Gas-Phase Interactions of Lithium Ions and Dipeptides

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Abstract: FAB and tandem mass spectrometry have been used to investigate the gas-phase interactions of lithium ions and dipeptides. Lithiated dipeptides decompose as metastable ions, producing two amino acid ions, those corresponding to the N-terminus ($[B_1 + Li + OH]^+$) and the C-terminus ($[Y_1 + Li + H]^+$). The lithium ion interacts with the carboxylate anion of the C-terminus in a first step. The relative abundances of the fragment ions depend upon the site of protonation, the stability of the lithiated amino acid formed, and the stability of the imine lost. For aliphatic dipeptides, the relative abundances of the two amino acid fragment ions are approximately equal; however, if one amino acid contains a side chain of high proton affinity (e.g., His, Arg, Lys), that amino acid fragment ion (as a $[M + Li]^+$ ion) dominates. Other side chains such as Phe, Met, and Ser show intermediate behavior. Upon collisional activation, the production of the $[M + Li]^+$ of the C-terminus amino acid becomes more dominant, indicating this reaction is kinetically favored for higher energy ions. $[M + Li]^+$ ions of His, Arg, and Lys, however, still dominate, making sequencing difficult. Structure determination, however, is still possible by using less abundant ions (e.g., $[A_1 + Li - H]^+$ ions and those from side chain losses). The interactions of sodium and potassium ions with peptides are similar to that of lithium; however, the lower polarizing power of K^+ dramatically reduces the formation of the N-terminus amino acid ion.

Many of the metal ions that are important in enzymatic reactions in biology are bound by proteins.[1] The study of the metal ion–protein interactions is, therefore, of fundamental importance. Because of the complexity of these enzyme systems, it is necessary to investigate simple molecules still containing the essential features and reactive sites of the larger proteins. Mass spectrometry offers one opportunity.

The advantages of FAB and tandem mass spectrometry for peptide sequencing and for analysis of peptide mixtures are better-known than its applicability for assessing metal ion interactions.[2] The structural utility of mass-selecting the cationized peptide species (i.e., $[M + Cat]^+$ where Cat is an alkali metal ion) in MS/MS studies has been reported by us[3] and by others.[4-7]

Mallis and Russell[4a] were the first to suggest that the decompositions of a gas-phase peptide $[M + Na]^+$ ion should reveal the site of interaction of the metal ion. Specifically, the attachment of Na^+ to the tripeptide benzoyl-GlyHisLeu enhances specific fragmentations that are complementary in information content to those from collisional activation of the $[M + H]^+$ species. The results suggest that the binding of a metal ion to the peptide is more selective compared to that for a proton, and, more specifically, that the type of fragment ions point to metal ion attachment at the N-terminus. Decompositions of protonated peptides, likewise, have been explained by correlating specific peptide fragmentations to the site of protonation.[8,9] Metal ion directed fragmentations have also been observed upon CA of alkali cationized sugars,[10] nucleosides,[11] and nucleotides.[4c,12]

Russell and co-workers[4b] recently extended their study to include di- and tripeptides containing basic amino acid residues. Decompositions of $[M + H]^+$ and $[M + Na]^+$ ions were explained again by charge retention (i.e., protonation or metal ion attachment) by fragment ions containing the basic amino acid. The attachment of the Na^+ ion at sites of high proton affinity was thought to be consistent with the observed fragment ions although no mechanisms for their formation were explicitly proposed. For small peptides containing histidine, lysine, arginine, or tyrosine residues, the Na^+ ion was postulated to interact with the amino terminus, the amide nitrogen, and the nitrogens of the basic side chain. Formation of the fragment ions was viewed as simple cleavages of bonds at the C-terminus, a segment of the peptide that does not interact with the metal ion.

Unfortunately, some of the key fragment ions reported in Russell's work[4] were not assigned correctly, possibly because of mass measurement difficulties in the MIKES experiments. These mass assignment errors (±1 u) prevented the correct assignment of fragment structures; thus, a reasonable mechanism for formation of these ions could not be formulated, and, in our opinion, the nature of the metal ion interaction was incorrectly deciphered.[3] In addition, peptide $[M + Cat]^+$ ions desorbed by FAB and SIMS decompose as metastable ions,[3,6] and these were not addressed in Russell's work.

Standing and co-workers,[6] on the other hand, did study the metastable ion decompositions of leucine- and methionine-enkephalin $[M + Na]^+$ ions in a time-of-flight (TOF) mass spectrometer. On the basis of labeling studies, they correctly identified the principal decomposition as the loss of the C-terminal residue and proposed a mechanism involving association of the sodium ion at the C-terminus. In our previous paper,[3] we also gave substantial evidence in support of a similar mechanism.[5] Moreover, we demonstrated that the C-terminal loss also occurs upon CA and that CA induces migration of the metal ion from the C-terminus to give interactions with specific amino acid residues.[3] The importance of this gas-phase chemistry for understanding metal ion–enzyme interactions was also suggested.[3]

It is now clear on the basis of metastable ion spectra that the

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(1) Hay, R. W. *Bio-Inorganic Chemistry*; Ellis Horwood Limited: Chichester, 1984. Eichhorn, G. L., Ed. *Inorganic Biochemistry*; Elsevier: Amsterdam, 1973; pp 203-548.

(2) (a) Hunt, D. F.; Bone, W. M.; Shabanowitz, J.; Rhodes, J.; Ballard, J. M. *Anal. Chem.* **1981**, *53*, 1704-1706. 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. (b) Lippstreu-Fisher, D. L.; Gross, M. L. *Anal. Chem.* **1985**, *57*, 1174-1180. (c) Biemann, K.; Martin, S. A. *Mass Spectrom. Rev.* **1987**, *6*, 1-76. Biemann, K.; Scoble, H. A. *Science* **1987**, *237*, 992-998. Biemann, K. *Anal. Chem.* **1986**, *58*, 1288A-1300A. Martin, S. A.; Biemann, K. *Int. J. Mass Spectrom. Ion Proc.* **1987**, *78*, 213-228.

(3) Grese, R. P.; Cerny, R. L.; Gross, M. L. *J. Am. Chem. Soc.* **1989**, *111*, 2835-2842.

(4) (a) Mallis, L. M.; Russell, D. H. *Anal. Chem.* **1986**, *58*, 1076-1080. (b) Russell, D. H.; McGlohon, E. S.; Mallis, L. M. *Anal. Chem.* **1988**, *60*, 1818-1824. (c) Mallis, L. M.; Russell, D. H. *Int. J. Mass Spectrom. Ion Proc.* **1987**, *78*, 147-178.

(5) Renner, D.; Spiteller, G. *Biomed. Mass Spectrom.* **1988**, *15*, 75-77.

(6) Tang, X.; Ens, W.; Standing, K. G.; Westmore, J. B. *Anal. Chem.* **1988**, *60*, 1791-1799. Standing, K. G.; Beavis, R.; Ens. W.; Tang, X.; Westmore, J. B. In *The Analysis of Peptides and Proteins by Mass Spectrometry*; McNeal, C. J., Ed.; Wiley: New York, 1988; pp 267-278.

(7) Leary, J. A.; Williams, T. D.; Bott, G. *Rapid Comm. Mass Spectrom.* **1989**, *3*, 192.

(8) Hunt, D. F.; Buko, A. M.; Ballard, J. M.; Shabanowitz, J.; Giordani, A. B. *Biomed. Mass Spectrom.* **1981**, *9*, 397-408.

(9) Williams, D. H.; Bradley, C. V.; Santikarn, S.; Bojesen, G. *Biochem. J.* **1982**, *201*, 105-117.

(10) Cerny, R. L.; Tomer, K. B.; Gross, M. L. *Org. Mass Spectrom.* **1986**, *21*, 655-660.

(11) Gross, M. L.; Tomer, K. B.; Cerny, R. L.; Giblin, D. E. In *Mass Spectrometry in the Analysis of Large Molecules*; McNeal, C. J., Ed.; Wiley: New York, 1986; pp 171-190.

(12) Mallis, L. M.; Raushel, F. M.; Russell, D. H. *Anal. Chem.* **1987**, *59*, 980-984.

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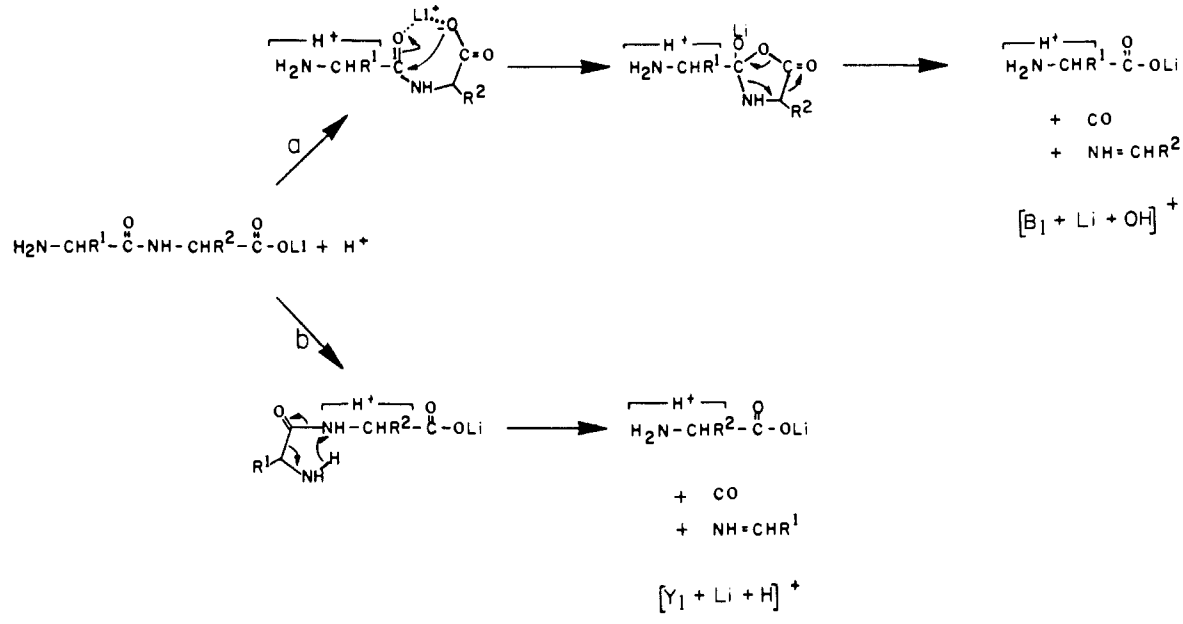
Scheme I



most favorable, intrinsic interaction of a peptide and alkali metal ion is at the C-terminal carboxylate. When Li^+ or Na^+ are the metal ions, the peptide–metal ion complex decomposes by loss of the C-terminal amino acid residue (as CO and an imine). The K^+ ion is less effective in promoting this loss. Addition of internal energy (e.g., by collision) causes the peptide–metal ion complex to isomerize to a new complex in which the metal ion is bound to a deprotonated amide nitrogen (see Scheme IV in ref 3). Decomposition now leads to an immonium ion with the N–H replaced by an N–metal bond (an A-type ion according to Roepstorff notation[13]). This interaction is particularly stable if the metal ion can also be coordinated by the adjoining side chains of histidine, arginine, and aromatic amino acids.

In this paper, we report a study of the gas-phase interactions of dipeptides and alkali metal ions; furthermore, we examine the utility of MS/MS of cationized dipeptides for sequence information. Dipeptides are the simplest substances containing a peptide bond, and the vacuum is the simplest medium. Therefore, dipeptides are the starting point for establishing the ground rules for interactions of peptides and metal ions in the gas phase. Prior to this study, the collisional activation decompositions of only two cationized dipeptides, the $[M + Na]^+$ ions of gly-his and gly-lys, were reported.[4b]

Dipeptides contain at least three possible coordination sites: the N-terminal amino group, the C-terminal carboxyl group, and the one peptide bond. Dipeptides composed of one or two amino acids containing chelatable side chains may offer additional sites for metal ion binding. These additional sites may play an important role in defining active centers of metalloenzymes in biological systems.

The effects of side chains on the interaction of dipeptides and transition metals, especially Cu^{2+}, in solution are of interest.[14] Recently the binding of dipeptides to immobilized Ni^{2+} was studied to define the influence of side chains in immobolized metal affinity chromatography (IMAC).[15] The retention behavior of a series of dipeptides on columns containing immobolized Co^{2+}, Ni^{2+}, and Cu^{2+} identified a dipeptide sequence that can be used to derivatize the N-terminus of proteins to facilitate their removal by IMAC.[16] Amino acid residues implicated in those studies include histidine, cysteine, tryptophan, and tyrosine.

The identification of dipeptides in mixtures is also important in medicine and biology. For example, the presence of targeted dipeptides in the urine of patients was used as a clinical indication of several abnormal metabolic conditions.[17] In addition, the sequencing of large peptides by examination of the dipeptide mixture resulting from enzymatic digestion by dipeptidylaminopeptidase I[18] is a possible alternative to more classical Edman degradation methods.[19]

Various mass spectrometric methods have been used for analysis of dipeptides; these include the following: electron ionization (EI),[20] chemical ionization (CI),[17a,19b,21] gas chromatography/mass spectrometry (GC/MS),[17a,19a] and fast-atom bombardment (FAB).[22] Of these methods, only FAB does not require derivatization prior to analysis; moreover, FAB of dipeptides provides molecular weight and amino acid information but not sequence, especially if the sample is contaminated with amino acids. FAB combined with MS/MS offers an attractive method for unambiguously sequencing dipeptides. A strategy for sequencing dipeptides by using the metastable and CA mass spectra of the protonated species was recently reported.[22]

Results and Discussion

Metastable Decompositions of Dipeptide $[M + Li]^+$ Ions. In our first paper on peptide–metal ion interactions,[3] we presented evidence that lithiated tri- to nonapeptides (i.e., $[M + Li]^+$) decompose as metastable ions by loss of the C-terminal amino acid residue, thus producing a new peptide shortened by one less amino acid. These fragments, denoted as $[B_n + Li + OH]^+$, are generally the most abundant ions in the metastable ion spectra and can be used analytically to identify the C-terminal amino acid. In the decomposition of a lithiated dipeptide, the resulting fragment ion is the lithiated N-terminal amino acid.

(13) Roepstorff, P.; Fohlman, J. *Biomed. Mass Spectrom.* **1984**, *11*, 601.
(14) Yamauchi, O.; Tsujide, K.; Odani, A. *J. Am. Chem. Soc.* **1985**, *107*, 659–666. Rabenstein, D. L.; Daignault, S. A.; Isab, A. A.; Arnold, A. P.; Shoukry, M. M. *J. Am. Chem. Soc.* **1985**, *107*, 6435–6439. Bonomo, R. P.; Cali, R.; Cucinotta, V.; Impellizzeri, G.; Rizzarelli, E. *Inorg. Chem.* **1986**, *25*, 1641–1646. Kiss, T.; Szücs, Z. *J. Chem. Soc., Dalton Trans.* **1986**, 2443–2447.
(15) Hemdan, E. S.; Porath, J. *J. Chromatogr.* **1985**, *323*, 265–272.
(16) Smith, M. C.; Furman, T. C.; Pidgeon, C. *Inorg. Chem.* **1987**, *26*, 1965–1969.
(17) (a) Charpentier, C.; Johnstone, R. A. W.; Lemonnier, A.; Myara, I.; Rose, M. E.; Tuli, D. *Clin. Chim. Acta* **1984**, *138*, 299–308. (b) Kamerling, J. P.; Aarsen, G. J.; Duran, M.; De Bree, P. K.; van Sprang, F. J. *Clin. Chim. Acta* **1980**, *102*, 137–145. (c) Faull, K. F.; Schier, G. M.; Schlesinger, P.; Halpern, B. *Clin. Chim. Acta* **1976**, *70*, 313–321.
(18) McDonald, J. K.; Zeitman, B. B.; Reilly, T. J.; Ellis, S. *J. Biol. Chem.* **1969**, *244*, 2693–2709.
(19) (a) Caprioli, R. M.; Seifert, W. E. Jr.; Sutherland, D. E. *Biochem. Biophys. Res. Commun.* **1973**, *55*, 67–75. Schier, G. M.; (b) Bolton, P. D.; Halpern, B. *Biomed. Mass Spectrom.* **1976**, *3*, 32–40.
(20) Kraft, R.; Büttner, D.; Franke, P.; Etzold, G. *Biomed. Environ. Mass Spectrom.* **1987**, *16*, 5–7.
(21) Nagasawa, H. T.; Magnan, D. J.; Foltz, R. L. *Biomed. Mass Spectrom.* **1982**, *9*, 252–256.
(22) Kulik, W.; Heerma, W. *Biomed. Environ. Mass Spectrom.* **1988**, *17*, 173–180.

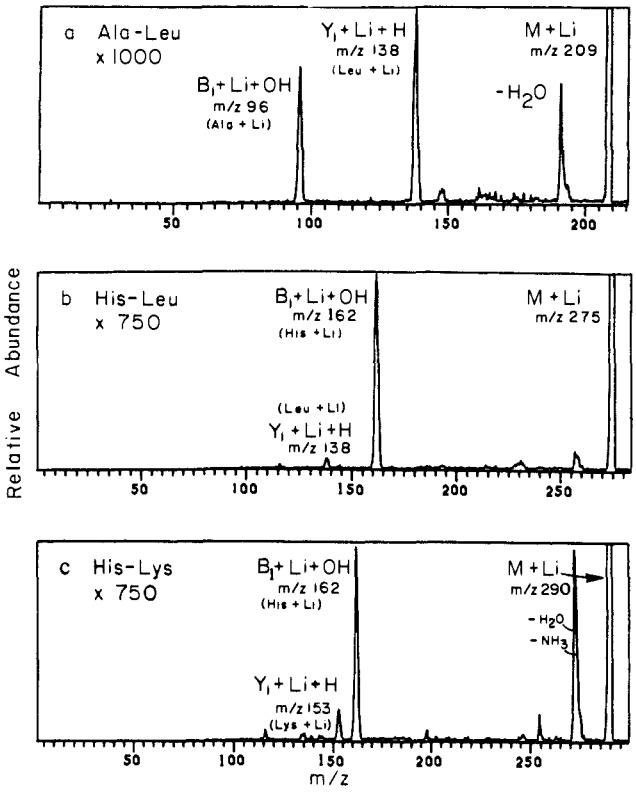


Figure 1. Metastable ion MS/MS spectra of lithiated dipeptides: (a) $[M + Li]^+$ ion of Ala-Leu, m/z 209; (b) $[M + Li]^+$ ion of His-Leu, m/z 275; and (c) $[M + Li]^+$ ion of His-Lys, m/z 290.

In contrast to the general fragmentation of larger peptides, dipeptide $[M + Li]^+$ ions decompose to give both the lithiated C-terminal amino acid (denoted as $[Y_1 + Li + H]^+$) and the N-terminal amino acid (see Scheme I). The metastable ion MS/MS spectra of three dipeptide $[M + Li]^+$ ions, presented in Figure 1, are typical examples.

To investigate the nature of the competing reactions, we examined the decompositions of 21 lithium ion–dipeptide complexes containing various amino acids. The relative abundances of the N- and C-terminus amino acid ions are listed in Table I. For convenience, Table I is divided into three sections: one for dipeptides containing aliphatic side chains, another for those with nonaliphatic side chains (Ser, Met, and Phe), and another for those with basic side chains (Arg, His, and Lys).

Aliphatic dipeptide $[M + Li]^+$ ions decompose to give equal abundances of both the lithiated N-terminal and C-terminal amino acid ions. The metastable decompositions of the $[M + Li]^+$ ion of Ala-Leu (Figure 1a) are representative of the aliphatic dipeptides. Slightly more $[Y_1 + Li + H]^+$ (lithiated leucine, m/z 138) than the $[B_1 + Li + OH]^+$ ion (lithiated alanine, m/z 96) is produced. The isomer, Leu-Ala, likewise produces slightly more $[Y_1 + Li + H]^+$. If one of the amino acids is a glycine, however, the ion of m/z 82 (lithiated glycine) is generally more abundant presumably because of product stability: the imine from glycine, $NH{=}CH_2$, is less stable than imines from the other amino acids.

Dipeptides composed of an aliphatic amino acid and a nonaliphatic amino acid also decompose producing both N- and C-terminal lithiated amino acids; however, the nonaliphatic amino acid residue becomes the more abundant fragment ion. For example, the $[M + Li]^+$ ion of His-Leu decomposes to yield predominantly lithiated histidine (m/z 162) (Figure 1b). More revealing is comparison of the decompositions of the isomers listed in Table I (Phe-Leu/Leu-Phe, Lys-Val/Val-Lys, and His-Ala/Ala-His). For all of these dipeptides, the more abundant fragment ion corresponds to the nonaliphatic residue.

The third group of dipeptides is those containing two nonaliphatic amino acids. Depending on the amino acids present, one residue may dominate, as is the case with His-Lys, which decomposes as a metastable ion to produce primarily the histidine ion (Figure 1c). In comparison, isomers Phe-Met and Met-Phe both yield the $[M + Li]^+$ of the C-terminal amino acid as slightly more abundant.

Table I. Relative Abundances of Fragment Ions from Metastable Decomposition of Dipeptide $[M + Li]^+$ Ions[a]

dipeptide	N-terminus amino acid[b]	C-terminus amino acid[c]	other abundant ions[d]
Ala-Gly	18	100	-H_2O
Gly-Ala	100	13	-H_2O
Leu-Gly	14	100	-H_2O
Gly-Leu	100	20	-H_2O, -CO_2
Ala-Ile	100	99	-H_2O
Ala-Leu	70	100	-H_2O
Leu-Ala	47	100	-H_2O
Gly-Ser	13	100	-H_2O
Gly-Phe	23	100	Phe-OCOOH
Hip-Phe	100	15	
Leu-Phe	15	100	
Phe-Leu	100	17	
Met-Phe	75	100	
Phe-Met	96	100	
Val-Lys	5	100	-NH_3
Lys-Val	100	4	-H_2O
Ala-His	5	100	
His-Ala	100	25	
His-Leu	100	5	
His-Lys	100	16	-NH_3
Arg-Ala	100	8	-NH_3, -$NH_2C(NH)NH_2$, Arg-HCOOH
Arg-Asp	100		-H_2O, -$2H_2O$, -CO_2

[a] All fragment ions contain Li^+. [b] $[B_1 + Li + OH]^+$. [c] $[Y_1 + Li + H]^+$. [d] Resolution of MIKES insufficient to completely resolve NH_3 and H_2O losses.

N-Terminus vs C-Terminus Amino Acid Ions. The mechanisms for formation of the N- and C-terminus ions are shown in Scheme I. We propose that interaction of the alkali cation occurs at the carboxylic group and that the decomposition that occurs depends on the site of protonation. If protonation occurs on the N-terminal residue (amine or R^1), reaction Ia proceeds with loss of CO and the imine to give the lithiated N-terminal amino acid. The reaction is driven by complexation of the alkali metal at the C-terminus and polarization of the carbonyl of the amide bond. This interaction enables the nucleophilic attack of the negative carboxylate oxygen and subsequent formation of the $[B_1 + Li + OH]^+$ ion.

On the other hand, if protonation occurs at the C-terminal residue (amide nitrogen or R^2), then reaction Ib occurs with hydride ion transfer form the amine and subsequent loss of CO and the imine to give the lithiated C-terminal amino acid ion. The hydride transfer is similar to that proposed for the formation of $[Y_n + 2H]^+$ ions from protonated peptides.[23] Mueller et al.[23] have suggested that the neutral lost from peptide $[M + H]^+$ ions may be a 3-membered ring cyclic amide instead of the combination of CO and an imine proposed here. The $[Y_n + Li + H]^+$ or C-terminal amino acid ions are of low abundance or not observed for larger peptides because the transfer of the hydride from an N-terminus *amine* involves an unfavorably large ring and the transfer of an amide hydride is also not favored. Decompositions of Hip-Phe (benzoyl-Gly-Phe) $[M + Li]^+$ ion support this hypothesis; the fragmentation gives predominantly the N-terminus amino acid ion (Table I).

The formation of abundant "Y-type" ions from the collisional decompositions of dilithiated penta- and hexapeptides (i.e., $[M - H + 2Li]^+$), recently reported by Leary et al.,[7] is consistent with our mechanism. We suggest, however, that both lithium ions bind at the C-terminus and that the formation of the "Y-type" ions occurs via hydride transfer remote to the C-terminal charge site. Although this process is disfavored for $[M + Li]^+$ ions, it occurs for $[M - H + 2Li]^+$ because the alternate pathway (i.e., loss of the C-terminal residue) is prevented owing to the presence of both

(23) Mueller, B. R.; Eckersley, M.; Richter, W. T. *Org. Mass Spectrom.* **1988**, *23*, 217–222.

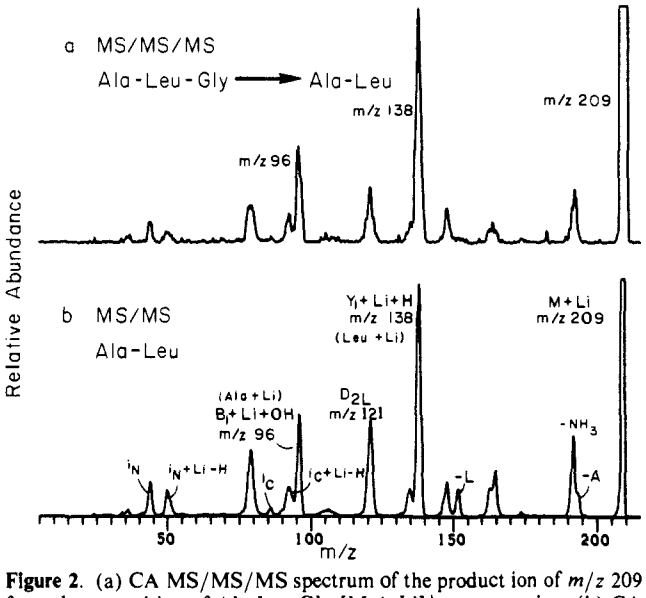


Figure 2. (a) CA MS/MS/MS spectrum of the product ion of m/z 209 from decomposition of Ala-Leu-Gly $[M + Li]^+$ precursor ion; (b) CA MS/MS spectrum of Ala-Leu $[M + Li]^+$ ion, m/z 209.

lithiums at the C-terminus. The formation of the C-terminus amino acid ions from $[M + Li]^+$ of dipeptides in which the C-terminal side chain is protonated occurs via a similar charge-remote mechanism.

The consecutive reactions of tripeptides as studied by MS/MS/MS are also consistent with this mechanism. Ala-Leu $[M + Li]^+$ ion produced by collisional activation (CA) of the tripeptide Ala-Leu-Gly decomposes in a nearly identical manner as that of the authentic lithiated dipeptide (Figure 2). This is consistent with the interaction of the metal ion at the C-terminus in both reactions.

The collisionally induced decompositions of the lithiated amino acid ion products are also identical with those of suitable references. For example, CA of $[M + Li]^+$ ions of Leu-Phe and Phe-Leu gives an ion of m/z 172. MS/MS/MS experiments show that the structure of this product ion is identical with the $[M + Li]^+$ of phenylalanine, in accord with the proposed mechanism.

The competition between the formation of the two amino acid ions from dipeptides is a function of the stabilities of the ion formed and the neutral lost and the proton affinities of the amino acid residues. When arginine, histidine, or lysine are present in a dipeptide, their basic sites are protonated and it is the $[M + Li]^+$ ions of these amino acids that are formed. Biemann and co-workers[24] recently concluded that for peptides containing a basic amino acid, the majority of the $[M + H]^+$ ions have a proton at that residue. The greater part of the fragmentations are then thought to occur remote to the charge site.

This mechanism is in contrast to conclusions put forth by Russell and co-workers,[4] who proposed that the side chains of basic amino acids interact with the metal ion. Their proposal is based on the hypothesis that side chain proton affinities reflect alkali metal ion affinities and on the alleged facile fragmentations to lose pieces of the non-interacting C-terminus. These N-terminal amino acids were mistakenly mass assigned and identified in the Russell study as arising from losses of the pieces of the C-terminus rather than from the rearrangement given in Scheme Ia. We have included in this study two dipeptides, AlaHis and ValLys, that are similar to those studied by Russell and co-workers. When complexed to Li^+, the dipeptides give both the C-terminal and the N-terminal amino acid $[M + Li]^+$ ions. The latter ions are best explained by interaction of the Li^+ at the C-terminal carboxylate.

It is especially interesting that the $[M + Li]^+$ ion of His-Lys decomposes to give predominantly the histidine ion (Figure 1c). This indicates that the gas-phase proton affinity of the imidazole group of histidine is greater than that of lysine, even though in solution the basicity of the lysine side chain is four orders of magnitude larger than that of the histidine side chain (pK_a' 10.53 and 6.0, respectively).[25] This is consistent with the findings of Bojesen,[26] who ordered the basic amino acids by their gas-phase proton affinities as Arg > His > Lys > Trp. It was suggested that the anomalously high proton affinity of histidine residues may be due to geometric factors between the pyridine nitrogen of the imidazole ring and an amide nitrogen.[4b]

Table II. Literature Gas-Phase Proton Affinities of Various Amino Acids

amino acid	PA,[a] kcal/mol	amino acid	PA,[a] kcal/mol
glycine	211.6, 208.2[b]	leucine	218.1, 214.5
alanine	214.8, 212.2	isoleucine	218.9
phenylalanine	216.5, 215.1	methionine	221.4
aspartic acid	216.7	lysine	230.3
serine	216.8	histidine	231.9
valine	217.0, 213.9	arginine	*c*

[a] Reference 27. [b] Reference 28. [c] PA value not available.

According to the mechanism for decomposition of dipeptides, proton affinities should be useful for predicting which amino acid will dominate the fragmentation. Literature values of the gas-phase proton affinities of the amino acids contained in the dipeptides in this study are listed in Table II.[27,28] For example, the gas-phase proton affinity of lysine was estimated to be greater than that of valine.[27] If this is true, protonation of the isomers Lys-Val and Val-Lys should predominantly be at the lysine residue and the subsequent metastable decompositions should produce abundant lithiated lysine (m/z 153).

Several of the decompositions of the dipeptides cannot be explained solely by the relative proton affinities of the amino acid residues. For example, we have already discussed the effect of the low stability of the glycine imine that is responsible in the low abundances of the other amino acid in aliphatic dipeptides containing glycine.

The dominance of the phenylalanine fragments in the decomposition of $[M + Li]^+$ ions of Phe-Leu and Leu-Phe is surprising because the proton affinity of phenylalanine is not much larger than that of leucine. The abundant Phe fragment ions, however, may result because of an enhanced stability of the amino acid–metal ion complex owing to a weak attractive interaction between the aromatic side chain and the metal ion. Similar attractive interactions have been used to explain the greater stability of Cu^{2+} and phenylalanine, tyrosine, and tryptophan in solution.[29] We suspect that the stability of the histidine–metal ion complex, likewise, is a result of not only the increased proton affinity but also the "anchoring" effect of the imidazole on the alkali metal ion. Interaction of the imidazole ring with the metal ion in the collisional activation of peptide $[M + Li]^+$ ions was used to explain the abundant $[A_n + Li - H]^+$ product ions formed at histidine residues.[3]

Collisional Activation of Cationized Dipeptides. Although the metastable decompositions of lithiated dipeptides can be explained in terms of relative proton affinities, it would be difficult to sequence an unknown dipeptide on the basis of this interaction. Collisional activation (CA), on the other hand, provides additional sequence information.

Generally, the abundances of the C-terminus amino acid ion increase more than those of the N-terminus ion upon collisional activation. The formation of the former ion (Scheme Ib) is kinetically favored for higher internal energy ions. Dipeptides containing basic amino acids at the N-terminus (e.g., Arg, Lys,

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

(25) Lehninger, A. L. *Principles of Biochemistry*; Worth Publishers: New York, 1982; p 107.

(26) Bojesen, G. *J. Am. Chem. Soc.* **1987**, *109*, 5557–5558. Bojesen, G. *J. Chem. Soc., Chem. Commun.* **1986**, 244–245.

(27) Lias, S. G.; Liebman, J. F.; Levin, R. D. *J. Phys. Ref. Data* **1984**, *13*, 695–808.

(28) Meot-Ner (Mautner), M.; Hunter, E. P.; Field, F. H. *J. Am. Chem. Soc.* **1979**, *101*, 686–689.

(29) Martin, R. B. In *Metal Ions in Biological Systems*; Sigel, H., Ed.; Marcel Dekker, Inc.: New York, 1979; Vol. 9, pp 1–39.

Table III. Relative Abundances of Sequence Ions Produced by Collisional Activation of $[M + Li]^+$ of Dipeptides[a]

dipeptide	N-terminus amino acid[b]	C-terminus amino acid[c]	-N[g]	-C[h]	i_N[i]	i_N + Li - H[j]	i_C[k]	i_C + Li - H[l]
Ala-Gly	15	100	17	22	20	16	1	7
Gly-Ala	88	100	68	5	12	11	7	32
Leu-Gly	39	100	*d*	0	21	18	4	5
Gly-Leu	93	100	0	*d*	1	8	6	22
Ala-Ile	58	100	11	15	12	8	6	25
Ala-Leu	44	100	10	12	15	11	3	13
Leu-Ala	21	100	21	4	17	12	9	18
Gly-Ser	25	100	38	41	8	8	4	16
Gly-Phe	31	100		6	2	5	15	79
Leu-Phe	15	100	19	9	7	5	6	17
Phe-Leu	100	56	24	11	18	24	1	5
Met-Phe	40	100	49	10	3	5	4	16
Phe-Met	65	100	46	27	16	23	3	13
Val-Lys	14	100	50	12	14	18	1[e]	33
Lys-Val	100	80	43	12	0[e]	76	4	16
Ala-His	7	100	7	3	2	1	3	24
His-Ala	83	25	40	9	25	100	1	3
His-Leu	100	23	29	21	15	64	5	5
His-Lys	77	24	32	35	24	100	30[e]	48
Arg-Ala	84	33	87	25	4[f]	100	2	15
Arg-Asp	100	77	69	23	*f*	50		

[a] Other ions not listed such as $[M + Li - H_2O]^+$, $[M + Li - NH_3]^+$ may be more abundant than those ions listed in this table. [b] $[B_1 + Li + OH]^+$. [c] $[Y_1 + Li + H]^+$. [d] Loss of C_4H_9 from leucine is the same mass as loss of glycine residue. [e] Relative abundance of ion of m/z 84 instead of less abundant immonium ion of m/z 101. [f] Relative abundance of ion of m/z 70 instead of less abundant immonium ion of m/z 129. [g] -N: ion due to loss of N-terminus side chain. [h] -C: ion due to loss of C-terminus side chain. [i] i_N: immonium ion of N-terminal amino acid. [j] i_N + Li - H: lithiated immonium ion of N-terminus amino acid (=$[A_1 + Li - H]^+$). [k] i_C: immonium ion of C-terminal amino acid. [l] i_C + Li - H: lithiated immonium ion of C-terminus amino acid.

His) fragment upon CA to give more abundant N-terminus ions; however, the abundances of the C-terminus ions increase substantially upon CA. For example, the N-terminus amino acid ion from the $[M + Li]^+$ species of Lys-Val increases by a factor of 7 upon CA whereas the C-terminus amino acid ion increases by a factor of 150. It is likely that increasing the internal energy allows the proton to shift from basic sites (e.g., at Lys) to less basic sites (e.g., the peptide bond) permitting fragmentation to give lithiated Val. Table III lists the relative abundances of the ions produced by CA of the $[M + Li]^+$ ions of the dipeptides studied.

The CA mass spectrum of His-Leu $[M + Li]^+$ (Figure 3a) is representative of the collisionally induced decompositions of lithiated dipeptides. The ions of m/z 162 and 138 are the N- and C-terminus amino acid ions, respectively. The dominance of the histidine residue is expected because of the large difference between the gas-phase proton affinities of histidine (231.9 kcal/mol) and leucine (218.1 kcal/mol). Fragment ions in the range m/z 50 to 150 are from further decomposition of the N- and C-terminus amino acid ions and are consistent with CA decompositions of lithiated histidine and leucine. The ions of m/z 110 and 116 are the immonium ion and the lithiated immonium ion originating from the histidine residue. The ion of m/z 116 may also be denoted as an $[A_1 + Li - H]^+$ ion and is an abundant ion in the CA of dipeptides containing N-terminal Lys, Arg, His, and Phe groups. The lithiated immonium ion originating from the leucine residue (m/z 92) is also produced.

Higher mass ions of m/z 258, 231, and 229 are due to losses of NH_3, CO_2, and HCOOH, respectively. The ions of m/z 194 and 218 are formed from the losses of the amino acid side chains of histidine and leucine, respectively, as radicals. These ions are labeled in the spectra with the appropriate amino acid single letter code preceded by a minus sign in accordance with the nomenclature used by Martin and Biemann.[30] Mass assignments of these unusual radical losses were confirmed by using CA with linked scans at constant B/E. Generally, the ion from loss of the N-terminal residue is more abundant and thus indicative of the sequence. The ion of m/z 187, a so-called D_{2L} fragment formed by the loss of 88 amu, identifies the C-terminus as a leucine residue.[24] The loss of 88 amu is also seen in the CAD of the Ala-Leu $[M + Li]^+$ ion to give the ion of m/z 121 (Figure 2); in contrast, collisional activation of Ala-Ile $[M + Li]^+$ produces an abundant ion of m/z 135, identifying isoleucine as the C-terminal residue in that dipeptide.

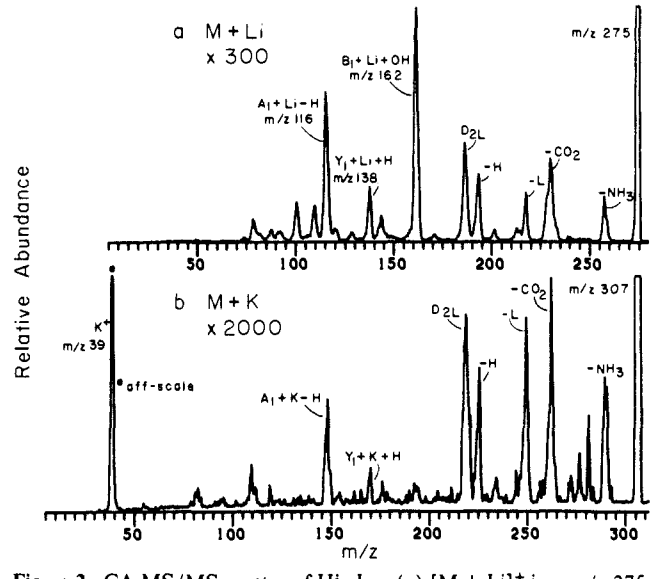
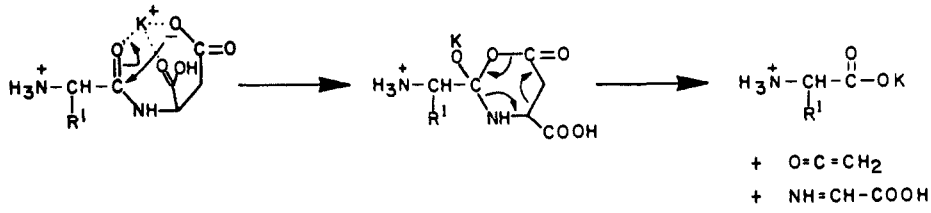


Figure 3. CA MS/MS spectra of His-Leu (a) $[M + Li]^+$ ion, m/z 275, and (b) $[M + K]^+$ ion, m/z 307.

Interaction with Other Alkali Metals. In addition to decompositions of $[M + Li]^+$ ions, we also investigated those of dipeptides cationized with other alkali metal ions. Generally, the decomposition of $[M + Na]^+$ ions is similar to those of the $[M + Li]^+$ species. For complexes of dipeptides and other Group I metals, however, the most abundant fragmentation is usually the release of the metal ion, an indication of the decreasing bond energies as one descends the alkali series (Li^+, Na^+, K^+). Mallis and Russell[4a] showed that the binding energy of alkali metal ions to peptides correlates well with the ionic radius of the metal. The decompositions of cationized dipeptides follow similar trends; the potassium ion is preferentially released upon collisional activation of dipeptide $[M + K]^+$ ions.

(30) Martin, S. A.; Biemann, K. *Int. J. Mass Spectrom. Ion Proc.* **1987**, *78*, 213–228.

Scheme II



The CA mass spectra of His-Leu $[M + K]^+$ and $[M + Li]$ ions (Figure 3, a and b) reveal that the N- and C-terminus amino acid ions are considerably attenuated for the $[M + K]^+$ species. The interaction of the potassium with the dipeptide is similar to that of lithium as evidenced by the formation of the C-terminus amino acid ion and similar minor fragment ions from both species. In the decomposition of the $[M + K]^+$ ion, the dramatic reduction in the formation of the N-terminus amino acid ion reveals that potassium is less effective in polarizing the carbonyl of the amide bond, and the abundant K^+ ion reveals that the binding with the carboxylate group is weaker. Potassium ions are considerably larger and have less polarizing power than lithium ions (2.80 and 0.56 z/r^2 for K^+ and Li^+, respectively).[31] In addition, the lower polarizing power of potassium may also be a result of a different potassium–carboxylate conformation. Recent studies modeling metal ion–carboxylate interactions suggest that the larger alkali metal ions prefer a syn conformation and may bind out of the plane of the carboxyl group.[32] In accord with our mechanism (Scheme I), the reduced polarizing power of potassium affects the formation of the N-terminus amino acid ion more than that of the C-terminus ion.

Although the $[B_1 + K + OH]^+$ and $[Y_1 + K + H]^+$ ions are of low abundance, the sequence can still be determined. The m/z 226 and 250 ions due to loss of the side chain radicals point to the presence of His and Leu (or isoleucine) residues. The abundant ion of m/z 148 is identified as the $[A_1 + K - H]^+$ of the histidine residue, indicating histidine as the N-terminus. C-terminal leucine is confirmed by the D_{2L} ion of m/z 219 (loss of 88 amu), as discussed above.

The metastable ion decompositions of dipeptide $[M + K]^+$ ions are generally weak and uninformative. An exception is the metastable decomposition of the $[M + K]^+$ ion of Arg-Asp. Although the most abundant ion is due to loss of H_2O (100% relative abundance) from the molecular species, a $[B_1 + K + OH]^+$ (m/z 213) ion (50% relative abundance) and a $[B_1 + K - H]^+$ (m/z 195) ion (15% relative abundance) are seen. The $[B_n + K - H]^+$ ions are abundant metastable ions in decompositions of larger peptides containing arginine residues adjacent to the C-terminus.[3] CA of Arg-Asp $[M + K]^+$ yields the N-terminal amino acid ion ($[B_1 + K + OH]^+$) as most abundant ion (100% relative abundance) and less abundant $[M + K - NH_3]^+$ (84% relative abundance), $[A_1 + K - H]^+$ (35% relative abundance), and K^+ (45% relative abundance) ions. The release of K^+ is considerably less, indicative of stronger complexation of K^+ by this dipeptide compared to the others studied here.

A mechanism analogous to Scheme Ia explains the loss of the aspartic acid residue from this dipeptide. The potassium ion interacts with the carboxylate anion of the aspartic acid side chain and the amide carbonyl oxygen in addition to the C-terminal carboxylate as in Scheme II. Oxygen transfer occurs to the carbonyl carbon, and a six-membered intermediate structure is formed (not the five-membered structure as in Scheme Ia). Subsequent losses of the imine and ketene (CO in Scheme Ia) yield the $[B_1 + K + OH]^+$ ion. The six-membered intermediate and loss of ketene are responsible in part for the more favorable loss of the C-terminus in this peptide. More important is the supplemental binding of K^+ by the additional carboxylate group at the C-terminus that promotes this C-terminus loss and suppresses the release of K^+.

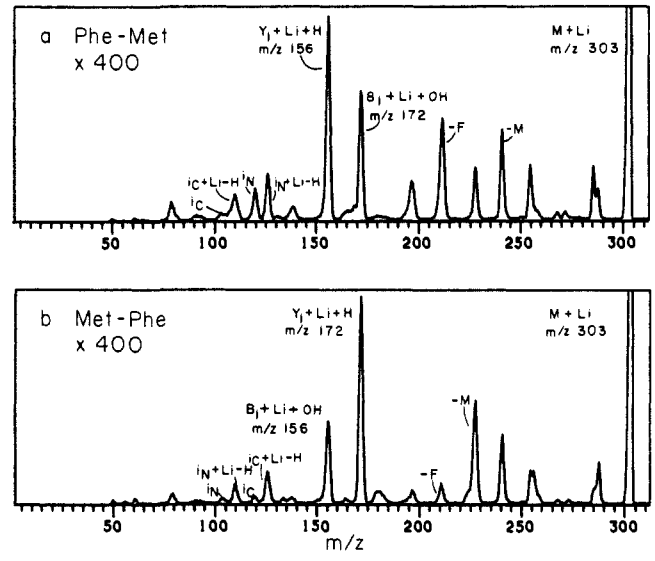


Figure 4. CA MS/MS spectra of (a) Phe-Met and (b) Met-Phe $[M + Li]^+$ ions of m/z 303.

Determination of Dipeptide Sequence. To be structurally useful, the decompositions of dipeptide $[M + Li]^+$ ions must allow one to distinguish the sequence of isomeric dipeptides. The utility of the CA decompositions of lithiated dipeptides is illustrated for the isomeric Phe-Met and Met-Phe in Figure 4. The presence of methionine and phenylalanine in these dipeptides is readily established on the basis of the ions of m/z 156 and 172 in both spectra. In Figure 4a, the abundant m/z 156 ion identifies the C-terminus as methionine. Confirmation of this sequence is made by comparing the abundances of the side chain losses. The more abundant ion of m/z 212, compared to the ion of m/z 228, is consistent with N-terminal phenylalanine. Figure 4a is therefore identified as the Phe-Met isomer. By using a similar approach, Figure 4b is identified as the spectrum of the Met-Phe isomer. These isomers are also easily sequenced by CA of the protonated species by using the strategy designed by Kulik and Heerma.[22]

Experimental Section

Reagents and Procedures. The peptides were obtained from either Sigma Chemical Co. (St. Louis, MO) or Chemical Dynamics Corp. (South Plainfield, NJ) and were used as received. Glycerol, thioglycerol, and the alkali iodides were obtained from Aldrich Chemical Co. (Milwaukee, WI). The glycerol/thioglycerol/alkali iodide matrix was prepared by mixing equal weights of glycerol and thioglycerol and saturating with the alkali iodide. The DT matrix was prepared by mixing 25 g of dithiothreitol with 5 g of dithioerythritol (>97%, Aldrich) and heating at 40 °C.

For FAB–MS/MS experiments, approximately 1 μg of the peptide was mixed on a copper tip with either DT, for production of $[M + H]^+$ ions, or glycerol/thioglycerol/alkali iodide, for production of $[M + Cat]^+$ ions.

Instrumentation. CA and metastable mass spectra were obtained by using a Kratos (Manchester, UK) MS-50 triple analyzer mass spectrometer of EB/E design, which was previously described.[33] MS-I is a standard high-resolution Kratos MS-50 (ESA and magnet). MS-II is

(31) Birch, N. J. In *Metal Ion in Biological Systems*; Sigel, H., Ed.; Marcel Dekker, Inc.: New York, 1982; Vol. 14, pp 257–313.

(32) Carrell, C. J.; Carrell, H. L.; Erlebacher, J.; Glusker, J. P. *J. Am. Chem. Soc.* **1988**, *110*, 8651–8656.

(33) 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.

a second electrostatic analyzer (ESA-II). The field-free regions (FFR), located between the source and ESA-I (1st FFR) and between the magnet and ESA-II (3rd FFR), are equipped with standard collision cells. An Ion Tech saddle-field atom gun (Ion Tech, Middlesex, England) was used for producing 7 to 8 keV Ar atoms for FAB desorption in a commercially available Kratos FAB source.

For FAB-MS/MS experiments, an ion of interest was selected by using MS-I at a mass resolution of approximately 1000 (width at 10% height). Mass selected ion kinetic energy spectra (MIKES) were obtained by scanning MS-II. Twenty scans were averaged by using software written in this laboratory. CA experiments were done by activating the mass selected ion in the 3rd FFR by using a helium pressure that gave a 50% main beam suppression.

For MS/MS/MS experiments,[34] source-produced ions were activated in the first field free region. The fragment ion of interest was then transmitted to the third FFR by setting both the first ESA and the magnet at the appropriate values. The selected product ion was collisionally activated and the resultant fragment ions were analyzed by scanning the final electric sector.

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

For linked scans at constant B/E, source-produced ions were activated in the first FFR. ESA-I and the magnet were scanned at a constant B/E value determined by the ratio of the field strengths necessary to transmit precursor ions of the desired m/z ratio holding the accelerating potential (V) constant. Fragmentations occurring in the first FFR were observed in this way. The voltage applied to ESA-II followed that of ESA-I and the final detector (after ESA-II) was used.

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Registry No. Ala-Gly, 687-69-4; Gly-Ala, 3695-73-6; Leu-Gly, 686-50-0; Gly-Leu, 869-19-2; Ala-Ile, 29727-65-9; Ala-Leu, 3303-34-2; Leu-Ala, 7298-84-2; Gly-Ser, 7361-43-5; Gly-Phe, 3321-03-7; Hip-Phe, 744-59-2; Leu-Phe, 3063-05-6; Phe-Leu, 3303-55-7; Met-Phe, 14492-14-9; Phe-Met, 15080-84-9; Val-Lys, 22677-62-9; Lys-Val, 20556-11-0; Ala-His, 3253-17-6; His-Ala, 16874-75-2; His-Leu, 7763-65-7; His-Lys, 37700-85-9; Arg-Ala, 40968-45-4; Arg-Asp, 15706-88-4; Li^+, 17341-24-1.

Solvent Effects on the Interionic Structure of Ion Pairs. Electric Dipole Moments and Infrared Spectra of *N*-Allyl-*N*-ethyl-*N*-methylanilinium *p*-Toluenesulfonate Ion Pairs in a Series of Solvents[1]

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Abstract: Molar dielectric increments for *N*-allyl-*N*-ethyl-*N*-methylanilinium *p*-toluenesulfonate ion pairs (AEM^+Ts^-), measured at concentrations up to 3.5 mM, gave the following apparent dipole moments: 4.9 ± 0.2 D in benzene (ϵ 2.275); 8.1 ± 0.1 D in octanoic acid (HOct; ϵ 2.46); 7.0 ± 0.5 D in anisole (ϵ 4.33); 11.2 ± 0.3 D in chloroform (ϵ 4.72); 7.4 ± 0.5 D in chlorobenzene (ϵ 5.61); and 8.6 ± 0.2 D, both in 0.95 M HOct in benzene and in 1.00 M benzene in HOct. In non-hydrogen-bonding solvents ranging from benzene to dimethyl sulfoxide (ϵ 46.7), the S-O stretching absorption of Ts^- consisted of two nearly coalesced bands with maxima at 1220 ± 5 and 1198 ± 5 cm^{-1}. In formic, acetic, and octanoic acids this band shifted to 1145-1160 cm^{-1} owing to acceptance of a hydrogen bond by the SO_3^- group. Analysis of the carboxyl C=O stretching vibration in benzene indicated the formation of a 1:1 complex, formally AEM^+Ts^-·HOct, with an association constant of 280 ± 40 M^{-1}. In $CHCl_3$ the centroid of the S-O stretching absorption shifted little or not at all and gave no real evidence for hydrogen bonding. The marked variability of the ion-pair dipole moment is attributed at least in part to specific solvent-induced changes in the *average* interionic geometry, the ion pair being regarded as a solvent-dependent mixture of interionic structural isomers that differ in interionic tightness. Such changes are facilitated by the asymmetric structure of the AEM^+ cation, and the variation of tightness calls to mind the "intimate" and "solvent-separated" ion-pair isomers proposed[6] as intermediates in solvolysis.

In liquid solutions the interionic structure of an ion pair can be perturbed, relative to that in the gas phase, by physical and chemical solvation mechanisms.[2-8] In the chemical mechanisms, the cations and/or anions in the ion pairs form molecular complexes with solvent molecules, typically by donor-acceptor and/or hydrogen bonds.[2,4,6-8] In the physical mechanisms the solvent is represented as a dielectric continuum with specific properties or as a densely packed ensemble of specific molecules whose presence around the ion pair perturbs the interionic forces.[3,5] We shall take

(1) (a) This contribution is dedicated to the memory of Saul Winstein. (b) Work supported by the U.S. National Science Foundation.

(2) (a) Grunwald, E.; Highsmith, S.; I, T.-P. *Ions and Ion Pairs in Organic Reactions*; Szwarc, M., Ed.; Wiley-Interscience: New York, 1974; Vol. 2, Chapter 5. (b) Grunwald, E.; I, T.-P. *Pure Appl. Chem.* **1979**, *51*, 53; *J. Am. Chem. Soc.* **1974**, *96*, 2879.

(3) (a) Gilkerson, W. R. *J. Chem. Phys.* **1956**, *26*, 1199. (b) Fuoss, R. M. *J. Am. Chem. Soc.* **1958**, *80*, 5059. (c) Bottcher, C. F. J.; van Belle, O. C.; Bordewijk, P.; Rip, A. *Theory of Electric Polarization*, 2nd ed.; Elsevier: Amsterdam, 1973; Vol. 1.

(4) Davis, M. M. *Acid-Base Behavior in Aprotic Organic Solvents*; National Bureau of Standards Monograph 105; U.S. Government Printing Office: Washington, DC, 1968.

(5) Grunwald, E. *Anal. Chem.* **1954**, *26*, 1696.

(6) Winstein, S.; Clippinger, E.; Fainberg, A. H.; Robinson, G. C. *J. Am. Chem. Soc.* **1954**, *76*, 2597. Winstein, S.; Clippinger, E.; Fainberg, A. H.; Heck, R.; Robinson, G. C. *Ibid.* **1956**, *78*, 328.

(7) (a) Hogen-Esch, T. E.; Smid, J. *J. Am. Chem. Soc.* **1965**, *87*, 669. Roberts, R. C.; Szwarc, M. *Ibid.* **1965**, *87*, 5542. Carvajal, C.; Tolle, K. J.; Smid, J.; Szwarc, M. *Ibid.* **1965**, *87*, 5548. (b) Smid, J. *Ions and Ion Pairs in Organic Reactions*; Szwarc, M., Ed.; Wiley-Interscience: New York, 1972; Vol. 1, Chapter 3. Edgell, W. F. *Ibid.* Chapter 4. (c) Flora, H. B.; Gilkerson, W. R. *J. Phys. Chem.* **1976**, *80*, 679. (d) Onishi, S.; Farber, H.; Petrucci, S. *J. Phys. Chem.* **1980**, *84*, 2923.

(8) (a) de Boer, E.; Sommerdijk, J. L. *Ions and Ion Pairs in Organic Reactions*; Szwarc, M., Ed.; Wiley-Interscience: New York, 1972; Vol. 1, Chapters 7 and 8. (b) Sharp, J. H.; Symons, M. C. R. *Ibid.* Chapter 5. (c) Szwarc, M. *Ibid.* Appendix. (d) Greenberg, M. S.; Popov, A. I. *J. Solution Chem.* **1976**, *5*, 653. (e) Chuang, H.-J.; Soong, L.-L.; Leroi, G. E.; Popov, A. I. *Ibid.* **1989**, *18*, 759.

0002-7863/90/1512-5104$02.50/0