Infrared Spectroscopy of Cationized Lysine and ϵ-*N*-methyllysine in the Gas Phase: Effects of Alkali-Metal Ion Size and Proton Affinity on Zwitterion Stability

Matthew F. Bush,[†] Matthew W. Forbes,[‡] Rebecca A. Jockusch,[‡] Jos Oomens,[§] Nick C. Polfer,[§] Richard J. Saykally,[†] and Evan R. Williams*,[†]

Department of Chemistry, University of California, Berkeley, California 94720-1460, Department of Chemistry, University of Toronto, Ontario M5S 3H6, Canada, and FOM Institute for Plasma Physics "Rijnhuizen", Edisonbaan 14, 3439 MN Nieuwegein, The Netherlands

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The gas-phase structures of protonated and alkali-metal-cationized lysine (Lys) and ϵ-*N*-methyllysine (Lys(Me)) are investigated using infrared multiple photon dissociation (IRMPD) spectroscopy utilizing light generated by a free electron laser, in conjunction with ab initio calculations. IRMPD spectra of Lys·Li^+ and Lys·Na^+ are similar, but the spectrum for Lys·K^+ is different, indicating that the structure of lysine in these complexes depends on the metal ion size. The carbonyl stretch of a carboxylic acid group is clearly observed in each of these spectra, indicating that lysine is nonzwitterionic in these complexes. A detailed comparison of these spectra to those calculated for candidate low-energy structures indicates that the bonding motif for the metal ion changes from tricoordinated for Li and Na to dicoordinated for K, clearly revealing the increased importance of hydrogen-bonding relative to metal ion solvation with increasing metal ion size. Spectra for Lys(Me)·M^+ show that Lys(Me), an analogue of lysine whose side chain contains a secondary amine, is nonzwitterionic with Li and zwitterionic with K and both forms are present for Na. The proton affinity of Lys(Me) is 16 kJ/mol higher than that of Lys; the higher proton affinity of a secondary amine can result in its preferential protonation and stabilization of the zwitterionic form.

Introduction

Amino acid structure depends on both the side-chain functionality and the surrounding environment. Basic amino acids, such as arginine and lysine, often participate in intra- and intermolecular salt bridges with acidic residues. Whether these ionic interactions result in the stabilization of a conformer or complex depends on the solvent accessibility of these residues, the identity and position of nearby residues, and other factors. An important advantage of gas-phase measurements is that effects of individual interactions, whether with water molecules, other residues, or metal cations, can be measured separately to deduce the relative contributions of each of these interactions. Although none of the naturally occurring amino acids are zwitterionic in isolation, zwitterionic forms can be preferentially stabilized by cations or by water molecules. Extensive work has been done to investigate the effects of cations on the stability of amino acid zwitterions.[1−10] Divalent ions can be more effective at stabilizing the zwitterionic form of amino acids.[1−6] The stability of the zwitterionic (ZW) form relative to the nonzwitterionic (NZ) form of selected sodiated amino acids with aliphatic side chains is directly related to the proton affinity of the amino acid.[7,8] However, the effect of proton affinity can be far less direct for amino acids with basic side chains; heteroatoms in the side chain can also solvate the metal ion and substantially stabilize the nonzwitterionic form of the amino acid.[9−12,34]

Arginine and lysine are the two most basic naturally occurring amino acids.[35] On the basis of their proton affinities, the sodiated forms of these amino acids were predicted to be zwitterionic.[7] For alkali-metal-cationized arginine, low-energy dissociation experiments,[9] kinetic method experiments,[16,17] and infrared multiple photon dissociation (IRMPD) action spectroscopy experiments[10] indicate that the structure of arginine depends on the metal ion size. Results from the latter experiments provide detailed information about the structure and show that cationized arginine is predominantly nonzwitterionic with Li, but predominately zwitterionic with Na and K.[10]

Lysine (Lys) has the second highest proton affinity of the naturally occurring amino acids.[35] Kinetic method experiments, in which cationized dimers consisting of Lys and lysine methyl ester were dissociated, indicate that Lys·M^+, M = Li, Na, and K, is nonzwitterionic.[17] A complicating factor in deducing structural information from these experiments is that the structure of the amino acid in a cationized heterodimer may not be the same as that of the isolated, cationized amino acid. For example, analogous experiments indicate that lithiated proline is nonzwitterionic,[17] but results from ab initio calculations[17−20] and binding energies determined using guided ion beam (GIB)[21,36] and blackbody infrared radiative dissociation (BIRD)[18] experiments indicate that the zwitterionic form is most stable. Calculations indicate that the lowest energy forms of Lys·Li^+ and Lys·Ag^+ are nonzwitterionic.[11,26] Water binding energies to singly hydrated lithiated Lys, ϵ-*N*-methyllysine (Lys(Me)), and additional structural analogues indicate that the amino acids in these complexes are nonzwitterionic, although detailed structural information could not be deduced from these experiments alone.[11] The side chain of Lys(Me) contains a secondary

* To whom correspondence should be addressed Fax: (510) 642-7714. E-mail: williams@cchem.berkeley.edu.
[†] University of California.
[‡] University of Toronto.
[§] FOM Institute for Plasma Physics "Rijnhuizen".

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amine, and the calculated proton affinity of isolated Lys(Me) is 16 kJ/mol higher than that of Lys.[11]

IRMPD action spectroscopy, using light from either free electron lasers (typically 5−10 μm)[28−31,37−39] or benchtop laser systems based on nonlinear frequency conversion (typically 2.5−4 μm),[10,27,32,40,41] has emerged as a powerful method to probe ion structure in the gas phase. Kapota et al. reported signatures for the nonzwitterionic and zwitterionic forms of cationized aliphatic amino acids in the 5−10 μm region based on the spectra of sodiated glycine and proline, respectively.[31] Polfer et al. have investigated the structures of selected cationized amino acids with aromatic side chains.[28−30] The amino acids in these complexes are nonzwitterionic, and the metal ion is typically coordinated with the N-terminal amino group, the carbonyl oxygen, and the aromatic ring.[28−30] For cationized tryptophan, a second nonzwitterionic population in which the metal ion is coordinated to the carbonyl oxygen and the aromatic ring, and in which the N-terminal amino group accepts a hydrogen bond from the carboxylic acid, was also observed for complexes containing larger metal ions.[28] These results for cationized aromatic amino acids are consistent with calculations[23,25] and GIB experiments.[23]

Here, we report IRMPD spectra of Lys·M^+ and Lys(Me)·M^+, M = H, Li, Na, and K. By comparing these measured spectra to those calculated for low-energy structures, detailed information about the structures of these ions is obtained. These results show that the propensity of these ions to form a zwitterionic structure depends explicitly on the metal ion size and on the proton affinity of the protonation site.

Methods

Mass Spectrometry and Photodissociation. Experiments were performed using a 4.7 T Fourier-transform ion cyclotron resonance mass spectrometer. The instrument[42] and general experimental methods[29] are described elsewhere. The amino acids Lys and Lys(Me) were obtained from Aldrich Chemical Co. (St. Louis, MO) and Bachem AG (Bubendorf, Switzerland), respectively. Cationized amino acids were formed by electrospray ionization from a solution of 1 mM amino acid and 1 mM alkali-metal chloride or hydroxide in 80:20 MeOH/H_2O using solution flow rates ranging from 15 to 40 μL/min. Tunable radiation for the photodissociation experiments is generated by a free electron laser for infrared experiments (FELIX).[43] Due to a broken shutter, ions were continuously irradiated in the ion cell, which included 2 s prior to precursor ion isolation and 3 s prior to ion detection.

Computational Chemistry. Low-energy structures of Lys·M^+ and Lys(Me)·M^+, M = H, Na, and K, were generated by Monte Carlo conformational searching with the MMFF94 force field using MacroModel 9.1 (Schrödinger, Inc., Portland, OR). For the initial search, no constraints were placed on the geometry, and 5000 structures of each ion were generated. Additional structures were generated through further conformation searching and modifying previously generated structures. The resulting low-energy structures were grouped into families with similar noncovalent interactions. Representative structures from each family were energy-minimized using hybrid method density functional calculations (B3LYP) performed using Jaguar v. 6.5 (Schrödinger, Inc., Portland, OR). Structures were fully optimized using the 6-31G* and 6-31++G** basis sets, and the corresponding LACVP effective core potentials were used for potassium. Low-energy structures for Lys·Li^+ were obtained using similar methods and have been reported previously.[11] Structures A0, B0, C0, D0, E0, F0, and G0 in the previous study[11] correspond to $N_{SC}N_TO$-NZ, $N_{SC}O$-NZ, N_TO-NZ, OO^{α}-ZW, OO^{β}-ZW, N_TO-ZW, $N_{SC}OO$-ZW in this study. Note that previous attempts to energy minimize N_TO-NZ structures of Lys·Li^+ all resulted in isomerization to N_TO-ZW;[11] here a stable N_TO-NZ structure of Lys·Li^+ was found by substituting the metal ion in the N_TO-NZ structure found for Lys·Na^+.

TABLE 1: Product Ions Observed from Photodissociation of Lys·M^+ and Lys(Me)·M^+, M = Li, Na, K, and H

	Li	Na	K	H
Lys·M^+	$-NH_3$, $-H_2O$	$-NH_3$, $-H_2O$	K^+	$-NH_3$
Lys(Me)·M^+	$-H_2O$, $-H_3CNH_2$	$-NH_3$, $-H_2O$, $-H_3CNH_2$	K^+	$-H_3CNH_2$

Vibrational frequencies and intensities were calculated using the harmonic oscillator approximation and numerical derivatives of the 6-31++G** energy-minimized Hessian. All frequencies were scaled by 0.975. This factor was reported to provide good agreement between IRMPD action spectra and calculations done at a similar level of theory.[28] The calculated vibrational frequencies were positive for all minimized structures, indicating that these geometries are all local-minimum structures. In the calculated absorbance spectra, all oscillators were broadened using 20 cm^{-1} fwhm Gaussian distributions.

Results and Discussion

IRMPD Action Spectroscopy. Photodissociation of Lys·M^+ and Lys(Me)·M^+, M = H, Li, Na, and K, results in the loss of small neutral molecules or the metal ion depending on the complex (Table 1). For M = Li and Na, two or more competing fragmentation pathways are observed. No significant effect of the laser frequency on the branching ratio of the fragmentation pathways was observed over the range of wavelengths investigated. IRMPD action spectra are obtained from the sum of the relative intensity of the fragments, are corrected for laser power,[29,37,38] and are shown in Figure 1.

Low-Energy Structures. The nitrogen atom on the side chain of Lys·M^+ or Lys(Me)·M^+, M = Li, Na, and K, can accept a proton, form hydrogen bonds with other heteroatoms, or solvate the metal cation. The large number of possible interactions involving the side chain results in many possible NZ and ZW structures that are close in energy. These structures have been grouped into families and are discussed in detail hereafter. Note that the low-energy structures of Lys(Me)·M^+ are similar to those identified for Lys·M^+ (Figure 2), although the relative energies of these structures differ (Table 2).

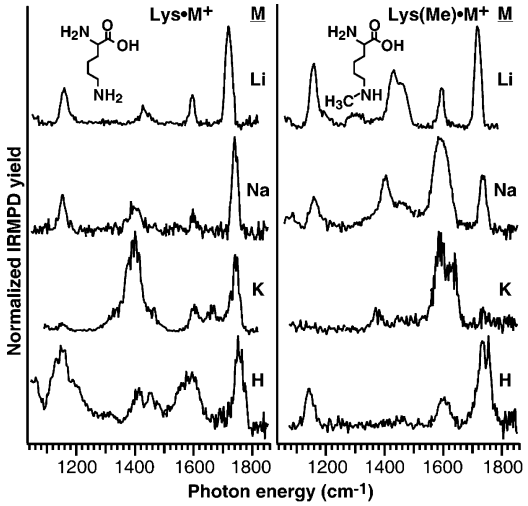

Figure 1. IRMPD action spectra of Lys·M^+ and Lys(Me)·M^+, M = Li, Na, K, and H.

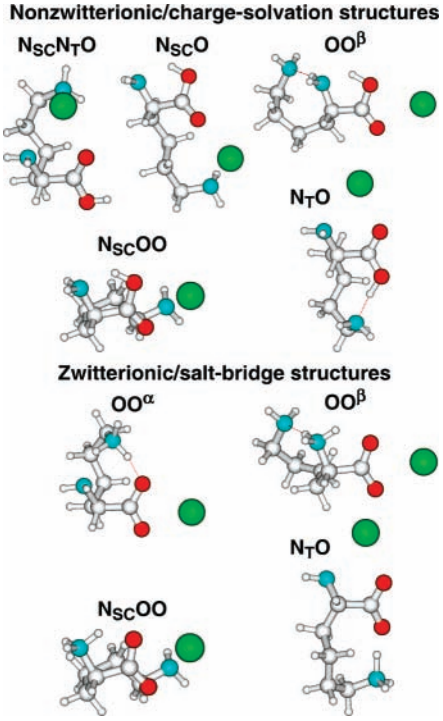

Figure 2. Energy-minimized structures of low-energy nonzwitterionic and zwitterionic conformers of Lys·K^+ from B3LYP/6-31++G** calculations. Energy-minimized structures of Lys·Li^+ have been reported previously.[11]

In the lowest energy nonzwitterionic structures of Lys·M^+ and Lys(Me)·M^+, M = Li and Na, the metal ion is tricoordinated and interacts with both amino groups and an oxygen atom of the carboxylic acid ($N_{SC}N_TO$-NZ). This structure is similar to that reported for the lowest energy nonzwitterionic forms of Gln·M^+, M = Li and Na,[34] and Arg·M^+, M = Li, Na, and K.[10] The analogous structures in which the hydroxyl oxygen of the carboxylic acid group solvates the metal ion instead of the carbonyl oxygen is 30 kJ/mol higher in energy than structure $N_{SC}N_TO$-NZ of Arg·Na^+. The metal ion in the lowest energy nonzwitterionic structures of Lys·K^+ and Lys(Me)·K^+ is dicoordinated with two heteroatoms, the side-chain nitrogen and one oxygen of the carboxylic acid group ($N_{SC}O$-NZ). In this structure, the carboxylic acid donates a hydrogen bond to the N-terminal amino group. The analogous structure in which the N-terminal amino group donates a hydrogen bond to the carboxylic acid group instead of the converse (structure $N_{SC}O$-NZ) is 21 kJ/mol higher in energy for Lys·K^+. The binding energy to the metal ion decreases with increasing alkali-metal ion size, resulting in a greater relative contribution of a hydrogen bond to the stability of these ions. The N_TO-NZ structure, in which the metal ion is coordinated similarly to that in the lowest energy form of aliphatic amino acids with small metal ions,[1,7,13,14] is higher in energy. For Lys(Me)·Li^+ and Lys(Me)·Na^+, this structure is not stable and minimized to the zwitterionic form with similar metal ion binding (N_TO-ZW). Some other nonzwitterionic structures also minimized to the corresponding zwitterionic structures (Table 2).

The lowest energy zwitterionic forms of Lys·Li^+ and Lys(Me)·Li^+ are similar to N_TO-NZ, but the acidic proton of the carboxylic acid is localized on the side-chain amino nitrogen, resulting in a metal ion/carboxylate/protonated amine salt bridge (N_TO-ZW). The metal ion in the lowest energy zwitterionic forms of Lys·Na^+ and Lys·K^+ is OO-coordinated, and the protonated N-terminal amino group donates a hydrogen bond to the side-chain amino group (OO^{β}-ZW). The lowest energy zwitterionic form of Lys(Me)·Na^+ and Lys(Me)·K^+ has the same metal ion coordination, but the side chain is protonated and donates hydrogen bonds to both the N-terminal amino group and one oxygen atom of the carboxylate group (OO^{α}-ZW). Both OO-coordinated structures are stable for all complexes. The OO^{α}-ZW structures are favored over the corresponding OO^{β}-ZW structures for Lys(Me)·M^+ at 298 K, whereas the converse is true for Lys·M^+, consistent with the higher proton affinity of a secondary amine. Zwitterionic structures with $N_{SC}OO$-coordinated metal ions are also stable ($N_{SC}OO$-ZW), but the nonzwitterionic forms of this structure are more stable for the potassiated complexes.

The side-chain amino group (N_{SC}) is the preferred site of protonation for both Lys·H^+ and Lys(Me)·H^+ (Figure 3) and is favored over the N-terminal amino group ($N_T \rightarrow N_{SC}$) by 9 and 16 kJ/mol for Lys·H^+ and Lys(Me)·H^+ at 298 K, respectively. N_{SC} can donate hydrogen bonds to the N-terminal amino group ($N_{SC} \rightarrow N_T$), the carbonyl oxygen ($N_{SC} \rightarrow O$), or both acceptor sites ($N_{SC} \rightarrow N_TO$). The $N_T \rightarrow N_{SC}$ structures of Lys(Me)·H^+ that were evaluated energy minimized to $N_{SC} \rightarrow N_T$ at the B3LYP/6-31++G** level of theory, indicating a strong preference for protonation of the secondary amine. $N_{SC} \rightarrow N_TO$ was found to be lowest in energy for both ions, consistent with previous reports on the structures of these ions.[11,35,44] However, the energies of the higher energy structures are all close to that of $N_{SC} \rightarrow N_TO$ at this level of theory.

Gibbs free energies were calculated at both 0 and 298 K; the latter value corresponds to the approximate starting temperature of the ions in this experiment. The effects of temperature on the relative energies of these structures are relatively small. The lowest energy structures are the same at both temperatures, and the relative energies differ by an average and maximum of 1.4 and 4.8 kJ/mol, respectively. These differences are likely within the uncertainties of the calculations, and only the 298 K free energies are discussed subsequently.

Lys·M^+, M = Li, Na, and K. The IRMPD action spectra of lithiated and sodiated lysine are similar, whereas the spectrum for potassiated lysine differs significantly (Figure 1). This indicates that the structures of these complexes depend on the metal ion size. The most intense band in the spectrum of Lys·Li^+ is at 1720 cm^{-1}, and a weaker band is observed at 1595 cm^{-1}. These bands are assigned to the carbonyl stretch of the carboxylic acid group and the NH_2 scissor bends, respectively, on the basis of spectral assignments made for other metal ion amino acid complexes[28−31] and our calculated absorption spectra for the nonzwitterionic structures that have these modes ($N_{SC}O$-NZ and $N_{SC}N_TO$-NZ, Figure 4). These results strongly indicate that lysine is nonzwitterionic in this complex. The nonzwitterionic structure N_TO-NZ also has a carboxylic acid group, but the acidic hydrogen in this structure is hydrogen bonded to the side-chain amino group, which results in a significant red shift of the carboxylic acid OH stretch to 1832 cm^{-1} and of the carbonyl stretch to 1687 cm^{-1}. Some of the other calculated spectra also contain bands from hydrogen stretches or bands that are strongly coupled to hydrogen stretches. These bands are indicated by an asterisk in Figure 4 and correspond to significantly red-shifted modes of strongly bonded NH_3 or OH groups. The potential wells for these vibrational modes are very sensitive to minor perturbations to the ion structure. In many cases, the corresponding vibrational modes for slightly higher energy structures in the same structural families were considerably to the blue and outside of the measured spectral range. Although these bands are calculated to have high integrated oscillator strengths, the absence of these

TABLE 2: Relative Gibbs Free Energies (kJ/mol) for the NZ and ZW Conformers of Lys·M$^+$ and Lys(Me)·M$^+$, M = Li, Na, and K, at T = 0 and 298 K[a]

			Lys·M$^+$			Lys(Me)·M$^+$		
			Li	Na	K	Li	Na	K
0 K	NZ	$N_{SC}N_TO$	0	0	8.0	0	6.8	18.9
		$N_{SC}O$	19.4	5.5	4.0	20.5	12.1	14.0
		N_TO	29.4	16.1	11.9	*b*	*b*	21.0
		$N_{SC}OO$	*b*	19.7	5.3	*b*	*b*	14.7
		OO^β	*b*	*b*	17.9	*b*	*b*	26.1
	ZW	OO^α	34.5	9.3	4.6	18.3	0	0
		OO^β	34.9	5.5	0	29.6	6.1	5.0
		N_TO	29.9	25.3	24.9	12.8	12.9	22.2
		$N_{SC}OO$	36.9	21.0	22.7	35.8	26.5	31.2
	E(ZW−NZ)		29.9	5.5	−4.0	12.8	−6.8	−14.0
298 K	NZ	$N_{SC}N_TO$	0	0	8.0	0	6.4	16.5
		$N_{SC}O$	16.3	4.1	2.6	17.5	11.5	11.8
		N_TO	30.4	17.7	12.2	*b*	*b*	20.9
		$N_{SC}OO$	*b*	18.7	5.0	*b*	*b*	12.9
		OO^β	*b*	*b*	14.5	*b*	*b*	21.3
	ZW	OO^α	34.0	10.6	5.7	19.2	0	0
		OO^β	31.7	3.4	0	26.3	3.1	3.7
		N_TO	29.5	26.3	25.1	12.7	12.6	21.3
		$N_{SC}OO$	38.3	22.9	24.2	38.2	28.1	31.3
	E(ZW−NZ)		29.5	3.4	−2.6	12.7	−6.4	−11.8

[a] All values are from B3LYP/6-31++G** calculations (the LACVP effective core potential used for K) and include zero-point energy corrections. [b] These NZ forms energy minimized to the corresponding ZW forms.

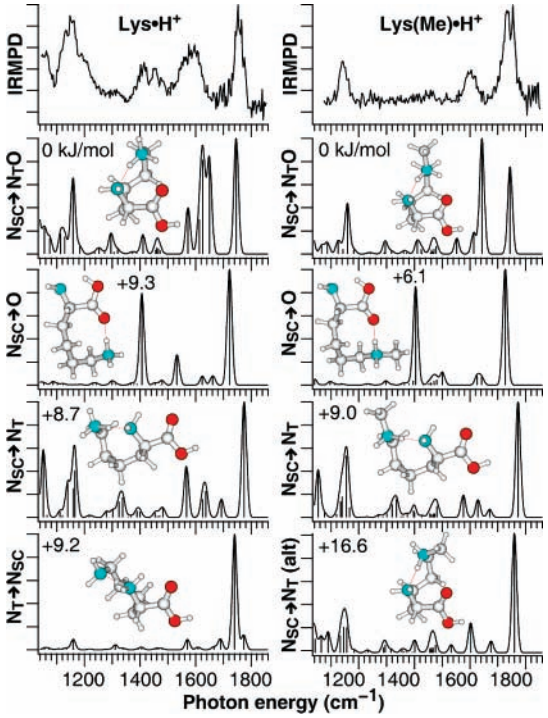

Figure 3. IRMPD spectra and B3LYP/6-31++G**-calculated absorption spectra for candidate conformers of Lys·H$^+$ and Lys(Me)·H$^+$. Structure names are based on (proton-accepting group) → (hydrogen bond acceptors from the proton-accepting group). A higher energy N_{SC} → N_T structure (N_{SC} → N_T(alt)) is provided for comparison. Gibbs free energies at 298 K are in kilojoules per mole and are reported relative to that of the lowest energy structure for each complex.

bands in an IRMPD spectrum may be attributable to an artifact of the harmonic oscillator approximation used in these calculations, making it difficult to infer information about structure from the absence of these bands.

The two bands near 1600 and 1720 cm^{-1} for Lys·Li$^+$ persist in the spectra of Lys·Na$^+$ and Lys·K$^+$, although the carboxylic acid carbonyl stretches are noticeably blue-shifted with increasing metal ion size. This shift in frequency with metal ion size is also clearly indicated from the calculated spectra of the two lowest energy nonzwitterionic structures. By comparison to the spectra calculated for the nonzwitterionic structures for Lys·Li$^+$ and Lys·Na$^+$, the experimental spectra of these two ions most closely resemble those for $N_{SC}N_TO$-NZ. Although we cannot rule out the presence of other structures, the very good match suggests that the abundance of any other structure is likely minor.

The persistence of the carboxylic acid carbonyl stretch band at ~1740 cm^{-1} in the spectrum of Lys·K$^+$ indicates that this complex is predominately nonzwitterionic, whereas significant differences between this spectrum and those obtained for Lys·Li$^+$ and Lys·Na$^+$ suggest differences in structure. Notably, the most intense band in the spectrum of Lys·K$^+$ is at 1400 cm^{-1}, which is similar to that observed previously for Trp·M$^+$, M = K, Rb, and Cs; this band was assigned to the OH bend of a carboxylic acid that is hydrogen bonded to the N-terminal amino group.[28] The spectrum does not match that for $N_{SC}N_TO$-NZ, indicating that the structure of this complex is different from that of Lys·Li$^+$ or Lys·Na$^+$. The remaining nonzwitterionic structures generally resemble the measured spectrum of Lys·K$^+$; they all contain strong bands near 1400 cm^{-1} (OH bend from bonded carboxylic acid) and 1740 cm^{-1} (carbonyl stretch of a carboxylic acid). There is a small feature at ~1660 cm^{-1} that is not present in the spectra for Lys·Li$^+$ or Lys·Na$^+$ nor in the calculated spectra of the four lowest energy nonzwitterionic forms of Lys·K$^+$. OO^β-NZ has NH_2 scissor bends at 1621 and 1664 cm^{-1}, whereas these modes for the remaining four nonzwitterionic structures of Lys·K$^+$ range from 1616 to 1629 cm^{-1}. For comparison, the NH_2 scissor bends in the IRMPD spectra of Lys·Li$^+$ and Lys·Na$^+$ appear as a single band at 1600 cm^{-1}. This may indicate the presence of OO^β-NZ, despite this structure having a calculated free energy 14 kJ/mol higher than that of the lowest energy nonzwitterionic structure ($N_{SC}O$-NZ). Alternately, the feature at ~1660 cm^{-1} may correspond to a carboxylate asymmetric stretch; this frequency is slightly red-shifted from the band observed for

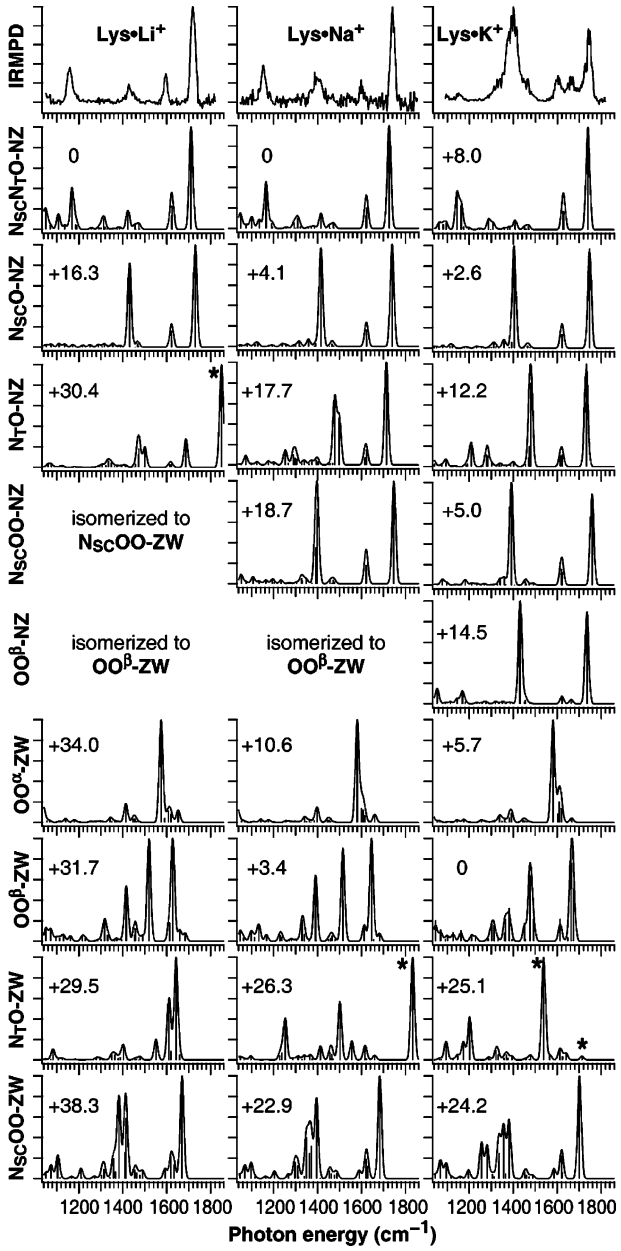

Figure 4. IRMPD spectra and B3LYP/6-31++G**-calculated absorption spectra for candidate conformers of Lys·M$^+$, M = Li, Na, and K. Bands marked by an asterisk are hydrogen stretches or are significantly coupled to hydrogen stretches. Gibbs free energies at 298 K are in kilojoules per mole and are reported relative to that of the lowest energy structure for each complex.

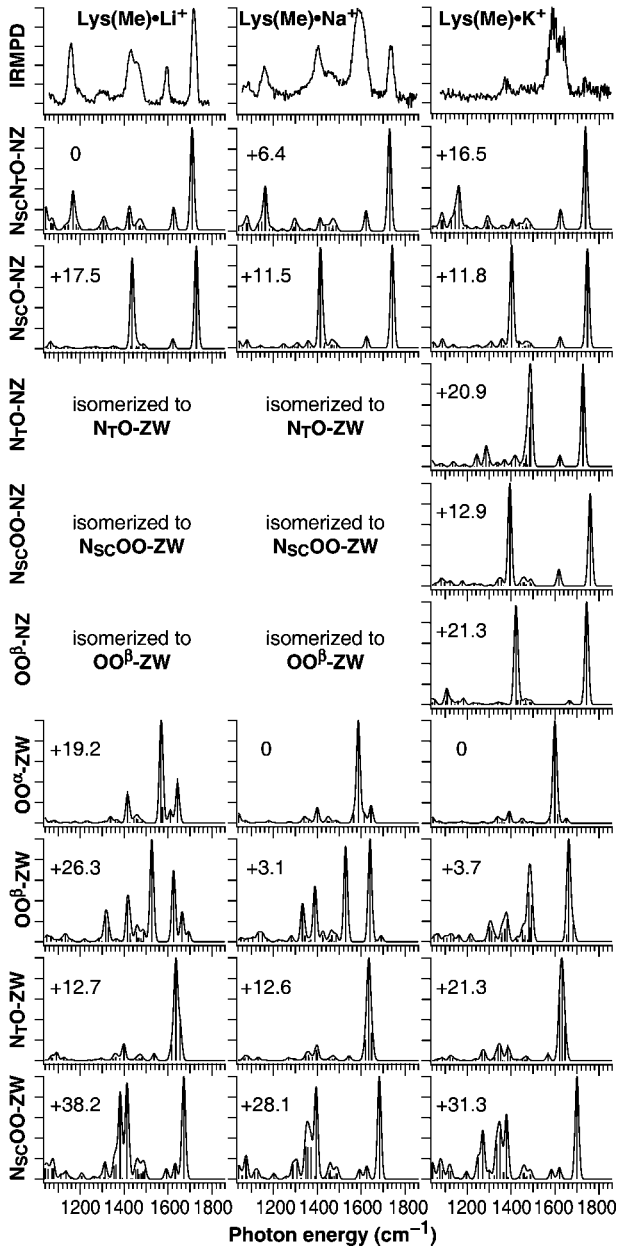

Figure 5. IRMPD spectra and B3LYP/6-31++G**-calculated absorption spectra for candidate conformers of Lys(Me)·M$^+$, M = Li, Na, and K. Gibbs free energies at 298 K are in kilojoules per mole and are reported relative to that of the lowest energy structure for each complex.

proline·Na$^+$ (1698 cm^{-1}).[31] The lowest energy zwitterionic structure (OO$^\beta$-ZW), which is calculated to be the global minimum, has the carboxylate asymmetric stretch at this frequency and another large band at 1477 cm^{-1} corresponding to the bonded NH bend of the protonated amino group. A band at this latter frequency is also observed in the measured spectrum. These results suggest that the lysine in Lys·K$^+$ is predominantly nonzwitterionic, but some population of the zwitterionic form OO$^\beta$-ZW may also be present.

Lys(Me)·M$^+$, M = Li, Na, and K. The IRMPD spectrum of Lys(Me)·Li$^+$ is generally similar to that of Lys·Li$^+$. The band at ~1720 cm^{-1}, corresponding to the carbonyl stretch of a carboxylic acid, strongly indicates that Lys(Me) is nonzwitterionic when lithiated. Compared to that of Lys·Li$^+$, the spectrum of Lys(Me)·Li$^+$ has significantly more relative photodissociation near 1450 cm^{-1}; a minor band at ~1300 cm^{-1} that was not observed for Lys·Li$^+$ is also present. This suggests that the structures of these two ions differ or that an additional structure may contribute to the IRMPD spectrum of Lys(Me)·Li$^+$. The calculated spectra for the $N_{SC}N_TO$-NZ structures of these two lithiated complexes are very similar (Figures 4 and 5). The other low-energy structure identified for Lys(Me)·Li$^+$ ($N_{SC}O$-NZ) has a major band at ~1420 cm^{-1}; the presence of some of this structure could account for the enhanced intensity observed at this frequency in the measured spectrum. The $N_{SC}O$-NZ structures are calculated to be 16 and 17 kJ/mol higher in energy than $N_{SC}N_TO$-NZ for Lys·Li$^+$ and Lys(Me)·Li$^+$, respectively. The reason why the former structure is present for Lys(Me)·Li$^+$, but not for Lys·Li$^+$, is unclear. A population of $N_{SC}OO$-ZW could also account for the higher photodissociation intensity near 1440 cm^{-1}, but the free energies of these structure are quite high for both Lys·Li$^+$ and Lys(Me)·Li$^+$ (+38 kJ/mol for both complexes). In addition, the absence of a band

corresponding to a carboxylate group appears to eliminate the possibility of a significant population of zwitterionic structures. The band at 1300 cm^{-1} could correspond to out-of-plane CH bends in $N_{SC}N_TO$-NZ, although the reason for its absence [below the signal-to-noise ratio (S/N)] in the IRMPD spectrum of Lys·Li^+ is unclear. Although a mixture of the $N_{SC}O$-NZ and $N_{SC}N_TO$-NZ structures appears to best account for the measured spectrum of Lys(Me)·Li^+, it is possible that other structures that were not identified in our conformational searching may be present.

The IRMPD spectrum of Lys(Me)·K^+ contains no significant photodissociation above 1700 cm^{-1}, indicating that the vast majority of the ions do not contain a carboxylic acid group and lysine in this complex is therefore zwitterionic. The majority of the photodissociation in this spectrum is centered near 1600 cm^{-1}, consistent with the carboxylate group of a zwitterionic amino acid. This band is quite broad and appears to have multiple unresolved features, which may indicate the presence of multiple zwitterionic forms. Making detailed structural assignments from this spectrum is challenging due to the low signal-to-noise ratio and the limited number of resolved features, although both OO^{α}-ZW and N_TO-ZW are consistent with the experimental spectrum.

The IRMPD spectrum of Lys(Me)·Na^+ appears to be a superposition of the spectra obtained for Lys(Me)·Li^+ and Lys(Me)·K^+, suggesting that both nonzwitterionic and zwitterionic populations of Lys(Me)·Na^+ exist under the conditions of the experiment. This is consistent with the observation that the spectrum does not match any single calculated spectrum, but has elements from several of these spectra. Without higher quality reference spectra and information regarding the relative stabilities and relative cross sections of each population, such as that obtained previously for metal-cationized arginine,[10] it is difficult to provide even a crude estimate of the relative abundances of the zwitterionic and nonzwitterionic forms in the experiment. However, given that the free energy difference between the zwitterionic and nonzwitterionic forms of Lys(Me)·Na^+ is likely very small, the structures of analogous complexes may serve as very sensitive probes of zwitterion stability. Subtle modifications to the amino acid or additional ligand binding will likely have a large effect on the relative population of the zwitterionic and nonzwitterionic forms of the amino acid in the complex.

Lys·H^+ and Lys(Me)·H^+. The most intense bands in the IRMPD spectra of Lys·H^+ and Lys(Me)·H^+ occur near 1740 cm^{-1} and are assigned to the carbonyl stretches of carboxylic acid groups (Figure 3). This indicates that these ions are nonzwitterionic, consistent with calculations by Bleiholder et al. indicating that the zwitterionic form of Lys·H^+ is 38 kJ/mol higher in energy than the nonzwitterionic form.[35]

Although the IRMPD spectra of Lys·H^+ and Lys(Me)·H^+ both contain carboxylic acid carbonyl stretches, the lower frequency regions of the spectra clearly differ. The IRMPD spectrum of Lys·H^+ resembles the calculated absorbance spectrum of $N_{SC} \rightarrow N_TO$, although the photodissociation near 1450 cm^{-1} may indicate that additional conformers, such as $N_{SC} \rightarrow O$, contribute to the observed photodissociation. The experimental spectrum of Lys(Me)·H^+ does not resemble the calculated spectrum of $N_{SC} \rightarrow N_TO$. The most intense mode in this region of the calculated spectrum of $N_{SC} \rightarrow N_TO$ is due to the NH_2 scissor bend of the side-chain amino group (1643 cm^{-1}); the corresponding band in the IRMPD spectrum is very weak relative to that of the carboxylic acid carbonyl stretch. The lack of a strong bonded OH bend band (1400 cm^{-1}) present

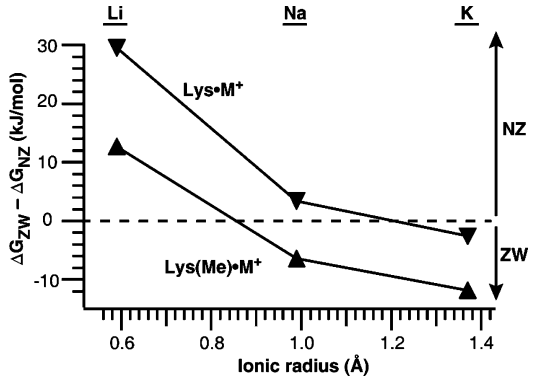

Figure 6. Gibbs free energy differences at 298 K between the lowest energy ZW and NZ structures of Lys·M^+ (▼) and Lys(Me)·M^+ (▲), M = Li, Na, and K, plotted as a function of the metal ion size.

in the calculated absorption spectrum of $N_{SC} \rightarrow O$ indicates that this structure is not likely populated. The $N_{SC} \rightarrow N_T$ structure is consistent with the experimental data. However, the calculated absorption spectrum of this structure has multiple, weak, NH bending modes that are well spaced in frequency (1576, 1629, and 1670 cm^{-1}), whereas the IRMPD spectrum contains a single band near 1600 cm^{-1}. It is possible that an alternate $N_{SC} \rightarrow N_T$ structure, such as $N_{SC} \rightarrow N_T$(alt), may better match the experimental spectrum. A superposition of the $N_{SC} \rightarrow N_T$ and $N_{SC} \rightarrow N_TO$ spectra also appears to provide a reasonable fit to the experimental data.

Effects of Metal Ion Size. The difference in free energy between the lowest energy zwitterionic and nonzwitterionic forms of both Lys·M^+ and Lys(Me)·M^+ depends on the metal ion size, with the zwitterionic form preferentially stabilized for the larger metal ions. For Lys·M^+, this difference at 298 K is calculated to be +29, +3, and −3 kJ/mol, for M = Li, Na, and K, respectively (Figure 6). For Lys(Me)·M^+, the respective differences are +13, −6, and −12 kJ/mol. A similar trend in increasing relative zwitterionic stability with increasing metal ion size was observed previously for Arg·M^+, M = Li, Na, and K,[9,10] but the opposite trend was reported for sodiated and rubidiated glycine, alanine, and analogues of these two amino acids.[7]

For Lys·Li^+ and Lys·Na^+, the complexes adopt a structure in which the metal ion is tricoordinated ($N_{SC}N_TO$-NZ), whereas the metal ion in Lys·K^+ is dicoordinated and a strong hydrogen bond is formed between the carboxylic acid and the N-terminal amino group ($N_{SC}O$-NZ). The ligand binding energies of lithium to these functional groups are expected to be the highest of the alkali metals. For the larger alkali-metal ions that have weaker ligand binding energies, formation of an intramolecular hydrogen bond becomes increasingly favored relative to metal ion solvation, resulting in a change in metal ion coordination with increasing alkali-metal ion size.

Previous IRMPD spectra of tryptophan complexed with small cations indicated that the metal ions in these complexes are coordinated to the N-terminal amino group, the carbonyl oxygen, and the aromatic ring (N/O/ring), whereas the metal ions in complexes with larger cations are coordinated to the carbonyl oxygen and the aromatic ring, and the N-terminal amino group accepts a hydrogen bond from the carboxylic acid (O/ring).[28] This was attributed to improved cation−π interactions in the O/ring structure of tryptophan in complexes with larger cations.[28] The N/O/ring and O/ring structures of tryptophan·M^+ are generally analogous to the $N_{SC}N_TO$ and $N_{SC}O$ structures of Lys·M^+, respectively, and primarily differ in that the metal ions in these structures are solvated by the aromatic ring in the former

and the side-chain amino group in the latter. Because similar trends with metal ion size are observed for cationized complexes of both aromatic (tryptophan) and basic (lysine) amino acids, this suggests that hydrogen bonding may drive the formation of O/ring structures of tryptophan·M^+ with increasing alkali-metal ion size.

Effects of Proton Affinity. The proton affinities of Lys (997 kJ/mol) and Lys(Me) (1013 kJ/mol) differ by 16 kJ/mol.[11] Zwitterionic structures in which the side-chain amino group is protonated (OO^{α}-ZW and N_TO-ZW) were found to be most stable for Lys(Me)·M^+, although structures in which the N-terminus is protonated are very close in energy in the case of Na and K. For Lys·M^+ at 298 K, protonation of the N-terminal amino group is favored, although the energy difference between these two zwitterionic forms is small for all cations. Thus, the secondary amine in the side chain of Lys(Me)·M^+ is sufficiently more basic than the primary amine in the side chain of lysine that the proton is directed to this site in lowest energy zwitterionic forms.

The extent to which proton affinity preferentially stabilizes the zwitterionic forms of these ions can be obtained from the difference in the relative zwitterionic and nonzwitterionic stabilities for these two species. The lowest energy zwitterionic forms of Lys(Me)·M^+ are preferentially stabilized by 17, 10, and 9 kJ/mol, for M = Li, Na, and K, respectively, compared to Lys·M^+ and at 298 K (Figure 6). For Li, the 16 kJ/mol higher proton affinity of Lys(Me)·M^+ corresponds to an essentially direct preferential stabilization of the zwitterionic form. By comparison, this preferential stabilization of the zwitterionic form is somewhat less for the sodiated and potassiated complexes. These trends with proton affinity are generally similar to those observed previously for sodiated complexes of aliphatic amino acids.[7,8]

The relationship between proton affinity and zwitterion stability can be complicated by competing effects of ion stabilization, such as ion solvation or hydrogen bonding. *N*-methyllysine (NMeLys) is a derivative of lysine in which the N-terminal amino group is methylated, forming a secondary amine at this site. The proton affinity of NMeLys is only 2.2 kJ/mol higher than that of Lys, but this modification preferentially stabilizes the lowest energy zwitterionic form of NMeLys·Li^+ by 14.2 kJ/mol relative to Lys·Li^+.[11] Methylation of the N-terminal amino group of lysine has a relatively minor effect on proton affinity because the side-chain amino group is the preferred protonation site in Lys·H^+.[11] However, despite the minor effect on proton affinity, methylation of the N-terminal amino group preferentially stabilizes the zwitterionic form to a degree comparable to that of methylation of the side-chain amino group.[11] It is even more challenging to infer zwitterionic stability on the basis of differences in proton affinity between amino acids with very different side chains. For example, the proton affinity of arginine is 160 kJ/mol higher than that of glycine,[35] yet the nonzwitterionic forms of lithiated arginine and lithiated glycine are both 16 kJ/mol more stable than the corresponding zwitterionic forms.[10,13] In contrast to trends established for aliphatic amino acids,[7,8] the zwitterionic form of lithiated arginine is no more stable than that of lithiated glycine, despite the 160 kJ/mol higher proton affinity of the former.

Conclusions

IRMPD spectra in the 1150−1850 cm^{-1} region provide useful information about the structures of cationized Lys and Lys(Me). The spectra of Lys·Li^+ and Lys·Na^+ are similar, whereas the spectrum for Lys·K^+ is different, indicating that the structure of lysine in these complexes depends on the metal ion size. The presence of a carbonyl oxygen stretch of a carboxylic acid group at ∼1720 cm^{-1} provides strong evidence that lysine is nonzwitterionic in all three ions. By comparison to spectra calculated for low-energy candidate structures, more detailed information about the structure is obtained. These results show that the metal ion binding to lysine changes from tricoordinate for Li and Na to dicoordinate with K, a result consistent with the increasing importance of hydrogen bonding relative to metal ion solvation to ion stability with increasing metal ion size. Although calculations at the B3LYP/6-31++G** level indicate that the zwitterionic form of Lys·K^+ is most stabile, the spectral data show that the majority of the ion population is nonzwitterionic.

Information about the effects of proton affinity on zwitterionic stability is obtained by comparing results from Lys(Me) to Lys. The structure of Lys(Me) changes from nonzwitterionic for lithiated complexes to zwitterionic for potassiated complexes, on the basis of spectral bands corresponding to the carbonyl stretches of a carboxylic acid (1720 cm^{-1}) and a carboxylate group (∼1600 cm^{-1}), respectively. Lys(Me)·Na^+ appears to be a mixture of both nonzwitterionic and zwitterionic forms. The higher proton affinity of Lys(Me) compared to Lys results in a direct stabilization of the zwitterionic form relative to the nonzwitterionic form for Li, but the relative stabilization is much less for Na and K. These results suggest that the proton affinity of the amino acid is important for zwitterionic stability, but other factors, such as metal ion solvation and hydrogen bonding, also contribute significantly.

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Supporting Information Available: Cartesian coordinates for all structures. This material is available free of charge via the Internet at http://pubs.acs.org.

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