Binding Energies of Water to Doubly Hydrated Cationized Glutamine and Structural Analogues in the Gas Phase

Andrew S. Lemoff, Chih-Che Wu, Matthew F. Bush, and Evan R. Williams*

Department of Chemistry, University of California, Berkeley, California 94720-1460

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The modes of metal-ion and water binding in doubly hydrated complexes of lithiated and sodiated glutamine (Gln) are probed using blackbody infrared radiative dissociation experiments and density functional theory calculations. Threshold dissociation energies, E_0, for loss of a water molecule from these complexes are obtained from master-equation modeling of these data. The values of E_0 are 36 ± 1 and 38 ± 2 kJ/mol for the lithiated and sodiated glutamine complexes, respectively, and are consistent with calculated water binding energies for the nonzwitterionic form of these complexes. Calculated water binding energies for the zwitterionic forms of these complexes are significantly higher. In contrast, calculations indicate that the zwitterionic form of Gln in these complexes is more stable than the nonzwitterionic form by 8 and 15 kJ/mol when lithiated and sodiated, respectively. Doubly hydrated lithiated and sodiated complexes of asparagine methyl ester (AsnOMe), asparagine ethyl ester (AsnOEt), and glutamine methyl ester (GlnOMe) were also studied for comparison to Gln. Although these clusters lack the acidic group of Gln and therefore have different water coordination behavior, these results further support the conclusion that Gln is nonzwitterionic in these clusters. Surprisingly, the complexes containing sodium are more stable than those containing lithium, a result that is attributed to subtle differences in how these two metal ions bind to the amino acid esters in these complexes.

Introduction

Naturally occurring amino acids are nonzwitterionic in the gas phase, even though they exist predominantly in zwitterionic forms in aqueous solutions at neutral pH. Although the effects of bulk solution on molecular structure have been extensively investigated, obtaining a detailed molecular understanding of how solvent molecules interact with and influence molecular structure is still an important topic of current studies.[1−3] By studying how water molecules interact with smaller biomolecules, such as amino acids, and how these interactions affect molecular structure, a better understanding of how hydration effects the structure of larger biomolecules, such as proteins, can, in principle, be obtained.

Calculations indicate that the zwitterionic and nonzwitterionic forms of an amino acid can be made nearly isoenergetic in the gas phase by the addition of only a few water molecules.[4−8] In the absence of water, the nonzwitterionic form of glycine is ∼90 kJ/mol lower in energy than the zwitterionic form, and it is not a minimum on the potential energy surface.[9,10] The presence of two water molecules can stabilize the zwitterionic form as a local minimum,[10] but this complex is still ∼50 kJ/mol higher in energy than the nonzwitterionic form. However, between three and six water molecules have been reported to make the zwitterionic form of glycine comparable in energy to the nonzwitterionic form.[11−14]

The effects of water on the structure of cationized amino acids have been investigated previously using blackbody infrared radiative dissociation (BIRD) experiments,[4,5,15−19] guided ion beam studies,[20] and theory.[4,5,15−17,20] BIRD experiments and density functional theory calculations indicate that complexes of lithiated valine with one or two water molecules attached have charge-solvated nonzwitterionic structures, with the metal ion binding to the amine nitrogen and carbonyl oxygen (NO coordination).[4,5,15] The addition of a third water molecule results in a change in the metal-ion position so that it interacts with the two oxygen atoms of valine (OO coordination). Although these results suggest a zwitterionic structure of valine, the structure of this complex could not be unambiguously determined.[4,5] Similar experiments on sodiated valine complexes indicate that the mode of metal-ion binding changes from NO coordination to OO coordination upon the addition of a second water molecule to the singly hydrated complex, although it is unclear whether valine is a zwitterion in these complexes.[5,15,16] Both lithiated and sodiated complexes of valine with six water molecules are more stable than those with five, suggesting that valine is zwitterionic in the six-water complex.[5] Armentrout and co-workers reported the same change in metal-ion binding for hydrated complexes of sodiated glycine upon the addition of two water molecules.[20] They also found that sodiated glycine is nonzwitterionic when solvated by zero to four water molecules.[20]

The mode of metal-ion binding to amino acids depends on cation size. For nonzwitterionic aliphatic amino acids, smaller metal ions tend to be NO-coordinated, whereas larger metal ions tend to be OO-coordinated.[15,21−26] The metal ion is typically OO-coordinated in the zwitterionic form of amino acids.[4,7,16−18,26−28]

In addition to intermolecular effects between an amino acid, a metal ion, and water molecules, intramolecular interactions can also affect the structure of an amino acid. The stability of the zwitterionic form of aliphatic amino acids is directly related their proton affinity (PA),[16,26] but this relationship is not as straightforward for amino acids with heteroatom-containing side chains. For example, lysine (PA = 238 kcal/mol) and arginine (PA = 251.2 kcal/mol)[29] both have proton affinities that should result in gas-phase zwitterionic conformations when sodiated

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* To whom correspondence should be addressed. E-mail: williams@cchem.berkeley.edu. Fax: (510) 642-7714.

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based on the results for aliphatic amino acids.[26] However, the structures of these complexes are nonzwitterionic.[25,30]

We recently performed a study of the effects of metal-ion and water molecule coordination on the structure of glutamine (Gln) by determining water binding energies for Gln·$Li^+(H_2O)$ and Gln·$Na^+(H_2O)$ complexes using BIRD experiments and density functional theory calculations.[18] Glutamine in these singly hydrated complexes is nonzwitterionic, and the metal ion interacts with the amine nitrogen, carbonyl oxygen, and amide oxygen of the side chain (NOO coordination). The water molecule binds directly to the metal ion and does not affect the structure of the cationized amino acid. These modes of metal-ion and water binding are also present in three model complexes: glutamine methyl ester (GlnOMe), asparagine methyl ester (AsnOMe), and asparagine ethyl ester (AsnOEt). However, the shorter side chain of the asparagine complexes weakens metal-ion binding, resulting in a stronger interaction between the metal ion and the water molecule.

Herein, we report the threshold dissociation energies for loss of a water molecule for Gln·$Li^+(H_2O)_2$, Gln·$Na^+(H_2O)_2$, and the three nonzwitterionic structural analogues used previously.[18] For Gln, results indicate that one water molecule binds directly to a NOO-coordinated metal ion, just as in the singly hydrated complexes, and the other water molecule binds to the hydrogen of the carboxylic acid group. For the analogue complexes, which do not have carboxylic acid groups, one water molecule binds directly to the metal ion, and the other water molecule disrupts the NOO coordination and acts as an acceptor−donor bridge between the metal ion and the amino acid. The difference in water threshold dissociation energies measured for the one-water Gln and Asn complexes is negligible in the complexes with two water molecules.

Experimental Methods

Chemicals. Glutamine (Gln), asparagine methyl ester (AsnOMe), glutamine methyl ester (GlnOMe), asparagine ethyl ester (AsnOEt), lithium hydroxide (LiOH), and sodium hydroxide (NaOH) were purchased from Sigma Chemical Co. (Saint Louis, MO), Bachem California Inc. (Torrance, CA), Oakwood Products (West Columbia, SC), Maybridge Chemical Company Ltd. (Trevillett, Tintagel, Cornwall, U.K.), Aldrich Chemical Co. (Milwaukee, WI), and Fischer Scientific (Fair Lawn, NJ), respectively. All chemicals were used without further purification. The composition of the electrospray solutions were prepared by optimizing signal for AA·$M^+(H_2O)_2$ (AA = 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 a concentration of 3.0 mM Gln and 1.0 mM LiOH or NaOH.

Mass Spectrometry. All experiments were performed on a home-built Fourier transform mass spectrometer with a 2.8-T superconducting magnet. The instrument and experimental methods are described in detail elsewhere.[16,31,32] Ions were generated by nanoelectrospray ionization and were accumulated in an ion cell for 5−8 s. The ion of interest was isolated by ejecting other ions from the cell using stored waveform inverse Fourier transform (SWIFT) and chirp excitation waveforms. This isolated ion then underwent unimolecular dissociation for times ranging between 0 and 300 s. The abundances of the precursor and fragment ions were measured as a function of time. The temperature of the cell was controlled by cooling the copper jacket surrounding the cell with liquid nitrogen[32] and was allowed to equilibrate for at least 8 h prior to the start of each experiment. This ensured that the copper jacket surrounding the cell had reached an equilibrium temperature and that ions were exposed to a steady-state radiative energy distribution.

Computational Details. The structures of AA·$M^+(H_2O)$ with AA = Gln, AsnOMe, GlnOMe, and AsnOEt and M = Li and Na have been reported previously.[18] Structures of AA·$M^+(H_2O)_2$ 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 50 000 conformations were generated with a Monte Carlo simulation. All unique structures within 50 kJ/mol of the lowest-energy structure were used as starting structures for hybrid method density functional calculations (B3LYP) performed using Jaguar v. 5.5 (Schrödinger, Inc., Portland, OR) with increasingly large basis sets. Full geometry optimizations were performed at the 6-31G* and 6-31++G** levels. Vibrational frequencies and intensities were calculated using numerical derivatives of the 6-31++G** energy-minimized Hessian. Structures were minimized to geometries yielding all positive-frequency vibrational modes, indicating that all structures reported here are local minima. Water binding energies, including zero-point energies and enthalpies at 298 K, were calculated from these low-energy structures.

The AA·$M^+(H_2O)_2$ complexes studied here are too small to be in the rapid-energy-exchange limit.[33−35] To extract water threshold dissociation energies for these complexes, master-equation modeling of the experiments was performed. This modeling has been described in detail previously.[16,36] Briefly, the measured water dissociation rate constant depends on the rates of infrared photon absorption and emission, the transition-state entropy of the complex, and the threshold dissociation energy of the water molecule. The radiative rates are obtained by combining Einstein coefficients determined from calculated absorption spectra for the clusters and a blackbody energy field calculated for the temperature of the experiment. Loss of a water molecule from these clusters is expected to proceed through a relatively loose transition state, although a wide range of transition-state entropies were modeled because this value is not explicitly known. The water threshold dissociation energy used to calculate the water dissociation rate constants was varied in the model in order to fit the dissociation rate constants. In addition, transition dipole moments were scaled by a factor between 0.6 and 1.2 to account for uncertainties inherent in the calculated absorbance cross sections. The fit was considered successful if the experimentally determined Arrhenius values of E_a and A agreed with the modeled values within experimental error and the dissociation rate constants were within a factor of 2 of the experimental values.

Results

The structures of Gln·$M^+(H_2O)_2$, M = Li and Na, are deduced from both blackbody infrared radiative dissociation (BIRD) experiments and density functional theory calculations for these and related clusters. Kinetic data for the loss of a water molecule from the complexes were measured over a ∼30−50 °C temperature range and subsequently modeled using the master-equation formalism. This process yielded a threshold dissociation energy, E_0, that could be compared to calculated binding energies for these complexes, as well as experimental and calculated values determined for the nonzwitterionic model complexes containing asparagine methyl ester (AsnOMe), glutamine methyl ester (GlnOMe), and asparagine ethyl ester (AsnOEt). The structures of these molecules are shown in Chart 1.

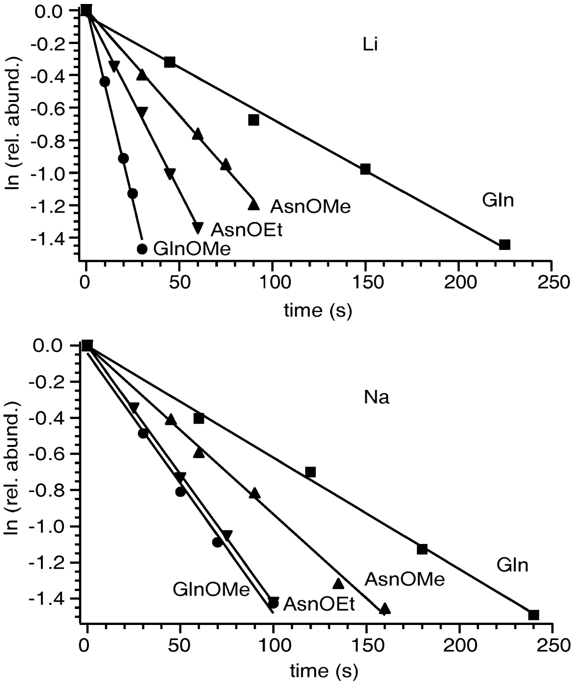

Figure 1. Blackbody infrared radiative dissociation kinetics for the loss of a water molecule from AA•Li$^+$(H_2O)$_2$ and AA•Na$^+$(H_2O)$_2$ clusters at $T = -92$ °C.

Blackbody Infrared Radiative Dissociation. BIRD rate constants for the loss of a water molecule from AA•M$^+$(H_2O)$_2$, AA = Gln, AsnOMe, GlnOMe, and AsnOEt, are measured with the copper jacket that surrounds the ion cell at temperatures between −92 and −65 °C for M = Li and between −100 and −50 °C for M = Na. Representative kinetic plots are shown in Figure 1. All of the data can be fit well by straight lines with correlation coefficients ≥0.99, indicating first-order kinetics. Experiments were performed at pressures of $<10^{-8}$ Torr (zero-pressure limit), so that the internal energy of the ions was controlled by radiation absorption and emission.[31−37]

Arrhenius plots obtained from the zero-pressure-limit (ZPL) rate constants for the loss of a water molecule from AA•M$^+$(H_2O)$_2$, AA = Gln, AsnOMe, GlnOMe, AsnOEt; M = Li and Na, are shown in Figure 2. Correlation coefficients for these data are >0.99, except that for AsnOMe•Li$^+$(H_2O)$_2$, which is 0.98. The measured ZPL Arrhenius activation energies (E_a) and preexponential factors (A) obtained from the Arrhenius plots are reported in Table 1.

To obtain water threshold dissociation energies (E_0) from these Arrhenius data, master-equation modeling of the BIRD data was performed. A detailed description of this modeling process is provided elsewhere.[16,34] The values of E_0 obtained from this modeling for the AA•M$^+$(H_2O)$_2$ complexes are reported in Table 2. The values are similar for all of the complexes (33−38 kJ/mol), except that for GlnOMe•Li$^+$(H_2O)$_2$, which is significantly lower.

CHART 1

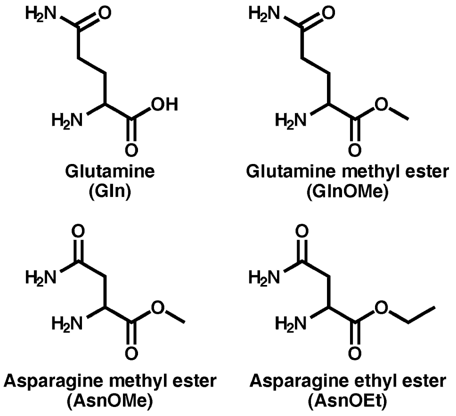

TABLE 1: Zero-Pressure-Limit Arrhenius Parameters for Loss of Water from AA•M$^+$(H_2O)$_2$, M = Li and Na

AA	E_a (kJ/mol)	log A
	M = Li	
Gln	19 ± 1	3.2 ± 0.3
AsnOMe	16 ± 1	2.7 ± 0.3
GlnOMe	12 ± 1	2.1 ± 0.1
AsnOEt	17 ± 1	3.2 ± 0.1
	M = Na	
Gln	19 ± 1	3.4 ± 0.2
AsnOMe	17 ± 1	3.0 ± 0.2
GlnOMe	16 ± 1	2.9 ± 0.2
AsnOEt	16 ± 1	2.9 ± 0.2

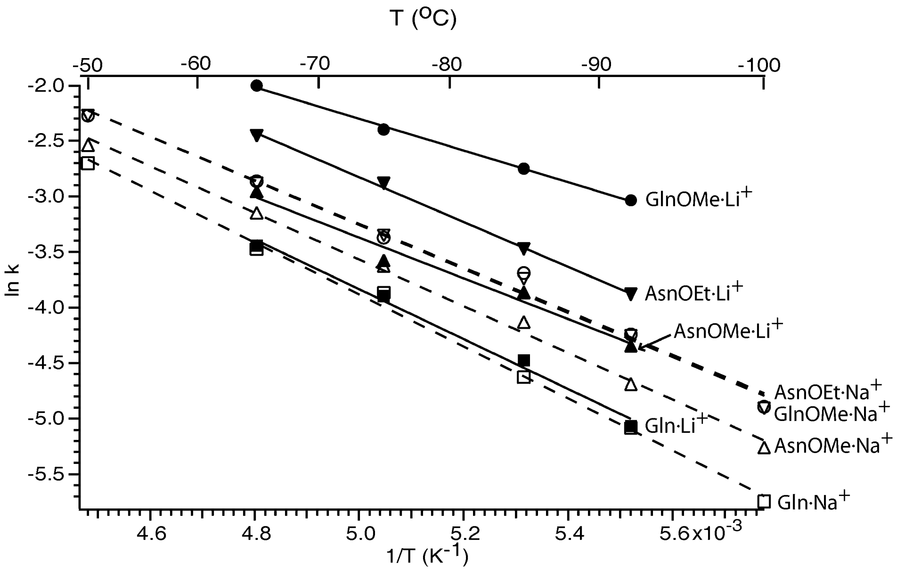

Figure 2. Arrhenius plots for the loss of a water molecule from AA•M$^+$(H_2O)$_2$. The data are fit between −92 and −65 °C for M = Li and between −100 and −50 °C for M = Na.

TABLE 2: Threshold Dissociation Energies (E_0) and Binding Enthalpies (in kJ/mol) for Loss of Water from $AA{\cdot}M^+(H_2O)_2$ Determined from Master-Equation Modeling of BIRD Kinetics Data

	E_0		binding enthalpy	
AA	Li	Na	Li	Na
Gln	36 ± 1	38 ± 2	39 ± 1	40 ± 2
AsnOMe	33 ± 3	35 ± 1	34 ± 3	37 ± 1
GlnOMe	27 ± 2	36 ± 2	29 ± 2	39 ± 2
AsnOEt	33 ± 1	35 ± 1	36 ± 1	38 ± 1

Lowest-Energy Structures. Potential structures of these clusters are explored computationally to obtain parameters necessary for master-equation modeling and to provide insights into the binding motifs possible to these clusters, which all have considerable conformational flexibility. Water binding energies were calculated from these structures for comparison to the experimentally determined values. Low-energy structures and relative energies of $Gln{\cdot}Li^+(H_2O)_2$ that represent significant metal-ion and water coordination motifs are shown in Figure 3. These same motifs were identified for the sodiated complex, and the relative energies of the analogous clusters are also shown in this figure.

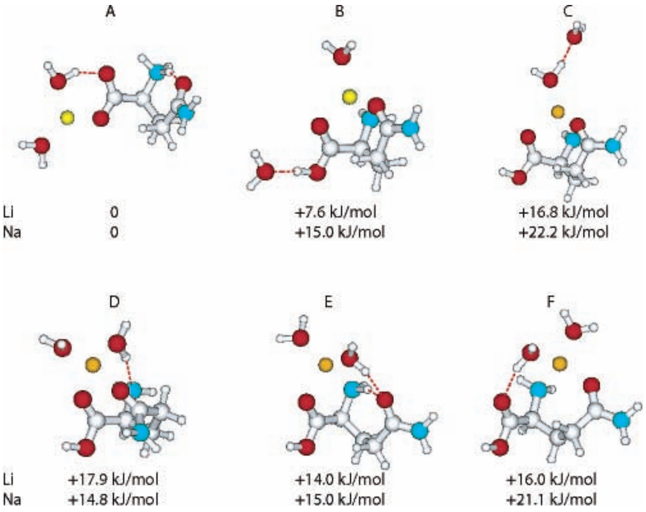

Figure 3. Lowest-energy structures of $Gln{\cdot}Li^+(H_2O)_2$ complexes at the B3LYP/6-31++G** level of theory, with relative energies for these and the similar sodiated complexes.

Calculations indicate that the lowest-energy structure of $Gln{\cdot}M^+(H_2O)_2$ (structure A, Figure 3) is one in which the metal ion is OO-coordinated to zwitterionic glutamine. The lowest-energy nonzwitterionic structure of $Gln{\cdot}Li^+(H_2O)_2$, structure B, is 7.6 kJ/mol higher in energy than structure A. In structure B, the metal ion interacts with the amine nitrogen, carbonyl oxygen, and amide oxygen (NOO coordination) of Gln. One water molecule interacts directly with the metal ion, and the second water molecule interacts with the hydrogen of the carboxylic acid. For $Gln{\cdot}Na^+(H_2O)_2$, structure B is 15.0 kJ/mol higher in energy than structure A and is essentially isoenergetic with structures D and E. In these latter structures, both water molecules interact directly with the metal ion, thereby disrupting one of the interactions between the metal ion and the amino acid.

The lowest-energy structures of $AA{\cdot}M^+(H_2O)_2$, AA = AsnOMe, GlnOMe, and AsnOEt, are shown in Figure 4. Each of the ester-containing complexes have four low-energy structures within 9 kJ/mol, an energy range that is likely comparable to the uncertainty at this level of theory. These structures have the same coordination motifs as glutamine structures C−F in Figure 3. In comparison to the lowest-energy structures reported previously for the one-water clusters of these cationized amino acid analogues,[18] the second water molecule can form a second solvation shell (structure C) or alternatively can act as an acceptor/donor bridge, disrupting the interaction between the metal ion and the amine nitrogen (structure D), the amide oxygen (structure E), or the carbonyl oxygen (structure F). Relative energies for these four structures for each of the amino acid analogues are reported in Table 3.

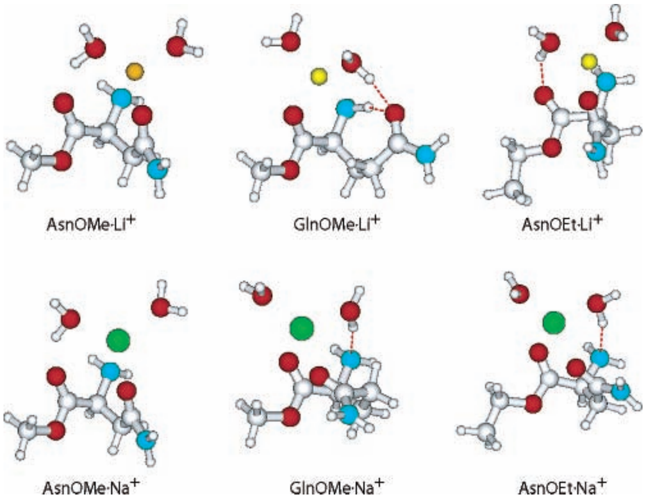

Figure 4. Lowest-energy structures of the nonzwitterionic $AA{\cdot}M^+(H_2O)_2$ model complexes at the B3LYP/6-31++G** level of theory.

TABLE 3: Relative Energetics (in kJ/mol) of the Low-Energy Structures of $AA{\cdot}M^+(H_2O)_2$, AA = AsnOMe, GlnOMe, and AsnOEt; M = Li and Na, at the B3LYP/6-31++G Level of Theory, Including Zero-Point Energy and ΔH(298 K) Corrections**

	structure[a]			
AA	C	D	E	F
		M = Li		
AsnOMe	2.5	8.6	2.8	0
GlnOMe	2.4	4.7	0	1.6
AsnOEt	1.2	7.4	1.2	0
		M = Na		
AsnOMe	0.7	0.2	0.5	0
GlnOMe	6.0	0	3.2	8.3
AsnOEt	2.2	0	1.0	0.4

[a] Structure designations refer to geometries with water coordination analogous to those presented in Figure 3.

Calculations indicate that the second water molecule binds differently in the lowest-energy structures of the lithiated ester-containing complexes versus those of the sodiated analogues. For the lowest-energy $AsnOMe{\cdot}Li^+(H_2O)_2$ and $AsnOEt{\cdot}Li^+(H_2O)_2$ complexes, the second water molecule bridges the metal ion and the carbonyl oxygen, whereas this water molecule bridges the metal ion and the amide oxygen in the lowest-energy $GlnOMe{\cdot}Li^+(H_2O)_2$ complex, with a hydrogen bond present between the amide oxygen and a hydrogen of the amine group. A water molecule binds to the amine group in $AsnOEt{\cdot}Na^+(H_2O)_2$ and $GlnOMe{\cdot}Na^+(H_2O)_2$, whereas in $AsnOMe{\cdot}Na^+(H_2O)_2$, a water molecule binds to the carbonyl oxygen. However, all four lowest-energy structures of the $AsnOMe{\cdot}Na^+(H_2O)_2$ complex are within 0.7 kJ/mol of each other.

Calculated Water Binding Energies. Adiabatic and select diabatic water binding energies, including zero-point energies and enthalpies at 298 K, were calculated from the low-energy $AA{\cdot}M^+(H_2O)$ and $AA{\cdot}M^+(H_2O)_2$ structures and are reported

TABLE 4: Binding Energies of Water for $AA{\cdot}M^+(H_2O)_2$ (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	42	64[a]/50	37	32	34
Na	40[a]/39	55[a]/54	35	36	32

[a] Diabatic binding energy.

in Table 4. An adiabatic binding energy corresponds to the enthalpy for a dissociation reaction from the lowest-energy reactant to the lowest-energy products. This value is an appropriate comparison to the experimental value when the structure of the transition state for dissociation resembles that of the lowest-energy products. However, if the lowest-energy products have very different structures than the reactant, then a diabatic binding energy can be a better comparison. For these clusters, the barrier for interconversion between the zwitterionic and nonzwitterionic forms of the cluster is expected to be higher than that for the loss of a water molecule. Thus, the lowest-energy product ion will not necessarily be formed in these kinetically controlled experiments. For example, calculations by Hoyau and Ohanessian indicate that the interconversion barrier between NO-coordinated and OO-coordinated nonzwitterionic glycine is ~80 kJ/mol.[22] The effects of side-chain interactions and water coordination are not known but might increase this barrier because of a greater degree of structural rearrangement required. This suggests that, in cases involving significant changes in metal-ion binding, e.g., NOO coordination versus OO coordination, the isomerization barrier between these structures will be greater than the threshold dissociation energy for the loss of a second water molecule. Furthermore, the direct loss of a water molecule should be entropically favored over structural isomerization. In cases where metal-ion coordination of the reactant and product differ, a diabatic binding energy, which corresponds to the energy difference between the reactant ion and the lowest-energy product ion with the same metal-ion coordination, should be the best comparison to the experimental value.

The experimentally determined E_0 values were converted into binding enthalpies for direct comparison of these values to the calculated water binding energies. The binding enthalpies of water to $AA{\cdot}M^+(H_2O)_2$ are reported in Table 2. These values are all 1−3 kJ/mol higher than the threshold dissociation energies and are within ±7 kJ/mol of the calculated binding energies. Previous water binding energies for similar complexes determined using B3LYP calculations can be higher than experimentally obtained values by 8 kJ/mol or more.[4,16,17,38−40]

Discussion

Zwitterionic versus Nonzwitterionic Structure. The calculations presented here clearly indicate that the lowest-energy form of Gln in the $Gln{\cdot}M^+(H_2O)_2$ clusters is zwitterionic. The lowest-energy nonzwitterionic form is 7.6 and 14.8 kJ/mol higher in energy for M = Li and Na, respectively. In striking contrast, our experimental data are not consistent with this structure. Three pieces of experimental evidence suggest the nonzwitterionic form. First, our measured binding energies are in excellent agreement with those calculated for the nonzwitterionic form, whereas calculated values for the zwitterionic form are much higher. Second, similar experimental dissociation kinetics for the lithiated and sodiated complexes suggest that the second water molecule is not directly coordinated to the metal ion, a result that is not consistent with the zwitterionic form. Third, the binding energies of the second water molecule in these clusters are similar to those of the model compounds that cannot adopt zwitterionic forms. This evidence is further strengthened through comparisons of these data with those previously obtained for cationized betaine, a zwitterionic cluster calculated to have water coordination similar to that of the zwitterionic form of Gln.

Absolute Binding Energies. The calculated adiabatic binding energies of the second water molecule in the nonzwitterionic forms of these complexes are 42 and 39 kJ/mol for Li^+ and Na^+, respectively. These values are in excellent agreement with the experimentally derived values of 39 and 40 kJ/mol, respectively. In contrast, the calculated diabatic binding energies for the zwitterionic form of Gln in these complexes are 64 and 55 kJ/mol. The 25 and 15 kJ/mol difference between the calculated and experimental values for Li^+ and Na^+, respectively, are much larger than the expected deviations between the theory and the experiments for similar complexes.[4,16,20,39−41] Thus, these results are most consistent with the loss of a water molecule from a cluster containing the nonzwitterionic form of Gln.

Water coordination to the zwitterionic form of glutamine in these complexes is remarkably similar to that calculated previously for two water molecules bound to cationized betaine (Bet), a zwitterionic molecule.[4,5] Although Gln has heteroatoms in its side chain that can solvate the protonated amine in the zwitterionic form, this has little effect on the binding energy of a water molecule to the zwitterionic form of this amino acid.[18] Because of factors described previously, the mode of metal-ion coordination is not expected to change at the transition state in these experiments. Therefore, diabatic binding energies were used to consider the loss of a water molecule from the clusters containing glutamine in its zwitterionic form. For lithiated clusters of the zwitterionic forms of Gln and Bet, the respective second water binding energies are calculated to be 64 and 68 kJ/mol.[4] By contrast, the respective experimentally determined 298 K corrected binding enthalpies for Gln and Bet in these clusters are 39 ± 1 and 69 ± 3 kJ/mol! The nearly identical measured and calculated water binding energies for Bet but not for Gln provides strong evidence that the water coordination of Gln is not like that of Bet. This result provides compelling evidence for the nonzwitterionic form of glutamine in these clusters.

Calculations indicate that adiabatic binding energies of the second water molecule to the zwitterionic form of lithiated and sodiated Gln differ by 9 kJ/mol. This difference is not reflected in the experimental data. By comparison, the dissociation kinetics of cationized Bet are very sensitive to metal-ion size. Kinetics at 293 K reveal that $Bet{\cdot}Na^+(H_2O)_2$ dissociates nearly 7 times faster than $Bet{\cdot}Li^+(H_2O)_2$. Master-equation modeling of a limited data set using BIRD rates measured at 273,[5] 293, and 328 K[42] indicates that the threshold dissociation energy for the loss of a second water molecule from sodiated Bet is roughly 17 kJ/mol lower than the value measured for lithiated Bet under similar conditions (67 ± 2 kJ/mol).[4] Again, the clear differences between Gln and Bet provide further evidence that glutamine is not zwitterionic in these clusters.

Relative Dissociation Kinetics of Lithiated and Sodiated Species. In the gas phase, both Li^+ and Na^+ are optimally coordinated or solvated by four water molecules.[43,44] Additional water molecules are added to a second solvent shell and do not interact directly with the metal ion. For NOO-coordinated $Gln{\cdot}M^+(H_2O)$, the water molecule is directly coordinated to the metal ion, making the metal ion tetracoordinated. If the lithiated and sodiated complexes have comparable structures, the location of the second water molecule in these complexes can be indirectly inferred from the experimental data and the calculated structures.

The water binding energies for the AA•$M^+(H_2O)_2$ complexes obtained from the master-equation modeling of the experimental data are all similar. The range of values reflects both experimental error and uncertainty in values used in the modeling process. In contrast, the Arrhenius plots themselves, which directly reflect the kinetic stabilities of these complexes, are significantly different for some of these complexes and can be used to infer structure.

In contrast to the Arrhenius data obtained for the singly hydrated clusters,[18] where the water molecule coordinates directly to the metal ion, the Arrhenius plots for Gln•$Li^+(H_2O)_2$ and Gln•$Na^+(H_2O)_2$ are nearly the same (Figure 2). These two complexes should absorb and emit radiation at nearly the same rates, and the dissociation processes for loss of a water molecule from these two complexes should also be essentially the same. The similar kinetic stabilities of these two ions over the temperature range studied indicate that the second water molecule does not interact directly with the metal ion. If this water molecule interacts directly with the metal ion, one would expect that the lithiated complex would be more stable, as is the case for the one-water-molecule complex. This suggests that the structure of Gln•$M^+(H_2O)_2$ is not likely D, E, or F (Figure 3). The second water molecule in structures B and C does not directly interact with the metal ion. For structure C, one would expect to observe a difference in binding energy between lithiated and sodiated ions; the binding energies of water molecules in the second solvent shell of Li^+ are larger than those of Na^+. For example, the binding energies of a water molecule in $Li^+(H_2O)_5$ and in $Li^+(H_2O)_4$ are 6.6 and 10.7 kJ/mol greater, respectively, than the sodiated counterparts.[43] Therefore, structure B, in which the metal ion is NOO-coordinated to nonzwitterionic Gln, one water molecule binds directly to the metal ion, and the other water molecule binds to the hydrogen of the carboxylic acid group, is the most likely structure for the Gln•$M^+(H_2O)_2$ complex.

Comparison between Glutamine and the Ester-Containing Complexes. The threshold dissociation energies for the loss of a second water molecule from the cationized nonzwitterionic analogues, excluding GlnOMe•$Li^+(H_2O)_2$, which is discussed separately below, are very similar to those for glutamine. The values of E_0 for the sodiated species are ~2 kJ/mol higher than those of the lithiated species. The values of E_0 for these nonzwitterionic analogues are systematically lower than those measured for glutamine by ~3 kJ/mol. This trend is particularly significant given that water binding to a cationized ester of an amino ester is expected to be weaker than that to a cationized nonzwitterionic amino acid, because of the relative strengths of the metal ion−amino acid/amino acid methyl ester interaction.[25]

The threshold dissociation energy obtained for GlnOMe•$Li^+(H_2O)_2$ is significantly lower than the values measured for the other complexes. We infer the origin of this difference from the calculated lowest-energy structures for these complexes, which indicate that an internal hydrogen bond between the amine and amide oxygen atom occurs in the GlnOMe•$Li^+(H_2O)_2$ complex. Such an internal hydrogen bond, which reduces the strength of the amide−metal ion interaction, is not observed in the AsnOMe•$Li^+(H_2O)_2$ and AsnOEt•$Li^+(H_2O)_2$ complexes. Nor is it observed in the lithiated glutamine complex, in which the second water molecule interacts with the amine nitrogen or carbonyl oxygen, suggesting that the second water molecule bridges the amide oxygen and the metal ion.

As was the case for Gln, the threshold dissociation energies for the loss of a water molecule from the other lithiated complexes are similar to those of the sodiated complexes. In fact, the Arrhenius plots show that the kinetic stabilities of the lithiated complexes are lower than those of the corresponding sodiated complexes. This is initially surprising, because water typically binds more strongly to lithiated amino acid complexes than to their sodiated counterparts.[4,16−18] For example, the binding energies of a water molecule in the singly hydrated lithiated glutamine and model complexes are ~10 kJ/mol greater than those in the corresponding sodiated complexes.[18]

It is not possible to unambiguously determine how water is bound in the model complexes because of the similarity in threshold dissociation energies. It is likely that the second water molecule is not binding directly to the first water molecule (as in structure C, Figure 3), because this would result in a higher binding energy for the lithiated than for the sodiated complexes, which is clearly not the case. The presence of a structure such as B (Figure 3) is precluded because of the absence of an acidic hydrogen in these complexes. Therefore, it is likely that one of the water molecules in the doubly hydrated model complexes bridges the metal ion to a heteroatom of the AA. It is difficult to determine the heteroatom with which the water molecule interacts from the lowest-energy structures, because all of the possible structures are within 9 kJ/mol of the lowest-energy structure. Furthermore, it is possible that multiple conformations of these complexes are present during the experiment.

An interesting trend in the lowest-energy structures of the model complexes is the higher propensity for a water molecule to form a hydrogen bond with the nitrogen atom of the amine group versus other heteroatoms. This can be rationalized by how the metal ion is bound in the lithiated versus the sodiated complexes. The strongest interaction between the metal ion and a heteroatom in these complexes is that with the amide oxygen.[40,45] The water molecule will therefore bridge the metal ion to either the carbonyl oxygen or the amine nitrogen. The amine nitrogen is closer to the amide oxygen, making it possible for the smaller lithium ion to interact with both of these heteroatoms and the water molecule to form a bridge between the metal ion and the carbonyl oxygen. This interaction is present in the lowest-energy structures of AsnOMe•$Li^+(H_2O)_2$ and AsnOEt•$Li^+(H_2O)_2$.

In contrast, this interaction is not present in the lowest-energy structures of most of the sodiated complexes. A larger binding pocket is necessary to bind a sodium ion than a lithium ion, so the sodium ion interacts with the amide oxygen and carbonyl oxygen. The water molecule then forms a bridge between the sodium ion and the amine nitrogen. This type of structure is the lowest in energy for AsnOEt•$Na^+(H_2O)_2$ and GlnOMe•$Na^+(H_2O)_2$ and is essentially isoenergetic with the lowest-energy structure of AsnOMe•$Na^+(H_2O)_2$.

Although the second water molecule binds to different heteroatoms in the lithiated and sodiated complexes, this water molecule still interacts directly with the metal ion and would be expected to bind more strongly to lithium than to sodium. However, calculations indicate that a water molecule binds more strongly to an amine group (CH_3NH_2) than to a similar carbonyl group (CH_3CHO) by ~6 kJ/mol.[46] The water molecules in the lithiated complexes tend to interact with the carbonyl group, whereas the water molecules in the sodiated complexes tend to interact with the amine group. This difference in hydrogen bonding energies might compensate for the difference in water-

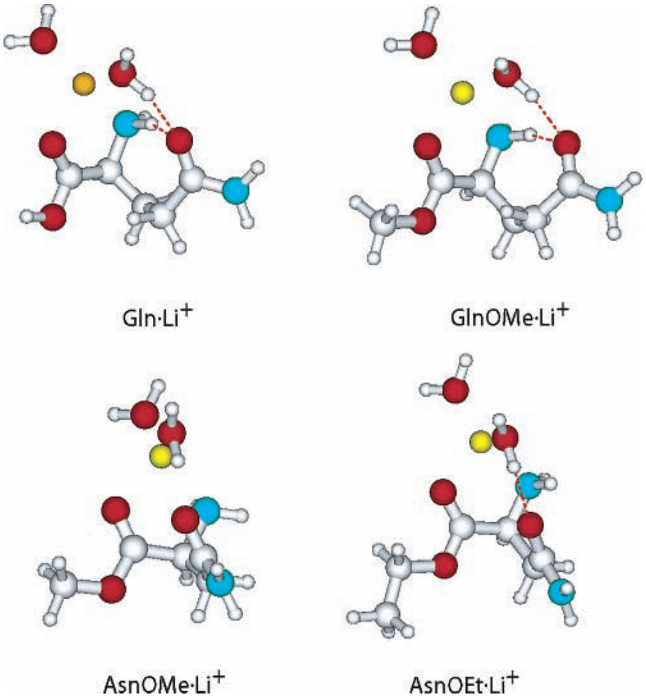

Figure 5. Structures of AA·$Li^+(H_2O)_2$ complexes that have a water molecule interacting with the amide oxygen at the B3LYP/6-31++G** level of theory.

metal-ion interaction energy, resulting in similar water binding energies for the lithiated and sodiated complexes.

Effects of Side Chain on Water Binding. It is interesting to note that the mode of water binding observed for GlnOMe·$Li^+(H_2O)_2$ is not favorable for AsnOMe·$Li^+(H_2O)_2$ and AsnOEt·$Li^+(H_2O)_2$. An investigation of low-energy structures of AA·$Li^+(H_2O)_2$ that have the same modes of metal-ion and water binding as GlnOMe·$Li^+(H_2O)_2$ (Figure 5) indicates that the internal hydrogen bond between the hydrogen of the amine group and the amide oxygen atom that is observed for GlnOMe and Gln complexes does not occur in AsnOMe and AsnOEt complexes because of the ring strain associated with the shorter side chain in these latter complexes.

In the singly hydrated complexes, the water threshold dissociation energies for the AsnOMe and AsnOEt complexes are, on average, slightly higher than those for the Gln and GlnOMe complexes.[18] The modes of metal-ion and water binding are similar in all of these singly hydrated complexes, but the shorter side chain of Asn relative to Gln results in less effective solvation of the charge of the metal ion, resulting in E_0 values that are 0−3 kJ/mol greater for the Asn complexes.[18] In contrast, the value of E_0 for GlnOMe·$Na^+(H_2O)_2$ is the same as the values for AsnOMe·$Na^+(H_2O)_2$ and AsnOEt·$Na^+(H_2O)_2$, within error. The metal ion in these doubly hydrated complexes interacts with only two of the heteroatoms of the AA. The water molecule, which forms a bridge between the metal ion and the third heteroatom, results in increased conformational flexibility of the complex, making possible more effective solvation of the metal ion.

Conclusions

The structures of Gln·$M^+(H_2O)_2$, M = Li and Na, and related compounds were investigated using BIRD experiments and density functional theory calculations. Although the lowest-energy form of Gln in both the lithiated and sodiated complexes is calculated to be zwitterionic, results from these experiments indicate that Gln in these complexes is nonzwitterionic. Structures are inferred from the experimental data based on the binding energies of a water molecule to these complexes, which are calculated to be significantly different for the clusters that contain these two different forms of Gln. This discrepancy between theory and experiment is not likely due to kinetic trapping in the experiment. This would be expected to result in preferential formation of the zwitterionic form of the amino acid, which is most stable in solution.

It is important to note that diabatic binding energies are often used for comparison to experiment. In the dissociation process, the lowest-energy form of the complex with one fewer water molecule will not necessarily be formed at the transition state. This is particularly true for cases where the metal ion has to significantly change coordination, e.g., from OO coodination in a zwitterionic structure to NOO coodination in a nonzwitterionic structure, because the barrier required for this rearrangement to occur is expected to be much larger than that for the loss of a water molecule. In these cases, calculated diabatic binding energies should be the most direct comparison with experiment. Conversely, if rearrangement to the lowest-energy product occurs at the transition state, then the adiabatic binding energy provides the best comparison to the experimental values. For the sodiated clusters, the diabatic and adiabatic values are essentially the same, so that this issue does not effect conclusions for these clusters. However, for Li, the diabatic binding energy is 14 kJ/mol higher than the adiabatic value. Nevertheless, the measured value is still much closer to that of the nonzwitterionic form. It should also be noted that guided ion beam experiments by Armentrout and co-workers indicate that the energy required to remove a water molecule from Gly·$Na^+(H_2O)_2$ is not great enough to change the position of the metal ion.[20] This result indicates that the use of diabatic energies is more appropriate.

Comparisons were made between experimental data obtained for the glutamine-containing clusters and those containing amino acid analogues to deduce information about structures. These related clusters, all of which lack the acidic group present in glutamine, have fewer potential conformations. With the careful use of calculations, these additional data coupled with the similarities and differences between glutamine and the amino acid analogues provide additional insights into the structures of these clusters.

Detailed information about the structures is difficult to infer from the experimental results alone. The binding energies of a water molecule in these complexes are all very similar. However, it appears that a structure for Gln·$M^+(H_2O)_2$ that is most consistent with the data is one in which the metal ion is NOO-coordinated to the nonzwitterionic form of Gln, one water molecule interacts directly with the metal ion, and the other water molecule accepts a hydrogen bond from the acidic hydrogen of the carboxylic acid group. Furthermore, these studies assist in resolving ambiguities in the previous study of Gln·$Na^+(H_2O)$.[18] The results for this complex with two water molecules indicate that Gln is in its nonzwitterionic form. One would also expect that Gln in the one-water sodiated complex is also nonzwitterionic.

Finally, it is important to recognize that the stability of the lithiated clusters is lower than that of the corresponding sodiated clusters, a result that has not been observed previously for cationized amino acids. This result is attributed to differences in how these two metal ions bind to the amino acid and related compounds.

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