this analysis, we suggest that the addition product observed by Nibbering is a covalently bound tetrahedral anion.

The addition reaction of methoxide–methanol plus benzaldehyde proceeds easily, whereas the corresponding reaction of ethoxide–ethanol does not. This result can be understood in terms of Scheme I, the thermochemical cycle for formation of the tetrahedral adduct from the separated nucleophile and carbonyl compound. According to this model, the heat of reaction to form the addition product approximately equals the difference in the electron affinities between the attacking nucleophile and the tetrahedral adduct. The electron affinity of the alkoxide–benzaldehyde adduct would be expected to depend only slightly on the nature of the alkoxide due to its distance from the oxygen bearing the negative charge.[37] The difference in the heats of reaction to form methoxide–benzaldehyde and ethoxide–benzaldehyde from the free alkoxide then reduces to the difference in the electron affinities of methoxide and ethoxide.[8] The heat of reaction to form ethoxide–benzaldehyde is therefore ~3 kcal/mol less exothermic than the heat of reaction to give methoxide–benzaldehyde. This small difference is apparently enough to decrease the tetrahedral adduct formation energy just below the ethanol–ethoxide well depth, ~28 kcal/mol,[44] thereby making addition unfavorable.

The reaction of ethoxide–methanol with benzaldehyde results in formation of both methoxide–benzaldehyde and ethoxide–benzaldehyde. The energetics to give these two products are almost identical, since the methoxide and ethoxide adducts are so similar structurally. The slightly lower stability of ethoxide–methanol compared with ethoxide–ethanol[45] is apparently sufficient to make the overall reaction exothermic.

These results suggest that the energy differences between the hydrogen-bonded alcohol–alkoxide complexes and the corresponding tetrahedral adducts are extremely small. Slight perturbations such as substitution of ethanol for methanol in the hydrogen-bonded complex are sufficient to make addition endothermic. This hypothesis is supported by equilibrium measurements by Nibbering and co-workers for the addition reaction of methoxide–methanol with benzaldehyde,[19] eq 12; these workers report $\Delta G°_{t2} = -0.93$ kcal/mol.

V. Conclusions

Deprotonated 2-hydroxytetrahydropyran has been observed to rearrange to a more stable form. The isomerized structure is assigned as an enolate ion stabilized by an intramolecular hydrogen bond, on the basis of thermochemical arguments and proton-exchange experiments. This stabilized enolate ion is the intramolecular equivalent to the addition product of methoxide and acetaldehyde, observed by Bowie and co-workers.[18]

The large acidity of 2-hydroxytetrahydropyran indicates the stability of deprotonated hemiacetal anions. This acidity is used as a model for the stability of the tetrahedral reaction intermediate formed by addition of an alkoxide to an aldehyde with no enolizable protons. By use of thermochemical arguments to estimate the enthalpy of addition, the formation of the tetrahedral species was found to be exothermic by over 30 kcal/mol. This result suggests that the methoxide–benzaldehyde addition product observed by Nibbering[19] is a tetrahedral species rather than an ion–dipole complex.

Acknowledgment. We are grateful to the National Science Foundation for support of this work. S.B. gratefully acknowledges graduate fellowship support from the Evelyn McBain Fund. We also thank Dr. Page O. Stoutland for stimulating discussion.

(44) The EtOHOEt⁻ stabilization energy was extracted from the MeOHOMe⁻ stabilization energy and the difference in energy between MeOHOMe⁻ and EtOHOEt⁻, measured by Bartmess and co-workers.[45]

(45) Caldwell, G.; Rozeboom, M. D.; Kiplinger, J. P.; Bartmess, J. E. *J. Am. Chem. Soc.* **1984**, *106*, 4660.

Fragmentations of Gas-Phase Complexes between Alkali Metal Ions and Peptides: Metal Ion Binding to Carbonyl Oxygens and Other Neutral Functional Groups

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Abstract: Collision-induced fragmentations of gas-phase $(M + Cat)^+$ complexes between 55 structurally diverse peptides and 5 alkali metal ions, Li^+, Na^+, K^+, Rb^+, and Cs^+, are reported. Mechanisms for fragmentation indicate that neither N-terminal $(a_{n-m} + Cat - H)^+$ nor C-terminal $(y_{n-m} + Cat + H)^+$ fragment ions arise from complexes that contain the alkali metal ion bonded to a deprotonated (zwitterionic) carboxylate terminus. In cases in which there is no strongly interacting side chain, the metal ion is most likely bonded to an amide oxygen. Another reaction, which necessitates the metal ion being bonded toward the N terminus as opposed to being bonded to a carboxylate anion, differentiates C-terminal amino acids such as leucine and isoleucine. Decompositions of $(M + Cat)^+$ complexes of C-terminal amides generally provide more extensive sequence information than C-terminal carboxylates. Fragmentation patterns, which change with increasing size of the alkali metal ion, are related to more favorable coordinative multisite binding between the larger metal ions and several amide oxygens, in analogy to known chemistry. The fragmentations thus do not reflect aqueous-phase bonding to a zwitterionic species but instead reflect the types of interactions that could occur between a metal ion and binding sites in less hydrophilic interiors of proteins.

Alkali metal ions, especially Na^+ and K^+, interact with a variety of biologically important peptides and proteins. Some macrocyclic antibiotic ionophores selectively bind and transport the ions through cell membranes.[1,2] Examples are the cyclic antibiotic peptide valinomycin, which selectively binds K^+, and monensin, which binds Na^+. In contrast, dianemycin nonselectively binds any of the alkali metal ions.[1a] Linear peptides such as the pentadecapeptide gramicidin also bind alkali metal ions and transport them across cell membranes.[1a,c] Alkali metal ions activate some enzymes, and ATPase requires both Na^+ and K^+ in various steps

(1) (a) Hughes, M. N. *The Inorganic Chemistry of Biological Processes*; 2nd ed.; Wiley: New York, 1972; pp 89–124, 257–295. (b) Hanzlik, R. P. *Inorganic Aspects of Biological and Organic Chemistry*; Academic: New York, 1976; pp 29–37. (c) Pressman, B. C. In *Inorganic Biochemistry*; Eichhorn, G. L., Ed.; Elsevier Scientific: Amsterdam: 1973; pp 203–226.

(2) Lehn, J.-M. In *Structure and Bonding*; Dunitz, J. D., Hemmerich, P., Ibers, J. A., Jørgensen, C. K., Neilands, J. B., Reinen, D., Williams, R. J. P., Eds.; Springer-Verlag: New York, 1973; Vol. 4, pp 53–64.

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of the mechanism for hydrolysis of ATP.[1a,2]

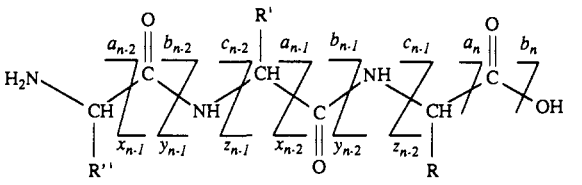
Both the size of the metal ion and the size of the metal ion binding site determine the selectivity of peptides or proteins for different alkali metal ions. In addition, metal ion induced conformational changes can alter the binding site and affect the resultant stability of the complex. Valinomycin's cavity is size-selective for K^+, but it still binds the other alkali metal ions with an order of preference of $K^+ > Rb^+ > Cs^+ > Na^+ > Li^+$.[2] Complexes that contain the smaller Na^+ and Li^+ ions are believed to be unstable because metal ion induced conformational changes can result in energetically unfavorable intraligand repulsions.[2] In contrast, favorable cation-specific conformational changes of ATPase are associated with the involvement of Na^+ and K^+ in its catalytic activity.[1a] Two important factors thus can affect binding between alkali or other metal ions and sites in less hydrophilic interiors of proteins. One is the relative intrinsic bond strengths between the metal ion and the intramolecular ligands. The other is the specific conformation of the protein that maximizes binding between the intramolecular ligands and the metal ion.[2,3]

Gas-phase ion chemistry potentially can address intrinsic (nonsolvated) bond strengths and conformational interactions between a metal ion and a peptide or small protein. Cody et al.[4] first demonstrated the potential analytical utility of collisional activation of peptides complexed with alkali metal ions: They showed that gas-phase $(M + K)^+$ complexes of gramicidin D and S give structurally informative fragmentations. It was Mallis and Russell,[5a] however, who studied complexes of *N*-benzoyl-Gly-His-Leu (Hip-His-Leu) and proposed that the gas-phase ion decompositions reflect multisite bonding between Na^+ and different peptide functional groups. They concluded[5b,c] that the alkali ion either is bonded intramolecularly to amide nitrogens and to the amino terminus or is bonded to amide nitrogens and side-chain substituents. Several other researchers[6-9] extended the gas-phase studies to complexes between Li^+, Na^+, or K^+ and a larger number of peptides. Their interpretations, however, are not in agreement with those of Mallis and Russell. Westmore and co-workers[6] studied fragmentations of $(M + Na)^+$ and $(M + K)^+$ complexes of three peptides and concluded that the metal ion is intramolecularly bonded to amide carbonyl oxygens. In complete contrast, Renner and Spiteller[7] proposed that the metal ion is complexed to a deprotonated carboxylate terminus. Gross and co-workers,[8] who performed the most comprehensive studies of both metastable ion and collision-induced dissociations of $(M + Li)^+$ complexes, also proposed that the metal ion is bonded to a deprotonated C-terminal carboxylate. Leary and co-workers[9] concluded that the gas-phase fragmentations reflect a combination of the above ideas. Whereas gas-phase metal ion binding to amide carbonyls and side-chain substituents could reflect intrinsic interactions in more anhydrous interiors of proteins where bulk aqueous solvation is absent, binding to a zwitterionic, deprotonated C terminus would instead reflect aqueous-phase (solvated) chemistry.

In a different investigation, we addressed the gas-phase chemistry of $(M + Cat^{2+} - H)^+$ complexes between 36 peptides and the alkaline earth metal ions Mg^{2+}, Ca^{2+}, and Ba^{2+}.[10] The locations of the alkaline earth metal ions were directly deduced from mechanisms of reaction, and the most abundant fragment ions arose from complexes in which the metal ions were preferentially bonded to deprotonated amide groups. Indeed, deprotonation of the C terminus was only important in cases in which the carboxylate had been converted into an amide. These and other results did not reflect aqueous-phase bonding to a deprotonated carboxylate terminus but instead reflected more intrinsic (gas-phase) interactions between the alkaline earth metal ions and the peptides.

As a result of these findings, we decided to examine complexes between alkali ions and a large series of peptides. Our results, presented here, provide evidence that the binding of alkali and alkaline earth metal ions to peptides is similar and reflects primarily intrinsic (gas-phase) interactions between the metal ion and the peptides. This observation contrasts with conclusions reached in previous work on the alkali systems, in which a primary binding site was proposed to be the deprotonated C-terminal carboxylate[7-9a] and in which the gas-phase chemistry was proposed to reflect aqueous-phase chemistry.[8a]

Table I. Sequence Ions Formed from Collision-Induced Decompositions of $(M + Cat)^+$ and $(M + H)^+$ Ions of Peptides



fragment ions from $(M + Cat)^+$ [a]	isoelectronic fragment ions from $(M + H)^+$ [a]
$(a_{n-m} + Cat - H)^+$	$(a_{n-m})^+$
$(H_2N\text{-peptide-CO-NH-CHR} - H + Cat^+)^+$	$(H_2N\text{-peptide-CO-NH-CHR})^+$
$(b_{n-m} + Cat - H)^+$	$(b_{n-m})^+$
$(H_2N\text{-peptide-CO-NH-CHR-CO} - H + Cat^+)^+$	$(H_2N\text{-peptide-CO-NH-CHR-CO})^+$
$(y_{n-m} + Cat + H)^+$	$(y_{n-m} + 2H)^+$
$(NH\text{-CHR-CO-peptide-CO}_2H + H + Cat^+)^+$	$(NH\text{-CHR-CO-peptide-CO}_2H + 2H^+)^+$

[a] Here, n refers to the total number of amino acids in the peptide and m is from 0 to $n - 1$.

Results

Fast atom bombardment (FAB) and collisional activation (CA) were used to study gas-phase $(M + Cat)^+$ complexes between 55 peptides and Li^+, Na^+, K^+, Rb^+, and Cs^+. The complexes were formed in the FAB ion source by bombarding a mixture of a peptide and an alkali iodide in a FAB matrix. The gas-phase ions were then accelerated to 8 keV and collided with He in the first field-free region of a normal-geometry mass spectrometer. Linked B/E scans, which provide fragment ion resolution of approximately 1500 and better than unit mass measurement, were used to obtain collision-induced decomposition (CID) spectra. The sizes and structures of the peptides varied: They ranged in size from di- to heptapeptides and some contained either a C-terminal carboxylate, amide, methyl ester, or alcohol group. Others contained an N-terminal benzoyl or *tert*-butyloxycarbonyl (*t*-BOC) group. Some had been N-acetylated, unless previously N-benzoylated, and then permethylated.

Spectra of $(M + Cat)^+$ complexes of *N*-benzoyl-Gly-His-Leu (Figure 1) show the general types of fragmentation patterns and

(3) (a) Cohen, J. S.; Hughes, L. J.; Wooten, J. B. In *Magnetic Resonance in Biology*; Cohen, J. S., Ed.; Wiley-Interscience: New York, 1983; Vol. 2, pp 130–247. (b) Jensen, L. H. In *Metal-Ligand Interactions in Organic Chemistry and Biochemistry*; Pullman, B., Goldblum, N., Eds.; Reidel: Dordrecht, Holland, 1977; Part 1, pp 229–242. (c) Colman, P. M.; Jansonius, J. N.; Matthews, B. W. *J. Mol. Biol.* **1972**, *70*, 701–724.

(4) Cody, R. B.; Amster, I. J.; McLafferty, F. W. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 6367–6370.

(5) (a) Mallis, L. M.; Russell, D. H. *Anal. Chem.* **1986**, *58*, 1076–1080. (b) Russell, D. H. *Mass Spectrom. Rev.* **1986**, *5*, 167–189. (c) Russell, D. H.; McGlohon, E. S.; Mallis, L. M. *Anal. Chem.* **1988**, *60*, 1818–1824.

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

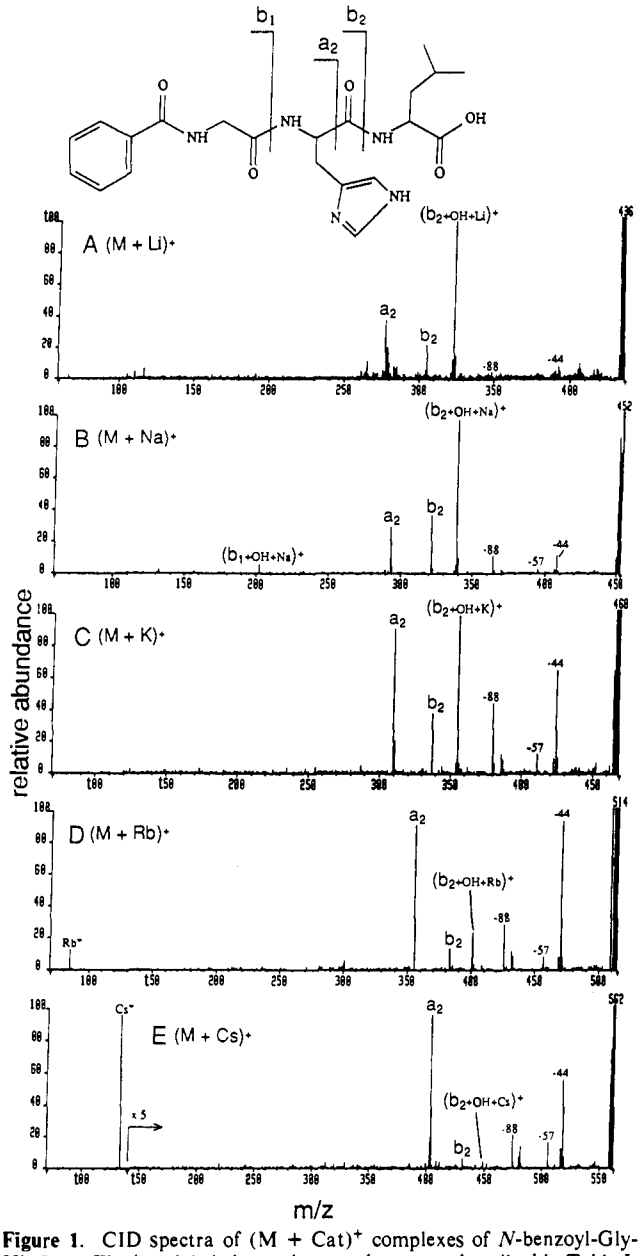


Figure 1. CID spectra of $(M + Cat)^+$ complexes of *N*-benzoyl-Gly-His-Leu. The ions labeled a_{n-m}, b_{n-m}, and y_{n-m} are described in Table I.

sequence ions that characterize complexes of tripeptides and larger peptides that contain a C-terminal carboxylate. The spectra are similar to those from energy scans,[5,8] except that linked B/E scans provide at least unit resolution and give better mass assignments than energy scans.[tt] A disadvantage, however, is that losses of Li^+, Na^+, or K^+, which are detected in energy scans,[5,8] cannot be detected in the linked scans. Other problems of B/E scans, which involve low-resolution mass selection of precursor ions and chemical noise, can be overcome, however, by carefully selecting FAB matrices and by subtracting background spectra.[12] The $(M + Cat)^+$ complexes generally fragment to give less structurally informative ions than either $(M + H)^+$ or $(M + Cat^{2+} - H)^+$ complexes that contain alkaline earth metal ions.[t0] Some sequence ions from $(M + Cat)^+$ complexes are isoelectronic with ions both from $(M + H)^+$ (Table I) and from alkaline earth containing $(M + Cat^{2+} - H)^+$ complexes.[t0] The $(a_{n-m} + Cat - H)^+$ and $(b_{n-m} + Cat - H)^+$ fragment ions contain the N terminus: The N-terminal $(b_{n-t} + Cat + OH)^+$ fragment ion (see Figure 1) is not listed in Table I. Spectra of tripeptides and larger peptides generally show weakly abundant or missing C-terminal $(y_{n-m} + Cat + H)^+$ fragment ions, although complexes of some dipeptides give an abundant $(y_t + Cat + H)^+$ ion.[8b]

Fragmentation patterns and fragment ion abundances usually change significantly with increasing size of the alkali metal ion, especially in transition from Na^+ to K^+.[13a] The $(b_{n-t} + Cat + OH)^+$ ion, which is highly abundant in complexes of Li^+ or Na^+, becomes of less absolute abundance in complexes of K^+ or the larger alkali metal ions (Figure 1).[t3b] It virtually disappears in spectra of $(M + Cs)^+$ complexes. In comparison, absolute abundances of the $(a_{n-1} + Cat - H)^+$ ion and ions from cleavages of side chains increase with increasing size of metal ion. Losses of 44 and 57 u in Figure 1 arise from cleavages of the Leu side chain. (Loss of 44 u also can be loss of CO_2, as seen in spectra of peptides that do not contain Leu.) Loss of 88 u involves a special rearrangement reaction to be discussed later. The $(M + K)^+$ complexes can be formed by FAB as easily as the other $(M + Cat)^+$ complexes, but they generally do not undergo as facile CIDs as the other complexes.

Spectra of C-terminal acids and C-terminal amides (Figure 2) show some important differences and some similarities. There is no counterpart in spectra of amides (Figure 2C,D) to the $(b_{n-1} + Cat + OH)^+$ ion in spectra of carboxylates (Figure 2A,B).[8a] Complexes of amides, however, fragment to give highly abundant C-terminal $(y_{n-m} + Cat + H)^+$ ions, which provide significantly more information about the structure of the peptide chain. Amides fragment also to give more abundant C-terminal $(x_{n-m} + Cat - H)^+$ ions and $(z_{n-m} + Cat)^{\cdot+}$ ions. Such abundant C-terminal fragment ions also characterize spectra of alkaline earth containing $(M + Cat^{2+} - H)^+$ complexes of peptide amides.[t0] Spectra of carboxylates and amides are similar in that amides also give increasing absolute abundances of the $(a_{n-t} + Cat - H)^+$ ion and side-chain cleavages with increasing size of the alkali metal ion (compare losses of 44 and 107 u in parts C and D of Figure 2). The $(M + K)^+$ complexes of C-terminal amides also undergo less facile fragmentations as discussed earlier for the carboxylates.

Fragmentations of C-terminal methyl esters (Figure 3) are similar to fragmentations of amides. There likewise is no counterpart in spectra of esters (Figure 3B) to the $(b_{n-t} + Cat + OH)^+$ ion in spectra of carboxylates (Figure 3A).[8a] Esters fragment also to give C-terminal $(y_{n-m} + Cat + H)^+$ and $(z_{n-m} + Cat)^{\cdot+}$ ions, but their absolute abundances are intermediate between the carboxylates and the amides. Increasing size of metal ion also results in increased absolute abundances of the $(a_{n-t} + Cat - H)^+$ ion and side-chain cleavages. In Figure 3, losses of 16 and 43 u are from cleavages of the Ala and Val side chains, respectively. The $(M + K)^+$ complexes of esters also undergo less facile fragmentations.

Although spectra of most $(M + Cat)^+$ complexes change significantly with increasing size of alkali metal ion, spectra of $(M + Cat)^+$ complexes of His-Leu-Gly-Leu-Ala-Arg (HLGLAR) are virtually identical [see Figure 4 for the $(M + Li)^+$ complexes]. The $(M + Cat)^+$ complexes all fragment to give the typical $(b_{n-t} + Cat + OH)^+$, $(a_{n-m} + Cat - H)^+$, and $(b_{n-t} + Cat - H)^+$ sequence ions. The Arg side chain cleaves to lose 17, 44, 60, and 87 u, and a special rearrangement reaction to be discussed later causes loss of 131 u. The spectrum in Figure 4 can be compared to the spectrum of $(M + Ca^{2+} - H)^+$ complexes of HLGLAR shown in ref 10. The Ca^{2+}-containing complexes fragment similarly to complexes that contain either Mg^{2+} or Ba^{2+} and produce a complete series of N-terminal $(c_{n-m} + Ca^{2+})^+$ ions. A series of $(a_{n-m} + Ca^{2+} - 2H)^+$ ions, except for the $(a_3 + Ca^{2+} - 2H)^+$ ion, is formed also. The alkaline earth metal ion complexes decompose also to lose 60 u from cleavage of the Arg side chain. In comparison, the $(M + Cat)^+$ complexes of HLGLAR (Figure

(11) Problems with assigning masses from energy scans are evident in spectra published previously.[5,8a]

(12) Contado, M. J.; Adams, J. *Anal. Chim. Acta*, in press.

(13) (a) We originally showed that significant changes in fragmentation patterns occur with increasing size of alkali metal ion in going from Li^+ through Cs^+, in the Proceedings of the 1989 ASMS Conference (p 907), as noted in the Acknowledgement. Gross and co-workers[8] also have recently pointed out some aspects of this chemistry. (b) The absolute abundances of the different sequence and other organic fragment ions are compared relative to total fragment ion current without including loss of the alkali cation as Cat^+.

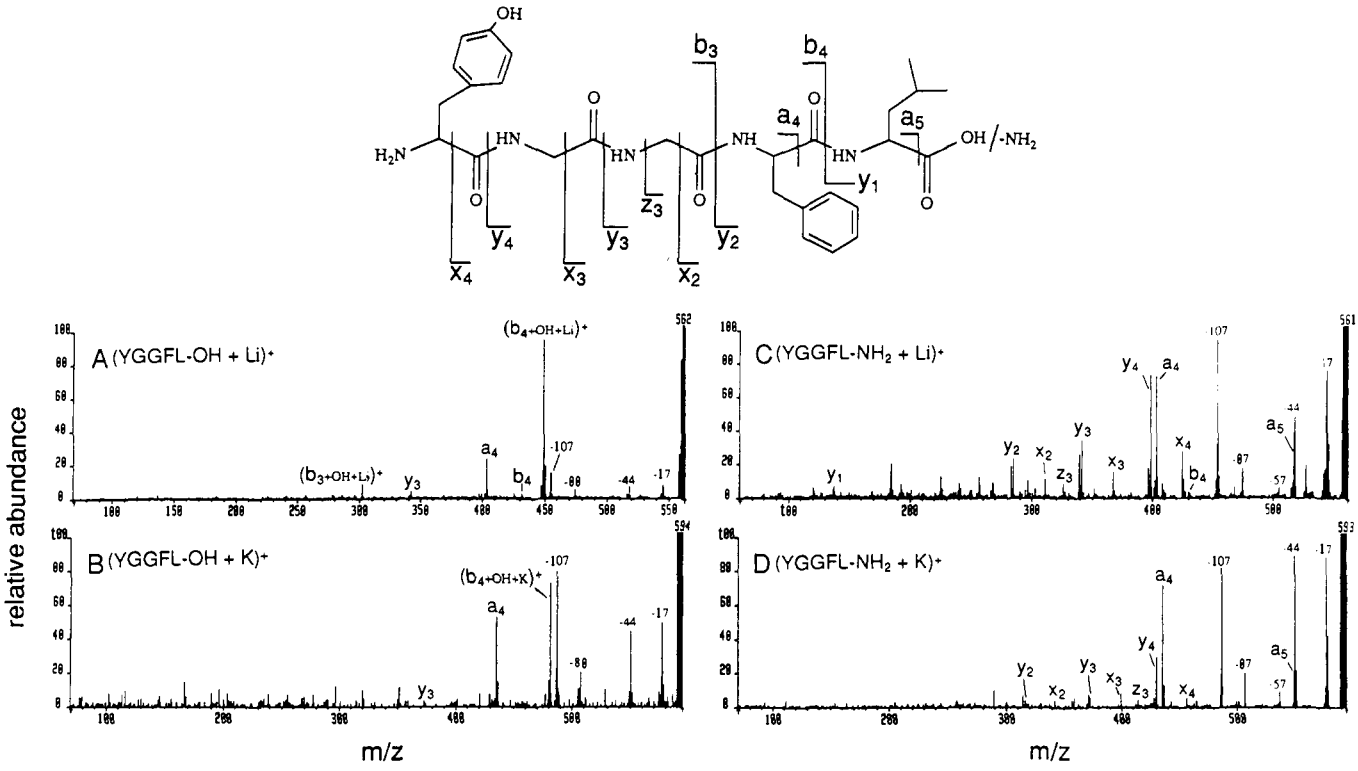


Figure 2. CID spectra of $(M + Cat)^+$ complexes of Leu-enkephalin, Try-Gly-Gly-Phe-Leu-OH (A and B), and Leu-enkephalin amide, Try-Gly-Gly-Phe-Leu-NH_2 (C and D). The ions labeled a_{n-m}, b_{n-m}, and y_{n-m} are described in Table I. The x_{n-m} and the z_3 ions are $(x_{n-m} + Cat - H)^+$ and $(z_3 + Cat)^{\cdot+}$ ions, respectively.

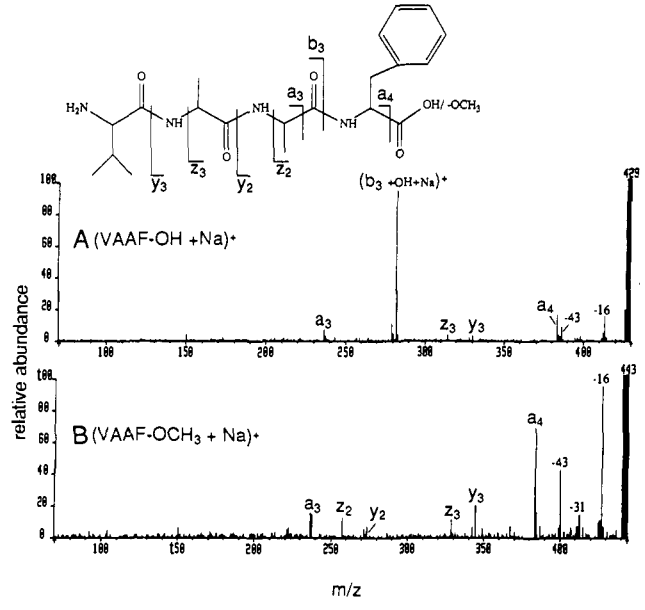


Figure 3. CID spectra of $(M + Na)^+$ complexes of Val-Ala-Ala-Phe-OH (A) and Val-Ala-Ala-Phe-OCH_3 (B). The ions labeled a_{n-m}, b_{n-m}, and y_{n-m} are described in Table I. The z_{n-m} ions are $(z_{n-m} + Na)^{\cdot+}$ ions.

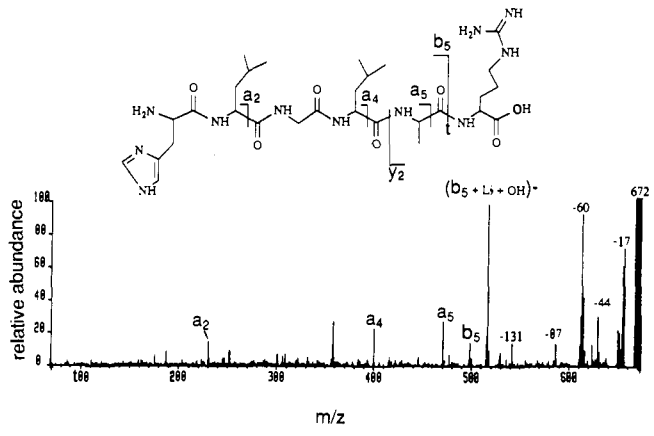


Figure 4. CID spectrum of $(M + Li)^+$ complexes of His-Gly-Leu-Gly-Ala-Arg. The ions labeled a_{n-m}, b_{n-m}, and y_{n-m} are described in Table I. We do not know the origin of the ion that arises from loss of 60 u, other than it involves the Arg side chain.

4) also yield a series of isoelectronic $(a_{n-m} + Cat - H)^+$ ions, except neither $(a_3 + Cat - H)^+$ nor $(a_t + Cat - H)^+$ ions are formed. More significantly, no N-terminal c_{n-m}-type fragment ions arise from alkali metal ion containing complexes. Abundant $(b_{n-t} + Cat + OH)^+$ fragment ions are seen in spectra of the alkali metal ion complexes, but analogous fragment ions are missing in spectra of the alkaline earth metal ion complexes.

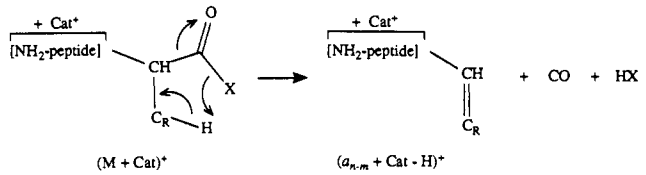
Discussion

CID spectra shown here in Figures 1–4, and in other published spectra,[4-9] show that increasing the size of the metal ion, exchanging a C-terminal carboxylate for an amide or a methyl ester, or changing the amino acid sequence affects the types and abundances of fragment ions. To understand these changes, it is first necessary to understand the mechanisms for formation of the different fragment ions. The mechanisms also can provide information about the specific location of the metal ion in the gas-phase complexes, as shown in our study of peptides and alkaline earth metal ions.[10]

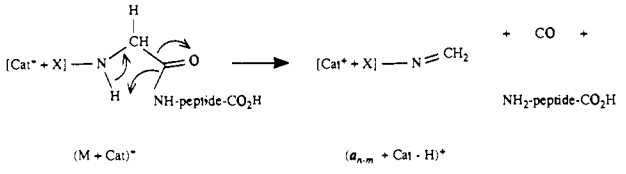
Our approach to elucidating mechanisms of fragmentation differs from previous approaches. In one study,[8a] mechanisms were proposed from changes in metastable ion vs collision-induced fragment ion abundances. Relationships between relative ion abundances and amino acid proton affinities were used to support other mechanisms.[8b] Other investigations used changes in fragmentations of $(M + Li)^+$ vs $(M + 2Li - H)^+$ ions as evidence for mechanisms,[9] and another[9b] used precursor ion scans to elucidate fragmentation paths. Others[9b,14] prepared deuterium-exchanged

(14) Mueller, D. R.; Eckersley, M.; Richter, W. J. *Org. Mass Spectrom.* **1988**, *23*, 217–221.

Scheme I



Scheme II



peptides and drew mechanistic conclusions from the results. There are problems, however, with this latter approach that may make the results ambiguous.[15] Consequently, we instead began our research here by simply using a variety of structurally diverse peptides to determine the source of hydrogen that is transferred in the different fragmentation reactions.[16] Transfer of hydrogen can arise from several locations in a peptide ion (see structure in Table I), and simply changing the structure of the peptide provides a way to systematically eliminate each source for hydrogen transfer. An amide NH can be eliminated if the $(M + Cat)^+$ complex contains either the amino acid proline or a methylated amide group. Substitution of Gly (R = H) for another amino acid eliminates the possibility of hydrogen transfer from the amino acid side chain (R). Alanine ($R = CH_3$) eliminates hydrogen transfer from a side-chain carbon other than the side-chain α-carbon. Derivatizing the N-terminal amine to an *N*-benzoyl amide removes the C(H)R group as a source for hydrogen transfer, and peralkylation of this or *N*-acetyl species completely eliminates all remaining NH and OH groups. Conversion of the C-terminal carboxylate into an ester eliminates the carboxyl OH as a source for hydrogen transfer.

Formation of $(a_{n-m} + Cat - H)^+$ Sequence Ions. Cleavages through HRC–CO bonds (Table I) produce the N-terminal $(a_{n-m} + Cat - H)^+$ ions, and hydrogen must be transferred from the fragment ion to a neutral leaving group. Fragmentations of $(M + Cat)^+$ complexes that contain Pro provide one important piece of evidence in this regard. For example, complexes of Ile-Pro-Ile fragment to give $(a_2 + Cat - H)^+$ ions from cleavage of the Pro(C)–(CO)Ile bond.[17a] Because Pro has no amide NH, the amide NH in the $n - 1 = 2$ amino acid cannot be the source for hydrogen transfer. Others[8a] also presented several examples of peptides that contain Pro as the $n - 1$ amino acid, and each of them also fragment to produce abundant $(a_{n-1} + Cat - H)^+$ ions. Proline in other $n - m$ locations beside the $n - 1$ position likewise does not affect formation of $(a_{n-m} + Cat - H)^+$ ions.[17b]

Fragmentations of peptides that contain either Gly (R = H) or Ala ($R = CH_3$) provide other information about the mechanism. Glycine as the $n - m$ amino acid inhibits formation of $(a_{n-m} + Cat - H)^+$ ions. One example is illustrated in Figure 4 in which the $(a_3 + Li - H)^+$ ion is completely missing from the a-ion series. Others[8a] also presented two excellent examples in which the inhibitory effect of Gly as the $n - m$ amino acid is evident: Ala-Gly-Phe–Leu preferentially gives the typically abundant $(a_3 + Li - H)^+$ ion, whereas Pro-Phe–Gly-Lys instead gives the not so typically abundant $(a_2 + Li - H)^+$ ion.[17c] Figure 4 also shows, however, that Gly as either the $n - m + 1$ or the $n - m - 1$ amino acid does not affect formation of $(a_{n-m} + Cat - H)^+$ ions. In contrast to Gly, neither Ala nor other peptides that contain hydrogen on the α-carbon of the $n - m$ amino acid side chain inhibit the reaction (see the a_5 ion in Figure 4 in which Ala is the $n - m = 5$ amino acid). These results indicate that hydrogen transfer is not from a C(H)R group of the fragment ion but is from the α-carbon of the $n - m$ amino acid side chain.

The above changes in peptide structure provide evidence about the source for hydrogen transfer in the mechanism. The changes unfortunately provide no evidence regarding the exact location of the alkali metal ion in relation to the site of fragmentation. Both C-terminal amides (Figure 2C,D) and methyl esters (Figure 3B), however, give abundant $(a_{n-m} + Cat - H)^+$ ions. This clearly shows that $(M + Cat)^+$ complexes that contain the alkali metal ion bonded to a deprotonated C-terminal carboxylate are not required for the mechanism.

A mechanism (Scheme I) in which the alkali metal ion is bonded on the N-terminal side of the site of cleavage, and in which X = OH, NH_2, OCH_3, or NH peptide, reconciles our experimental observations and others[5-9] (also see footnote 15). This mechanism is analogous to mechanisms for thermolytic fragmentations of acyl chlorides to give CO, an alkene, and HCl, which have activation energies of 50–55 kcal mol^{-1} and Arrhenius *A* factors of $\sim 10^{14}$ s^{-1}.[18] A heterolytic cleavage to lose Li^+ from $(M + Li)^+$ ions of $CH_3CONH(C_2H_5)$ would require approximately 55 kcal mol^{-1} and would have an *A* factor of $\sim 10^{15}$–10^{17} s^{-1}.[19] Loss of Li^+, however, is not a major fragmentation route,[8] and consequently the reaction in Scheme I probably has a lower activation energy than the thermolytic analogies.

The mechanism in Scheme I, which describes the most facile formation of $(a_{n-m} + Cat - H)^+$ fragment ions, is identical with the one that describes the most facile reaction to give a-ions from $(M + Cat^{2+} - H)^+$ complexes between alkaline earth metal ions and peptides.[10] In another analogy to the alkaline earth ion complexes, some of the alkali ion complexes also can decompose to give weakly abundant $(a_{n-m} + Cat - H)^+$ ions even in cases in which Gly is the $n - m$ amino acid. For example, weakly

(15) Deuterium-exchanged peptides were used originally[14] to try to elucidate mechanisms for formation of $(y_{n-m} + 2H)^+$ ions from $(M + H)^+$ precursors and more recently[9b] to address formation of $(a_{n-m} + Cat - H)^+$ ions from $(M + Li)^+$ and $(M + 2Li - H)^+$ complexes of peptides. In the former study,[14] the researchers obtained identical results from a deuterium-exchanged tripeptide that was prepared in situ by using reactions either with glycerol-d_3 in a FAB ion source or with ND_3 in a CI ion source. In the latter study,[9b] the researchers used FAB to desorptively ionize "pre-exchanged" peptides from deuterium-exchanged FAB matrices. The difficulty we find with this overall approach is that one does not really know the true location of the exchanged deuterium atoms in the gas-phase ions: In the gas phase, the most exchangeable (acidic) protons *are not* the acid, amide, and *amine* protons, but *are instead* the acid, amide, *α-carbon*, and *phenylalanine benzyl* protons. The peptide functional groups, in decreasing order of exchangeability (acidity), are as follows: $NH_2CH_2CO_2^-$ acid anion, ΔH_{acid} = 342.5 kcal mol^{-1};[15b] $CH_3CON(CH_3)^-$ amide anion, ΔH_{acid} = 362.3 kcal mol^{-1};[15c] $CH_2CON(CH_3)_2^-$ α-carbon anion, ΔH_{acid} = 373.5 kcal mol^{-1};[15d] $C_6H_5CH_2^-$ benzyl anion, ΔH_{acid} = 380.7 kcal mol^{-1};[15b] and $NHCH(CH_3)_2^-$ amine anion, ΔH_{acid} = 397.2 kcal mol^{-1}.[15b] Thus in the latter study,[9b] the benzyl protons of Phe in the four peptides investigated may actually be the ones exchanged for deuterium instead of the N-terminal amine protons, and this would entirely change the conclusions that were drawn regarding the mechanism for formation of the $(a_{n-m} + Cat - H)^+$ ions. (b) Lias, S. G.; Bartmess, J. E.; Liebman, J. F.; Holmes, J. L.; Levin, R. D.; Mallard, W. G. *J. Phys. Chem. Ref. Data* **1988**, *17*, Suppl. 1. (c) Meot-Ner, M. *J. Am. Chem. Soc.* **1988**, *110*, 3071–3075. (d) Bartmess, J. E.; McIver, B. T. In *Gas Phase Ion Chemistry*; Bowers, M. T., Ed.; Academic: New York, 1979; Vol. 2, pp 87–122.

(16) Our method is somewhat akin to "steric blocking" described in: Levsen, K. *Fundamental Aspects of Organic Mass Spectrometry*; Verlag Chemie: Weinheim, 1978; Vol. 4, pp 164–165.

(17) (a) The absolute abundances, relative to total fragment ion current, of sequence ions in the CID spectrum of IPI $(M + Li)^+$ complexes are $(b_2 + Li + OH)^+$, 64%; $(a_2 + Li - H)^+$, 5%; $(b_3 + Li - H)^+$, 5%; $(b_2 + Li - H)^+$, 5%; $(y_1 + Li + H)^+$, 5%. (b) Other $(M + Li)^+$ complexes of Pro-containing peptides also fragment to give $(a_{n-m} + Li - H)^+$ ions (in percent abundance relative to total fragment ion current): RYLPT gives a_4 (1%), a_3 (2%), a_2 (6%), and a_1 (1%) ions; VHLTP gives a_5 (12%), a_4 (9%), a_3 (3%), and a_2 (7%) ions; YPFPG gives a_5 (3%) and a_4 (4%) ions; GPGG gives a_4 (2%), a_3 (7%), and a_2 (18%) ions; and GPRP gives a_4 (3%) and a_3 (1%) sequence ions. (c) Another striking case is GPGG that gives a significantly less abundant a_3 ion (7%) vs the a_2 ion (18%).

(18) (a) Robinson, P. J.; Holbrook, K. A. *Unimolecular Reactions*; Wiley-Interscience: London, 1972; pp 231–236. (b) Lennon, B. S.; Stimson, V. R. *J. Am. Chem. Soc.* **1969**, *91*, 7562–7564. (c) Lennon, B. S.; Stimson, V. R. *Aust. J. Chem.* **1970**, *23*, 525–531.

(19) The theoretically calculated bond dissociation energy is from: (a) Hinton, J. F.; Beeler, A.; Harpool, D.; Briggs, R. W.; Pullman, A. *Chem. Phys. Lett.* **1977**, *47*, 411–415. The *A* factor is from direct homolytic cleavages from: (b) Benson, S. W.; O'Neal, H. E. *Kinetic Data on Gas Phase Unimolecular Reactions* (*NSRDS-NBS 21*); National Bureau of Standards, U.S. Government Printing Office: Washington, DC, 1970.

abundant $(a_t + Cat - H)^+$ and $(a_2 + Cat - H)^+$ ions are found in spectra of $(M + Cat)^+$ complexes of *N*-benzoyl-Gly-Gly-Gly. We[10] previously proposed a mechanism for what must be a less facile reaction to give a_{n-m} fragment ions from Gly-containing complexes between peptides and alkaline earth metal ions. This mechanism (Scheme II) also explains formation of the analogous ions here. In Scheme II, X can be either H_2N–peptide–CO or benzoyl, the source for hydrogen transfer is instead the Gly amide NH, and the alkali metal ion is bonded on the N-terminal side of the site of reaction. A combination of a higher activation energy and a competitive shift may account for the weaker abundances of fragment ions formed by this route vs those formed as depicted in Scheme I.

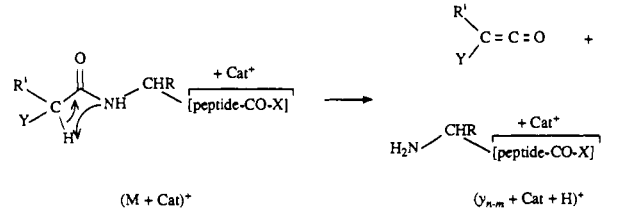
As mentioned above and in contrast to complexes between alkaline earth metal ions and peptides,[10] the reactions in Schemes I and II provide no evidence about the exact location of the alkali metal ion in relation to the site of reaction. We do know, however, that gas-phase Li^+ affinities of amide oxygens are greater than Li^+ affinities of analogous amines.[19a,20] Thus, amide oxygens and not the N-terminal amine would be the preferred site of gas-phase cationization in the peptides, at least in cases in which there is no strongly interacting side chain. In spectra of peptides such as shown in Figures 1–3, the high-mass $(a_{n-t} + Cat - H)^+$ ion is usually the most abundant ion in its series. Bonding to one particular amide thus does not seem related to preferential formation of this ion, but instead a sterically or otherwise favored loss of the smallest neutral appears to control the chemistry.

The mechanisms proposed in Schemes I and II contrast with mechanisms that others[8a,9] have proposed. Grese et al.[8a] proposed an isomerization reaction via an eight-membered ring in which the $n - 1$ amide NH is the source for hydrogen transfer and the C terminus is a deprotonated and cationized carboxylate anion. Their[8a] mechanism does not explain either the chemistry of C-terminal esters or fragmentations of peptides in which Pro is the $n - 1$ amino acid, as discussed above. Leary and co-workers[9] proposed that the $(a_{n-m} + Li - H)^+$ ions arise via several mechanisms, one of which involves an intermediate radical cation in a two-step reaction. This latter mechanism is a two-step version of Scheme I and is thus entirely consistent with our experimental results.

Formation of $(y_{n-m} + Cat + H)^+$ Sequence Ions. The C-terminal $(y_{n-m} + Cat + H)^+$ ions result from cleavages through amide bonds (Table I) and require transfer of hydrogen from a neutral leaving group to the fragment ion. Neither Gly[21a] nor Pro[21b] inhibits formation of $(y_{n-m} + Cat + H)^+$ ions. Thus, the mechanism neither involves transfer of an α-hydrogen from a side chain nor involves transfer of a hydrogen from an amide NH. Two remaining sources for hydrogen transfer are either the N-terminal amine or a C(H)R group. Converting the N-terminal amine into an *N*-benzoyl amide and permethylation of *N*-acetyl derivatives of peptides still allow formation of $(y_{n-m} + Cat + H)^+$ ions.[22] Thus, the N-terminal *amine* cannot be the source for hydrogen transfer, and hydrogen must be transferred to the fragment ion from the C(H)R group of the $n - m + 1$ amino acid.

The above results do not provide any evidence about the exact location of the alkali metal ion in the gas-phase complexes. The $(y_{n-m} + Cat + H)^+$ ions are abundant in spectra of C-terminal amides (Figure 2) and methyl esters (Figure 3), however. This clearly shows that $(M + Cat)^+$ complexes that contain the alkali metal ion bonded to a deprotonated C-terminal carboxylate are not required for the mechanism.

Scheme III



A mechanism (Scheme III), which is identical with one that Hunt and co-workers[23] proposed for formation of isoelectronic $(y_{n-m} + 2H)^+$ ions from $(M + H)^+$ ions of *N*-acetyl-*N*,*O*-permethylated peptides, reconciles both our results and those of others.[8b] This mechanism also is identical with one we[10] proposed for formation of isoelectronic $(y_{n-m} + Cat^{2+})^+$ ions from $(M + Cat^{2+} - H)^+$ complexes between alkaline earth metal ions and peptides. The 1,2 elimination, in which X = OH, NH_2, or OCH_3 and Y = NH_2–peptide, C_6H_5CO–NH, or CH_3CO–$N(CH_3)$, is analogous to the thermolytic 1,2 elimination of NH_3 and isobutene from *tert*-butylamine, which has an activation energy of 67 kcal mol^{-1} and an *A* factor of $\sim 10^{14}$ s^{-1}.[18a] As discussed above for the a-ions, the reaction in Scheme III probably has a lower activation energy than the thermolytic analogy because competitive loss of Li^+ is not detectable.[8]

The reaction in Scheme III only differs from the one we[10] proposed previously in that here the alkali metal ion is not bonded to either a deprotonated C-terminal carboxylate or amide. It is bonded nonetheless on the C-terminal side of the reaction, preferably to a carbonyl oxygen,[19a,20] at least in cases in which there is no strongly interacting side chain. The $(y_{n-m} + Cat + H)^+$ ions increase in absolute abundances with C-terminal acids < methyl esters < amides,[24] and these orders parallel both theoretical and gas-phase Li^+ and Na^+ affinities of each carbonyl moiety.[19a,20,25] These data suggest that y-ions arise from $(M + Cat)^+$ complexes that preferentially contain the metal ion bonded to the carbonyl oxygen of the C-terminal amino acid. This would explain the high abundances of y-ions in spectra of amides: Because a C-terminal amide oxygen would compete most effectively for the metal ion against the other amide oxygens, more $(M + Cat)^+$ complexes that produce $(y_{n-m} + Cat + H)^+$ ions would be formed. This hypothesis assumes that the reaction kinetics [i.e., log $k(E)$ curves] are the same for all three types of peptides.

Grese and Gross[8b] first noted that tripeptides and larger peptides that contain a C-terminal carboxylate produce either weakly abundant or no low-mass $(y_1 + Li + H)^+$ ions, and they used this as evidence for their mechanism. We find, however, that *all* $(y_{n-m} + Li + H)^+$ ions are either weakly abundant or missing from spectra of tripeptides and larger carboxylates, although side-chain structure dictates their abundances. For instance, the $(y_{n-2} + Li + H)^+$ ion is barely detected in Figure 2A, the $(y_{n-t} + Li + H)^+$ ion is barely detected in Figure 3A, but $(y_{n-2} + Li + H)^+$ and $(y_{n-t} + Li + H)^+$ ions occur in higher and approximately the same abundances for Gly-Gly-Val. Even in spectra of C-terminal amides, in which y-ions are more abundant, the smallest ions in the y-series, and in all other sequence ion series, are usually the weakest in abundance (see Figure 2C). These data suggest that in cases in which there is no strongly interacting side chain, unfavorable losses of larger neutrals influence the abundances of smaller mass y-ions, as discussed above for the a-ions.

(20) (a) Kollman, P.; Rothenberg, S. *J. Am. Chem. Soc.* **1977**, *99*, 1333–1342. (b) Staley, R. H.; Beauchamp, J. L. *J. Am. Chem. Soc.* **1975**, *97*, 5920–5921.

(21) (a) Although there are no detectable y_2 peaks in Figure 1 or y_3 peaks in Figure 4, Gly-containing GGV and GPGG fragment to give y_2 and y_1 ions, and other peptides that contain Gly also undergo this chemistry. We believe that the overall lack of C-terminal sequence ions in Figures 1 and 4 is a result of other factors that are related to preferential metal ion complexation with the basic His residue. (b) The $(M + Li)^+$ complexes of several Pro-containing peptides fragment to give y-sequence ions (in percent abundance relative to total fragment ion current): IPI gives y_1 (5%) ions; GPGG gives y_2 (3%) and y_1 (1%) ions; RYLPT gives y_4 (1%) and y_2 (1%) ions; VHLTP gives y_3 (2%), y_2 (1%), and y_1 (2%) ions; and YPFPG gives y_4 (10%) ions.

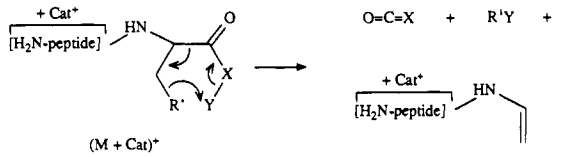
(22) We observe $(y_{n-m} + Cat + H)^+$ ions from permethylated *N*-acetyl-GGV and permethylated *N*-acetyl-YGGFL-NH_2.

(23) Hunt, D. F.; Buko, A. M.; Ballard, J. H.; Shabanowitz, J.; Giordani, A. B. *Biomed. Environ. Mass Spectrom.* **1981**, *8*, 397–408.

(24) It is reasonable to argue that a competitive shift that is caused by preferential formation of the $(b_{n-1} + Cat + OH)^+$ ion causes the $(y_{n-m} + Cat + H)^+$ ions to be in low absolute abundances in spectra of C-terminal carboxylates. This argument does not, however, explain the increases in absolute abundances of $(y_{n-m} + Cat + H)^+$ ions in changing from C-terminal esters to amides.

(25) Perriduct, M.; Pullman, A. *FEBS Lett.* **1973**, *34*, 222–226.

Scheme IV



Our experimental results and the mechanism shown in Scheme III contrast with mechanisms that others[8b,9a] have proposed. Grese and Gross[8b] and, for $(M - H + 2 Li)^+$ ions, Leary et al.[9a] proposed mechanisms that require transfer of a hydride ion either from the N-terminal amine NH_2[26] or from the $n - m + 1$ amide NH of the departing neutral[14] (also see footnote 15), which also require the precursor ion to contain a deprotonated and cationized C-terminal carboxylate. Their[8b,9a] mechanisms do not explain either the chemistry of C-terminal esters, fragmentations of peptides that contain Pro, or decompositions of *N*-acetyl-*N*,*O*-permethylated peptides.

Mechanism for Differentiating C-Terminal Amino Acids. A special fragmentation reaction differentiates C-terminal amino acids that have alkyl side chains, such as Leu and Ile.[27] C-Terminal Leu carboxylates lose 88 u (Figures 1 and 2A,B), whereas amides and methyl esters lose 87 (Figure 2C,D) and 102 u, respectively. In contrast, Ile carboxylates lose 74 u. C-Terminal Met leads to loss of 106, Val to loss of 60, and Arg to loss of 131 u (Figure 4). Neither C-terminal Ala (R = CH_3), Phe (R = $CH_2C_6H_5$), nor His (R = $CH_2C_3H_3N_2$), however, fragments to yield analogous ions.

A mechanism (Scheme IV) that is similar to the mechanism for pyrolysis of β,γ unsaturated carboxylic acids[t8a,28] explains these results.[29] Here, X–Y is O–H, NH–H, or O–CH_3. The exact location of the alkali metal ion is unknown other than it must be on the N-terminal side of the cleavage reaction, presumably bonded to an amide carbonyl oxygen. The metal ion cannot be bonded to a deprotonated C-terminal carboxylate because then neither C-terminal esters nor carboxylates themselves could undergo this chemistry.

Grese and Gross[8b] also showed excellent examples of dipeptides that fragment to give these losses. Although they neither proposed a mechanism nor showed the generality of the reaction, they labeled these fragment ions as d-ions, analogous to those[30] formed from peptide $(M + H)^+$ ions. The structures of the fragment ions in Scheme IV are indeed formally analogous to those of d-ions although the mechanisms for formation of the two types of ions are completely different. The similarity between the two types of reactions involves the location of the metal ion and the proton[30] in the two respective precursor ions: Both are located on the N-terminal side of the cleavage reactions.

Effects of Increasing Size of Alkali Metal Ion on Fragmentation Patterns. The $(M + Cat)^+$ complexes between peptides and alkali metal ions generally fragment to give few informative sequence ions so that evidence about preferential sites of alkali metal ion bonding is relatively scarce. Furthermore, the mechanisms for fragmentation provide no direct evidence about the location of the metal ion. In addition, steric or other factors, and not variations in potential metal ion binding sites, appear to control formation of the most abundant ions, except in cases in which there are specific side-chain interactions. As discussed above, the higher mass fragment ions are generally the most abundant, and this suggests that the transition-state energies for the mechanisms shown in Schemes I–III are lowest for losses of the smallest neutral fragments.

Chemistry of complexes studied here contrasts with chemistry of peptide–alkaline earth metal ion complexes,[10] which fragment to give significantly more abundant and consistent sequence ions (compare spectra of Leu-enkephalin in Figure 2 to analogous spectra in ref 10). Although higher mass $(a_{n-1} + Cat^{2+} - 2H)^+$ ions in spectra in ref 10 also generally are the most abundant a-ions, relative abundances of a variety of other types of ions change significantly with increasing size of the metal ion. Marked changes in fragmentation patterns with increasing size of alkaline earth metal ion provided evidence in support of possible metal ion induced conformational changes.[t0]

Changes in fragmentation patterns also occur here with increasing size of alkali metal ion (see Figures 1 and 2) unless there is a strongly interactive side chain. Although spectra of $(M + Li)^+$ and $(M + Na)^+$ complexes are virtually identical, there is a marked transition in going from Na^+ to K^+ and to the larger metal ions.[13a] The intrinsic binding interactions between the peptides and the larger alkali metal ions must be changing. Grese and Gross[8b] were the first to propose that the decrease in the polarizing ability of K^+ accounts for decreased abundances of $(b_{n-1} + K + OH)^+$ ions in spectra of $(M + K)^+$ complexes of dipeptides. We find, in addition, that as the absolute abundances of $(b_{n-t} + Cat + OH)^+$ ions decrease, the absolute abundances of other ions such as $(a_{n-1} + Cat - H)^+$ ions generally increase.[13b] This is better demonstrated in spectra of C-terminal amides for which there is no b-ion, but the a-ion nevertheless increases in absolute abundance in going from Li^+ to K^+ (see Figure 2C,D).

The transition in our spectra between complexes that contain Na^+ vs K^+ is interesting because it is known that larger intramolecular binding centers in enzymes favor K^+ over Na^+,[31,32] and stabilities of chelated complexes of biuret and glyoxal significantly increase in going from Na^+ to K^+.[33] Furthermore, as mentioned earlier in this paper, multidentate binding between amide oxygens of valinomycin and the smaller Li^+ or Na^+ cations is believed to be less favorable than binding to K^+ because the smaller cations can induce excessive contraction of the peptide molecule that would result in intraligand repulsions.[2] Even in linear systems, more energy is intrinsically required for a smaller alkali metal cation to contract an open-chain structure into a cyclic species, which would then be optimal for multidentate metal ion binding.[34] Consequently, we expect gas-phase metal ion induced conformational changes of peptides to be more energetically favorable for the larger alkali metal ions, and we wonder if more favorable multidentate interactions between K^+ and several amide oxygens cause the changes in the spectra and if the $(a_{n-t} + Cat - H)^+$ ions preferentially arise from such species. All of these effects can be related both to a large relative increase in cation size[35] and

(26) The hydride ion affinity (HIA) of NH_2^+ can be calculated to be 346 kcal mol^{-1}.[15b] Unfortunately, thermochemical data are not available to calculate the HIA of an amide nitrogen, which would allow a thermodynamic evaluation of the hydride-transfer mechanism proposed by others.[8b,9a]

(27) We originally showed that the reaction in Scheme IV is analytically useful for differentiating Leu from Ile, in the Proceedings of the 1989 ASMS Conference (p 907), as noted in the Acknowledgement. Gross and co-workers[8b] also have recently pointed out some aspects of this chemistry.

(28) (a) Smith, G. G.; Blau, S. E. *J. Phys. Chem.* **1964**, *68*, 1231–1234. (b) Bigley, D. B.; Thurman, J. C. *J. Chem. Soc.* **1965**, 6202–6205. (c) Bigley, D. B.; Thurman, J. C. *J. Chem. Soc. B* **1966**, 1076–1077. (d) Bigley, D. B.; May, R. W. *J. Chem. Soc. B* **1967**, 557–559.

(29) It should be noted that we observe, in some cases, losses that could conceivably correspond to reaction intermediates. For instance, C-terminal Met also gives fragment ions that arise from separate losses of 44 and 62 u (to give a total of 106 u according to Scheme IV), Ile gives separate losses of 44 and 30 u (total of 74 u), and Val gives separate losses of 44 and 16 u (total of 60 u). Analogous "intermediates" are not observed, however, either for C-terminal amides (there is no loss of 43 u, or OCNH) or for C-terminal methyl esters (there is neither loss of 58 u, or C_4H_{10}, for Leu-methyl ester nor loss of 30 u, or C_2H_6, for Val-methyl ester). Furthermore, some peptides undergo side-chain losses, in addition to a separate loss of CO_2, even though the side chain is not at the C terminus. In these latter cases, however, formation of a fragment ion from loss of the *total* neutral masses shown in Scheme IV does not occur. These data thus support a concerted, as opposed to a two-step mechanism such as shown in Scheme IV.

(30) Johnson, R. S.; Martin, S. A.; Biemann, K. *Int. J. Mass Spectrom. Ion Processes* **1988**, *86*, 137–154.

(31) (a) Pressman, B. C. In *Inorganic Biochemistry*; Eichhorn, G. L., Ed.; Elsevier Scientific: Amsterdam, 1973; pp 203–226.

(32) Williams, R. J. P. *Adv. Chem. Ser.* **1971**, *No. 100*, 155–173.

(33) (a) Rode, B. M.; Gstrein, K. H. *J. Chem. Soc., Faraday Trans. 2* **1978**, *74*, 889–895. (b) Rode, B. M.; Kraft, H. G. *Chem. Phys. Lett.* **1979**, *61*, 410–412.

(34) Rode, B. M.; Honnongbua, S. V. *Inorg. Chim. Acta* **1985**, *96*, 91–97.

(35) Morris, D. F. C. In *Structure and Bonding*; Jørgensen, C. K., Neilands, J. B., Nyholm, R. S., Reinen, D., Williams, R. J. P., Eds.; Springer-Verlag: New York, 1968; Vol. 4, pp 63–82.

to a significant increase in cation polarizability[36] in going from Na^+ to K^+.

The formation of multidentate, cyclic $(M + Cat)^+$ complexes could explain why loss of the smallest neutral to give the higher mass $(a_{n-1} + Cat - H)^+$ ion is generally the most favored reaction of its type. To lose larger neutrals, energy would be needed both to disrupt bonding in the cyclic chelate and to overcome any steric hindrance to attainment of transition-state structure. Thus, the greater abundances of higher mass a-ions could be a combined result of more structurally specific $(M + Cat)^+$ complexes undergoing the fragmentation and an increase in the rate of reaction. Although interactions with amide carbonyls in the cyclic species would be less specific, they would appear to favor the N-terminal end of the peptide chains: Note the decreased absolute abundances of C-terminal y- and x-type ions in going from Li^+ to K^+ in Figure 2C,D.

Effects of Substituents on Fragmentation Patterns. More structurally specific binding interactions between the alkali metal ions and certain peptide functional groups could cause changes in fragmentation patterns with changing side-chain structure (Figure 4) and with changing the C-terminal carboxylate into an amide or ester (Figures 2 and 3). Similar structural changes in complexes between alkaline earth metal ions and peptides produce more pronounced changes in fragmentation patterns.[10] Such changes provided evidence that alkaline earth metal ions may preferentially bond intramolecularly to some side chains and to some specific amide carbonyls.

Others[5,8] have discussed the importance of intramolecular side-chain interactions in fragmentations of complexes between peptides and alkali metal ions. Some of the most important interactions are those that involve basic amino acid side chains.[5a,c,8] Interpretations about the exact nature of the interactions have been quite different, however. Mallis and Russell[5a,c] proposed that the precursor $(M + Cat)^+$ complexes preferentially contain the alkali metal ion bonded intramolecularly to the basic side-chain substituent and amide nitrogens. In contrast, Gross and co-workers[8] proposed that the precursor complexes contain the metal ion bonded to the C-terminal carboxylate and that intramolecular stabilizing interactions between the side chain and the metal ion occur in the final fragment ion.[8a] They also proposed[8] that both the precursor complexes and the final fragment ions contain the metal ion bonded to the C-terminal carboxylate and that there is a single-site interaction between the side chain and a proton that instead controls the chemistry.

We showed above that the site of metal ion attachment in $(M + Cat)^+$ complexes that give rise to $(a_{n-m} + Cat - H)^+$ ions is most likely not at a deprotonated C-terminal carboxylate. Consequently, our data in principle support the general proposal of Mallis and Russell[5a,c] in which the preferential site of metal ion binding in $(M + Cat)^+$ complexes of peptides such as shown in Figure 4 may at least partially involve a strongly interacting side chain such as the basic imidazole of His. Such a specific and preferential interaction could explain why the spectrum in Figure 4 shows the extensive series of a-sequence ions instead of simply the highest mass member of the series. It is important to note, however, that other factors are important because $(M + Cat)^+$ complexes of RVYVHPF (Figure 5) also fragment to give a relatively extensive series of a-sequence ions and no C-terminal sequence ions are detected: Here, Arg instead of His is the N-terminal amino acid, and His instead of Arg is located toward the C terminus. It also should be quite clear from Figures 4 and 5 that the types and abundances of fragment ions have no simple relationship to either solution- or gas-phase proton or lithium ion affinities of the two basic amino acids.[37]

Conclusions

Experimental results presented here provide evidence for mechanisms for fragmentation of peptide $(M + Cat)^+$ ions to give

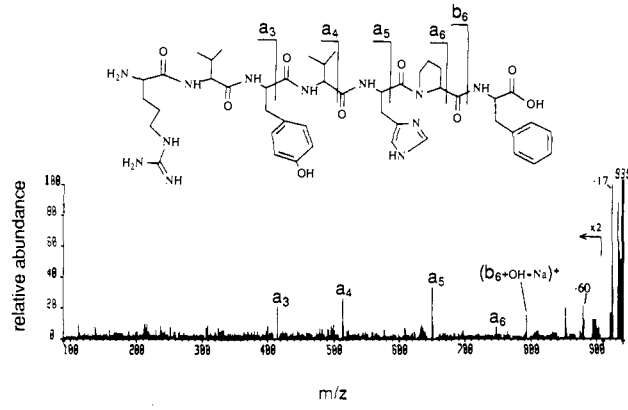


Figure 5. CID spectrum of $(M + Na)^+$ complexes of Arg-Val-Tyr-Val-His-Pro-Phe. The ions labeled a_{n-m} are described in Table I.

N-terminal $(a_{n-m} + Cat - H)^+$, C-terminal $(y_{n-m} + Cat + H)^+$, and another fragment ion from a special rearrangement reaction. Our evidence has basis in changes in fragment ion abundances with removal or addition of sources of hydrogen that can be transferred in the reactions. Further substantiation of the mechanisms is needed, which requires specific deuterium-labeling (as opposed to deuterium-exchange[t5]) experiments, determinations of kinetic isotope effects, and evaluations of changes in reaction energetics with changing substituents. The mechanisms proposed here nevertheless explain the current experimental data, and they are completely analogous to mechanisms for formation of isoelectronic ions from $(M + Cat^{2+} - H)^+$ complexes between peptides and alkaline earth metal ions.[t0] Further research either by us or by others may reveal aspects of the hydrogen-transfer reactions that are not reconcilable by our proposals, but such research can only improve our fundamental understanding of the gas-phase chemistry of peptides complexed with metal(I) ions.

One of the most important aspects of our results is that the mechanisms for formation of both a- and y-sequence ions from $(M + Cat)^+$ complexes clearly do not require precursor species that contain a deprotonated and cationized C-terminal carboxylate anion. Instead, and in accord with gas-phase ion chemistry, the alkali metal ion would most likely be bonded to basic carbonyl oxygens,[t9a,20] unless there were an amino acid that contained a strongly interacting side chain. Indeed, changes in fragmentation patterns with increasing size of the alkali metal ion can be related to intramolecular, multidentate bonding between the peptide and the metal ion, and such binding interactions have analogies in known chemistry.

All our results thus indicate that fragmentations of $(M + Cat)^+$ complexes investigated here reflect binding of the alkali metal ions to the types of sites that could be accessible in less hydrophilic interiors of proteins. Our results also support the general interpretation of Westmore and co-workers,[6] who proposed that the metal ions can interact nonspecifically and intramolecularly with several carbonyl oxygens of the peptide chain. Our results only partially support interpretations of Russell and co-workers[5] because, in contrast to their proposals, the site of alkali metal ion bonding would *not be at an amide nitrogen*, but *would be at an amide oxygen*, which is the basic site in the amide moiety.[t9a,20]

Analytically, our results provide new information about the utility of using CA of peptide–alkali metal ion $(M + Cat)^+$ complexes to determine peptide structure. Fragmentations of complexes that contain C-terminal amides yield more useful information than fragmentations of carboxylates because the amides decompose to give increased abundances of the $(y_{n-m} + Cat + H)^+$ series of sequence ions. Furthermore, the product ion from the rearrangement reaction shown in Scheme IV can be used to differentiate C-terminal amino acids that contain alkyl chains, especially Leu from Ile.

Experimental Section

Peptides were obtained either from Sigma or from the Emory University Microchemistry Center. Standard techniques[38] were used to pre-

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pare C-terminal methyl esters and permethylated derivatives. The peptides included RVYVHPF; YGGFLK and HLGLAR; RYLPT, VHLTP, YPFPG, YGGFM, FFFFF, *t*-Boc-YAG[*N*-Me]FG-ol, YGGFL, YGGFL-NH_2, FLEEI, and FLEEL; ALAL, ALAL-OCH_3, FFFF, GPRP, *N*-acetyl-GPRP-OCH_3, GPGG, VAAF, VAAF-OCH_3, AGFL, GGFL, AGFM, VIHN, GGFM, and GGFM-NH_2; ALG, GLY, MLF, GLF, GGV, GGV-OCH_3, FFF, IPI, *N*-benzoyl-GGG, *N*-benzoyl-GHL, *p*-OH-*N*-benzoyl-GHL, and AAA-OCH_3; IN, AL, LG, LG-OCH_3, FF, LL, LL-OCH_3, GH, *N*-benzoyl-GK, *N*-benzoyl-GF, and GL-NH_2; and permethylated *N*-benzoyl-GF, *N*-acetyl-LG, *N*-acetyl-GGV, *N*-acetyl-YGGFL-NH_2, and *N*-acetyl-GGFM-NH_2.

Matrices used for fast atom bombardment (FAB) were 3-nitrobenzyl alcohol, 5:1 dithiothreitol/dithioerythritol, and 2:1 thioglycerol/glycerol and were obtained from Aldrich. The $(M + Cat)^+$ complexes were prepared by mixing small amounts (micrograms) of the peptides with one of the FAB matrices, which had been previously saturated with an alkali iodide.

Mass spectrometric experiments were performed by using a VG 70-S, normal-geometry mass spectrometer. The mass spectrometer is equipped with an Ion Tech saddle-field FAB gun and a commercial FAB ion source. Precursor ions were produced by bombarding the sample with 7-keV Ar atoms at an atom gun current of 2 mA. Ions produced were accelerated to 8-keV translational energy.

Fragment ions that were formed by collision-induced decomposition (CID) in the first field-free region between the ion source and the ESA were observed by using B/E linked scans. Helium was used as collision gas at a pressure of 1×10^{-6} Torr (~50% beam reduction), as measured by the ion gauge in the first field-free region. Experiments were performed at a fragment ion resolution of approximately 1500 (10% valley), and magnet calibration was performed from a mixture of LiI, NaI, RbI, and CsI in H_2O. All spectra were acquired by using VG software, and CID spectra are the result of averaging 10-20 scans. Background spectra were acquired for all experiments to eliminate artifact fragment ions that might arise from chemical noise.

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Evidence for Facile and Selective Desulfurization: The Reactions of 2,5-Dihydrothiophene on Mo(110)

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Abstract: The reactions of 2,5-dihydrothiophene (2,5-DHT) on Mo(110) have been studied under ultrahigh vacuum conditions by using temperature-programmed reaction and X-ray photoelectron spectroscopies and found to be similar to those observed in organometallic complexes. Following adsorption of a saturation exposure of 2,5-DHT at 120 K, approximately 75% of the reactant molecules remain intact, while the remaining 25% immediately undergo sulfur elimination, yielding atomic sulfur and either weakly adsorbed butadiene (~12%) or an unidentified strongly bound hydrocarbon intermediate (~12%). During temperature-programmed reaction, the weakly adsorbed butadiene is desorbed above 140 K, while the strongly bound hydrocarbon nonselectively decomposes to gaseous dihydrogen and surface carbon. Surface sulfur is produced from both reactions. The primary reaction pathway for initially intact adsorbed 2,5-DHT is gaseous butadiene elimination. Molecular desorption near 200 K, and nonselective, irreversible decomposition beginning below 300 K are relatively minor competing pathways. Roughly two-thirds of the irreversibly adsorbed 2,5-DHT yields gas-phase butadiene and surface S in an intramolecular process, while one-third decomposes completely to H_2, surface carbon, and surface sulfur. The reactivity of 2,5-DHT is qualitatively different from the other four-carbon sulfur cycles studied under similar conditions, tetrahydrothiophene and thiophene. This is rationalized in terms of the degree of reorganization required in the transition state for intramolecular elimination, which makes such a process kinetically favorable for 2,5-DHT.

Introduction

A large number of studies aimed at advancing the understanding of industrially significant HDS reactions have employed thiophene (*c*-C_4H_4S) as a model substrate. Despite these efforts, the mechanism for thiophene desulfurization is not yet understood in detail, and a considerable amount of debate has been engendered over the principal reaction models. In one scheme, partial or full hydrogenation of the thiophene ring precedes desulfurization.[1] Alternatively, hydrogenolysis of the carbon-sulfur bonds may directly occur, forming 1,3-butadiene.[2] In a third model, best described as dehydrosulfurization, surface hydrogen is not required at all for thiophene desulfurization, as β-hydrogen elimination can produce diacetylene as the initial hydrocarbon product.[3]

On the basis of a recent series of organometallic studies, the mechanistic pathway for thiophene hydrodesulfurization processes has been proposed to proceed via nuclephilic attack at the 2-position by hydride species, followed by protonation at the 3-position to give an η^3-2,3-dihydrothiophene intermediate.[4,5] This intermediate isomerizes to a more thermodynamically favorable sulfur-bound 2,5-dihydrothiophene (2,5-DHT), which can then eliminate butadiene.[6,7]

Previous ultrahigh vacuum studies in our laboratory have examined the reactions of thiophene (*c*-C_4H_4S)[8,9] and tetrahydro-

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