different mechanisms lead to different temperature dependences of the relative sensitivities, as shown in Figure 2.

It was shown earlier that the relative sensitivities of aliphatic alcohols and ethers that undergo dissociative addition reactions with $(CH_3)_3Si^+$

$$(CH_3)_3Si^+ + ROH \rightarrow (CH_3)_3SiOH_2^+ \quad (1)$$

$$(CH_3)_3Si^+ + ROR \rightarrow \begin{cases} (CH_3)_3SiOR^+ & (2a) \\ R^+ & (2b) \end{cases}$$

are independent of temperature (*10*, *15*), as shown for *n*-pentyl ether, *sec*-butyl ether, and phenetole in Figure 2. The Si-containing ions, produced in the above reactions, have been shown to react by trimethylsilyl ion transfer with a second sample molecule to form the adduct ion $(M + 73)^+$.

However, the relative sensitivities for those compounds that form adduct ions by collisionally stabilized addition

$$(CH_3)_3Si^+ + M \rightleftharpoons [(CH_3)_3SiM^{+*}] \xrightarrow{X} (CH_3)_3SiM^+ \quad (3)$$

have strong negative temperature coefficients as seen in Figure 2 for anisole, tetrahydropyran, and 2,5-dimethylfuran, in agreement with earlier work on association reactions on simple systems (*17*). Consequently, the calculated relative sensitivities for these compounds will be reliable only at relatively low temperatures when the rate of sample ion formation is determined by the rate of collisions of the trimethylsilyl ion with the samples.

Additional problems may be encountered because the relative sensitivities may not be independent of the source configuration, although this would not affect the rate constants. More experimentation with different instruments is needed to test this possible source of error.

In conclusion, we have given preliminary results on the use of calculated rate constants for the prediction of relative sensitivities. The agreement between the calculated and experimental values of relative sensitivities for reactions of the trimethylsilyl ion with a number of compounds with a variety of functional groups is quite good; the average deviation was found to be 9.2% for the Langevin model and 5.8% for the Su and Chesnavich model. These errors are slightly smaller than the sum of the errors in the experiments, and the calculated values usually lie within one standard deviation of the experimental value. Reliable values of relative sensitivities can be obtained from rate constants calculated from the Langevin model, which requires only the polarizabilities, and not the dipole moments. Since strong negative temperature coefficients are observed for the relative sensitivities of those compounds with which the trimethylsilyl ions form adduct ions only by associative addition, reaction 3, this method is limited to low temperatures for reliable calculations of values for the relative sensitivities of these compounds. Because the relative rate constants can be calculated for the reactant ions from any CI reagent gas, we suggest the use of this method for other systems.

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Fast-Atom Bombardment Tandem Mass Spectrometry Studies of Organo-Alkali-Metal Ions of Small Peptides. Competitive Interaction of Sodium with Basic Amino Acid Substituents

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The fragment ions obtained from the $[M + Na]^+$ ion of model peptides (formed by fast-atom bombardment desorption ionization) are rationalized by binding of the Na^+ ion to a specific site of the peptide. The dissociation reactions of the $[M + Na]^+$ ions of peptides containing titratable R groups are consistent with the site of attachment of Na^+ being dependent upon the relative alkali-metal ion affinity of the basic side chain. Also, the proton and alkali-metal ion affinity of histidine is shown to be higher than expected based on the pK_a of the imidazole N. The anomalously high proton and alkali-metal ion affinity is rationalized by formation of an ionic complex involving the imidazole and amide nitrogens.

Although fast-atom bombardment (FAB) desorption ionization has proven to be a versatile and sensitive ionization method for many compounds (*1-7*), the FAB mass spectrometry (MS) spectra are frequently complicated by the presence of impurities and/or adduct ions of the sample and liquid matrix (*8-11*). To eliminate interferences from impurities and background signals, it has been proposed that FAB ionization be combined with tandem mass spectrometry for structural characterization of biomolecules (*3*, *12-15*). A practical limitation for the combined use of FAB ionization and tandem mass spectrometry is the low yield for structurally significant fragment ions (e.g., fragment ions characteristic of the amino acid sequence of peptides) when dissociation is induced by collisional activation (*14*). We recently described a potentially useful method for enhancing the structural information obtained by collision-induced dissociation (*16*, *17*). The method

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relies upon comparing the collision-induced dissociation spectra of $[M + H]^+$ ions and organo-alkali-metal ions of the form $[M + xA - (x - 1)H]^+$, where A is the alkali metal and $x = 1$–3. Owing to the fact that molecules such as peptides, sugars, nucleotides, etc. contain highly polar functional groups and that these functional groups have different H^+ and A^+ ion affinities, it follows that the binding sites of H^+ and A^+ with the organic molecule may differ (*17–20*). The site of A^+ attachment to the molecule as well as the nature of the organo-alkali-metal ion complex was shown to enhance specific dissociation reactions for the $[M + A]^+$ (A = Li, Na, K, Rb, Cs) ions of hippurylhistidylleucine (*16*) (benzoyl-Gly-His-Leu) and uridine triphosphate (*17*).

An additional factor influencing the dissociation reactions of organo-alkali-metal ions is the relative molecule/alkali-metal ion binding energy and the energetics for dissociation of the $[M + A]^+$ ionic complex (*17*). For example, an organo-alkali-metal ion complex can dissociate via two competitive reaction channels (reactions 1 and 2). The relative abundance of A^+ in the collision-induced dissociation (CID) spectra of $[M + A]^+$ ions of benzoyl-Gly-His-Leu was shown to follow the trend $Li^+ < Na^+ < K^+ < Rb^+ < Cs^+$ (*16*). This is the expected trend based on the relative binding energies ($D°(M–A^+)$) of the alkali-metal ion to the organic molecule (*21, 22*). Because Li^+ has the largest $D°(M–A^+)$ (*22, 23*), and the appearance energy for reaction 1 is high (relative to Na^+ or K^+ for the corresponding $[M + Na]^+$ or $[M + K]^+$ ions), the $[M + Li]^+$ ion can accommodate more internal energy before dissociating to M and Li^+ (*17*). The occurrence of reaction 2 will depend upon the relative energetics for reactions 1 and 2, and the only fragmentation reactions (reaction 2) that can occur are those with appearance energies less than the $[M–A^+]$ binding energy.

$$[M + A]^+ \longrightarrow M + A^+ \quad (1)$$

$$[M + A]^+ \longrightarrow [F_i + A]^+ + N \quad (2)$$

In the present studies, the interaction of Na^+ with basic amino acid residues (e.g., histidine, arginine, lysine, etc.) is studied by examining the CID spectra of the $[M + Na]^+$ ions of small peptides. First, the interaction of the Na^+ ion with glycylhistidine and glycyllysine is examined, and the observed fragment ions are discussed with particular emphasis on the sites of attachment of the Na^+ ion to the peptide. These two model compounds were chosen because both contain C-terminus amino acids having high solution basicities. Although we view the products of dissociation, $[F_i + A]^+$, of the organo-alkali-metal ion as indicating the site of attachment of A^+ to M, the product ions that are formed are an indication of the relative A^+ ion affinity of the fragmentation products. That is, when $[M + A]^+$ dissociates (reaction 2), $[F_i + A]^+$ will be formed if the A^+ ion affinity of F_i is greater than that for N. For instance, if the $[M + Na]^+$ ion of a dipeptide such as Gly-His dissociates to form $[Gly-Na]^+$, this would indicate that the Na^+ ion affinity of Gly is greater than the Na^+ ion affinity of His. Thus, the relative abundance of the various $[F_i + Na]^+$ ions in the CID spectrum of a particular $[M + Na]^+$ ion can be used to obtain relative Na^+ ion affinities analogous to similar experiments used to measure relative proton affinities (*23, 24*).

The correlation between the relative abundance of the various $[F_i + Na]^+$ fragment ions and Na^+ ion affinity is examined further by using the model compounds Gly-Gly-His, Gly-His-Gly, His-Gly-Gly, Gly-Ala-Tyr, Gly-Tyr-Ala, and Tyr-Ala-Gly. Second, the CID spectrum of the $[M + Na]^+$ ions of glycylhistidylarginylproline (Gly-His-Arg-Pro) is interpreted in terms of competitive interaction of the Na^+ ion with the most basic amino acid residues (e.g., histidine and arginine). Because the interaction of the Na^+ ion in the organo-alkali-metal cluster ions is proposed to be with the most basic sites of the organic molecule (*16, 17*), a correlation between the relative binding energy of the Na^+ ion and the pK_a of the basic nitrogen atom is expected. However, the relative Na^+ ion binding energy for the histidine imidazole N is anomalously high. The anomalously high Na^+ ion affinity for the imidazole N is explained in terms of binding of the Na^+ to both the imidazole and the amide N's.

EXPERIMENTAL SECTION

The studies reported here were performed with a Kratos MS-50 triple analyzer (*25*), in the fast-atom bombardment (FAB) ionization mode. The FAB ion source used for these studies is the standard Kratos system, equipped with an Ion Tech 11-NF saddle field atom gun. Xenon was used for the bombarding fast atom beam; typical operating conditions were beam energies of 6–7 keV and neutral beam currents equivalent to 20–30 μA as measured on an Ion Tech (Model B 50) current and voltage regulator/meter.

Collision-induced dissociation (CID) studies were performed in the mass-analyzed ion kinetic energy (MIKE) scan mode (*25*), with helium target gas and an incident ion energy of 8 keV. All CID spectra were recorded with a collision gas pressure corresponding to a 20% attenuation of the main ion beam. To improve the signal-to-noise ratio, all CID spectra were signal-averaged (eight scans at a rate of 20 s/scan) with a Nicolet Instrument Corp. (Model 172/2) signal averager. Spectra were plotted on a Houston Instruments 2000 X-Y recorder. In all cases the peak heights were used to calculate the percent total fragment ion yields. As noted by a referee, the mass resolution for the CID spectra is ca. one part in 200, thus it is difficult to make absolute mass assignments involving hydrogen transfer, e.g., Y_1 as opposed to $Y_1 + H$ or $Y_1 - H$. We have attempted to, where possible, make such distinctions, but it should be noted that the peak widths and the energy loss accompanying CID make such assignments suspect (*26*).

The peptides used in these studies [glycylhistidine (G-1502); glycyllysine (G-2127); Gly-Gly-His, Gly-His-Gly, His-Gly-Gly; Gly-Ala-Tyr, Gly-Tyr-Ala, Tyr-Ala-Gly; glycylhistidylarginylproline (G-8636); (A-9525)] were purchased from Sigma Chemical Co. and used without additional purification. A solution of each peptide was prepared by dissolving approximately 1.5 mg of sample in 500 μL of HPLC grade methanol (Fischer Chemical Co.; A-452) or distilled water. Typically, 2 μL of this solution is placed on a brass probe tip and air-dried. To this sample, approximately 3 μL of glycerol (liquid matrix) and 1 μg of sodium iodide were admixed on the probe tip.

RESULTS AND DISCUSSION

In our original paper on the dissociation reactions of organo-alkali-metal ions it was proposed that the fragment ions observed in the collision-induced dissociation spectrum of hippurylhistidylleucine (benzoyl-Gly-His-Leu) $[M + Na]^+$ ion could be attributed to the strong interaction of Na^+ with the basic nitrogens of the molecule (*16*). That is, the $[M + Na]^+$ ion can be viewed as an ionic complex in which the Na^+ ion is chelated by the lone pair of electrons on the basic nitrogens. The mechanism for formation of $[M + Na]^+$ type ions is complex, and the relative yield of $[M + Na]^+$ ions is sensitive to many factors, e.g., pH of the glycerol matrix, the Na^+ counterion (Cl^-, I^-, HCO_3^-, and etc.), the ratio of organic compound to alkali-metal salt, and etc. (*17*). On the other hand, the dissociation reactions of the $[M + Na]^+$ ion are insensitive to the manner by which it is formed (*17*).

In order to investigate further the interaction site of an alkali-metal ion (Na^+) with peptides containing basic amino acid residues, $[M + Na]^+$ ions of glycylhistidine (Gly-His), glycyllysine (Gly-Lys), tripeptides containing Gly, His, Ala, and Tyr, and glycylhistidylarginylproline (Gly-His-Arg-Pro) were formed by FAB and analyzed by use of tandem mass spectrometry with collision-induced dissociation. These compounds were selected because histidine, tyrosine, lysine, and arginine contain nitrogens of varying solution basicity

Table I. Collision-Induced Dissociation Data for $[M + H]^+$ and $[M + Na]^+$ Ions of Gly-His

$[M + H]^+$ m/z 213			$[M + Na]^+$[a] m/z 235		
m/z	cleavage	rel abund, %	m/z	cleavage	rel abund, %
196	-OH	20	218	-OH	5
168	-COOH	20	205	$-(NH_2CH_2)$	5
156	$Y_1 + 2H$	100	190	-COOH	60
110	$(Y_1 + H -$ COOH)	70	178	$Y_1 + H$	50
			162	Z_1	70
94	(imid + C_2H_3)	15	132	$Y_1 - COOH$	100
82	$R_1 + H$	30	117	$Z_1 - COOH$	20
30	NH_2CH_2	15	104	$(R_2$[b] $- CH_2) + Na$	25
			97	$(C_1 + H)$	95
			95	$(C_1 - H + Na)$	30

[a] All fragment ions contain Na^+ unless indicated. [b] m/z 104 corresponds to the $[R_2 + Na]^+$ ion, i.e., (imid - CH_2Na^+).

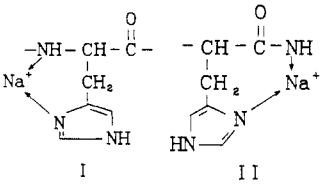
(pK_a = 6.0, 10.11, 10.5, and 12.5, respectively; pK_a values are for the R group substituent nitrogen) and (presumably) high alkali-metal ion affinities. The basicities of the side chain nitrogens increase in the series His < Tyr ~ Lys < Arg, but the geometrical factors influencing formation of an ionic complex between the amino acids and Na^+ differ greatly. If we use the same model as developed for benzoyl-Gly-His-Leu, the Na^+ ion interacts with the N-terminal NH_2 group, the amide bond N, and the His imidazole N. The basicity (solution) of the N-terminal Gly NH_2 group is relatively high (pK_a = 9.60); thus we would expect this group to have a relatively large alkali-metal ion affinity. Conversely, the histidine residue of a peptide can more easily accommodate a Na^+ ion (structures I and II) than can lysine or arginine. On the basis of basicity alone, one might expect Lys and Arg to have higher Na^+ ion affinities; however geometrical factors also play an important role. In fact, the data reported by Bojesen suggest that His has a higher proton affinity than does Lys (*23*).

In order to simplify the identification and discussion of the dissociation reactions of the peptides, the Roepstorff method for bond identification will be used throughout the text (*27*). In some cases the fragment ions in the CID spectrum are formed by consecutive dissociative reactions involving loss of N-terminus and C-terminus residues. For convenience, these fragment ions will be denoted as combinations of such cleavage processes, e.g., A_i/Y_j denotes cleavage of both the A_i and Y_j bonds; however, this notation is not intended to suggest the order in which the events occur. In all cases peak heights were used to calculate the percent of total fragment ion yield for each ion in the spectrum. More details regarding this notation are presented later.

The major fragmentations (>5% relative abundance) observed in the CID spectrum of the $[M + Na]^+$ ions of glycylhistidine are given in Table I. For the sake of comparison the same data for the $[M + H]^+$ ion are also given in the same table. (Note that all the fragment ions observed in the CID spectrum of the $[M + Na]^+$ contain Na^+. To simplify the notation used the presence of Na^+ is not indicated.) The fragment ions that are formed provide insight into the attachment site of the Na^+ ion to the peptide. For example, fragment ions at m/z 190, 132, 117, and 104 correspond to loss of the carboxyl group, cleavage of the amide bond combined with loss of CO_2 (e.g., $(Y_1 - CO_2)$), cleavage of the NH–CH bond combined with loss of OH (e.g., Z_1 - COOH)), and the histidyl R group plus Na^+ (i.e., $[R_2 + Na]^+$), respectively. These four fragment ions account for approximately 25% of the total fragment ion yield. Two additional fragment ions are observed at m/z 97 and 95. These ions correspond to cleavage (C_1 + H + Na) and (C_1 - H + Na), respectively, and ions account for approximately 15% of the total fragment ion yield.

The observed fragment ions suggest that the Na^+ ion strongly interacts with the imidazole ring nitrogen (e.g., note the relative abundance of the m/z 104 ion, $[R_2 + Na]^+$) and the amide bond nitrogen and weakly interacts with the N-terminus (e.g., note the absence of fragment ions corresponding to $[Gly-Na]^+$ type ions). Such an assignment is also consistent with the fact that approximately 80% of the total fragment ion yield observed in the CID spectrum of the $[M + Na]^+$ ions of Gly-His can be rationalized by involving interaction of the Na^+ ion with the imidazole ring nitrogen and the amide bond nitrogen. Furthermore, the most abundant fragment ion in the CID spectrum of the $[M + Na]^+$ ions of Gly-His is m/z 132, which corresponds to cleavage reaction with subsequent loss of COOH, e.g., $[Y_1 - COOH + Na]^+$. The proposed reaction sequence is consistent with the collision-induced spectrum of the m/z 178 observed in the normal FAB mass spectrum. For example, the m/z 178 ion undergoes loss of COOH as a metastable ion reaction and upon collisional activation.

Note that unlike the case for benzoyl-Gly-His-Leu (*16*), the CID spectra of the $[M + H]^+$ and $[M + Na]^+$ ions of glycylhistidine are quite similar. For example, for both the $[M + H]^+$ and $[M + Na]^+$ ions, fragment ions that correspond to cleavage $[(Y_1 - CO_2H) + H]^+$ or $[(Y_1 - CO_2H) + Na]^+$ and $[Y_1 + 2H]^+$ or $[Y_1 + H + Na]^+$ are important. In addition, the loss of COOH is observed for both ions. The similarities between the $[M + H]^+$ and $[M + Na]^+$ ions suggest that the proton affinity of the His residue is greater than that of the Gly residue. Thus, the fragmentation reactions of the $[M + H]^+$ ions occur at or near the site of protonation. As noted previously the anomalously high proton affinity of the His residue (*23*) may be influenced by geometrical factors such as those illustrated in structures I and II. The differences in the relative abundances of the fragment ions in the $[M + H]^+$ and $[M + Na]^+$ ion spectra as well as the general appearance of the CID spectra suggest that the internal energies of the $[M + H]^+$ and $[M + Na]^+$ ions differ significantly. The different internal energies arise from the maximum internal energy that the ion can receive upon collisional activation. If the $[M + Na]^+$ ion is activated to an energy in excess of the Na^+ ion binding energy, the dominant dissociation reaction channel will be formation of Na^+ (reaction 1). For example, formation of the histidyl R-group fragment ion, $[R_2 + H]^+$, is more important for the $[M + H]^+$ ion (15% of the total fragment ion yield). The corresponding fragmentation channel for the $[M + Na]^+$ ion accounts for less than 2% of the total fragment ion yield in the $[M + Na]^+$ ion spectrum.



I II

The absence of the $[R_2 + H]^+$ ion in the CID spectrum of the $[M + Na]^+$ ion suggests that the appearance energy for this fragment ion exceeds the Na^+ binding energy. Similar types of fragmentation reactions are observed in the CID spectrum (Table II) of the $[M + Na]^+$ ions of glycyl-lysine. For example, the most abundant fragment ions in the spectrum occur at m/z 154, 151, and 123. These ions are assigned as $[Z_1 + Na + H]^+$, $[X_1 + Na - COOH]^+$, and $[Y_1 + Na - COOH]^+$, respectively. It is also possible that the m/z 151 ion corresponds to cleavage Y_1–OH. Owing to the low mass resolution for the CID spectra, it is not possible to distinguish the two possible product ions. The actual identity of the two

Table II. Collision-Induced Dissociation Data for $[M + H]^+$ and $[M + Na]^+$ Ions of Gly-Lys

$[M + H]^+$ m/z 204			$[M + Na]^+$[a] m/z 226		
m/z	cleavage	rel abund, %	m/z	cleavage	rel abund, %
187	-OH	70	209	-OH	25
147	$Y_1 + 2H$	50	181	-COOH	20
130	Z_1	100	168	Y_1	30
101	(Y_1 + H - COOH)	15	154	$Z_1 + H$	95
			151	X_1 - COOH (or Y_1 - OH)	100
84	Y_1 - (COOH + NH_3) + H	100	123	Y_1 - COOH	90
56	X_1 - (R_2 + COOH)	15	97	$C_1 + H$	20
30	NH_2CH_2	90			

[a] See footnote *a* in Table I.

possible product ions (X_1 - COOH or Y_1 - OH) does not alter the arguments regarding site of Na^+ ion attachment.

Two additional fragment ions are observed at m/z 167 and 97. These fragment ions are assigned as $[Y_1 + Na]^+$ and $[C_1 + Na + H]^+$. The observed fragment ions suggest that the sites of alkali-metal ion attachment are to the nitrogen of the lysine R-group and the amide nitrogen. Again, for the sake of comparison, the CID spectrum of the $[M + H]^+$ ion of Gly-Lys is also given (Table II). Note that the CID spectra for the $[M + H]^+$ and $[M + Na]^+$ ions of Gly-Lys show the same general trends as observed for Gly-His. A notable difference, however, is the Z_1 fragment ion, which is observed for both $[M + H]^+$ and $[M + Na]^+$, whereas the $X_1 - CO_2H$ fragment is not observed for the $[M + H]^+$ ion. Again, we attribute the differences in the observed fragment ions to internal energies of the $[M + H]^+$ and $[M + Na]^+$ ions.

The fragmentation reactions for a series of tripeptides, Gly-Gly-His, Gly-His-Gly, and His-Gly-Gly, illustrate the effects of changes in the position of the amino acid residue containing the basic R-group. Even though the imidazole group is not a strong base (solution $pK_a = 6.0$), the His residue has a high proton (*23*) and A^+ ion affinity. The CID spectra for the $[M + H]^+$ and $[M + Na]^+$ ions of Gly-Gly-His, Gly-His-Gly, and His-Gly-Gly are reported in Table III. The most abundant fragment ions in the CID spectrum of [His-Gly-Gly + $Na]^+$ are observed at m/z 247, 233, 155, and 133 and are assigned as $[M + Na - CO_2H]^+$ and cleavage reactions C_2, Y_2, and A_1, respectively. Note that the dominant ions correspond to cleavage reactions of A, B, or C type, i.e., charge retention (Na^+) by the N-terminus. Also, the fragment ion formed by cleavage Y_2 corresponds to loss of the N-terminal glycyl residue and charge retention (Na^+) by the His-Gly moiety.

Clearly in all three spectra, fragment ions formed due to interaction of Na^+ with the Gly amino terminus are of low relative abundance. In fact, in each case the preferred cleavage reactions occur in the vicinity of the His residue. Note that this general statement is true for both the $[M + H]^+$ and $[M + Na]^+$ ions. For example, the A_2, $Y_2 + H$, and $B_2 + H$ cleavage reactions dominate the spectrum for the $[M + Na]^+$ ion of Gly-His-Gly, and these same cleavage reactions are important for the $[M + H]^+$ ion. Note also, that the combination cleavage reactions of the Gly-His-Gly $[M + H]^+$ ion also occur in the vicinity of the his residue. The fact that combination cleavage reactions are only observed for the [M + $H]^+$ ion and not for the $[M + Na]^+$ ion is another indication

Table III. Collision-Induced Dissociation Data for $[M + H]^+$ and $[M + Na]^+$ Ions of His-Gly-Gly, Gly-His-Gly, and Gly-Gly-His

	$[M + H]^+$ m/z 270			$[M + Na]^+$[a] m/z 292		
	m/z	cleavage	rel abund, %	m/z	cleavage	rel abund, %
His-Gly-Gly	253	-OH	20	275	-OH	35
	225	-COOH	18	247	-COOH	30
	195	B_2	50	233	C_2	100
	167	A_2	15	218	B_2	20
	138	B_1	12	210	-(R_1 + H)	85
	110	A_1	100	190	A_2	15
	82	$[R_1 + H]^+$	10	176	C_1	12
				155	(Y_2 + H)	20
				133	(A_1 - H + Na)	30
Gly-His-Gly	253	-OH	20	275	-OH	30
	239	-(NH_2CH_2)H	25	261	-(NH_2CH_2)H	15
	236	(-NH_3 + OH)	30	235	(Y_2 + H)	35
	225	-COOH	8	219	(B_2 + H)	100
	213	(Y_2 + 2 H)	70	212	-R_2 + H	15
	197	B_2 + 2 H	100	203	(C_2/X_2)	10
	181	(C_2/X_2)	20	190	A_2	60
	167	A_2	80			
	154	(Y_2 + 2H/C_2)	25			
	122	(Z_2/B_2)	25			
	110	(Y_2/A_2) + H	45			
Gly-Gly-His	253	-OH	15	275	-OH	10
	239	-(NH_2CH_2)H	30	261	-(NH_2CH_2)H	10
	236	-(NH_3 + OH)?	15	247	-COOH	30
	225	-COOH	15	235	(Y_2 + H)	10
	213	(Y_2 + 2H)	60	218	(Z_2 + H)	10
	196	Z_2	10	190	(X_1 - OH + 2H)	10
	167	Y_2 + H - COOH	10	178	(Y_1 + H)	
	156	Y_1 + 2H	100	155	(C_2 + 2H)	100
	151	(Z_2 - COOH)	10	132	(Y_1 - COOh)	25
	136	(Y_1 - H_2O)	15	110	A_2	
	122	(Z_1 - OH)	10			
	110	(Y_1 - CO_2)	30			

[a] See footnote *a* in Table I.

Table IV. Collision-Induced Dissociation Data for $[M + H]^+$ and $[M + Na]^+$ Ions of Gly-Ala-Tyr, Gly-Tyr-Ala, and Tyr-Ala-Gly

	$[M + H]^+$ m/z 310			$[M + Na]^+$[a] m/z 332		
	m/z	cleavage	rel abund, %	m/z	cleavage	rel abund, %
Gly-Ala-Tyr	293	-OH	15	315	-OH	40
	265	-COOH	5	287	-COOH	25
	252	$Y_2 + H$	20	274	Y_2	20
	182	$(Y_1 + 2H)$	100	258	$Z_2 - H$	25
	163	$(Z_1 - 2H)$	15	226	$-(R_3 - H)$	15
	146	$(C_2 + 2H)$	5	223	$-(R_3 + 2H)$	
	135	$(Y_1 - COOh)$	20	203	$Y_1 - H$	40
	129	B_2	30	167	C_2	100
	107	$-R_3$	15	121	$(X_2/B_2) - H$	
	101	A_2	15			
Gly-Tyr-Ala	293	-OH	10	315	-OH	25
	265	-COOH	<5	287	-COOH	15
	237	$C_2 + H$	5	260	$C_2 + H$	100
	222	B_2	100	244	B_2	25
	193	A_2	70	214	Z_2-COOH	60
	148	$(Z_2/B_2) - 2H$	20	108	$(R_2 + H)$	10
	135	(Y_2/A_2)	60			
	120	(Z_2/A_2)	10			
Tyr-Ala-Gly	293	-OH	20	315	-OH	85
	265	-COOH	10	287	-COOH	20
	237	$B_2 + H$	80	273	C_2	70
	209	$A_2 + H$	20	256	$C_2 - OH$	
	147	$(Y_2 + 2H)$	30	229	$A_2 - H$	70
	135	$(A_1 - H)$	100	224	$-(R_1 + H)$	100
	119	$(A_1 - NH_3)$	15	212	$A_2 - H_2O$	
	106	$(R_1 - H)$	10	166	$Y_2 - 2H$	20
				154	$Z_2 + H$	35
				130	R_1	10
				93	C_6H_5O	20

[a] See footnote *a* in Table I.

that the relative internal energies of the $[M + H]^+$ and $[M + Na]^+$ ions are quite different. Specifically, $[M + Na]^+$ ions that have high internal energy, sufficient energy to undergo consecutive dissociation reactions, eliminate M to form Na^+ (reaction 1).

It is also instructive to examine in detail the chemistry of these combination cleavage reactions of the $[M + H]^+$ ions. For instance, the cleavage reaction for the Gly-His-Gly $[M + H]^+$ ion denoted $(Y_2 + 2H/C_2)$ (Table III) occurs by formation of the $Y_2 + 2H$ ion (m/z 213) followed by a C_2 cleavage to eliminate CH_2COOH to form the radical cation (m/z 154) as illustrated by reaction 3 in Scheme I. The proposed reaction scheme is consistent with the generally observed dissociation reactions of peptide $[M + H]^+$ ions (*28–30*). Although the proposed reaction sequence was confirmed by performing collision-induced dissociation on the $Y_2 + 2H$ (m/z 213) ion, it is not possible to unequivocally establish the product ions depicted in Scheme I. For example, the signal observed at m/z 154 occurs at m/z 153.4 in the CID spectrum of the Gly-His-Gly $[M + H]^+$ and at m/z 153.6 in the CID spectrum of the $Y_2 + 2H$ (m/z 213) ion. The m/z values were obtained by assuming (i) the energy loss accompanying collisional activation is negligible in the mass range less than 200–300 and (ii) the centroid of the signal is not significantly distorted by competing reaction channels differing by a hydrogen atom (e.g., competing loses of CH_2COOH and CH_3COOH) or H loss occurs for the CID product ion. It is certainly possible that energy loss accompanying collisional activation causes a shift to low mass in the m/z value for the CID product ion (*26*), but it is also reasonable that the m/z 213 ion loses both CH_2COOH and CH_3COOH or that the $(Y_2 + 2H/C_2)$ fragment ion eliminates H to form what would formally be a $(Y_2 + 2H/C_2 - H)$ fragment ion.

A similar mechanism can be used to rationalize the m/z 181 (C_2/X_2) fragment ion observed in the CID spectrum of Gly-His-Gly $[M + H]^+$ ion, e.g., reaction 4, Scheme I. Note that the two dissociation pathways for the Gly-His-Gly $[M + H]^+$ depicted in Scheme I differ only in the configuration of the ion, viz. structure I versus structure II (the $[M + H]^+$ ion is probably best represented by a combination of these two forms). Thus, dissociation of the structure I representation produces the $Y_2 + 2H$ ion, whereas dissociation of the structure II representation produces the C_2 (m/z 211) ion. A CID product ion corresponding to a C_2 cleavage reaction is not detected (e.g., m/z 211); however the limited mass resolution of the CID spectrum does not permit us to unequivocally state that such an ion is not formed. The details of this proposed mechanism are presently being probed further by using other model peptides. Specifically, model peptides where the nature of the R-groups adjacent to the his residue may alter the relative contribution of structures I and II are being studied.

One last point is to be made regarding the combination cleavage reactions. Note that such reactions are observed only in cases where a relatively basic residue is in a nonterminal (either N- or C-terminus) position. This is yet another example that illustrates the importance of the site of protonation on the type of dissociation channels that are observed (*28–30*).

It is interesting to compare the fragmentation reactions observed for Gly-Gly-His, Gly-His-Gly, and His-Gly-Gly $[M + H]^+$ and $[M + Na]^+$ ions with those for Gly-Ala-Tyr, Gly-Tyr-Ala, and Tyr-Ala-Gly. The tripeptides composed of Gly, Tyr, and Ala do not contain side chains with appreciable basicities. Although the A^+ ion affinity of the hydroxybenzyl side chain of Tyr is relatively large (assuming relative Na^+ ion affinities follow the same trend as Li^+ ion affinities) (*21*), the preferred site for Na^+ attachment is to the amide and N-terminal NH_2 nitrogens. For instance, in the CID spectra of all three Gly-, Tyr-, Ala-containing peptides (Table IV), the fragment ions can be explained by assuming Na^+ ion

Scheme I

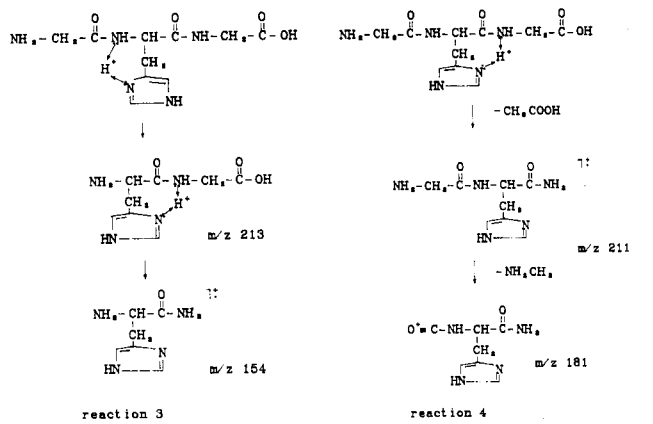


Table V. Collision-Induced Dissociation Data for [M + H]$^+$ and [M + Na]$^+$ Ions of Gly-His-Arg-Pro

[M + H]$^+$ m/z 466			[M + Na]$^+$ [a] m/z 488		
m/z	cleavage	rel abund, %	m/z	cleavage	rel abund, %
421	-COOH	55	390	$-(R_2 + OH)$	20
368	$-(R_2 + OH)$	70	374	$(B_3 + H)$	100
352	$B_3 + H$	50	347	$A_3 + H$	15
325	Z_3 - imid	60	332	$B_3 - CH_2N_2$	40
195	B_2	85	278	Z_2	18
167	A_2	85	261	$(Z_2 - OH)$	16
110	$(Y_3 - A_2 + H)$	90	233	C_2	16
86	$A_2 - R_2$	95	190	A_2	20
70	Y_1 - COOH	100			

[a] See footnote *a* in Table I.

attachment to the amide nitrogens. Note the favored loss of the Tyr C-terminus for Gly-Ala-Tyr [M + Na]$^+$, e.g., cleavage reaction C_2, and the loss of C_7H_7O for the N-terminus of Tyr-Ala-Gly. Also, for the [M + Na]$^+$ ions the dominant fragment ions are formed by A, B, or C cleavage reactions, and in each case these cleavage reactions occur at the C-terminal residues. For example, cleavage C_2 dominates the spectrum of Gly-Ala-Tyr, cleavage B_2 and C_2 dominate the spectra of Gly-Tyr-Ala, and cleavage A_2 and C_2 dominate the spectra of Tyr-Ala-Gly. Note the same trends hold for the [M + H]$^+$ ion, except for Gly-Ala-Tyr where cleavage Y_1 is dominant, suggesting that protonation occurs at the Tyr residue. Again, the combination cleavage reactions can be rationalized by a similar reaction sequence as shown in Scheme I.

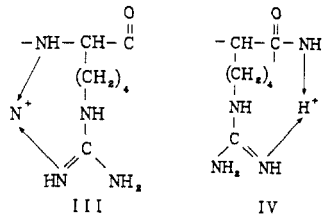
The dissociation reactions for the [M + Na]$^+$ ions can be extended to more complex systems such as glycylhistidylarginylproline (Gly-His-Arg-Pro). The Gly-His-Arg-Pro system is of interest for several reasons. The His, Arg, and Pro residues are relatively strong (solution) bases: pK_a = 10.6 for the Pro residue; His pK_a = 6.0 (imide N), 9.15 (amino N); Arg pK_a = 13.2 (guanidino), 9.0 (amino N). The CID spectrum of the [M + H]$^+$ ion is dominated by N-terminal fragments (Table V). The major fragment ions in the CID spectrum of the [M + Na]$^+$ ions of Gly-His-Arg-Pro suggest that the Na$^+$ ion interacts with the histidyl and arginyl R-groups. For example, the most abundant fragment ion in the CID spectrum is m/z 374, corresponding to cleavage of the amide linkage (B_3) at the proline residue. Fragment ions are observed at m/z 347 and m/z 332, which correspond to cleavage A_3 and B_3 with loss of 42 mass units, presumably CH_2N_2 from the guanidino group of the Arg residue. (The collision-induced dissociation spectrum of the m/z 332 ion observed in the normal FAB spectrum is consistent with this assignment. Similar fragment ions are observed for other Arg-containing peptides, and the mechanism of this reaction is being explored further. Presumably the driving force for this fragmentation channel is formation of the six-membered ring cyclic amide.) Fragment ions at m/z 278 and m/z 261 are also observed in the CID spectrum of the [M + Na]$^+$ ions of Gly-His-Arg-Pro. These two fragment ions correspond to cleavage Z_2 and Z_2 minus OH. The ions at m/z 233 and m/z 190 correspond to C_2 and A_2 minus H, respectively.

Analogous to the dipeptide studies, the fragment ions in the CID spectrum of the [M + Na]$^+$ ions of Gly-His-Arg-Pro can be attributed to the sites of the Na$^+$ ion attachment to the peptide. The fragment ions at m/z 233 and 190 account for roughly 16% of the total fragment ion yield and are assigned to Na$^+$ ion interaction predominantly at the histidyl R-group (pK_a = 6.0). Owing to the higher basicity of the arginyl R-group (pK_a = 12.5), the fragment ions at m/z 374, 347, 330, 278, and 261 (approximately 71% of the total fragment ion yield) are attributed to Na$^+$ ion interaction predominantly at the arginyl R-group. On the basis of the observed fragment ions, we propose that the Na$^+$ ion is strongly bound to the guanadino group of arginine while interacting with the amide nitrogen of the arginylhistidyl peptide bond and the imidazole ring nitrogen of the histidine.

We find it interesting that fragment ions corresponding to Na$^+$ ion attachment at the Pro N (pK_a = 10.6) are not observed. This may be due to the relatively high Na$^+$ ion affinity of Arg and His or that Pro is the C-terminal residue. The lack of Na$^+$ ion attachment at the Pro amide N could also be due to the rigidity of the Pro residue preventing folding of the structure in a form that which would favor interaction. We are in the process of preparing a series of model peptides containing Pro and other basic residues, e.g., Lys, His, Arg, and etc. These model peptides will allow us to probe such questions as changes in the Na$^+$ ion affinity with positional changes and competition for Na$^+$ between two basic sites where one is Pro.

In the case of the dipeptides the CID spectra for the [M + Na]$^+$ and [M + H]$^+$ ions are quite similar. Conversely, the CID spectra for the [M + Na]$^+$ and [M + H]$^+$ ions of Gly-His-Arg-Pro are dramatically different. Note (Table V) the strong preference for cleavage A_2 for the [M + H]$^+$ ion. That is, for the [M + H]$^+$ ions cleavage at the CH–CO histidine bond is favored, whereas cleavage reactions A_3 and B_3 (between the arginine and proline residues) are preferred for the [M + Na]$^+$ ion. Again, these differences can be attributed to the difference in the average internal energies of the [M + H]$^+$ and [M + Na]$^+$ ions and the difference in the location of the charge site. Although more detailed studies are required, the clear preference for A type cleavage reactions of the [M + H]$^+$ ion suggests that the charge site of the ion is distributed over the Gly-His portion of the molecule. This can be understood if we assume that H$^+$ is competitively bound to the imidazole N, the histidyl amide nitrogen, and the glycine N-terminus. This situation corresponds to charge distribution via a six- and seven-membered ring interaction (structures I and II), whereas protonation at the guanidino N and subsequent charge distribution to other portions of the molecule would require much less favorable interactions (structures III and IV). These ideas and concepts are presently being studied with other model systems. Again, it is evident from these data that the gas-phase proton affinity of His is larger than expected based on solution pK_a values.

Because a major consideration for determining the interaction site of the Na$^+$ ion with peptides is the relative Na$^+$ ion affinity of the amino acid R-groups present within the molecule (see discussion above), a direct relationship between solution pK_a and relative alkali ion affinity should exist. Recently Bojesen measured the relative gas-phase proton



III IV

affinities for a series of amino acids (*23*) using the bracketing method of Cooks and Kruger (*24*), relative proton affinities Arg > His > Lys > Trp. More detailed studies on the relationship between amino acid R-group and the relative alkali-metal binding energy are presently under way. Preliminary results suggest a similar scale for alkali-metal ion affinities, e.g., Arg > His > Lys > Trp. Although the solution pK_a for the amino terminus of histidine is 10^3 times greater than that for the imidazole N, it appears that A^+ is preferentially attached at the imidazole nitrogen. Likewise, for model peptides containing both His and Arg, it appears that the Na^+ ion binding energy to the His imidazole N is quite high, in spite of the much higher (ca. 10^6) basicity of the guanidino N. For example, compare the fragment ions observed for Gly-His-Arg-Pro $[M + Na]^+$ ion. Also, the data for Gly-Gly-His, Gly-His-Gly, and His-Gly-Gly clearly show the change in type and relative abundance of fragment ions formed due to the change in position of the imidazole group and thereby the change in position of the cation.

Although this paper emphasizes the role of the site of interactions between M and Na^+ on the fragment ions formed by dissociation of $[M + Na]^+$, the dissociation energetics and the averaged-internal energies of the ions are also important. For example, we previously noted the different fragment ions formed from gramicidin S desorbed (Cs^+ ion desorption) from gold and stainless steel surfaces. Although more detailed studies are under way, it appears that $[M + Na]^+$ ions of gramicidin S desorbed from stainless steel are formed with higher averaged-internal energies than the same ions formed by desorption from a gold surface (*31*). In an effort to probe both the effects of Na^+ attachment site and dissociation energetics laser-ion beam photodissociation studies of model $[M + Na]^+$ ions are being performed. In these studies the site of Na^+ attachment can be controlled (e.g., by examining Gly-Gly-His vis-a-vis His-Gly-Gly), and the energy of the dissociating ion can be varied by changing the laser wavelength (*32*). Such studies will allow us to probe independently the role of attachment site and reaction energetics.

CONCLUSIONS

The interaction of alkali-metal ions with peptides is determined by the most basic sites of the molecule. For small peptides that contain only one basic amino acid residue (e.g., glycylhistidine, glycyllysine), the Na^+ ion interacts with the amino terminus, the amide nitrogen of the peptide bond, and the nitrogen(s) of the R-group. The proposed sites for Na^+ ion attachment are consistent with studies on Zn^{2+} with glycylhistidine and alanylhistidine analyzed by use of NMR (*33*).

It is also interesting to note that the fragment ions observed in the $[M + Na]^+$ ion collision-induced dissociation spectra of peptides are A-type fragment ions with retention of charge by the N-terminus of the molecule. Exceptions to this occur when the basic amino acid is the C-terminal residue (e.g., Gly-Gly-His), and (presumably) for larger systems this same observation would hold true (*30*). However, there does not appear to be any preference for Na^+ ion attachment at a C-terminal proline residue.

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