

# Alkali Metal Ion Binding to Glutamine and Glutamine Derivatives Investigated by Infrared Action Spectroscopy and Theory

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The gas-phase structures of alkali-metal cationized glutamine are investigated by using both infrared multiple photon dissociation (IRMPD) action spectroscopy, utilizing light generated by a free electron laser, and theory. The IRMPD spectra contain many similarities that are most consistent with glutamine adopting nonzwitterionic forms in all ions, but differences in the spectra indicate that the specific nonzwitterionic forms adopted depend on metal-ion identity. For ions containing small alkali metals, the metal ion is solvated predominantly by the amino group, the carbonyl oxygen of the carboxylic acid group, and the carbonyl oxygen of the amide group. With increasing alkali-metal-ion size, additional structures are present in which the carboxylic acid group donates a hydrogen bond to the amino group and the metal ion is solvated only by the amide and carboxylic acid groups. The effects of alkylation of the amino and amide groups on the proton affinity of isolated glutamine and the relative zwitterion stability of sodiated glutamine were examined computationally. Methylation of the amino group increases the proton affinity of isolated glutamine and preferentially stabilizes the zwitterionic form of sodiated glutamine by roughly 20 kJ/mol. Ethylation and isopropylation of the amide group each increase the proton affinity of isolated glutamine by roughly 13 kJ/mol but preferentially stabilize the zwitterionic form of sodiated glutamine by less than 3 kJ/mol. These results indicate that effects of proton affinity on relative zwitterion stability compete with effects of metal-ion solvation.

## Introduction

The gas-phase stabilities of zwitterionic relative to nonzwitterionic forms of selected sodiated amino acids with aliphatic side chains are directly related to the proton affinity of the amino acid.<sup>1,2</sup> On the basis of trends in zwitterion stability and proton affinity found for sodiated glycine, alanine, and derivatives of these amino acids, the zwitterionic forms of sodiated arginine (Arg), glutamine (Gln), and lysine (Lys) were predicted to be lowest in energy.<sup>2</sup> Calculations indicate that the zwitterionic form of Arg•Na<sup>+</sup> is lowest in energy,<sup>3–5</sup> consistent with infrared (IR) multiple photon dissociation (IRMPD) spectra in both the hydrogen-stretch<sup>4</sup> and 5–10  $\mu\text{m}^5$  regions, although these spectra indicate the presence of small nonzwitterionic populations and that both forms of Arg are competitive under these experimental conditions. In contrast, calculations and IRMPD spectroscopy in the 5–10  $\mu\text{m}$  region of Lys•Na<sup>+</sup> indicate that the nonzwitterionic form is lowest in energy.<sup>6</sup> The IRMPD spectrum of sodiated *N*<sub>ε</sub>-methyl-lysine, a derivative of Lys with a proton affinity that is 16 kJ/mol greater than that of Lys, indicates that populations of both the nonzwitterionic and zwitterionic forms of the amino acid are present under the conditions of the experiment.<sup>6</sup>

The structures of lithiated and sodiated Gln solvated by up to two water molecules have been investigated by blackbody IR radiative dissociation (BIRD).<sup>7,8</sup> For Gln•Na<sup>+</sup>(H<sub>2</sub>O), the measured threshold dissociation energy for the loss of a water molecule is consistent with adiabatic binding energies calculated for both the zwitterionic and nonzwitterionic forms of Gln.<sup>7</sup>

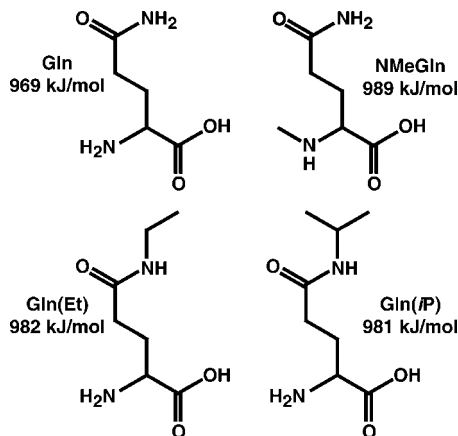
Comparisons with values calculated for a simple model system, Gly•Na<sup>+</sup>,<sup>9</sup> suggest that water loss barriers are lower than isomerization barriers and that the calculated diabatic binding energy to the zwitterionic form, which is ~7 kJ/mol higher than the corresponding adiabatic value, is likely a better comparison with experiment.<sup>7</sup> On the basis of these results, lithiated and sodiated Gln were assigned to nonzwitterionic structures, both with and without one or two water molecules.<sup>7,8</sup> First and second water binding energies to sodiated Gln were recently determined by high-pressure mass-spectrometry experiments, and these values<sup>10</sup> are consistent with those previously measured by BIRD.<sup>7,8</sup> Sodium binding affinities of Gln have also been measured.<sup>11–13</sup>

Action spectroscopy using light from either free electron lasers (typically 5–10  $\mu\text{m}$ )<sup>5,6,14–27</sup> or benchtop laser systems based on optical parametric generation (typically 2.5–4  $\mu\text{m}$ )<sup>4,28–33</sup> has emerged as a powerful method to directly probe the gas-phase structures of cationized amino acids and amino acid analogues,<sup>4–6,14–20,22,24,27,30,32</sup> peptides,<sup>15,24,26</sup> amino acid multimers,<sup>25,29,31</sup> and even proteins.<sup>21,28</sup> Action spectra of gas-phase hydrogen/deuterium exchange<sup>34</sup> and collisionally activated dissociation<sup>35</sup> products have provided insights into the mechanisms of these reactions. Oomens and Steill recently reported IRMPD spectra of deprotonated benzoate derivatives, which are the first IR signatures for free carboxylate groups.<sup>23</sup> Here, we report the IRMPD spectra of Gln•M<sup>+</sup>, M = Li, Na, K, and Cs. By comparing these experimental spectra to each other, those obtained previously for other alkali-metal cationized amino acids, and those calculated for candidate low-energy structures, detailed structural information is obtained. These results indicate that the propensity of these ions to adopt different structures depends on metal-ion identity. Additional calculations indicate that any relationship between proton affinity and relative

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**Figure 1.** Structures and MP2/6-311++G(2d,2p)//B3LYP/6-31++G(d,p) proton affinities of Gln,  $N_{\alpha}$ -methyl-glutamine (NMeGln), theanine ( $N_{\gamma}$ -ethyl glutamine, Gln(Et)), and  $N_{\gamma}$ -isopropyl glutamine (Gln(iP)).

zwitterion stability is only indirect for derivatives of Gln with alkylated amine or amide groups.

## Methods

**Mass Spectrometry and Photodissociation.** Experiments were performed by using a 4.7 T Fourier-transform ion cyclotron resonance mass spectrometer. The instrument<sup>36</sup> and general experimental methods<sup>17</sup> are described elsewhere. Cationized amino acids were formed by electrospray ionization from a solution of 1 mM amino acid and 1 mM alkali-metal hydroxide in 70:30 MeOH/H<sub>2</sub>O infused at a rate of 20  $\mu$ L/min. After isolation by using a stored waveform inverse Fourier transform pulse, ions were irradiated for 3 s by using tunable radiation generated by the FELIX free electron laser.<sup>37</sup>

**Computational Chemistry.** Low-energy structures of isolated and cationized Gln, theanine ( $N_{\gamma}$ -ethyl-glutamine, (Gln(Et)),  $N_{\gamma}$ -isopropyl-glutamine (Gln(iP)), and  $N_{\alpha}$ -methyl-glutamine (NMeGln), Figure 1, were generated by Monte Carlo multiple minimum conformational searching by using MacroModel 9.1 (Schrödinger, Inc. Portland, OR), the MMFFs or OPLS force fields, and methods described previously.<sup>6</sup> The resulting low-energy structures were grouped into families with similar metal-ion binding and hydrogen-bonding motifs. Representative structures from each family were energy-minimized by using hybrid method density functional calculations (B3LYP), performed by using Jaguar v. 6.5 (Schrödinger, Inc., Portland, OR), with the LANL2DZ effective core potential (ECP) for K, Rb, and Cs, and the 6-31G(d,p) basis set for all remaining atoms. Vibrational frequencies and intensities were calculated by using the double-harmonic approximation and analytical derivatives of the 6-31++G(d,p) energy minimized Hessian (CRENBL ECP used for Rb and Cs) by using Q-Chem v.3.0.<sup>38</sup> All frequencies were scaled by 0.975 and convolved by using a 20  $\text{cm}^{-1}$  fwhm Gaussian distribution, which yielded good agreement between experiment and theory for Lys $\cdot$ M<sup>+</sup> and Lys(Me) $\cdot$ M<sup>+</sup>.<sup>6</sup> Single-point energies for the B3LYP/6-31++G(d,p) geometries were also calculated at both the B3LYP and MP2 levels of theory by using the CRENBL ECP for Rb and Cs and the 6-311++G(2d,2p) basis set for all remaining elements.

## Results and Discussion

**IRMPD Action Spectra.** Photodissociation of Gln $\cdot$ M<sup>+</sup>, M = Li, Na, K, and Cs, results in the loss of a small neutral molecule or the amino acid (Table 1). For example, photodissociation of Gln $\cdot$ Na<sup>+</sup> results in the loss of either a neutral water

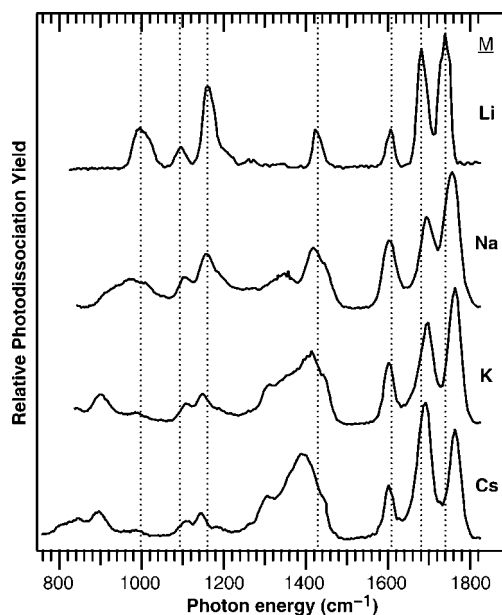
**TABLE 1: Fragmentation Pathways and Branching Ratios Observed from Photodissociation of Gln $\cdot$ M<sup>+</sup>**

M	Li	Na	K	Cs
neutral loss	NH <sub>3</sub> :H <sub>2</sub> O	NH <sub>3</sub> :H <sub>2</sub> O	NH <sub>3</sub> :H <sub>2</sub> O	Gln:H <sub>2</sub> O
branching ratio	1:1	1:1	1:5	2:3

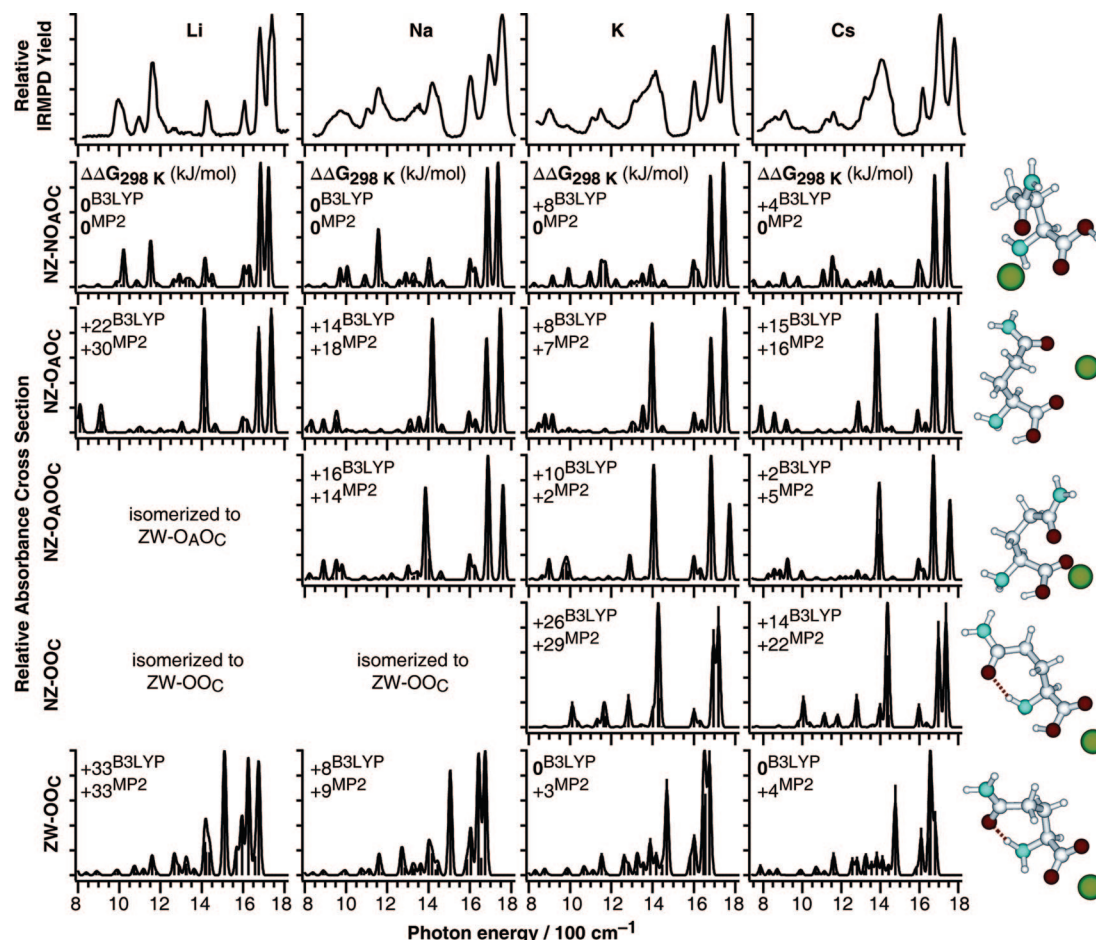
or ammonia molecule, which are the two lowest-energy dissociation channels observed from collision-induced dissociation of Gln $\cdot$ Na<sup>+</sup>.<sup>12</sup> IRMPD action spectra (Figure 2) are obtained from the photodissociation yield at each frequency.<sup>5</sup> Photodissociation yields are corrected linearly for laser power,<sup>17,39,40</sup> but IRMPD is a multiple photon process under these conditions, and spectral intensities therefore do not necessarily reflect those obtained from quantum mechanical calculations.

The IRMPD spectra of Gln $\cdot$ M<sup>+</sup> have intense bands at frequencies greater than 1700  $\text{cm}^{-1}$ . The IRMPD spectra of Arg,<sup>5</sup> Lys,<sup>6</sup> and tryptophan<sup>16</sup> complexed with lithium contain diagnostic bands near 1720  $\text{cm}^{-1}$  that are assigned to the carbonyl stretches of carboxylic acid groups. The presence of a carboxylic acid functional group indicates that these amino acids adopt nonzwitterionic structures when complexed with lithium. The presence of bands in this region of the IRMPD spectra of Gln $\cdot$ M<sup>+</sup> indicates that populations of ions in which Gln is nonzwitterionic are present under experimental conditions for ions containing each of the alkali metals studied.

The IRMPD spectra of Gln $\cdot$ M<sup>+</sup>, M = Na, K, and Cs, contain many bands at frequencies close to those exhibited for Gln $\cdot$ Li<sup>+</sup>, but these features evolve with increasing alkali-metal-ion size, and additional features become more pronounced. For example, the relative intensities of the bands near 1000 and 1160  $\text{cm}^{-1}$  for Gln $\cdot$ Li<sup>+</sup> decrease with increasing alkali-metal-ion size, whereas the relative photodissociation intensities below 950  $\text{cm}^{-1}$  and from 1200 to 1400  $\text{cm}^{-1}$  increase. These spectral changes indicate that the structures of these ions change with increasing alkali-metal-ion size, but the persistence of many bands, particularly the carbonyl stretch of a carboxylic acid group, indicates that many structural elements or populations of structures exist for all ions. However, the carbonyl stretch band for the carboxylic acid group of Gln $\cdot$ Cs<sup>+</sup> exhibits significantly lower relative intensity than those in the other



**Figure 2.** IRMPD action spectra of Gln $\cdot$ M<sup>+</sup>, M = Li, Na, K, and Cs.



**Figure 3.** IRMPD action spectra and selected calculated spectra of  $\text{Gln}\cdot\text{M}^+$ ,  $\text{M} = \text{Li}, \text{Na}, \text{K},$  and  $\text{Cs}$ . Energy minimized structures are shown for  $\text{Gln}\cdot\text{K}^+$ . Relative 298 K free energies are reported at the B3LYP/6-311++G(2d,2p)//6-31++G(d,p) and MP2/6-311++G(2d,2p)//B3LYP/6-31++G(d,p) levels of theory.

spectra, suggesting the presence of significant populations of ions with very different structure. Assignments for the remaining IRMPD bands and additional insights into ion structures are determined through comparisons with the calculated linear absorbance spectra for candidate low-energy structures.

**Low-Energy Structures of  $\text{Gln}\cdot\text{M}^+$ .** The metal ion in the nonzwitterionic (NZ) and zwitterionic (ZW) forms of alkali-metal cationized Gln can be solvated by the N-terminal amino group (N), the oxygen of the amide group ( $\text{O}_A$ ), and one ( $\text{O}_C$ ) or two ( $\text{OO}_C$ ) oxygen atoms of the carboxylate/carboxylic acid group. Depending on alkali-metal-ion identity, four to six important low-energy structures with unique amino-acid/metal-ion interactions were identified, and those for  $\text{Gln}\cdot\text{K}^+$  are shown in Figure 3. Similar structures were also identified for the other alkali-metal cationized Gln ions, but some of those nonzwitterionic structures energy minimized to analogous zwitterionic structures with similar amino-acid/metal-ion interactions. Zwitterionic structures analogous to  $\text{NZ-O}_A\text{OO}_C$  were also stable for  $\text{M} = \text{Li}$  and  $\text{Na}$ , but these structures are significantly higher in energy. The relative Gibbs free energies of these structures at 0 and 298 K, determined by using B3LYP and MP2 calculations, are given in Table 2, and selected data as a function of cation size are plotted in Figure 4. The latter temperature corresponds to the approximate temperature of the ions prior to laser irradiation. Structural changes during ion heating by photon absorption can potentially occur prior to dissociation.

In the lowest-energy structures that contain a smaller alkali-metal ion, the ion is solvated by three different functional groups

of a nonzwitterionic form of the amino acid ( $\text{NO}_A\text{O}_C$  coordinated). With increasing metal-ion size, additional structures that contain hydrogen bonds are preferentially stabilized. These alternate low-energy structures include a zwitterionic structure in which the metal ion is  $\text{OO}_C$  coordinated and the protonated N-terminal amino group donates a hydrogen bond to the amide group ( $\text{ZW-OO}_C$ ) and nonzwitterionic structures in which the carboxylic acid group donates a hydrogen bond to the amino group. In these nonzwitterionic structures, the metal ion is solvated by just the carboxylic acid group ( $\text{NZ-OO}_C$ ) or both the carboxylic and amide groups ( $\text{NZ-O}_A\text{O}_C$  and  $\text{NZ-O}_A\text{OO}_C$ ). The relative energies of these structures depend on the level of theory, but these calculations indicate that zwitterionic structures are lowest in energy or competitive with the lowest-energy nonzwitterionic structure for  $\text{M} = \text{K}, \text{Rb},$  and  $\text{Cs}$  (Figure 4).

Many of these structures were also identified previously for  $\text{Gln}\cdot\text{Li}^+$  and  $\text{Gln}\cdot\text{Na}^+$ ,<sup>7</sup> including most low-energy nonzwitterionic structures ( $\text{NZ-NO}_A\text{O}_C$ ,  $\text{NZ-O}_A\text{O}_C$ , and  $\text{NZ-OO}_C$ ) and the lowest-energy zwitterionic structure ( $\text{ZW-OO}_C$ ). The new structures identified here are  $\text{NZ-O}_A\text{OO}_C$ , which is stable for all ions except  $\text{M} = \text{Li}$ , and  $\text{ZW-O}_A\text{OO}_C$ , which was recently reported by Heaton et al. as a low-energy structure for  $\text{Gln}\cdot\text{Na}^{+12}$  and is only stable for  $\text{M} = \text{Li}$  and  $\text{Na}$ . Interestingly,  $\text{NZ-OO}_C$  was previously identified as a local minimum structure for  $\text{Gln}\cdot\text{Li}^+$  and  $\text{Gln}\cdot\text{Na}^+$ ,<sup>7</sup> but these structures energy-minimized to  $\text{ZW-OO}_C$  in these calculations. This may be attributable to differences in the DFT grids used or subtle differences in the initial candidate structures identified in the two studies. Heaton



TABLE 2: Relative Gibbs Free Energies of Gln•M<sup>+</sup> Conformers in kJ/mol

	B3LYP/6-311++G(2d,2p)//6-31++G(d,p)					MP2/6-311++G(2d,2p)//B3LYP/6-31++G(d,p)				
	Li	Na	K	Rb	Cs	Li	Na	K	Rb	Cs
	0 K									
	NZ									
NO <sub>A</sub> O <sub>C</sub>	0	0	7.6	11.6	8.7	0	0	0	6.7	1.0
O <sub>A</sub> O <sub>C</sub>	23.4	14.4	11.6	9.5	19.0	31.6	17.8	11.1	3.5	16.2
O <sub>A</sub> OO <sub>C</sub>	<sup>-a</sup>	17.5	10.5	6.8	6.0	<sup>-a</sup>	15.1	3.2	3.2	5.2
OO <sub>C</sub>	<sup>-a</sup>	<sup>-a</sup>	28.3	17.2	16.6	<sup>-a</sup>	<sup>-a</sup>	32.3	17.9	20.9
	ZW									
OO <sub>C</sub>	34.3	9.1	0	0	0	34.5	9.6	3.2	0	0
O <sub>A</sub> OO <sub>C</sub>	54.0	34.1	<sup>-b</sup>	<sup>-b</sup>	<sup>-b</sup>	48.5	29.5	<sup>-b</sup>	<sup>-b</sup>	<sup>-b</sup>
	298 K									
	NZ									
NO <sub>A</sub> O <sub>C</sub>	0	0	8.1	10.3	3.8	0	0	0	5.4	0
O <sub>A</sub> O <sub>C</sub>	21.6	14.2	8.4	8.3	15.3	29.7	17.6	7.5	2.4	16.3
O <sub>A</sub> OO <sub>C</sub>	<sup>-a</sup>	16.3	10.2	6.6	2.2	<sup>-a</sup>	13.9	2.4	3.1	5.3
OO <sub>C</sub>	<sup>-a</sup>	<sup>-a</sup>	26.0	15.6	13.9	<sup>-a</sup>	<sup>-a</sup>	29.5	16.2	22.0
	ZW									
OO <sub>C</sub>	32.6	8.2	0	0	0	32.8	8.8	2.6	0	3.9
O <sub>A</sub> OO <sub>C</sub>	51.9	33.9	<sup>-b</sup>	<sup>-b</sup>	<sup>-b</sup>	46.3	29.3	<sup>-b</sup>	<sup>-b</sup>	<sup>-b</sup>

<sup>a</sup> Energy minimized to the corresponding zwitterionic forms. <sup>b</sup> Energy minimized to the corresponding nonzwitterionic forms.

et al. also reported multiple structures with different methylene group dihedral angles for several families of structures with identical hydrogen bonding and metal binding motifs,<sup>12</sup> whereas here, we only report the lowest-energy structure identified for each family. In general, these small conformational differences have small effects on the calculated absorbance spectra in this spectral region.

**Structure of Gln•Li<sup>+</sup>.** The IRMPD spectrum of Gln•Li<sup>+</sup> has a band at 1740 cm<sup>-1</sup>, which is close in frequency to the carbonyl stretch of the carboxylic acid groups in structures NZ-NO<sub>A</sub>O<sub>C</sub> and NZ-O<sub>A</sub>O<sub>C</sub> (1723 and 1737 cm<sup>-1</sup>, respectively). In contrast, structure ZW-OO<sub>C</sub> does not have a carboxylic acid functional group, and consequently, its spectrum does not have any vibrational modes at frequencies greater than 1680 cm<sup>-1</sup> in this

TABLE 3: Band Assignments for Gln•Li<sup>+</sup>

IRMPD freq. <sup>a</sup>	assignment	structure NZ-NO <sub>A</sub> O <sub>C</sub>	
		calc. freq. <sup>b</sup>	calc. int. <sup>c</sup>
1740	COOH asym. stretch	1723	393
1680	C=O <sub>A</sub> stretch	1684	407
—	N <sub>T</sub> H <sub>2</sub> scissor	1630	71
1605	N <sub>A</sub> H <sub>2</sub> bend	1604	69
1425	COOH sym. stretch	1416	88
1160	OH bend	1152	153
1095	CN <sub>T</sub> stretch/CH <sub>2</sub> wag	1084	20
1000	N <sub>T</sub> H <sub>2</sub> wag/CH <sub>2</sub> twist	1022	121

<sup>a</sup> Experimental IRMPD band frequencies in cm<sup>-1</sup>. <sup>b</sup> Calculated band frequencies in cm<sup>-1</sup>. <sup>c</sup> Calculated band intensities in km/mol.

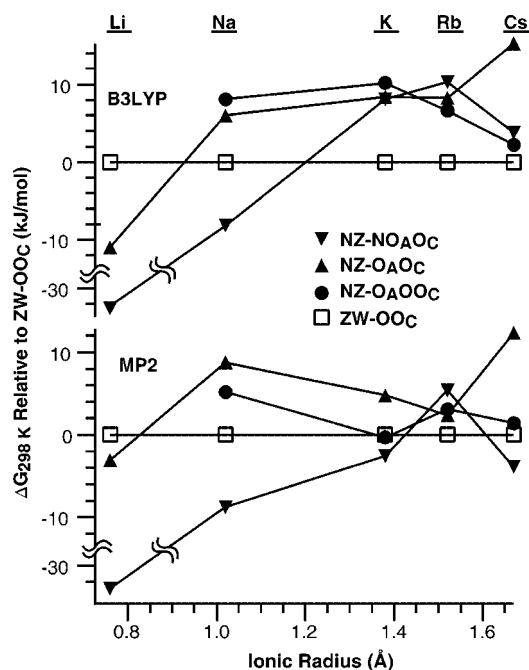


Figure 4. Relative 298 K free energies of selected Gln•M<sup>+</sup> conformers at the B3LYP/6-311++G(2d,2p)//6-31++G(d,p) and MP2/6-311++G(2d,2p)//B3LYP/6-31++G(d,p) levels of theory. Structure NZ-O<sub>A</sub>OO<sub>C</sub> energy minimized to ZW-O<sub>A</sub>OO<sub>C</sub> for Gln•Li<sup>+</sup>.

spectral range. Instead, the calculated absorbance spectrum of this zwitterionic structure has strong bands at 1416 and 1625 cm<sup>-1</sup>, which correspond to the symmetric and asymmetric stretches of the carboxylate group, respectively, and at 1510 cm<sup>-1</sup>, which corresponds to the umbrella bend of the protonated amino group. These comparisons, coupled with previous results for other alkali-metal cationized amino acids,<sup>5,6,14,16</sup> provide compelling evidence that Gln is nonzwitterionic in the lithiated ion.

In addition to the carbonyl stretch of the carboxylic acid group, the IRMPD spectrum of Gln•Li<sup>+</sup> contains many other bands that are in good agreement with the absorbance spectrum calculated for structure NZ-NO<sub>A</sub>O<sub>C</sub>. This comparison and band assignments are summarized in Table 3. The agreement with structure NZ-O<sub>A</sub>O<sub>C</sub> is poor—the calculated absorbance spectrum contains oscillators at 810 and 909 cm<sup>-1</sup> that are not observed experimentally and lacks any intense oscillators from 950 to 1350 cm<sup>-1</sup>. Additionally, the relative intensity of the OH bend, calculated to occur at 1411 cm<sup>-1</sup>, is considerably greater than that of the corresponding band in the IRMPD spectrum. Therefore, the IRMPD spectrum is most consistent with structure NZ-NO<sub>A</sub>O<sub>C</sub>.

**Structures of Gln•Na<sup>+</sup> and Gln•K<sup>+</sup>.** The IRMPD spectra of Gln•Na<sup>+</sup> and Gln•K<sup>+</sup> contain many bands at frequencies close to those exhibited for Gln•Li<sup>+</sup>. For example, the IRMPD spectra of Gln•Na<sup>+</sup> and Gln•K<sup>+</sup> exhibit carbonyl stretches of carboxylic acid groups near 1755 and 1760 cm<sup>-1</sup>, respectively. These frequencies are slightly blue-shifted and have slightly greater

relative intensities than those of  $\text{Gln}\cdot\text{Li}^+$  ( $1723\text{ cm}^{-1}$ ). These frequencies for  $\text{Gln}\cdot\text{Na}^+$  and  $\text{Gln}\cdot\text{K}^+$  are closest to those calculated for structures  $\text{NZ}\cdot\text{O}_A\text{O}_C$  ( $1748\text{ cm}^{-1}$  for both ions); those for structures  $\text{NZ}\cdot\text{NO}_A\text{O}_C$  are slightly lower ( $1736$  and  $1743\text{ cm}^{-1}$ , respectively), and those for structures  $\text{NZ}\cdot\text{O}_A\text{OO}_C$  are slightly higher ( $1760$  and  $1772\text{ cm}^{-1}$ , respectively). In addition, the relative intensities of the carbonyl stretches of the carboxylic acid groups of  $\text{Gln}\cdot\text{Na}^+$  and  $\text{Gln}\cdot\text{K}^+$  are greater than those for the corresponding carbonyl stretches of the amide groups ( $1695\text{ cm}^{-1}$  for both ions). These relative intensity ratios are generally consistent with those calculated for structures  $\text{NZ}\cdot\text{NO}_A\text{O}_C$  and  $\text{NZ}\cdot\text{O}_A\text{O}_C$  of these ions, whereas those ratios are inverted for structures  $\text{NZ}\cdot\text{O}_A\text{OO}_C$ .

However, there are substantial spectral differences that indicate that additional structures contribute to the IRMPD spectra of these ions containing larger alkali metals. With increasing alkali-metal size, the IRMPD spectra of  $\text{Gln}\cdot\text{M}^+$  exhibit increased relative photodissociation from  $1250$  to  $1450\text{ cm}^{-1}$ . Additionally, photodissociation in this region evolves from a single sharp band at  $1425\text{ cm}^{-1}$  into at least two poorly resolved, broad bands. These spectral changes are most consistent with the presence of structures containing carboxylic acid groups that donate a hydrogen bond to the amino group, such as structures  $\text{NZ}\cdot\text{O}_A\text{O}_C$ ,  $\text{NZ}\cdot\text{O}_A\text{OO}_C$ , or  $\text{NZ}\cdot\text{OO}_C$ . These structures are all calculated to contain intense oscillators corresponding to the OH bend of the carboxylic acid group. For  $\text{Lys}\cdot\text{M}^+$ ,  $\text{M} = \text{Li}, \text{Na}, \text{and K}$ , the increasing relative intensity for the OH-bend band is a key spectral signature for growing populations of nonzwitterionic structures with intramolecular hydrogen bonds with increasing alkali-metal-ion size.<sup>6</sup> In addition, structures  $\text{NZ}\cdot\text{O}_A\text{O}_C$  and  $\text{NZ}\cdot\text{O}_A\text{OO}_C$  contain additional oscillators from  $800$  to  $1000\text{ cm}^{-1}$  that are consistent with the increased relative photodissociation observed at low frequencies. These modes correspond to the  $\text{NH}_2$  wag of the amino groups and the out-of-plane OH bend of the carboxylic acid groups.

These results indicate that multiple structures contribute to the observed photodissociation spectra. In all cases, these results are most consistent with Gln adopting nonzwitterionic structures. Structure  $\text{NZ}\cdot\text{NO}_A\text{O}_C$  is the predominant structure for ions containing smaller alkali-metal ions, perhaps exclusively so for  $\text{M} = \text{Li}$ , and structures in which the carboxylic acid group donates a hydrogen bond to the amino group are increasingly populated with increasing alkali-metal-ion size. Because of spectral congestion and other factors, the specific structures contributing to the later class of nonzwitterionic structure is ambiguous, but the new spectral features are consistent with populations of ions of structure  $\text{NZ}\cdot\text{O}_A\text{O}_C$  and, to a somewhat lesser extent, of structure  $\text{NZ}\cdot\text{O}_A\text{OO}_C$ .

**Structure of  $\text{Gln}\cdot\text{Cs}^+$ .** The carbonyl stretch of the carboxylic acid group ( $1765\text{ cm}^{-1}$ ) of  $\text{Gln}\cdot\text{Cs}^+$  is significantly less intense than that of the band at slightly lower frequency ( $1690\text{ cm}^{-1}$ ), which is assigned solely to the carbonyl stretch of the amide groups for the ions containing smaller alkali-metal ions. This ratio is significantly different than those observed for  $\text{M} = \text{Li}, \text{Na}, \text{and K}$ , suggesting that the structure of  $\text{Gln}\cdot\text{Cs}^+$  differs from those of the other ions. Although subtle differences in the relative intensities of bands in IRMPD spectra are generally considered less reliable than the band frequencies for structural assignment because of nonlinear effects of IRMPD, a comparison of the IRMPD and matrix isolation spectra of polycyclic aromatic hydrocarbon cations indicates that relative band intensities resulting from IRMPD can be in fair agreement with those from linear absorbance.<sup>39</sup> Although dissociation energies

for  $\text{M} = \text{Cs}$  are likely the smallest of all these ions, loss of  $\text{H}_2\text{O}$  is the predominant fragmentation pathway for both  $\text{M} = \text{K}$  and  $\text{Cs}$  and suggests comparable dissociation energies for both ions. Therefore, it is likely that the observed differences in photodissociation efficiencies for these Gln-containing ions reflect structural differences between these ions.

A population of ions in which Gln is zwitterionic would engender a change in relative intensity for the two highest frequency bands in the IRMPD spectrum—such ions would not exhibit a carbonyl stretch band for a carboxylic acid group but would have a carbonyl stretch band for an amide group. The spectrum of structure  $\text{ZW}\cdot\text{OO}_C$  has no modes in this region higher than  $1685\text{ cm}^{-1}$  but does have an intense carboxylate asymmetric stretch at  $1658\text{ cm}^{-1}$  that is only slightly to the red of that calculated for the carbonyl stretch of the amide group  $1681\text{ cm}^{-1}$ . However, this structure is also calculated to have an intense protonated amino umbrella bend ( $1477\text{ cm}^{-1}$ ), a frequency at which essentially no IRMPD intensity is observed. This mode, which is a spectral signature for ions containing zwitterionic amino acids, was central to the assignment of tryptophan complexed with divalent barium to a zwitterionic structure.<sup>18</sup> The lack of photodissociation in this region ostensibly limits the possibility that zwitterionic structures are present under the conditions of the experiment.

Alternatively, the change in relative intensity for the two highest frequency bands in the IRMPD spectrum may be attributable to the presence of additional nonzwitterionic structures. For example, the carbonyl stretch of the carboxylic acid group of structure  $\text{NZ}\cdot\text{O}_A\text{OO}_C$  is calculated to be less intense than the corresponding carbonyl stretch of the amide group, whereas the opposite is true for the remaining nonzwitterionic structures. The calculated spectrum of  $\text{NZ}\cdot\text{O}_A\text{OO}_C$  is also consistent with the broad IRMPD band near  $1380\text{ cm}^{-1}$  and the weaker bands below  $1000\text{ cm}^{-1}$ . However, the IRMPD spectrum of  $\text{Gln}\cdot\text{Cs}^+$  also exhibits bands near  $1110$  and  $1140\text{ cm}^{-1}$ , a region where the calculated spectrum of  $\text{NZ}\cdot\text{O}_A\text{OO}_C$  has only very weak modes. These IRMPD bands suggest the presence of additional structures, such as  $\text{NZ}\cdot\text{NO}_A\text{O}_C$ .

**Effect of Proton Affinity on Zwitterion Stability.** Post-translational modification can affect the binding properties and activity of biomolecules in vivo. The effects of three post-translational modifications, N-terminal methylation, NMeGln, amide ethylation, Gln(Et), and amide iso-propylation, Gln(*i*P), on the structures of the isolated, protonated, and sodiated amino acids were studied here by using computational chemistry. Because the stability of the zwitterionic form relative to the nonzwitterionic form of selected sodiated amino acids with aliphatic side chains is directly related to the proton affinity of the amino acid,<sup>1,2</sup> it is interesting to consider whether these differences in proton affinity result in different lowest-energy structures for the modified amino acids. The conformation spaces of the Gln derivatives complexed with sodium were explored by using the same methodology used for sodiated Gln, and these results are summarized in Table 4.

Because the PAs of the Gln derivatives have not been reported, these values were calculated by using methods reported previously<sup>41</sup> and are given in Table 4. This methodology was also used to calculate the PA of Gln, and these values are consistent with many values reported in the literature. For example, Bleiholder et al. reported 0 K PAs of  $970$  and  $964\text{ kJ/mol}$  determined by using B3LYP and G2(MP2) calculations,<sup>42</sup> whereas our B3LYP and MP2 calculated 0 K PAs for Gln are  $971$  and  $962\text{ kJ/mol}$ , respectively. Experimental values between  $933$  and  $988\text{ kJ/mol}$  have been reported.<sup>43–45</sup>

**TABLE 4: Proton Affinities (PA) of the Isolated Amino Acids and Relative Free Energies of the Sodiated Amino Acids at 298 K in kJ/mol**

	Gln	Gln(Et) B3LYP <sup>b</sup>	Gln( <i>i</i> P)	NMeGln
	PA (298 K)			
	978	991	991	999
<b>NZ<sup>a</sup></b>				
<b>NO<sub>A</sub>O<sub>C</sub></b>	<b>0</b>	<b>0</b>	<b>0</b>	9.8
<b>O<sub>A</sub>O<sub>C</sub></b>	14.2	13.3	12.1	19.0
<b>O<sub>A</sub>OO<sub>C</sub></b>	16.3	16.5	19.3	25.7
<b>ZW</b>				
<b>OO<sub>C</sub></b>	8.2	7.0	5.7	<b>0.0</b>
<b>O<sub>A</sub>OO<sub>C</sub></b>	33.9	42.3	40.7	21.1
	MP2 <sup>c</sup>			
	PA (298 K)			
	969	982	981	989
<b>NZ<sup>a</sup></b>				
<b>NO<sub>A</sub>O<sub>C</sub></b>	<b>0</b>	<b>0</b>	<b>0</b>	11.1
<b>O<sub>A</sub>O<sub>C</sub></b>	17.6	18.9	14.9	24.0
<b>O<sub>A</sub>OO<sub>C</sub></b>	13.9	15.3	16.8	16.1
<b>ZW</b>				
<b>OO<sub>C</sub></b>	8.8	9.6	6.1	<b>0.0</b>
<b>O<sub>A</sub>OO<sub>C</sub></b>	29.3	38.6	35.9	16.8

<sup>a</sup> NZ-OO<sub>C</sub> energy minimized to ZW-OO<sub>C</sub> for all ions.

<sup>b</sup> Calculated at the B3LYP/6-311++G(2d,2p)//6-31++G(d,p) level of theory. <sup>c</sup> Calculated at the MP2/6-311++G(2d,2p)//B3LYP/6-31++G(d,p) level of theory.

The PA of NMeGln is 20–21 kJ/mol greater than that of Gln. The lowest-energy zwitterionic form of Gln•Na<sup>+</sup> is 8–9 kJ/mol higher in free energy than the lowest-energy nonzwitterionic form, whereas the lowest-energy zwitterionic form of NMeGln•Na<sup>+</sup> is 10–11 kJ/mol lower in free energy than the lowest-energy nonzwitterionic form. The zwitterionic form of the derivative is preferentially stabilized by 18–20 kJ/mol relative to that for Gln•Na<sup>+</sup>, a value that is almost identical to the difference in PAs of the two amino acids. These results indicate that the relationship between PA and relative zwitterion stability for Gln•Na<sup>+</sup> and NMeGln•Na<sup>+</sup> is essentially identical to that established for selected aliphatic amino acids.<sup>1,2</sup>

The PAs of the Gln derivatives with alkylated amide groups are 13–15 kJ/mol greater than that of Gln. The zwitterionic forms of Gln(Et)•Na<sup>+</sup> and Gln(*i*P)•Na<sup>+</sup> are 6–10 kJ/mol higher in free energy than the lowest-energy nonzwitterionic forms. The zwitterionic forms of Gln(Et)•Na<sup>+</sup> and Gln(*i*P)•Na<sup>+</sup> are preferentially stabilized by less than 3 kJ/mol relative to that for Gln•Na<sup>+</sup>. Interestingly, the nonzwitterionic form of Gln(Et)•Na<sup>+</sup> is preferentially stabilized by 1 kJ/mol relative to Gln•Na<sup>+</sup> at the MP2 level of theory, although that difference is certainly within the uncertainty of the calculations. In contrast to the results established for selected aliphatic amino acids,<sup>1,2</sup> these results indicate that effects of PA on relative zwitterion stability for Gln(Et)•Na<sup>+</sup> and Gln(*i*P)•Na<sup>+</sup> are competitive with other effects, such as solvation of the metal ion.<sup>46</sup> Thus, the relationship between proton affinity and zwitterion stability is more difficult to predict for amino acids or their derivatives that contain heteroatoms in their side chains.

## Conclusions

IRMPD spectra in the 800–1820 cm<sup>-1</sup> region provide useful information about the structure of alkali-metal cationized Gln.

These IRMPD spectra contain many similarities, and the persistent carbonyl stretch band at high frequency indicates that Gln is nonzwitterionic, but differences in the spectra indicate that the metal-ion binding motif depends on metal-ion identity. For ions containing small alkali metals, the metal ion is solvated predominantly by the amino group, the carbonyl oxygen of the carboxylic acid group, and the carbonyl oxygen of the amide group. With increasing alkali-metal-ion size, additional structures in which the carboxylic acid donates a hydrogen bond to the amino group and the metal ion is solvated only by the amide and carboxylic acid groups are preferentially stabilized. These results are consistent with the changes in metal-ion coordination and hydrogen-bond formation observed with increasing metal-ion size for alkali-metal cationized Arg,<sup>4,5,32</sup> Lys,<sup>6</sup> serine,<sup>19</sup> threonine,<sup>20</sup> and tryptophan.<sup>16</sup>

Effects of metal-ion size on the relative energies of different structures at the B3LYP and MP2 levels of theory are similar, except that nonzwitterionic structures are systematically favored relative to zwitterionic structures at the MP2 level of theory, and those calculations are thus more consistent with the experimental results. However, for complexes containing all but the smallest alkali-metal ion, lithium, both levels of theory indicate that both zwitterionic and nonzwitterionic structures are within 10 kJ/mol of free energy of the lowest-energy structure.

Methylation of the amino group increases the proton affinity and preferentially stabilizes the zwitterionic form of the sodiated amino acid by roughly 20 kJ/mol. In contrast, ethylation and isopropylation of the amide group increases the proton affinity by roughly 13 kJ/mol but preferentially stabilizes the zwitterionic form of the sodiated amino acid by less than 3 kJ/mol. These results indicate that making structural predictions based on proton affinity is challenging, even for closely related molecules, and that the effects of proton affinity on relative zwitterion stability must be considered in conjunction with effects of metal-ion solvation, hydrogen bonding, and other factors.

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