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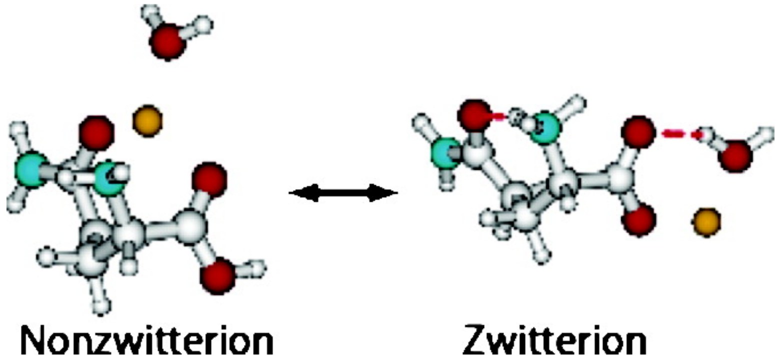

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Structures and Hydration Enthalpies of Cationized Glutamine and Structural Analogues in the Gas Phase

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Abstract: The structures of lithiated and sodiated glutamine, both with and without a water molecule, are investigated using experiment and theory. Loss of water from these complexes and from lithiated and sodiated complexes of asparagine methyl ester, asparagine ethyl ester, and glutamine methyl ester is probed with blackbody infrared radiative dissociation experiments performed over a wide temperature range. Threshold dissociation energies, E_o, for loss of a water molecule from these complexes are obtained from master equation modeling of these data. The values of E_o are 63 ± 1 and 53 ± 1 kJ/mol for the lithiated and sodiated glutamine complexes, respectively. These values are similar to those for the nonzwitterionic model complexes and are in excellent agreement with calculated values. In contrast, water binding to the zwitterionic form is calculated to be significantly higher. These results indicate that glutamine in these lithiated and sodiated complexes with a water molecule are nonzwitterionic. Complexes with the asparagine side chain have slightly higher E_o values than those with the glutamine side chain, a result consistent with more effective solvation of the metal ion due to the slightly longer side chain of glutamine. Calculations indicate that lithiated and sodiated glutamine are nonzwitterionic, with the metal ion interacting with the amine nitrogen and carbonyl oxygen from the amino acid backbone and the amide oxygen of the side chain. Addition of a water molecule does not affect the lowest-energy structure of lithiated glutamine, whereas, for sodiated glutamine, the lowest-energy zwitterionic and nonzwitterionic structures are essentially isoenergetic.

Introduction

The structure of a molecule in solution is influenced by intrinsic intramolecular interactions, such as hydrogen bonding in alpha helices or in beta sheets, as well as interactions with surrounding molecules and ions. For example, peptides with high valine content are more likely to form helices than their alanine analogues in the gas phase, but the opposite is observed in aqueous solution.[1] Obtaining a fundamental understanding of the contribution each of these types of interactions has on molecular structure is a challenging task. One way to simplify experimental investigations of intermolecular effects is to probe interactions between the molecule and one or more of the species of interest in the gas phase. Gas-phase studies of both hydration and metal ion interactions of biomolecules have provided new insight into how solvent and electrostatic interactions influence biomolecular structure.

Amino acids, the building blocks of peptides and proteins, have been extensively investigated. The structures and energetics of cationized amino acids have been studied using a wide range of methods, including ion mobility,[2,3] blackbody infrared radiative dissociation (BIRD),[4−9] the kinetic method,[10,11] H/D exchange,[12] computation,[13−35] spectroscopy,[36] and guided ion beam mass spectrometry.[37−41] The focus of many of these studies is how metal ion size and charge affect the propensity for zwitterion formation in the gas phase. All naturally occurring

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amino acids exist in their nonzwitterionic form in the gas phase, but the zwitterionic form can be preferentially stabilized by the addition of a metal ion. For example, attachment of a singly charged metal cation to glycine can stabilize the zwitterionic form of glycine by as much as 85 kJ/mol relative to the nonzwitterionic form, although the latter form is still the lowest in energy.[3,39,40,42] In contrast, attachment of a doubly charged cation, such as the larger alkaline earth metals, can make the zwitterionic form of glycine more stable.[13−15]

For simple aliphatic amino acids complexed to singly charged cations, the structure of the nonzwitterionic form can depend on cation size. Small metal cations typically bind to the amine nitrogen and carbonyl oxygen (NO coordination), whereas larger metal ions tend to bind to the two carboxylic acid oxygens (OO coordination).[3,4,10,21] For the zwitterionic form, the metal is typically bound to the two carboxylate oxygens (OO coordination).[3,6,14,15] In general, the size of the cation plays a role in the relative stability of the nonzwitterionic versus zwitterionic forms of the amino acid. For example, kinetic method experiments by Wesdemiotis and co-workers indicate that lithiated, sodiated, and potassiated complexes of alanine, valine, leucine, and isoleucine may be nonzwitterionic but cesiated complexes are zwitterionic.[10] A similar trend was reported for divalent alkaline earth cationized glycine[13] and alkali-metal cationized arginine.[9,10] In contrast, calculations by Bowers and co-workers indicated that rubidium ions stabilize the nonzwitterionic form of an amino acid more than smaller sodium ions.[3]

In addition to the effects of metal ion size and charge state, the proton affinity of the protonation site can also play an important role on the relative stabilities of the nonzwitterionic and zwitterionic forms of an amino acid.[3,7,43,44] For five aliphatic amino acids and amino acid analogues, Bowers and co-workers reported that there was a fairly linear relationship between the proton affinity and the increased relative stability of the zwitterionic forms versus nonzwitterionic forms of these complexes.[3] However, predicting zwitterionic stability for all amino acids based on proton affinity alone is complicated by the potential competing effect of preferential stabilization of the nonzwitterionic form by any heteroatoms in side chains, an effect particularly pronounced with small cations such as lithium. For example, the proton affinity of arginine is 164 kJ/mol higher than that of glycine,[45] yet the zwitterionic forms are ∼15 kJ/mol and ∼90 kJ/mol higher in energy than the nonzwitterionic forms for isolated arginine[70,71] and isolated glycine,[42] respectively. Attachment of a cation further reduces the effect that proton affinity has on the relative stability of these two forms of the amino acid. This is due to the preferential stabilization of the nonzwitterionic form due to the ability of an amino acid to "solvate" the charge via interactions with heteroatoms in the side chains. Calculations indicate that the nonzwitterionic form of sodiated glycine is ∼10 kJ/mol lower in energy than the zwitterionic form,[3,20,31] whereas the zwitterionic form of sodiated arginine is ∼4 kJ/mol lower in energy than the nonzwitterionic form.[9] Despite a 164 kJ/mol difference in proton affinity, the differences in relative stability between the zwitterionic and nonzwitterionic form of these two amino acids is only ∼14 kJ/mol. Interestingly, results from experiments suggest that sodiated arginine is nonzwitterionic, indicating that the proton affinity alone is a poor indicator of structure for amino acids with heteroatom-containing side chains.[9,10]

Water molecules can also preferentially stabilize the zwitterionic form of an amino acid; in bulk solution under physiological conditions, amino acids exist predominantly in forms in which the C-terminal carboxylic acid is deprotonated and the N-terminal amine is protonated. In the gas phase, attachment of only a few water molecules can result in the zwitterionic form being energetically comparable to the nonzwitterionic form.[5,6,14,46−48] For glycine, ∼3−5 water molecules can make the zwitterionic form the most stable.[49,50] Recent experimental evidence on ionic species also indicates that very few water molecules are necessary.[5,6,47] The effects of water on the structure of cationized amino acids have been investigated using BIRD and theory.[4−8] For example, BIRD experiments indicate that lithiated valine with one and two water molecules has a charge-solvated nonzwitterionic structure where the metal ion is NO coordinated, with the water molecules interacting only with the metal ion.[4−6] Addition of a third water molecule makes valine a zwitterion in these clusters, with the metal ion shifting to OO coordination. Two water molecules bind to the lithium ion and carboxylate oxygens, with the third water molecule binding directly to the protonated amine nitrogen.[5,6] Proline in lithiated and sodiated complexes is zwitterionic both with[8] and without[8,10,17,41] a single water molecule attached. This preference for the zwitterionic form for proline is due to the high proton

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affinity of the secondary amine of proline and minimal charge solvation in the nonzwitterionic form.

A key remaining challenge is to be able to accurately predict how "amino acids with heteroatoms in the side chain might act to preferentially stabilize charge solvation structures".[3] Glutamine (Gln) appears to be an excellent amino acid to investigate further: the structure of sodiated glutamine has been reported to be both nonzwitterionic[11] and zwitterionic.[3] The lowest-energy structure of Gln•Ag$^+$ is nonzwitterionic, with the silver ion interacting with the amine nitrogen and carbonyl oxygen from the amino acid backbone and the amide oxygen of the side chain (NOO coordination). In contrast, a zwitterionic structure for sodiated glutamine was predicted to be lowest in energy based on the proton affinity of glutamine.[3] Calculations of the proton affinity of glutamine differ with those measured using the kinetic method by as much as 50 kJ/mol.[51,52] This deviation is attributed to protonated Gln having a strong hydrogen bond between the protonated amine nitrogen and heteroatoms in the side chain. This interaction is disrupted in the kinetic method measurements. The kinetic method has also been used to investigate the sodium binding affinity of Gln and 16 other amino acids.[11] Based on the poor relationship between the proton affinity and the measured sodium ion affinity of these amino acids, the authors concluded that all the sodiated amino acids, except proline, have nonzwitterionic structures. A complicating factor in these kinetic measurements, however, is that the structure of an amino acid may be different in a cationized dimer versus a cationized monomer, a result that can obfuscate the conclusions. For example, kinetic measurements indicate that lithiated proline is nonzwitterionic,[10] whereas BIRD measurements[8] and theory[8,17] both indicate that proline in this complex is zwitterionic. Lithiated proline is apparently nonzwitterionic when present in a lithium ion bound dimer with proline methyl ester.[10]

Here, we measure threshold dissociation energies of a water molecule bound to lithiated and sodiated glutamine and three nonzwitterionic structural analogues. We show that glutamine bound to a lithium or sodium ion forms an NOO-coordinated nonzwitterionic complex, with or without a water molecule attached. We also show that the asparagine ester complexes, which have a shorter side chain (one less methylene group), bind the metal ion less strongly than the glutamine complexes and that this effect of side-chain length, although small, is reflected in the experimental data.

Experimental Methods

Chemicals. Glutamine (Gln) was obtained from Sigma Chemical Co. (Saint Louis, MO). Asparagine methyl ester (AsnOMe) was purchased from Bachem California Inc. (Torrance, CA). Glutamine methyl ester (GlnOMe) was purchased from Oakwood Products (West Columbia, SC). Asparagine ethyl ester (AsnOEt) was purchased from Maybridge Chemical Company Ltd. (Trevillett, Tintagel, Cornwall, UK). Lithium hydroxide was purchased from Aldrich Chemical Co. (Milwaukee, WI). Sodium hydroxide was purchased from Fischer Scientific (Fair Lawn, NJ). All chemicals were used without further purification. Electrospray solutions were made to optimize signal for AA•M$^+$ (amino acid or amino acid analogue) and were typically 1.0 mM AA and 2.0 mM LiOH or NaOH. Glutamine solutions were typically made to 3.0 mM Gln and 1.0 mM LiOH or NaOH.

Mass Spectrometry. All dissociation experiments were performed on a home-built Fourier transform mass spectrometer with a 2.7 T superconducting electromagnet. The instrument and experimental methods are discussed in detail elsewhere.[7,53,54] Briefly, ions generated by nanoelectrospray are accumulated in the ion cell for a period of 3−6 s. Unwanted ions are ejected from the cell using a series of stored waveform inverse Fourier transform (SWIFT) and chirp excitation waveforms. The hydrated ion cluster of interest then undergoes unimolecular dissociation for times ranging from 0 to 540 s. Dissociation kinetics are obtained by measuring the abundance of the parent and daughter ions as a function of reaction time. The temperature of the cell is controlled by heating the vacuum chamber with electrically resistive heating blankets[53] or by cooling the copper jacket surrounding the cell with liquid nitrogen.[54] Prior to all experiments, the temperature is allowed to equilibrate overnight (> 8 h) to ensure that ions are exposed to a steady-state radiative energy distribution from infrared photons emitted from the walls of the copper jacket and vacuum chamber.

Computational Details. Possible low-energy structures of Gln, AsnOMe, GlnOMe, and AsnOEt are determined by using a combination of conformational searching and chemical intuition. Structures of AA•M$^+$ and AA•M$^+$(H$_2$O) clusters were generated using Monte Carlo conformation searching with the MMFF94 force field using Maestro 6.5 (Schrödinger, Inc. Portland, OR). For the initial search, no constraints were placed on the molecules, and 5000 conformations were generated with a Monte Carlo simulation. No additional structures within 50 kJ/mol were identified upon generation of an additional 5000 conformations, indicating that the majority of low-energy structures obtainable from these molecular mechanics calculations were identified. Starting structures for higher-level calculations were chosen from this group of structures. In several instances, additional structures which were more than 50 kJ/mol less stable than the lowest-energy structure were also chosen. In no instance were these additional higher energy structures found to be the most stable structure at higher levels of theory.

After identifying lowest-energy structures from the mechanics calculations, hybrid method density functional calculations (B3LYP) were performed using Jaguar v. 5.0 and 5.5 (Schrödinger, Inc., Portland, OR) with increasingly large basis sets. Full geometry optimizations were performed at the 6-31G*, 6-31+G*, and 6-31++G** levels.

Adiabatic water binding energies were calculated from these lowest-energy structures, and include electronic energies, zero-point energies, and enthalpies at 298 K. Nonadiabatic water binding energies are also calculated for the zwitterionic Gln•M$^+$(H$_2$O) complexes. This nonadiabatic binding energy is used because the dissociative transition state will likely share the same metal ion binding mode as the lowest-energy zwitterionic structure of Gln•M$^+$, not the lowest-energy Gln•M$^+$ structure, which is nonzwitterionic.

In the BIRD experiments, the measured dissociation rate depends on the rates of radiative absorption and emission, the transition state entropy of the dissociation, and the binding energy of the water to the cluster. We can numerically simulate the experimentally measured kinetic data by modeling these processes using a master equation formalism. This is discussed in detail elsewhere.[55] Briefly, radiative rates are obtained by combining Einstein coefficients determined from calculated absorption spectra for the clusters and a blackbody energy field at the temperature of the experiment. Dissociation processes are included in the model by using microcanonical dissociation rate constants calculated with RRKM theory. The transition state entropy of the dissociation is not accurately known, so we model a range of transition state entropies which results in a range of dissociation rate constants. The binding energy used to calculate the RRKM rate

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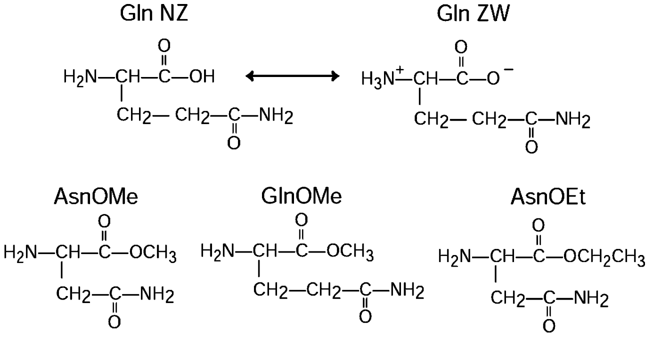
Scheme 1



constants is also varied in the model. Modeling was done for each of the isomers assuming both a "neutral" and "loose" transition state (Arrhenius pre-exponentials of 10^{13} and 10^{17} s^{-1}, respectively). Loss of a water molecule from these clusters is expected to proceed through a relatively loose transition state, but a wider range of transition state entropies was used to better assess the effect of this parameter. In addition, a transition dipole moment multiplication factor between 0.8 and 1.2 was used to estimate effects of uncertainties in the calculated values.

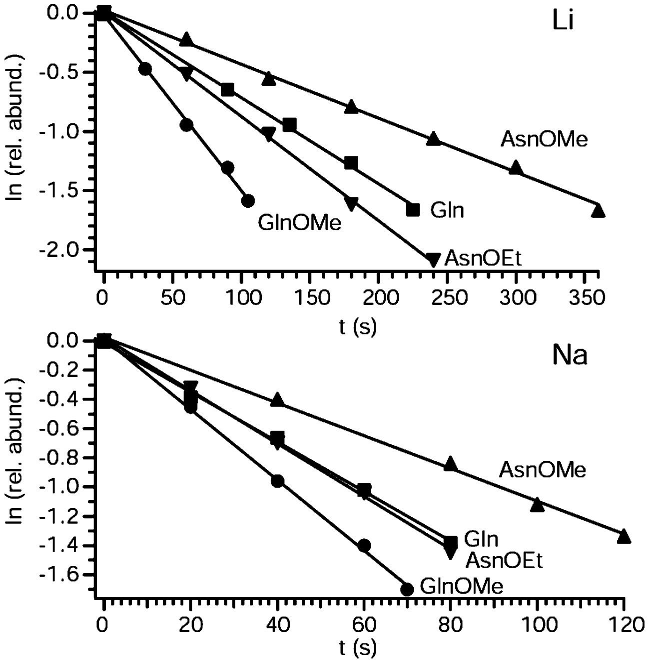


Figure 1. Blackbody infrared radiative dissociation kinetics for the loss of a water molecule from $AA{\cdot}Li^+(H_2O)$ clusters at $T = 0$ °C and from $AA{\cdot}Na^+(H_2O)$ clusters at $T = -27.5$ °C.

Results

Structural Model Complexes. Information about the structure of cationized glutamine ($Gln{\cdot}M^+$, M = Li and Na) is determined from the threshold dissociation energy, E_o, for loss of a water molecule from $Gln{\cdot}M^+(H_2O)$. This value is obtained from blackbody infrared radiative dissociation (BIRD) experiments and compared to values of E_o for similar molecules with known structure. Asparagine methyl ester (AsnOMe) is a structural isomer of glutamine and is used as a model of the nonzwitterionic form of Gln. These molecules are structurally similar, but the side chain of AsnOMe is one methylene group shorter than that of Gln. Glutamine methyl ester (GlnOMe), though not an isomer of Gln, has the same side chain and should have similar modes of metal ion and water binding as nonzwitterionic Gln. Asparagine ethyl ester (AsnOEt) is a structural isomer of GlnOMe and is also nonzwitterionic. For structures in which the metal ion and water binding of these four complexes involve interactions with the side chain, the longer side chains of Gln and GlnOMe may cause the metal ion and water molecule to be bound differently than in the complexes of AsnOMe and AsnOEt. The structures of all four molecules are given in Scheme 1.

Dissociation Kinetics. The loss of a water molecule from $AA{\cdot}M^+(H_2O)$, AA = Gln, AsnOMe, GlnOMe, AsnOEt, was measured as a function of time over a temperature range of −5 to 60.3 °C for M = Li and from −40 to 31.8 °C for M = Na. All experiments were performed at pressures $< 10^{-8}$ Torr, so that all these kinetics were measured in the zero-pressure limit (ZPL).[56−58] Representative plots of $\ln \{[AA{\cdot}M^+(H_2O)]/([AA{\cdot}M^+(H_2O)] + [AA{\cdot}M^+])\}$ as a function of time are shown in Figure 1. Correlation coefficients for all of these data are ≥0.99, indicating first-order kinetics.

Arrhenius Plots. Arrhenius plots obtained from the ZPL rate constants for the loss of water from $AA{\cdot}M^+(H_2O)$, AA = Gln, AsnOMe, GlnOMe, AsnOEt and M = Li and Na, are shown in Figure 2. Arrhenius parameters from these data are given in Table 1. The data are not fit over the entire temperature range due to curvature in the Arrhenius data at high temperatures. The curvature is due to an increased depletion of the high energy tail of the distribution as the temperature is increased. This phenomenon is described in more detail elsewhere.[7,55,59] Correlation coefficients for the fit data are all >0.994. The Arrhenius plots for $Gln{\cdot}M^+(H_2O)$ are clearly different from $AsnOMe{\cdot}M^+(H_2O)$, as are the plots for $GlnOMe{\cdot}M^+(H_2O)$ and $AsnOEt{\cdot}M^+(H_2O)$. However, the difference between the $Gln{\cdot}M^+(H_2O)$ and $AsnOMe{\cdot}M^+(H_2O)$ plots is similar to that between the $GlnOMe{\cdot}M^+(H_2O)$ and $AsnOEt{\cdot}M^+(H_2O)$ plots for both M = Li and Na.

Threshold Dissociation Energies. For complexes of the size studied here, the measured Arrhenius parameters are smaller than those measured if these complexes were in the rapid energy exchange limit.[55,59] To obtain accurate threshold dissociation energies (E_o) from these measurements, master equation modeling of the BIRD data is performed. A detailed description of this modeling process is provided elsewhere.[7,59] Values of E_o from this modeling for $AA{\cdot}M^+(H_2O)$ are given in Table 2. These values are all within a 3 kJ/mol range around 64 and 51 kJ/mol for the lithiated and sodiated complexes, respectively.

Lowest-Energy Structures. Calculations were performed to identify the lowest-energy structures of $AA{\cdot}M^+(H_2O)_n$, AA = Gln, AsnOMe, GlnOMe, AsnOEt, M = Li and Na, $n = 0$ and 1. These structures were used to obtain parameters necessary for the master equation modeling processes and to determine if the metal ion and water molecule are bound similarly in Gln and the model complexes. Water binding energies, which can be compared to E_o, are also calculated from these structures. The lowest-energy structures for $Gln{\cdot}Li^+$ and the lithiated model complexes are given in Figure 3. The structures of $AA{\cdot}M^+$ do

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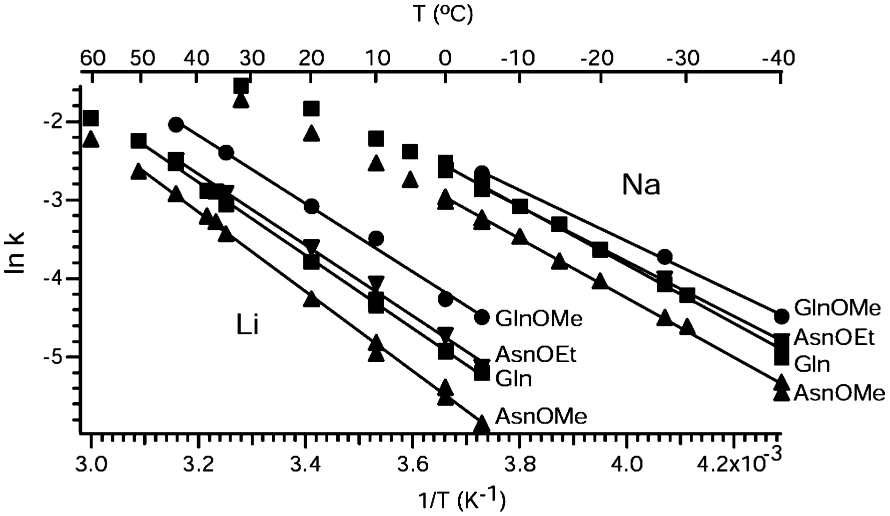

Figure 2. Arrhenius plots for the loss of a water molecule from AA•$M^+(H_2O)$. The data are fit between 0° and 50 °C for M = Li and between −40° and 0 °C for M = Na.

Table 1. Zero-Pressure Limit Arrhenius Parameters, E_a (in kJ/mol), and A (in s^{-1}), for Loss of Water from AA•$M^+(H_2O)$, M = Li and Na

M	AA	E_a	log A
Li	Gln	39 ± 1	5.2 ± 0.1
	AsnOMe	42 ± 1	5.6 ± 0.2
	GlnOMe	36 ± 1	5.1 ± 0.2
	AsnOEt	38 ± 1	5.1 ± 0.2
Na	Gln	31 ± 1	4.8 ± 0.1
	AsnOMe	32 ± 1	4.7 ± 0.1
	GlnOMe	27 ± 1	4.1 ± 0.2
	AsnOEt	29 ± 1	4.4 ± 0.1

Table 2. Threshold Dissociation Energies (E_o) and Binding Enthalpies (in kJ/mol) for Loss of Water from AA•$M^+(H_2O)$ Determined from Master Equation Modeling of BIRD Kinetics Data

	E_o		binding enthalpy	
AA	Li	Na	Li	Na
Gln	63 ± 1	53 ± 1	64 ± 1	52 ± 1
AsnOMe	66 ± 1	53 ± 1	66 ± 1	53 ± 1
GlnOMe	63 ± 2	50 ± 1	63 ± 2	49 ± 1
AsnOEt	65 ± 1	53 ± 1	65 ± 1	52 ± 1

not significantly depend on whether the metal ion is lithium or sodium. The metal ion is bound similarly in nonzwitterionic Gln, AsnOMe, GlnOMe, and AsnOEt, interacting with the amine nitrogen, carbonyl oxygen, and the amide oxygen of the side chain (NOO coordination). In zwitterionic glutamine, the metal ion is bound to both carboxylate oxygens (OO coordination), and protonation of the amine group is energetically favored over alternate protonation sites on the side chain. Zwitterionic Gln•M^+ is 36.0 and 8.0 kJ/mol higher in energy than the nonzwitterionic form for M = Li and Na, respectively, at the B3LYP/6-31++G** level of theory, including zero-point energy and ΔH(298 K) corrections (Table 3).

Seven additional low-energy structures of Gln•Li^+ are given in Figure 4, with their relative energetics along with those for the corresponding sodiated structures at the B3LYP/6-31G* level of theory. These structures illustrate the energetic effects associated with different metal ion interactions. There are additional structures with energies similar to those in Figure 4; however, the structures shown in the figure are the lowest in energy for each class of structures with a specific heteroatom−metal ion interaction. The three lowest-energy structures of Gln•Li^+ (A, B, C) all have the metal ion undergoing NOO coordination. This binding motif is destabilized in structures of Gln•Na^+, with structures E (OO coordination) and D (OOO coordination) both being lower in energy than the NOO-coordinated B and C structures.

The lowest-energy structures of AA•$Li^+(H_2O)$ are shown in Figure 5. The nonzwitterionic sodiated structures are similar to the lithiated structures. The water molecule interacts directly with the metal ion and does not perturb the AA•Li^+ structure. In contrast, the water molecule in the zwitterionic form of AA•$Na^+(H_2O)$ shifts the position of the metal ion such that the water molecule interacts with both the metal ion and a carboxylate oxygen (Structure ZW B, Figure 5), though the difference in energies between structures ZW A and ZW B is small. One would expect the barrier for interconversion between these structures to also be small, so if zwitterionic Gln•$Na^+(H_2O)$ is present in the experiment, both forms would likely be present and indistinguishable.

Zwitterionic Gln•$Li^+(H_2O)$ (ZW A) is 19.2 kJ/mol higher in energy than the nonzwitterionic form at the B3LYP/6-31++G** level of theory, including zero-point energy and ΔH(298 K) corrections (Table 3). The zwitterionic form of Gln•$Na^+(H_2O)$ (ZW B) is 0.7 kJ/mol lower in energy than the nonzwitterionic form at this level of theory, although this small energy difference is well within the expected error in these calculations. The metal ion is NOO coordinated in Gln•$M^+(H_2O)$ and the three nonzwitterionic model complexes, with the water molecule binding to the metal ion. AsnOMe•$M^+(H_2O)$, GlnOMe•$M^+(H_2O)$, and AsnOEt•$M^+(H_2O)$ appear to be good model complexes for the metal ion and water binding in nonzwitterionic Gln•$M^+(H_2O)$, except for minor differences due to the shorter side chains of AsnOMe and AsnOEt. The metal ion in zwitterionic Gln•$M^+(H_2O)$ is OO coordinated, and the water molecule binds either directly to the metal ion (ZW A) or with both the metal ion and a carboxylate oxygen (ZW B).

Calculated Water Binding Energies. From the lowest-energy AA•M^+ and AA•$M^+(H_2O)$ structures, water binding energies are calculated and are given in Table 4. For the model complexes and the nonzwitterionic form of Gln, the calculated binding energies are adiabatic values, while both adiabatic and

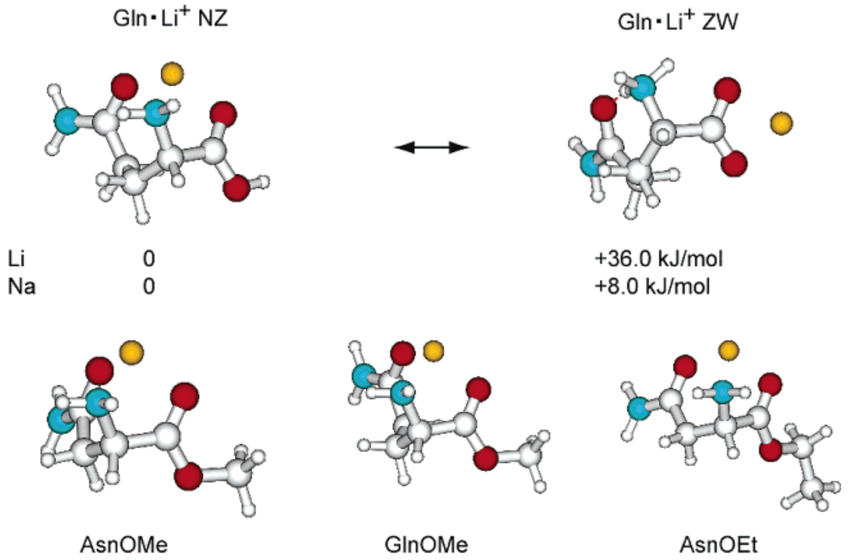

Figure 3. Lowest-energy structures of AA•Li$^+$ complexes at the B3LYP/6-31++G** level of theory.

Table 3. Relative Energies, in kJ/mol, of Gln•M$^+$ and Gln•M$^+$(H$_2$O), M = Li and Na, at Various Levels of Theory[a]

method/basis set	Gln•Li$^+$ NZ	Gln•Li$^+$ ZW	Gln•Li$^+$(H$_2$O) NZ	Gln•Li$^+$(H$_2$O) ZW A	Gln•Li$^+$(H$_2$O) ZW B
B3LYP/6-31G*	0	43.6	0	22.4	29.8
B3LYP/6-31+G*	0	33.0	0	12.6	17.0
B3LYP/6-31++G**	0	37.7	0	19.7	25.5
ΔZPE	0	−2.1	0	−0.3	2.4
Δ*H*(298 K)	0	0.4	0	−0.3	−2.7
total B3LYP/6-31++G**	0	36.0	0	19.2	25.2

method/basis set	Gln•Na$^+$ NZ	Gln•Na$^+$ ZW	Gln•Na$^+$(H$_2$O) NZ	Gln•Na$^+$(H$_2$O) ZW A	Gln•Na$^+$(H$_2$O) ZW B
B3LYP/6-31G*	0	11.7	0	4.7	2.0
B3LYP/6-31+G*	0	2.6	5.9	0	0.5
B3LYP/6-31++G**	0	8.1	0.4	0	0.5
ΔZPE	0	0.7	−4.6	0	−4.4
Δ*H*(298 K)	0	−0.8	3.9	0	1.1
total B3LYP/6-31++G**	0	8.0	0.7	1.0	0

[a] NZ = nonzwitterionic form, ZW = zwitterionic form.

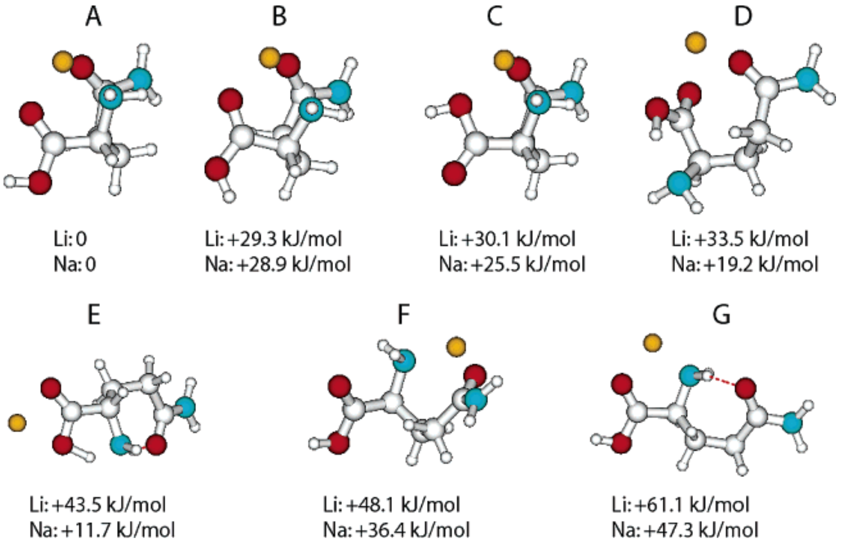

Figure 4. Low-energy structures of nonzwitterionic AA•Li$^+$ complexes at the B3LYP/6-31G* level of theory, with relative energies for these and the similar sodiated complexes (in kJ/mol).

nonadiabatic values are given for the zwitterionic form of Gln. Isomerization of zwitterionic Gln•M$^+$ to the most stable structure, which is nonzwitterionic, is likely to occur after the transition state of the dissociating complex.

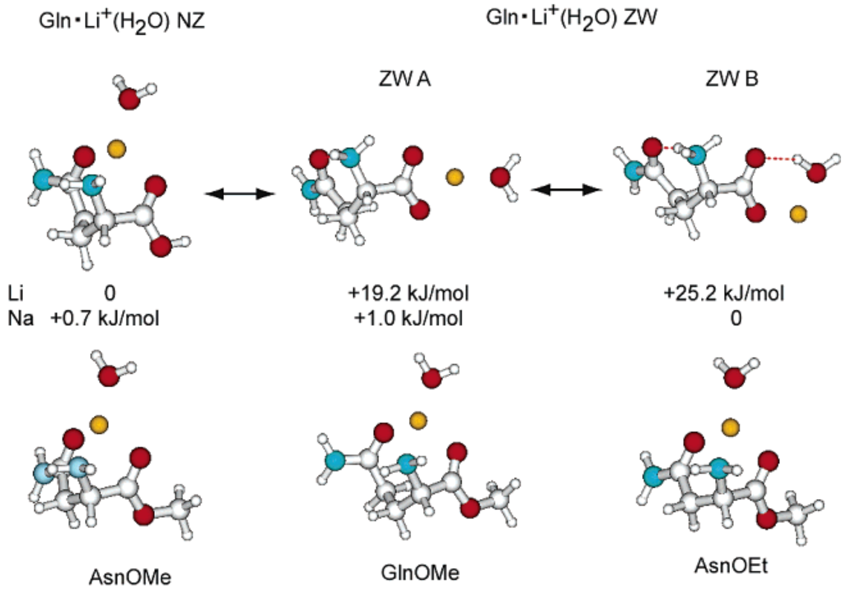

Figure 5. Lowest-energy structures of AA•$Li^+(H_2O)$ complexes at the B3LYP/6-31++G** level of theory.

Table 4. Binding Energies of Water for AA•$M^+(H_2O)$ (in kJ/mol) from Density Functional Calculations at the B3LYP/6-31++G** Level of Theory, with Zero-Point Energy and ΔH (298 K) Corrections

M	Gln NZ	Gln ZW	AsnOMe	GlnOMe	AsnOEt
Li	67	87[a]/51	70	64	70
Na	55	62[a]/54	55	55	57

[a] Nonadiabatic binding energy.

The calculated barrier for conversion from the zwitterionic form of sodiated glycine to its lowest-energy nonzwitterionic form is ~70 kJ/mol;[21] this value for the hydrated complex is unknown. The measured binding energy of a water molecule to nonzwitterionic sodiated glycine is ~75 kJ/mol.[37] Thus, the barrier for isomerization and water loss may be comparable for sodiated glycine. This brings up the possibility that these two forms of glycine may interconvert prior to the water loss, although the loss of a water molecule is expected to be strongly entropically favored. For glutamine, the barrier for interconversion between the zwitterionic and nonzwitterionic forms is not known, nor is that for the hydrated complex. It is possible that the barrier for Gln•M^+ is even higher than that for Gly•M^+ due to the reorientation of the side chain in going from the zwitterionic form (interaction with the protonated amine) to the nonzwitterionic form (interaction with the metal ion). In addition, the water binding energy for nonzwitterionic Gln•$Na^+(H_2O)$ (~55 kJ/mol) is much lower. As is the case for glycine, water loss is expected to be entropically favored. Thus, water loss is likely to occur prior to isomerization between the zwitterionic and nonzwitterionic forms. The nonadiabatic binding energy should be more comparable to the experimentally determined values for reaction pathways that have such a large barrier to isomerization.[6]

The binding energies of water to nonzwitterionic Gln•Li^+ and the three nonzwitterionic model complexes are all within 6 kJ/mol. However, the nonadiabatic water binding energy to zwitterionic Gln•Li^+ is 20 kJ/mol greater than that to the nonzwitterionic form, whereas the adiabatic value is 16 kJ/mol less. For the sodiated complexes, the binding energies of all of the nonzwitterionic structures are within 2 kJ/mol. The nonadiabatic water binding energy to zwitterionic Gln•Na^+ is 7 kJ/mol larger than that to nonzwitterionic Gln•Na^+, whereas the adiabatic value is 1 kJ/mol smaller.

To directly compare these calculated binding energies to the BIRD experiments, the experimentally determined threshold dissociation energies must be converted into binding enthalpies. If there is no significant reverse activation barrier for loss of water from these clusters, binding enthalpies can be calculated using the following equation:

$$\Delta H(T) = E_o + E_{vib}{}^T(AA{\cdot}M^+) + E_{vib}{}^T(H_2O) - E_{vib}{}^T(AA{\cdot}M^+(H_2O)) + 4RT \quad (1)$$

where $T = 298$ K and $E_{vib}{}^T$ is the vibrational energy at temperature T. The binding enthalpies of water for AA•$M^+(H_2O)$ are given in Table 2. All the binding enthalpies are 1.0−6.5 kJ/mol lower in energy than the calculated binding energies for nonzwitterionic Gln and the model complexes. Previous water binding energies on similar metalated complexes calculated using B3LYP calculations are typically higher than experimentally obtained values by 8 kJ/mol or more.[6−8,60−62]

Discussion

Metal Ion Size. Lithium ions are smaller and have higher charge densities than sodium ions. Thus, lithium ions bind more strongly to both amino acids[11,63−67] and water molecules.[68] For the molecules investigated here, the metal ion size has little effect on the lowest-energy nonzwitterionic structures with or

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without a water molecule (Figures 3 and 5), an effect observed previously for valine, α-methyl-proline, and nonzwitterionic models of these complexes.[4−8] There is a subtle difference in the zwitterionic form of Gln•M^+ when a single water molecule is attached. For M = Li, structure ZW A (Figure 5) is the lowest-energy zwitterionic structure, whereas, for M = Na, structure ZW B is lowest in energy. This difference in structure between the zwitterionic forms of AA•$Li^+(H_2O)$ and AA•$Na^+(H_2O)$ has been reported previously.[4,6−8] The energy difference between ZW A and ZW B is small (+6.0 and −1.0 kJ/mol for lithiated and sodiated Gln, respectively). The energy barrier for interconversion between these two structures is also likely to be small, so both structures would rapidly interconvert under these experimental conditions should the zwitterionic form of Gln•$M^+(H_2O)$ be present.

In contrast to this minor effect of metal ion size on structure, the effects of metal ion size on the relative stabilities of the zwitterionic versus nonzwitterionic forms of Gln are dramatic. At the temperature corrected B3LYP/6-31++G** level of theory, the nonzwitterionic form of Gln•M^+ is more stable than its zwitterionic form by 36.0 and 8.0 kJ/mol for M = Li and Na, respectively. The nonzwitterionic form of Gln•M^+ is tremendously stabilized by the smaller cation. A similar effect is observed for singly hydrated species, but the difference between metal ions is slightly smaller (∼22 kJ/mol). Interestingly, theory indicates that attachment of a single water molecule makes the zwitterionic form of Gln•Na^+ marginally more stable (by 0.7 kJ/mol), but the experiment indicates that the nonzwitterionic form is more stable (vide infra).

The preferential stabilization of a nonzwitterionic form with smaller cations has also been reported previously for many amino acids,[10] including cationized glycine,[13] valine,[4,6,7] arginine,[9] proline,[17] and α- and *N*-methyl proline.[8] The difference in zwitterionic stability between Gln•Li^+ and Gln•Na^+ is significantly larger than that for these lithiated and sodiated amino acid complexes. In contrast, calculations by Bowers and co-workers indicated that the nonzwitterionic forms of glycine and 4 methylated analogues were better stabilized by a rubidium ion than by a smaller sodium ion.[3]

Nonzwitterionic Versus Zwitterionic Form. For the lithiated Gln complex, both experiment and theory provide compelling evidence that the nonzwitterionic form is more stable than the zwitterionic form. Calculations indicate that the compounds used to model the nonzwitterionic form of Gln•$Li^+(H_2O)$ (AsnOMe•$Li^+(H_2O)$, GlnOMe•$Li^+(H_2O)$, and AsnOEt•$Li^+(H_2O)$) have essentially the same mode of metal ion and water binding (Figure 5). The effects of the different side chain lengths in the Gln and Asn complexes, although subtle, can be observed in the experimental data (vide infra). The binding enthalpies of water obtained from the BIRD measurements of these four complexes range from 63 to 66 kJ/mol. This range of energies is slightly lower than, but in excellent agreement with, the calculated binding energies of water to the nonzwitterionic reference molecules (64−70 kJ/mol) and to the nonzwitterionic form of Gln (67 kJ/mol). B3LYP calculations have been previously shown to yield binding energies that are systematically higher than experimental binding enthalpies: Armentrout and co-workers reported a difference of ∼8 kJ/mol for select ligand−metal complexes[60,62] and Williams and co-workers reported differences between 5 and 15 kJ/mol for water bound to lithiated and sodiated valine, α-methyl-proline, and isomeric model compounds.[6−8]

Unfortunately, a good isomeric structural model for the zwitterionic form of Gln•$Li^+(H_2O)$ was not found. Thus, we do not have a good experimental measurement for the difference in water binding energy between the zwitterionic and nonzwitterionic forms of Gln in these clusters. Previous studies on valine and α-methyl proline, where good structural model compounds are available, show that this difference in energy can be small (3−5 kJ/mol) but easily measured in these experiments. The calculations, however, indicate that the nonadiabatic binding energy of a water molecule to the zwitterionic form of Gln•Li^+ is significantly higher (87 kJ/mol) than the corresponding value for the nonzwitterionic form (67 kJ/mol). This difference is even larger than that calculated or measured for other systems. Although initially surprising, this result can be directly attributed to effects of charge solvation by the side chain (vide infra). The very similar water binding energies measured for Gln•$Li^+(H_2O)$ and the nonzwitterionic analogues provide strong support for Gln being nonzwitterionic in this cluster.

For the sodiated complexes, the differences in energy between the two forms of Gln are much smaller than observed for the lithiated complexes. For Gln•Na^+, the nonzwitterionic form is more stable by only 8 kJ/mol, and attachment of a water molecule results in the zwitterionic form (ZW B, Figure 3) being more stable by 0.7 kJ/mol. This difference in energy is very small and almost certainly within the uncertainty of our calculations. But this result does suggest that the two forms of the molecule are nearly isoenergetic in the hydrated cluster.

In contrast, the experimental results indicate that Gln•$Na^+(H_2O)$ is nonzwitterionic. The binding energies of water obtained from the BIRD measurements for the four sodiated complexes range from 49 to 53 kJ/mol. Calculated binding energies for the nonzwitterionic forms of these complexes range from 55 to 57 kJ/mol. As is the case with the lithiated species, the calculated values are in excellent agreement with the measured values, especially since water binding energies calculated at this level of theory tend to be slightly high.[6−8,60,62] The calculated nonadiabatic binding energy of water to the zwitterionic form is higher (62 kJ/mol). The relative difference in binding energy between the zwitterionic and nonzwitterionic forms is much smaller for sodiated complexes (7 kJ/mol) than for lithiated complexes (20 kJ/mol). Nevertheless, this difference can be readily resolved in the experiment, and the similar binding energies measured for Gln•Na^+ and the nonzwitterionic model compounds indicates that Gln is nonzwitterionic in these clusters.

Side-Chain Effects on the Water Binding to the Nonzwitterionic Form. In the nonzwitterionic forms of simple amino acids with alkyl side chains, smaller metal ions are typically solvated by two heteroatoms: the amine nitrogen and the carbonyl oxygen, resulting in a structure where the metal ion is NO coordinated. In addition to these interactions, the metal ion in the nonzwitterionic forms of the Gln and Asn complexes is solvated by the oxygen of the amide side chain (NOO coordination). This side-chain interaction with the metal ion results in a significantly lower water binding energy compared to clusters where this interaction is not present. The experimentally obtained threshold dissociation energies of water to lithiated valine and a nonzwitterionic analogue in which the metal ion is NO coordinated are ~85 kJ/mol.[6] For nonzwitterionic lithiated proline methyl ester, the value of E_o is ~80 kJ/mol.[8] In contrast, the water threshold dissociation energies for Gln•$Li^+(H_2O)$ and the nonzwitterionic model compounds are ~15−20 kJ/mol lower.

Similarly, the interaction of the side chain in Gln with the metal ion lowers the water threshold dissociation energy in the sodiated complexes by ~5−15 kJ/mol compared to clusters where the metal ion is just NO coordinated.[7,8] The interaction of the amide-containing side chain with the metal ion in Gln and the model complexes clearly causes the metal ion to be bound more strongly than in amino acids that do not have heteroatoms in the side chain. This increased solvation of the ion results in a shift of the charge density in the metal ion toward the amino acid and away from the water molecule, resulting in a lower binding energy of water.

The relative solvating abilities of the Gln and Asn side chain compared to a single water molecule can be deduced by a comparison of the E_o value for Gln•$Li^+(H_2O)$ (63 kJ/mol) and nonzwitterionic valine•$Li^+(H_2O)_2$ (58 kJ/mol).[6] In the latter cluster, the extra water molecule solvates the metal ion similarly to the oxygen of the amide side chain of Gln. The lower E_o value for the valine cluster suggests that a water molecule is only marginally more effective than the amide oxygen at solvating the metal ion. The water binding energy for NO-coordinated nonzwitterionic valine•$Li^+(H_2O)$ (87 kJ/mol) is slightly less than the value for $Li^+(H_2O)_3$ (94 ± 4 kJ/mol),[69] indicating that NO coordination is more effective than two water molecules at solvating the charge of a lithium ion. Interestingly, the difference between these two binding energies is similar to the difference between Gln•$Li^+(H_2O)$ (63 kJ/mol) and $Li^+(H_2O)_4$ (71 ± 5 kJ/mol),[69] indicating that the oxygen in the amide side chain of Gln solvates the lithium ion about as effectively as a water molecule does. Overall, these results suggest that the extent of solvation provided by the amide oxygen in these clusters is comparable to that provided by a water molecule. By comparison, the binding energy of *N*,*N*-dimethyl-formamide to Na^+ is much larger than that of water.[62,67] This suggests that the orientation of the side-chain amide in these clusters is constrained to a less than optimal geometry for solvation of the metal ion.

Side-Chain Effects on Water Binding to the Zwitterionic Form. Although there is no measured value for the water binding energy to a zwitterionic form of Gln, our calculations indicate that the difference in water binding energies between the zwitterionic and nonzwitterionic forms of Gln is surprisingly high (+20 kJ/mol for the lithiated complex). By comparison, the binding energy for lithiated betaine, a zwitterionic isomer of valine, is 5 kJ/mol *lower* than nonzwitterionic lithiated valine. A similar difference was measured for zwitterionic α-methyl-proline and its nonzwitterionic isomer, proline methyl ester. In fact, the calculated binding energy of water in zwitterionic Gln•$Li^+(H_2O)$ (87 kJ/mol) is similar to those values calculated for betaine (87 kJ/mol) and the zwitterionic form of valine, α-methyl-proline, and *N*-methyl-proline (84−91 kJ/mol). Thus, these calculations indicate that charge solvation of the ammonium group by the Gln side chain has very little effect on water binding to the zwitterionic form of this amino acid.

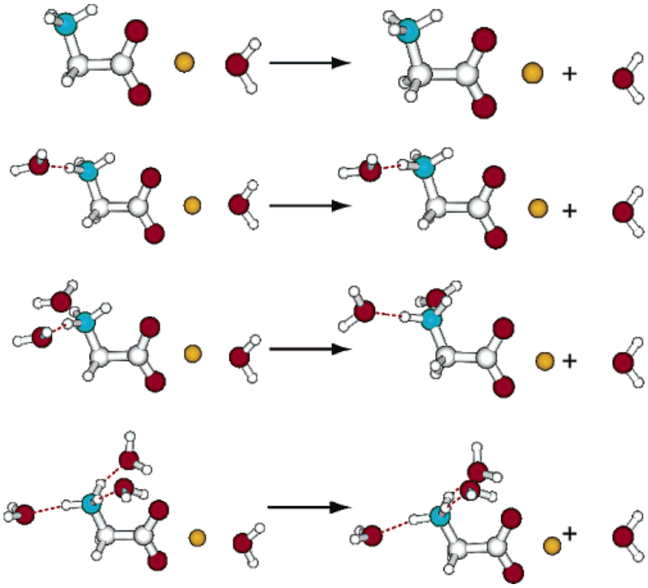

Figure 6. Energy-minimized structures of the zwitterionic form of lithiated glycine with 0−3 water molecules bound to the protonated amine. Note that these structures are not global minima.

Table 5. Binding Energy of a Water Molecule (in kJ/mol) to Structures of Gly•Li^+ Shown in Figure 6 Calculated at the B3LYP/6-31++G** Level of Theory, Including Zero-Point Energy and Δ*H*(298 K) Corrections, with Varying Numbers of Water Molecules Interacting with the Amine Nitrogen of Glycine

no. of waters	binding energy
0	93.3
1	89.1
2	86.2
3	87.0

To further investigate why charge solvation by the side chain does not significantly affect water binding energies to the zwitterionic form, a computational study of water binding to the zwitterionic form of selectively hydrated glycine was done. Low-energy structures of the zwitterionic form of lithiated glycine with 0−3 water molecules bound to the protonated amine group were identified. Geometry optimized structures of these complexes at the B3LYP/6-31++G** level of theory (including zero-point energy and Δ*H*(298 K) corrections) are shown in Figure 6. These structures are not the lowest-energy structures for these complexes but rather were generated to determine effects of solvating the protonated amine in the zwitterionic form. The nonadiabatic binding energies of the water molecule interacting with the OO-coordinated lithium ion were calculated and are given in Table 5. Note that this may not be the most weakly bound water molecule, but it is the one of interest for determining the effects of side-chain solvation on the binding energy of a single water molecule to zwitterionic lithiated glutamine.

With no water molecules bound to the amine group, the water binding energy is ~6 kJ/mol higher than that calculated for the Gln complex. Attachment of a single water molecule to the protonated amine lowers this binding energy by only 4 kJ/mol. Similarly, the binding energy only decreases by a few kJ/mol

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Table 6. Bond Angles Involving the Atoms Labeled in Figure 7 for the Lowest-Energy Structures of AA·M$^+$, AA = Gln, AsnOMe, GlnOMe, AsnOEt, M = Li and Na, at the B3LYP/6-31++G** Level of Theory[a]

AA·M$^+$	angle ADB	angle ADC	angle BDC	angle ABC
Gln·Li$^+$	99.3°	115.3°	84.6°	68.5°
GlnOMe·Li$^+$	99.8°	116.2°	84.9°	68.5°
AsnOMe·Li$^+$	94.7°	103.6°	84.4°	64.7°
AsnOEt·Li$^+$	95.0°	103.9°	83.9°	64.7°
Gln·Na$^+$	84.2°	103.0°	71.3°	73.2°
GlnOMe·Na$^+$	84.8°	104.8°	71.7°	73.7°
AsnOMe·Na$^+$	81.9°	89.6°	70.8°	66.3°
AsnOEt·Na$^+$	82.1°	90.2°	71.0°	66.5°

[a] A = amide oxygen. B = amine nitrogen. C = carbonyl oxygen. D = metal ion.

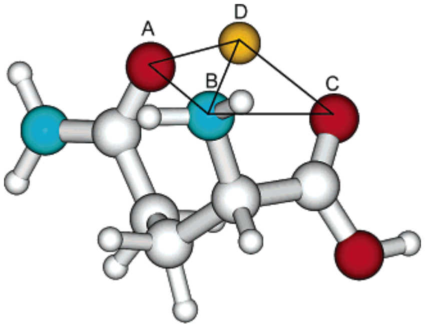

Figure 7. Labels of heteroatoms used in bond angle determination (bond angles in Table 6). A = amide oxygen, B = amine nitrogen, C = carbonyl oxygen, D = metal ion.

upon attachment of a second and third water molecule to this site. These results indicate that extensive solvation of the protonated amine in the zwitterionic form of this ion has very little effect on the interaction of the water molecule bound to the metal ion. *Thus, a significantly larger difference in water binding energy between the nonzwitterionic and zwitterionic forms of amino acids may occur for those amino acids with heteroatoms in the side chains.* This effect is significantly less for species with sodium or larger cations because of the reduced solvation provided in the nonzwitterionic form compared to that in the zwitterionic form. It should be noted that heteroatoms in the side chain of a cationized amino acid may not always interact with the protonated amine in its lowest-energy zwitterionic form. This presumably depends on many factors, including the conformational flexibility and functional-group identity of the side chain.

Gln vs Asn: Effects of Side-Chain Length. Asn differs from Gln in that it has one less methylene group in the side chain. The dissociation kinetic data for clusters containing these two molecules are remarkably different. For both lithiated and sodiated clusters, the dissociation rate constants for Gln and GlnOMe are larger than those for their respective isomers, AsnOMe and AsnOEt, at all temperatures (Figure 2). The threshold dissociation energies for loss of water from lithiated Gln and GlnOMe are about 2 kJ/mol lower than those from AsnOMe and AsnOEt (Table 2). This difference is very small and marginally within the error bars reported for our measured E_o values. However, this small difference appears to be significant and is reflected by differences observed in the kinetic data (Figure 2). Similar results are obtained for the sodiated species although the difference appears to be even smaller for Gln. Again, the kinetic data are consistent with a lower binding energy of water for Gln and GlnOMe versus the respective Asn isomeric species.

In the NOO-coordinated nonzwitterionic cationized species, the metal ion is bound slightly differently in Asn than in Gln because of the longer side chain in Gln which results in an eight-member ring with the metal ion vs a seven-member ring for Asn (Figure 3). Although the water molecule is bound directly to the metal ion, its interaction with the metal ion appears to be perturbed by the small difference in side-chain length. The slightly different metal ion interaction in Gln vs Asn is indicated by the bond angles given in Table 6 between the heteroatoms and the metal ion in these complexes labeled in Figure 7. The largest and apparently most significant difference is the amide oxygen−metal ion−carbonyl oxygen angle (ADC), which is ∼11°−15° larger in Gln than in Asn. The longer chain length in Gln makes possible a more favorable orientation of the side-chain oxygen for solvating the metal ion. This results in a better interaction between the metal ion and the amino acid which results in a slight reduction in the water binding energies for the Gln versus the Asn based clusters.

Effect of a Water Molecule on Structure. As discussed earlier, addition of a single water molecule does not result in a change in the structure of the nonzwitterionic forms of the lithiated or sodiated clusters, but a minor change in the mode of metal/water binding is observed in the lowest-energy sodiated zwitterionic form of the cluster (ZW A vs ZW B; Figure 5). The energy difference in the ZW A and ZW B forms is very small (1.0 kJ/mol), and almost certainly both structures would be present if the zwitterionic forms were energetically competitive with the nonzwitterionic forms. The nonzwitterionic form of Gln·Li$^+$ is clearly more stable than the zwitterionic form, although attachment of a single water molecule increases the relative stability of the zwitterionic form by about 11 kJ/mol. A similar increase in stability upon attachment of a water molecule to the sodiated clusters is observed.

This additional stabilization makes the zwitterionic form slightly more stable in these calculations. However, the experimental data indicate that this is not the case.

It is important to note that these BIRD experiments are not a direct probe of structure, but rather structural information is deduced from the kinetic data for loss of a single water molecule from these clusters and related clusters of known structure. These kinetic data depend on the threshold dissociation energy which reflects the difference in energy between the hydrated cluster and the transition state for the loss of a water molecule. To the extent that the transition state is similar to the product, i.e., there is no significant reverse activation barrier, the threshold dissociation energy reflects the binding energy of water to the cluster. It is possible that the lowest-energy cluster is not formed in these experiments, but rather higher energy forms of the clusters could potentially be kinetically trapped upon solvent evaporation that takes place in the ion cell. This would presumably result in preferential formation of the zwitterionic form which appears not to be the case. It is also possible that Gln isomerizes prior to or at the transition state. If zwitterionic Gln·M$^+$(H_2O) completely isomerizes to its nonzwitterionic form at the transition state, then the measured water binding energy would be an adiabatic value and would be lower than the binding energies measured for the model complexes. However, the measured water threshold dissociation energies for Gln·$M^+(H_2O)$ are similar to those for the nonzwitterionic model complexes. We cannot rule out the possibility that some isomerization prior to the transition state occurs. This would result in a measured binding energy that is between the adiabatic and nonadiabatic values. However, the energy barrier for conversion from the zwitterionic form of Gln·$M^+(H_2O)$ to its nonzwitterionic form, though not known, is likely to be larger than the threshold dissociation energy, so significant isomerization is not expected to occur.

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