Na^+ Binding to Cyclic and Linear Dipeptides. Bond Energies, Entropies of Na^+ Complexation, and Attachment Sites from the Dissociation of Na^+-Bound Heterodimers and ab Initio Calculations

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Abstract: The Na^+ affinities of simple cyclic and linear dipeptides and of selected derivatives are determined in the gas-phase based on the dissociations of Na^+-bound heterodimers [peptide + B_i]Na^+, in which B_i represents a reference base of known Na^+ affinity (kinetic method). The decompositions of [peptide + B_i]Na^+ are assessed at three different internal energies; this approach permits the deconvolution of entropic contributions from experimentally measured free energies to thus obtain affinity (i.e. enthalpy or bond energy) values. The Na^+ affinities of the peptides studied increase in the order (kJ mol^{-1}) *cyclo*-glycylglycine (143) < *cyclo*-alanylglycine (149) < *cyclo*-alanylalanine (151) < *N*-acetyl glycine (172) < glycylglycine (177) < alanylglycine (178) < glycylalanine (179) < alanylalanine (180) < glycylglycine ethyl ester (181) < glycylglycine amide (183). The method used provides quantitative information about the difference in bond entropies between the peptide−Na^+ and B_i−Na^+ bonds, which is most significant when Na^+ complexation alters rotational degrees of freedom either in the peptide or in B_i. From the relative bond entropies, it is possible to appraise absolute entropies of Na^+ attachment, which are ∼104 and ∼116 J mol^{-1} K^{-1} for the cyclic and linear molecules, respectively. The combined affinity and entropy data point out that the cyclic dipeptides bind Na^+ in a monodentate fashion through one of their amide carbonyl oxygens, while the linear molecules coordinate Na^+ in a multidentate arrangement involving the two carbonyl oxygens and, possibly, the N-terminal amino group. High-level ab initio calculations reveal that the most stable [glycylglycine]Na^+ structure arises upon bidentate chelation of Na^+ by the two carbonyls and concomitant formation of a hydrogen bond between the amino group and the amide nitrogen. Such a structure agrees very well with the experimental enthalpy and entropy trends observed for the linear molecules. According to theory, zwitterionic forms of [glycylglycine]Na^+ are the least stable isomers, as also suggested by the experimental results.

Introduction

Sodium ion is among the most abundant metal ions in biological systems. As a bulk electrolyte, Na^+ not only helps regulate energy metabolism and signaling mechanisms through transmembrane concentration gradients, but also serves to stabilize the structure of proteins and membranes.[1−4] Although Na^+ is mainly an extracellular cation, it is also required in specific stoichiometries for intracellular processes to promote enzyme activity and protein function, as in the cases of Na^+/K^+-ATPase and oxaloacetate decarboxylase.[4]

Na^+ must interact with the appropriate peptides or proteins in order to carry out its regulatory or structural functions. These interactions can be quite complex, because peptides and proteins are multifunctional molecules with several potential binding sites. An important factor for the selection of a certain location for Na^+ attachment is the bond strength (affinity) between the donor groups available at this location and Na^+. Reciprocally, the sodium ion affinity of a potential binding site in a biomolecule is greatly affected by the site's structural features, in particular the coordination it can offer, inductive and steric effects, and the types of ancillary, intramolecular, noncovalent interactions that can be supported at or near the binding site. Knowledge about the intrinsic Na^+ binding modes to simple protein components, such as dipeptides, would significantly enhance our understanding of how this metal ion interacts with larger biomolecules in real living systems. To address this aspect, the sodium ion affinities of model cyclic and linear dipeptides composed of glycine and/or alanine residues are probed here by tandem mass spectrometry (MS/MS).[5] The solvent-free environment of the mass spectrometer provides an ideal medium for measuring these important intrinsic properties in the absence of complicating or interfering solvent effects. Further, by the choice of peptides with glycine and alanine, which bear no functionalized side chains, this study focuses on the binding properties of the backbone, i.e., of amine, amide, and carboxyl substituents.

Due to the immense importance of alkali metal ions in living systems,[1−4] the gas-phase chemistry of ionic peptide−alkali metal complexes has been an area of intensive research.[6−19] Most studies so far have concentrated on the collisionally

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activated dissociation (CAD) of these complexes and its analytical use in the determination of peptide sequences. On the basis of the fragments observed in CAD spectra, MS^3 and labeling experiments, and theory, it has been proposed that alkali metal ions bind at the most basic site of the peptide (N-terminus or side chain),[7,19] or at the carbonyl O-atoms,[8,11,12,16] or at the carboxylate function of the zwitterionic peptide,[9,10] or at several of these sites.[11,19] The preferred binding site might depend on the types of amino acid residues present and could, therefore, vary among different peptides.[19] Additionally, the metal ion could migrate during collision-induced fragmentation, thus yielding misleading information about its original position. Here, the enthalpies and entropies of Na^+ attachment to peptides are examined as an alternative, nondissociative approach for interrogating the structures of metal ion coordinated peptides. The only study so far about the thermochemistry of Na^+−peptide complexes was reported by Kebarle et al.,[20] who determined the Na^+ affinity of GG via threshold CAD experiments.[21−23] The present study closely reproduces Kebarle's value by using a modified variant of the Cooks kinetic method (see below),[24] measures the Na^+ affinities of several other peptides, and identifies the most likely binding mode of Na^+ to simple dipeptides, based on the derived thermochemical data. In addition, ab initio calculations are performed to both corroborate as well as help explain the experimental results.

Na^+ Affinities Based on the Dissociation of Na^+-Bound Dimers at Different Internal Energies. The sodium ion affinity ($\Delta H°_{Na^+}$) of a peptide (P) is defined as the enthalpy change of reaction 1 and corresponds to the dissociation energy of the P−Na^+ bond. The kinetic method, developed by Cooks and co-workers, measures *relative* sodium affinities. Specifically, the method compares the rates of dissociation of a Na^+-bound dimer to each of the individual sodiated monomers to estimate the difference in Na^+ affinities between the two monomers (i.e. their *relative* sodium ion affinity). For heterodimers between a dipeptide, P, and a series of reference bases, B_i, the pertinent dissociations are shown in eqs 2a and 2b.

$$[P]Na^+ \rightarrow P + Na^+ \qquad \Delta H°_{rxn} = \Delta H°_{Na^+} \tag{1}$$

$$[P + B_i]Na^+ \xrightarrow{k} [P]Na^+ + B_i \tag{2a}$$

$$[P + B_i]Na^+ \xrightarrow{k_i} P + [B_i]Na^+ \tag{2b}$$

Using transition state theory,[25] the ratio of the corresponding rate constants is given by eq 3,[26] where Q* and Q_i* are the partition functions of the activated complexes for reactions 2a and 2b, respectively, $\epsilon°$ and $\epsilon_i°$ are the corresponding activation energies, and T_{eff} is the effective temperature[27] of the dissociating dimer ion $[P + B_i]Na^+$. If the reverse activation energies of reactions 2a and 2b are negligible, which can be assessed by measuring the kinetic energy releases upon these dissociations,[28,29] $\epsilon_i° - \epsilon°$ can be approximated by $\Delta H°_{Na^+}(P) - \Delta H°_{Na^+}(B_i) = \Delta(\Delta H°_{Na^+})$, i.e., by the relative Na^+ affinity of the two bases comprising the sodium ion-bound dimer (eq 4).

$$\ln(k/k_i) = \ln(Q^*/Q_i^*) + (\epsilon_i° - \epsilon°)/RT_{eff} \tag{3}$$

$$\ln(k/k_i) = \ln(Q^*/Q_i^*) + \Delta(\Delta H°_{Na^+})/RT_{eff} \tag{4}$$

In the absence of reverse activation energies, the term $\ln(Q^*/Q_i^*)$ is equivalent to the difference in entropy change between reactions 2a and 2b, viz. $-\Delta(\Delta S°_{Na^+})/R$.[25,30,31] Substitution of $\ln(Q^*/Q_i^*)$ by $-\Delta(\Delta S°_{Na^+})/R$ leads to eq 5,[31]

$$\ln(k/k_i) = -\Delta(\Delta S°_{Na^+})/R + \Delta(\Delta H°_{Na^+})/RT_{eff} = \Delta(\Delta G°_{Na^+})/RT_{eff} \tag{5}$$

$$\ln(k/k_i) = [\Delta H°_{Na^+}(P)/RT_{eff} - \Delta(\Delta S°_{Na^+})/R] - \Delta H°_{Na^+}(B_i)/RT_{eff} \tag{6}$$

$$\Delta G^{app}{}_{Na^+}(P) = \Delta H°_{Na^+}(P) - T_{eff}\,\Delta(\Delta S°_{Na^+}) \tag{7}$$

from which it is evident that the kinetic measurement provides free energies, not enthalpies, unless the entropic parameter is negligible. Usually, $\Delta(\Delta S°) \approx 0$ (i.e. $Q^* \approx Q_i^*$) when the two bases contained in the dimer are structurally similar compounds forming the same type of bond(s) to the metal ion bridging them.[32,33] Unfortunately, a set of reference bases (B_i) with *both* known sodium ion affinity *and* peptide-like structures does not exist. Hence, $\Delta(\Delta S°_{Na^+}) \neq 0$; nevertheless, this entropy parameter can be kept constant if B_i are chosen to be chemically similar with each other, coordinating Na^+ in an analogous mode; they still may differ from P. In such a case, eq 5 can be

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expanded to eq 6, where the terms remaining constant within a series of heterodimers $[P + B_i]Na^+$ have been combined in brackets. The quantity in brackets represents an apparent gas-phase basicity of P toward Na^+, $\Delta G^{app}_{Na^+}(P)$, as defined by eq 7. The rate constant ratio k/k_i is experimentally measured and is equal to the ratio of the abundances of $[P]Na^+$ and $[B_i]Na^+$ in the MS/MS spectra of $[P + B_i]Na^+$. A plot of $\ln(k/k_i)$ versus the known sodium ion affinity of the reference bases, $\Delta H°_{Na^+}(B_i)$, leads to a regression line whose slope and intercept provide T_{eff} and $\Delta G^{app}_{Na^+}(P)$, respectively. The entropic and enthalpic parts of $\Delta G^{app}_{Na^+}(P)$ can be deconvoluted by determining the rate constant ratios at several effective temperatures.[30,31] T_{eff} is a measure of the internal energy of $[P + B_i]Na^+$ and can be changed by collisional excitation.[27,30,31] Application of eq 7 to metastable dimer ions (MI) as well as to dimer ions that have been subjected to CAD with He and Ar targets furnishes three effective temperatures (T_{MI}, T_{He}, and T_{Ar})[34] and three apparent sodium ion basicities ($\Delta G^{app}_{Na^+}$) for each dipeptide. A plot of $\Delta G^{app}_{Na^+}/RT_{eff}$ versus $1/RT_{eff}$ gives a new regression line which, according to eq 7, will supply $\Delta(\Delta S°_{Na^+})$ (from the intercept) and $\Delta H°_{Na^+}(P)$, i.e., the sodium ion affinity of the dipeptide in question (from the slope).

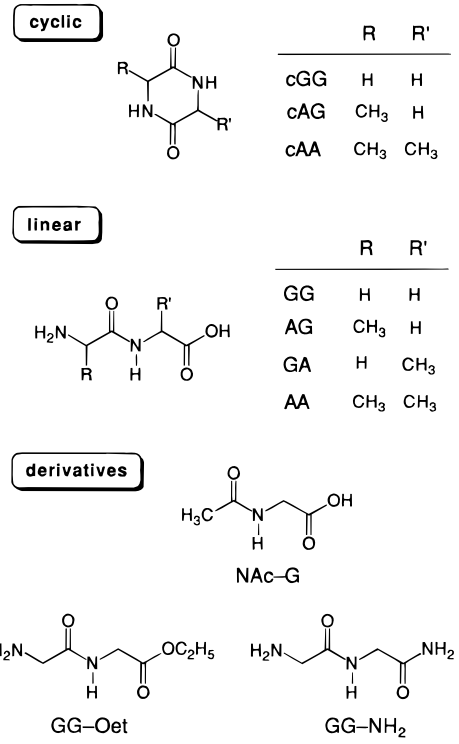
The described approach has successfully been used by Fenselau and co-workers to measure proton affinities[30,35] and, more recently, by us to measure the Li^+, Na^+, and K^+ affinities of the fundamental DNA and RNA nucleobases.[31] The latter study[31] showed that the assessment of relative entropies, $\Delta(\Delta S°_{M^+})$, not only yields better enthalpy data but also reveals structural insight about the rotational flexibility of the metalated species. This investigation provides further evidence for the usefulness of entropy in the detection of noncovalent interactions, and thus tertiary structure details, in the neutral as well as the sodiated peptides.

Methods

Experimental Procedures. All experiments were conducted with the Akron VG AutoSpec tandem mass spectrometer of E_1BE_2 geometry.[36] The sodium ion-bound heterodimers $[P + B_i]Na^+$ were generated by FAB with use of ~12 keV Cs^+ ions as bombarding particles and monothioglycerol as the matrix. The $[P + B_i]Na^+$ precursor ion of interest was accelerated to 8 keV, mass selected by MS-1 (E_1B), and allowed to dissociate spontaneously or by collision in the field free region between the magnet and the second electrostatic sector (FFR-3). The fragments thus produced were dispersed by MS-2 (E_2) and recorded in the corresponding metastable ion (MI) or collisionally activated dissociation (CAD) mass spectrum, respectively. For CAD, the target gas (He or Ar) was introduced in one of the two collision cells situated in FFR-3 until the precursor ion beam was attenuated by 20%. The MI and CAD spectra measured are multiscan summations and reproducible to ±5%. Kinetic energy releases of MI signals were calculated from peak widths at half-height ($T_{0.5}$) by established procedures.[28,29]

The samples were prepared from saturated solutions (in the matrix) of the appropriate dipeptide, reference base, and sodium acetate. To generate the desired heterodimer ion, ca. 0.5 mL aliquots of the individual stock solutions were mixed and a few microliters of the resulting mixture were transferred onto the FAB probe tip. This procedure maximized the intensity of $[P + B_i]Na^+$. The abundance of the heterodimer ions in the FAB spectrum was approximately 2−10% of the base peak (usually $[P + H]^+$ or $[B_i + H]^+$). All substances were purchased from Sigma and were used without any modification.

Scheme 1. Peptides Studied



Theory. The calculations were run on IBM RISC 6000 workstations and a Cray C94, using the Gaussian 94 package.[37] Initially, the semiempirical AM1 method[38] was used to explore the potential energy surfaces of the $[GG]Na^+$ complex and of neutral GG, for pre-optimization of the geometries of a number of potential $[GG]Na^+$ isomers and GG conformers. The AM1 geometries were re-optimized at the HF/3-21G level to get close to the true minima. Further optimization followed at the HF/6-31G* level, at which point the corresponding vibrational frequencies and entropies were also obtained. The final energies of the two most stable structures of $[GG]Na^+$ and GG were then calculated at the MP2/6-311+G(2d,2p) level by using the HF/6-31G* geometries. Basis set superposition errors (BSSE) were estimated at the MP2/6-311+G(2d,2p) level with the counterpoise method; this procedure leads to errors smaller than 10−15 kJ mol^{-1} according to a recent systematic study of the Li^+ complexes of small molecules.[39] Zero point vibrational corrections were added to yield D_0 values.[40] Thermal corrections, obtained at the HF/6-31G* level, gave D_{298}; combination of the latter with computed entropies finally led to ΔG_{298} values.

Results and Discussion

The reference bases, B_i, utilized to determine the sodium ion affinities of the dipeptides and derivatives studied (Scheme 1) are the nucleobases guanine, cytosine, and adenine; their Na^+ affinities have recently been established and are summarized in Table 1.[31] In accord with the prerequisites of the modified kinetic method (vide supra), the members of this reference set

(34) Compared with He, collisions with Ar are associated with a much larger center-of-mass energy and impart, on average, higher internal energies even in the keV domain.[5]

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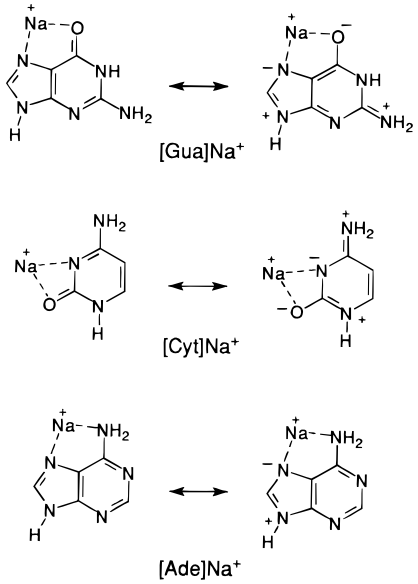
Table 1. Na^+ Affinities of Reference Bases

bases (B_i)	$\Delta H°_{Na^+}(B_i)$,[a] kJ mol^{-1}	ref
nucleobases		
guanine (Gua) [b]	182	31
cytosine (Cyt)[b]	177	31
adenine (Ade)[b]	172	31
thymine (Thy)[c]	144	31
uracil (Ura)[c]	141	31
amino acids		
valine (V)[b]	172	32
alanine (A)[b]	165	32
glycine (G)[b]	159	32

[a] Determined by using the kinetic method[31,32] and the 298 K Na^+ affinity of *N*,*N*-dimethyl formamide as absolute anchor.[32] Hence, the $\Delta H°_{Na^+}(B_i)$ values listed correspond to 298 K affinities. [b] Bidentate ligands. [c] Monodentate ligands.

Scheme 2. Structures of $[B_i]Na^+$, B_i = Gua, Cyt, Ade[31]



are chemically similar to each other, coordinating Na^+ in a common, bidentate manner (Scheme 2).[31]

The affinity order obtained for the linear species based on the above B_i set was then validated by comparing selected peptides with each other, i.e., by assessing the dissociations of heterodimers $[P_1 + P_2]Na^+$, in which peptide P_1 served as the unknown and was paired with a series of peptides P_2 that were designated as the reference bases. For the cyclic dipeptides, two alternative sets of reference bases, namely (i) the amino acids glycine, alanine, and valine and (ii) the nucleobases thymine and uracil, were additionally used to corroborate the internal consistency of the deduced enthalpic and entropic data. Table 1 includes relevant $\Delta H°_{Na^+}(B_i)$ values[31,32] as well as the acronyms, by which the selected B_i molecules are typically abbreviated.[41]

Dissociation of the Na^+-Bound Heterodimers. Irrespective of the peptide or reference base involved, the predominant fragments in the MI spectra of $[P + B_i]Na^+$ and $[P_1 + P_2]Na^+$ are the corresponding Na^+-attached monomers, generated according to eq 2. This is attested in Figure 1a for $[GA + Cyt]Na^+$. The shapes of all metastable $[P]Na^+$ and $[B_i]Na^+$ peaks are Gaussian and the corresponding kinetic energy releases ($T_{0.5}$) about 20 meV or less. These characteristics are consistent with endothermic cleavages without appreciable reverse activation energy,[28,29] a prerequisite for applying eqs 6−7 to derive thermochemical data (vide supra). Because the $P-Na^+$ and B_i-Na^+ bonds are of electrostatic nature,[1,42] the reactions of eq 2 involve simple electrostatic bond dissociations, which commonly proceed with no reverse activation energy,[42] justifying our experimental observation.

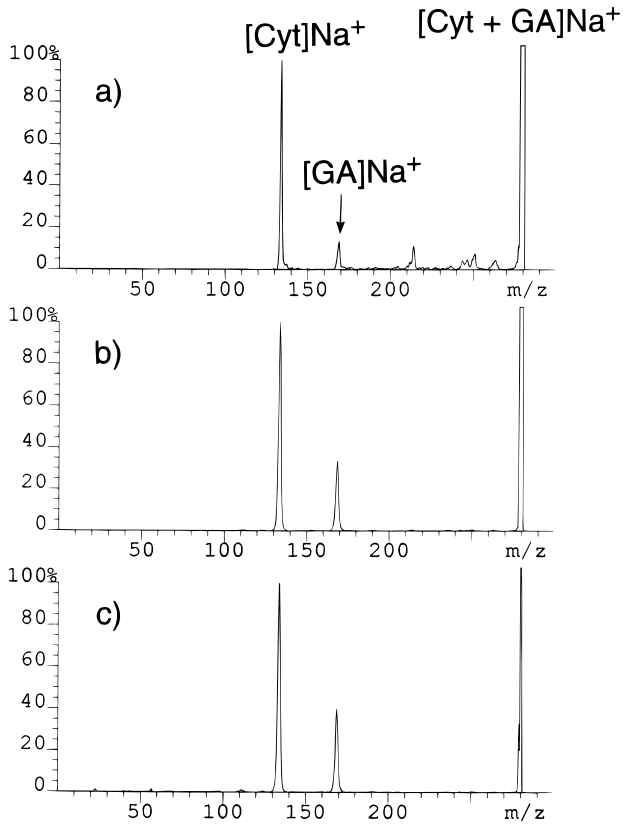


Figure 1. (a) MI, (b) CAD/He, and (c) CAD/Ar spectra of heterodimer $[GA + Cyt]Na^+$.

The competitive eliminations of one P and/or B_i unit (eq 2) remain as the main decomposition channel also after collisional activation, as documented in spectra b and c in Figure 1 by the He and Ar CAD spectra of the heterodimer $[GA + Cyt]Na^+$. No other significant fragmentation takes place, substantiating that the two bases forming the dimers are weakly bridged via a central Na^+ ion, viz. $P-Na^+-B_i$ or $P_1-Na^+-P_2$. Such a connectivity must be fulfilled before using the kinetic method to quantitatively determine bond energies (vide supra).[24,26,30−33]

Sodium Ion Affinities. The dissociations of $[P + B_i]Na^+$ were assessed for metastable ions as well as for ions undergoing CAD with He and Ar targets. These MS/MS spectra sample precursor ions of different internal energies,[5] corresponding to the effective temperatures T_{MI}, T_{He}, and T_{Ar}, respectively.[27] Each $[P + B_i]Na^+$ series includes three MS/MS spectra per effective temperature and peptide, which were replicated three times. Figure 2 shows a set of CAD/He spectra for dimers $[AG + B_i]Na^+$. Plotting the $\ln(k/k_i)$ values from these spectra versus $\Delta H°_{Na^+}(B_i)$ affords, according to eq 6, a regression line (Figure 3) whose slope and intercept render the effective temperature

(41) The kinetic method probes *relative* affinities and *relative* entropies of Na^+ complexation (eq 5), which are fairly independent of temperature because of the very similar heat capacity changes upon the competitive dissociations 2a and 2b. The relative affinities are converted to absolute $\Delta H°_{Na^+}$ data by anchoring them to the absolute affinities of the reference bases. The latter correspond to 298 K values (Table 1) and, thus, lead to the 298 K affinities of the peptides studied (Tables 2−4). Similarly, the 298 K values of $\Delta S°_{Na^+}(B_i)$ are used to assess 298 K entropies of Na^+ attachment from relative entropies (Table 8).

(42) See, for example: More, M. B.; Ray, D.; Armentrout, P. B. *J. Phys. Chem. A* **1997**, *101*, 831−839.

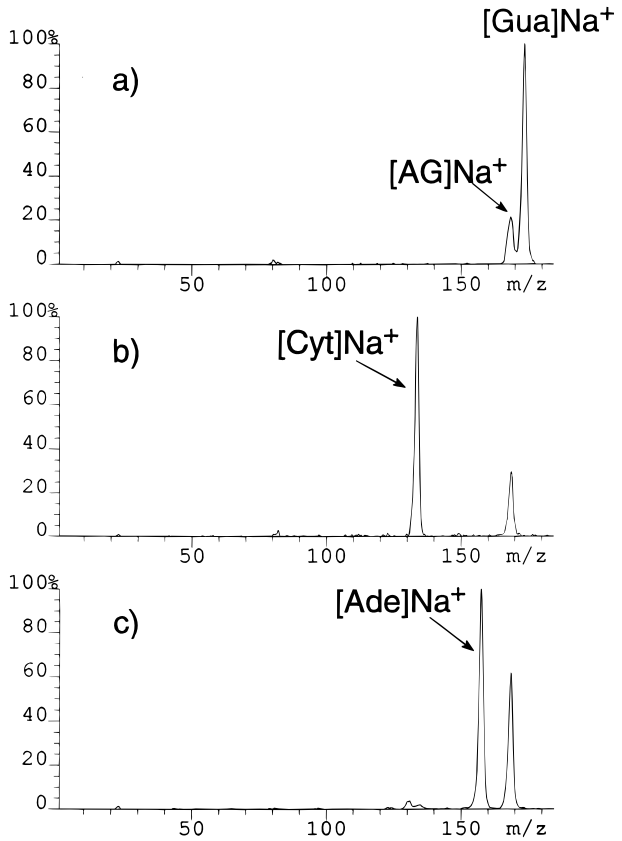


Figure 2. CAD/He spectra of the heterodimer series [AG + B_i]Na^+, B_i = Gua, Cyt, Ade: (a) [AG + Gua]Na^+; (b) [AG + Cyt]Na^+; and (c) [AG + Ade]Na^+.

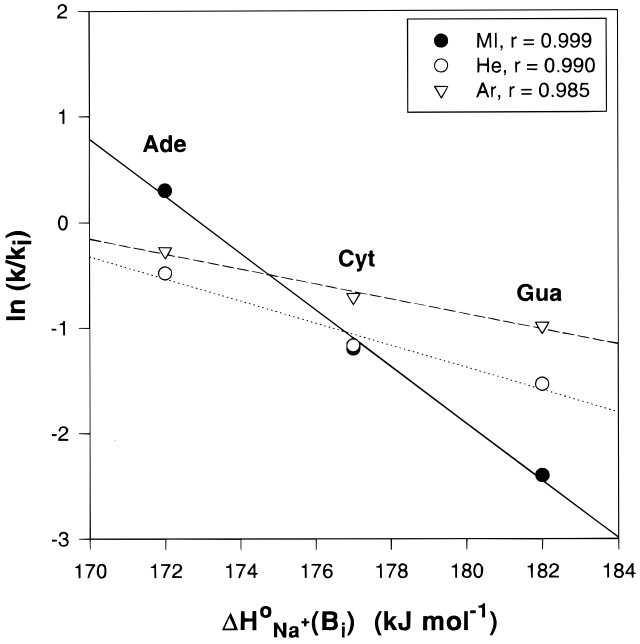


Figure 3. Plot of ln(k/k_i) vs $\Delta H°_{Na^+}(B_i)$ at three effective temperatures for heterodimers [AG + B_i]Na^+, B_i = Gua, Cyt, Ade. The rate constant ratio k/k_i is equal to the ratio of the abundances of [AG]Na^+ and [B_i]-Na^+ in the corresponding MI (filled circles), CAD/He (open circles), and CAD/Ar spectra (open triangles). The slopes of these regression lines provide the effective temperatures T_{MI}, T_{He}, and T_{Ar}, respectively, while their intercepts yield the corresponding apparent Na^+ affinities of AG, i.e $\Delta G^{app}{}_{Na^+}(AG)_{Teff}$.

of the dimers (i.e. T_{He} of [AG + B_i]Na^+) and the apparent Na^+ basicity of AG (i.e. $\Delta G^{app}{}_{Na^+}(AG)_{He}$), respectively. Similar

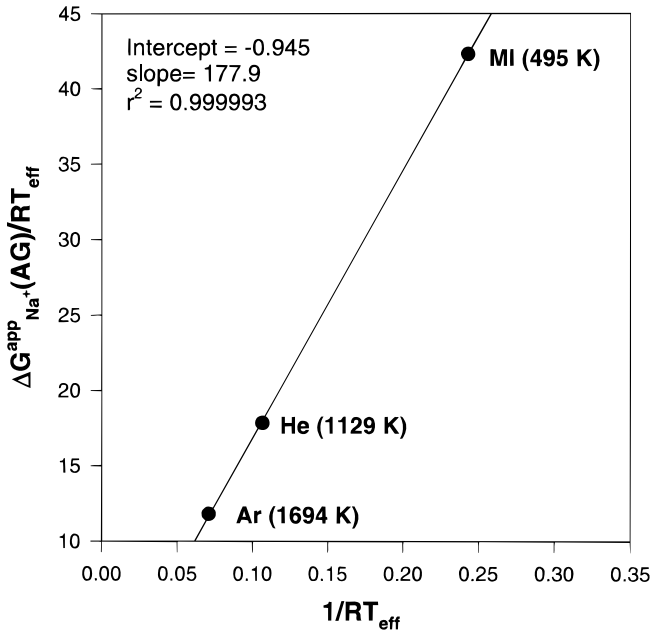


Figure 4. Plot of $\Delta G^{app}{}_{Na^+}(AG)_{Teff}/RT_{eff}$ vs $1/RT_{eff}$ for heterodimers [AG + B_i]Na^+, B_i = Gua, Cyt, Ade (equivalent to plot of the intercepts vs slopes of the regression lines shown in Figure 4). The slope of this regression line provides the Na^+ affinity of AG, $\Delta H°_{Na^+}(AG)$, and its intercept provides the relative entropy between the AG−Na^+ and B_i−Na^+ bonds, $\Delta(\Delta S°_{Na^+})$.

treatment of the MI and CAD/Ar dissociations of [AG + B_i]-Na^+ supplies T_{MI}, T_{Ar}, $\Delta G^{app}{}_{Na^+}(AG)_{MI}$, and $\Delta G^{app}{}_{Na^+}(AG)_{Ar}$ (Figure 3). On the basis of these data, a plot of $\Delta G^{app}{}_{Na^+}(AG)/RT_{eff}$ versus $1/T_{eff}$ (eq 7) can be constructed (Figure 4) to provide the Na^+ affinity of AG, viz. $\Delta H°_{Na^+}(AG)$, from the slope, and the relative entropy of the AG−Na^+ and B_i−Na^+ bonds, viz. $\Delta(\Delta S°_{Na^+})$, from the intercept. The excellent linear correlation of the latter plots ($r^2 > 0.99999$), as exemplified by Figure 4, affirms that $\Delta(\Delta S°_{Na^+})$ remains constant for chemically similar reference bases (as assumed above) and that it is independent of T_{eff}.

Tables 2−4 summarize the Na^+ affinities and relative bond entropies resulting from this procedure for the dipeptides and derivatives studied. The error limits are ±7 kJ mol^{-1} for $\Delta H°_{Na^+}$, ±<1 kJ mol^{-1} for $\Delta(\Delta H°_{Na^+})$, and ±2 J mol^{-1} K^{-1} for $\Delta(\Delta S°_{Na^+})$. The kinetic method can sense very small differences in affinities and, therefore, provides relative thermochemical data with high accuracy;[24,26,30−33] the error limits of absolute values are, however, greater due to the uncertainty in $\Delta H°_{Na^+}(B_i)$. It is worth noting at this point that the Na^+ affinity of GG measured here (177 kJ mol^{-1}) is in excellent agreement with the value reported by Kebarle et al. (179 kJ mol^{-1})[20] in a recent threshold CAD study.

Replicate measurements of the same [P + B_i]Na^+ set lead to effective temperatures lying within ca. ±50 K for MI and ±100 K for CAD spectra. As shown in Table 2, T_{eff} of different peptides can vary beyond these experimental reproducibilities. The effective temperature is not a true thermodynamic temperature because the isolated fragmenting ions are not in thermal equilibrium;[43] it represents the average temperature of hypothetical Boltzman distributions of [P + B_i]Na^+ which decompose to give [P]Na^+ and [B_i]Na^+ in the same abundance ratio as observed in the experiment.[27a] T_{eff} reflects the internal energy of the dissociating [P + B_i]Na^+ precursor ions.[24,30,31] Brauman

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Table 2. Na^+ Affinities and Relative Bond Entropies of the Linear Dipeptides and Derivatives (P), Deduced from the Dissociation of $[P + B_i]Na^+$ Heterodimers (B_i = Gua, Cyt, Ade)

peptide (P)	$\Delta G^{app}_{Na^+}(P)_{MI}$, kJ mol^{-1}	T_{MI}, K	$\Delta G^{app}_{Na^+}(P)_{He}$, kJ mol^{-1}	T_{He}, K	$\Delta G^{app}_{Na^+}(P)_{Ar}$, kJ mol^{-1}	T_{Ar}, K	$\Delta(\Delta S^\circ_{Na^+})$, J mol^{-1} K^{-1}	$\Delta H^\circ_{Na^+}(P)$, kJ mol^{-1}
NAc-G	169.6	411	167.7	881	165.3	1145	+5.4	172
GG	173.9	499	171.7	881	169.1	1432	+6.0	177
AG	174.1	495	167.6	1129	166.2	1694	+7.9	178
GA	174.9	544	168.9	1142	166.9	1694	+8.1	179
AA	175.1	491	169.7	1336	163.9	1522	+8.9	180
GG-Oet	177.2	481	170.6	1307	165.3	1795	+8.6	181
GG-NH_2	178.1	536	174.9	1064	168.3	1795	+7.4	183

Table 3. Na^+ Affinities and Relative Bond Entropies of the Cyclic Dipeptides (cP), Deduced from the Dissociation of $[cP + B_i]Na^+$ Heterodimers

	cGG		cAG		cAA	
B_i set	$\Delta H^\circ_{Na^+}(cP)$, kJ mol^{-1}	$\Delta(\Delta S^\circ_{Na^+})$, J mol^{-1} K^{-1}	$\Delta H^\circ_{Na^+}(cP)$, kJ mol^{-1}	$\Delta(\Delta S^\circ_{Na^+})$, J mol^{-1} K^{-1}	$\Delta H^\circ_{Na^+}(cP)$, kJ mol^{-1}	$\Delta(\Delta S^\circ_{Na^+})$, J mol^{-1} K^{-1}
Thy, Ura	141	+1.0	147	+0.8	148	−1.2
Gua, Cyt, Ade	143	−6.6	152	−6.0	155	−6.2
V, A, G	145	−11.2	149	−12.7	150	−12.8
av	143		149		151	

Table 4. Na^+ Affinities and Relative Bond Entropies of the Linear Dipeptides (P), Deduced from the Dissociation of $[P_1 + P_2]Na^+$ Heterodimers

peptide P_1	ref peptides P_2	$\Delta H^\circ_{Na^+}(P_1)$, kJ mol^{-1}	$\Delta(\Delta S^\circ_{Na^+})$, J mol^{-1} K^{-1}
GG	GA, AG, AA	175	+1.0
AG	GG, AA	178	+1.9
GA	GG, AA	179	+0.5
AA	GG, AG, GA	181	+0.5

et al. have suggested that T_{eff} can be viewed as the average number of excess energy quanta per oscillator in the reacting precursor ion, viz. $(n - m)/s$,[27b] where s is the number of oscillators in the ion, n is the total number of quanta in all oscillators (i.e. the total energy of the system), and m is the average number of quanta in the reaction coordinate necessary to decompose. The quantities n, m, and s (and, hence, T_{eff}) depend on several factors, including the internal energy distribution upon ionization, the size and structure of peptide and reference base, and (in CAD) the collisional cross-sections of the dimer ions with the target gases used; in addition, T_{eff} is affected by the solubility and concentration of the sample in the FAB matrix.[44] Even under constant experimental conditions, some of these variables cannot be controlled and may cause the observed fluctuations.

The difference in Na^+ affinity between several dipeptides (i.e. their relative affinity) is only 1 kJ mol^{-1} and, thus, very close to the experimental error. For this reason, we cross-checked the derived affinity order of GG, AG, GA, and AA by comparing them directly to each other. In the so examined heterodimers $[P_1 + P_2]Na^+$, one peptide acted as the unknown (P_1) and the other 2−3 as the reference bases (P_2), as given in Table 4. The enthalpy values resulting from these experiments are presented in Table 4 and are in very good agreement with those obtained with the reference bases (Table 2). More importantly, the order of $P-Na^+$ bond strengths remains unchanged, i.e., GG < AG < GA < AA. Comparison of the cyclic dipeptides among themselves similarly verified the order deduced by using as B_i the nucleobases Ade, Cyt, and Gua. The affinity trend cGG < cGA < cAA was further reproduced with two new sets of reference bases, namely the nucleobases Thy and Ura and the amino acids G, A, and V (Table 3). Although the sodium ion affinity remains independent of the B_i set used, it is clearly evident from Tables 2−4 that the relative entropy term $\Delta(\Delta S^\circ_{Na^+}) = \Delta S^\circ_{Na^+}(P) - \Delta S^\circ_{Na^+}(B_i)$, which gauges the entropy difference between the $P-Na^+$ and B_i-Na^+ bonds, strongly depends on the nature of the reference bases, as expected. The variations observed in $\Delta(\Delta S^\circ_{Na^+})$ and their significance are discussed in detail later.

The Na^+ affinities determined in this study increase in the order cGG < cGA < cAA ≪ NAc-G < GG < AG < GA < AA < GG-Oet < GG-NH_2 (Tables 2−4), revealing the trend $\Delta H^\circ_{Na^+}$(cyclic dipeptide) < $\Delta H^\circ_{Na^+}$(amino acid) < $\Delta H^\circ_{Na^+}$(linear dipeptide or derivative). Evidently, the more flexible linear molecules, which can offer multiple binding sites simultaneously, form stronger bonds. The known proton affinities of the four linear dipeptides follow a somewhat different order, namely GG < GA < AG < AA.[45] The proton generally binds to the single most basic site available as a result of its size and charge density; in contrast, Na^+ favors multidentate coordination when possible. This difference in preferred binding modes can account for the different relative affinities of GA and AG for Na^+ vs H^+.

Ab initio calculations by Bouchonnet and Hoppilliard,[46] Jensen,[47] and Hoyau and Ohanessian[48] have shown that the most stable isomer of $[G]Na^+$ contains the metal ion complexed between the carbonyl and amine groups in a bidentate mode (**1** in Scheme 3). The lower affinities of the cyclic peptides (cP) versus glycine are thus consistent with a monodentate ligation of Na^+ in $[cP]Na^+$, as in **2** (Scheme 3). A similar coordination was proposed by Kebarle et al. for the Na^+ adduct of *N*-methyl acetamide, $CH_3CONHCH_3$.[20] This unbranched secondary amide closely resembles cGG (Scheme 1). It is therefore not surprising that our $\Delta H^\circ_{Na^+}$(cGG) value (143 kJ mol^{-1}) is very similar with $\Delta H^\circ_{Na^+}$(*N*-methylacetamide) = 145 kJ mol^{-1}.[20]

(44) De Pauw, E. *Methods Enzymol.* **1990**, *193*, 201−214.

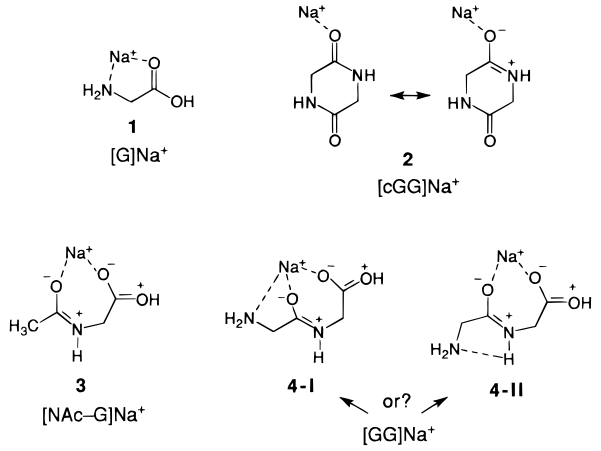
(45) (a) Cassady, C. J.; Carr, S. R.; Zhang, K.; Chung-Phillips, A. *J. Org. Chem.* **1995**, *60*, 1704−1712. (b) Zhang, K.; Zimmerman, D. M.; Chung-Phillips, A.; Cassady, C. J. *J. Am. Chem. Soc.* **1993**, *115*, 10812−10822.

(46) Bouchonnet, S.; Hoppilliard, Y. *Org Mass Spectrom.* **1992**, *27*, 71−76.

(47) Jensen, F. *J. Am. Chem. Soc.* **1992**, *114*, 9533−9537.

(48) Hoyau, S.; Ohanessian, G. Presented at the Gordon Conference on Structures, Energetics and Dynamics of Gaseous Ions, Ventura, CA, February 23−27, 1997; Monday poster No. 3.

Scheme 3. Na^+ Coordination by Glycine, *N*-Acetylglycine, and Glycine-Based Dipeptides



Na^+ attachment to the amide O-atom enhances capto-dative resonance,[49] creating ion pairs that are similar to the salt bridges encountered in zwitterionic species (cf. **2** in Scheme 3).[49−51] By promoting the participation of salt bridge-like resonating structures, such as those shown in Schemes 2 and 3 (and in later schemes), capto-dative effects increase the partial negative charges on the atoms attached to Na^+, thereby incurring extra stabilization through electrostatic attraction forces.[52]

The Na^+ affinity of NAc-G is substantially higher than that of glycine ($\Delta(\Delta H°_{Na^+}) = +13$ kJ mol^{-1}), indicating that conversion of the N-terminal amine to an amide, i.e., addition of a new carbonyl O-atom, strengthens the bond to sodium ion. Such behavior is in keeping with the oxyphilic character of Na^+ in solution.[1] Bidentate coordination of Na^+ between the two carbonyl O-atoms of NAc-G can account for the higher affinity of the latter molecule vis-à-vis glycine, cf. **3** vs **1** in Scheme 3. A further, but not as large affinity rise is observed from NAc-G to GG ($\Delta(\Delta H°_{Na^+}) = +5$ kJ mol^{-1}). Both these molecules possess one amide and one carboxyl substituent, while GG also carries an N-terminal amine group. The extra amine group in GG can participate either in the coordination of Na^+, as in **4-I**, or in an auxiliary hydrogen bond, as in **4-II** (Scheme 3). In this context, the term "auxiliary" (or "ancillary") refers to interactions that do not involve directly the sodium ion. The higher $\Delta H°_{Na^+}$ value of GG vs NAc-G may be the result of tri- vs bidentate complexation, cf. **4-I** vs **3**. Alternatively, it could be due to a larger charge-induced dipole interaction in **4-I** or **4-II** vis-à-vis **3**; such interactions increase with the size of the ligand and, thus, should be larger for GG than NAc-G. The current experimental results would support either structure **4-I** or **4-II**. Theory provides a more definitive answer about their relative stability and, hence, likelihood of production (vide infra).

Dipeptide GG contains a free carboxyl group and could bind Na^+ via its zwitterionic form, providing another rationale for its higher Na^+ affinity. In such a case, $[GG]Na^+$ would actually be a protonated carboxylate, $^+H_3NCH_2CONHCH_2COO^-Na^+$, as had been suggested for sodium ion cationized peptides based on their CAD fragmentation patterns.[9,10] The Na^+ affinity increases, however, for the derivatives GG-Oet and GG-NH_2, in which the C-terminus cannot form a carboxylate, clearly excluding a zwitterionic complexation.

It is noteworthy that derivatization of the C-terminus influences significantly the strength of the P−Na^+ bond, pointing out a closer proximity to the C- than N-terminus for sodium ion. This is true with isomer **4-II**, but would also apply to isomer **4-I** if the H_2N-Na^+ bond is weaker and, hence, longer than the bonds between Na^+ and the carbonyl O-atoms (vide infra). The $\Delta H°_{Na^+}$ ranking GG < GG-Oet < GG-NH_2 can be attributed to an increased participation of stabilizing salt bridges when the free C-terminus is converted to an ester or amide which have superior capto-dative character.[53] Additional evidence that Na^+ binds close to the C-terminus is supplied by the inductive effects observed for AG, GA, and AA (Tables 2 and 4), where the glycine residues are successively substituted by alanines which are better ligands (Table 1). Replacing the C-terminal glycine (GG → GA and AG → AA) leads to a larger affinity increment than replacing the N-terminal glycine (GG → AG and GA → AA); the differences are small, but beyond experimental error. Besides substantiating coordination near the C-terminus, these data also indicate that Na^+ thermochemistry recognizes small changes in the peptide's sequence.

Ab Initio Calculations. Due to the polyfunctional nature of the linear dipeptides, several Na^+-coordination possibilities exist, and it is difficult to decide the structure of the $[P]Na^+$ complex from the experimental data alone. To obtain more information on the nature of the P−Na^+ bond, ab initio calculations were performed on the simplest sodium cationized dipeptide, viz. $[GG]Na^+$, as well as on neutral GG.

Several plausible $[GG]Na^+$ isomers and GG conformers were considered; their optimized structures are summarized in Figures 5 and 6, respectively. Table 5 presents the levels of theory used and the relative energies obtained at each level for the various $[GG]Na^+$ isomers. Table 6 contains the relative energies for the different GG conformers studied. The calculated Na^+ affinity, based on the most stable structures of $[GG]Na^+$ and GG, is reported in Table 7.

Structures **I** and **II** (Figure 5) are the two most stable $[GG]Na^+$ isomers (Table 5). In **I**, Na^+ is ligated in a tridentate fashion by the two carbonyl oxygens and the amino group. Structure **II** displays bidentate Na^+ coordination by the two carbonyl groups, while the N-terminus is involved in a hydrogen bond with the amide nitrogen. The difference between the D_{298} binding energies of these two isomers is only 0.5 kJ mol^{-1}. Such a small difference indicates that the lower degree of complexation in **II** (vis-à-vis tridentate **I**) is counter-balanced by the ancillary HN---H hydrogen bond.

Simultaneous attachment of Na^+ to both carboxylic oxygens, the amide oxygen, and the amino terminus was attempted. Upon geometry optimization, this arrangement collapsed to structure **III**, in which the hydroxyl oxygen is not bound to Na^+ (Figure 5). The binding interactions in **III** are similar to those in **I**; however, **III** is significantly less stable than **I** at the HF/6-31G* level (by 26 kJ mol^{-1}, see Table 5) because of the unfavorable trans conformation of the carboxylic function.

Complexation of Na^+ to the amino terminus and the amide oxygen was examined, in analogy to the most favorable structure of $[G]Na^+$.[46−48] This yielded structures **IV** and **V** (Figure 5), with **IV** being significantly more stable (Table 5). **IV** bears a *cis*-carboxyl group and a NH---O hydrogen bond, while **V** involves a less favorable OH---O linkage over a seven-

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(52) The reported Na^+ affinity of acetone, which lacks capto-dative substitution, is 140 kJ mol^{-1} (obtained by high-pressure mass spectrometry): Guo, B. C.; Cocklin, B. J.; Castleman, A. W., Jr. *J. Am. Chem. Soc.* **1989**, *111*, 6506−6510.

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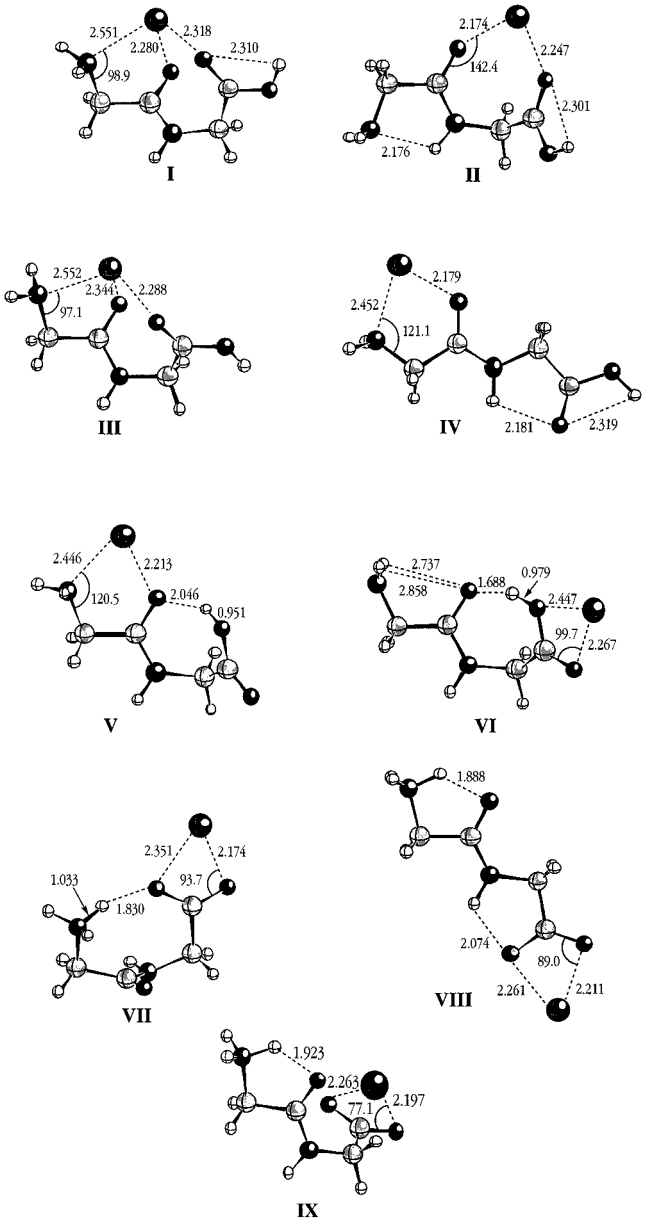


Figure 5. Optimized structures of the [GG]Na^+ isomers **I**−**IX**; the corresponding relative energies are given in Table 5. The bond lengths and bond angles marked are for HF/6-31G* geometries.

membered pseudocycle, which requires a trans conformation of the carboxyl group. **IV** is the third most stable structure of [GG]Na^+, 17 kJ mol^{-1} higher in energy than **II**.

Interaction of Na^+ with the C-terminus was also considered. The initial structure of **VI** (Figure 5) contained a salt bridge between the protonated amide oxygen and the carboxylate group, but this structure collapsed onto **VI** which involves a OH---O bond between the carboxyl and amide oxygens of neutral GG. This structure is of similar energy to **V**, lying ∼74 kJ mol^{-1} above **II** (Table 5).

Finally, complexation of Na^+ with the carboxylate terminus of amino-protonated zwitterionic GG was investigated, cf. **VII**−**IX** in Figure 5. Structure **VII** involves a direct interaction between the positively charged ammonium and negatively charged carboxylate termini, while structure **VIII** has a linear conformation of GG that allows two hydrogen bonds to be formed. **VIII** is the most stable zwitterionic [GG]Na^+ structure found. When additional complexation of Na^+ to the amide oxygen was attempted, structure **IX** emerged instead, in which

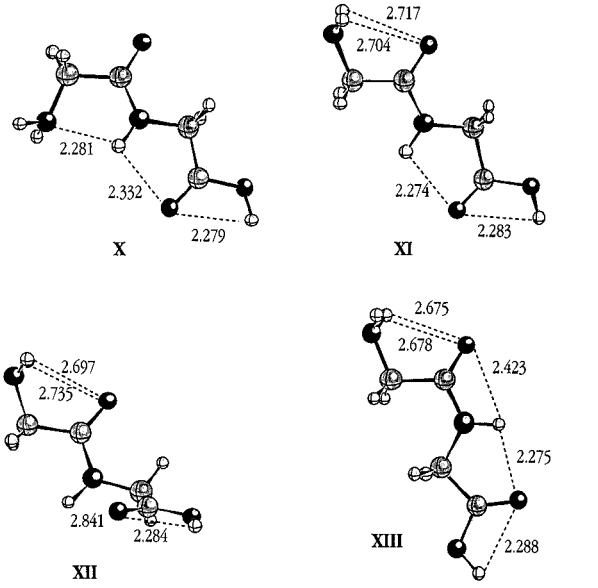


Figure 6. Optimized structures of the GG conformers **X**−**XIII**; the corresponding relative energies are given in Table 6. The bond lengths and bond angles marked are for HF/6-31G* geometries.

Na^+ is again attached to the carboxylate group only. This latter structure, which contains a less favorable NH---O hydrogen bond than **VII**, is the least stable isomer. All of the [GG]Na^+ structures based on zwitterionic GG lie ∼100 kJ mol^{-1} higher in energy than **I** and **II** (Table 5). This result clearly indicates that the gaseous [dipeptide]Na^+ ions considered cannot contain the zwitterionic form of the peptide.

To obtain a computational value for the Na^+ affinity of GG, the most stable structure of neutral GG was also investigated. Figure 6 shows the four optimized GG conformers considered and Table 6 presents the levels of theory used and the relative energies obtained for each conformer. The most stable GG conformer found at the MP2/6-311+G(2d,2p)//HF/6-31G* level is structure **X** (Figure 6). This structure involves two hydrogen bonds over five-membered rings plus a *cis*-carboxyl group. Conformer **X** is more stable than conformer **XI** at all levels of theory examined; at the highest level, viz. MP2/6-311+G(2d,-2p)//HF/6-31G*, **X** lies 6.7 kJ mol^{-1} below **XI**, which is the isomer that had been reported as the global minimum by Cassady et al.[45a] **XII**, another conformation formerly proposed by the latter researchers,[45b] is slightly less stable than **XI** (Table 6). **X**, **XI**, and **XII** contain a trans peptide linkage; to inquire whether a cis linkage could bring upon a lower energy, structure **XIII** was optimized, but found to be 23.8 kJ mol^{-1} less stable than **X**, despite its four hydrogen bonds.

By using structure **X** as the most stable GG conformer, the Na^+ affinity of GG is calculated to be 189.5 kJ mol^{-1} for generation of **I** and 190.0 kJ mol^{-1} for generation of **II** (Table 7). These values result after BSSE correction and adjustment to 298 K with calculated vibrational frequencies. The calculated affinities are somewhat higher than our or Kebarle's experimental affinities (177 and 179 kJ mol^{-1}, respectively) but, nevertheless, fall closely within the error margin of the experimental method used here (±7 kJ mol^{-1}).

It is instructive to look at the impact of computational level on D_e of **I** and **II**. At either the HF or MP2 level, there is a very large diminution of D_e when larger basis sets are used. This trend is expected for electrostatic complexes, such as those encountered here, for which BSSE is the main drawback of using small bases. A large improvement is already obtained with 6-31G*, but the use of diffuse functions on GG is

Table 5. Relative Energies of [GG]Na^+ Isomers (kJ mol^{-1})

computational level	[GG]Na^+ Isomer I	II	III	IV	V	VI	VII	VIII	IX
HF/3-21G	0.0	3.8	33.5	36.4	93.7	86.6	108.4	108.4	125.5
HF/6-31G*	1.3	0.0	27.2	16.7	74.1	74.5	98.8	92.9	104.2
MP2/6-31G*//HF/6-31G*	0.0	9.6		21.8					
HF/6-311+G(2d,2p)//HF/6-31G*	5.0	0.0		15.5					
MP2/6-311+G(2d,2p)//HF/6-31G*	0.0	1.7		13.4					
MP2/6-311+G(2d,2p)//HF/6-31G* + BSSE	0.4	0.0							

Table 6. Relative Energies of GG Conformers (kJ mol^{-1})

computational level	GG Conformer X	XI	XII	XIII
HF/3-21G	0.0	8.4	*a*	29.3
HF/6-31G*	0.0	3.8	7.1	23.8
HF/6-311+G(2d,2p)//HF/6-31G*	0.0	2.9		
MP2/6-311+G(2d,2p)//HF/6-31G*	0.0	6.7		

[a] Unstable.

Table 7. Binding Energy between GG and Na^+ in Structures **I** and **II** as a Function of Computational Level[a]

computational level	I	II
D_e HF/3-21G	304.6	300.8
D_e HF/6-31G*	230.5	231.8
D_e HF/6-311+G(2d,2p)//HF/6-31G*	205.0	210.5
D_e MP2/6-31G*//HF/6-31G*	248.9	239.3
D_e MP2/6-311+G(2d,2p)//HF/6-31G*	207.5	205.9
D_e MP2/6-311+G(2d,2p)//HF/6-31G* + BSSE	195.8	196.2
best D_0	188.7	190.0
best D_{298}	189.5	190.0
ΔS_{298} (J mol^{-1} K^{-1})	133.1	118.4
ΔG_{298}	149.8	154.8

[a] All values given are in kJ mol^{-1} (unless indicated otherwise).

mandatory to reduce BSSE to low levels. Even with bases as large as 6-311+G(2d,2p), the BSSE cannot be neglected (10−12 kJ mol^{-1}). As could be expected, BSSE is larger for tridentate complex **I** than for bidentate **II**, leading to a reversal of relative energies. It appears that with a large enough basis set, correlation corrections become quite small, making HF/large basis the appropriate compromise between accuracy and tractability for the computation of alkali cation−peptide complexes.

Comparison of Experiment and Theory. Structures **4-I** and **4-II** (Scheme 3), which reconcile the experimental trends observed, correlate excellently with the two most stable isomers predicted for [GG]Na^+ by theory, viz. **I** and **II** (Figure 5), respectively. In **I**, the CO−Na^+ bonds to the carboxyl and amide groups are of similar length (2.31 and 2.32 Å) and markedly shorter than the H_2N−Na^+ bond (2.55 Å), consistent with a closer proximity of Na^+ to the C- than the N-terminus, as indicated by the affinity order (vide supra). Because of the very similar energy levels of **I** and **II**, both structures could be sampled in the experiment.

In the [P + B_i]Na^+ heterodimers (Table 2), linear dipeptides are tri- or bidentate ligands, depending on whether they adopt structure **I** or **II**, respectively. The reference bases (B_i) used with the linear molecules, viz. adenine, cytosine, and guanine, are bidentate ligands (Scheme 2).[31] Hence, the overall coordination number is 4−5. Similarly, in heterodimers with two linear peptides (Table 4), the total coordination number can vary between 4 and 6. Since sodium ion readily accommodates 6 ligands in solution,[1] there should be no significant crowding effects in the studied dimers. This expectation is affirmed by the self-consistency of the affinity order of the linear dipeptides resulting from [P + B_i]Na^+ vs [P_1 + P_2]Na^+ dimers (Tables 2 and 4) which have different degrees of crowding.[54]

Relative Bond Entropies. Inspection of Tables 2−4 reveals that the relative bond entropy, $\Delta(\Delta S^\circ_{Na^+})$, can be considerable, making the measured $\Delta G^{app}_{Na^+}$ values significantly different from the desired affinities, particularly at the higher effective temperatures. The overall entropic contributions to $\Delta G^{app}_{Na^+}$, viz. T_{eff} $\Delta(\Delta S^\circ_{Na^+})$, are comparable to the relative affinity, $\Delta(\Delta H^\circ_{Na^+})$, between individual peptides. Hence, $\Delta(\Delta S^\circ_{Na^+})$ can obscure the affinity rankings of the dipeptides and must be taken into account for assessing the correct order (and values) of the peptide−Na^+ bond enthalpies.

$\Delta(\Delta S^\circ_{Na^+}) = \Delta S^\circ_{Na^+}(P) - \Delta S^\circ_{Na^+}(B_i)$ depends on the set of reference bases used, as is evident from Table 3 for the cyclic and Tables 2 and 4 for the linear peptides, respectively. For a given [P + B_i]Na^+ series, a positive $\Delta(\Delta S^\circ_{Na^+})$ is the result of a larger entropy increase upon breakup of the P−Na^+ than the B_i−Na^+ bond (eqs 2b and 2a); on the other hand, a negative $\Delta(\Delta S^\circ_{Na^+})$ results from the reverse situation. When cyclic and linear dipeptides are compared against the same set of reference bases (Ade, Cyt, Gua), the former show negative $\Delta(\Delta S^\circ_{Na^+})$ values of ca. −6 to −7 J mol^{-1} K^{-1} (Table 3), while the latter show quite similar positive $\Delta(\Delta S^\circ_{Na^+})$ values ranging between 6 and 9 J mol^{-1} K^{-1} (Table 2). As discussed in the following sections, these net entropy changes provide valuable information about where and how Na^+ is coordinated by the cyclic vs linear peptides.

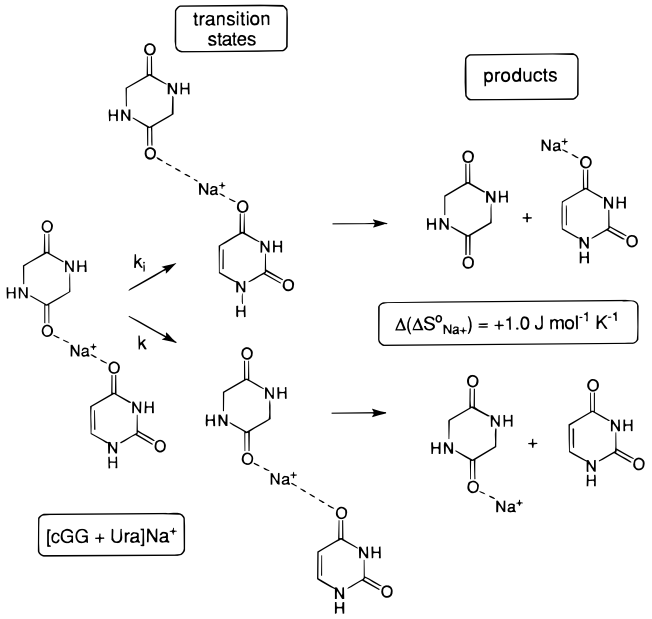
$\Delta(\Delta S^\circ_{Na^+})$ encompasses translational, vibrational, and rotational entropy.[55,56] Electronic entropy is not considered, because the FAB-generated dimer ions and their dissociation products are assumed to exclusively be in their closed shell ground electronic states. There is no net change in translational entropy because the products of reactions 2a and 2b have identical total mass and temperature. Because these two channels dissociate bonds of common nature (electrostatic) and comparable strength, the vibrational portion of $\Delta(\Delta S^\circ_{Na^+})$ is also expected to be small, leaving rotational entropy as the principal contributor to $\Delta(\Delta S^\circ_{Na^+})$.[57] The term "rotational entropy" is used here to describe global molecular rotation as well as internal rotations within the ligands; depending on their barrier, the latter can range from free rotations to torsional vibrations. Several of the dipeptides and reference bases investigated here are capable of multidentate metal ion chelation and H-bonding, both of which can hinder internal rotations (cf. Schemes 2 and 3 and Figure 5). The resulting $\Delta(\Delta S^\circ_{Na^+})$ reflects the extent of such rotation-

(54) The kinetic method probes the difference in transition state energies of the heterodimer dissociations (Δe°), which becomes equivalent to the relative Na^+ affinity, $\Delta(\Delta H^\circ_{Na^+})$, in the absence of reverse barriers.[24] Δe° and $\Delta(\Delta H^\circ_{Na^+})$ do not depend on the depth of the dimer well. Even if the dimer is crowded, hindering some stabilizing interactions between Na^+ and the peptide, the correct $\Delta(\Delta H^\circ_{Na^+})$ should be obtained as long as all interactions are fully restored in the product-like transition states and final products.

(55) Searles, S. K.; Kebarle, P. *Can. J. Chem.* **1969**, *47*, 2619−2627.

(56) Woodin, R. L.; Beauchamp, J. L. *J. Am. Chem. Soc.* **1978**, *100*, 501−508.

Scheme 4. Dissociations of Heterodimer [cGG + Ura]Na^+



restricting noncovalent interactions in the neutral vs the Na^+-cationized peptides (vide infra).

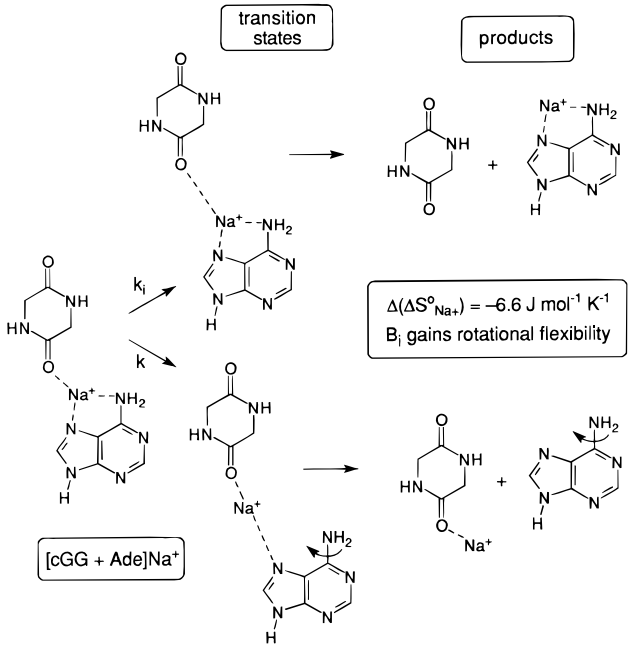
It is important to reemphasize that kinetic method experiments examine the activated complexes of reactions 2a and 2b and, thus, actually measure relative *activation entropies*, $\Delta(\Delta S^{\ddagger}_{Na^+}) = -R \ln(Q^*/Q_i^*)$ (eq 3). However, for the loosely bonded, product-like complexes traversed upon the dissociation of [P + B_i]Na^+ and [P_1 + P_2]Na^+ dimers, $\Delta(\Delta S^{\ddagger}_{Na^+})$ is very similar to the corresponding relative *reaction entropies*, $\Delta(\Delta S^{\circ}_{Na^+})$.[25] To visualize this similarity, the ensuing decomposition schemes show the change in rotational entropy (which is the main source of the observed entropy effects) in both the product-like transition states and the final products.

Entropy Effects with Cyclic Dipeptides (cP). Dimers of the cyclic dipeptides with the nucleobases uracil and thymine lead to negligible $\Delta(\Delta S^{\circ}_{Na^+})$ values (Table 3). Ura or Thy and cP are chemically similar species that ligate Na^+ in an analogous monodentate manner, as exemplified for [cGG + Ura]Na^+ in Scheme 4. As a result, the entropies of Na^+ attachment to cP and to Ura or Thy are very similar and the entropy changes associated with the competing dissociations 2a and 2b essentially cancel.

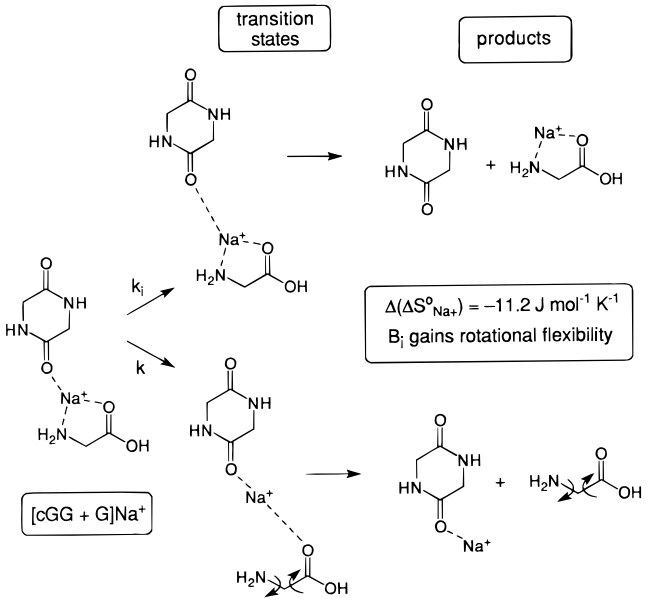
When the reference base in [cP + B_i]Na^+ is changed to the nucleobases adenine, cytosine, and guanine, a negative relative entropy of 6−7 J mol^{-1} K^{-1} is observed for all three cyclic peptides. Now, the reference set consists of bidentate ligands bearing an amino group whose rotational flexibility is inhibited upon complexation to Na^+, cf. Scheme 2.[31] The repercussions for comparing cP to such B_i molecules are illustrated in Scheme 5 for [cGG + Ade]Na^+: the nucleobase recovers one rotation (around the $-NH_2$ bond) when it is released from the Na^+-bound dimer, while the cyclic dipeptide recovers none. The smaller entropy increase upon formation of cP + [B_i]Na^+ vs [cP]Na^+ + B_i (Scheme 5) leads to a negative relative entropy, i.e., $\Delta(\Delta S^{\circ}_{Na^+}) = \Delta S^{\circ}_{Na^+}(cP) - \Delta S^{\circ}_{Na^+}(B_i) \approx -6$ J mol^{-1} K^{-1}, reflecting the gain of rotational freedom in the dissociating reference base.

(57) Also in ligand exchange reactions with simple Lewis bases, such as $B_1M^+ + B_2 \rightarrow B_1 + B_2M^+$, the overall change in rotational entropy has been shown to be more significant than the changes in either translational or vibrational entropy.[55,56] Note that the entropy change upon the above ligand exchange reaction is identical with the relative entropy of the $B_1-M^+-B_2$ dimer.

Scheme 5. Dissociations of Heterodimer [cGG + Ade]Na^+



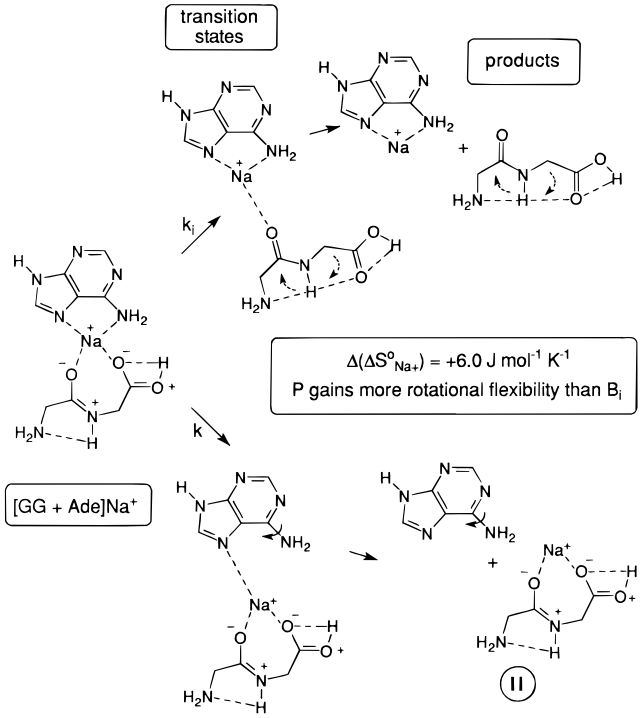
Scheme 6. Dissociations of Heterodimer [cGG + G]Na^+



Negative $\Delta(\Delta S^{\circ}_{Na^+})$ values are also observed when the cyclic peptides are paired with the amino acids glycine, alanine, and valine (last row in Table 3). Na^+ attachment to these amino acids freezes rotation about their H_2N-C^a and C^a-CO_2H bonds. As depicted in Scheme 6 for [cGG + G]Na^+, these rotations are recovered upon elimination of the amino acid (B_i); in contrast, elimination of cP recovers no rotations. The result is a negative relative entropy of approximately −11 to −13 J mol^{-1} K^{-1} (Table 3). Notice that the overall change is ∼2 times larger than with Ade, Cyt, and Gua, consistent with the gain of more rotational flexibility in the amino acids than the nucleobases upon detachment of the metal ion.

The entropy results discussed here suggest a relationship between the number of internal rotations affected in the formation or dissociation of Na^+-bound heterodimers and the quantity of $\Delta(\Delta S^{\circ}_{Na^+})$. According to Table 3, the entropy gain (loss) per recovered (restricted) rotor is estimated to be 6−7

Scheme 7. Dissociations of Heterodimer [GG + Ade]Na^+ (Na^+ Coordination by GG via Structure **II** (cf. Figure 5))



(±2) J mol^{-1} K^{-1}. Similar changes were reported by Meot-Ner[58] and Kebarle et al.[59] for protonation reactions.

Entropy Effects with Linear Dipeptides and Derivatives. The four linear peptides were compared internally by pairing each one of them (P_1) with 2−3 of the remaining three (reference set P_2). The [P_1 + P_2]Na^+ heterodimers resulting this way (Table 4) give very small $\Delta(\Delta S^\circ_{Na^+})$ values, confirming once more that entropic effects virtually cancel out for dimers composed of *chemically similar* ligands. This result further proves that Na^+ is complexed in the same fashion by all four linear dipeptides.

The relative entropies determined with dimers [P + B_i]Na^+ (B_i = Ade, Cyt, Gua) are summarized in Table 2. Now, all species investigated give significant, positive relative entropies ranging within 5.4 and 8.9 (i.e. 7 ± 2) J mol^{-1} K^{-1}. The similar quantities suggest comparable net changes in rotational entropy; based on the foregoing discussion, ∼7 J mol^{-1} K^{-1} corresponds to the net recovery of one rotation. The positive sign further reveals that the increase of rotational flexibility occurs in the linear peptide and not in B_i (the opposite was true with cP, vide supra). The reference bases adenine, cytosine, and guanine gain one rotation upon cleavage of the B_i−Na^+ bond in [P + B_i]-Na^+ (cf., for example, Scheme 5).[31] Since $\Delta(\Delta S^\circ_{Na^+}) = \Delta S^\circ_{Na^+}(P) - \Delta S^\circ_{Na^+}(B_i)$, the competitive cleavage of the P−Na^+ bond must recover the equivalent of two rotations to justify the observed net entropy change of ca. +7 J mol^{-1} K^{-1}.

The theoretically predicted most stable structures of [GG]-Na^+ and GG can explain quite satisfactorily the measured entropy change. As demonstrated in Scheme 7 for dimer [GG + Ade]Na^+ involving isomer **II**, Na^+ attachment to GG freezes rotations about *six* backbone bonds, viz. H_2N−CH_2−C(O)−NH−CH_2−C(O)−OH, due to coordination, resonance, or ancillary H-bonding. Only the equivalent of *two* new rotations is, however, regained upon dissociation to GG + [Ade]Na^+ (vide supra). Consequently, neutral GG must have a rotationally rigid conformation, in agreement with the extensive H-bonding predicted by ab initio theory (Figure 6). Assuming that dissociation of [GG + Ade]Na^+ preferentially yields the most stable GG conformer **X**, as shown in Scheme 7, the experimental $\Delta(\Delta S^\circ_{Na^+})$ result is best rationalized in terms of the overall better rotational flexibility expected for neutral **X** (in which only *neutral* H-bonds are possible) vis-à-vis the rotational flexibility of GG in **II** and in [GG + Ade]Na^+ (in which *ionic* noncovalent interactions prevail). The increased rotational/torsional freedom in *neutral* GG is symbolized by the broken arrows in Scheme 7. Isomer **I**, in which all *six* backbone bonds of GG are rotationally rigid, too, can similarly account for the observed entropy data.

According to Scheme 7, rearrangement must take place within the [GG + Ade]Na^+ heterodimer to arrive at the transition state producing GG + [Ade]Na^+. Existing thermochemical data (see below) suggest that conformational rearrangement of GG within the dimer ions is fast at the energies needed to reach the product-like activated complex shown in Scheme 7 and, thus, should not have any significant impact on the measured dissociation kinetics of the heterodimers and the thermochemical data derived from them.

In the rate-determining, loose transition state shown in Scheme 7, the bond between GG and [B_i]Na^+ is elongated and the conformation of GG closely resembles that found in the dissociated molecule. At long GG-----Na^+ distances, multidentate coordination is not feasible and the peptide should interact with Na^+ just through the substituent that provides the highest stabilization. Because Na^+ binds stronger to amides than amines, carboxylic acids, or ketones,[20] the amide group of GG is the most probable center of interaction with Na^+ at long distances.[60] The Na^+ affinities of simple amides range about 150 kJ mol^{-1} (Table 3).[20] $\Delta H^\circ_{Na^+}$(GG), on the other hand, is 177 kJ mol^{-1} (Table 2); multiple coordination increases the bond strength by ∼20%. On the basis of these data, breakup of the weaker interactions to form a monodentate complex between GG and Na^+, in which conformational rearrangements within the dipeptide can take place, should cost only a fraction (∼20%) of the energy needed to completely release the peptide. As a result, such rearrangements should be facile at the energies needed for complete dissociation, as assumed above. The detailed discussion presented here for GG also applies to the remaining linear molecules, all of which yield comparable $\Delta(\Delta S^\circ_{Na^+})$ values.

Entropies of Na^+ Attachment to Dipeptides. Our experimental method supplies relative entropies, viz. $\Delta(\Delta S^\circ_{Na^+}) = \Delta S^\circ_{Na^+}(P) - \Delta S^\circ_{Na^+}(B_i)$. From these values it is possible to derive actual P−Na^+ bond entropies if $\Delta S^\circ_{Na^+}(B_i)$ is known. Using $\Delta S^\circ_{Na^+}(G) = 116.5$ J mol^{-1} K^{-1}, a recent computational value,[48] the B_i−Na^+ bond entropies of the reference bases employed in this study have been appraised and are listed in Table 8. These $\Delta S^\circ_{Na^+}(B_i)$ and the experimental $\Delta(\Delta S^\circ_{Na^+})$ data of Tables 2 and 3 allow us to obtain representative values for $\Delta S^\circ_{Na^+}$ of cyclic and linear dipeptides, which are included in Table 8. The entropy of Na^+ attachment to the linear molecules is expectedly higher (by ∼12 J mol^{-1} K^{-1}) due to the loss of rotational/torsional freedom experienced upon complexation of the metal ion. Nevertheless, $\Delta S^\circ_{Na^+}$(linear peptide) is compa-

(58) Meot-Ner (Mautner), M. *J. Am. Chem. Soc.* **1983**, *105*, 4906−4911 and 4912−4915.

(59) Sharma, R. B.; Blades, A. T.; Kebarle, P. *J. Am. Chem. Soc.* **1984**, *106*, 510−516.

(60) In such a loose transition state, transient interactions between Na^+ and the other basic sites are possible, and the experiment most likely samples an average of transition state structures.

Table 8. Estimated Entropies (J Mol^{-1} K^{-1}) of Na^+ Complexation of Representative Cyclic (cGG) and Linear Dipeptides (GG and GG-NH_2)

		$\Delta S°_{Na^+}$(dipeptide)		
			linear	
B_i set	$\Delta S°_{Na^+}(B_i)$	cyclic cGG[d]	GG	GG-NH_2
V, A, G	116.5[a]	105.3		
Thy, Ura	103.7[b]	104.7		
Gua, Cyt, Ade	109.1[c]	102.1	115.1[e]	116.5[e]
			118.4[f]	

[a] $\Delta S°_{Na^+}$(amino acid) assigned the value of $\Delta S°_{Na^+}(G) = 116.5$ J mol^{-1} K^{-1};[48] anchoring relative entropies to this 298 K value furnishes 298 K entropies of Na^+ complexation. [b] Calculated by using the known relative bond entropies of Thy and Ura vs the amino acid set V, A, and G.[31] [c] Calculated by using the known relative bond entropies of Gua, Cyt, and Ade vs the amino acid set V, A, and G.[31] [d] Based on the relative entropy vs the B_i set listed in the same row (Table 3). [e] Based on the relative entropy vs Gua, Cyt, and Ade (Table 2). [f] Computed for structure **II** at the HF/6-31G* level (see also Table 7).

rable to $\Delta S°_{Na^+}$(glycine), despite the fact that dipeptides have many more rotors that can potentially be restricted. This reaffirms the conclusion that the linear dipeptides are folded by intramolecular hydrogen bonding and, hence, do not rotate freely about *all* their backbone bonds.

The HF/6-31G* calculated entropies of Na^+ attachment to GG are 133 and 118 J mol^{-1} K^{-1} for forming **I** and **II**, respectively. The latter is in very good agreement with the value of 115 J mol^{-1} K^{-1} deduced from our experimental $\Delta(\Delta S°_{Na^+})$, as outlined above and in Table 8. This striking similarity strongly suggests that **II** is the structure probed in our experiments. Notice that the smaller GG$-Na^+$ bond entropy of **II** brings upon a more favorable free energy of formation for this isomer (Table 7), justifying its preferred ("entropy driven") production.

Conclusions

This study has shown that, with proper modification, the Cooks kinetic method can be used to obtain the Na^+ affinities of dipeptides, for which no chemically similar reference bases exist. The variant employs heterodimers [peptide + B_i]Na^+, in which the peptide is paired with a set of reference molecules that are structurally similar to each other but not (necessarily) to the peptide under investigation. Assessment of the competitive dissociations of these dimers at two or more internal energies provides both the desired Na^+ affinity (i.e. the peptide$-Na^+$ bond enthalpy) and the relative bond entropy between the peptide$-Na^+$ and B_i-Na^+ bonds. The method discerns very small changes in Na^+ affinity and, thus, is capable of distinguishing peptides of very similar affinity.

The enthalpic and entropic data measured here are consistent with the cyclic dipeptides being monodentate ligands, analogous to aliphatic amides. On the other hand, linear dipeptides (and linear derivatives) coordinate Na^+ in a multidentate fashion near their C-terminus and through their neutral, not zwitterionic form. The entropy data further reveal the presence of stabilizing H-bonds both in the Na^+-cationized and the neutral peptide. These conclusions are supported by ab initio theory, which is essential for obtaining exact coordination geometries.

The combined theoretical and experimental results point out the existence of (at least) two isomers of very similar stability for the [linear dipeptide]Na^+ complexes. In both structures, Na^+ is coordinated by the carbonyl O-atoms of the amide and carboxyl groups. The difference lies in the function of the N-terminus: in one isomer, the N-terminal amine supplies an extra binding site to Na^+, leading to an overall tridentate complex. In the other isomer, the N-terminus is involved in an ancillary H-bond to the amide H-atom. This situation applies to peptides composed of amino acids with no side chain substituents. Addition of such substituents as well as an increase of the peptide size could create more low-energy isomers. The presence of several such isomers could in turn explain the controversy about the location of Na^+ in earlier CAD studies (see introduction).

Finally, it is encouraging to find that thermochemistry offers an alternative to dissociative MS/MS for determining structures. The role of entropy for detecting the presence and extent of H-bonds and other types of noncovalent interactions[61] will be demonstrated for protonated and metal ion cationized polyglycols, polyglymes, and crown ethers in an upcoming publication. Current, parallel studies focus on side-chain functionalized dipeptides, larger alanine- and glycine-based peptides, and diastereomeric saccharides.

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