The Sodium Ion Affinity of Glycylglycine

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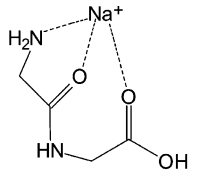
The sodium ion affinity of the simplest peptide, glycylglycine (GlyGly), is determined by the kinetic method, from the dissociation kinetics of Na^+-bound dimers of GlyGly and amino acid reference bases. The dimer ions are formed by electrospray ionization and their competitive dissociations to the metalated monomers are probed by collisionally activated dissociation in a quadrupole ion trap mass spectrometer. The experimental value derived is 203±8 kJ mol^{-1} (ΔH_{Na} at 298 K). Concurrent high-level ab initio calculations predict a GlyGly−Na^+ bond enthalpy of 200 kJ mol^{-1}, in excellent agreement with the experimental result. The most stable structure of the GlyGly−Na^+ complex found by the ab initio calculations involves solvation of the metal charge by the multidentate GlyGly ligand. The Na^+ affinity reported here lies substantially above older literature values (177−181 kJ mol^{-1}). Plausible reasons for the earlier underestimations are briefly discussed.

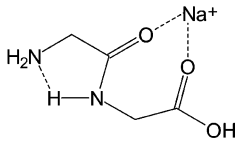
Introduction

The intrinsic chemistry of the Na^+ complexes of peptides has been studied extensively for many years.[1−20] One reason is the importance of Na^+−protein interactions in biology,[21,22] which can be modeled by examining smaller and, hence, more manageable Na^+−peptide systems; comparison of the solution to the intrinsic reactivity of such systems allows for a better understanding of solvent effects in biological environments. One other reason is the analytical potential of using the intrinsic chemistry of [peptide + Na]$^+$ complexes to elucidate unknown peptide sequences. The vast majority of studies so far have concerned the dissociation characteristics and structures of the sodiated peptides.[1−8,10,12−14,16−20] In contrast, there are very few references about the thermochemistry of Na^+−peptide bonds.[9,11,15] Here, we report new experimental and computational data about the intrinsic binding enthalpy of sodium ion, ΔH_{Na} (also called Na^+ affinity or binding energy), to the simplest peptide, dipeptide glycylglycine.

The first thermochemical data for the [GlyGly + Na]$^+$ complex were reported by Klassen et al.,[9] who employed threshold collision-induced dissociation[23] to measure a Na^+ affinity of 179 ±10 kJ mol^{-1} for GlyGly at 298 K. A very similar value (177 ±10 kJ mol^{-1}) was derived two years later by Cerda et al.[11] via the kinetic method,[24,25] on the basis of the competitive dissociations of Na^+-bound heterodimers of GlyGly with the nucleobases adenine, cytosine, or guanine (reference bases). The latter study[11] also examined computationally the structure and energetics of [GlyGly + Na]$^+$ at the MP2/6-311+G(2d,2p)//HF/6-31G(d)+BSSE level of ab initio theory. Complexes **I** and **II** were found to be the lowest energy isomers and of approximately equal stability, the calculated GlyGly−



I



II

Na^+ binding energies (ΔH_{Na}) and entropies (ΔS_{Na}) at 298 K being 189.5 kJ mol^{-1} and 133 J mol^{-1} K^{-1} for **I** and 190.0 kJ mol^{-1} and 118 J mol^{-1} K^{-1} for **II**, respectively.[11] **I** involves a tridentate interaction of the metal ion with the two carbonyl oxygens and the amino group, while **II** results from a bidentate chelation of the metal by the two carbonyl oxygens; these charge solvation arrangements are more stable than zwitterionic isomers.[11] A subsequent kinetic method study by Feng et al.[15] assessed a 300 K free energy of 143 kJ mol^{-1} for the GlyGly−Na^+ bond (ΔG_{Na}). With use of the mean of the calculated GlyGly−Na^+ bond entropies for structures **I** and **II**, the free energy of Feng et al. corresponds to a ΔH_{Na} affinity of 181 kJ mol^{-1}, which is in very good agreement with the experimental Na^+ affinities of Klassen et al.[9] and Cerda et al.[11] It is noteworthy, however, that all experimental values fall below the predicted ab initio value.

The ab initio Na^+ affinities of Cerda et al. were obtained after correction for basis set superposition errors (BSSE). Recent literature has found that non-BSSE-corrected affinities lie closer to true binding energies, if a sufficiently high computational level is used, similar to the one quoted above.[26−28] If the BSSE corrections are removed from the calculated Na^+ affinities for complexes **I** and **II** of GlyGly, the affinities increase by 10−12 kJ mol^{-1}, bringing the predicted ΔH_{Na} value close to 200 kJ mol^{-1}.[11] In this case, all experimental Na^+ affinities reported would be severely underestimated. Corroborating evidence that this might be true comes from recently reported experimental studies concerning the aromatic amino acids phenylalanine,

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tyrosine, and tryptophan.[28−30] Their Na^+ affinities from kinetic method experiments with adenine, cytosine, or guanine as reference bases (these molecules were also paired with GlyGly, vide supra) were substantially smaller than Na^+ affinities deduced also by the kinetic method but with amino acid reference bases,[28,29] or by gas-phase ligand-exchange equilibria.[30] The present study attempts to clarify these discrepancies by calculating the Na^+ binding energy of GlyGly at a higher ab initio level than previously used and by conducting a new series of kinetic method experiments that replace the nucleobase reference bases found to yield underestimated Na^+ affinities with amino acid reference bases.

Experimental Section

Materials and Methods. All experiments were performed with a Bruker Esquire-LC ion trap mass spectrometer (Billerica, MA). The sodium ion bound heterodimers of GlyGly with amino acids (GlyGly−Na^+−AA_i) were produced in the gas phase by electrospray ionization (ESI). The dissociation kinetics of these heterodimer ions were determined by collisionally activated dissociation (CAD). For the formation of the GlyGly−Na^+−AA_i dimers, 1 mg of each amino acid, GlyGly, or sodium trifluoroacetate was dissolved in 1 mL of solvent (2:1 mixture of water and methanol). The amino acid, GlyGly, and salt solutions were mixed in the ratio 1:1:0.75 and introduced into the source at a rate of 300 μL/h with a syringe pump. Nitrogen was used as the nebulizing gas (10 psi) and drying gas (8 L/min, 160 °C) and helium was used as the buffer gas in the trap. CAD tandem mass spectra were acquired by isolating the dimer ion of interest and exciting it to fragment with a radio frequency that was resonant with the dimer ion's frequency of motion in the trap. The excitation time was 40 ms and the resonance excitation amplitude was adjusted to 0.40, 0.45, 0.50, 0.55, 0.60, or 0.65 V. Thirty scans were averaged per spectrum and the experiments were performed in triplicate. The chemicals and solvents (HPLC grade) were purchased from Aldrich or Sigma (Milwaukee, WI) and were used in the condition received.

Kinetic Method. The Na^+ affinity of GlyGly was determined from the dissociation behavior of GlyGly−Na^+−AA_i heterodimers, where AA_i represents an amino acid of known Na^+ affinity. CAD of the dimers causes dissociation into the individual sodiated monomers according to eq 1.

$$\text{GlyGly}-\text{Na}^+ + \text{AA}_i \overset{k}{\leftarrow} \text{GlyGly}-\text{Na}^+-\text{AA}_i \overset{k_i}{\rightarrow} \text{GlyGly} + \text{Na}^+-\text{AA}_i \quad (1)$$

The abundance ratio of the monomer peaks (GlyGly−Na^+ and Na^+−AA_i) in the CAD spectrum represents an approximate measure of the rate constant ratio of these competing dissociations (k/k_i). This assumption presumes that other competing pathways and mass discrimination effects are negligible. The natural logarithm of k/k_i is a function of the relative free energy of activation of the two competing dissociations of the heterodimers based on the thermodynamic formulation of transition state theory (eq 2),[31]

$$\ln(k/k_i) = -[\Delta G^{\ddagger} - \Delta G^{\ddagger}_i]/RT_{\text{eff}} = [\Delta S^{\ddagger} - \Delta S^{\ddagger}_i]/R - [\Delta H^{\ddagger} - \Delta H^{\ddagger}_i]/RT_{\text{eff}} \quad (2)$$

where R is the ideal gas constant and T_{eff} is the effective temperature of the dissociating dimer ions.[32] The free energies in eq 2 include entropy and enthalpy components, as shown. Since the reactions of eq 1 cleave electrostatic bonds, reverse activation energies are expected to be negligible,[28−30,33] and the relative enthalpy of activation becomes equivalent (with opposite sign) to the difference in Na^+ binding enthalpy between GlyGly and AA_i (eq 3)

$$\ln(k/k_i) = -[\Delta S_{\text{Na}}{}^{\text{app}}(\text{GlyGly}) - \Delta S_{\text{Na}}{}^{\text{app}}(\text{AA}_i)]/R + [\Delta H_{\text{Na}}(\text{GlyGly}) - \Delta H_{\text{Na}}(\text{AA}_i)]/RT_{\text{eff}} = -\Delta(\Delta S_{\text{Na}})^{\text{app}}/R + [\Delta H_{\text{Na}}(\text{GlyGly}) - \Delta H_{\text{Na}}(\text{AA}_i)]/RT_{\text{eff}} \quad (3)$$

where the Na^+ binding enthalpy or Na^+ affinity is defined as the enthalpy change, ΔH_{Na}, of the reaction GlyGly−Na^+ (or AA_i−Na^+) → GlyGly (or AA_i) + Na^+. The relative activation entropy is replaced with an apparent relative entropy of activation of the GlyGly−Na^+ and AA_i−Na^+ bonds.[34] An effective temperature and apparent entropy difference are used instead of a thermodynamic temperature and entropy difference, because the Na^+-bound dimers are not in thermal equilibrium with their surroundings and their internal energy distributions are not Boltzmann-shaped.[28,34,35] On the basis of recent studies, $\Delta(\Delta S_{\text{Na}})^{\text{app}}$ depends on the identity of the decomposing dimer ion and on T_{eff} and its value can range from ~0 to the corresponding actual (thermodynamic) entropy difference of the bonds compared in the Na^+-bound dimer.[28,34−37]

$$\ln(k/k_i) = [\Delta H_{\text{Na}}(\text{GlyGly}) - T_{\text{eff}}\Delta(\Delta S_{\text{Na}})^{\text{app}}]/RT_{\text{eff}} - \Delta H_{\text{Na}}(\text{AA}_i)/RT_{\text{eff}} \quad (4)$$

$$\text{slope} = -1/RT_{\text{eff}} \quad (5)$$

$$x\text{-intercept} = \Delta H_{\text{Na}}(\text{GlyGly}) - T_{\text{eff}}\Delta(\Delta S_{\text{Na}})^{\text{app}} \approx \Delta H_{\text{Na}}(\text{GlyGly}) \quad (6)$$

$$\ln(k/k_i) \approx [\Delta H_{\text{Na}}(\text{GlyGly}) - \Delta H_{\text{Na}}(\text{AA}_i)]/RT_{\text{eff}} \quad (7)$$

The AA_i reference bases used (vide infra) were shown to have very similar apparent Na^+ binding entropies;[28] hence, $\Delta(\Delta S_{\text{Na}})^{\text{app}}$ is approximately constant and eq 3 can be modified to eq 4, which contains all variables that do not depend on AA_i in brackets. The experiment provides the k/k_i ratio. Plotting $\ln(k/k_i)$ vs the known sodium ion affinities of the reference bases, $\Delta H_{\text{Na}}(\text{AA}_i)$, leads to a regression line whose slope (eq 5) and x-intercept (eq 6) yield $1/RT_{\text{eff}}$ and $\Delta H_{\text{Na}}(\text{GlyGly}) - T_{\text{eff}}\Delta(\Delta S_{\text{Na}})^{\text{app}}$, respectively. If $\Delta(\Delta S_{\text{Na}})^{\text{app}} \approx 0$, the x-intercept is the desired affinity (eq 6). Otherwise, the enthalpic and entropic terms of the x-intercept can be deconvoluted by acquiring CAD spectra as a function of effective temperature, varied by changing, for example, the collision energy.[25,28,35,37] When $\Delta(\Delta S_{\text{Na}})^{\text{app}} \approx 0$, eq 4 is simplified to eq 7, which is the classical kinetic method equation;[24,25] on the other hand, eqs 3 and 4 are known as the extended kinetic method.

Calculations. All computations were carried out with the Gaussian 98 program package.[38] The geometries of the sodiated complexes and neutral ligands were determined by full optimization followed by a vibrational frequency analysis, both at the MP2(full)/6-31G(d) level, where "(full)" indicates that there were no electrons frozen in the MP2 calculations. Final energetics were obtained at the MP2(full)/6-311+G(2d,2p) level with use of the MP2(full)/6-31G(d) geometries. No basis set superposition error (BSSE) correction was applied to the results.[26−28] Thermal corrections at 298 K and zero-point vibrational energies were obtained from the vibrational analysis and used to derive the 298 K binding enthalpies reported. Binding entropies were also obtained at the MP2(full)/6-31G(d) level. All calculations used five-component d sets and seven-

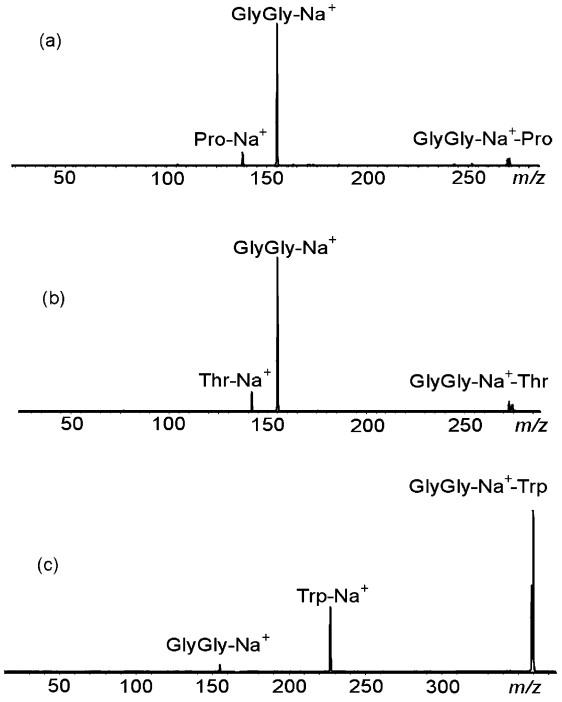

Figure 1. CAD mass spectra of (a) GlyGly−Na^+−Pro (*m*/*z* 270), (b) GlyGly−Na^+−Thr (*m*/*z* 274), and (c) GlyGly−Na^+−Trp (*m*/*z* 359) heterodimers measured at an excitation amplitude of 0.4 V.

component f sets. On the basis of the available data,[26,27,39−41] Na^+ binding energies and entropies computed by the described procedure agree to within ±4 kJ mol^{-1} and ±8 J mol^{-1} K^{-1}, respectively, with well-established experimental values.

Results and Discussion

The Na^+ Affinity of GlyGly. The CAD spectra of 16 GlyGly−Na^+−AA_i heterodimers were measured but only five of them, viz. those from the dimers of GlyGly with AA_i = serine (Ser), proline (Pro), threonine (Thr), phenylalanine (Phe), and tryptophan (Trp), showed an acceptable signal/noise ratio as well as measurable abundances for both sodiated monomers. The CAD spectra of these five dimers were acquired at six different collision energies, corresponding to resonance excitation amplitudes (V_{p-p}) of 0.40, 0.45, 0.50, 0.55, 0.60, and 0.65 V. Representative spectra, resulting from GlyGly−Na^+−Pro, GlyGly−Na^+−Thr, and GlyGly−Na^+−Trp at 0.40 V, are shown in Figure 1. The dimer ions undergo fragmentation to yield the metalated monomers; however, a few dimers coproduce additional fragments at higher collision energies. Specifically, GlyGly−Na^+−Phe loses CO_2 and CO_2 + H_2O at ≥0.60 V; GlyGly−Na^+−Trp loses NH_3, CH_2NH, and CO_2 at ≥0.60 V; and GlyGly−Na^+−Ser undergoes CH_2O loss at every amplitude studied, most prominently at 0.55 V where the sequential elimination of CH_2O + CO_2 also occurs. The relative abundances of the additional fragments are much lower than those of the metalated monomers and susceptible to the amplitude used for CAD, in contrast to the abundance ratio of GlyGly−Na^+ to Na^+−AA_i. It is therefore highly unlikely that the minor fragments influence the ln(*k*/k_i) values from which the sodium ion affinity of GlyGly is derived.

The ln(*k*/k_i) ratios were found to be insensitive to the collision energy (excitation amplitude), presumably because of the appreciable Na^+ binding energies of the GlyGly and AA_i ligands. The collision energy mainly affects the relative abundance of the precursor dimer ions, which decreases with collision energy. The mean values of ln(*k*/k_i) from all six collision energies employed are listed in ladder form in Figure 2 and bracket the Na^+ affinity of GlyGly between the Na^+ affinities of Phe (198 kJ mol^{-1})[28] and Trp (210 kJ mol^{-1}).[28]

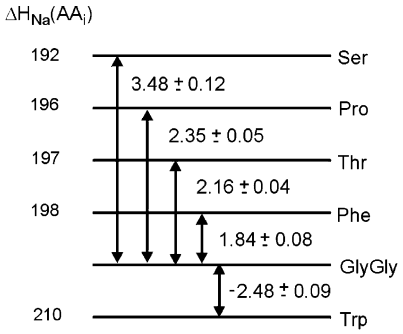

Figure 2. Ln(*k*/k_i) values calculated from the abundances of the GlyGly−Na^+ and Na^+−AA_i fragments in the CAD spectra of GlyGly−Na^+−AA_i. Arrows connect the GlyGly/AA_i dimers investigated. The ratios given are the mean values from the six collision energies used and are followed by the corresponding standard deviations. The Na^+ affinities (ΔH_{Na}) of the AA_i reference bases given at the left side (kJ mol^{-1}) are from Kish et al.[28] and have relative and absolute uncertainties of ±4 and ±8 kJ mol^{-1}, respectively.

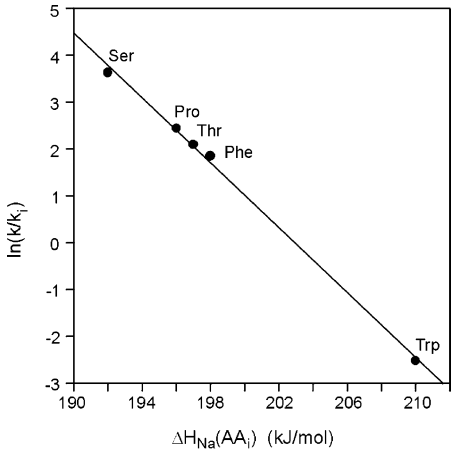

Figure 3. Plot of ln(*k*/k_i) vs $\Delta H_{Na}(AA_i)$ for heterodimers GlyGly−Na^+−AA_i (eq 4). AA_i represents the amino acids Ser, Pro, Thr, Phe, and Trp. The *k*/k_i ratios were calculated from the abundances of GlyGly−Na^+ and Na^+−AA_i in CAD spectra measured at an excitation amplitude of 0.40 V. The correlation coefficient of the regression line (r^2) is 0.998.

At each collision energy, the ln(*k*/k_i) values obtained from the CAD spectra of the GlyGly−Na^+−AA_i series were plotted vs $\Delta H_{Na}(AA_i)$ according to eq 4. The Na^+ affinities of the reference bases Ser, Pro, Thr, Phe, and Trp were taken from the work of Kish et al. (given in Figure 2).[28] The regression line resulting from data acquired at V_{p-p} = 0.40 V is shown in Figure 3. The slope (eq 5) and intercept (eq 6) of this line and the lines constructed for the other five collision energies used are listed in Table 1 together with the corresponding standard deviations.

Because the ln(*k*/k_i) ratios are insensitive to collision energy (vide supra), neither the slopes nor the *x*-intercepts of the ln(*k*/k_i) vs $\Delta H_{Na}(AA_i)$ regression lines change outside experimental error in the collision energy range sampled between V_{p-p} = 0.40 and 0.65 V (Table 1). Evidently, the internal energy (and hence T_{eff}) of GlyGly−Na^+−AA_i does not vary sufficiently within this range to affect the GlyGly−Na^+ vs Na^+−AA_i abundance ratios. A wider range of internal energies could not be sampled because no dimer dissociation is observed below 0.40 V while all dimer ions are depleted by dissociation and/or scattering above 0.65 V.

TABLE 1: Effective Temperature (T_{eff}) and Sodium Ion Affinity (ΔH_{Na}) of GlyGly, Deduced from the Slope and *x*-Intercept of Regression Lines Constructed According to Eq 4 from CAD Spectra of GlyGly−Na^+−AA_i Dimers at Different Collision Energies[a]

	collision energy, V_{p-p} (in V) =					
	0.40	0.45	0.50	0.55	0.60	0.65
slope of eq 4	−0.346(0.010)	−0.349(0.011)	−0.332(0.019)	−0.326(0.026)	−0.332(0.008)	−0.342(0.013)
T_{eff} (K) from eq 5[b]	348	345	363	369	362	351
x-intercept of eq 4	202.9(0.2)	202.8(0.2)	203.0(0.2)	202.9(0.6)	203.0(0.2)	202.8(0.3)
ΔH_{Na}(GlyGly) (kJ mol^{-1}) from eq 6			mean value of *x*-intercept = 202.9(0.3)			

[a] The numbers in parentheses are standard deviations. The linear correlation coefficients (r^2) of the regression lines are 0.998 (0.40 V), 0.997 (0.45 V), 0.991 (0.50 V), 0.982 (0.55 V), 0.998 (0.55 V), and 0.996 (0.65 V). [b] The mean value of the six slopes and the corresponding pooled standard deviation, 0.338(0.016), correspond to a mean effective temperature of 356(17) K.

The mean value of the slopes given in Table 1 is 0.338 ± 0.016, which leads to a mean T_{eff} of 356 ± 17 K for the dissociating GlyGly−Na^+−AA_i heterodimers. The mean value of the *x*-intercepts in Table 1 is 202.9 ± 0.3 kJ mol^{-1}, or 203 ± 8 kJ mol^{-1} if the uncertainty in $\Delta H_{Na}(AA_i)$[28] is also considered. In the absence of entropy effects, this value is equal to the Na^+ affinity of GlyGly, cf. eq 6. The dependence of ln(k/k_i) on T_{eff} would have revealed whether significant entropy effects operate upon the dissociation of GlyGly−Na^+−AA_i, i.e., whether the apparent relative entropy of the GlyGly−Na^+ and Na^+−AA_i bonds is negligible (see eq 6). This dependence could not be measured (vide supra). Fortunately, information about $\Delta(\Delta S_{Na})^{app}$ is provided by the ladder of Figure 2. From the measured ln(k/k_i) data, the relative affinity between any two amino acid reference bases can be estimated by addition or subtraction. For example, $\ln(k_{Trp}/k_{Ser}) = \ln(k_{Trp}/k_{GlyGly}) + \ln(k_{GlyGly}/k_{Ser}) = 2.48(\pm0.09) + 3.48(\pm0.12) = 5.96(\pm0.15)$. On the basis of eq 7 and the T_{eff} value determined above, this ln(k_{Trp}/k_{Ser}) ratio sets the relative Na^+ affinity of Trp vs Ser at $\ln(k_{Trp}/k_{Ser})RT_{eff} = 5.96(\pm0.15) \times 8.3145 \times 356(\pm17) = 17(\pm1)$ kJ mol^{-1}, which agrees well with the relative affinity of 18(±4) kJ mol^{-1} measured independently by Kish et al. from the dissociation of Na^+-bound amino acid dimers AA_1−Na^+−AA_2.[28] Similarly, the scale of Figure 2 predicts that $\ln(k_{Phe}/k_{Thr}) = 2.16(\pm0.04) - 1.84(\pm0.08) = 0.32(\pm0.09)$, which leads to a Na^+ affinity difference between Phe and Thr of $0.32(\pm0.09) \times 8.3145 \times 356(\pm17) = 0.9(\pm0.3)$ kJ mol^{-1}, while the independent comparison of amino acids indicated an affinity difference of 1(±4) kJ mol^{-1}. The relative Na^+ affinities of all other amino acids in Figure 2, calculated indirectly from the dissociation characteristics of GlyGly−Na^+−AA_i dimers, match within experimental error the relative affinities established earlier[28] from the dissociation characteristics of AA_1−Na^+−AA_2 dimers. This self-consistency, or independence on pathway in which the relative affinity is probed, provides evidence that the apparent relative entropy of the GlyGly−Na^+ and Na^+−AA_i bonds, $\Delta(\Delta S_{Na})^{app}$, is indeed negligible,[35] for appreciable $\Delta(\Delta S)^{app}$ quantities have been shown to cause disagreement between relative affinities determined through different thermodynamic cycles.[35,42,43] Under these conditions, the Na^+ affinity of GlyGly derived from the data of Figure 2 and Table 1 is 203 ± 8 kJ mol^{-1} (vide supra). This Na^+ affinity is assigned to GlyGly at 298 K, as it was determined using 298 K affinities of the reference bases.

Computational Results. Complexes **I** and **II** were found to be the most stable [GG + Na]$^+$ isomers in earlier ab initio calculations at the MP2/6-311+G(2d,2p)//HF/6-31G(d) level of theory.[11] Here, the structures of **I** and **II** were optimized further at the MP2(full)/6-31G(d) level, and the energetics of the resulting geometries were recalculated at the MP2(full)/6-311+G(2d,2p) level. The results are summarized in Table 2.

TABLE 2: Thermochemical Data for the Dissociation GlyGly−Na^+ → GlyGly + Na^+, Calculated at the MP2(full)/6-311+G(2d,2p)//MP2(full)/6-31G(d) Level of Theory[a]

quantity	GlyGly	GlyGly−Na^+ **I**	GlyGly−Na^+ **II**
total energy[b]	−491.614488	−653.490569	−653.490052
rel energy[c]		0	1.4[i]
D_e[d]		207.1[j]	205.8[k]
D_0[e]		199.6	199.5
D_{298}[f]		197.8	196.7
ΔH_{298}[g] = $D_{298} + RT$		200.3	199.2
ΔS_{298}[h]		140.0	124.7

[a] Energies and entropies calculated at this level generally differ less than ±4 kJ mol^{-1} and ±8 J mol^{-1} K^{-1}, respectively, from well-established experimental values.[26,27,39−41] [b] Hartrees. [c] kJ mol^{-1}. [d] Electronic bond dissociation energy in kJ mol^{-1}. [e] Bond dissociation energy at 0 K in kJ mol^{-1}. [f] Bond dissociation energy at 298 K in kJ mol^{-1}. [g] Bond enthalpy (affinity) at 298 K in kJ mol^{-1}. [h] Bond entropy at 298 K in J mol^{-1} K^{-1}. [i] 1.7 at MP2/6-311+G(2d,2p)//HF/6-31G(d). [j] 207.5 at MP2/6-311+G(2d,2p)//HF/6-31G(d). [k] 205.9 at MP2/6-311+G(2d,2p)//HF/6-31G(d).

Isomer **I** remains slightly more stable than isomer **II**, binding Na^+ more strongly. The corresponding 298 K binding enthalpies are 200.3 kJ mol^{-1} for **I** and 199.2 kJ mol^{-1} for **II**. These values are in excellent agreement with the above-discussed new experimental result of 203 ± 8 kJ mol^{-1}. It should be mentioned that the computed Na^+ affinities calculated at the lower level, which was used previously, deviate from the new values by <1 kJ mol^{-1}; hence the lower level furnishes adequate energetics. On the other hand, BSSE corrections, which were employed previously but not now, would have reduced the affinities by 10−12 kJ mol^{-1}, causing disagreement between theory and experiment.

Comparison of Results with Experimental Literature Values. The present experimental and (average) computational Na^+ affinities of GlyGly (203 ± 8 and 200 kJ mol^{-1}, respectively) agree favorably, but are substantially larger than the affinities reported by Klassen et al.[9] (179 kJ mol^{-1}) and Cerda et al.[11] (177 kJ mol^{-1}). The reference bases used by Cerda and co-workers in their kinetic method study were the rigid and planar nucleobases adenine, cytosine, and guanine, which could have caused an underestimation in the Na^+ affinity of GlyGly by preventing the formation of the most stable (and hence, most strongly bound) [GlyGly + Na]$^+$ conformer from GlyGly−Na^+−nucleobase heterodimers.[28] Crowding in the dimeric complex due to the rigid nucleobase ligand could force GlyGly to attach as a monodentate ligand, resulting into the formation of a monodentate GlyGly−Na^+ complex upon heterodimer dissociation; such a complex would have a significantly smaller Na^+ binding energy.[11]

Recent studies by Holmes et al.,[44] our laboratory,[35] and Bouchoux et al.[36,37] have shown that the extended kinetic method

yields underestimated proton affinities for bifunctional molecules, if the latter are paired with reference bases of significantly lower protonation entropy. This entropy effect should also operate in Na^+ affinity determinations. The calculated average entropy of Na^+ complexation lies at 105 J mol^{-1} K^{-1} for adenine, cytosine, and guanine,[45] at 121 J mol^{-1} K^{-1} for the amino acid reference bases used here,[46,47] and at 132 J mol^{-1} K^{-1} for GlyGly to form isomers **I** and **II** (Table 2). Based on these data, $\Delta(\Delta S_{Na}) = 27$ J mol^{-1} K^{-1} with GlyGly$-Na^+-$nucleobase dimers and 11 J mol^{-1} K^{-1} with GlyGly$-Na^+-AA_i$ dimers. The relative entropy with the nucleobases exceeds the amount found to result in underestimated proton affinities (>20 J mol^{-1} K^{-1})[35] and could, thus, be an additional cause of the low ΔH_{Na}(GlyGly) value derived by Cerda et al.[11]

The underestimation of ΔH_{Na}(GlyGly) in Klassen's experiments[9] is attributed to an incomplete thermalization of the GlyGly$-Na^+$ ions subjected to threshold collision-induced dissociation and/or the use of incorrect parameters in the model fitting performed on the experimental data to correct the kinetic shift operating upon the GlyGly$-Na^+$ bond cleavage. Finally, the Na^+ affinity of GlyGly reported by Feng et al.[15] (181 kJ mol^{-1}) was deduced from a free energy (ΔG_{Na}) measurement, assuming a temperature of 300 K for ions dissociating in an ion trap. In this case, the underestimated ΔH_{Na} value could be the result of using a temperature lower than the actual temperature for the $\Delta G_{Na} \rightarrow \Delta H_{Na}$ conversion.

Conclusions

The kinetic method was applied to Na^+-bound heterodimers of GlyGly with amino acids of known Na^+ affinity to determine the Na^+ affinity of the dipeptide. The value obtained, 203 ± 8 kJ mol^{-1}, agrees excellently with the Na^+ affinity predicted by ab initio theory at the MP2(full)/6-311+G(2d,2p)//MP2(full)/6-31G(d) level (200 kJ mol^{-1}). The experimental value is 24−26 kJ mol^{-1} higher than literature values, measured by the kinetic method or threshold collision-induced dissociation. The underestimation is attributed to entropy effects and crowding in the dimers used in the earlier kinetic method experiments, and to incomplete thermalization and/or imperfect modeling of the threshold results. Our study further confirms recent findings that ion energetics from high level ab initio calculations lie closer to experimental energetics without BSSE corrections.

It is interesting to compare the Na^+ affinity of GlyGly with the Na^+ affinity of Gly, which has been reexamined most recently via threshold collision-induced dissociation by Moision and Armentrout (166 ± 6 kJ mol^{-1}),[48] via the kinetic method by Kish et al. (161 ± 8 kJ mol^{-1}),[28] and via ligand exchange equilibria by Gapeev and Dunbar (161 ± 12 kJ mol^{-1}).[47] Expanding Gly to the GlyGly dipeptide enhances the binding energy by ~40 kJ mol^{-1}; this substantial increase reflects both the higher intrinsic basicity of the amide group present in GlyGly and, mainly, a better charge solvation by the larger number of functional groups present in the dipeptide.

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