. Chem.* **1986**, *58*, 1076-1080. (c) Renner, D.; Spiteller, G. *Biomed. Environ. Mass Spectrom.* **1988**, *15*, 75-77. (d) Tang, X. J.; Ens, W.; Standing, K. G.; Westmore, J. B. *Anal. Chem.* **1988**, *60*, 1791-1799. (e) Grese, R. P.; Cerny, R. L.; Gross, M. L. *J. Am. Chem. Soc.* **1989**, *111*, 2835-2842. (f) Grese, R. P.; Gross, M. L. *J. Am. Chem. Soc.* **1990**, *112*, 5098-5104. (g) Leary, J. A.; Zhou, Z. R.; Ogden, S. A.; Williams, T. D. *J. Am. Soc. Mass Spectrom.* **1990**, *1*, 473-480. (h) Teesch, L. M.; Adams, J. *J. Am. Chem. Soc.* **1991**, *113*, 812-820. (i) Teesch, L. M.; Orlando, R. C.; Adams, J. *J. Am. Chem. Soc.* **1991**, *113*, 3668-3675.

(3) Thorne, G. C.; Ballard, K. D.; Gaskell, S. J. *J. Am. Soc. Mass Spectrom.* **1990**, *1*, 249-257.

(4) Teesch, L. M.; Adams, J. *J. Am. Chem. Soc.* **1990**, *112*, 4110-4120.

(5) Hu, P. F.; Gross, M. L. *J. Am. Chem. Soc.*, preceding paper in this issue.

0002-7863/92/1514-9161$03.00/0 

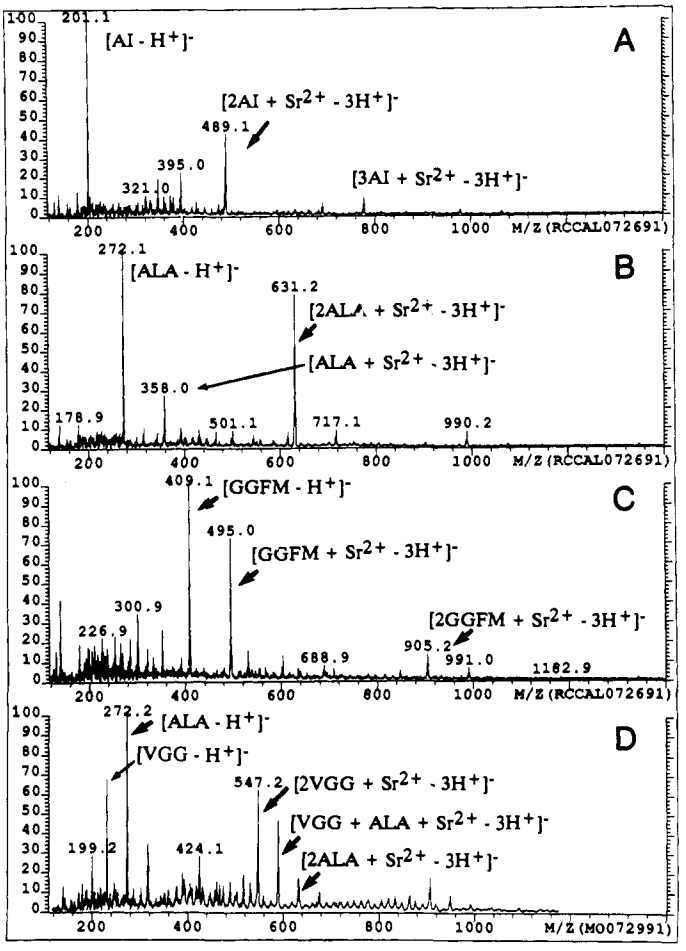



Figure 1. Negative ion FAB spectra of (A) Ala-Ile, (B) Ala-Leu-Ala, (C) Gly-Gly-Phe-Met, and (D) a mixture of Ala-Leu-Ala and Val-Gly-Gly (ca. 1:1). The matrix used for acquiring these spectra was Sr-$(OH)_2$–saturated glycerol/thioglycerol (1:1).

ion to peptide concentration ratio is 1:1. A change in the metal ion to peptide concentration ratio (1:1 to 1:6) does not change the pattern significantly. When the metal ion to peptide concentration ratio is higher than 2:1, the relative abundance of the metal-bound peptide matches that of the metal–bis(peptide) complex. The overall production of metal-ion–peptide complexes is then suppressed, and the background or matrix ions dominate the desorption. These results indicate that the desorption of anionic peptide complexes by FAB is not a pure statistical event. The deprotonation of the peptide, which is the key step in the complex formation, and the stabilities of the complexes control, at least in part, the relative abundance of the metal/peptide complexes. The stable metal–bis(peptide) complex is desorbed more readily than both the metal-bound peptide, in which the peptide is triply deprotonated,[5] and the metal–tris(peptide) complex, in which each of the peptides is likely to be singly deprotonated.

A mixed peptide/metal complex can also be formed from two different peptides. The two peptides form two bis(peptide) complexes (e.g., the $[2VGG + Sr^{2+} - 3H^+]^-$ and $[2ALA + Sr^{2+} - 3H^+]^-$ ions of $m/z$ 547 and 631, respectively), and a mixed peptide/metal complex of two different peptides, $[ALA + VGG + Sr^{2+} - 3H^+]^-$, of $m/z$ 589 (see Figure 1D).

In acquiring FAB spectra of VGG, similar results were acquired when $Ca^{2+}$ and $Ba^{2+}$ were used as the complexing metal ion respectively in the experiment. The metal–bis(peptide) complex is generally the most abundant among the three complexes, whereas the metal-bound peptide and tris(peptide) complexes have similarly low abundances. There is no significant metal dependence.

**Structure of Metal–Bis(peptide) Complexes.** The first observation that gives insight into the structure of metal–bis(peptide) complexes is the contrast in the abundances of metal-bound peptide, formed by loss of the peptides via either CAD or metastable ion fragmentation. The metal-bound peptide is the most abundant ion in the CAD of metal–bis(tripeptide) complexes (Figure 2A and Table I), but it is only moderately abundant in the fragmentation of metal–bis(dipeptide) complexes (Figure 2B and Table II). The abundance of the metal-bound peptide diminishes to an insignificant level in metastable ion fragmentation of both metal–bis(tripeptide) and metal–bis(dipeptide) complexes. Furthermore, the relative abundance of the metal-bound peptide increases with the collision cell pressure. This evidence suggests that the energy requirement for formation of $[pept + Met^{2+} - 3H^+]^-$ is higher than or at the same level as those of fragmentations involving the peptide backbone. The structural implication is that the metal ion bonds coordinate covalently to both constituent peptides and not strongly to one (as in a metal-bound peptide) and weakly to the other. Given a total of three deprotonation sites, one peptide must be doubly deprotonated and the other singly deprotonated.

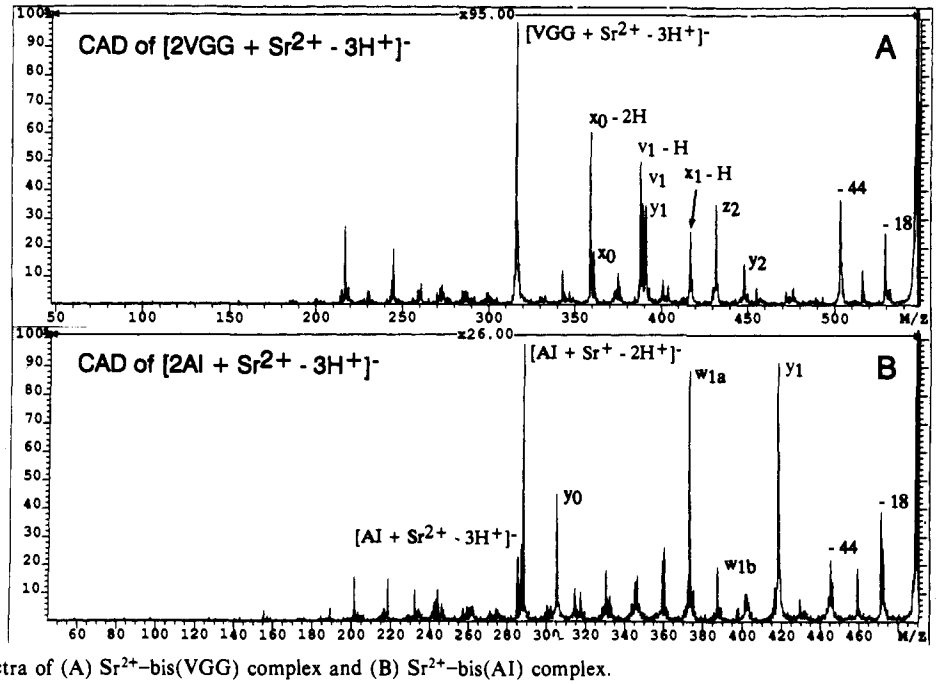



Figure 2. CAD spectra of (A) $Sr^{2+}$–bis(VGG) complex and (B) $Sr^{2+}$–bis(AI) complex.

**Table I.** Major Fragments in the CAD of $Sr^{2+}$–Bis(tripeptide) Complexes

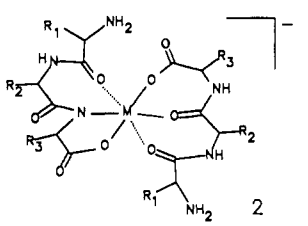
| peptide | MBP[a] | $x_1/x_2$ | $y_1/y_2$ | $z_1/z_2$ | $w_1/w_2$ | $x_0/x_0$-2H | $M^b - 17/18$ | other ions |
|---|---|---|---|---|---|---|---|---|
| AAA | 100 | 5/15 | 50/19 | 78/22 | n/a[c] | 36/43 | nd[d]/25 | $v_1$ (50)<br>$z_1 - H$ (37)<br>$z_2 - H$ (13)<br>404 (36)<br>245 (51) |
| ALA | 100 | 16/5 | 48/6 | 46/37 | nd/31 | 9/38 | 9/10 | M – 44 (44)<br>457 (46) |
| APG | nd | nd/nd | 100/nd | nd/nd | nd/nd | nd/nd | nd | |
| GGV | 100 | nd/nd | 13/nd | nd/nd | 48/nd | 28/49 | nd/nd | $v_1$ (42)<br>$v_1 - H$ (20) |
| GFA | 90 | nd/11 | 25/nd | 100/16 | nd/nd | 27/48 | nd/nd | $v_1$ (26)<br>$z_1 + H$ (35)<br>$z_1 - H$ (38)<br>$z_2 - 1$ (27) |
| GFS | 54 | nd/nd | nd/nd | nd/nd | 24/nd | 9/8 | nd/nd | $z_1 - H$ (21)<br>M – 30 (100)<br>M – 60 (21)<br>MBP – 30 (50)<br>M – 92 (8) |
| GLA | 100 | nd/nd | 31/10 | 14/nd | nd/10 | 8/9 | 4/8 | $z_1 + H$ (15)<br>$z_1 - H$ (15)<br>M – 44 (11)<br>443 (24) |
| GPA | 6 | nd/nd | 100/nd | nd/nd | nd/nd | nd/nd | nd/nd | |
| GSF | 4 | nd/nd | nd/nd | nd/nd | nd/nd | nd/nd | nd/nd | M – 60 (100)<br>M – 30 (64)<br>MBP – 30 (13) |
| MAS | 100 | nd/nd | nd/nd | nd/nd | 47/nd | 21/26 | nd/nd | $z_1 - H$ (47)<br>M – 30 (80)<br>M – 62 (14)<br>MBP – 30 (51) |
| PGG | 51 | 8/nd | 38/23 | nd/13 | nd/nd | 58/24 | nd/16 | MBP – 2H (100)<br>$v_1$ (27)<br>$x_1 - H$ (26)<br>$x_2 + H$ (14)<br>M – 43/42 (28/17)<br>245/244 (42/23)<br>283 (22)<br>217 (22) |
| VGG | 100 | 8/14 | 36/15 | nd/34 | nd/nd | 18/59 | 3/24 | $x_1 - H$ (25)<br>$v_1$ (35)<br>$v_1 - H$ (49)<br>M – 44 (37)<br>217 (28)<br>245 (19) |

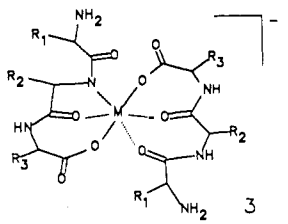
[a] MBP, metal-ion-bound peptide, $[pept + Sr^{2+} - 3H^+]^-$. [b] The M before a minus sign is used to denote the metal-ion-bound peptide. [c] Not applicable. [d] Not detected.

C-Terminal derivatized peptides, such as AAA-$OCH_3$ and PLA-$NH_2$, do not form detectable anionic metal–bis(peptide) complexes, implying that a free C-terminal carboxylate group is essential to the formation of the metal–bis(peptide) complex. In solution, a metal ion needs "a primary ligating site" or "anchor" to chelate a peptide.[6] It is possible that this "anchor" is the C-terminal carboxylate for the formation of the gas-phase metal–peptide complexes studied here. This point will be discussed more fully by us in a forthcoming paper on transition metal/peptide complexes. The production of $y_0$ and $x_0$ ions in the CAD of these bis(peptide) complexes further confirms that the C-terminal COOH group is deprotonated (see later discussions).

For metal–bis(peptide) complexes, structure **2** is the most probable structure. Supporting evidence is in the following observations. The dipeptide PA forms a bis(peptide) complex that fragments like other bis(peptide) complexes containing no proline. The dipeptide AP, however, does not form any detectable anionic bis(peptide) complexes because there is only one acidic site that can be deprotonated (compare the abundances of the $m/z$ 457 ion in Figures 3A and B). AP does form a mixed peptide/metal complex with another peptide that does not have proline at the C-terminus (see Figure 3C). Thus, only one peptide of the pair must have both a free C-terminal carboxylate group and an NH group as part of the adjoining peptide bond. The other peptide need only have a free C-terminal carboxylate group.

For metal–bis(peptide) complexes, the doubly deprotonated peptide has two possible structures differing in the deprotonated peptide nitrogen. One has a deprotonated peptide nitrogen adjoining the C-terminus, and the other has a deprotonated amide adjoining the N-terminus. Both GGP and GPA form metal–bis(peptide) complexes, suggesting that either peptide nitrogen in the deprotonated form of a tripeptide facilitates the formation of a metal–bis(peptide) complex. Therefore, metal–bis(tripeptide) complexes can exist as two structures, **2** and **3**.




The occurrence of water loss from bis(peptide) complexes may indicate that a small population of bis(peptide) complexes has a structure in which the C-terminus is not deprotonated. The possibility cannot be excluded that water loss is a result of structural isomerization to give an intermediate in which the C-terminal COOH is not deprotonated. Isomerization is in accord with the observation that peptides without a C-terminal COOH

(6) Sigel, H.; Martin, R. B. *Chem. Rev.* **1982**, *82*, 385–426.

**Table II.** Major Product Ions in the CAD of $Sr^{2+}$–Bis(dipeptide) Complexes

| peptide | $y_1/z_1$ | $w_1$ | M(I)/M(II)[a] | $M^b - 17/18$ | $y_0$ | $M^b - 44$ | others |
|---|---|---|---|---|---|---|---|
| AG | 100/34 | n/a[c] | 39/13 | 31/24 | nd[d] | 31 | $x_1$ (21) |
| AI | 96/8 | 95(a)<br>18(b) | 100/25 | 27/40 | 42 | 22 | $z_1$ – H (9)<br>M(II) – 2H (20)<br>$x_1$ (14) |
| AL | 46/6 | 100 | 48/16 | 13/14 | 34 | 31 | |
| GA | 100/37 | n/a | 89/13 | nd/68 | 51 | 10 | $x_1$ – H (45)<br>$z_1$ – H (36)<br>274 (39)<br>285 (38) |
| GF | 35/21 | nd | 51/99 | nd/44 | 38 | 36 | $v_1$ (100)<br>$z_1$ – H (16)<br>M – 92 (57)<br>M – 195 (57)<br>M – 179 (39)<br>M – 151 (46) |
| GL | 100/22 | 92 | 51/22 | nd/64 | 31 | 34 | $x_0$ – 2H (18)<br>$x_1$ – H (35)<br>$z_1$ + H (14)<br>$z_1$ – H (9)<br>332 (30) |
| GS | nd/nd | 5 | 4/5 | nd/18 | nd | nd | $w_1$ – 18 (46)<br>M – 30 (100)<br>M – 48 (54)<br>M – 60 (6)<br>M – 78 (15) |
| FM | 10/nd | 33 | 5/nd | 100/nd | nd | nd | M – 92 (10)<br>$z_1$ – H (21) |
| LA | 100/28 | n/a | 28/nd | 8/13 | 15 | 9 | $z_1$ – H (28) |
| LG | 100/44 | nd | 54/11 | 9/13 | nd | 17 | $z_1$ – H (24)<br>$z_1$ + H (11)<br>235 (43)<br>233 (31) |
| PA | 100/4 | n/a | 17/nd | nd/8 | 11 | nd | $x_1$ (7)<br>$z_1$ – H (9)<br>M – 2H (53) |

[a] M(I) and M(II) represent respectively metal(I)-bound peptide and metal(II)-bound peptide. [b] The M before a minus sign is used to denote the metal-ion bound peptide. [c] Not applicable. [d] Not detected.

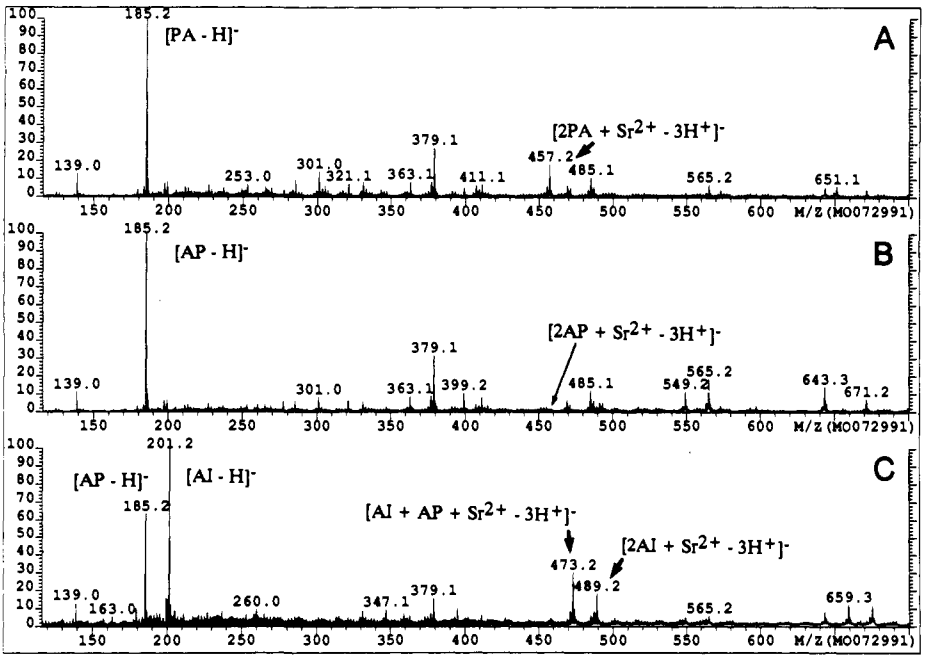



**Figure 3.** Negative ion FAB spectra of (A) Pro-Ala, (B) Ala-Pro, and (C) a mixture of Ala-Ile and Ala-Pro (ca. 1:1) in $Sr(OH)_2$–saturated glycerol/thioglycerol (1:1).

do not form detectable amount of metal–bis(peptide) complexes.

$Ca^{2+}$ is usually involved in a high number of coordinations, 6, 7, and 8.[7,8] Given the availability of binding sites of two di-/tripeptides and the rigidity of the peptide chain induced by the deprotonation of amide groups (the hybridization of amide nitrogens changes in part from $sp^3$ to $sp^2$), the metal in a bis(peptide) complex may have a maximum coordination number of six. Each

(7) (a) Snyder, E. E.; Buoscio, B. W.; Falke, J. J. *Biochemistry* **1990**, *29*, 3937–3943. (b) Strynadka, N. C. J.; James, M. N. G. *Annu. Rev. Biochem.* **1989**, *58*, 951–998.

(8) Dayan, I.; Libman, J.; Shanzer, A.; Felder, C. E.; Lifson, S. *J. Am. Chem. Soc.* **1991**, *113*, 3431–3439.

peptide contributes three binding sites. We, however, do not have evidence for the exact coordination number.

With regard to the spatial arrangement of the ligands of metal–bis(peptide) complexes, precedent can be found in an investigation of the structure of $Ca^{2+}$–tris(peptide) complexes.[8] The peptides are so-called tripode ligands and have three arms, each of which donates two ion binding sites. The strain imposed by the ligand's stem affects the geometry of the metal complexes. Ligands that have a long stem and, therefore, more conformational freedom form octahedral-type complexes. Shorter ligands, which have less conformational freedom, form prismatic or distorted $Ca^{2+}$ complexes. For gas-phase, alkaline-earth-metal–bis(peptide) complexes, each peptide contributes three bonding sites to the metal ion, leaving little ligand conformational freedom. An octahedral geometry can be achieved only if the portions of the peptides that contain the binding sites are held planar and the two peptide planes are perpendicular to each other. This requirement for an octahedral geometry cannot be satisfied on the basis of a total deprotonation number of three for the two peptides. Thus, the peptides in bis(peptide) complexes may be regarded as analogous to the shorter ligand tris(peptides), and the geometry of the gas-phase bis(peptide) complexes may also be prismatic or distorted. Alkaline earth metal ions have inherently high flexibility for adopting different ligation geometries.[8] The formation of abundant metal–bis(peptide) complexes and their fragmentation may be a result of the coordination geometry plasticity of alkaline earth metal ions because, by way of comparison, dipositively charged transition metal ions interact with peptides differently, as will be discussed in a forthcoming paper.[9]

In summary, the bonding interaction of alkaline earth metal ions and deprotonated amide groups has not been characterized. On the basis of the limited precedent, we propose three rules for describing the structure of metal–bis(peptide) complexes. (1) A deprotonated amide group binds to metal ions via the amide nitrogen, which is well-established in solid-phase chemistry and in solution chemistry.[10] That the amide groups in a peptide never exist as the alternative form in which the amide hydrogen resides on the oxygen supports this idea. (2) The carbonyl oxygen of a neutral amide group is favored for metal binding because it is more basic than the amide nitrogen.[6,8,10] (3) Because $Ca^{2+}$ usually has a high coordination number in binding as described above, a coordination number of six is proposed for the metal ion in bis(peptide) complexes.

**Collisionally Activated Decomposition.** Upon collisional activation, metal–bis(peptide) complexes decompose extensively (Figure 2 and Tables I and II). A common product is formed by the loss of an intact peptide molecule, and its abundance varies with peptide size. The mechanism for its formation is discussed later.

In contrast to the CAD of positively charged alkaline-earth-metal-cationized peptides[4] and protonated peptides,[1] which produce both N-terminal and C-terminal fragment ions, alkaline-earth-metal–bis(peptide) complexes produce predominantly upon CA ions possessing the C-terminus (i.e., x, y, and z ions; see Figure 2 and Tables I and II).[11] The y ion series is usually complete and can be used for peptide sequencing, at least for the simple peptides studied here. One advantage of using metal–bis(peptide) complexes is the simplicity brought about by the formation of only C-terminal sequence ions. A similar advantage also applies to the fragmentation of $[M + H]^+$ ions of peptides that have a basic amino acid located at either termini.[1a-c] Another way to induce this kind of behavior is to derivatize the peptide by introducing an ionic group at one of the termini,[1d] a procedure requiring time and sample. The general applicability to peptide sequencing remains to be established and will be addressed in future studies.

A hallmark of the fragmentation of metal–bis(peptide) complexes is the capability for forming fragments, such as w, z – H, and v ions, by high energy processes. The w ions and v ions are also observed in the high energy CAD of protonated peptides and can be applied to the differentiation of leucine and isoleucine.[1d,e] The w ion is one of the most abundant fragments from metal–bis(peptide) complexes provided the peptide contains an amino acid residue that supports its formation and that is not located at the N-terminus. Examples of w ions are the $w_2$ ion in the fragmentation of $Sr^{2+}$–bis(ALA) complexes (Table I) and the $w_1$ ion in the fragmentation of $Sr^{2+}$–bis(GL) complex (Table II). If the peptide in the complex contains amino acids that are able to form two w ions, usually both of them are produced (e.g., the $[2AI + Sr^{2+} - 3H^+]^-$ ion forms $w_{1a}$ and $w_{1b}$, see Figure 2B). The $v_1$ ion is the most abundant in the CAD of $Sr^{2+}$–bis(GF) complex. In the CAD of $Sr^{2+}$–bis(VGG) complex there is also a $v_1 - 1$ ion whose mechanism of formation and structure are not yet understood.

A common fragment ion in the CAD and metastable ion fragmentation of metal–bis(peptide) complexes is produced by the loss of 44 mass units (apparently $CO_2$). Like the product formed by water loss, it likely involves the decomposition of the C-terminus.

Certain side chains of amino acids affect the fragmentation of the metal–bis(peptide) complexes. For a $Sr^{2+}$–bis(GSF) complex, the loss of 60 mass units (apparently two molecules of $CH_2O$, one from each serine) gives the most abundant product (Table I). The ion from the loss of one serine side chain is the second most abundant ion, whereas that from the loss of an intact peptide (i.e., $Sr^{2+}$-bound GSF) is only a minor ion. The ion that is 30 mass units lower than the mass of the metal-bound peptide is even more abundant than the metal-bound peptide itself. The $Sr^{2+}$–bis(GFS) complex, however, expels a single $CH_2O$ most readily, and the losses of $2(CH_2O)$ form the second most abundant ion (Table I). It appears that the central serine residue is more vulnerable to fragmentation than the one at the C-terminus. The mechanisms for side chain losses from bis(peptide) complexes should be similar to those proposed in the accompanying paper[5] for metal-bound peptides.

A $Sr^{2+}$–bis(MAS) complex shows a very similar fragmentation pattern to that of a $Sr^{2+}$–bis(GFS) complex (Table I). The loss of $CH_2O$ is again the most facile fragmentation for the $Sr^{2+}$–bis(GS) complex (Table II). The easy fragmentation of the serine side chain is consistent with the strong metal–peptide bonding, which makes the peptide chain relatively resistant to cleavage. The presence of serine also affects the deprotonated dipeptide fragmentation, as was reported recently.[12] If serine is the C-terminal amino acid, the fragmentation is dominated by the loss of $CH_2O$.

The unimolecular decompositions of metal–bis(peptide) complexes show similar features to CAD spectra described above, except that the ions of high energy processes, such as w, v, and metal-bound peptide are not formed.

**Loss of a Peptide.** The formation of metal-bound peptides from bis(peptide) complexes requires that one proton be transferred from the already doubly deprotonated peptide to the singly deprotonated peptide so that an intact peptide molecule can be eliminated. If the transferred proton is from the second amide NH of the doubly deprotonated peptide in structure **2**, the structure of the product ion should be identical to that of the source-produced, metal-bound peptide whose structure (**1**) was

(9) In a forthcoming paper, the collisionally induced fragmentations of transition-metal-ion-bound peptides and bis(peptide) complexes will be discussed in comparison to those of alkaline earth metal ions. Hu, P.; Gross, M. L., submitted for publication.

(10) Freeman, H. C. In *Advances in Protein Chemistry*; Anfinsen, C. B.; Anson, M. L.; Edsall, J. T.; Richards, F. M., Eds.; Academic Press: New York, 1967; Vol. 22, p 342.

(11) Roepstorff, P.; Fohlman, J. *Biomed. Mass Spectrom.* **1984**, *11*, 601. The convention used here to denote fragment ions is the same as that proposed by Roepstorff and Fohlman and modified by Biemann and Martin.[1a] Small letters are used to denote the fragmentation sites, and the subscript number specifies the number of amino acid residues. The number and the sign that follow a letter indicate the net number and the direction of hydrogen transfer, respectively. All these fragment ions contain a doubly deprotonated second peptide, and for convenience of notation this is ignored in the code [i.e., the metal ion and doubly deprotonated peptide are considered to be equivalent to the proton in $(M + H)^+$ ions].

(12) Waugh, R. J.; Eckersley, M.; Bowie, J. H.; Hayes, R. N. *Int. J. Mass Spectrom. Ion Proc.* **1990**, *98*, 135–145.

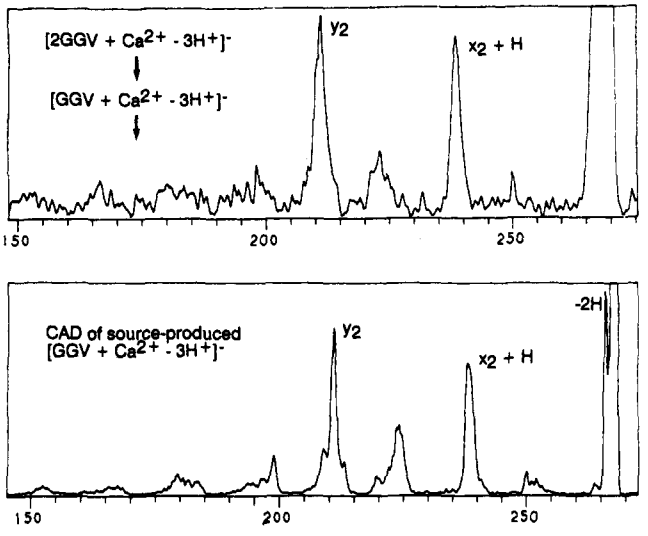


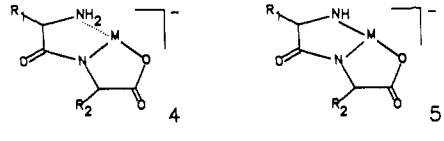

Figure 4. (A)CAD spectrum of $Ca^{2+}$-bound GGV produced from $Ca^{2+}$–bis(GGV) complex in the third field-free region of Kratos MS-50 instrument.[13] (B)CAD spectrum of source-produced $Ca^{2+}$-bound GGV.

reported in the accompanying paper.[5] Collisional activation of the $Ca^{2+}$-bound GGV, formed from the collisionally activated $Ca^{2+}$–bis(GGV) complex (conducted by an MS/MS/MS experiment[13]), gives a nearly identical CAD spectrum to that of source-produced $Ca^{2+}$-bound GGV (Figure 4). The $x_2$ + H and $y_2$ ions are the major fragments, and the ion formed by a loss of 44 mass units is of comparable abundance to that produced from the authentic $Ca^{2+}$-bound GGV. Therefore, the structures of source-produced [pept + $Met^{2+}$ – $3H^+$]$^-$ ion and the corresponding product of the bis(peptide) complex are the same.

Additional support includes the results of collisional activation of metal-bound peptides with proline as the central amino acid. APG and GPA form the metal–bis(peptide) complexes with structure **2** as expected, but the bis(peptide) complexes do not give metal-bound APG and GPA upon CA. Instead, the $y_1$ ion is the only dominant ion formed upon CA. Because peptides with proline as the central amino acid do not have a second amide hydrogen, the metal–bis(peptide) complexes of APG or GPA cannot undergo the process to form the required structure of an anionic metal-bound peptide. On the basis of this evidence, a mechanism for the formation of the [pept + $Met^{2+}$ – $3H^+$]$^-$ ion from structure **2** is proposed in Scheme I.

The mechanism can also apply to structure **3**, except that the hydrogen transferred is from the amide group adjoining the C-terminus. The nature of the hydrogen transfer from the amide to the C-terminal $COO^-$ of the singly deprotonated peptide is the same as that in Scheme I, and the products are also identical.

**Change of the Oxidation State of the Metal Ion.** The metal–carboxylate oxygen bond of the singly deprotonated peptide can be cleaved homolytically or heterolytically. The likely consequence of the homolytic cleavage is the reduction of the metal ion from II to I and concurrent formation of a metal(I)-bound peptide [pept + $Met^+$ – $2H^+$]$^-$ (structure **4**), whose molecular weight is 1 mass unit higher than that of the metal(II)-bound peptide (**5**, usually designated as metal-bound peptide in this paper). Most noticeably, the reduction of the metal occurs in the fragmentation of metal–bis(dipeptide) complexes. The relative abundance of the metal(II)- with respect to the metal(I)-bound peptide for the dipeptide complexes studied here ranges from 20 to 40% (e.g., for AI, 31%; for GL, 41%; for LG, 20%; for AL, 42%; for AG, 30%). The GF complex, however, shows a reversal in the relative abundances (Table II). The interaction of the metal ion and the phenylalanine side chain may stabilize the product of the heterolytic reaction and, in so doing, decrease the energy of the transition structure for the reaction.



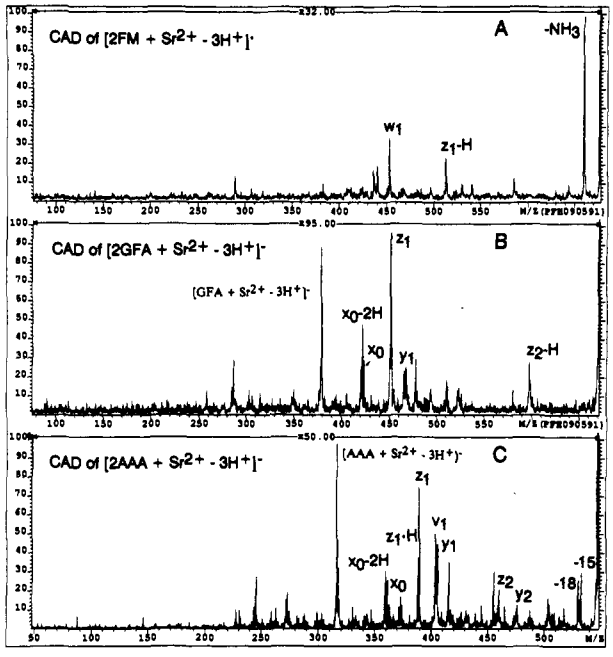
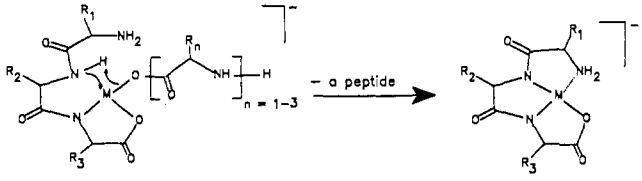



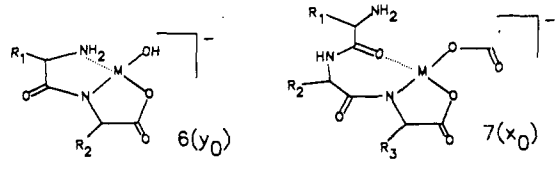

Figure 5. CAD spectra of (A) the [2FM + $Sr^{2+}$ – $3H^+$]$^-$ ion, (B) the [2GFA + $Sr^{2+}$ – $3H^+$]$^-$ ion, and (C) the [2AAA + $Sr^{2+}$ – $3H^+$]$^-$ ion.

Scheme I




For metal–bis(tripeptide) complexes, the homolytic cleavage and accompanying reduction of the metal ion occur less readily upon CA than the heterolytic cleavage. The Sr(I)-bound tripeptides are usually minor ions (less than 10% with respect to the abundances of the Sr(II)-bound tripeptides). The product stability apparently affects the relative competitiveness of the heterolytic and homolytic cleavage. For metal–bis(tripeptide) complexes, the product of the heterolytic cleavage of the metal–oxygen bond accompanied by proton transfer is a stable metal-bound tripeptide.[5] This pathway, therefore, dominates. For metal–bis(dipeptide) complexes, the corresponding product is a less stable metal-bound dipeptide. The homolytic cleavage now dominates, and the metal(I)-bound peptide is formed more readily.

**Cleavages at the C-Terminus of the Singly Deprotonated Peptide.** Cleavage of the C-terminal CO–O bond of the singly deprotonated peptide of the metal-bound peptides accompanied by hydrogen transfer also occurs upon CA to form a product ion that may have structure **6** (according to the nomenclature used in this paper, this ion can be denoted as $y_0$ to indicate the fragmentation site; see the note in ref 11). The formation process for **6** may be analogous to that for the y ions. These latter ions are equivalent to the y + 2 ions formed in the CAD of protonated peptides. To produce each, one rearrangement is required (see the note in ref 11).




(13) Burinsky, D. J.; Cooks, R. G.; Chess, E. K.; Gross, M. L. *Anal. Chem.* **1982**, *54*, 295–299.

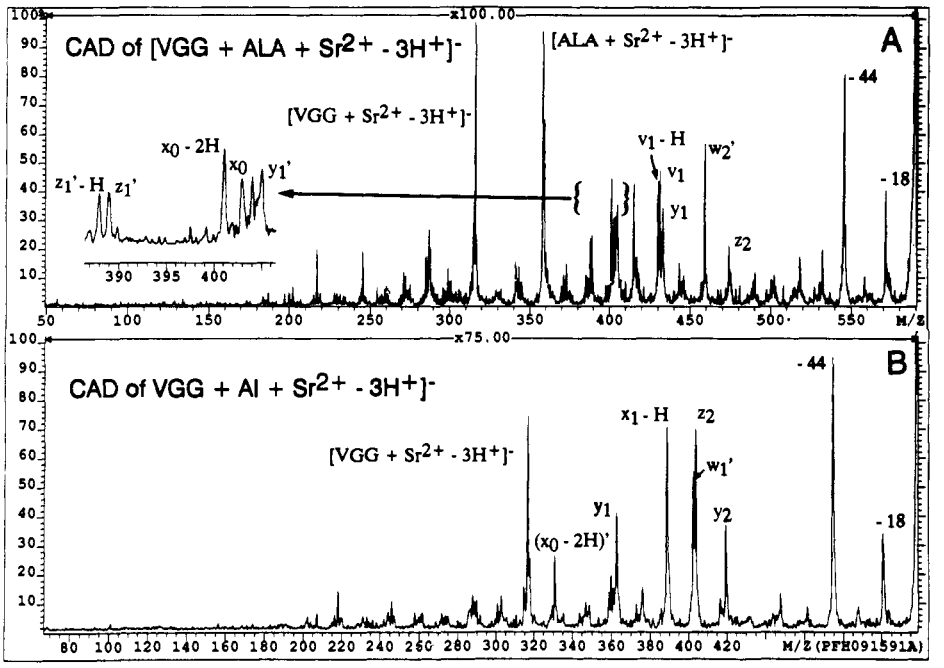



**Figure 6.** CAD spectra of mixed peptide–metal complexes of (A) Ala-Leu-Ala and Vgg-Gly-Gly and (B) Ala-Ile and Val-Gly-Gly. A (') in spectrum A indicates that the ion is formed from the fragmentation of Ala-Leu-Ala chain and in spectrum B indicates that the ion is formed from the Ala-Leu chain.

Simple cleavage of the C-terminal Cα–CO bond gives an ion of structure **7**, which is denoted as $x_0$ in accord with the nomenclature. This ion is also a radical ion and contains a double deprotonated peptide. An $x_0 - 2H$ ion is also produced (see Figure 2 and Tables I and II). To form the latter, the doubly deprotonated peptide must be dehydrogenated, which may occur at either the Cα–Cβ bond of the side chains or the Cα–NH (amine or amide) bond as for the dehydrogenation of metal-bound tripeptides.[5]

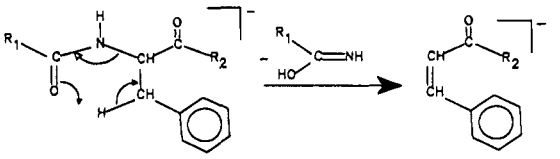
Interestingly, $x_0$ is usually less abundant than the $x_0 - 2H$ ion. The $y_0$ ion is generally prominent in the CAD of metal–bis(dipeptide) complexes, but it is insignificant in those of the bis(tripeptide) complexes. On the contrary, the $x_0$ and $x_0 - 2H$ ions are usually formed abundantly in the decompositions of metal–bis(tripeptide) complexes but are less significant in those of the bis(dipeptide) complexes.

The formation of $y_0$, $x_0$, and $x_0 - 2H$ ions, discussed above, is unique to metal–bis(peptide) complexes, owing to the presence of a second peptide. Their formation further underscores the strong metal binding of the peptides and is consistent with the structure proposed for metal–bis(peptide) complexes.

**Formation of z – H Ions.** The CAD of metal–bis(peptide) complexes of peptides that contain phenylalanine show abundant z – H ions (Figure 5). If a peptide has an N-terminal phenylalanine, the loss of $NH_3$ dominates (Figure 5A). When phenylalanine is the central amino acid of a tripeptide, a $z_2 - H$ ion forms (Figure 5B). Ions of the z – H type are not observed in the fragmentation of protonated peptides[1] and metal-ion-cationized peptides.[2,4] The formation from metal–bis(peptide) complexes may be the result of a charge-remote elimination[14] (see Scheme II). The structure of the product ion has an enlarged π-system, extending to the benzene ring of the phenylalanine residue and giving stability to the product ion as well as reducing the energy of the transition structure for the reaction.

Alanine in peptides similarly promotes the formation of z – H ions (e.g., in the fragmentation of the metal–bis(peptide) complexes of Gly-Ala, Ala-Leu-Ala, Gly-Leu-Ala, etc.; see Tables I and II) except when alanine is at the N-terminus. The mechanism for z – H ion formation from phenylalanine-containing peptides can be applied here. The double bond is again conjugated to the amide carbonyl, stabilizing the product ion.

**Scheme II**[a]



[a] $R_2$ represents the C-terminal part of the peptide which is bound via the metal ion to a dinegatively charged second peptide.

The structural requirements for formation of z – H ions are similar to those for w ions; both ions result from 1,2-elimination-type fragmentation. An alkyl group is eliminated in w ion formation, whereas a hydrogen is eliminated in z – H ion formation. The z – H ion can be produced from both alanine- and phenylalanine-containing peptides. Alanine has no substitution on the side chain to enable w ion formation, whereas phenylalanine does. The competitive formation of z – H ion may be explained by considering the mechanism for w ion formation. As shown by Biemann and co-workers,[1e] w ions are formed via z + H ions, which are distonic ions. It is the radical located at the α-carbon of the now N-terminal amino acid in the z + H ion that induces the cleavage of part of the side chain leading to the w ion formation. If a w ion is to be formed from a phenylalanine residue, the bond to be cleaved would be vinylic, which is not favored.[15] Thus the formation of z – H ion by way of elimination of a H from the β-carbon is more favored. For peptides that contain nonaromatic side chains, the w ion formation is more favorable.

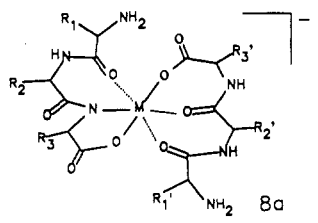
Enhanced $z_n$ ion production is also observed in the CAD of some metal–bis(peptide) complexes that have an alanine residue as the *n*th amino acid (Figure 4C and Table II). An understanding of the dependence of the abundance of $z_n$ ion on the structural features requires further study.

**Metal Complexes of Two Different Peptides.** As is expected from the structure of the metal–bis(peptide) complexes, two different peptides should also form a mixed peptide–metal complex. When a tripeptide is doubly deprotonated, it can exist as two structures in which the sites of deprotonation are different.

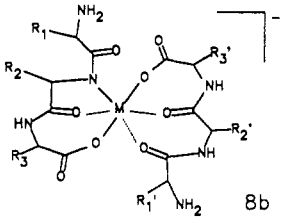
(14) Jensen, N. J.; Tomer, K. B.; Gross, M. L. *J. Am. Chem. Soc.* **1985**, *107*, 1863–1868.

(15) (a) McLafferty, F. W. *Interpretation of Mass Spectra*, 3rd Ed.; University Science Books: Mill Valley, California, 1980; pp 179–180. (b) Hu, P. F.; Gross, M. L.; Yuan, S. Q.; Wei, T. T.; Lu, Y. Q. *Org. Mass Spectrom.* **1992**, *27*, 99–104.

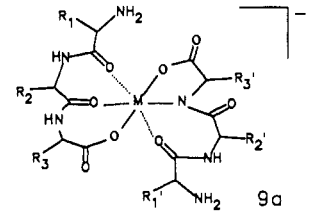
When this peptide and another that is singly deprotonated form a complex with a metal, two structures of the complex are produced. If the charge roles of the two peptides are reversed (i.e., the doubly deprotonated peptide becomes singly deprotonated and the one bearing a 1-charge becomes 2-), then two more structures are possible. These four structures are pictured as **8a**, **8b**, **9a**, and **9b**). The CAD spectrum of [ALA + VGG + $Sr^{2+}$ - $3H^+$]$^-$



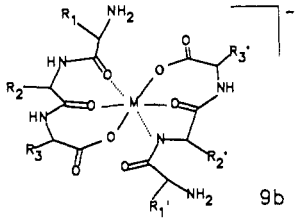
8a



8b



9a



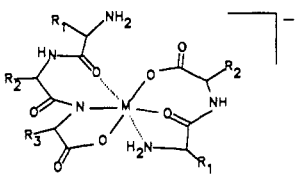
9b

illustrates the point: in one pair of structures $Sr^{2+}$ binds to doubly deprotonated VGG and singly deprotonated ALA, and in the other pair the metal binds to singly deprotonated VGG and doubly deprotonated ALA (see Figure 6A). Both $Sr^{2+}$-bound ALA, formed by the loss of a VGG molecule, and $Sr^{2+}$-bound VGG, formed by the loss of an ALA molecule, are produced in comparable abundances. The other fragment ions are produced in the decompositions of $Sr^{2+}$-bis(VGG) and $Sr^{2+}$-bis(ALA) complexes. When a dipeptide is doubly deprotonated, only one form exists and only two different structures of a mixed dipeptide–metal complex can form.

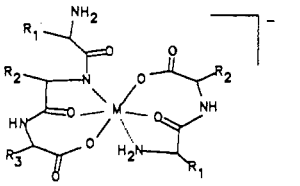
The collisional activation of mixed peptide–metal complexes provides an estimate of the relative affinities of peptides for the metal ion. The relative population of the two structures of the mixed peptide–metal complexes is reflected in part by the relative abundances of the two product metal-bound peptides, if we can assume that each of the two metal-bound peptide products is formed from a precursor in which that peptide is doubly deprotonated. The relative populations in turn are determined by the ease of deprotonating the peptide and by the metal ion affinity of the doubly deprotonated peptide. One difficulty is that the fragmentation propensities of the two structures are likely to be different. Thus, metal ion affinities from this approach are at best an estimate.

Peptides of different size also form mixed peptide–metal complexes. A mixed peptide–metal complex consisting of a tripeptide and a dipeptide has three structures (**10a**, **10b**, and **11**). The fragmentations of these complexes, however, are expected to have different constraints than those of bis(peptide) complexes formed from peptides of the same size. The metal-bound tripeptide product ion will usually be more abundant than the dipeptide counterpart simply because tripeptides are more readily triply deprotonated than are dipeptides (see Figure 6B).

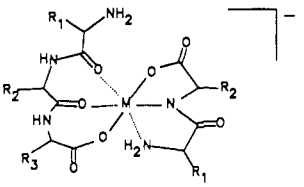
Fragments from both peptide chains are observed for mixed peptide–metal complexes (compare, for example, the CAD spectra of metal–bis(AI) and metal–bis(VGG) complexes in Figure 2 and those of the mixed peptide–metal complexes of the two peptides in Figure 6). The relative abundances of the fragments from each peptide chain depend on the nature of the two peptide chains. The VGG chain product ions are more abundant than those of the AI chain (see Figure 5). Some products are enhanced and others are suppressed (e.g., the product formed by loss of $CO_2$ is not abundantly produced from $Sr^{2+}$-bis(AI) complex or from $Sr^{2+}$-bis(VGG) complex, but it is the most abundant fragment from the mixed peptide/metal complex). The $y_1$ ion of the AI



10a



10b



11

chain fragmentation is only weakly formed upon collisional activation of the $Sr^{2+}$ complex of AI and VGG, whereas it is one of the most abundant ions from the bis(AI) complex.

## Conclusion

Alkaline-earth-metal–bis(di- and tripeptide) complexes can be readily introduced into the gas phase by FAB or LSIMS. The metal ion binds to both a doubly deprotonated and a singly deprotonated peptide in these complexes. The deprotonation sites include both C-terminal carboxyl groups and an amide group of one peptide. The bonding between the metal ion and both peptides is sufficiently strong that collisional activation of this complex produces both extensive peptide chain and side chain fragments.

The structure of the metal–bis(peptide) complex dictates the fragmentation processes. Because the C-termini of the peptides are tightly bound by the metal ion and the charge is confined to the C-terminus region, most of the fragmentation processes occur away from the C-terminus and may be charge-remote in nature.[14] Major fragment ions comprise a doubly deprotonated peptide and the C-terminal part of the second peptide. Furthermore, high energy fragments, such as w ions, z - H ions, and v ions, are also produced. The z - H ions are observed for the first time.

Although most of the results presented in this paper are for $Sr^{2+}$ complexes, similar results were obtained for $Ca^{2+}$ and $Ba^{2+}$ complexes. Their CAD spectra are qualitatively the same as those of $Sr^{2+}$–bis(peptide) complexes. The subtle effect of metal ion influence awaits more detailed studies.

Gas-phase interactions of metal ions and peptide ligands are considered intrinsic. There may be a similarity between the gas-phase and condensed-phase metal ion–peptide interactions such as those involving metal ion and their binding loops inside a protein or an enzyme. The bonding of both alkaline-earth-metal-bound peptides and bis(peptide) complexes is a useful reference point for launching future investigations of strong gas-phase metal ion–peptide interactions.

## Experimental Section

**Reagents.** Peptides used in this work were obtained 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 acquisition of both mass spectra and tandem mass spectra were a Kratos MS-50 and a VG four-sector ZAB-T. The Kratos MS-50 is a triple analyzer mass spectrometer of EBE design, which was previously described.[16] It was 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. Both the field-free region between ESA-1 and the magnet and that 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

(16) Gross, M. L.; Chess, E. K.; Lyon, P. A.; Crow, F. W.; Evans, S.; Tudge, H. *Int. J. Mass Spectrom. Ion Phys.* **1982**, *42*, 243–254.

ion and a spectrum was obtained by scanning the electric field of ESA-2 to obtain the product ion spectrum. Consecutive collisional activation experiments (MS/MS/MS) were conducted as previously described.[13]

The ZAB-T four-sector tandem mass spectrometer consisted of two high-mass, double-focusing mass spectrometers.[17] The design of MS-2 was a reverse geometry, Mattauch–Herzog type (BE). The instrument was equipped with a Cs ion gun and was capable of producing a 50-keV $Cs^+$ beam. For the negative ion SIMS experiment, the Cs gun was operated at ca. 17 kV so that the overall energy of the $Cs^+$ beam was approximately 25 keV. When full mass spectra were obtained, 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).

(17) Gross, M. L. Tandem Mass Spectrometry: Multisector Magnetic Instrument. In *Methods in Enzymology*; McCloskey, J. A., Vol. Ed.; Academic Press: San Diego, CA, 1990; Vol. 193, pp 131–153.

**Procedures.** For acquiring a full scan mass spectrum, a few micrograms of the peptide was mixed with ca. 3 μL of the alkaline earth metal ion hydroxide–saturated glycerol/thioglycerol (1:1) on the tip of the FAB probe. The probe then was exposed to the 25-keV $Cs^+$ beam to cause desorption. In a typical experiment, the first ten 20-s scans following sample introduction were acquired and the signal averaged.

For FAB-MS/MS experiments, a few micrograms of the peptide was mixed on the tip of a FAB probe with the alkaline earth metal ion hydroxide or acetate in glycerol/thioglycerol (1:1). The tip was then exposed to a 25-keV $Cs^+$ ion beam on the ZAB-T instrument to cause desorption of the various metal-bound peptide ions.

For FAB-MS-MS-MS experiments,[13] 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.

**Acknowledgment.** This work was supported by the U.S. National Science Foundation, Grant No. CHE9017250.

# Molecular Complexes between Potassium and Ethylenediamine: Photoionization and ab Initio Molecular Orbital Studies

**Y.-H. Liau[†] and T.-M. Su*[,†,‡]**

*Contribution from the Department of Chemistry, National Taiwan University, Taipei, Republic of China, and The Institute of the Atomic and Molecular Sciences, Academia Sinica, Taipei, Republic of China.* *Received March 25, 1992*

**Abstract:** The molecular complexes formed between potassium and ethylenediamine (en), K(en) and $K_2$(en), were generated in a flow reactor and studied by photoionization mass spectrometry. The photoionization efficiencies of these complexes were analyzed with Watanabe plots, and the photoionization threshold energies were determined. Ab initio molecular orbital calculations were also performed for the neutral complexes and their corresponding positive ions. Supported by the calculation results, several conformers of K(en) in the photoionization efficiency spectrum were assigned. The photoionization threshold energies were measured to be 3.34, 3.54, and 3.64 ± 0.01 eV for three types of K(en) conformers and 3.57 ± 0.05 eV for the cyclic conformer of $K_2$(en), respectively. The upper bound of the enthalpy of the complex dissociation of $K_2$(en) into $K_2$ and ethylenediamine was determined to be 10.4 ± 1.5 kcal/mol. The conformational analysis of the potassium–ethylenediamine molecular complexes using photoionization spectrometry was reported for the first time. On the basis of the molecular orbital study, the structures and the nature of the bonding of these molecular complexes were also described.

## 1. Introduction

The molecular complexes formed between alkali metal atoms and Lewis base molecules have been under intensive investigation for the past decade or so.[1-16] Theoretical calculations were centered on the stabilities and the nature of these bonds.[1-11] To date, the gas-phase experiments have mainly concerned the measurement of the photoionization threshold energies and the photoionization efficiencies of these complexes.[12-16] A few of the experimental bond energies of the molecular complexes were deduced from these experiments.[12-14] Some high clusters containing sodium or cesium atoms were also reported.[12,13,15,16] Despite these theoretical and experimental efforts, the properties of the molecular complexes formed between alkali metal atoms and polyfunctional Lewis base molecules, such as the ethylenediamine molecule (en), were still not available. The interplay between the interactive forces of the complex bonding of alkali atom–ethylenediamine and the internal hydrogen bonding of the two amino groups of ethylenediamine had also not been touched.

In this article, we report the photoionization measurements and molecular orbital calculations on the molecular complexes formed

[†] National Taiwan University.
[‡] Academia Sinica.

(1) Nicely, V. A.; Dye, J. L. *J. Chem. Phys.* **1970**, *52*, 4795–4803.
(2) Trenary, M.; Schaefer, H. F., III; Kollman, P. A. *J. Am. Chem. Soc.* **1977**, *99*, 3885–3886.
(3) Trenary, M.; Schaefer, H. F., III; Kollman, P. A. *J. Chem. Phys.* **1978**, *68*, 4047–4050.
(4) Curtiss, L. A.; Frurip, D. J. *Chem. Phys. Lett.* **1980**, *75*, 69–74.
(5) Bentley, J.; Carmichael, I. *J. Phys. Chem.* **1981**, *85*, 3821–3826.
(6) Bentley, J. *J. Am. Chem. Soc.* **1982**, *104*, 2754–2759.
(7) Broughton, J. Q.; Bagus, P. S. *J. Chem. Phys.* **1982**, *77*, 3627–3634.
(8) Curtiss, L. A.; Pople, J. A. *J. Chem. Phys.* **1985**, *82*, 4230–4235.
(9) Curtiss, L. A.; Kraka, E.; Gauss, J.; Cremer, D. *J. Phys. Chem.* **1987**, *91*, 1080–1084.
(10) Wurthwein, E.-U.; Schleyer, P. v. R.; Pople, J. A. *J. Am. Chem. Soc.* **1984**, *106*, 6973–6978.
(11) Hsiao, Y.-W.; Chang, K.-M.; Su, T.-M. *Chem. Phys.* **1992**, *162*, 335–348.
(12) Schulz, C. P.; Haugstatter, R.; Tittes, H. U.; Hertel, I. V. *Phys. Rev. Lett.* **1986**, *57*, 1703–1706.
(13) Schulz, C. P.; Haugstatter, R.; Tittes, H. U.; Hertel, I. V. *Z. Phys. D: At., Mol. Clusters* **1988**, *10*, 279–290.
(14) Kuan, T.-C.; Jiang, R.-C.; Su, T.-M. *J. Chem. Phys.* **1990**, *92*, 2553–2558.

0002-7863/92/1514-9169$03.00/0 