J. Phys. Chem. A **2005,** *109,* 1903−1910

Structures of Cationized Proline Analogues: Evidence for the Zwitterionic Form

Andrew S. Lemoff, Matthew F. Bush, and Evan R. Williams*

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

Received: July 26, 2004; In Final Form: November 19, 2004

The structures of lithiated and sodiated α-methyl-proline (α-Me-Pro) and structural isomers, both with and without a water molecule, are investigated using blackbody infrared radiative dissociation (BIRD) and density functional theory. From the BIRD kinetic data measured as a function of temperature, combined with master equation modeling of these data, threshold dissociation energies for the loss of a water molecule from these clusters are obtained. These energies are 77.5 ± 0.5 and 53 ± 1 kJ/mol for lithiated and sodiated α-Me-Pro, respectively. For the nonzwitterionic isomer, proline methyl ester, these values are 3.0−4.5 kJ/mol higher. These results provide compelling experimental evidence that α-Me-Pro is zwitterionic in these clusters. Theory at the temperature corrected B3LYP/6-311++G**//B3LYP/6-31++G** level indicates that the salt-bridge or zwitterionic forms of lithiated and sodiated α-Me-Pro are between 17 and 23 kJ/mol lower in energy than the nonzwitterionic or charge-solvated forms and that attachment of a single water molecule does not significantly change the structure or the relative energies of these clusters. The proton affinity of proline is 8 kJ/mol higher than that of α-Me-Pro, indicating that lithiated and sodiated singly hydrated proline should also be zwitterionic.

Introduction

The in vivo structure of biomolecules is the result of both *intra*molecular interactions intrinsic to the molecule and *inter*molecular interactions with surrounding molecules and ions. These effects are each significant and often favor radically different structures. For example, amino acids in aqueous solution are zwitterions over a wide pH range, even though nonzwitterionic structures are energetically favored in the gas phase. Clearly, water preferentially stabilizes the zwitterionic form of amino acids. While this general concept is well understood, the full structural impact of water on biomolecular structure remains poorly characterized. In principle, gas-phase studies of biomolecules, such as amino acids and their hydrated clusters, should reveal how water interacts with and influences the structure of such molecules.

Many studies have used ab initio theory to investigate the structures of hydrated clusters of amino acids. For glycine, the simplest amino acid, the nonzwitterionic form is 92 ± 8 kJ/mol lower in energy than the zwitterionic form, which is not even a local minimum on the potential energy surface at the thermodynamically calibrated BAC/MP4/6-31G**//6-31G* level of theory.[1] Calculations indicate that the addition of two water molecules makes the zwitterionic form of glycine a local minimum on the potential energy surface, although the nonzwitterion is still favored by ∼50 kJ/mol.[2] Theory suggests that the addition of a third water molecule makes the two forms of glycine nearly equal in energy.[1] Ai et al. recently used theory to investigate the role of hydration and charge in stabilizing zwitterionic zinc/glycine clusters.[3] Calculations of Gly•Zn^+ show that an NO-coordinated, nonzwitterionic structure is 10.9 kJ/mol lower in energy than the lowest-energy zwitterionic structure. Single-point energy calculations of Hartree−Fock optimized geometries show that the addition of water preferentially stabilizes the zwitterionic form of Gly•Zn^+: one water molecule makes the zwitterionic and nonzwitterionic forms of glycine nearly equal in energy, and two water molecules make the zwitterion more stable by 24−28 kJ/mol. For Gly•Zn^{2+}, an OO-coordinated zwitterionic structure is favored and the addition of a single water molecule has a negligible impact on the geometry and relative energies of the low-energy conformations investigated.[3]

There are relatively few experimental studies of how water molecules influence the structures of gas-phase amino acids. The structures of hydrated, cationized valine clusters have been investigated using blackbody infrared radiative dissociation (BIRD).[4−7] Lithiated valine with one and two water molecules attached is nonzwitterionic, but with three water molecules, lithiated valine becomes a zwitterion.[5,6] Sodiated valine with a single water molecule was found to be nonzwitterionic, but a close structural analogue with a 31.0 kJ/mol higher proton affinity was found to be zwitterionic.[7] Simons and co-workers have used resonant two-photon ionization (R2PI), UV hole burn, and IR dip experiments to investigate the structure of tryptophan in the gas phase, both with[8] and without[9] water. Although their spectroscopic data of thermally evaporated tryptophan with zero to three water molecules show no evidence for zwitterionic tryptophan, their R2PI generated mass spectrum of laser ablated tryptophan shows a “uniquely stable ion cluster” with three water molecules, which may be indicative of a kinetically trapped, zwitterionic form of tryptophan formed under hot ablation conditions.[8] Bowen and co-workers recently reported results from photoelectron spectroscopy indicating that glycine with at least five water molecules can form a stable cluster with a dipole-bound electron.[10] From this observation, Bowen and co-workers concluded that a minimum of five water molecules is needed to form the glycine zwitterion. Similarly, tryptophan and phenylalanine each required at least four water molecules to form a stable dipole-bound electron species, suggesting a zwitterionic form of these amino acids in the cluster.[10]

---

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

10.1021/jp0466800 CCC: $30.25

Although none of the naturally occurring amino acids are zwitterionic in the gas phase, the zwitterionic form of these molecules is generally stabilized for amino acids with high gas-phase proton affinities. For arginine, the most basic amino acid, the zwitterionic form of the molecule is only ~15 kJ/mol less stable than the nonzwitterionic form,[11,12] and solvation by an extra electron makes the zwitterionic form lowest in energy.[13] Predicting structures of cationized amino acids based solely on proton affinity is complicated by the countering effects of charge solvation.[14] For molecules in which charge solvation does not play a significant role, zwitterionic forms are enhanced with increasing proton affinity and gas-phase basicity.[7,15−17] For the aliphatic amino acids and some amino acid analogues, Bowers and co-workers reported that there is a “fairly linear relationship” between proton affinity and the relative zwitterionic stability.[15]

Determining the structure of gas-phase cationized proline has been the subject of several recent investigations. Proline is unique among the natural amino acids in that the N-terminus is a secondary amine which makes this site more basic than the corresponding sites in other amino acids that are not involved in hydrogen bonding.[18,19] Isolated proline is nonzwitterionic.[20−23] The pyrrolidine ring of proline, which contains the N-terminus of the amino acid, reduces the ability of the amino acid to solvate an ion in a charge-solvated form.[24] Shoeib et al. found that the OO-coordinated zwitterionic or salt-bridge structure of $Pro{\cdot}Ag^+$ is 9.2 kJ/mol more stable than the NO-coordinated nonzwitterionic or charge-solvated structure using B3LYP with the DZVP basis set.[25] In contrast, calculations of Hoyau and Ohanessian indicate that the NO-coordinated nonzwitterionic structure of $Pro{\cdot}Cu^+$ is 14.2 kJ/mol more stable than the OO-coordinated zwitterionic structure.[26] Shoeib et al. attribute this metal ion dependent relative zwitterionic stability to the preference of Cu^+ to primarily coordinate to one oxygen atom of the carboxylate anion, which preferentially destabilizes salt-bridge structures.[25] By comparison, the nonzwitterionic forms of $Gly{\cdot}Ag^+$ and $Gly{\cdot}Cu^+$ are respectively 18.8 and 38.5 kJ/mol more stable than the zwitterionic forms.[27] Möller−Plesset calculations by Wesdemiotis, Ohanessian, and co-workers show that the salt-bridge structures of the proline−alkali metal ion complexes are 16−28 kJ/mol more stable than the charge-solvated structures.[28] Marino et al. investigated the structures and energetics of proline complexed with Li^+, Na^+, and K^+ using B3LYP calculations.[29] In all three cases, the lowest-energy structure is zwitterionic, with the metal ion OO-coordinated. The lowest-energy nonzwitterionic structures of $Pro{\cdot}Li^+$ (which is +5.4 kJ/mol higher in energy than the lowest-energy zwitterion) and $Pro{\cdot}Na^+$ (+17.6 kJ/mol) have the metal ion NO-coordinated to the amino acid. Nonzwitterionic $Pro{\cdot}K^+$ (+14.6 kJ/mol) prefers an OO-coordinated structure with the hydrogen of the carboxylic acid interacting with the amino nitrogen.

Wesdemiotis and co-workers used the kinetic method to investigate the structure of cationized proline and other amino acids.[28] Cationized (M = Li, Na, K, or Cs) heterodimers of an amino acid (AA) and its associated methyl ester (AAOMe, a nonzwitterion) were formed by fast atom bombardment and dissociated in a mass spectrometer to give $AA{\cdot}M^+ + AAOMe$ or $AA + AAOMe{\cdot}M^+$. The branching ratio ($[AA{\cdot}M^+]$ versus $[AAOMe{\cdot}M^+]$) was used to infer structure: a greater abundance of $AA{\cdot}M^+$ suggests that the metal ion was involved in a strong salt-bridge interaction with a zwitterionic amino acid, whereas a greater abundance of $AAOMe{\cdot}M^+$ suggests that the metal ion was solvated by two nonzwitterionic molecules and preferentially bound to the methyl ester because of its electron enriched carbonyl oxygen. Their experimental data indicate that sodiated, potassiated, and cesiated proline are zwitterionic, whereas lithiated proline is nonzwitterionic. The result for lithiated proline was in disagreement with their theoretical data, and they attributed this to a different structure of proline when present in the cationized heterodimer. The authors argued that proline methyl ester preferentially destabilizes the $Pro{\cdot}Li^+$ salt bridge by competing for charge transfer from the small metal ion. Larger metal ions exhibit reduced levels of charge transfer in their amino acid binding; therefore, this competitive effect is less significant for the larger cations. Recently, Ohanessian and co-workers measured an infrared spectrum of sodiated proline using a free electron laser and an ion cyclotron resonance mass spectrometer and found this spectrum to be consistent with a zwitterionic form of proline.[30] There are no known experimental results indicating that lithiated proline is zwitterionic.

Here, the threshold dissociation energies of water to lithiated and sodiated proline analogues are measured with high precision using BIRD experiments. By comparing these threshold dissociation energies to those measured for model molecules of known structure, the mode of water binding in these clusters is determined. From these results, the structure of the proline and its analogues in each of these clusters is deduced.

Experimental Methods

Chemicals. α-Methyl-proline (α-Me-Pro) was obtained from Bachem Califonia Inc. (Torrance, CA). The monohydrate of *N*-methyl-proline (*N*-Me-Pro), the hydrochloride salt of proline methyl ester (ProOMe), and lithium hydroxide were obtained from Aldrich Chemical Co. (Milwaukee, WI). Sodium hydroxide was purchased from Fischer Scientific (Fair Lawn, NJ). All chemicals were used without further purification. Electrospray solutions of α-Me-Pro and *N*-Me-Pro were made to 1.0 mM AA (*a*mino *a*cid or *a*mino *a*cid analogue) and 1.0 mM of the inorganic base. Solutions with ProOMe were made to 1.0 mM ProOMe·HCl and 3.0 mM of the inorganic base. Solution concentrations were chosen to optimize signal for the cationized hydrated species.

Mass Spectrometry. All blackbody infrared radiative dissociation (BIRD) experiments were performed on a home-built Fourier transform mass spectrometer with a 2.7 T magnet. The instrument and experimental methods are discussed in detail elsewhere.[7,31,32] Briefly, hydrated, cationized clusters are formed by nanoelectrospray ionization[33] and accumulated in the ion cell for 3−5 s. The hydrated ion cluster of interest is isolated using a series of stored waveform inverse Fourier transform and chirp excitation waveforms. The hydrated ion cluster then undergoes unimolecular dissociation for times ranging from 0 to 360 s prior to detection. Dissociation kinetics are obtained from the abundances of the precursor and fragment ions as a function of reaction time. The temperature of the cell is controlled by heating the vacuum chamber using electrically resistive heating blankets[31] or by cooling the copper jacket surrounding the cell with liquid nitrogen.[32] Prior to all experiments, the cell temperature is allowed to equilibrate for more than 8 h to ensure that the ions in the cell are exposed to a radiative energy field given by Planck’s distribution law.

Computational Details. Candidate low-energy structures of proline, α-Me-Pro, *N*-Me-Pro, and ProOMe are determined using molecular mechanics conformational searching. Structures of AA, $AA{\cdot}H^+$, $AA{\cdot}M^+$, and $AA{\cdot}M^+(H_2O)$ clusters were generated using Monte Carlo conformation searching with the MMFF94 force field using Macromodel 7.1 (Schrödinger, Inc., Portland, OR). For the initial search, no constraints were placed on the molecules and 1000 conformations were generated. Upon generating an additional 5000 conformations, no new structures within 50 kJ/mol of the lowest-energy structure found in the initial search were identified. After identifying many low-energy structures from the mechanics calculations, hybrid method density functional calculations (B3LYP) were performed using Jaguar version 5.0 (Schrödinger, Inc., Portland, OR) with incrementally larger basis sets. Structures were fully optimized using the 6-31G*, 6-31+G*, and 6-31++G** basis sets, and a single-point energy calculation at the 6-311++G** level based on the 6-31++G** optimized geometry was performed.

In these BIRD experiments, the measured dissociation rate depends on the rates of radiative absorption and emission, the binding energy of the water molecule to the ion, and to a limited extent, and the transition state entropy of the dissociation. The experimental data are modeled using a master equation formalism that takes into account all of these processes. This is discussed in detail elsewhere.[34] Briefly, radiative rates are obtained by combining Einstein coefficients determined from calculated absorption spectra for the clusters and a blackbody energy field at the temperature of the experiment. A transition dipole moment multiplication factor (μ) is used to take into account uncertainties in the calculated values. This factor is varied between 0.8 and 1.4, and threshold dissociation energies (E_0) that result in fits to the data within established criteria are found. Here, the data could only be fit with values of μ between 0.8 and 1.2. Dissociation processes are included in the model by using microcanonical dissociation rate constants calculated with RRKM theory. The transition state entropy of the dissociation is not modeled explicitly. Instead, a range of transition state entropies are modeled. Modeling was done for each of the isomers assuming both a "neutral" and "loose" transition state (Arrhenius A-factors of 10^{13} and 10^{17} s^{-1}, respectively). Dissociation of water from these clusters is expected to proceed by a relatively loose transition state, but a wider range of transition state entropies is used to better assess the effects of this parameter. Because the modeled kinetic rates in the small molecule, weakly bound cluster limit have a weak dependence on transition state entropy, modeling a wide range of A-factors does not lead to significant uncertainty in the binding energy.[35] The threshold dissociation energy is determined by varying this value in the model over a range that results in a fit to the experimental data.

Results and Discussion

Structure from Threshold Dissociation Energies. Information about the structure of an ion of interest is obtained from experimentally measured threshold dissociation energies (E_0) for the loss of a water molecule from a cluster using blackbody infrared radiative dissociation (BIRD). Molecules with known structure, that is, zwitterionic or nonzwitterionic, that bind the water molecule similarly to the two forms of the molecule of interest are used as reference molecules. Previous studies have shown the difference in water binding between zwitterionic and nonzwitterionic cationized species can be very small (~5 kJ/mol).[6,7] Small energy differences are most accurately measured for ions that absorb and emit radiation at similar rates. This reduces the effects of any uncertainties that may be present in the calculated transition dipole moments and vibrational frequencies that are used in the master equation modeling process. For this reason, similar molecules that are structural isomers provide the best potential for accurately measuring very small differences in water binding energy. For all clusters, extensive modeling is done in order to establish the extent to which these reference molecules represent good model compounds for the zwitterionic and nonzwitterionic forms of the molecule in question; that is, they have the same mode of water and metal ion binding.

TABLE 1: Comparison of Calculated Water Dissociation Rate Constants for AA·M$^+$(H$_2$O), M = Li or Na, Modeled at T = 324 K Using a Threshold Dissociation Energy of 77 kJ/mol for M = Li and 53 kJ/mol for M = Na and Transition State Entropies Corresponding to A-Factors of 10^{13} and 10^{17} s^{-1} [a]

M	A-factor (s^{-1})	AA	k (s^{-1})	% difference
Li	10^{13}	α-Me-Pro ZW	0.00579	
		ProOMe	0.00598	+3.3%
		N-Me-Pro ZW	0.00648	+11.9%
	10^{17}	α-Me-Pro ZW	0.00672	
		ProOMe	0.00684	+1.8%
		N-Me-Pro ZW	0.00748	+11.3%
Na	10^{13}	α-Me-Pro ZW	0.0185	
		ProOMe	0.0203	+9.7%
		N-Me-Pro ZW	0.0179	−3.2%
	10^{17}	α-Me-Pro ZW	0.0185	
		ProOMe	0.0204	+10.3%
		N-Me-Pro ZW	0.0179	−3.2%

[a] The percent difference is reported relative to AA = α-Me-Pro.

For each of the complexes investigated here, a wide range of transition state entropies are modeled to take into account differences in dissociation pathways or transition states for water molecules that are bound in these complexes. Arrhenius A-factors of 10^{13} and 10^{17} s^{-1}, corresponding to neutral and loose transition states, respectively, are used in the modeling. Loss of a water molecule is expected to occur via a loose transition state, so that our modeling should overestimate the uncertainty due to differences in dissociation entropy. Photon absorption rates are modeled explicitly using calculated transition dipole moments and vibrational frequencies. To determine how similar the photon absorption and emission rates are for the complexes investigated here, water dissociation rates from the lithiated complexes were modeled using a temperature of 324 K, a water binding energy of 77 kJ/mol, and A-factors of 10^{13} and 10^{17} s^{-1}. With these parameters, the water dissociation rate constants for the isomeric clusters are within 12% of each other (Table 1), indicating that these complexes all have similar photon absorption and emission rates.

Model of Proline. Suitable isomeric models of proline were not identified. However, α-methyl-proline (α-Me-Pro) is a very similar molecule and proline methyl ester (ProOMe) is an excellent isomeric model of a nonzwitterionic form of this molecule (Scheme 1). Similarly, *N*-methyl-proline (*N*-Me-Pro) is potentially a good model compound for the zwitterionic form of α-Me-Pro because the tertiary nitrogen should make this protonation site more basic. In the absence of other effects, such as solvation by polarizable side chains, the relative stability of the zwitterionic form relative to the nonzwitterionic form increases with the proton affinity of the molecule.[7,15−17]

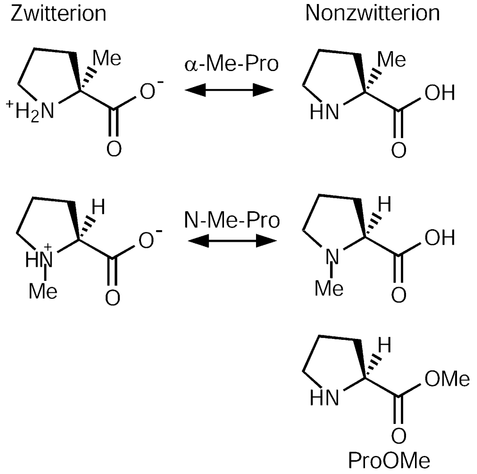
Although both experimental and theoretically derived values of the proton affinity (PA) and gas-phase basicity (GB) of proline have been reported,[18,19,36,37] these values for the proline analogues studied here have not. These values were calculated for proline as well as the three isomers described above. The proton affinities were calculated using eq 1

$$\mathrm{PA}_{298} = -\Delta H_{298} = [E_{\mathrm{el}}(\mathrm{AA{\cdot}H^+}) - E_{\mathrm{el}}(\mathrm{AA})] + [\mathrm{ZPE}(\mathrm{AA{\cdot}H^+}) - \mathrm{ZPE}(\mathrm{AA})] + [H_{298}(\mathrm{AA{\cdot}H^+}) - H_{298}(\mathrm{AA})] - (^5/_2)R^*(298\ \mathrm{K}) \quad (1)$$

TABLE 2: Values of Proton Affinity (PA) and Gas-Phase Basicity (GB) (in kJ/mol) for Pro, α-Me-Pro, *N*-Me-Pro, and ProOMe at 298 K Calculated at the B3LYP/6-31++G Level of Theory**

	Pro	α-Me-Pro	*N*-Me-Pro	ProOMe
PA	941.8	933.5	959.0	971.1
GB	910.0	903.7	927.2	936.8

SCHEME 1



where E_{el} is the electronic energy and ZPE is the zero-point energy. Gas-phase basicities are determined using eq 2

$$\mathrm{GB}_{298} = -\Delta G_{298} = \mathrm{PA}_{298} - (298\ \mathrm{K})*[S(\mathrm{AA{\cdot}H^+}) - S(\mathrm{AA}) - S(\mathrm{H^+})] \quad (2)$$

where *S* is the entropy. The resulting values are given in Table 2. For proline, the PA and GB values are in excellent agreement with those recently reported by others.[19,36,37] The PA and GB values for α-Me-Pro are ~8 kJ/mol lower than those for Pro, indicating that α-Me-Pro should be a good model for Pro. These values for *N*-Me-Pro are significantly higher. The relative stability of the zwitterionic form of this ion compared to the nonzwitterionic form would be expected to be increased by a similar factor.[7,15−17]

BIRD Kinetic Data. The loss of a single water molecule from $\mathrm{AA{\cdot}M^+(H_2O)}$, AA = α-Me-Pro, *N*-Me-Pro, ProOMe, M = Li or Na, is measured as a function of time at temperatures ranging from 50 to 110 °C for lithiated species and from −5 to 37 °C for sodiated species. These experiments are conducted at pressures $\leq 10^{-8}$ Torr, and kinetic parameters obtained from these experiments are in the zero-pressure limit (ZPL).[38,39] Typical plots of $\ln\{[\mathrm{AA{\cdot}M^+(H_2O)}]/([\mathrm{AA{\cdot}M^+(H_2O)}] + [\mathrm{AA{\cdot}M^+}])\}$ versus time are shown in Figure 1 for both lithiated and sodiated clusters. ZPL rate constants for the loss of a water molecule from these complexes are determined from the slopes of these data and are given in Table 3. Correlation coefficients for all these data are ≥0.992, indicating an excellent fit to first-order kinetics.

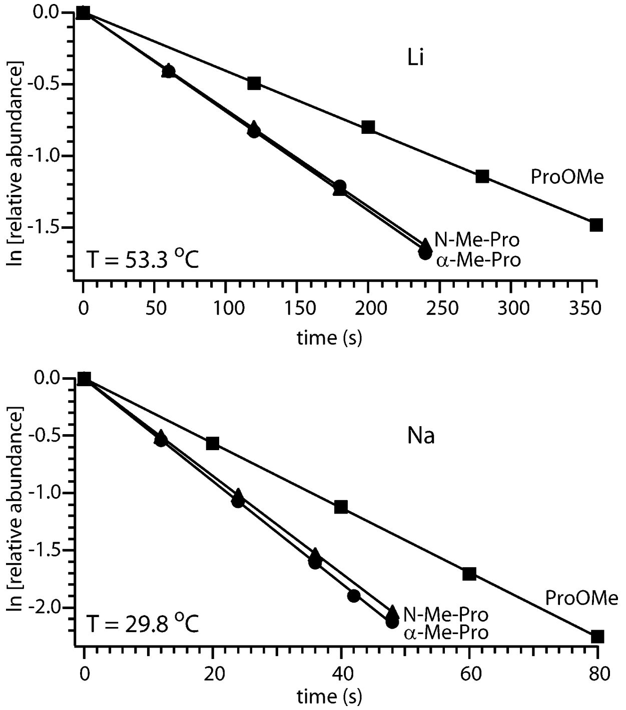


Figure 1. Blackbody infrared radiative dissociation kinetics for the loss of a water molecule from $\mathrm{AA{\cdot}Li^+(H_2O)}$ clusters at *T* = 53.3 °C and from $\mathrm{AA{\cdot}Na^+(H_2O)}$ clusters at *T* = 29.8 °C. The identity of the AA is labeled on the figure.

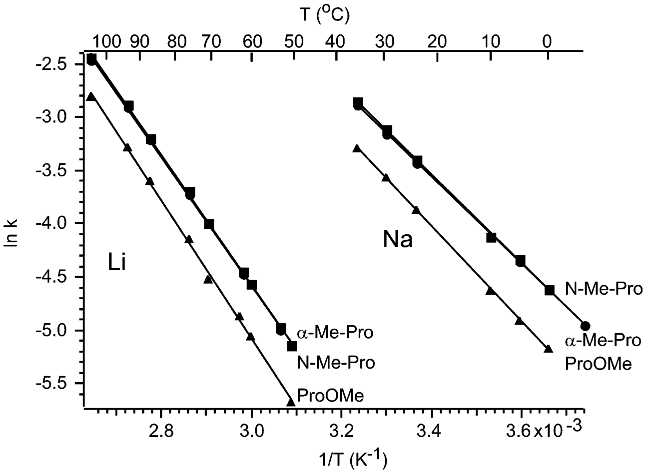


Figure 2. Arrhenius plot for the loss of a water molecule from $\mathrm{AA{\cdot}M^+(H_2O)}$, M = Li or Na. The data are fit between 51 and 105 °C for $\mathrm{AA{\cdot}Li^+(H_2O)}$ and between 0 and 36 °C for $\mathrm{AA{\cdot}Na^+(H_2O)}$ for AA = ProOMe and α-Me-Pro (−5 and 36 °C for *N*-Me-Pro$\cdot\mathrm{Na^+(H_2O)}$).

These ZPL rate constants measured as a function of temperature are used to construct an Arrhenius plot (Figure 2) from which ZPL Arrhenius parameters are obtained. These values for each cluster of interest are given in Table 4. As clearly seen in Figure 2, the dissociation rate constants for α-Me-Pro and *N*-Me-Pro are indistinguishable at all temperatures studied. The ZPL activation energies are also indistinguishable. In striking contrast, the rate constants for ProOMe are significantly lower, with ZPL activation energies ~3 kJ/mol higher than those of the other two species for both Li and Na. On the basis of these kinetic measurements alone, it is clear that the water molecule binds nearly identically in both cationized α-Me-Pro and *N*-Me-Pro. Furthermore, the water molecule is bound differently in the cationized ProOMe species.

Threshold Dissociation Energies. For small, weakly bound clusters, such as those investigated here, the ZPL Arrhenius parameters are lower than those measured if these ions were in the rapid energy exchange limit.[35] This is due to a depletion of the higher-energy tail of the thermal population. To obtain E_0 for the loss of a water molecule from these species, these experimental data can be modeled using a master equation modeling approach. Parameters that go into this model include the infrared transition dipole moments and vibrational frequencies that are obtained from candidate low-energy structures (vide

TABLE 3: Measured BIRD Kinetic Rate Constants for the Loss of a Water Molecule from $\mathrm{AA{\cdot}M^+(H_2O)}$, M = Li ($T$ = 53.3 °C) or Na (T = 29.8 °C)[a]

M	AA	k	% difference
Li	ProOMe	0.00410 ± 0.00005	−40.6%
	α-Me-Pro	0.0069 ± 0.0002	
	N-Me-Pro	0.00679 ± 0.00005	−1.5%
Na	ProOMe	0.0283 ± 0.0002	−36.5%
	α-Me-Pro	0.0446 ± 0.0003	
	N-Me-Pro	0.0426 ± 0.0001	−4.5%

[a] The percent difference is reported relative to AA = α-Me-Pro$\cdot$M$^+$(H$_2$O).

TABLE 4: ZPL Arrhenius Parameters for the Loss of a Water Molecule from $\mathrm{AA{\cdot}M^+(H_2O)}$, M = Li or Na

M	AA	E_a (kJ/mol)	log A
Li	ProOMe	53 ± 1	6.2 ± 0.1
	α-Me-Pro	50 ± 1	5.9 ± 0.1
	N-Me-Pro	50 ± 1	6.0 ± 0.1
Na	ProOMe	38 ± 1	4.8 ± 0.1
	α-Me-Pro	35 ± 1	4.6 ± 0.1
	N-Me-Pro	34 ± 1	4.5 ± 0.1

TABLE 5: Threshold Dissociation Energies and Binding Enthalpies (in kJ/mol) for the Loss of a Water Molecule from $\mathrm{AA{\cdot}M^+(H_2O)}$, M = Li or Na, Determined from Master Equation Modeling of the BIRD Experimental Data, Using Transition Dipole Moment Multiplication Factors (*μ*) Ranging from 0.8 to 1.2

$\mathrm{AA{\cdot}M^+(H_2O)}$		μ				
M	AA	0.8	1.0	1.2	E_0	ΔH_{298}
Li	α-Me-Pro	77−78	77−78		77.5 ± 0.5	73.0 ± 0.5
	N-Me-Pro		76−78	76−78	77 ± 1	72 ± 1
	ProOMe		80−81	80	80.5 ± 0.5	75.0 ± 0.5
Na	α-Me-Pro	52−53	52−54		53 ± 1	48 ± 1
	N-Me-Pro	52−53	53−54		53 ± 1	46 ± 1
	ProOMe	57−58			57.5 ± 0.5	51.0 ± 0.5

infra). The values of E_0 that are obtained from this modeling do not strongly depend on these parameters for different low-energy structures of the same species.[40] The E_0 values for each of these clusters is given in Table 5. For both the lithiated and sodiated species, the values of E_0 for both α-Me-Pro and *N*-Me-Pro are indistinguishable. In contrast, these values for ProOMe are 3 and 5 kJ/mol higher for the Li and Na cationized species, respectively. It is important to emphasize that the reported error bars do not take into account systematic errors that may be present in our modeling parameters. However, any systematic error that may be present should be similar for each of these three isomers so that the *difference* in threshold dissociation energy, although very small, can be clearly resolved in these experiments.

In a previous study using the same instrument and experimental conditions as the present work, an E_0 value of 80−85 kJ/mol was reported for alanine ethyl ester$\cdot$Li$^+$(H$_2$O) and 76−81 kJ/mol was reported for betaine$\cdot$Li$^+$(H$_2$O).[6] These values are comparable to the value 80 ± 1 kJ/mol measured for ProOMe$\cdot$Li$^+$(H$_2$O) and the value 77 ± 1 kJ/mol measured for α-Me-Pro and *N*-Me-Pro. Lithiated betaine forms a salt-bridge structure in which betaine is a zwitterion. In contrast, alanine ethyl ester and proline methyl ester do not have acidic hydrogen atoms and cannot form salt-bridge or zwitterionic structures. These structures form charge-solvated structures instead. The E_0 value measured for lithiated alanine ethyl ester is ∼4 kJ/mol higher than that for lithiated betaine. Similarly, E_0 for proline methyl ester is ∼3 kJ/mol higher than that for either α-Me-Pro or *N*-Me-Pro. The higher E_0 for the nonzwitterionic model compound provides compelling evidence that both α-Me-Pro and *N*-Me-Pro form salt-bridge structures in which these molecules are zwitterionic. The slightly lower E_0 values for the lithiated proline analogues compared to the lithiated valine analogues investigated previously could be due to the effects of the secondary amine in proline. However, this effect was too small to measure in a study of sodiated isomers.[7] Because α-Me-Pro is less basic than proline, we conclude that lithiated proline with one water molecule should also be zwitterionic.

In contrast, experimental results of Wesdemiotis and co-workers in which Li$^+$-bound dimers of proline and proline methyl ester are dissociated indicated that lithiated proline is nonzwitterionic.[28] Their calculations, however, indicated that Pro$\cdot$Li$^+$ forms a salt-bridge structure in which Pro is zwitterionic, consistent with the results presented here. They attributed this discrepancy between their experiments and theory to proline methyl ester inducing a structural change in proline in the cluster. In our experiments, the structures and the relative energetics of the clusters may also be affected by the presence of a single water molecule, but our calculations suggest that this effect is very small (vide infra).

For sodium cationized species, threshold dissociation energies reported for the loss of a water molecule from sodiated sarcosine ethyl ester and alanine ethyl ester were 67 ± 1 kJ/mol.[7] For sodiated betaine, this value was 63 ± 1 kJ/mol. For these sodiated clusters, E_0 for the nonzwitterionic species is ∼4 kJ/mol higher than that for the zwitterionic species. Here, the E_0 value for ProOMe$\cdot$Na$^+$ is ∼4 kJ/mol higher than those values measured for the corresponding clusters with α-Me-Pro and *N*-Me-Pro. The lower E_0 value for the loss of a water molecule again provides strong evidence that both these latter species are zwitterionic in these clusters. On the basis of the lower PA of α-Me-Pro versus Pro, we expect that Pro would be zwitterionic in these clusters as well. This result is consistent with previous theoretical studies and the kinetic method experiments of Wesdemiotis and co-workers[28] and the spectroscopic studies of Ohanessian and co-workers.[30]

Low-Energy Structures. It is necessary to calculate low-energy structures for each of these isomers to obtain parameters used in the master equation modeling process and to determine how water binds to the cluster, that is, if the water binding in a reference molecule is similar to the corresponding form of the unknown. It is also useful to elucidate the extent to which a water molecule affects the structure and relative energetics of different forms of the amino acid of interest in the hydrated species. Lowest-energy structures calculated at the B3LYP/6-31++G** level of theory for AA$\cdot$Li$^+$ are shown in Figure 3; structures for the sodiated species have the same mode of metal ion binding. Relative energies for α-Me-Pro$\cdot$M$^+$ at various levels of theory are given in Table 6. The lowest-energy structures of α-Me-Pro$\cdot$M$^+$ are similar to those found previously for Pro$\cdot$M$^+$.[28,29] It is possible that the structures shown here do not have the correct “ring pucker”, but the endo versus exo ring structures differ in energy by <2 kJ/mol,[29] an insignificant difference that should not affect any of our conclusions. The metal ions in ProOMe$\cdot$M$^+$ and in the nonzwitterionic forms of α-Me-Pro$\cdot$M$^+$ and *N*-Me-Pro$\cdot$M$^+$ are bound to both the amine nitrogen and the carbonyl oxygen atoms (NO coordination). Thus, ProOMe appears to be an excellent model for the nonzwitterionic forms of these other two isomers. For the zwitterionic forms of α-Me-Pro$\cdot$M$^+$ and *N*-Me-Pro$\cdot$M$^+$, the metal ions interact with both oxygen atoms of the carboxylate group (OO coordination).

At the B3LYP/6-311++G**//B3LYP/6-31++G** level of theory, including zero-point energy and ΔH_{298} corrections,

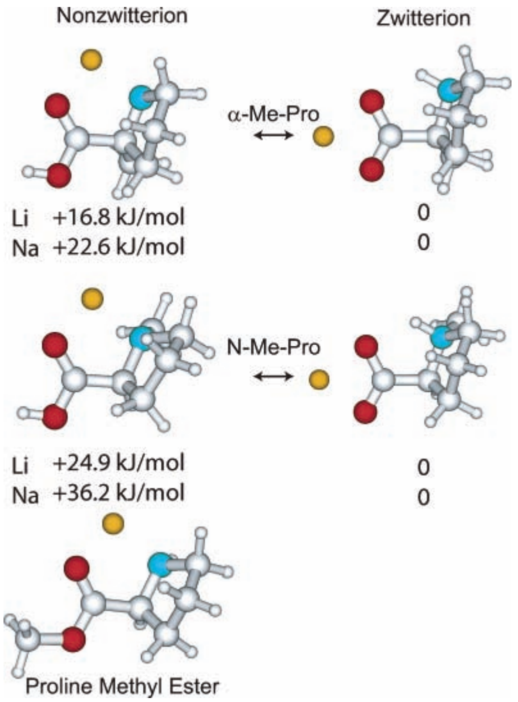


Figure 3. Lowest-energy structures of AA·M^+ complexes at the B3LYP/6-31++G** level of theory. The relative energies (in kJ/mol) are from single-point energy calculations at the B3LYP/6-311++G** level of theory and include zero-point energy and ΔH_{298} corrections.

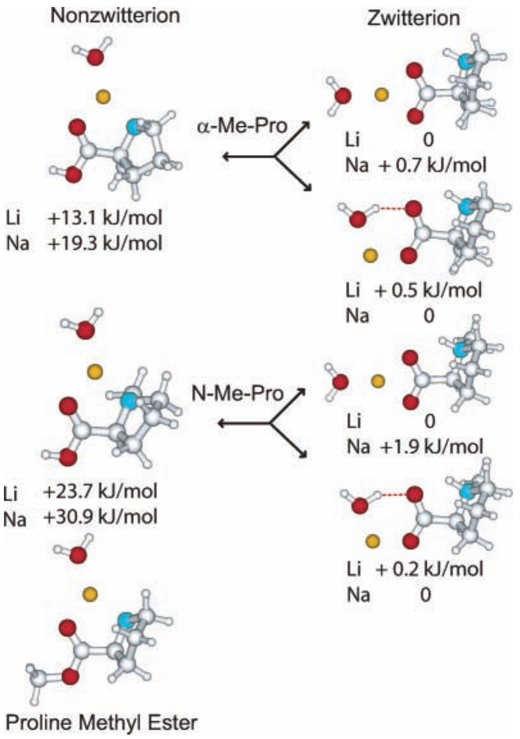


Figure 4. Lowest-energy structures of AA·$M^+(H_2O)$ complexes at the B3LYP/6-31++G** level of theory. The relative energies (in kJ/mol) are from single-point energy calculations at the B3LYP/6-311++G** level of theory and include zero-point energy and ΔH_{298} corrections.

TABLE 6: Energy (in kJ/mol) of the Lowest-Energy Nonzwitterionic Form of α-Me-Pro·M^+ and α-Me-Pro·$M^+(H_2O)$, M = Li or Na, Relative to the Lowest-Energy Zwitterionic Form at Various Levels of Theory[a]

method/basis set	α-Me-Pro·Li^+	α-Me-Pro·$Li^+(H_2O)$
B3LYP/6-31G*	21.3	17.2
B3LYP/6-31+G*	25.1	21.3
B3LYP/6-31++G**	18.2	15.8
ΔZPE	−1.9	−1.8
ΔH_{298}	−0.06	−0.4
total B3LYP/6-31++G**	16.3	14.1
B3LYP/6-311++G**[b]	17.8	15.5
ΔZPE	−0.7	−2.0
ΔH_{298}	−0.3	−0.4
total B3LYP/6-311++G**[b]	16.8	13.1

method/basis set	α-Me-Pro·Na^+	α-Me-Pro·$Na^+(H_2O)$
B3LYP/6-31G*	28.0	23.8
B3LYP/6-31+G*	32.6	30.5
B3LYP/6-31++G**	25.4	24.7
ΔZPE	−2.6	−4.3
ΔH_{298}	0.1	−0.03
total B3LYP/6-31++G**	22.8	20.3
B3LYP/6-311//G**[b]	24.7	20.8
ΔZPE	−2.1	−3.3
ΔH_{298}	0.09	1.8
total B3LYP/6-311++G**[b]	22.6	19.3

[a] Total = $\Delta E_{\text{electronic}}$ + ΔZPE + ΔH_{298}. [b] Single-point energy calculation using the B3LYP/6-31++G** geometry.

zwitterionic *N*-Me-Pro·M^+ is 24.9 and 36.2 kJ/mol more stable than the nonzwitterionic form for M = Li and Na, respectively. The α-Me-Pro·M^+ zwitterion is more stable than the nonzwitterionic form by 16.8 and 22.6 kJ/mol for M = Li and Na, respectively. The difference in the relative zwitterionic stability of α-Me-Pro·Li^+ and *N*-Me-Pro·Li^+ is 8.1 kJ/mol, whereas, for α-Me-Pro·Na^+ and *N*-Me-Pro·Na^+, the difference is 13.6 kJ/mol. These differences are somewhat less than the 26 kJ/mol difference in proton affinities between the two molecules. Marino et al. reported that the Pro·M^+ zwitterion is more stable than the nonzwitterion by 5.4 and 17.6 kJ/mol for M = Li and Na, respectively, at the B3LYP/6-311++G** level of theory.[29]

For the clusters with one water molecule, the lowest-energy structures calculated at the B3LYP/6-31++G** level of theory are shown in Figure 4. The relative energies of the α-Me-Pro·M^+ structures at various levels of theory are given in Table 6. In each of the clusters, the water molecule binds directly to the metal ion and there is no significant difference in the structures of the lowest-energy zwitterionic and nonzwitterionic forms of these molecules compared to the corresponding structures with no water molecules. The mode of water molecule and metal ion binding in ProOMe·$M^+(H_2O)$ is the same as that in the nonzwitterionic forms of α-Me-Pro·$M^+(H_2O)$ and *N*-Me-Pro·$M^+(H_2O)$. Thus, ProOMe·$Li^+(H_2O)$ appears to be an excellent model for the nonzwitterionic forms of these molecules. Similarly, the zwitterionic forms of *N*-Me-Pro·$M^+(H_2O)$ and α-Me-Pro·$M^+(H_2O)$ have the same mode of metal ion and water molecule binding. At the B3LYP/6-311++G**//B3LYP/6-31++G** level of theory, including zero-point energy and ΔH_{298} corrections, the α-Me-Pro·$M^+(H_2O)$ zwitterion is more stable than the nonzwitterionic form by 13.1 and 19.3 kJ/mol for M = Li and Na, respectively. Zwitterionic *N*-Me-Pro·M^+ is 23.7 and 30.9 kJ/mol more stable than the nonzwitterionic form for M = Li and Na, respectively. The results of these calculations are consistent with the experimental results that clearly show that the water molecule is bound differently in the nonzwitterionic isomer, ProOMe·M^+, than it is in the other two molecules and that the other two molecules are zwitterionic.

Water Binding Energies. To the extent that there is no significant reverse activation barrier for the loss of a water molecule from any of these clusters, the binding energy of a

TABLE 7: Binding Enthalpies at 298 K (Including Zero-Point Energy Corrections) of a Water Molecule to $AA\cdot M^+(H_2O)$ (in kJ/mol) from Density Functional Calculations at the B3LYP/6-311++G//B3LYP/6-31++G** Level of Theory**

M	α-Me-Pro NZ	α-Me-Pro ZW	ProOMe	*N*-Me-Pro NZ	*N*-Me-Pro ZW
Li	88	84	85	86	85
Na	65	61	66	66	61

water molecule to these clusters can be determined from the measured threshold dissociation energies from eq 3

$$\Delta H(T) = E_0 + E_{\mathrm{vib}}{}^{T}(\mathrm{AA}\cdot\mathrm{M}^+) + E_{\mathrm{vib}}{}^{T}(\mathrm{H_2O}) - E_{\mathrm{vib}}{}^{T}(\mathrm{AA}\cdot\mathrm{M}^+(\mathrm{H_2O})) + 4RT \quad (3)$$

where $T = 298$ K and $E_{\mathrm{vib}}{}^{T}$ is the vibrational energy at temperature T. These values are given in Table 5. The experimentally derived binding enthalpies for the α-Me-Pro·$M^+(H_2O)$ and *N*-Me-Pro·$M^+(H_2O)$ complexes are the same within error, and these values are 2−5 kJ/mol lower than those for the ProOMe·$M^+(H_2O)$ complexes. These enthalpies can be directly compared to the calculated binding energies determined from the lowest-energy structures (Table 7). All of the calculated binding energies at the B3LYP/6-311++G**//B3LYP/6-31++G** level of theory are ∼15 kJ/mol higher than the experimental binding enthalpies. Previous studies have also shown that B3LYP ligand−metal binding energies tend to be systematically higher than measured values,[6,7,41−43] although the magnitude of the deviation reported here is higher than that in other systems. Without a single-point energy calculation, this deviation is ∼19 kJ/mol. A full geometry optimization with the larger basis set could potentially reduce the systematic deviation further.[41] The calculated binding energies for the lithiated and sodiated complexes are all within 3 and 5 kJ/mol, respectively, and there appear to be no significant differences between nonzwitterionic and zwitterionic isomers given the error at this level of theory.

Conclusions

The structures of α-Me-Pro·$M^+(H_2O)$, M = Li or Na, and two isomers were investigated using both blackbody infrared radiative dissociation and theory. Both experiment and theory indicate that the lowest-energy structure of α-Me-Pro in this cluster is zwitterionic. Theory indicates that the lowest-energy structure is one in which lithium interacts with the carboxylate group and the water molecule interacts solely with the metal ion, whereas, in the lowest-energy structure with sodium, the metal ion interacts with only one carboxylate oxygen atom and the water molecule interacts with the other oxygen atom and the metal ion. The experimentally derived threshold dissociation energies show that the water binding energies to the nonzwitterionic or charge-solvated complexes are 3.0−4.5 kJ/mol higher than these values for the zwitterionic or salt-bridge complexes for both Li^+ and Na^+. The relative energy difference between the zwitterionic and nonzwitterionic forms of these ions is slightly lower than the difference in the molecular proton affinities. Because proline has a slightly higher proton affinity than α-Me-Pro, it is expected that lithiated and sodiated proline should also form salt-bridge structures in which proline is zwitterionic in these clusters.

In these experiments, the structures of these clusters are deduced from the threshold dissociation energies for the loss of a water molecule from the clusters. These results clearly show that both the dissociation kinetics and the thermodynamic binding energy of a water molecule to the cluster are exquisitely sensitive probes of zwitterionic versus nonzwitterionic structure in these clusters. Attachment of a water molecule to a cluster is likely to preferentially stabilize one form over the other. In cases where the zwitterionic and nonzwitterionic forms of a molecule are very close in energy, this may result in a different structure in the cluster with a water molecule versus the cluster without a water molecule. For the ions in this study, calculations indicate that the attachment of a water molecule to these cationized species does not have a significant effect on either the relative energies of the zwitterionic versus nonzwitterionic forms or their structures. In addition, the zwitterionic forms of both α-Me-Pro·$M^+(H_2O)$ and *N*-Me-Pro·$M^+(H_2O)$ are significantly more stable than the nonzwitterionic forms. Thus, the structures of the clusters in this study are likely the same both with and without a single water molecule.

Acknowledgment. Financial support was provided by the National Science Foundation (grant CHE-0415293). This work was partially supported by the National Computational Science Alliance under CHE0200959N and utilized the NSCA SGI/CRAY Origin2000 and IBM p690. A.S.L. gratefully acknowledges training support from the NIH T32GM08295.

References and Notes

(1) Kassab, E.; Langlet, J.; Evleth, E.; Akacem, Y. *J. Mol. Struct. (THEOCHEM)* **2000**, *531*, 267−282.

(2) Jensen, J. H.; Gordon, M. S. *J. Am. Chem. Soc.* **1995**, *117*, 8159−8170.

(3) Ai, H.; Bu, Y.; Han, K. *J. Chem. Phys.* **2003**, *118*, 10973−10985.

(4) Jockusch, R. A.; Lemoff, A. S.; Williams, E. R. *J. Am. Chem. Soc.* **2001**, *123*, 12255−12265.

(5) Jockusch, R. A.; Lemoff, A. S.; Williams, E. R. *J. Phys. Chem. A* **2001**, *105*, 10929−10942.

(6) Lemoff, A. S.; Williams, E. R. *J. Am. Soc. Mass Spectrom.* **2004**, *15*, 1014−1024.

(7) Lemoff, A. S.; Bush, M. F.; Williams, E. R. *J. Am. Chem. Soc.* **2003**, *125*, 13576−13584.

(8) Snoek, L. C.; Kroemer, R. T.; Simons, J. P. *Phys. Chem. Chem. Phys.* **2002**, *4*, 2130−2139.

(9) Snoek, L. C.; Kroemer, R. T.; Hockridge, M. R.; Simons, J. P. *Phys. Chem. Chem. Phys.* **2001**, *3*, 1819−1826.

(10) Xu, S.; Nilles, J. M.; Bowen, K. H., Jr. *J. Chem. Phys.* **2003**, *119*, 10696−10701.

(11) Rak, J.; Skurski, P.; Simons, J.; Gutowski, M. *J. Am. Chem. Soc.* **2001**, *123*, 11695−11707.

(12) Julian, R. R.; Beauchamp, J. L.; Goddard, W. A. *J. Phys. Chem. A* **2002**, *106*, 32−34.

(13) Skurski, P.; Rak, J.; Simons, J.; Gutowski, M. *J. Am. Chem. Soc.* **2001**, *123*, 11073−11074.

(14) Jockusch, R. A.; Price, W. D.; Williams, E. R. *J. Phys. Chem. A* **1999**, *103*, 9266.

(15) Wyttenbach, T.; Witt, M.; Bowers, M. T. *J. Am. Chem. Soc.* **2000**, *122*, 3458−3464.

(16) Strittmatter, E. F.; Wong, R. L.; Williams, E. R. *J. Phys. Chem. A* **2000**, *104*, 10271−10279.

(17) Strittmatter, E. F.; Williams, E. R. *Int. J. Mass Spectrom.* **2001**, *212*, 287−300.

(18) Gorman, G. S.; Speir, J. P.; Turner, C. A.; Amster, I. J. *J. Am. Chem. Soc.* **1992**, *114*, 3986−3988.

(19) Kuntz, A. F.; Boynton, A. W.; David, G. A.; Colyer, K. E.; Poutsma, J. C. *J. Am. Soc. Mass Spectrom.* **2002**, *13*, 72−81.

(20) Stepanian, S. G.; Reva, I. D.; Radchenko, E. D.; Adamowicz, L. *J. Phys. Chem. A* **2001**, *105*, 10664−10672.

(21) Lesarri, A.; Mata, S.; Cocinero, E. J.; Blanco, S.; Lopez, J. C.; Alonso, J. L. *Angew. Chem., Int. Ed.* **2002**, *41*, 4673−4676.

(22) Czinki, E.; Csaszar, A. G. *Chem. Eur. J.* **2003**, *9*, 1008−1019.

(23) Reva, I. D.; Stepanian, S. G.; Plokhotnichenko, A. M.; Radchenko, E. D.; Sheina, G. G.; Blagoi, Y. P. *J. Mol. Struct.* **1994**, *318*, 1−13.

(24) Shoeib, T.; Hopkinson, A. C.; Siu, K. W. M. *J. Phys. Chem. B* **2001**, *105*, 12399−12409.

(25) Shoeib, T.; Siu, K. W. M.; Hopkinson, A. C. *J. Phys. Chem. A* **2002**, *106*, 6121−6128.

(26) Hoyau, S.; Ohanessian, G. *C. R. Acad. Sci., Ser. IIc* **1998**, *1*, 795−799.

(27) Shoeib, T.; Rodriquez, C. F.; Siu, K. W. M.; Hopkinson, A. C. *Phys. Chem. Chem. Phys.* **2001**, *3*, 853−861.

(28) Talley, J. M.; Cerda, B. A.; Ohanessian, G.; Wesdemiotis, C. *Chem. Eur. J.* **2002**, *8*, 1377−1388.

(29) Marino, T.; Russo, N.; Toscano, M. *J. Phys. Chem. B* **2003**, *107*, 2588−2594.

(30) Kapota, C.; Lemaire, J.; Maitre, P.; Ohanessian, G. *J. Am. Chem. Soc.* **2004**, *126*, 1836−1842.

(31) Price, W. D.; Schnier, P. D.; Williams, E. R. *Anal. Chem.* **1996**, *68*, 859−866.

(32) Wong, R. L.; Paech, K.; Williams, E. R. *Int. J. Mass Spectrom.* **2004**, *232*, 59−66.

(33) Rodriguez-Cruz, S. E.; Klassen, J. S.; Williams, E. R. *J. Am. Soc. Mass Spectrom.* **1999**, *10*, 958−968.

(34) Price, W. D.; Schnier, P. D.; Williams, E. R. *J. Phys. Chem. B* **1997**, *101*, 664−673.

(35) Price, W. D.; Williams, E. R. *J. Phys. Chem. A* **1997**, *101*, 8844−8852.

(36) Pepe, C.; Rochut, S.; Paumard, J. P.; Tabet, J.-C. *Rapid Commun. Mass Spectrom.* **2004**, *18*, 307−312.

(37) Mezzache, S.; Afonso, C.; Pepe, C.; Karoyan, P.; Fournier, F.; Tabet, J.-C. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 1626−1632.

(38) Price, W. D.; Schnier, P. D.; Jockusch, R. A.; Strittmatter, E. F.; Williams, E. R. *J. Am. Chem. Soc.* **1996**, *118*, 10640−10644.

(39) Dunbar, R. C.; McMahon, T. B. *Science* **1998**, *279*, 194−197.

(40) Jockusch, R. A.; Williams, E. R. *J. Phys. Chem. A* **1998**, *102*, 4543−4550.

(41) Dunbar, R. C. *J. Phys. Chem. A* **2002**, *106*, 7328−7337.

(42) Armentrout, P. B.; Rodgers, M. T. *J. Phys. Chem. A* **2000**, *104*, 2238−2247.

(43) Armentrout, P. B. *Top. Curr. Chem.* **2003**, *225*, 233−262.