Proton Affinity and Zwitterion Stability: New Results from Infrared Spectroscopy and Theory of Cationized Lysine and Analogues in the Gas Phase

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The gas-phase structures of alkali metal cationized lysine (Lys), α-*N*-methyllysine (NMeLys), and ϵ-N,N-dimethyllysine ($Lys(Me)_2$) are investigated using infrared multiple photon dissociation (IRMPD) spectroscopy utilizing light generated by a free electron laser and *ab initio* calculations. The proton affinities of the compounds span a range of ~20 kJ/mol. For NMeLys•M^+, experiment and theory indicate that NMeLys is nonzwitterionic for M = Li and zwitterionic for M = Na and K. For $Lys(Me)_2$•M^+, experiment and theory indicate that $Lys(Me)_2$ is zwitterionic for M = Li, Na, and K. This is the first spectroscopic observation of the zwitterionic form of an amino acid complexed with Li^+. The results are compared with IRMPD spectra reported previously for Lys and ϵ-*N*-methyllysine (Lys(Me)) complexed with Li, Na, and K, and new calculations performed at higher levels of theory for those ions. The combined experimental and theoretical results indicate that protonation in the zwitterionic forms of the these amino acids is favored at the more basic methylated amine site, but that any relationship between the proton affinity of the amino acid and the relative zwitterion stability of the alkali metal cationized amino acid is only indirect. These results provide additional evidence that proton affinities are not a reliable indicator of zwitterion stability for cationized amino acids because side chains can have very different effects on the stability of different conformers in the neutral, protonated, and metal cationized forms.

Introduction

Naturally occurring amino acids are nonzwitterionic when isolated in the gas phase. Interactions with surrounding molecules and ions can preferentially stabilize the zwitterionic forms of amino acids in aqueous solution, including under physiological conditions. The proton affinity of a proton accepting group is an important factor in stabilizing zwitterionic structures in the gas phase. For example, zwitterionic structures of protonated trimers consisting of two organic bases and a trifluoroacetic acid molecule are increasingly stable for clusters containing bases with increasingly large proton affinities.[1,2] The proton affinities of derivatives of arginine with up to four additional methyl groups increase monotonically.[3] Zwitterionic forms are preferentially stabilized and lowest-in-energy for derivatives with up to three additional methyl groups, but the lowest-energy form of the derivative with four additional methyl groups is nonzwitterionic, despite having the highest proton affinity.[3] The stability of the zwitterionic form relative to the nonzwitterionic form of selected sodiated aliphatic amino acids is directly related to the proton affinity of the amino acid,[4,5] but results for sodiated glutamine,[6−8] lysine,[9,10] and tryptophan[11,12] show that these complexes are nonzwitterionic, despite the high proton affinities of these amino acids. These results illustrate some of the challenges in predicting relative zwitterionic stabilities of larger, more complex biomolecules based on trends established for simpler molecules.

Attachment of a metal cation to an amino acid can preferentially stabilize zwitterionic structures. Low-energy dissociation,[13] kinetic method,[14,15] and infrared multiple photon dissociation (IRMPD) action spectroscopy[16−18] experiments indicate that the structure of alkali metal cationized arginine depends on metal ion size. Results from the latter experiments provide detailed structural information and show that cationized arginine is predominantly nonzwitterionic with Li, but predominantly zwitterionic with Na and K.[16,17] IRMPD action spectra of Lys•M^+, M = Li, Na, and K, indicate that lysine in each of these complexes is predominantly nonzwitterionic, although a small zwitterionic population may exist for Lys•K^+.[10] These results are consistent with results from kinetic method dissociation of cationized heterodimers of Lys and lysine methyl ester,[15] experimental water binding energies to Lys•$M^+(H_2O)$,[9] and calculations.[9,10]

An important advantage of gas-phase studies 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. Vibrational action spectroscopy using light from either free electron lasers (typically 5−10 μm)[7,10,12,17,19−32] or benchtop laser systems based on optical parametric generation (typically 2.5−4 μm)[16,18,33−37] has emerged as a powerful method to directly probe the gas-phase structures of cationized amino acids and amino acid analogues,[7,10,12,16−24,26,28,31,32,35] peptides,[20,28,30] noncovalently bound multimers of amino acids,[29,34,36] and even proteins.[25,33] Here, we report IRMPD spectra of Lys•Cs^+, NMeLys•M^+, and $Lys(Me)_2$•M^+, M = Li, Na, and K. By comparing these experimental spectra to those reported previously for Lys•M^+ and Lys(Me)•M^+,[10] and those calculated for candidate low-energy structures, detailed information about the structures of these ions and the effects of selected amine methylation is obtained.

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TABLE 1: Product Ions Observed from Photodissociation of NMeLys•M^+ and Lys(Me)$_2$•M^+, M = Li, Na, and K

	Li[a]	Na	K
NMeLys	$-NH_3$, $-H_2O$	$-NH_3$, $-H_2O$, $-H_3CNH_2$, Na^+	K^+
Lys(Me)$_2$	$-(H_3C)_2NH$	$-NH_3$, $-(H_3C)_2NH$	K^+

[a] Any formation of Li^+ would not be observed in these experiments due to the *m*/*z* range of the mass spectrometer, but that is a high-energy dissociation pathway for other lithiated amino acids.[46,56]

Methods

Mass Spectrometry and Photodissociation. Experiments were performed on a 4.7 T Fourier-transform ion cyclotron resonance mass spectrometer[38] using general experimental methods that are described elsewhere.[21] 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 15−40 μL/min. Tunable radiation for the photodissociation experiments is generated by the *f*ree *e*lectron *l*aser for *i*nfrared e*x*periments (FELIX).[39]

Computational Chemistry. Low-energy structures of NMeLys•M^+ and Lys(Me)$_2$•M^+, M = Li, Na, and K, were generated by Monte Carlo multiple minima conformational searching methods described previously.[10] 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) as implemented in Jaguar v. 6.5 (Schrödinger, Inc., Portland, OR) using the LANL2DZ[40] effective core potential (ECP) for potassium and the 6-31G(d,p) basis set for all remaining atoms. Initial structures of Lys•Cs^+ were generated by substituting the metal ion in structures reported previously for Lys•K^+.[10] The lowest-energy structure in each family was energy minimized and vibrational frequencies were calculated at the B3LYP level of theory using the CRENBL[41] ECP for Cs and the 6-31++G(d,p) basis set for all remaining elements using Q-Chem v.3.0.[42] Single-point energies for the B3LYP/6-31++G(d,p) geometries were also calculated at both the B3LYP and MP2 levels of theory using the CRENBL ECP for Cs and the 6-311++G(2d,2p) basis set for all remaining elements. Single-point energies were also calculated for Lys•M^+ and Lys(Me)•M^+, M = Li, Na, and K, using geometries and thermochemical corrections calculated previously.[10] All frequencies for calculated absorption spectra were scaled by 0.975 and convolved using a 20 cm^{-1} fwhm Gaussian distribution, which yielded good agreement for Gln•M^+,[7] Lys•M^+,[10] and Lys(Me)•M^+,[10] and M = alkali metal ion.

Results

IRMPD Action Spectra. Photodissociation of NMeLys•M^+ and Lys(Me)$_2$•M^+, M = Li, Na, and K, results in the formation of the bare metal ion or the loss of a small neutral molecule from the complex (Table 1). Cs^+ is the only photodissociation product for Lys•Cs^+. Formation of bare metal ions is favored for complexes containing larger cations, presumably because of lower metal ion binding energies to the amino acid. Infrared multiple photon dissociation (IRMPD) action spectra for these ions are obtained from the sum of the relative intensities of the fragments and are shown in Figure 1 along with previously reported spectra of Lys•M^+ and Lys(Me)•M^+, M = Li, Na, and K, for comparison.[10] Photodissociation yields are corrected linearly for laser power.[21,43,44] Photodissociation in these experiments requires the absorption of multiple laser photons. Although band intensities for polycyclic aromatic hydrocarbon cations measured using IRMPD and matrix isolation experiments were in fair agreement,[43] spectral intensities in IRMPD experiments may not always reflect those obtained from quantum mechanical calculations or from linear absorbance measurements.

The IRMPD spectra of Lys•M^+, M = Li, Na, K, and Cs, NMeLys•Li^+, LysMe•Li^+, and LysMe•Na^+ each have an intense band above 1700 cm^{-1}. The IRMPD spectra of AA•Li^+, AA = arginine,[17] glutamine,[7] serine,[23] threonine,[24] and tryptophan,[12] have diagnostic bands near 1720 cm^{-1} corresponding to the carbonyl stretches of carboxylic acid groups. This band indicates that the amino acids in these lithiated complexes are nonzwitterionic. Hence, bands in this region of the IRMPD spectra of Lys•M^+, M = Li, Na, K, and Cs, NMeLys•Li^+, LysMe•Li^+, and LysMe•Na^+, indicate the presence of populations of ions in which the amino acids are nonzwitterionic under these conditions.

The carboxylic acid groups of zwitterionic forms of lysine are deprotonated and will not exhibit carbonyl stretches at frequencies greater than 1700 cm^{-1}. Instead, these structures are expected to yield carboxylate stretches as well as bending modes for the protonated amine group. For example, proline complexed with sodium is zwitterionic[19,45,46] and the IRMPD spectrum of that ion has an intense carboxylate asymmetric stretch band at 1698 cm^{-1}.[19] The IRMPD spectra of NMeLys•Na^+, NMeLys•K^+, Lys(Me)•K^+, Lys(Me)$_2$•Li^+, Lys(Me)$_2$•Na^+, and Lys(Me)$_2$•K^+ do not have carbonyl stretch bands, but do have at least one intense band in the range from 1600−1700 cm^{-1} that is consistent with a carboxylate asymmetric stretch and the presence of zwitterionic structures.

Low-Energy Structures. Because the side chains of Lys and the analogues each have the potential to accept a proton, form hydrogen bonds, and solvate metal ions, the amino acids in these alkali metal cationized complexes can adopt many potential nonzwitterionic (**NZ**) and zwitterionic (**ZW**) structures, many of which are similar in energy. The low-energy structures identified for NMeLys•M^+ and Lys(Me)$_2$•M^+, M = Li, Na, and K, are generally similar to each other and those identified previously for Lys•M^+ and Lys(Me)•M^+, M = Li, Na, and K.[10] Energy minimized structures for NMeLys•K^+ and Lys(Me)$_2$•K^+ are shown in Figures 3 and 4 respectively, and those for Lys•K^+ have been reported previously.[10] The relative energies of these structures depend on the level of theory and the identity of both the metal cation and the amino acid. The relative Gibbs free energies at 298 K, a temperature corresponding to the approximate starting temperature of ions in these experiments, for NMeLys•M^+, Lys(Me)$_2$•M^+, and Lys•Cs^+ are reported in Figures 3, 4, and 5, respectively. The relative Gibbs free energies for Lys•M^+, NMeLys•K^+, Lys(Me)•M^+, and Lys(Me)$_2$•K^+ at both 0 and 298 K are reported in Supporting Information Tables 1 and 2, respectively.

The metal ion in the lowest-energy **NZ** structures of AA•M^+, AA = Lys, NMeLys, Lys(Me), Lys(Me)$_2$, M = Li and Na, are solvated by the side-chain amine group, the N-terminal amine group, and the carbonyl oxygen of the carboxylic acid group (**$N_{SC}N_TO$-NZ**). This structure is analogous to the lowest-energy **NZ** forms of AA•M^+, AA = arginine,[13,16,17] glutamine,[6−8] serine,[23] threonine,[24] and tryptophan,[12] M = Li and Na; the metal ion in those structures is solvated by the N-terminal amino group, the carbonyl oxygen of the carboxylic acid group, and a heteroatom in the amino acid side chain. Alternate **NZ** structures are preferentially stabilized with increasing metal ion size; in

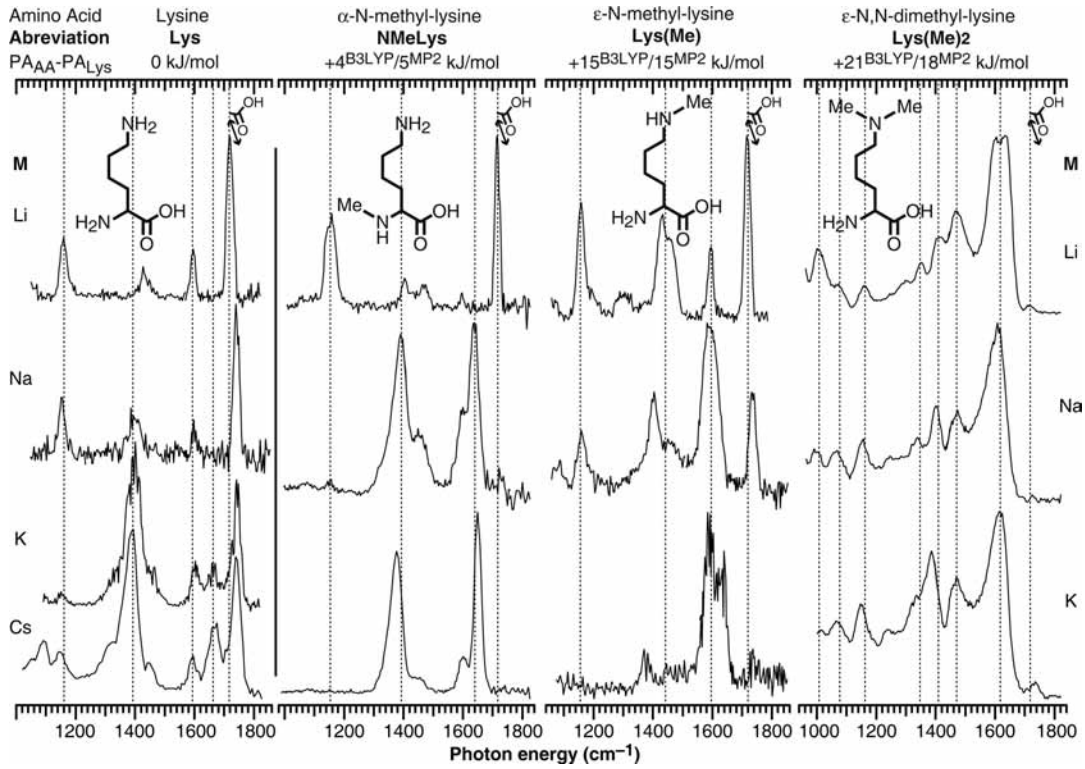

Figure 1. IRMPD action spectra of cationized amino acids. Spectra of Lys•M$^+$ and Lys(Me) M$^+$, M = Li, Na, and K, were reported previously.[10]

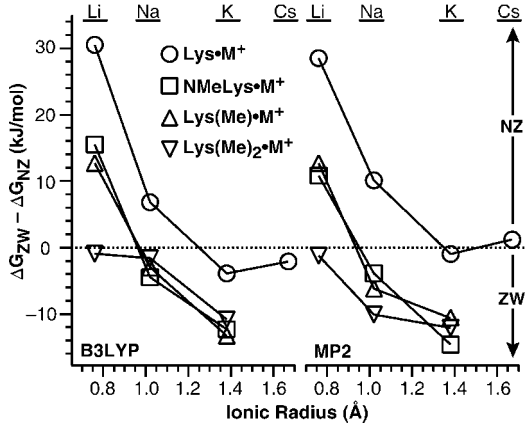

Figure 2. 298 K Gibbs free energy differences between the lowest-energy zwitterionic (**ZW**) and nonzwitterionic (**NZ**) forms of Lys•M$^+$, NMeLys•M$^+$, Lys(Me)•M$^+$, and Lys(Me)$_2$•M$^+$ Plotted as a function of metal ion size at B3LYP/6-311++G(2d,2p)//6-31++G(d,p) (*left*) and MP2/6-311++G(2d,2p)//B3LYP/6-31++G(d,p) (*right*) levels of theory.

these structures, the metal ion is solvated by one less amine group (**N$_{SC}$O-NZ**, **N$_{SC}$OO-NZ**, and **N$_T$O-NZ**) or by only the carboxylic acid group (**OO$^\beta$-NZ**). The carboxylic acid group donates a hydrogen bond to an amine group in these alternate structures, whereas that group does not donate a hydrogen bond in structure **N$_{SC}$N$_T$O-NZ**.

In each of the low-energy **ZW** structures, the carboxylic acid group is deprotonated and either the side chain or N-terminal amine groups is protonated. For a given metal ion, the relative stabilities of these different protonation sites depend strongly on the order (primary, secondary, or tertiary) of each amine group for the different amino acids. Some **ZW** structures of Lys(Me)$_2$•M$^+$ have subtle differences from those of the remaining amino acid complexes that can be attributed to the tertiary amine of the Lys(Me)$_2$ side chain, which is very basic, but can only donate one ionic hydrogen bond. In structure **OO$^\alpha$-ZW** of Lys(Me)$_2$•M$^+$, the protonated side chain donates a single hydrogen bond to the carbonyl oxygen, rather than two hydrogen bonds, one each to the carbonyl oxygen and the N-terminal amine group in the corresponding structure for NMeLys. In structure **OO$^\beta$-ZW** of Lys(Me)$_2$•Li$^+$ and Lys(Me)$_2$•Na$^+$, the side chain amine is the preferred site of protonation, rather than the N-terminal amine group that is preferred for analogous structures for the other amino acids studied. Relative to Lys•M$^+$, **ZW** structures with protonated N-terminal amine groups are preferentially stabilized for NMeLys•M$^+$ and those with protonated side chain amine groups are preferentially stabilized for Lys(Me)•M$^+$ and Lys(Me)$_2$•M$^+$.

The B3LYP and MP2 calculations result in very similar relative energies of the lowest-energy **NZ** and **ZW** forms, suggesting that calculations at the two levels of theory are moderately converged. These results generally indicate that zwitterionic forms are preferentially stabilized with increasing alkali metal ion size, and in many cases the zwitterionic and nonzwitterionic forms are very close in free energy.

Proton Affinities. Because the proton affinity (PA) of Lys(Me)$_2$ has not been reported, the PAs of all four amino acids (AA) were calculated at the same level of theory for comparison using eq 1

$$PA_{298\ K} = [E_{elec}(AA{\bullet}H^+) - E_{elec}(AA)] + [H_{298K}(AA{\bullet}H^+) - H_{298\ K}(AA) - (5/2)RT] \quad (1)$$

where E_{elec} is the electronic energy and $H_{298\ K}$ is the sum of the translational, rotational, and vibrational (including zero-point energies) enthalpies. The values calculated 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 are reported in Table 2. The PAs calculated at the two levels of theory are very close,

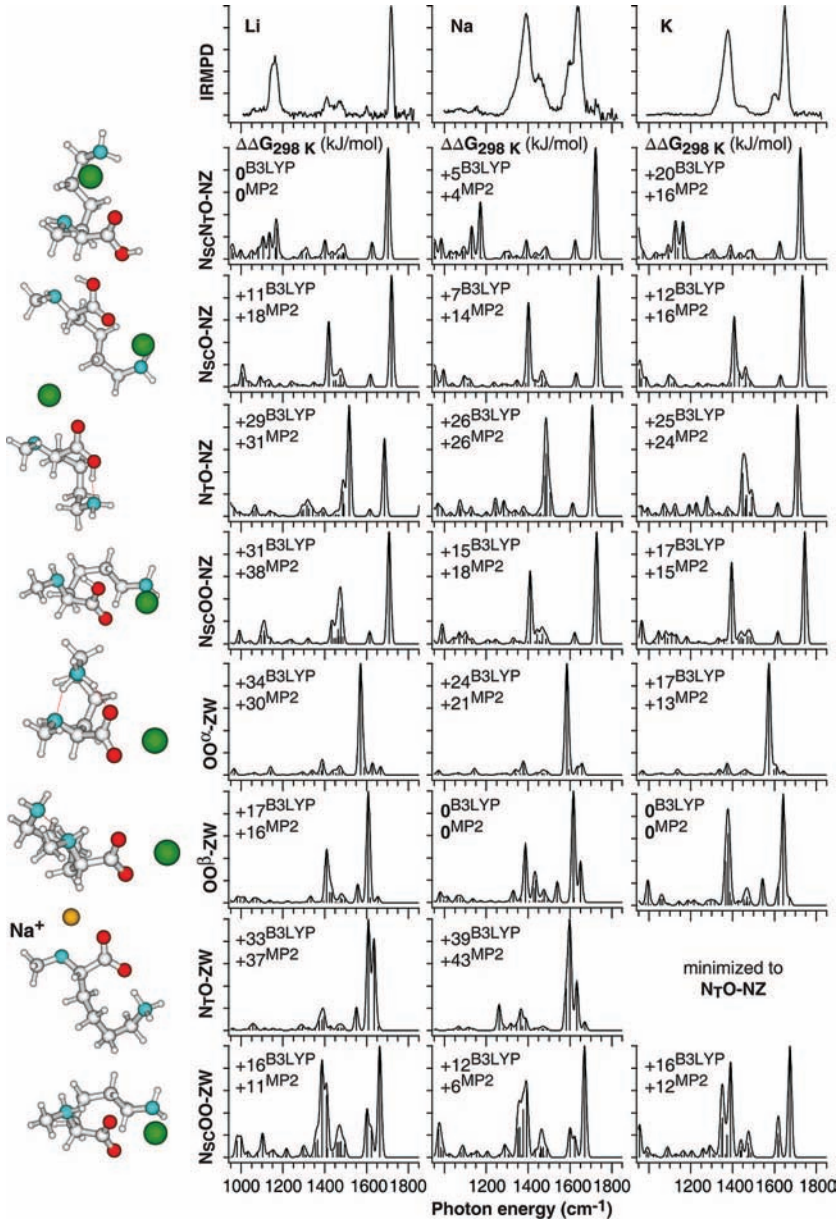

Figure 3. IRMPD spectra and B3LYP/6-31++G** calculated absorption spectra for candidate conformers of NMeLys•M^+, M = Li, Na, and K. Energy minimized structures are shown for NMeLys•K^+ (NMeLys•Na^+ for structure **N_TO-ZW**).

TABLE 2: Calculated 298 K Proton Affinities in kJ/mol

	AA	Lys	NMeLys	Lys(Me)	Lys(Me)$_2$
B3LYP[a]	PA_{AA}	993	997	1008	1014
	$PA_{AA}-PA_{Lys}$	0	+4	+15	+21
MP2[b]	PA_{AA}	989	994	1004	1007
	$PA_{AA}-PA_{Lys}$	0	+5	+15	+18

[a] B3LYP/6-311++G(2d,2p)//6-31++G(d,p). [b] MP2/6-311++G(2d,2p)//B3LYP/6-31++G(d,p).

although the values calculated at the B3LYP/6-311++G(2d,2p)//6-31++G(d,p) are systematically higher by 3−7 kJ/mol.

Although the PAs of the lysine analogues have not been measured, the PA of Lys has been determined using a variety of experimental and computational methods. The PAs calculated for Lys at the two levels of theory are very similar to that measured by Amster and co-workers using the bracketing method (988 ± 14 kJ/mol)[47] and slightly less than that measured by Poutsma and co-workers using the extended kinetic method (1007 ± 7 kJ/mol).[48] Bleiholder et al. reported 0 K PAs for lysine of 993 and 992 kJ/mol determined using B3LYP and G2(MP2) calculations.[49] Those PAs are slightly higher than our B3LYP and MP2 PAs adjusted to 0 K (986 and 983 kJ/mol, respectively). Lemoff et al. previously calculated the PAs of Lys, NMeLys, and Lys(Me) at the B3LYP/6-31++G(d,p) level of theory and obtained values of 996, 999, and 1013 kJ/mol, respectively.[9] Those PAs are similar to, but slightly greater than, those calculated here using B3LYP/6-311++G(2d,2p)//6-31++G(d,p).

Comparing relative PAs between Lys and the analogues ($PA_{AA}-PA_{Lys}$) should remove many systematic errors in these calculations and isolate the effects of the methylation. This is supported by the very close correlation between the relative PAs obtained at the two levels of theory (Table 2).

Discussion

Although considerable structural information (zwitterion versus nonzwitterion) is obtained from inspection of the IRMPD spectra (Figure 1) and comparisons with spectra reported

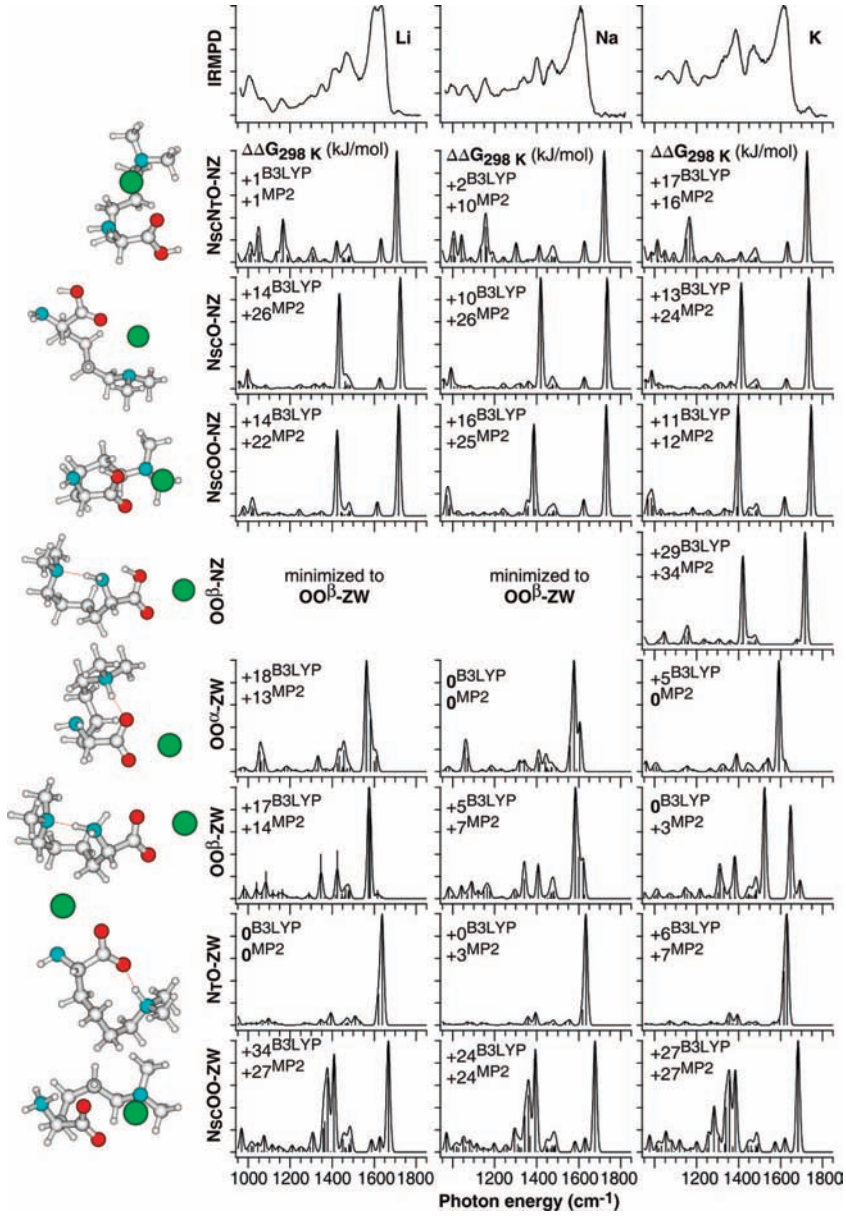

Figure 4. IRMPD spectra and B3LYP/6-31++G** calculated absorption spectra for candidate conformers of $Lys(Me)_2{\bullet}M^+$, M = Li, Na, and K. Energy minimized structures are shown for $Lys(Me)_2{\bullet}K^+$.

previously, additional structural information can be determined through comparisons with spectra calculated for low-energy candidate structures using density functional theory. However, there are the attendant uncertainties in comparing absorbance spectra calculated at zero K using the double-harmonic approximation with experimental action spectra obtained at finite temperatures and by absorption of multiple infrared photons.

NMeLys•M$^+$: Li, Na, and K. The IRMPD spectrum of NMeLys•Li$^+$ is very similar to that of Lys•Li$^+$, suggesting similar structures for both ions (Figure 1). The IRMPD spectrum of Lys•Li$^+$ was previously assigned to structure **$N_{SC}N_TO$-NZ**,[10] and the IRMPD spectrum of NMeLys•Li$^+$ is also very similar to the calculated spectrum for this structure (Figure 3). For example, IRMPD bands at 1720, 1600, 1470, 1410, and 1160 cm^{-1} correlate well with the carbonyl stretch (1703 cm^{-1}), $N_{SC}H_2$ scissor bend (1626 cm^{-1}), in-plane CH bends (~1460−1490 cm^{-1}), N_TH bend (1400 cm^{-1}), and the OH bend (coupled with multiple out-of-plane CH bends near 1160 cm^{-1}) calculated for this structure. These results are consistent with the structure deduced from binding energies measured for the loss of a water molecule from Lys•Li$^+$(H_2O) and NMeLys•Li$^+$(H_2O).[9]

The subtle differences between the IRMPD spectra of NMeLys•Li$^+$ and Lys•Li$^+$ can be attributed to the secondary amine in the former. For example, the relatively weak intensity of the NH_2 scissor bend for NMeLys•Li$^+$ is attributable the presence of only one NH_2 group for NMeLys, whereas the presence of only one band in spectrum of Lys•Li$^+$ in the region near 1400 cm^{-1} is attributable to the absence of a secondary amine for Lys.

The IRMPD spectra of NMeLys•Na$^+$ and NMeLys•K$^+$ are similar to each other, and very different from that of NMeLys•Li$^+$. Most notably, these spectra lack significant photodissociation at frequencies greater than 1700 cm^{-1}, which indicates that the amino acids in these complexes lack neutral carboxylic acid functional groups and are therefore zwitterionic. These spectra each contain intense bands near 1380 and 1640 cm^{-1}, and weaker bands near 1450 and 1600 cm^{-1}. The calculated spectra for structures **OO$^\alpha$-ZW** and **N_TO-ZW** have

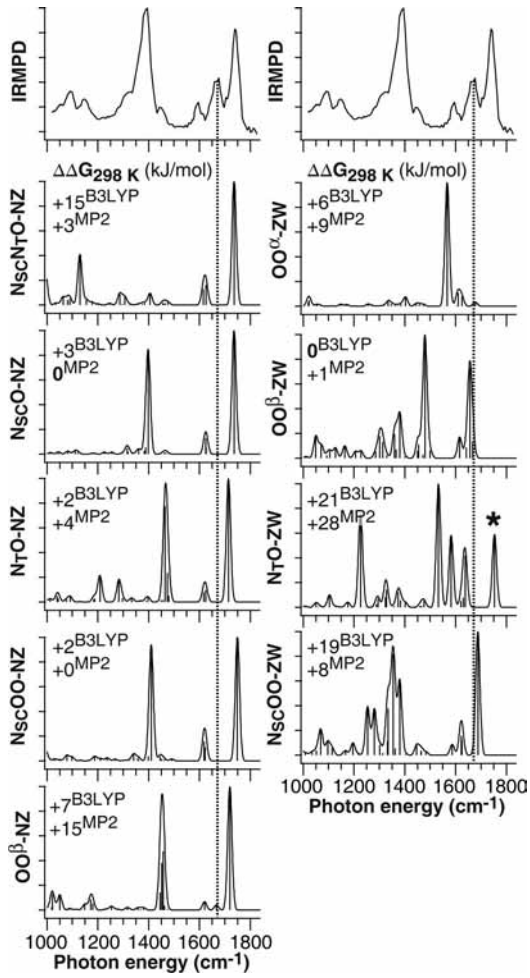

Figure 5. IRMPD spectra and B3LYP/6-31++G** calculated absorption spectra for candidate conformers of Lys•Cs^+. The band marked by a "*" for structure **N_TO-ZW** is a bonded hydrogen stretch.

very low absorbance below 1500 cm^{-1}, whereas the calculated spectra of structures **OO$^{\beta}$-ZW** and **N_{SC}OO-ZW** each have significance absorbance in both regions where intense photodissociation is observed experimentally.

For NMeLys•Na^+, the calculated spectrum of structure **OO$^{\beta}$-ZW** has a carboxylate symmetric stretch (1387 cm^{-1}), N_TH_2 out-of-plane bends (1427−1436 cm^{-1}, coupled with CH in-plane bends), and coupled carboxylate asymmetric stretching and N_TH_2 in-plane bending modes (1616 and 1641 cm^{-1}). The calculated spectrum of structure **N_{SC}OO-ZW** has coupled carboxylate symmetric stretching and N_TH_2 out-of-plane bending modes (1351−1393 cm^{-1}), an N_TH_2 in-plane bend (1600 cm^{-1}), and a carboxylate asymmetric stretch (1670 cm^{-1}). For NMeLys•K^+, the calculated spectrum of structure **OO$^{\beta}$-ZW** has a carboxylate symmetric stretch (1367 cm^{-1}), N_TH_2 out-of-plane bends (1380 cm^{-1}), bonded N_TH bend (1544 cm^{-1}), and carboxylate asymmetric stretch (1642 cm^{-1}) and the calculated spectrum of structure **N_{SC}OO-ZW** have coupled carboxylate symmetric stretching and N_TH_2 out-of-plane bending modes (1348−1390 cm^{-1}), NH_2 in-plane bends (1618 cm^{-1}), and a carboxylate asymmetric stretch (1674 cm^{-1}). Because the carboxylate asymmetric stretch of structure **N_{SC}OO-ZW** is calculated to occur ∼30 cm^{-1} to the blue of that observed experimentally and good agreement between the experimental and calculated frequencies of that mode have been reported previously,[19,21,22,31] the agreement between the IRMPD spectrum and the calculated spectrum for structure **OO$^{\beta}$-ZW** is better than that for structure **N_{SC}OO-ZW**.

Because the IRMPD spectra of NMeLys•Na^+ and NMeLys•K^+ have intense carboxylate symmetric stretches near 1380 cm^{-1} and carboxylate asymmetric stretches near 1640 cm^{-1}, which is a relatively low frequency for that band, these spectra are most consistent with structure **OO$^{\beta}$-ZW** and are least consistent with the spectra calculated for the **NZ** structures and structures **OO$^{\alpha}$-ZW** and **N_TO-ZW**. This indicates that these ions adopt zwitterionic structures with protonated N-terminal amino groups.

Lys(Me)$_2$•M^+, Li, Na, and K. The IRMPD spectra of Lys(Me)$_2$•M^+, M = Li, Na, and K, are similar to each other and all have very little photodissociation at frequencies greater than 1700 cm^{-1}, which is indicative of zwitterionic amino acids. These spectra contain a number of bands that persist as a function of metal ion size. The most intense bands in these spectra are centered near 1620 cm^{-1}, which is consistent with the asymmetric stretch of a carboxylate group. This frequency is very similar to those calculated for the asymmetric stretches of structures **N_TO-ZW** (1630−1640 cm^{-1}, Figure 4). The carboxylate asymmetric stretches of structures **OO$^{\alpha}$-ZW** are considerably red-shifted (1562−1591 cm^{-1}) from the IRMPD bands, whereas those stretches for structures **N_{SC}OO-ZW** are considerably blue-shifted (1670−1683 cm^{-1}). Interestingly, those stretches for structures **OO$^{\beta}$-ZW** are considerably red-shifted (1574−1584 cm^{-1}) for Lys(Me)$_2$•Li^+ and Lys(Me)$_2$•Na^+, but blue-shifted (1648 cm^{-1}) for Lys(Me)$_2$•K^+. This difference is likely attributable to different protonation sites calculated for the different complexes.

Bands below 1600 cm^{-1} appear to be superimposed on a broad photodissociation background, especially for the larger cations. Broad photodissociation has been observed in the IRMPD spectra of many gas-phase ions containing shared protons[50−53] and this has been attributed to finite temperature effects.[54] Stretching modes for such protons can occur at very low frequencies[55] and have very large integrated cross sections. At ambient temperatures, these modes are expected to be broad and likely couple with other vibrational transitions. This phenomenon makes spectral assignment especially challenging and may explain the poor agreement between the IRMPD bands at low frequencies and the vibrational mode calculated for candidate structures because photodissociation yields at specific wavelengths may depend strongly on coupling with shared hydrogen stretches.

Interestingly, the IRMPD spectra of Lys(Me)$_2$•Li^+ and Lys(Me)$_2$•K^+ have very weak bands at 1720 and 1730 cm^{-1}, respectively, whereas the spectrum of Lys(Me)$_2$•Na^+ has comparatively little photodissociation in this region. Bands in this region are consistent with the calculated frequencies of the carbonyl stretches of **NZ** structures, although the calculated relative energies provide no insights as to why small populations of **NZ** structures might be present for Lys(Me)$_2$•K^+, but less so for Lys(Me)$_2$•Na^+. The observed and calculated trends with metal ion size are inconsistent, but this difference is subtle and likely within the expected uncertainties in the calculations and experimental band intensities.

Lys•Cs^+. The IRMPD spectrum of Lys•Cs^+ is very similar to that reported previously for Lys•K^+[10] and calculated spectra for candidate structures of Lys•Cs^+ are shown in Figure 5. The IRMPD spectrum of Lys•Cs^+ has intense bands at 1390 and 1740 cm^{-1} that are consistent with the OH bend of a carboxylic acid group that donates a hydrogen bond to an amine group and the carboxylic acid carbonyl stretch, respectively.[10,12] These bands are spectral signatures for nonzwitterionic structures and

are the most intense features in this region of the spectra calculated for structures $\mathbf{N_{SC}O\text{-}NZ}$, $\mathbf{N_TO\text{-}NZ}$, $\mathbf{N_{SC}OO\text{-}NZ}$, and $\mathbf{OO^{\beta}\text{-}NZ}$.

However, the IRMPD spectrum also contains a strong band at 1660 cm^{-1} that is in poor agreement with the calculated spectra for the **NZ** structures and is instead indicative of a carboxylate asymmetric stretch. For example, structures $\mathbf{OO^{\beta}\text{-}ZW}$ and $\mathbf{N_{SC}OO\text{-}ZW}$ are calculated to have carboxylate asymmetric stretches at 1656 and 1687 cm^{-1} and the other intense modes at lower frequencies that are generally consistent with the observed IRMPD spectrum. B3LYP and MP2 calculations indicate that structure $\mathbf{N_{SC}OO\text{-}ZW}$ is 19 and 7 kJ/mol higher in free energy than structure $\mathbf{OO^{\beta}\text{-}ZW}$, respectively, but the spectrum calculated for structure $\mathbf{N_{SC}OO\text{-}ZW}$ is a slightly better fit to the data. These results indicate that substantial populations of both **NZ** and **ZW** structures of Lys•Cs^+ exist under the conditions of these experiments.

The IRMPD spectrum of Lys•K^+ contains a weak band at 1660 cm^{-1} that was attributed to the amine scissor bend of structure $\mathbf{OO^{\beta}\text{-}NZ}$ or the carboxylate asymmetric stretch of a $\mathbf{OO^{\beta}\text{-}ZW}$ structures.[10] Although the calculated spectrum of structure $\mathbf{OO^{\beta}\text{-}NZ}$ of Lys•Cs^+ has an amine scissor bend at 1666 cm^{-1}, the intensity of this mode is calculated to be ~5% of that of the carbonyl stretch and comparable to that for the other amine scissor bend at 1620 cm^{-1}. This comparison indicates that the IRMPD band for Lys•Cs^+ at 1660 cm^{-1} is most consistent with a carboxylate asymmetric stretch, and similarities between the IRMPD spectra of Lys•K^+ and Lys•Cs^+ suggest that the IRMPD band for Lys•K^+ at 1660 cm^{-1} may also be attributable to a small population of zwitterionic structures.

Effects of Amine Methylation. Although the addition of methyl groups to either of the amine groups of lysine results in an amino acid derivative with a proton affinity (PA) that is higher than that of Lys, the increase depends on more than just the number of methyl groups added (Table 2). The low-energy structures of AA and AA•H^+, AA = Lys, NMeLys, Lys(Me), and relative PAs have been discussed previously,[9] but have not been reported for AA = $Lys(Me)_2$. The PA of $Lys(Me)_2$ is the greatest of these AAs, but is only slightly greater than that of Lys(Me) (+6 and +3 kJ/mol at the B3LYP and MP2 levels of theory, respectively). Even though the side chain of $Lys(Me)_2$ is a tertiary amine, the protonated side chain in the lowest-energy form of $Lys(Me)_2$•H^+ can donate only one hydrogen bond, whereas that in Lys(Me)•H^+ can donate two.

The energy difference between the lowest-energy nonzwitterionic and zwitterionic forms of AA•M^+ for a given M does not appear to be well correlated with the PAs of the AAs (Figure 6). For example, the relative zwitterion stabilities of NMeLys•M^+ are at least 10 kJ/mol greater than those of Lys•M^+ for all M, even though the PA of the NMeLys is only roughly 5 kJ/mol greater than that of Lys. For M = Na and K at the B3LYP level of theory, the relative ZW stabilities of the three lysine analogues differ by less than 3 kJ/mol, even though the PAs of the three lysine analogues differ by 17 kJ/mol. These results indicate that any correlation between the relative zwitterion stabilities of the alkali metal cationized AAs and the PAs of the isolated AAs is only indirect.

One of the most interesting results from the IRMPD spectra is that the site of a single methyl group can have a substantial effect on the preferred site of protonation in zwitterionic forms. The IRMPD spectra of NMeLys•K^+ and Lys(Me)•K^+ have essentially no photodissociation at frequencies greater than 1700 cm^{-1} and indicate that these ions adopt zwitterionic structures. Additionally, the IRMPD spectrum of Lys(Me)•K^+ has very

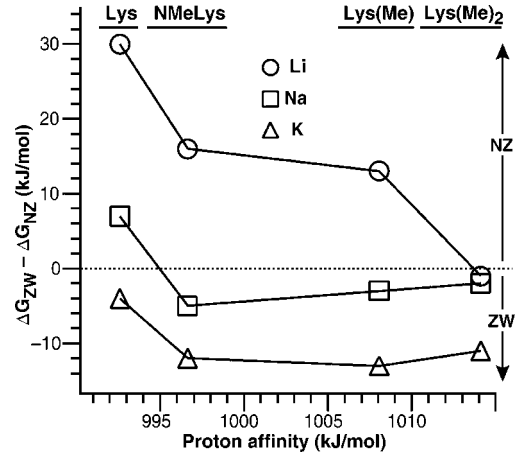

Figure 6. 298 K Gibbs free energy differences between the lowest-energy zwitterionic (**ZW**) and nonzwitterionic (**NZ**) forms of Lys•M^+, NMeLys•M^+, Lys(Me)•M^+, and $Lys(Me)_2$•M^+ plotted as a function of proton affinity at B3LYP/6-311++G(2d,2p)//6-31++G(d,p).

little photodissociation below 1500 cm^{-1} and is most consistent with structures $\mathbf{OO^{\alpha}\text{-}ZW}$ and $\mathbf{N_TO\text{-}ZW}$,[10] structures in which the side-chain amine group is protonated. In contrast, the IRMPD spectrum of NMeLys•K^+ has an intense band at 1380 cm^{-1} and is most consistent with structures $\mathbf{OO^{\beta}\text{-}ZW}$ and $\mathbf{N_{SC}OO\text{-}ZW}$, structures in which the N-terminal amino group is protonated. These results therefore indicate that the location of a single methyl group is sufficient to direct the preferred site of protonation in these two isomeric complexes.

Effects of Metal Ion Size. The IRMPD spectra indicate that zwitterionic structure are increasingly predominant with increasing alkali metal ion size for AA•M^+, AA = Lys, NMeLys, and Lys(Me) (Figure 1). Zwitterionic forms are predominant for $Lys(Me)_2$•M^+, M = Li, Na, and K, but weak photodissociation is observed above 1700 cm^{-1} in the IRMPD spectra for $Lys(Me)_2$•M^+ and may be indicative of a small fraction of nonzwitterionic structures. The intensity of this weak feature does not correlate with metal ion size.

There are subtle differences between the populations of structures predicted computationally and those apparent from the IRMPD spectra (Figures 1 and 2). For example, the spectrum of Lys(Me)•Na^+ exhibits substantial contributions from **NZ** structures, but the **ZW** form is calculated to be 3 and 6 kJ/mol lower in free energy at the B3LYP and MP2 levels of theory (Figure 2). However, the calculations do generally indicate that zwitterionic forms are preferentially stabilized for the larger alkali metal ions (Figures 2 and 6). One notable exception is that both the B3LYP and MP2 calculations indicate that **ZW** forms have greater stability relative to **NZ** forms for Lys•K^+ than the corresponding structures for Lys•Cs^+ (Figure 5). In contrast, the relative intensity of the carboxylate asymmetric stretch at 1660 cm^{-1} for Lys•Cs^+ is much greater than that observed for Lys•K^+, which suggests a greater relative abundance of **ZW** structures for Lys•Cs^+.

Conclusions

Even though nonzwitterionic forms of Lys•M^+ are predominant for M = Li, Na, and K, these results provide compelling evidence for substantial zwitterionic populations for NMeLys•M^+, M = Na and K, Lys(Me)•M^+, M = Na and K, and $Lys(Me)_2$•M^+, M = Li, Na, and K. These results indicate that the increased basicity of individual amine groups in the analogues results in a preferential stabilization of the zwitterionic forms and can direct the site of preferred site of protonation.

However, the relative proton affinities of lysine and the analogues are not well correlated with the structures. These effects compete with those of improved metal ion solvation in the nonzwitterionic form. For example, the IRMPD spectra of Lys•Na^+, NMeLys•Na^+ and Lys(Me)•Na^+ indicate that the amino acids in these complexes are predominantly nonzwitterionic, predominantly zwitterionic, and a mixture of nonzwitterionic and zwitterionic structures, respectively, whereas the proton affinities of NMeLys and Lys(Me) are roughly 5 and 15 kJ/mol greater than that of Lys, respectively. These results indicate that the proton affinity for amino acids are not a reliable indicator of zwitterion stability for cationized amino acids because side chains can have very different effects on the stability of different conformers in the neutral, protonated, and metal cationized forms.

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Supporting Information Available: Supporting Information Tables 1 and 2 and full citation for ref 42. This material is available free of charge via the Internet at http://pubs.acs.org.

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