SOME GENERAL ASPECTS OF THE CHEMISTRY OF ORGANO-ALKALI METAL IONS. AN OVERVIEW OF RECENT WORK

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ABSTRACT

Organo-alkali metal ions of the type \([M + xNa-(x-1)H]^+\) \((x = 1-3)\) are commonly observed in the desorption ionization mass spectra of polar organic molecules. An overview of our work on the formation and selected applications of organo-alkali metal ions is presented. We interpret our data as evidence that ions of the type \([M + A(AX)\text{,}]^+\) are formed from \([M+A]^-\) cluster ions. Another important aspect of this work deals with the dissociation reactions of organo-alkali metal ions. We have found that structurally significant fragment ions are observed in the collision-induced dissociation (CID) spectra of \([M+A]^-\) ions that are not observed in the \([M+H]^+\) ion CID spectrum. We attribute these differences to functional group basicity and organo-alkali metal ion binding energies. That is, alkali metal ions are bound to specific sites of the organic molecule and the fragment ions formed upon CID are indications of the structure of the ionic organo-alkali metal ion complex.

INTRODUCTION

Organo-alkali metal ions of the type \([M + xNa-(x-1)H]^+\) \((x = 1-3)\) are formed by all the desorption ionization methods developed for the analysis of polar organic molecules [1–9]. In fact, ion yields for field desorption (FD) ionization can be greatly enhanced by admixing alkali metal salts with the sample; the dominant molecular ions formed are \([M + Li]^+, [M + Na]^+, \text{etc.} [10–13]. Organo-alkali metal ions are also observed with \(^{252}\text{Cf}\) plasma desorption (PD) [14,15], laser desorption (LD) [16], secondary ion mass spectrometry (SIMS) [17], and fast atom bombardment (FAB) ionization [18].

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The mechanism for formation of \([M + xNa - (x - 1)H]^+\) type ions has been actively studied. Sichtermann and Benninghoven studied the formation of species such as \([M + A]^+\) and \([M + 2A - H]^+\) by solid-state SIMS [19]. These authors measured the relative yield for ions such as \([M + A]^+\) and \([M + 2A - H]^+\) from samples of small amino acids (e.g. leucine) mixed with alkali metal halide salts (e.g. LiCl) and the changes in secondary ion yield which result from annealing such mixtures. The studies showed that, over a range of beam current densities, the relative yields of \([M + Li]^+\) and \([M + 2Li - H]^+\) ions were similar and that only at high beam current densities (e.g. \(10 \times 10^{-6} \text{ A cm}^{-2}\)) did the yield for \([M + 2Li - H]^+\) deviate significantly from \([M + Li]^+\), suggesting a common mechanism for the formation of these ions [19].

Bursey et al. proposed that formation of \([M + Na]^+\) ions takes place as a consequence of the formation of cationized complexes of glycerol (solvent matrix) and the organic molecule and that the primary solvation of the alkali metal cation is by the matrix [20]. Puzo and Prome' studied several complexes of solvated sodiated hexose molecular ions and observed that unimolecular dissociation of the complex gives rise to sodiated hexose ions [5].

Our work on alpha- and beta-cyclodextrin and similar sugars showed that \([M + xA - (x - 1)H]^+\) ions are the dominant molecular ions formed in Cs+ desorption ionization FTMS. Detailed investigations of the cyclodextrin systems provided insight into the origin of these ions [21]. For example, it was found that the use of small sample sizes (nanogram samples loaded on to the sample probe) enhanced the relative yield of \([M + H]^+\) ions. In addition, operating the Cs+ ion gun at low-beam current density (\(1 \times 10^{-8} \text{ A cm}^{-2}\) or less) with short irradiation times (0.1–1 ms) gives higher yields of \([M + H]^+\) ions. At high Cs+ beam fluence (the product of beam current and irradiation time), formation of \([M + xA - (x - 1)H]^+\) (\(x = 1–3\)) is favored. Also, as the beam fluence is increased, the yield for organo-alkali metal ions increases, as does the yield for fragment ions containing sodium. A general model which can be used to describe these results has its basis with the schematic diagram in Fig. 1 [21]. This diagram depicts the relative yield \((Y)\) of the various secondary ions as a function of the Cs+ ion beam fluence \((Q)\). For example, at low Cs+ ion beam fluence, the yield for \([M + H]^+\) ions is high relative to fragment ions \((F_i^+)\) and \([M + xA - (x - 1)H]^+\) ions. As the Cs+ ion beam fluence is increased, the yield for \([M + H]^+\) ions decreases with a corresponding increase in the yield for fragment ions denoted \(F_i^+, F_j^+, \) and \([M + xA - (x - 1)H]^+\) ions.

Ionic alkali metal halide clusters of the type \(A(AX)_n^+\) are well known and have been studied by several workers [22–27]. Owing to the formation of \(A(AX)^+\) and \([G + A(AX)]^+\)-type ions \((G = \text{glycerol})\) in FAB mass spectrometry, a mechanism for the production of cationized glycerol was pro-
Fig. 1. Graphical summary of the DI mass spectrum of \( \beta \)-cyclodextrin. The parameter \( Q \) corresponds to increases in the Cs\(^+\) beam current density and/or longer sample irradiation times. (Reprinted from ref. 21.)

posed by Aubagnac et al. [28]. Because \([G + A(AX)]^+\)-type ions can be formed in the bulk solution, the proposed mechanism may be correct (i.e. formation of \([G + A]^+\) from \([G + A(AX)]^+\) ions), but based on thermodynamic considerations alone it is highly unlikely that simple substitution of H\(^+\) by Na\(^+\) is responsible for the formation of \([M + xA - (x - 1)H]^+\) (\(x = 2, 3\), etc.) ions. The heats of formation for H\(^+\) and Na\(^+\) are 365.2 and 144.2 kcal mol\(^{-1}\), respectively; thus formation of \([G + 2Na - H]^+\) via a simple substitution reaction would be ca. 220 kcal mol\(^{-1}\) endothermic [29]. Such an energetic reaction would lead to a highly excited ion which would readily undergo dissociation reactions. The low abundance of fragment ions in the FD spectra of \([M + xA - (x - 1)H]^+\)-type ions, for example, suggests that the molecular ions are not formed with appreciable amounts of internal energy. The supposition that \([M + xA - (x - 1)H]^+\) ions are not formed with excess internal energy is supported by the fact that thermal excitation (via IR laser) in laser-assisted FD induces some fragmentation of the organo-alkali metal ion species [30]. Thus, the results for laser-assisted FD suggest a low energy dissociation threshold for the fragmentation of \([M + xA - (x - 1)H]^+\) ions.

Fast atom bombardment (FAB) ionization is a versatile and sensitive ionization method for the analysis of biological compounds [31–41]. The
FAB mass spectra are frequently complicated by the presence of impurities and/or adduct ions of the sample and liquid matrix [5,42,43]. To minimize interferences from these adduct ions, the use of tandem mass spectrometry (TMS) in combination with FAB ionization for structural characterization of biomolecules has been proposed [44–48]. A limitation of this approach is that structurally significant fragment ions are either absent or of very low abundance in the FAB–TMS spectrum [38,46]. During the development of mass spectrometry, several methods for enhancing specific dissociation reactions have been proposed. These methods rely on producing dissociating ions with a narrow range of internal energies [22,49–58]. Alternatively, chemical derivatization of specific functional groups of the molecule can be employed to enhance specific dissociation reaction channels [59]. In previous work [41,60], we have shown that it is possible to enhance the structural information obtained by FAB–TMS by examining the dissociation reactions of \([M + xA - (x - 1)H]^+\) ions. Such results are analogous to remote-charge site fragmentation of long-chain fatty acids reported by Gross and co-workers [61–64].

In this paper, a general discussion of the chemistry of organo-alkali metal ions is presented. First, a mechanism for the formation of \([M + xA - (x - 1)H]^+\)-type ions is proposed. This general mechanism is based on data from FAB–TMS spectra of organo-alkali metal ions of the type \([G + A(AX)_n]^+\) and \([G + A + A(AX)_{n-1} - H]^+\) (where \(G = \) glycerol, \(A = \) alkali metal, \(X = \) halide counter anion, and \(n = 1–3\)). Second, a useful and simple method is described for enhancing the structural information obtained by collision-induced dissociation (CID) of small peptides. The method relies upon comparing the CID spectra of \([M + H]^+\) ions and organo-alkali metal ions of the form \([M + xA - (x - 1)H]^+\) where \(x = 1–3\). Owing to the fact that molecules such as peptides and/or sugars 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 \(A^+\) may differ. It has been shown that competition in SIMS among alkali metal cations in producing \([M + A]^+\) ions of organic molecules in liquid matrices does not appear to have an obvious dependence upon any single factor [5,20,28,65–67]. We will argue that functional group basicity plays a key role in determining the alkali metal binding energy as well as the number, type, and relative abundance of fragment ions observed in a corresponding desorption ionization mass spectrum.

Fast atom bombardment ionization is routinely used for the analysis of nucleotides [41,68–77]. Owing to the acidic nature of the phosphate chain, most FAB analysis of nucleotides have been performed for negative ions. Fragment ions formed by the dissociation of the nucleotide phosphate chain are not observed in the CID spectrum of the \([M - H]^-\) ions of ATP and
Finally, we will show that fragment ions formed by the dissociation of the nucleotide phosphate chain are readily observable in the CID spectrum of the [M + Na]+ ions of uridine triphosphate and that these fragment ions can be quite useful for studying isotope-exchange reactions of nucleotides.

MECHANISM FOR THE FORMATION OF [M + xA - (x - 1)H]+-TYPE IONS OF POLAR ORGANIC MOLECULES

A mechanism for the formation of organo-alkali metal ions that is consistent with all the experimental results reported to date involves alkali metal ion transfer via an organo-alkali metal halide cluster ion complex of the type [M + A(AX)n]+, viz.

\[
\begin{align*}
M + A(AX)_n^+ & \rightarrow [M + A(AX)_n]^+ \\
[M + A(AX)_n]^+ & \rightarrow [M + A]^+ + n \text{AX}
\end{align*}
\]

(1) (2)

The mechanism does not specify whether sodium transfer is a "surface" (or solution) reaction or a "gas-phase" reaction; however, the unimolecular metastable ion and collision-induced dissociation (CID) data clearly establish a link between [M + A(AX)n]+ ions and [M + xA - (x - 1)H]+ ions [21].

We have used FAB-TMS to provide insight into the mechanism for formation of the organo-alkali metal ions. [M + xA - (x - 1)H]+ ions are important fragment ions of ionic clusters of the type [M + A + A(AX)n - 1 - H]+ and these ions are formed by the elimination of HX from [M + A(AX)n]+-type ions [reaction (3)] [78].

\[
\begin{align*}
[M + A(AX)_n]^+ & \rightarrow [M + A + A(AX)_{n-1} - H]^+ + \text{HX} \\
[M + A + A(AX)_{n-1} - H]^+ & \rightarrow [M + 2A - H]^+ + (n - 1)\text{AX}
\end{align*}
\]

(3) (4)

It should be no surprise that relatively large anion and cation effects are observed for reactions (2)-(4) [79]. These effects can be summarized as follows. In the metastable ion (MI) spectra of [G + A(AX)n]+ (G = glycerol, A = alkali metal, X = halogen, and n = 1,2), the dominant fragment ions are [G + A]+ and A(AX)n+. For example, the [G + Na]+ (m/z 115) ion accounts for 87% of the total fragment ion yield in the MI spectrum of [G + Na(NaI)]+ (Fig. 2). Similarly, the production of [G + Na]+ accounts for 86% of the total product ion yield in the MI spectrum of [G + Na(NaI)]+ (Fig. 3). Conversely, in the MI spectrum of [G + Na(NaF)]+ (Fig. 4) the production of [G + Na]+ (m/z 115) accounts for only 11% of the total fragment ion yield and in the MI spectrum of [G + K(KI)]+ (Fig. 5), the production of [G + K]+ (m/z 131) accounts for only 7% of the total fragment ion yield.
The major fragment ions of $[G + Na(NaF)]^+$ and $[G + K(KI)]^+$ are $[Na(NaF)]^+$ ($m/z$ 65, 82% of the total fragment ion yield) and $[K(KI)]^+$ ($m/z$ 205; 68% of the total fragment ion yield), respectively.

Another important reaction channel for $[G + A(AX)_{n-1}]^+$-type ions is elimination of HX [reaction (3)] to form $[G + A + A(AX)_{n-1} - H]^+$. In the MI spectrum of $[G + Li(Li)]^+$ [Fig. 6(a)], elimination of HI to give $[G + 2Li - H]^+$ ($m/z$ 105) is an important reaction channel (57% of the total fragment ion yield); however, elimination of HI is a relatively minor reaction channel (14% of the total fragment ion yield) in the CID spectrum [Fig. 6(b)].
Fig. 3. Metastable ion spectrum of $[G + \text{Na(NaI)}]_2^+$ ($m/z$ 415).

The major product ions observed in the MI and CID spectra of ions such as $[G + A + A(AX)_{n-1} - H]^+$ (formed by HX elimination from $[G + A(AX)_n]^+$; $n = 2, 3$) are $[G + 2A - H]^+$-type ions. For example, in the MI spectra of $[G + A + A(AX)_{n-1} - H]^+$ ($A = \text{Li, Na, and K}$ and $X = \text{I}$) more than 80% of the total fragment ion yield corresponds to $[G + 2A - H]^+$ [80].

The formation of $[M + xA - (x - 1)H]^+$ ions ($x = 1, 2$) from $[G + A(AX)_n]^+$-type ions has been explained by using the following model [80]. The model considers the $[G + A(AX)_n]^+$ ions as alkali metal ion bound clusters of glycerol and alkali halide molecules (or clusters), e.g. $[aG \cdots \text{Na}^+ \cdots (\text{NaX})_n]$. According to the model, there are three ways for
the ions to dissociate: (1) loss of glycerol molecule; (2) loss of an alkali halide molecule; or (3) loss of HX. The dissociation pathway observed depends upon the relative binding energies associated with the organo-alkali metal ion system. For example, if the glycerol–alkali metal ion binding energy, $D^0(G-A^+)$, is greater than the alkali halide–alkali metal ion binding energy, $D^0(AX-A^+)$, the ion will dissociate to form $[G+A]^+$ (i.e. loss of AX will be the dominant process observed). If $D^0(AX-A^+)$ is greater than $D^0(G-A^+)$, the complex will dissociate to form $A(AX)^+$ (i.e. loss of G will be the dominant process observed). In those cases where the binding energies $D^0(G-A^+)$ and $D^0(AX-A^+)$ are approximately equal, i.e. where formation of neither $[G+A]^+$ or $[A(AX)_n]^+$ is favored, the organo-alkali metal ion system will dissociate by eliminating HX.

As a specific example, ions of the type $[G + 2Li - H]^+ (m/z 105)$ are formed by the elimination of HI from $[G + Li(LiI)]^+$. Thus, $D^0(G-Li^+)$ and $D^0(LiI-Li^+)$ are proposed to be approximately equal since HI elimination is the most abundant reaction channel. Upon collisional activation of $[G +
Li(LiI)]^+; the favored reaction channel is loss of LiI. The preferred loss of LiI upon collisional activation suggests that $D^0$(G−Li') is slightly greater than $D^0$(LiI−Li'). In addition, a strong dependence on the heat of formation ($\Delta H_f$) and IIX is observed. That is, as $\Delta H_f$ of HX decreases, the relative abundance of [G + 2Li − H]^+ as a fragment ion of [G + Li(LiX)]^+ increases (see Fig. 7).

This interpretation of the data is consistent with the fact that [G + Li(LiF)]^+ is not observed in the normal FAB mass spectrum of glycerol–LiF solutions. Owing to the low $\Delta H_f$ for HF (−65 kcal mol$^{-1}$), HF elimination is a facile reaction which precludes the observation of [G + Li(LiF)]^+-type ions in the normal FAB mass spectrum, i.e. [G + 2Li − H]^+ is formed.

[G + Na]^+ and $\Lambda$(AX)$_n$^+ ions are observed as important fragment ions in the MI spectrum of [G + Na(NaX)]^+. For example, 87% of the total fragment ion yield in the MI spectrum of [G + Na(NaI)]^+ corresponds to [G + Na]^+ ($m/z$ 115) ions, whereas formation of Na(NaX)$^+$ is the dominant
process observed in the MI spectra of \([G + \text{Na(NaX)}]^+\), where \(X = \text{Br, Cl, and F}\). Formation of \(A(AX)^+\) is observed as the major fragment ion of \([G + A(AX)]^+\), where \(A = \text{K}^+, \text{Rb}^+, \text{Cs}^+\) and \(X = \text{I, Br, Cl, F}\). Furthermore, formation of \(A(AX)_2^+\) is observed as the major fragment ion of \([G + A(AX)_2]^+\), where \(A = \text{K}^+, \text{Rb}^+, \text{Cs}^+\) and \(X = \text{I, Br, Cl, F}\). Thus, for all
alkali halide systems of K⁺, Rb⁺, and Cs⁺, $D^0(AX-A^+)$ must be greater than $D^0(G-A^+)$. Cluster ions of the type $[aG + A(AX)_n]^+ (a = 1−4; n = 1,2)$ and $[aG + A + A(AX)_{n-1}H]^+ (a = 1−4; n = 2,3)$ have a large effect on the chemistry of glycerol−alkali halide solutions. For example, in the MI spectrum of $[G + Li(LiI)]^+$ [Fig. 8(a)], the dominant dissociation channel is elimination of HI ($m/z$ 105; 57% of the total fragment ion yield). Conversely, in the MI spectrum of $[2G + Li(LiI)]^+$ [Fig. 8(b)], the elimination of HI ($m/z$ 197) accounts for only 27% of the total fragment ion yield. The major reaction

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Fig. 7. Graph of percent yield of $[G + 2Li-H]^+$ as a fragment ion of $[G + Li(LiX)]^+$ versus the heat of formation ($\Delta H_f$) of HX.
Fig. 8. (a) Metastable ion spectrum of $[G + Li(LiI)]^+$ ($m/z$ 233). (b) Metastable ion spectrum of $[2G + Li(LiI)]^+$ ($m/z$ 325). (c) Metastable ion spectrum of $[3G + Li(LiI)]^+$ ($m/z$ 417). (d) Metastable ion spectrum of $[4G + Li(LiI)]^+$ ($m/z$ 509).

channel for $[2G + Li(LiI)]^+$ corresponds to $[2G + Li]^+$ ($m/z$ 191). The $[2G + Li]^+$ ion accounts for 49% of the total fragment ion yield from $[2G + Li(LiI)]^+$. In the MI spectra of $[3G + Li(LiI)]^+$ [Fig. 8(c)] and $[4G + Li(LiI)]^+$ [Fig. 8(d)], the dominant process observed is the loss of a glycerol molecule.

The size of the glycerol cluster has the largest effect on the formation of $[G + A]^+$-type ions as fragment ions of $[aG + A(AX)]^+$ ($a = 2, 3, \ldots$). On the basis of the data shown in Fig. 8, we propose that the second, third, etc.,
glycerol molecules are only weakly bound to the cluster and comprise the primary solvation sphere. The formation of organo-alkali metal ions is thought to arise from \([G \cdots A^+ \cdots M]^+\)-type complex ions where primary solvation of the alkali metal ion occurs by the matrix via formation of \([G \cdots A^+ \cdots G]^+\)-type ions [20]. This conclusion is supported by the observed formation of \([2G + A]^+\) ions from \([aG + A(AX)]^+\). A specific example of this is shown in Fig. 8(b) where \([2G + Li]^+ (m/z 191)\) is observed as the preferred reaction channel in the MI spectrum of \([2G + Li(LiI)]^+\)-type ions.

**ORGANO-ALKALI METAL IONS OF SMALL PEPTIDES AND NUCLEOTIDES**

The dissociation reactions of several sugar molecules studied by FAB–TMS, including both \([M + H]^+\) and \([M + xA - (x - 1)H]^+\) (where \(x = 0–4\)), have been reported [21]. The dissociation reactions of the \([M + H]^+\) and \([M + Na]^+\) ions are quite similar, with the mass of the fragment ions being shifted by the mass of the alkali metal. Conversely, the dissociation reactions of the \([M + Na]^+\) ions of small peptides are markedly different from the dissociation reactions for the \([M + H]^+\) ions [60]. The CID spectra for the \([M + H]^+\) and \([M + Na]^+\) ions of hippuryl-histidyl-leucine (HHL) are shown in Fig. 9(a) and (b), respectively.

Two dominant ions in the CID spectrum of the \([M + H]^+\) ions of HHL are \(m/z\) 105 and \(m/z\) 110. The \(m/z\) 105 ion is indicative of a benzoyl group (e.g. \(C_7H_5O^+\)) and the \(m/z\) 110 ion is characteristic of peptides containing

![Image](image_url)

**Fig. 9.** Collision-induced dissociation (CID) spectra of (a) the \([M + H]^+ (m/z 430)\) ion and (b) the \([M + Na]^+ (m/z 452)\) ion of hippuryl-histidyl-leucine. (Reprinted from ref. 79.)
histidine residues (i.e. C₅H₈N₃⁺, cleavage b₁ + b₂, Fig. 10) [38]. Two additional structurally significant fragment ions are observed at m/z 269 and m/z 299. The m/z 269 ion is assigned to the loss of the benzoyl glycine (hippuryl) portion of the molecule, while the m/z 299 ion is formed by the loss of the leucine moiety, e.g. cleavage of the leu-his peptide bond. These two ions correspond to [Z₁ + H]⁺- and A²⁺-type fragment ions, respectively (Fig. 10).

The CID spectrum of the [M + Na]⁺ ion (m/z 452) of HHL contains fragment ions which are not observed in the [M + H]⁺ spectrum. In terms of structural information, the most important ions occur at m/z 363, 338, 321, and 294. The m/z 363 ion in the CID spectrum of the [M + Na]⁺ ion is assigned to the loss of CH(CH₃)₂ and CO₂H from the leucine portion of the molecule (denoted cleavage I, Fig. 10). The m/z 338 ion (cleavage c) arises from H transfer from the leucine residue (R group) followed by cleavage of the leucyl residue NH–CH bond. The two remaining ions at m/z 321 and
m/z 294 correspond to loss of the leucine residue and loss of leucine plus the adjacent (histidyl) carbonyl group (cleavages d and e, respectively). These ions account for ca. 70% of the total CID product ion current. Conversely, the two most structurally significant CID product ions (i.e. loss of hippuryl and leucine residues) in the spectrum of the [M + H]^+ ion account for less than 25% of the total product ions.

Attention is also drawn to the m/z 132 ion, which is assigned as the histidyl moiety plus the Na^+ ion (cleavages e and f). Owing to the basicity of the imidazole nitrogen (pK_a = 6), the Na^+ ion strongly interacts with this site of the molecule. This interaction is further stabilized by interaction between the amide nitrogens of glycyl and histidyl residues (see Fig. 10). It is also important to note that, in all cases, the CID product ions retain the Na^+ ion. On the basis of this information and studies on other systems, we proposed that specific binding of alkali metal ions to peptides can be employed to enhance specific, structurally informative dissociation reactions [60]. Investigation of the [M + Na]^+ ion of larger peptides (i.e. angiotensin I and II) containing both histidine and arginine indicates preferential attachment of Na^+ to the arginine residue [81]. The preferential binding of Na^+ to arginine is attributed to the fact that the R group of arginine (pK_a = 12) is ca. 10^6 times more basic than the R group of histidine (pK_a = 6) [82]. Highly specific attachment of Na^+ and K^+ ions is also indicated in the [M + Na]^+ and [M + K]^+ ions formed by Cs^+ desorption ionization of gramicidin S [83]. In this case, the alkali metal ion is preferentially bound to the ornithine residues; ornithine is the most basic site of the gramicidin S molecule.

Several studies on the analysis and structure elucidation of nucleotides and nucleosides by mass spectrometry have been reported [41,68–77,84–94]. One factor to consider in the case of FAB ionization of nucleotides is the greater sensitivity for negative ions. This is undoubtedly a result of the extent of dissociation of the acidic phosphate groups in the liquid matrix prior to particle bombardment [95].

The lowest energy dissociation reaction available to the collisionally activated [M – H]^+ ion is electron detachment. Although it would be preferable to characterize the nucleotide by positive ion FAB ionization, the phosphate groups of the nucleotide have relatively high alkali metal ion affinities and even trace impurities of sodium give rise to abundant [M + Na]^+ ions and only weak [M + H]^+ ions.

In an earlier paper, we used FAB–MS to monitor the positional isotope exchange (PIX) reaction of adenosine triphosphate (ATP)-[β-18O_2, β,γ-18O, γ-18O_3] (Scheme 1) [41]. In this study, incorporation of 18O at the alpha–beta position was followed by enzymatic degradation of the ATP to AMP followed by negative ion FAB–MS. Exchange of 18O into the alpha–beta bridging position of the ATP shifts the mass of the resulting
AMP by 2 mass units. By enzymatically stopping the PIX reaction at specific times, the percent of $^{18}$O exchange was determined by using FAB mass spectrometry [41].

A similar method cannot be used for UTP because an enzyme for UMP formation from UTP has not been reported. To determine the extent of PIX for $^{18}$O incorporation at the alpha-beta bridging position of UTP-$[^{18}$O$_2, \beta, \alpha, \gamma-^{18}$O], a specific reaction channel must be observed in the CID spectrum (see Scheme 2 for the PIX reaction for UTP) [47]. The most useful reaction channels involve cleavage of the $\alpha, \gamma$-pyrophosphate (i.e.
neutral loss of $\text{H}_3\text{P}_2\text{O}_6$ and $\text{H}_3\text{P}_2\text{O}_7$ [see Figs. 11(a) and (b)]. The specific fragment ions required to follow $^{18}\text{O}$ exchange into the alpha–beta bridging position may be observed in the CID spectrum of the $[\text{M} + \text{H}]^+$ ions of UTP; however, residual sodium present in the samples of UTP greatly reduces the yield for $\text{[M} + \text{H}]^+$ ions. Without performing extensive purification on each sample, CID analysis of the $[\text{M} + \text{H}]^+$ ions of UTP cannot be performed. Recall also that the fragment ions necessary to elucidate the position of $^{18}\text{O}$ incorporation are not observed in the CID spectrum of the
[\text{M} - \text{H}]^- \text{ ions of UTP, e.g. the lowest energy reaction channel for } [\text{M} - \text{H}]^- \text{ ions is electron detachment.}

In the CID spectrum of the [\text{M} + \text{Na}]^+ ions of a synthetic sample of UTP-[\beta^{18}\text{O}_2, \beta, \gamma^{18}\text{O}, \gamma^{18}\text{O}_3] (\text{Fig. 12}), fragment ions at m/z 345 and 329 are observed which correspond to the loss of \text{H}_3\text{P}_2^{18}\text{O}_6 \text{ and } \text{H}_3\text{P}_2^{18}\text{O}_6\text{O}, \text{respectively} [47]. The occurrence of these fragment ions is consistent with \text{^{16}O} \text{ being located at the alpha-beta bridging position (see Fig. 11).}

Following the PIX reaction, the fragment ion at m/z 345 should shift to higher mass due to the incorporation of \text{^{18}O} \text{ at the alpha-beta bridging position. Indeed, the CID spectrum of the [\text{M} + \text{Na}]^+ ions of UTP-[\alpha, \beta^{18}\text{O}, \beta^{18}\text{O}, \beta, \gamma^{18}\text{O}, \gamma^{18}\text{O}_3] (\text{Table 1}) contains fragment ions at m/z 347 and m/z 329, corresponding to the loss of \text{H}_3\text{P}_2^{18}\text{O}_5\text{O} \text{ and } \text{H}_3\text{P}_2^{18}\text{O}_6\text{O}, \text{respectively. The presence of these fragment ions indicates that } \text{^{18}O} \text{ has been incorporated at the alpha-beta bridging position.}

\text{Additional fragment ions are observed in the CID spectrum of the } [\text{M} + \text{Na}]^+ \text{ ions of UTP-[\beta^{18}\text{O}_2, \beta, \gamma^{18}\text{O}, \gamma^{18}\text{O}_3], which also indicate the interaction site of the } \text{Na}^+ \text{ ion with the nucleotide. For example, the ions at}
TABLE 1

Mass, proposed structural assignment, and relative abundance of observed ions in the [M + Na]+ CID spectrum of UTP-[\beta^{18}O_2, \beta, \gamma^{18}O, \gamma^{18}O_3]

<table>
<thead>
<tr>
<th>m/z a</th>
<th>Structure</th>
<th>Relative abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>518</td>
<td>[UTP + Na]+</td>
<td>–</td>
</tr>
<tr>
<td>431</td>
<td>[UTP + Na – H_2P^{18}O_3]+</td>
<td>45</td>
</tr>
<tr>
<td>413</td>
<td>[UTP + Na – H_2P^{18}O_4]^+</td>
<td>16</td>
</tr>
<tr>
<td>407</td>
<td>[UTP + Na – base]^+</td>
<td>22</td>
</tr>
<tr>
<td>390</td>
<td>[UTP + Na – (base + OH)]^+</td>
<td>41</td>
</tr>
<tr>
<td>345</td>
<td>[UTP + Na – H_3P_2^{18}O_6]^+</td>
<td>31</td>
</tr>
<tr>
<td>329</td>
<td>[UTP + Na – H_3P_2^{18}O_5O]</td>
<td>69</td>
</tr>
<tr>
<td>293</td>
<td>[H_3P_2^{18}O_6O_4Na]^2</td>
<td>100</td>
</tr>
<tr>
<td>213</td>
<td>[H_4P_2^{18}O_6ONa]^+</td>
<td>85</td>
</tr>
<tr>
<td>208</td>
<td>[H_3P_2^{18}O_4Na]+</td>
<td>48</td>
</tr>
<tr>
<td>129</td>
<td>[H_3P_3^{18}O_4Na]^+</td>
<td>14</td>
</tr>
<tr>
<td>121</td>
<td>[H_4PO_4Na]^+</td>
<td>13</td>
</tr>
<tr>
<td>102</td>
<td>[PO_3Na]^+</td>
<td>18</td>
</tr>
<tr>
<td>98</td>
<td>[H_2PO_4]^+</td>
<td>25</td>
</tr>
<tr>
<td>23</td>
<td>[Na]^+</td>
<td>24</td>
</tr>
</tbody>
</table>

a Masses were calculated using the equation \( m_i = m_1 \cdot \left( \frac{E_i}{E_0} \right) \) where \( E_i \) is the measured ESA voltage corresponding to \( m_i \) (the mass of the fragment ion) and \( E_0 \) is the measured ESA voltage corresponding to \( m_1 \) (the mass of the parent ion). The calculated masses are accurate to \( m/z \pm 1 \).

m/z 293, 213, and 208 correspond to \( H_3P_3^{18}O_6O_4Na \), \( H_4P_2^{18}O_6ONa \), and \( H_3P_2^{18}O_4O_3Na \) and the ions at m/z 129, 121, and 102 correspond to \( H_3P_3^{18}O_4Na \), \( H_3PO_4Na \), and \( PO_3Na \) (see Fig. 12) [8]. These ions account for approximately 53% of the total fragment ion yield, again suggesting a strong interaction of the Na⁺ ion with the phosphate chain of the molecule. We propose that the Na⁺ ion attachment to the phosphate portion of UTP gives rise to structurally significant fragment ions which are not observed in the CID spectrum of the [M + H]⁺ or [M – H]⁻ ions.

The studies cited above further illustrate the utility of alkali metal ion attachment to specific sites of a complex organic molecule to enhance structural information obtained by fast atom bombardment tandem mass spectrometry techniques. Investigations into the effect of binding energy and internal energy on the alkali metal ion interaction and the fragmentation behavior of larger peptides that contain basic amino acid residues (e.g. angiotensin II) are discussed in the following sections.

EFFECT OF FUNCTIONAL GROUP BASICITIES ON THE STRENGTH OF THE ORGANO-ALKALI METAL INTERACTION

To investigate further the site of interaction of the alkali metal ion (Na⁺) with peptides containing basic amino acid residues we have examined the
CID spectra of [M + Na]^+ ions of glycyl-histidine and glycyl-histidyl-arginyl-proline. These two compounds were selected because both histidine and arginine contain basic nitrogens which should have high alkali metal ion affinities [96].

Several important fragment ions are observed in the CID spectrum of the [M + Na]^+ ions of gly-his (Fig. 13). In addition to providing structural information, these fragment ions indicate the attachment site of the Na^+ ion to the peptide. For example, fragment ions at m/z 132, 117, and 104 correspond to the histidyl residue plus Na^+ minus the carboxyl terminus (i.e. cleavages at C_1 and A_1, see Fig. 14), the histidyl R group plus CH plus Na^+ (i.e. [R_2 + CH + Na]^+), and the histidyl R group plus Na^+ (i.e. [R_2 + Na]^+), respectively. In addition, fragment ions at m/z 98 and 81 correspond to the glycyl residue plus NHCH plus Na^+ and the glycyl residue plus Na^+ (i.e. [A_1 + Na]^+), respectively. Fragment ions at m/z 189 and 132 in the CID
Fig. 14. Proposed origin of fragment ions observed in the CID spectrum of the [M + Na]+ ions of glycyl-histidine.

spectra of the [M + Na]+ ions of gly-his are formed by cleavage of the carboxyl terminus, clearly suggesting that the Na+ ion does not interact at the C terminus. On the basis of the observed fragment ions, we propose that the Na+ ion strongly interacts with the imidazole ring nitrogen, the amide bond nitrogen, and the N terminus. Such an assignment is also consistent with the fact that 80% of the total fragment ion yield observed in the CID spectrum of the [M + Na]+ ions of gly-his are formed by interaction of the Na+ ion with the imidazole ring nitrogen, the amide bond nitrogen, and the N terminus. Furthermore, the most abundant fragment ion in the CID spectrum of the [M + Na]+ ions of gly-his is at m/z 132 which corresponds to loss of the carboxyl terminus plus the gly residue (i.e. [(C1−A1) + Na]+).

These observations can be extended to more complex systems such as gly-his-arg-pro. In the CID spectrum of the [M + Na]+ ions of gly-his-arg-pro (Fig. 15), fragment ions are observed which suggest that the Na+ ion interacts with the histidyl and arginyl R groups (see Fig. 16 for the structure of gly-his-arg-pro). For example, the most abundant fragment ion in the CID spectrum of the [M + Na]+ ions of gly-his-arg-pro is m/z 374. This ion is formed by cleavage of the amide linkage at the proline residue (i.e. [A3 + Na]+). Fragment ions are observed at m/z 374 and m/z 331, which correspond to cleavage of the amide linkage at the proline residue and expulsion of CO (i.e. [C2 + Na + H]+) and cleavage of the amide linkage at the proline residue with expulsion of CO plus the amino terminus (i.e. [(C2−Y0) + Na + H]+), respectively. Fragment ions at m/z 277 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 arg-pro plus Na (i.e. [Y2 + Na]+) and arg-pro plus Na+ minus OH (i.e. [Y2 + Na − OH]+). The ions at m/z 234 and m/z 189 correspond to the gly-his residue plus NH plus Na (i.e. [Y2 + Na − H]+) and the gly-his residue without CO plus Na (i.e. [C1 + Na − H]+), respectively.

The fragment ions in the CID spectrum of the [M + Na]+ ions of gly-his-arg-pro can be attributed to the site of the Na+ ion attachment to the
Fig. 15. CID spectrum of the [M+Na]^+ ions of gly-his-arg-pro (m/z 488).

Fig. 16. Proposed origin of fragment ions observed in the CID spectrum of the [M+Na]^+ ions of gly-his-arg-pro (m/z 488).
TABLE 2

Mass, proposed structural assignment, and relative abundance of observed fragment ions in the [M+Na]+ CID spectrum of angiotensin II

<table>
<thead>
<tr>
<th>m/z</th>
<th>Structure</th>
<th>Relative abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>953</td>
<td>[Z₁+Na]+</td>
<td>100</td>
</tr>
<tr>
<td>780</td>
<td>[C₅+Na]+</td>
<td>60</td>
</tr>
<tr>
<td>746</td>
<td>[(C₆-Y₁)+Na]+</td>
<td>58</td>
</tr>
<tr>
<td>530</td>
<td>[C₃+Na]+</td>
<td>50</td>
</tr>
<tr>
<td>485</td>
<td>[(C₃-R₃)+Na]+</td>
<td>44</td>
</tr>
</tbody>
</table>

*Masses were calculated using the equation $m_i = m_1 \times (E_i / E_0)$ where $E_i$ is the measured ESA voltage corresponding to $m_i$ (the mass of the fragment ion) and $E_0$ is the measured ESA voltage corresponding to $m_1$ (the mass of the parent ion). The calculated masses are accurate to $m/z \pm 1$.

peptide. The fragment ions at $m/z$ 234 and 189 account for roughly 16% of the total fragment ion yield and are assigned to Na⁺ ion attachment at the histidyl R group (pKₐ = 6.0). Owing to the higher basicity of the arginyl R group (pKₐ = 12.5), the fragment ions at $m/z$ 374, 347, 331, 277, and 261 (approximately 71% of the total fragment ion yield) are attributed to Na⁺ ion attachment at the arginyl R group. On the basis of the observed fragment ions, the Na⁺ ion is proposed to be strongly bound to the guanadino group of arginine while interacting with the amide nitrogen of the arg-his peptide bond and the imidazole ring nitrogen of the histidine.

The dissociation reactions of peptides containing several amino acids with titratable R groups follow the same trends as discussed above. That is, the relative abundance of the fragment ions which arise from interaction of the Na⁺ ion at a specific site of the peptide increases as the pKₐ of the R group increases. A good example of this is the [M + Na]+ ions of angiotensin II ($m/z$ 1069, Table 2). The five most abundant fragment ions in the CID spectrum are attributed to interaction of the sodium ion with a specific R group (i.e. arginine, pKₐ = 12.5; tyrosine, pKₐ = 10.1; histidine, pKₐ = 6.0; see Fig. 17 for the structure of angiotensin II). The fragment ions at $m/z$ 953 and $m/z$ 746 are assigned to the loss of the aspartic acid residue (i.e. [Z₁+Na]⁺) and the loss of the aspartic acid (and NH) and phenylalanine residues (i.e. [(C₆-Y₁)+Na]⁺), respectively; these two dissociation reactions suggest a strong interaction of the Na⁺ ion with the arginine R group. Fragment ions which correspond to Na⁺ ion interaction at the tyrosine R group are observed at $m/z$ 530 and $m/z$ 485 and are assigned as asp-arg-val-tyr (i.e. [C₅+Na]⁺) and asp-arg-val-tyr minus the valyl R group (i.e. [(C₃-R₃)+Na]⁺), respectively. The fragment ion at $m/z$ 780 is assigned to cleavage of the prolyl-phenylalanine residues (i.e. [C₅+Na]⁺), which corresponds to interaction of the sodium ion with the histidine R group.
Thus, the interaction of an alkali metal ion with polar organic molecules is determined by the relative alkali metal binding energy of the function groups present. For small peptides, which contain only one basic amino acid residue (e.g. gly-his), the Na\(^+\) ion interacts with the amino terminus, the amide nitrogen of the peptide bond, and the imidazole ring nitrogen of the histidine R group. Similar results have been reported for NMR of Zn\(^{2+}\) complexes with gly-his and ala-his [97]. For those peptides with multiple sites for interaction of the Na\(^+\) ion (i.e. peptides with more than one basic amino acid residue), the probability for observing fragment ions formed due to Na\(^+\) ion interaction at different locations is determined by the pK\(_a\) of the titratable R groups present in the molecule.

ENERGETIC CONSIDERATIONS FOR FRAGMENTATION PATHWAYS OF ORGANO-ALKALI METAL IONS FORMED BY FAB IONIZATION

An additional factor influencing the dissociation reactions of [M + A]\(^+\)-type ions is the binding energy of A\(^+\) to organic molecules [i.e. \(D^0(M-A^+)\)] and the energetics for dissociation of the [M + A]\(^+\) ion complex [98]. The [M + A]\(^+\) complex can dissociate via two competitive reactions, i.e. the formation of the alkali metal ion (A\(^+\)) or the formation of alkali metal attached fragment ions ([F, + A]\(^+\)), [reactions (5) and (6), respectively]. For example, the relative abundance of alkali metal ions (A\(^+\)) in the CID spectra of [M + A]\(^+\) ions of hippuryl-histidyl-leucine follows the trend Cs\(^+\) > Rb\(^+\) > K\(^+\) > Na\(^+\) > Li\(^+\) [101]. This trend is expected based on the relative binding energies \([D^0(M-A^+)]\) of the alkali metal ions to the organic molecule. Since Li\(^+\) has the largest \(D^0(M-A^+)\) [10,99,100], the appearance energy for reaction (5) is high (relative to Na\(^+\) or K\(^+\)) and the [M + Li]\(^+\) ion can
accommodate more internal energy before dissociating to M and Li\(^+\). The occurrence of reaction (6) will depend upon the relative energetics for reactions (5) and (6). Therefore, the only fragmentation reactions that can occur are those with appearance energies less than \(D'(M-A^+)\). Owing to the higher values for \(D'(M-A^+)\), where A = Li\(^+\) and Na\(^+\), the dissociation of the \([M + A]^+\) ions will favor reaction (6). As the internal energy of the \([M + A]^+\) ion decreases, the branching ratio for reactions (5) and (6) will depend upon the absolute values for \(D'(M-A^+)\) and the appearance energy.

**Fig. 18.** CID spectra of the \([M+A]^+\) ions of hippuryl-histidyl-leucine where A = Li (m/z 436), Na (m/z 452), K (m/z 468), Rb (m/z 514), and Cs (m/z 562). (Reprinted from ref. 79.)
Fig. 19. CID spectra of the [M + A]^+ ions of leucine enkephalin were A = Li (m/z 562), Na (m/z 578), K (m/z 594), Rb (m/z 640), and Cs (m/z 688).
for the \([F_i + A]^+ [AE^0(F_i + Na^+)]\) fragment ions.

\[
[M + A]^+ \rightarrow M + A^+ \rightarrow [F_i + A]^+ + N
\]  

These concepts follow the general observation that the desorption ionization mass spectra of compounds doped with \(K^+\) give high yields for \([M + K]^+\) and correspondingly low fragment ion yields. On the other hand, the spectra for compounds doped with \(Li^+\) or \(Na^+\) contain both \([M + A]^+\) and fragment ions. Therefore, by careful selection of the alkali metal cation, it is possible to preferentially cleave bonds of the molecule which have appearance energies less than \(D^0(M-A^+)\) [98].

![Breakdown graphs for sequence fragment ions observed for hippuryl-histidyl-leucine](image)

Fig. 20. Breakdown graphs for sequence fragment ions observed for hippuryl-histidyl-leucine, □, C-type fragment ions; ⊗, B-type fragment ions; △, A-type fragment ions.
There are three basic types of sequence fragment ions observed for collisionally activated organo-alkali metal ions of small peptides. These fragment ions correspond to cleavage at the amide bond (A-type), cleavage of the NH–CH bond (B-type), and cleavage of the CH–CO bond (C-type). All of these reactions occur with retention of charge towards the N terminus of the peptide [23,101]. If the cationizing species (e.g. Li⁺, Na⁺, H⁺, etc.) imposes an upper limit on the magnitude of the internal energy of the molecule, it is possible to determine the relative bond strength for the sequence fragment ions. That is, by calculating the percent total fragment ion yield of A-, B- and C-type fragment ions for a series of [M + A⁺] ions of peptides the relative strength of the three sequence ions can be determined.

A series of [M + A⁺] (A = H⁺, Li⁺, Na⁺, K⁺, Rb⁺, Cs⁺) ions of hippuryl-histidyl-leucine (HHL) and leucine enkephalin have been studied and the data are presented in Figs. 18 and 19. The purpose of this study was
to ascertain whether relative energetics could be obtained for the A, B, and C bond strengths. Breakdown graphs based on the relative abundance of the CID product ions versus the estimated value for $D^0(M-A^+)$ are presented in Figs. 20 and 21. On the basis of this information, the appearance energies for the fragment ions follow the trend $A > B > C$.

In general, the dominant dissociation reactions of $[M + Na]^+$ ions of peptides are cleavage of the C bond. Although all the possible bond-cleavage reactions are observed for angiotensin II, the cleavage of C-type bonds is favored. In the case of kemptide (a heptapeptide) all of the major fragment ions correspond to C type ions. In general, what this means is that the CID spectra of the $[M + A]^+$ ions of peptides are simpler (containing fewer ions) than CID spectra of $[M + H]^+$ ions. Thus, it may be possible to use the CID spectra for $[M + H]^+$ and $[M + A]^+$ in a complementary manner to elucidate subtle structural features.

**SUMMARY**

The role of cluster species in desorption ionization, from either liquid or solid matrices, is becoming increasingly important. It has been proposed that molecular ions are desorbed from the surface as large solvated molecule-ion clusters and the role solvation plays in obtaining an abundant molecular ion yield is beginning to emerge. For clusters such as $[aG + [M + A]^+]$ or $[aG + A(AX)_x]^+$, excess internal energy in the desorbed ion can be removed by successive loss of G and/or AX to form $[M + xA - (x - 1)H]^+$-type ions ($x = 1,2$). In the case of $[M + H]^+$, ionization involves competition among the solvent (G) and sample (S) for the proton, e.g. $[aG \cdot H^+ \cdot \cdot \cdot S_x]$. Thus, the number, type, and relative abundance of fragment ions observed for $[M + A]^+$ type ions of polar organic molecules correspond to those dissociation reactions associated with a lower appearance energy differing from those observed for dissociating $[M + H]^+$-type molecular ions.

Another aspect of the chemistry of ionic cluster systems involves the dissociation of such ions and the structural information that can be obtained for these species. In particular, preliminary studies reported here and elsewhere suggest that structural information about the molecule, viz. the location of basic amino acid residues in the molecule, can be readily obtained from the CID spectrum of the $[M + A]^+$ ions and the structural information obtained from the $[M + A]^+$ ion is complimentary to that obtained from the CID spectrum of the $[M + H]^+$ ions.

**REFERENCES**