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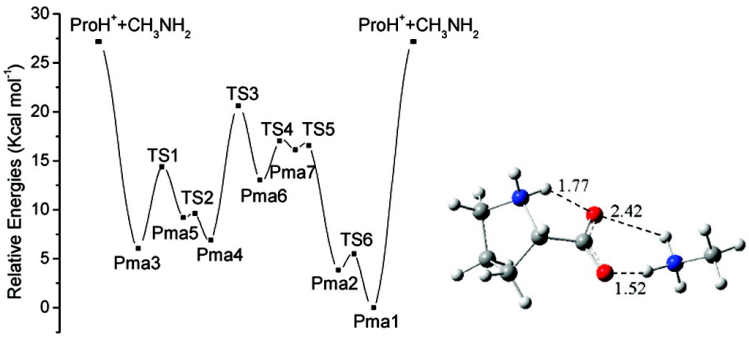

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Stabilization of Zwitterionic Structures of Amino Acids (Gly, Ala, Val, Leu, Ile, Ser and Pro) by Ammonium Ions in the Gas Phase

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Abstract: The thermochemistry of gas-phase ion−molecule interactions and structures of a variety of clusters formed between protonated amino acids and either ammonia or amines have been studied by pulsed ionization high-pressure mass spectrometry (HPMS) and ab initio calculations. The enthalpy changes for the association reactions of protonated Gly, Ala, Val, Leu, Ile, Ser, and Pro with ammonia have been measured as −23.2, −21.9, −21.0, −20.8, −20.6, −22.6, and −20.4 kcal mol^{-1}, respectively. A very good linear relationship exists between the enthalpy changes and the proton affinities (PAs) of the amino acids, with an exception of Ser, where the hydroxyl substituent forms an extra hydrogen bond with ammonia. For the association reaction of protonated proline and methylamine, the measured enthalpy and entropy changes are −26.6 kcal mol^{-1} and −30.1 cal mol^{-1} K^{-1}, respectively. The experimental and calculated results indicate that the zwitterionic structure of proline may be well stabilized by $CH_3NH_3^+$. For the first time, the interaction strengths between these amino acids and NH_4^+ have been obtained, and comparison with Na^+ is discussed. Stabilization of zwitterionic structures of a series of amino acids (Gly, Ala, Val, Ser, and Pro) by various ammonium ions (NH_4^+, $CH_3NH_3^+$, $(CH_3)_2NH_2^+$, and $(CH_3)_3NH^+$) has been investigated systematically. Energy decomposition analysis has been performed so that the salt bridge interaction strengths between zwitterionic amino acids and ammonium ions have been obtained. Some generalizations with respect to the relative stability of zwitterionic structures may be drawn. First, as the PA of an amino acid increases, within a series of Gly, Ala, Val, the zwitterionic structure becomes more energetically favorable relative to a non-zwitterionic isomer. Second, as the PA of an amine increases, the zwitterionic structure of a given amino acid within the complex becomes gradually less favorable. Third, compared to the other amino acids, Pro, the only secondary amine among the 20 naturally occurring amino acids, has a much more pronounced tendency to form the zwitterionic structure, which has been confirmed by the experimental results. Finally, substitutents on the amino acid backbone that may participate in additional hydrogen bond interactions in non-zwitterionic isomer may render it more stable, as seen in Ser. These organic ammonium ions are found to be able to very effectively stabilize the zwitterionic structure of amino acids, even more effectively than metal ions, which aids significantly in the understanding of why zwitterionic structures exist extensively in biological systems.

1. Introduction

Naturally occurring amino acids are well-known to exist as zwitterions in the solid state and in aqueous solution within a wide pH range. However the structures of amino acids in the gas phase are in the canonical form. In biological systems, the electric field caused by zwitterionic structures is the driving force for determination of the structure, function, and activity of amino acids, peptides, and proteins.[1−3] Therefore, it is of fundamental importance to study the zwitterionic structures of amino acids and salt bridge interactions in the isolated gas phase.

First, the stability of the zwitterionic form of an amino acid is a function of a number of factors such as the properties of the side chains. For the simplest amino acid, glycine, a zwitterionic structure is not even a local minimum on the gas-phase potential energy surface (PES)[4−6] and is about 20 kcal mol^{-1} in electronic energy higher than the most stable non-zwitterionic form.[7] However, an arginine zwitterion with a protonated side chain and deprotonated carboxyl group has been calculated to be either similar or only several kcal mol^{-1} higher in energy than the most stable canonical structure.[8−10] In comparison to the zwitterionic structure of glycine, the stability

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of zwitterions of arginine can be seemed to be due to the substantial basicity of the guanidine side chain.

Solvent plays an extremely important role in stabilizing the zwitterionic structure of amino acids, which results in the transformation from a non-zwitterionic to a zwitterionic structure when the isolated amino acid is dissolved in water or other solvents. It is of some interest to know the minimum number of water molecules needed to stabilize the zwitterionic structure of an amino acid in the gas phase. For glycine, Gordon et al.[11] reported that just two water molecules can stabilize the glycine zwitterion into a local minimum on the PES. Kassab et al.[7] found that three water molecules are sufficient to make the energy of a glycine zwitterion similar to its non-zwitterionic form. As many as six water molecules have been suggested to be necessary in another study.[12] Sized-selected photoelectron spectroscopic studies have shown that five water molecules are needed to transform glycine into its zwitterionic form, and four water molecules are required for phenylalanine and tryptophan.[13] Simons et al.[14] found that three water molecules cause the energy of the zwitterionic form of tryptophan to be only slightly higher than that of the non-zwitterionic counterpart. Suhai et al.[15] reported that four water molecules could stabilize the alanine zwitterion. Very recent experimental and computational results further indicate that the canonical and zwitterionic structures of Trp coexist in the complexes formed with five to six water molecules.[16] In addition, self-complex formation resulting in a neutral dimer can also stabilize the zwitterionic structure, most notably for arginine.[17]

The interactions between amino acids and different metal ions have also been studied extensively.[18−31] Bowers et al.[32] investigated the influence of proton affinity and alkali ion addition on the stabilization of zwitterionic structures of amino acids in the gas phase using ion mobility mass spectrometry combined with the theoretical calculations. The same methods were also used to study the interaction between arginine and Na^+ or Cs^+ ions, with the result that the salt bridge structures are found to be about 10 kcal mol^{-1} more stable than the charge solvated forms with both alkali ions.[33] Williams et al. used blackbody infrared radiative dissociation (BIRD) to study the effect of metal ion and water coordination on the zwitterionic structures of amino acids.[34−36] According to the order of the binding energies between metal ion and free acid, ester, or zwitterion (free acid < ester < zwitterion) and measured binding energies between arginine and alkali metal ions, Wesdemiotis et al. inferred that the Li^+ or Na^+ complex with arginine contains the free acid form of arginine (charge solvation), whereas the complex with larger K^+ or Cs^+ ions involves a zwitterionic arginine.[37] The combination of a free electron laser and a Fourier transfer ion cyclotron resonance (FT-ICR) or ion trap mass spectrometer is very powerful technique to record the vibrational spectra of gas-phase ions, thus permitting the ionic structure to be very clearly elucidated.[38−40] This method has been used to investigate Gly-Na^+ and Pro-Na^+, in which Gly is not a zwitterionic structure whereas Pro exists as a zwitterion.[41]

Organic ammonium ions, i.e., R_4N^+ (R═H, alkyl group), are very common and also exist extensively as entities in protonated amino acids, peptides, nucleic acid bases, and many other biological molecules, including the zwitterionic form of neutral amino acids and peptides. In biological systems, salt bridge interactions including ammonium ions are ubiquitous. However, their role in stabilizing the zwitterionic structures still remains elusive in the isolated state. Therefore, it is very interesting and meaningful to investigate the interactions between amino acids and organic ammonium ions and the stabilization of the zwitterionic structure of amino acids in the gas phase.

In the present work, the interactions between protonated amino acids (Gly, Ala, Val, Leu, Ile, Ser, and Pro) with ammonia or amines (methylamine, dimethylamine, and trimethylamine) have been studied by high-pressure mass spectrometry (HPMS) and *ab initio* calculations. Many isomers of these clusters have been calculated, and the potential energy surface for the interconversion of the isomers of protonated Pro and methylamine has been constructed. For the first time, the interaction strengths between these amino acids and NH_4^+ have been obtained, and their comparison with Na^+ is presented. The stabilization of the zwitterionic structure of a series of amino acids by various ammonium ions has been investigated systematically. Using energy decomposition methods, the salt bridge interaction strengths between zwitterionic amino acids and ammonium ions have also been obtained. Some generalizations with respect to the relative stability of zwitterionic structures of amino acids have been drawn. These will facilitate the further understanding of the structure, property, and function of zwitterions in biological systems.

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2. Experimental and Computational Section

2.1. Experimental. All experiments were carried out on a pulsed high-pressure mass spectrometer constructed at the University of Waterloo and whose general design has been described in detail elsewhere.[42−44] The instrument used in the present work is configured around a double focusing reversed geometry (B-E) magnetic sector mass spectrometer (VG ZAB-2F) mated to the home-built high-pressure ion source. Experiments were carried out in positive ion mode with an ion energy of 4 keV.

Gas mixtures were prepared in a 2.54 L reservoir using methane as the bath gas at a pressure of 500−1200 Torr. The partial pressure of ammonia or methylamine was typically in the range 0.1−50 Torr, depending upon the ion signal intensities desired for the particular equilibrium to be examined. A small amount of CCl_4 was added to the reservoir in order to increase the signal intensity at long ion source residence times by slowing the rate of ion diffusion to the source walls. The gas mixture was flowed into the ion source to a total pressure of 5−10 Torr. A solid sample of the amino acid of interest was introduced directly inside the high pressure source such that, when the high pressure source is heated, gaseous amino acid is present at its equilibrium vapor pressure. Ionization is initiated by a beam of energetic (2 keV) electrons, from an electron gun external to the ion source, focused onto the 100 μm electron entrance aperture of the ion source. Chemical ionization processes subsequently lead to formation of the desired ions.

In order to obtain equilibrium abundances the electron gun is operated in the pulsed mode. Mass selected ion intensity temporal profiles are monitored using a PC based multichannel scaler data acquisition system, typically configured between 10 and 30 μs dwell time per channel. A total of 1024 channels were acquired, and at least 3000 electron gun pulses were accumulated. The corresponding association reaction is described by eq 1, and the associated equilibrium constant is given by eq 2. The equilibrium constant can be calculated from the relative ionic

$$AlaH^+ + NH_3 \leftrightarrows Ala(NH_3)H^+ \quad (1)$$

$$K_{eq} = \frac{I_{Ala(NH_3)H^+}}{I_{AlaH^+}} \cdot \frac{1}{P_{NH_3}} \quad (2)$$

abundances and the partial pressure of ammonia. The relative ionic abundance is simply the constant ratio of intensities, measured by the HPMS experiment, at a sufficiently long reaction time in the ion intensity temporal profiles. The partial pressure of ammonia is readily determined from the known partial pressure of ammonia added to the gas mixture in the gas sample reservoir and the measured total pressure in the ion source. This pressure can be easily changed over several orders of magnitude by simply changing the partial pressure of ammonia in the gas sample reservoir. As the temperature is changed, K_{eq} can be determined at each temperature. Then, as given by the van't Hoff equation, eq 3, the enthalpy change, ΔH, and the entropy change, ΔS, for the reaction can be obtained from a plot of $\ln(K_{eq})$ vs reciprocal temperature.

$$\ln(K_{eq}) = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad (3)$$

2.2. Ab Initio Calculations. *Ab initio* calculations have been carried out using the Gaussian 03 program package.[45] The structures of the protonated L-amino acids (Gly, Ala, Val, Leu, Ile, Ser, and Pro) and their clusters with ammonia, methylamine, dimethylamine, and trimethylamine were calculated at the density functional theory level, employing the B3LYP exchange-correlation functional and the 6-311+G(d, p) basis set. The calculation of the potential energy surface for the transformation of the isomers of protonated proline clusters with ammonia has also been carried out using the same method. Vibrational frequencies were calculated for all structures using the harmonic approximation to verify that no imaginary frequencies are present for species determined to be minima on the potential energy surface. For the transition states obtained, only one imaginary frequency is found, and by examination of these imaginary frequencies, every transition state could be confirmed to be directly related to the corresponding reactant and product. Zero-point energies and thermal energy corrections at 298 K, based on harmonic frequencies, were also obtained. Basis set superposition error (BSSE) was computed using the counterpoise correction method after the final geometry optimization.[46] The entropies of the association reactions were also computed at the B3LYP/6-311+G(d, p) level of theory. In order to obtain more accurate interaction enthalpies, single-point energies have been computed for the global and local minima at the MP2(full)/6-311++G(2d,2p)//B3LYP/6-311+G(d, p) level of theory.[47]

3. Results

3.1. Equilibrium Measurements and Computational Energetics. The experimental van't Hoff plots for the association reactions of protonated Ala, Val, Leu, Ile, Ser, and Pro with ammonia and protonated Pro with methylamine investigated are shown in Figure 1, and the thermochemical data extracted from these van't Hoff plots are summarized in Table 1, together with the calculated values.

For the association reaction of protonated Ala with ammonia, eq 1, the measured enthalpy and entropy changes are −21.9 kcal mol^{-1} and −27.9 cal mol^{-1} K^{-1}, respectively. At the MP2-(full)/6-311++G(2d,2p)//B3LYP/6-311+G(d, p) level of theory, the computed enthalpy change for the formation of the most stable isomer, **AN1** (see Figure 2), is −23.5 kcal mol^{-1}, which is greater than the measured value. After BSSE correction, the calculated value obtained is −21.1 kcal mol^{-1}, which is slightly lower than the experimental value. The counterpoise correction often overestimates the BSSE, especially when the basis set used is not sufficiently large and when the structure computed is loose such as those previously reported.[48,49] The calculated entropy change for formation of **AN1** is −30.2 cal mol^{-1} K^{-1}, in good agreement with the experimentally determined value. Note that this discussion is limited to the computed values for the most stable isomers involved in each of these equilibria. A more detailed discussion follows in which many other structures are described.

The measured enthalpy and entropy changes for the association reaction of protonated valine and ammonia are −21.0 kcal mol^{-1} and −28.8 cal mol^{-1} K^{-1}. According to the single-point energy calculation, the binding energy of the most stable isomer, **VN1** (see Figure 3), is −22.1 kcal mol^{-1}. After correction for BSSE, the calculated value decreases to −20.0 kcal mol^{-1}. The experimental enthalpy change is in between these two computed values. For the corresponding association reactions of leucine and isoleucine, the experimental enthalpies of −20.8 and −20.6 kcal mol^{-1} are very close, as are their entropy changes of −28.1 and −28.8 kcal mol^{-1}. This is, of course, due to the similarity of their structures.

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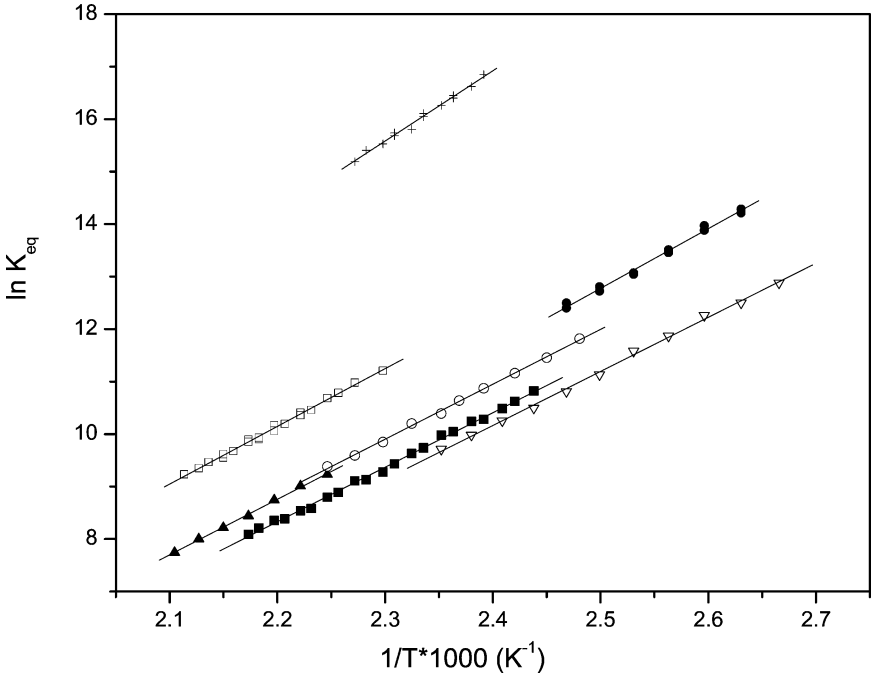

Figure 1. Van't Hoff plots for the association reactions: □, $Ala^+ + NH_3 \leftrightarrows Ala(NH_3)H^+$; ▲, $ValH^+ + NH_3 \leftrightarrows Val(NH_3)H^+$; ○, $LeuH^+ + NH_3 \leftrightarrows Leu(NH_3)H^+$; ■, $IleH^+ + NH_3 \leftrightarrows Ile(NH_3)H^+$; ●, $SerH^+ + NH_3 \leftrightarrows Ser(NH_3)H^+$; ▽, $ProH^+ + NH_3 \leftrightarrows Pro(NH_3)H^+$; +, $ProH^+ + CH_3NH_2 \leftrightarrows Pro(CH_3NH_2)H^+$.

Table 1. Experimental and Calculated Values of Enthalpy and Entropy Changes of the Association Reactions Measured by HPMS and Quantum Chemistry Calculations

	ΔH (kcal mol^{-1})		ΔS (cal mol^{-1} K^{-1})	
	expt	calcd[b]	expt	calcd[c]
$GlyH^+ + NH_3 \leftrightarrow Gly(NH_3)H^+$ [a]	−23.2	−24.4	−26.9	−30.9
$AlaH^+ + NH_3 \leftrightarrow Ala(NH_3)H^+$	−21.9	−23.5	−27.9	−30.2
$ValH^+ + NH_3 \leftrightarrow Val(NH_3)H^+$	−21.0	−22.1	−28.8	−29.5
$LeuH^+ + NH_3 \leftrightarrow Leu(NH_3)H^+$	−20.8	−21.6	−28.1	−29.3
$IleH^+ + NH_3 \leftrightarrow Ile(NH_3)H^+$	−20.6	−21.9	−28.8	−28.4
$SerH^+ + NH_3 \leftrightarrow Ser(NH_3)H^+$	−22.6	−23.5	−31.1	−32.7
$ProH^+ + NH_3 \leftrightarrow Pro(NH_3)H^+$	−20.4	−21.6	−28.9	−29.3
$ProH^+ + CH_3NH_2 \leftrightarrow Pro(CH_3NH_2)H^+$	−26.6	−27.5	−30.1	−33.1

[a] From ref 50. [b] The enthalpy changes are based on the most stable isomer calculated at the MP2(full)/6-311++G(2d,2p)//B3LYP/6-311+G(d,p) level of theory; ZPE, thermal energy corrections at 298 K. [c] Entropy changes are obtained at B3LYP/6-311+G(d,p) level of theory.

For the association reaction of protonated serine and ammonia, the experimentally measured enthalpy and entropy values are −22.6 kcal mol^{-1} and −31.1 cal mol^{-1} K^{-1}, respectively. These two values are very consistent with the calculated values for the association reaction of the most stable isomer, **SN1** (−23.5 kcal mol^{-1} and −32.7 cal mol^{-1} K^{-1}, respectively), in which NH_4^+ forms three intermolecular hydrogen bonds with neutral serine, as seen in Figure 3.

The experimental data for the association reaction of protonated proline with ammonia give the enthalpy and entropy changes of −20.4 kcal mol^{-1} and −28.9 cal mol^{-1} K^{-1}, respectively. For the association reaction of protonated proline with methylamine, the measured enthalpy and entropy changes are −26.6 kcal mol^{-1} and −30.1 cal mol^{-1} K^{-1}, respectively. The computed enthalpy changes from the single-point energies at the MP2(full)/6-311++G(2d,2p)//B3LYP/6-311+G(d, p) level of theory for the formation of the corresponding most stable isomers, **PN1** and **Pma1**, of −21.6 and −27.5 kcal mol^{-1}, respectively, are also in excellent agreement with their corresponding experimental data. The binding energy of protonated proline with methylamine is much higher than that with

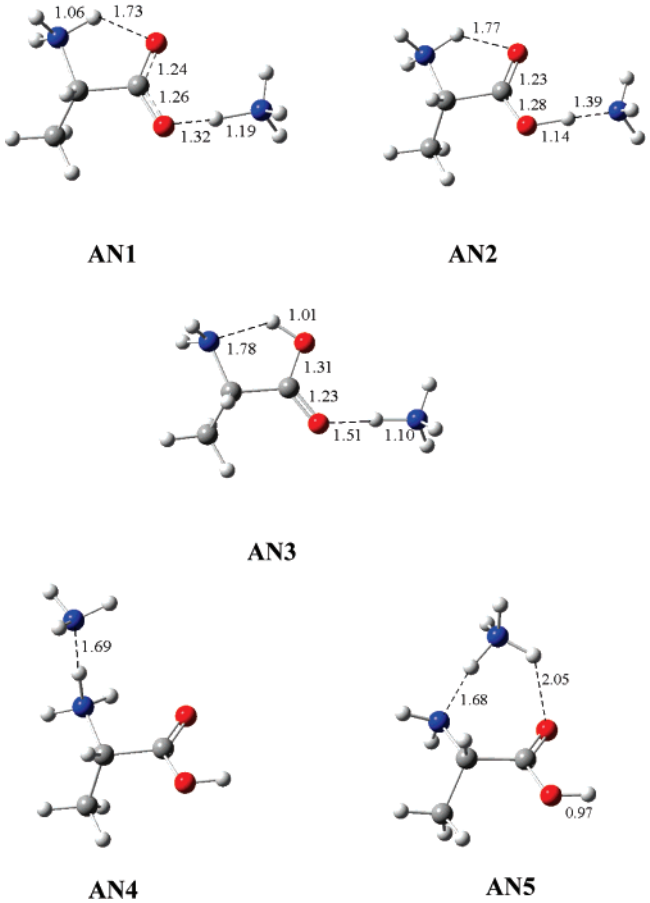

Figure 2. Structures of the five most stable isomers of the cluster of protonated alanine and ammonia obtained at the B3LYP/6-311+G(d,p) level of theory.

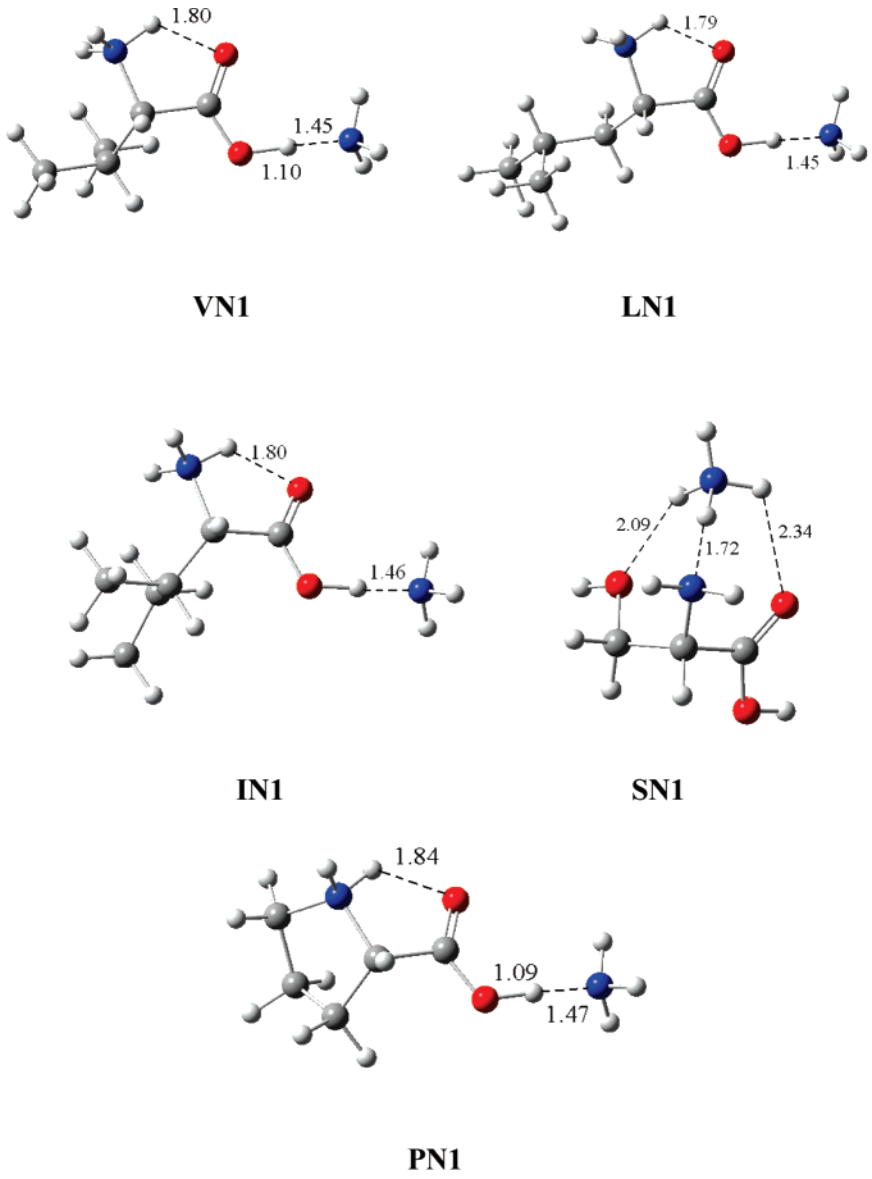

Figure 3. Structures of the most stable isomers of the cluster of protonated valine (**VN1**), leucine (**LN1**), isoleucine (**IN1**), serine (**SN1**), and proline (**PN1**) with ammonia obtained at the B3LYP/6-311+G(d,p) level of theory.

ammonia. The computed entropy changes are found to be −29.3 and −33.1 cal mol^{-1} K^{-1}, respectively, also in very good agreement with the measured values.

3.2. Computed Structures and Energetics of Clusters. 3.2.1. NH_3 Clusters. 3.2.1.1. Ala. Different possible isomers of the cluster of protonated alanine with ammonia have been optimized, and the five most stable isomers are shown in Figure 2, with the energies given in Table 2. At the B3LYP/6-311+G-(d, p) level of theory, at 0 K the most stable isomer is **AN3**, in which a proton is transferred from protonated alanine to ammonia despite the fact that the proton affinity of alanine is 11.5 kcal mol^{-1} greater than that of ammonia. A strong intermolecular H-bond forms between the carbonyl oxygen and ammonium ion. In addition, an intramolecular H-bond exists between the hydroxyl group and amino nitrogen of alanine. The resulting structure is an ammonium ion solvated by neutral alanine at the carbonyl oxygen. The 0 K binding energy for **AN3** is 24.1 kcal mol^{-1}, which is only 0.3 kcal mol^{-1} higher than that for either **AN1** or **AN2**. At the MP2(full)/6-311++G-(2d,2p)//B3LYP/6-311+G(d, p) level of theory, **AN1** is the most stable isomer, with a 298 K binding energy of 23.5 kcal mol^{-1}. This value is 1.3 kcal mol^{-1} higher than that for **AN3** and very close to that for **AN2** (23.4 kcal mol^{-1}).

AN1 may also be derived from separated reactants by proton transfer; however in this case only a single proton transfer occurs from the hydroxyl group of protonated alanine to ammonia. This leads to the novel structure of a gas phase ammonium ion solvated by a zwitterionic alanine molecule at one of its carboxylate oxygens. The two C−O bond lengths in this structure are very similar, 1.24 Å and 1.26 Å, in contrast to the values of 1.23 Å and 1.31 Å found in **AN3**. The hydrogen bonds in **AN1** appear to be particularly favorable. The bond length of 1.32 Å between the ammonium hydrogen and carboxylate oxygen is significantly reduced by 0.19 Å relative to the analogous bond in **AN3** while the N−H bond length involved increases by 0.09 Å. In addition, the intramolecular H-bond is also significantly enhanced with the length decreasing from 1.78 Å in **AN3** to 1.73 Å in **AN1**.

Table 2. Calculated Values of Enthalpy and Entropy Changes of the Association Reactions Related to Different Isomers with Ammonia

	B3LYP/6-311+G(d,p)			MP2(full)/6-311++G(2d,2p) //B3LYP/6-311+G(d,p)	
	ΔH_0 (kcal mol^{-1})	ΔH_{298} (kcal mol^{-1})	ΔS (cal mol^{-1} K^{-1})	ΔH_{298}[a] (kcal mol^{-1})	ΔH_{298} with BSSE[b] (kcal mol^{-1})
AN1	−23.8	−23.9	−30.2	−23.5	−21.1
AN2	−23.8	−24.0	−29.4	−23.4	−21.1
AN3	−24.1	−23.1	−28.8	−22.2	−20.3
AN4	−22.9	−21.5	−27.5	−21.8	−20.1
AN5	−22.7	−21.4	−32.7	−21.5	−19.4
VN1	−22.9	−22.6	−29.5	−22.1	−20.0
VN2	−21.8	−20.4	−27.0	−20.8	−19.1
VN3	−21.8	−21.0	−29.4	−20.1	−18.3
VN4	−20.7	−19.4	−33.1	−19.9	−17.7
SN1	−23.6	−22.1	−32.7	−23.5	−21.0
SN2	−23.3	−23.3	−28.9	−22.7	−20.5
SN3	−23.2	−23.1	−29.5	−22.8	−20.3
SN4	−21.8	−20.4	−25.2	−20.3	−18.6
SN5	−21.7	−20.8	−27.2	−20.2	−18.3
SN6	−21.0	−19.7	−31.0	−20.0	−17.7
SN7	−18.2	−16.7	−24.4	−16.0	−14.5
PN1	−22.4	−21.9	−29.3	−21.6	−19.6
PN2	−20.3	−18.8	−27.1	−19.3	−17.7
PN3	−17.0	−15.7	−27.6	−17.3	−15.4
PN4	−16.3	−15.8	−28.1	−15.3	−13.4

[a] ZPEs and thermal energy corrections are from the calculation results at the B3LYP/6-311+G(d,p) level of theory. [b] BSSE have been calculated at the MP2(full)/6-311++G(2d,2p) level of theory.

For **GN1** and **GN2** of glycine,[50] attempts were made to locate analogous structures in which the intermolecular proton transfer from protonated glycine to ammonia did not occur. All such attempts were unsuccessful however, indicating that this intermolecular proton transfer is a virtually barrierless process. However, for alanine, a local minimum was found, **AN2**, in which no proton transfer occurs. An intermolecular hydrogen bond forms between the carboxyl hydrogen of protonated alanine and ammonia with a H-bond length of 1.39 Å. Both **AN1** and **AN2** have very similar energies at the two different levels of theory.

AN4 is perhaps what might initially have been regarded as being the most intuitively logical species derived from protonated alanine and ammonia. Here ammonia functions as a hydrogen bond acceptor, forming an intermolecular hydrogen bond of 1.69 Å to a hydrogen of the protonated amine group of alanine. **AN5** is derived from **AN4** by a single proton transfer from the protonated amine group to ammonia, followed by the formation of the two intermolecular hydrogen bonds with the amino nitrogen and carbonyl oxygen, with lengths of 1.68 Å and 2.05 Å, respectively. The binding energies of **AN4** and **AN5** are very close at about 2 kcal mol^{-1} lower than that of the most stable isomer, **AN1**.

Presuming a Boltzmann distribution of possible isomers and using the enthalpy changes from the calculation at the MP2-(full)/6-311++G(2d,2p)//B3LYP/6-311+G(d, p) level of theory and the entropy changes calculated at the B3LYP/6-311+G(d, p) level of theory, the main species present throughout our experimental temperature range would be **AN1** (34%) and **AN2** (48%) with, however, as much as ∼9% of **AN3** and **AN4** present. Assuming such a statistical mixture of the different isomers, the overall enthalpy change becomes −23.2 kcal mol^{-1}, which is very close to the value determined for the most stable isomer.

3.2.1.2. Val, Leu, and Ile. Many isomers of the clusters of protonated valine, leucine, or isoleucine with ammonia have been optimized; however only the most stable isomers are displayed in Figure 3. The structures of several other most stable isomers for each cluster are given in the Supporting Information (Figure S1, S2, and S3). For valine, the most stable isomer is **VN1**, in which ammonia forms a strong H-bond with the hydroxyl group of protonated valine. The structure is analogous to **AN2**, but the H-bond length is longer in **VN1**, a result of the larger PA difference between valine and ammonia. The calculated binding energy of 22.1 kcal mol^{-1} at the single-point energy calculation is somewhat higher than the experimental value. Given the values of ΔH and ΔS for the different isomers summarized in Table 2 it would be predicted that, under thermal equilibrium conditions, the population of **VN1** and **VN2** would be 68% and 29%, respectively, with **VN3** and **VN4** having very minor contributions.

For leucine and isoleucine, the most stable isomers have nearly identical structures to that of valine, and the calculated binding energies of 21.6 and 21.9 kcal mol^{-1} are very similar to that of valine. In addition, as found for valine, a zwitterionic structure could not be found.

A comparison of the structures and energies of different isomers of the glycine, alanine, and valine clusters reveals some differences. For glycine and alanine, zwitterionic structures exist in the clusters (**GN2** and **AN1**); however, due to its higher proton affinity, a zwitterionic structure does not exist in the valine cluster. In addition, proton transfer is a barrierless process from the hydroxyl group of protonated glycine to ammonia. Therefore, for glycine a structure analogous to **AN2** or **VN1** could not be found. The energy order of the isomers is also different for these amino acids. The isomer, **AN1**, a zwitterionic structure, is the most stable isomer. For glycine, the analogous isomer, **GN2**, is isoenergetic with **GN1**. The most stable isomer of the valine cluster, **VN1**, involves protonated valine and neutral

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ammonia. **GN4**, **AN4**, and **VN2** have analogous structures, in which the protonated amine group of the amino acid acts as a proton donor to form a hydrogen bond with ammonia. The H-bond lengths increase from 1.67 to 1.69 to 1.71 Å proceeding from glycine to alanine to valine, which is consistent with the PA order and the change in their binding energies (22.4, 21.8, and 20.8 kcal mol^{-1} for **GN4**, **AN4**, and **VN2,** respectively).

3.2.1.3. Ser and Pro. Serine has an additional hydroxyl group substituent, which also may form intra- or intermolecular hydrogen bonds. The calculated structures of the seven most stable isomers are shown in Figure S4. **SN1** is the most stable isomer, in which a proton transfers from protonated serine to ammonia, followed by the formation of three intermolecular hydrogen bonds between NH_4^+ and the amino, carbonyl, and hydroxyl groups of neutral serine, whose hydrogen bond lengths are 1.72, 2.34, and 2.09 Å, respectively. The calculated binding energy of 23.5 kcal mol^{-1} is the same as that for the most stable isomer of alanine (**AN1**), and the experimental value is somewhat higher than that of alanine. This is due to the formation of an additional hydrogen bond. In **SN2**, ammonia forms an intermolecular H-bond with the carboxylic acid hydrogen and two intramolecular H-bonds between the protonated amino group and the hydroxyl substituent as well as the carbonyl oxygen. When a proton transfers from the carboxylic acid group to ammonia, the resulting isomer, **SN3**, involves a zwitterionic structure of serine. Both **SN2** and **SN3** have very similar energies to that of **SN1**. From the calculated values of ΔH and ΔS, the population of **SN1**, **SN2**, and **SN3** would be predicted to be 23%, 39%, and 33%, respectively, with **SN4** and **SN5** having very minor contributions under thermal equilibrium conditions.

Among the 20 naturally occurring amino acids, only proline has a secondary amine group, which is of particular interest because of its role in determining a protein secondary structure. Czinki et al.[51] have carried out an extensive investigation of the conformers of neutral proline, and Russo et al. have also investigated both neutral and protonated proline to obtain its proton affinity and gas-phase basicity.[52] The basic secondary amine group makes formation of a zwitterionic structure facile, relative to other amino acids.[53,54] The four most stable isomers of protonated proline and ammonia are shown in Figure S5. The most stable isomer, **PN1**, includes an intermolecular H-bond between ammonia and the carboxylic acid hydrogen and an intramolecular H-bond between the protonated amine group and the carbonyl oxygen. The calculated binding energy is 21.6 kcal mol^{-1}. In **PN2**, ammonia forms a H-bond with the protonated amine group, with a H-bond length of 1.77 Å. This value is larger than those of 1.71 Å for valine, 1.69 Å for alanine, and 1.67 Å for glycine in the analogue isomers. This is also consistent with their PA order. According to the calculated values of ΔH and ΔS summarized in Table 2, under thermal equilibrium conditions, the dominant species is **PN1** (94%), with **PN2** (5%) having a smaller contribution.

3.2.2. CH_3NH_2 Clusters. Several isomers of the methylamine clusters of protonated alanine, valine, serine, and proline have been calculated, and several of the most stable structures are shown in Figure S6, S7, and S8, respectively. Only the global energy minimum isomers are shown in Figure 4, and their corresponding binding energies are summarized in Table 3. Compared to the ammonia clusters, proton transfer from the protonated amino acids to methylamine is much more facile, because the PA of methylamine (214.9 kcal mol^{-1}) is larger than that of ammonia. Each of these most stable isomers involves a proton transfer to methylamine.

The most stable isomer of alanine, **Ama1**, has a similar structure to that of **AN3**. An intermolecular proton transfer occurs from the carboxylic acid group to methylamine with an intramolecular proton transfer from the protonated amino group to the carbonyl oxygen. The hydrogen length of 1.59 Å between protonated methylamine and neutral alanine is longer than the

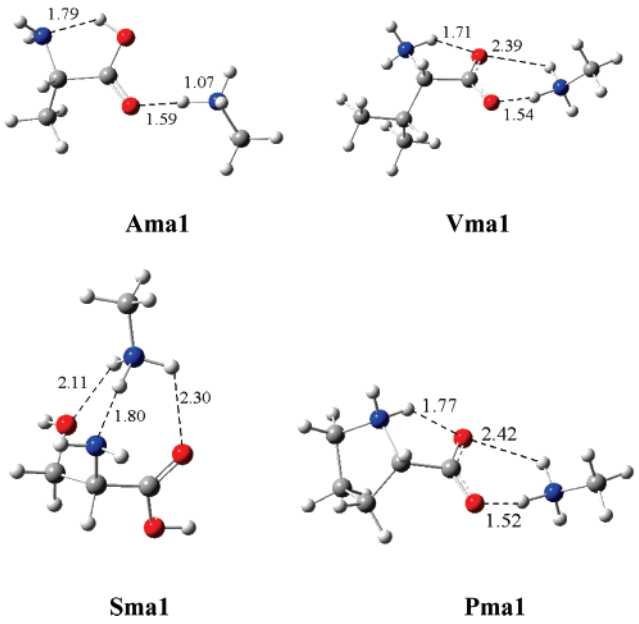

Figure 4. Structures of the most stable isomers of the cluster of protonated alanine (**Ama1**), valine (**Vma1**), serine (**Sma1**), and proline (**Pma1**) with methylamine obtained at the B3LYP/6-311+G(d,p) level of theory.

Table 3. Calculated Values of Enthalpy and Entropy Changes of the Association Reactions Related to Different Isomers with Methylamine

	B3LYP/6-311+G(d, p)			MP2(full)/6-311++G(2d,2p)//B3LYP/6-311+G(d, p)
	ΔH_0 (kcal mol^{-1})	ΔH_{298} (kcal mol^{-1})	ΔS (cal mol^{-1} K^{-1})	ΔH_{298}[a] (kcal mol^{-1})
Ama1	−31.2	−29.8	−30.9	−30.0
Ama2	−29.5	−28.1	−33.3	−29.5
Ama3	−29.4	−27.6	−35.0	−29.0
Vma1	−28.1	−26.6	−34.3	−28.2
Vma2	−28.9	−27.4	−32.8	−27.8
Vma3	−27.2	−25.4	−35.5	−27.2
Sma1	−30.1	−28.2	−34.4	−30.6
Sma2	−29.0	−27.3	−32.8	−28.4
Sma3	−28.8	−27.4	−31.2	−27.9
Sma4	−27.5	−25.7	−33.1	−27.4
Pma1	−27.2	−25.7	−33.1	−27.5
Pma2	−23.3	−22.2	−31.5	−22.9
Pma3	−21.1	−19.9	−30.8	−21.7
Pma4	−20.3	−18.9	−33.1	−21.3

[a] ZPEs and thermal energy corrections are from the calculation results at the B3LYP/6-311+G(d,p) level of theory.

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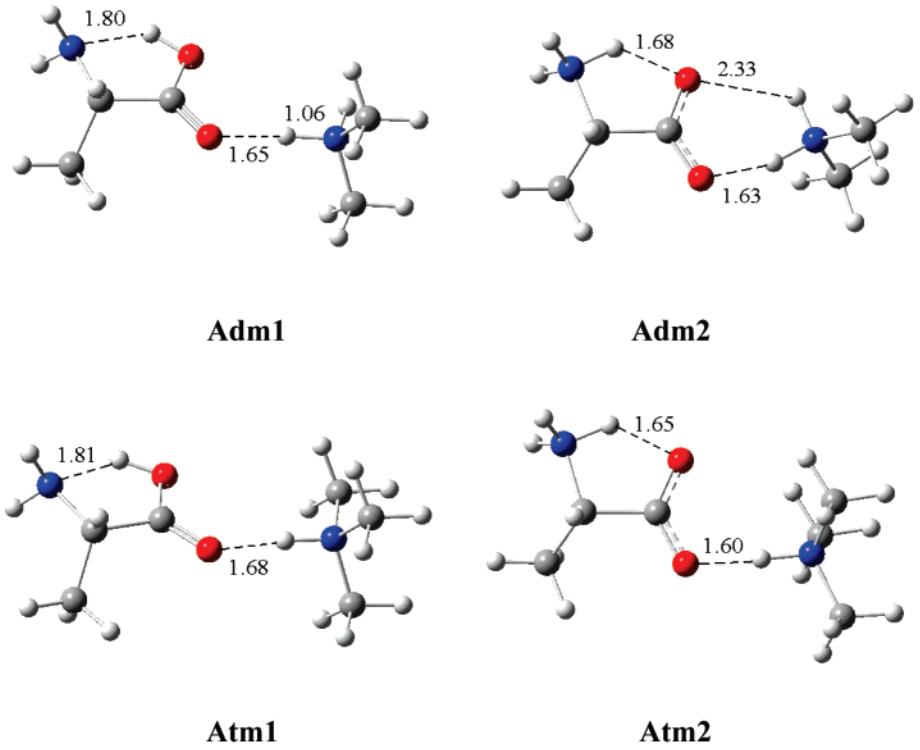

Figure 5. Structure of the most stable non-zwitterionic (**Adm1** and **Atm1**) and zwitterionic (**Adm2** and **Atm2**) isomers of the cluster of protonated alanine and dimethylamine and trimethylamine obtained at the B3LYP/6-311+G(d,p) level of theory.

corresponding length in **AN3**, which is due to the higher PA of methylamine. The binding energy of 30.0 kcal mol^{-1} between protonated alanine and methylamine is 6.5 kcal mol^{-1} greater than that for **AN3**. The second most stable isomer, **Ama2**, involving a zwitterionic alanine, has a very similar binding energy to that of **Ama1**.

Vma2 has an analogous structure to that of **Ama1**; however it is not the most stable isomer. For valine, the most stable isomer, **Vma1**, contains a zwitterionic structure of valine, which forms much stronger intramolecular and intermolecular hydrogen bonds. The calculated binding energy of 28.2 kcal mol^{-1} is about 2 kcal mol^{-1} less than that of **Ama1**.

When protonated methylamine forms H-bonds with both the amino nitrogen and the carbonyl oxygen of the neutral amino acids, the binding energies of the associated isomers, **Ama3** and **Vma3**, are about 1.0 kcal mol^{-1} lower than those of their corresponding most stable isomers, respectively.

The most stable isomer of protonated serine and methylamine, **Sma1**, has a similar structure to that of **SN1**, in which protonated methylamine forms three hydrogen bonds with the amino, carbonyl, and the substituent hydroxyl groups of serine, with the corresponding bond lengths of 1.80, 2.30, and 2.11 Å, respectively. Compared to **SN1**, the hydrogen bond length to the amine group of the amino acid is notably longer; however the other two are very similar. The binding energy of 30.6 kcal mol^{-1} is 7 kcal mol^{-1} greater than that for **SN1**. **Sma2** involves a zwitterionic structure of serine in which protonated methylamine forms two H-bonds with the two carboxylate oxygens, and in addition, there are two intramolecular H-bonds between hydrogens of the protonated amino acid nitrogen and both the substituent hydroxyl oxygen and one of the carboxylate oxygens. The energy of **Sma2** is about 2.0 kcal mol^{-1} higher than that of **Sma1**.

Among the clusters of protonated proline and methylamine, the most stable isomer (**Pma1**) involves a zwitterionic proline with a binding energy of 27.5 kcal mol^{-1} which is 4.6 kcal mol^{-1} greater than the most stable non-zwitterionic isomer (**Pma2**, Figure S8) at the MP2(full)/6-311++G(2d,2p)//B3LYP/6-311+G(d, p) level of theory. In **Pma2** and **Pma4**, a proton transfer occurs from protonated proline to methylamine; however this is absent in **Pma3**. **Pma3** and **Pma4** have very similar binding energies.

According to the calculated energies and assuming a Boltzmann distribution, the zwittionic isomer, **Pma1**, is essentially the exclusive species (99.8%) under thermal equilibrium conditions within our experimental temperature range. An experimental enthalpy change of −26.6 kcal mol^{-1} is in very good agreement with the calculated binding energy of **Pma1**. It is obviously much higher than the binding energy for the second most stable isomer. This is solid experimental evidence for the fact that the zwitterionic structure of proline is stabilized effectively by protonated methylamine in the gas phase.

3.2.3. $(CH_3)_2NH$ and $(CH_3)_3N$ Clusters. The structures for the clusters of protonated amino acids (glycine, alanine, valine, serine, and proline) with dimethylamine and trimethylamine have also been investigated extensively. Only the structures of the most stable non-zwitterionic and zwitterionic isomers of alanine are displayed in Figure 5.

The most stable non-zwitterionic isomers, **Adm1** and **Atm1**, have very similar structures and involve both inter- and intramolecular proton transfers. The binding energies are 35.6 and 39.1 kcal mol^{-1}, respectively, for **Adm1** and **Atm1**. The second most stable isomers (**Adm2** and **Atm2**) involve zwitterionic alanine; however their energies are 1.3 and 1.4 kcal mol^{-1} higher than those for **Adm1** or **Atm1**, respectively. When the analogous isomers for the clusters between protonated

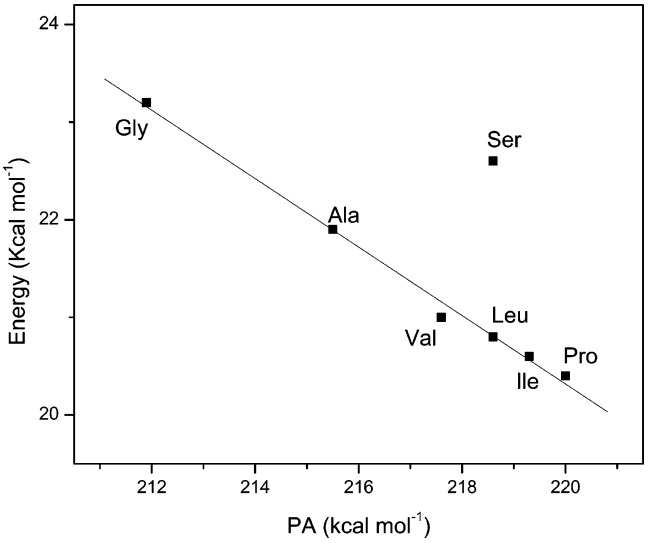

Figure 6. Binding energies between protonated amino acids and ammonia as a function of proton affinities of amino acids.

alanine and either ammonia, methylamine, dimethylamine, or trimethylamine are compared, the energy difference between **AN3** and **AN1** of −1.3 kcal mol^{-1} is reversed to 0.5, 1.3, and 1.4 kcal mol^{-1}, respectively, for the clusters of methylamine, dimethylamine, and trimethylamine. It is thus obvious that NH_4^+ is more effective in stabilizing zwitterionic alanine relative to the protonated amines. This is discussed below.

4. Discussion

4.1. Binding Energy and Proton Affinity. Numerous proton-bound dimers of the form $BH^+ \cdot A$ have been investigated,[55−57] and a good qualitative trend has been shown to exist between the binding energy and the PA difference between B and A ($\Delta PA = PA(B) - PA(A)$). The binding energy decreases as ΔPA increases. This may be understood as the result of the fact that H-bond formation in $BH^+ \cdot A$ may be viewed as a partial proton transfer from BH^+ to A in the cluster. Partial proton transfer will be facilitated when BH^+ becomes a more efficient proton donor, i.e., when the PA of neutral B decreases, or when A becomes a more efficient proton acceptor, i.e., when the PA of A increases. This trend has also been confirmed in many instances.[58−61] Desmeules et al.[62] have analyzed theoretically the relationship between the binding energy and the difference in PAs and partial proton transfer in the proton-bound dimers as measured by the elongation of the $A-H^+$ bond. The conclusion is consistent with the trend above.

The binding energies between protonated Gly, Ala, Val, Leu, Ile, Ser, or Pro and ammonia are shown in Figure 6, plotted as a function of the PAs of amino acids.[63] A very good linear relationship exists between the binding energies and the PA values, which is consistent with the trend discussed above. The exception to this relationship is serine, which may be attributed to the hydroxyl substituent of serine, leading to the formation of multiple H-bonds as seen for **SN1** shown in Figure 3. From this trend, it is obvious that the binding energy of protonated proline with methylamine is much higher than that with ammonia, because the PA difference between proline and methylamine is about 11 kcal mol^{-1} less than that for ammonia.

If the analogous isomers with ammonia (**AN3**), methylamine (**Ama1**), dimethylamine (**Adm1**), and trimethylamine (**Atm1**) are compared, the intermolecular H-bond lengths increase from 1.51 to 1.59, to 1.65, to 1.68 Å, which is consistent with the PA increase from ammonia (204) to methylamine (214.9) to dimethylamine (222.2) to trimethylamine (226.8 kcal mol^{-1}). When structures involving protonated alanine and neutral amine are compared, the calculated binding energies are 22.2, 30.0, 35.6, and 39.1 kcal mol^{-1}, respectively. When structures involving neutral alanine and protonated amine are considered, the calculated binding energies are 33.6, 30.3, 28.5, and 27.5 kcal mol^{-1}, respectively. The decreasing binding energy order in the latter case is consistent with the increase of the H-bond lengths from ammonia to trimethylamine, which also complies with the relationship between the binding energy and the PA difference.

4.2. Interactions between Neutral Amino Acids and NH_4^+ and Comparison with Na^+. Noncovalent interactions between neutral amino acids and organic or inorganic ions are ubiquitous and very important in biological systems. To date, although there has been a number of reports concerning interaction with metal ions,[18−33,64−69] the interaction with organic ions has been almost completely absent in the gas phase. Protonated amino groups are very common in biological systems, such as protonated amino acids, peptides, and proteins as well as DNA and RNA. The simplest ammonium ion, NH_4^+, may thus serve as a model for the investigation of the interactions between amino acids and organic ions leading to a more fundamental understanding of the characteristics and strengths of these kinds of interactions.

For Gly, Ala, Ser, and Pro, an examination of the structures of the most stable isomers reveals that a proton transfer has occurred from each of the protonated amino acids to ammonia even though the PAs of these amino acids are greater than that of ammonia (204 kcal mol^{-1}). This is likely due to the electrostatic interactions between the resulting ammonium ion and these neutral amino acids which will have appreciable local dipole moments thus favoring the endothermic proton transfer. Finally, the most stable structures include neutral amino acids interacting with the ammonium ion.

According to the experimental binding energies between the protonated amino acids and ammonia and the known PA difference between the amino acids and ammonia,[63] the interaction strengths between neutral amino acids and the ammonium ion may be obtained. These values are summarized in Table 4, together with the corresponding calculated binding energies between neutral amino acids and NH_4^+. The calculated values for NH_4^+ without BSSE are about 1.5 kcal mol^{-1} higher than

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Table 4. Comparison of the Binding Energy between NH_4^+ and Na^+ with Amino Acids

	NH_4^+ (kcal mol^{-1})		Na^+ (kcal mol^{-1})	
	expt[a]	calcd[b]	expt[c]	calcd[c]
Gly	31.1	32.6	38.5	39.2
Ala	33.4	34.9	39.9	39.9
Val	34.6	36.5	41.3	
Leu	35.4	36.5	41.8	
Ile	35.9	37.3	42.1	
Ser	37.2	37.6	45.9	47.8
Pro	36.4, 42.7[d]	42.0	46.8	46.6

[a] Binding energies between neutral amino acids and the corresponding NH_4^+ or $CH_3NH_3^+$ are based on the experimental enthalpy changes and proton affinity differences between the amino acid and ammonia or methylamine taken from the NIST database. [b] The enthalpy changes are based on the most stable isomer calculated at the MP2(full)/6-311++G(2d,2p)//B3LYP/6-311+G(d,p) level of theory; ZPE, thermal energy corrections, and entropy changes are from the calculations at B3LYP/6-311+G(d,p) level of theory. [c] From ref 29. Experimental method: kinetic method. Calculation method: MP2(full)/6-311+G(2d,2p)//MP2/6-31G(d) without BSSE corrections. [d] Proton affinity of proline is from ref 70.

the corresponding experimental values for Gly, Ala, Val, Leu, and Ile, respectively. However, with inclusion of BSSE, the calculated values become slightly lower than the experimental data. An exception is that of the calculated binding energy of 42.0 kcal mol^{-1} between proline and NH_4^+ which is markedly higher than the experimental value based on the NIST value of the PA of Pro (220 kcal mol^{-1}).[63] Recently, however, the PA of proline has been revisited and obtained as 226.3 kcal mol^{-1}.[70] Based on this value, the binding energy becomes 42.7 kcal mol^{-1}, which is in very good agreement with the calculated value. This also indicates that the PA of proline in NIST is likely an underestimate.

NH_4^+ is similar to Na^+ in that they have the same charge and a similar radius. Therefore, it is very interesting to compare their interactions with the amino acids. Some binding energies of Na^+ are available from literature data, and these values are also included in Table 4. It is apparent that the interactions between amino acids and Na^+ are much stronger than the corresponding values for NH_4^+ by ~7 kcal mol^{-1}. This may be attributed to a fundamental difference between NH_4^+ and Na^+ interactions with amino acids where, for example, the interactions between Na^+ and amino acids are mainly electrostatic while the interactions with NH_4^+ involve hydrogen bonds. This difference in the nature of the interaction also results in the structural distinction between the complexes of amino acids with Na^+ or NH_4^+. For Ala, in the most stable isomer with Na^+ obtained using the same level of theory (B3LYP/6-311++G(d, p)),[67] Na^+ interacts with both carbonyl oxygen and amino nitrogen with distances of 2.24 and 2.44 Å from sodium to the heavy atoms. The corresponding structure is analogous to **AN5**, whose energy is 1.4 kcal mol^{-1} higher than the most stable isomer of the NH_4^+ cluster. The ammonium ion interacts much more strongly with the amino nitrogen with a hydrogen bond length of 1.68 Å than with the carbonyl oxygen with a length of 2.05 Å. The most stable isomer is **AN3** at the B3LYP/6-311++G(d, p) level of theory; however the analogous structure is the fourth most stable isomer for Na^+. For the zwitterionic structure, the distances between Na^+ and the two carboxylate oxygens are very close (2.28 and 2.33 Å) and its energy is 1.2 kcal mol^{-1} higher than the most stable isomer. In the most stable zwitterionic isomer with an ammonium ion, **AN1**, NH_4^+ forms a very strong hydrogen bond with a length of 1.32 Å with one of the carboxylate oxygens, and its energy is only 0.3 kcal mol^{-1} higher at 298 K than the most stable isomer, **AN3**, at the B3LYP/6-311++G(d, p) level of theory.

4.3. Potential Energy Surfaces for Isomerization. Although *ab initio* calculations reveal that several different stable isomers of each of the cluster species investigated here are possible, it is not immediately evident whether all such species are energetically accessible under the experimental conditions employed. In order to answer this question an exhaustive search of the PES for the cluster of protonated proline with methylamine was undertaken to examine the interconversion of the various isomers. The PES for isomer transformation has been investigated at the B3LYP/6-311+G(d, p) level of theory, with the result shown in Figure 7. Figure 7a is the vibrationless 0 K PES, based on differences in electronic energies, for interconversion of the various Pro$(CH_3NH_2)H^+$ cluster species while Figure 7b shows the PES using 298 K enthalpy differences which include zero-point energy contributions and thermal energy corrections at 298 K. This surface is somewhat different from that at 0 K, and the energies of some transition states (for example, **TS2** and **TS6**) become lower than that of either of the two nearby minimum energy structures to which they are linked. However this constitutes a more accurate representation of the actual experimental situation. The structures of every stationary point located are shown in Figure 7c, including seven energy minima, **Pma1** to **Pma7**, and six transition states, **TS1** through **TS6**.

Two different approaches of CH_3NH_2 to protonated proline might be envisaged, either at the protonated amine group or at the hydroxyl hydrogen. In the former case, methylamine acts as a hydrogen bond acceptor and **Pma3** is formed. Alternatively, in the approach at the hydroxyl hydrogen, a barrierless proton transfer from protonated proline to methylamine occurs leading to **Pma1**, the most stable isomer, in which proline is a zwitterionic structure and protonated methylamine forms double H-bonds with the two oxygens of the carboxylate group.

In **Pma3** methylamine forms a H-bond with one hydrogen of the protonated amine group and, at the same time, the intramolecular H-bond with the other hydrogen still exists. Methylamine may form a H-bond with the other hydrogen as an acceptor, although the intramolecular H-bond is lost. At the same time methylamine may also act as a donor to form the other H-bond with the carbonyl oxygen. This isomer is **Pma5**, whose energy is 3.0 kcal mol^{-1} higher than that of **Pma3**. The barrier between **Pma3** and **Pma5** is 8.0 kcal mol^{-1} at 298 K. **Pma5** may undergo an intermolecular proton transfer from protonated proline to methylamine and arrive at **Pma4** *via* **TS2**. Although transition state **TS2** lies 0.4 kcal mol^{-1} above **Pma5** at 0 K, at 298 K it is 1.6 kcal mol^{-1} lower in energy than **Pma5** and has the same energy as **Pma4**. The conversion of **Pma4** to **Pma1** involves additional higher energy intermediates. Rotation about the C−OH bond leads to transformation of **Pma4** into the new species **Pma6** *via* **TS3**. As the hydrogen bond between protonated methylamine and the amino nitrogen is broken, a rotation about the O···H−N hydrogen bond transforms **Pma6** to **Pma7** *via* **TS4**. **Pma6** and **Pma7** are 12.9 and 15.7 kcal mol^{-1}, respectively, higher in energy than the most stable

(70) Mezzache, S.; Bruneleau, N.; Vekey, K.; Afonso, C.; Karoyan, P.; Fournier, F.; Tabet, J. C. *J. Mass Spectrom.* **2005**, *40*, 1300.

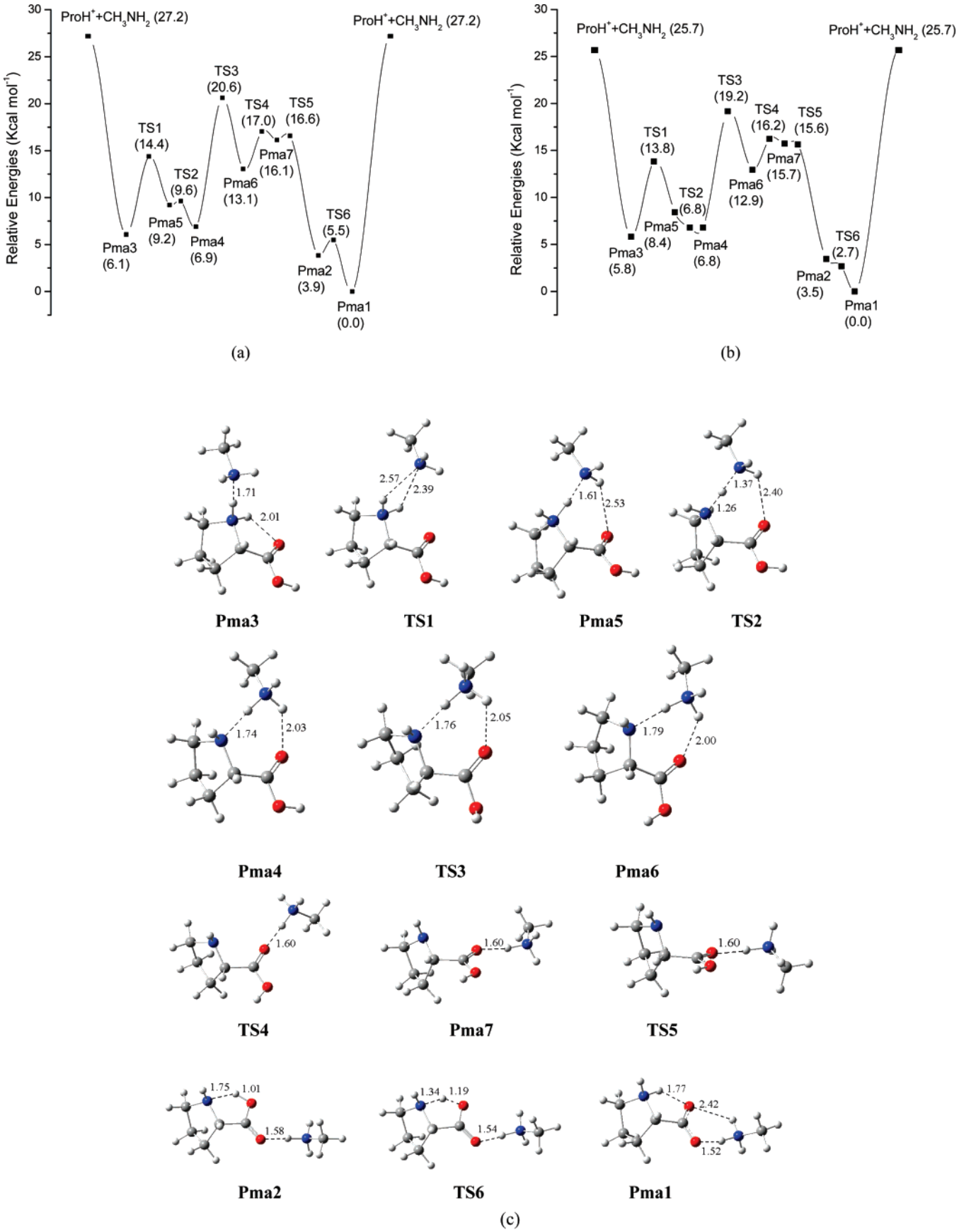

Figure 7. (a) 0 K, vibrationless potential energy surface for isomerization of protonated proline/methylamine clusters calculated at the B3LYP/6-311+G-(d,p) level of theory. The relative 0 K electronic energies of each stationary point are given in parentheses (kcal mol^{-1}). (b) Potential energy surface including zero-point energy contributions (ZPC) and thermal energy correction at 298 K; the relative enthalpies of each stationary point at 298 K are given in parentheses (kcal mol^{-1}). (c) The structures of each stationary point.

isomer, **Pma1**, while the barrier at **TS3** is 12.4 and 6.3 kcal mol^{-1}, respectively, higher in energy than either **Pma4** or **Pma6** which it separates. **TS4**, separating **Pma6** and **Pma7**, lies 3.3 kcal mol^{-1} higher in energy than the former and 0.5 kcal mol^{-1} above the latter. Finally, **Pma7** may become **Pma2** *via* rotation about the C−C bond through **TS5** which permits formation of the intramolecular hydrogen bond between the hydroxyl hydrogen and the amino nitrogen. This process has no barrier at 298 K. Finally, through the intramolecular proton transfer *via* **TS6**, **Pma2** may rearrange to the most stable isomer, **Pma1**, which is also a barrierless process at 298 K.

According to the calculation, the highest barrier is 19.2 kcal mol^{-1} higher than the most stable isomer, **Pma1**, however it is still much lower than the enthalpy change of the association reaction. Therefore, at the temperature of the HPMS experiments conducted here, the complete PES may be explored leading to a statistical distribution of isomeric species. The dominant species is **Pma1**, in which the zwitterionic proline may be very well stabilized by protonated methylamine. This has also been confirmed recently by infrared multiple photons dissociation (IRMPD) experiments.[71] In addition, it has been shown that the zwitterionic structure of proline also exists in the proton-bound proline dimmer.[72] These conclusions indicate that proline, a secondary amine, is very prone to formation of a zwitterionic structure and that organic ammonium ions may be very effective in stabilizing the zwitterionic structures of amino acids.

4.4. Stabilization of Zwitterionic Structure of Amino Acids. In the gas phase, isolated neutral amino acids are known to exist in the non-zwitterionic form. Interaction with other molecules and ions may stabilize the zwitterionic structure of amino acids. It is also of considerable interest to examine systematically the effect on stabilization of the zwitterionic structures of a series of amino acids by various ammonium ions. The relative stabilities of the zwitterionic and non-zwitterionic structures of the different amino acids (glycine, alanine, valine, serine, and proline) in their complexes with different ammonium ions (NH_4^+, $CH_3NH_3^+$, $(CH_3)_2NH_2^+$, and $(CH_3)_3NH^+$) are illustrated in Figure 8. The calculated relative zwitterionic stability is expressed as the difference in 0 K energies for the two forms obtained at the MP2(full)/6-311++G(2d,2p)//B3LYP/6-311+G(d, p) level of theory, such that a negative value indicates a greater stability of the zwitterionic form. These values are then plotted as a function of the proton affinities of ammonia and the amines. The calculated relative zwitterionic stability expressed as the difference in 298 K energies for the most stable zwitterionic and non-zwitterionic isomers is also given in Figure S9.

From the data summarized in Figure 8, it may be seen that complexation with NH_4^+ renders the zwitterionic form of alanine more stable. However, once the complexing agent increases in basicity, as in the cases of each of the alkyl ammonium ions, the energy of the zwitterionic isomer becomes progressively less favorable than the most stable non-zwitterionic isomer. In contrast, the zwitterionic structure of valine is more favorable in complexation with $CH_3NH_3^+$ although a zwitterionic structure of the complex of valine with NH_4^+ could not be found as a local minimum structure. If the additional interaction between the serine substituent (hydroxyl group) and an ammonium ion

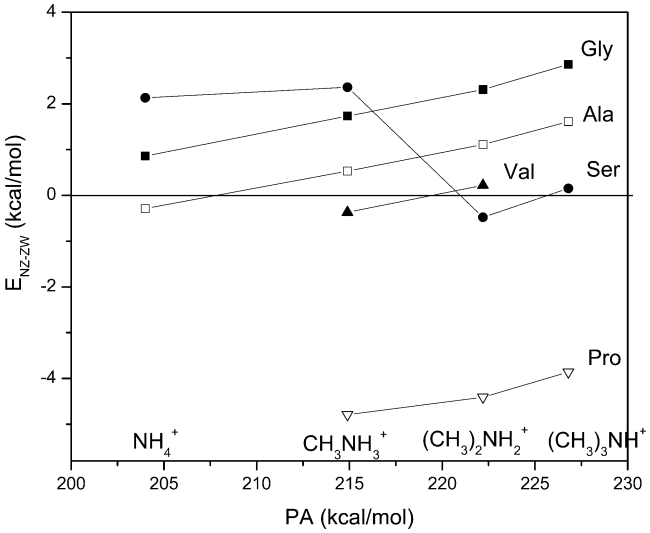

Figure 8. Relative stabilization of zwitterionic amino acids by ammonium ions, as expressed by the energy difference at 0 K between the most stable non-zwitterionic (NZ) and zwitterionic clusters (ZW) calculated at the MP2-(full)/6-311++G(2d,2p)//B3LYP/6-311+G(d,p) level of theory.

is excluded, it is evident that the complexes of each of NH_4^+, $CH_3NH_3^+$, and $(CH_3)_2NH_2^+$ with serine all lead to zwitterionic structures as the most stable isomers. However, because the hydroxyl group leads to formation of an additional strong hydrogen bond in the most stable non-zwitterionic isomer, this isomer remains as the most stable species in the clusters of NH_4^+ and $CH_3NH_3^+$. The lack of sufficient labile hydrogens for hydrogen bonding in the dimethyl and trimethyl ammonium ions allows the zwitterionic forms to be more competitive in stability. Significantly, for proline, the zwitterionic structures are all more stable in complexes with $CH_3NH_3^+$, $(CH_3)_2NH_2^+$, and $(CH_3)_3NH^+$.

Some generalizations with respect to the relative stabilities of ammonium ion−amino acid complexes can be drawn from these observations. First, as the proton affinity of the amino acid increases, within the series of glycine, alanine, and valine, the zwitterionic structure becomes more energetically favorable relative to the non-zwitterionic isomer. Second, as the proton affinity of the amine increases, the zwitterionic structure of a given amino acid within the complex becomes gradually less favorable. Third, compared to the other amino acids, proline, the only secondary amine among the 20 naturally occurring amino acids, has a much more pronounced tendency to form the zwitterionic structure. Finally, a substitutent on the amino acid side chain that may participate in additional hydrogen bond interactions in the non-zwitterionic isomer may render this isomer more stable, as seen in serine.

In order to understand further the stability of zwitterionic structures, energy decomposition analysis has been performed and the salt bridge interactions between zwitterionic amino acids and ammonium ions have also been investigated. A very useful quantity to aid in the understanding of the mechanism of the stabilization of zwitterionic forms is the deformation energy, which is defined as the energy needed to deform the amino acid and ammonium ion moieties from their equilibrium geometries to the geometric conformations adopted in the complex.[73,74]

(71) Wu, R. H.; McMahon, T. B. *J. Am. Chem. Soc.* **2007**, *129*, 4864.
(72) Wu, R. H.; McMahon, T. B. *Angew. Chem., Int. Ed.* **2007**, *46*, 3668.
(73) Ferenczy, G. G.; Angyan, J. G. *J. Comput. Chem.* **2001**, *14*, 1679.
(74) Cedeno, D. L.; Weitz, E.; Berces, A. *J. Phys. Chem.* **2001**, *105*, 8077.

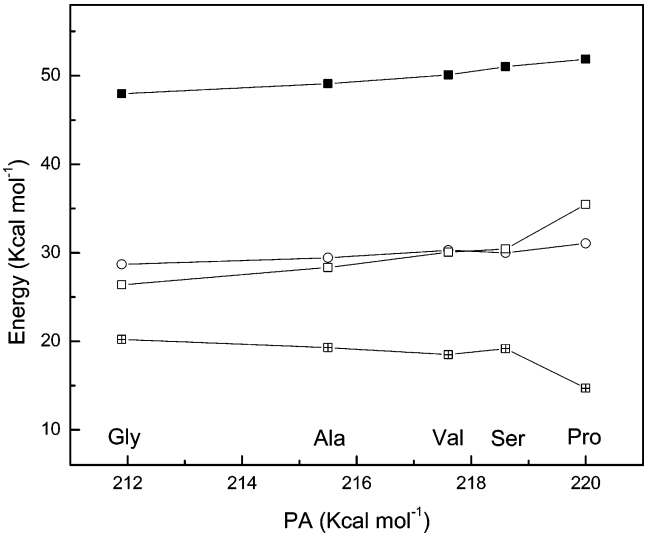

Figure 9. Analysis of the interactions between different amino acids and $(CH_3)_2NH_2^+$: ■, the salt bridge interaction energies between zwitterionic amino acids and $(CH_3)_2NH_2^+$; ⊞, the deformation energies of amino acids in the zwitterionic clusters; □, net binding energies in the zwitterions clusters, i.e., the salt bridge interaction energy minus the deformation energies of amino acid and dimethylaminium ion. ○, Net binding energies in the non-zwitterions clusters. The energies are calculated at the MP2-(full)/6-311++G(2d,2p)//B3LYP/6-311+G(d,p) level of theory.

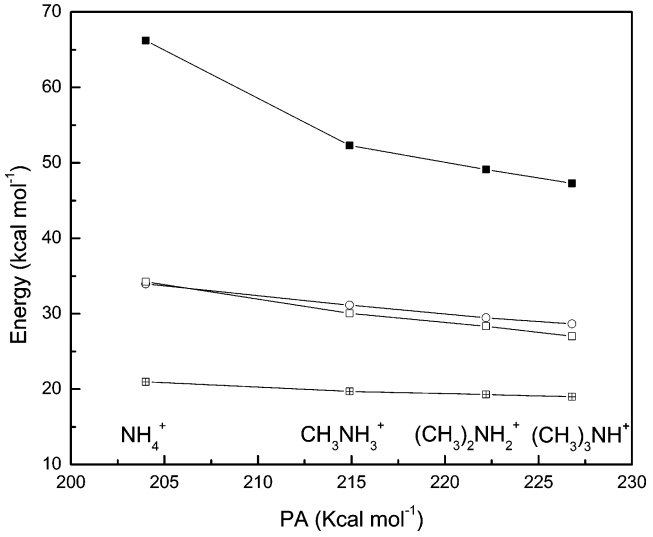

Figure 10. Interaction between alanine and different ammonium ions. ■, Salt bridge interaction energies between zwitterionic alanine and different ammonium ions; ⊞, deformation energies of amino acids in the zwitterionic clusters; □, net binding energies in the zwitterionic clusters. ○, Net binding energy in the non-zwitterionic clusters. Energies are calculated by at the MP2(full)/6-311++G(2d,2p)//B3LYP/6-311+G(d,p) level of theory.

The relative stability of zwitterionic structures of several amino acids in association with a dimethylammonium ion is illustrated in Figure 9. For the most stable zwitterionic cluster species, the salt bridge interaction energies between glycine, alanine, valine, serine, and proline and a dimethylaminium ion can be computed to be 48.0, 49.1, 50.1, 51.0, and 51.9 kcal mol^{-1}, respectively. This interaction energy increases gradually with the increase in proton affinity of the amino acids, as a result of the increased extent of proton transfer from the dimethylammonium ion to the amino acid with a high proton affinity. The deformation energies of the zwitterionic structures of glycine, alanine, valine, serine, and proline are 20.2, 19.3, 18.5, 19.2, and 14.7 kcal mol^{-1}, respectively. An early Fourier Transfer Ion Cyclotron Resonance (FTICR) study estimated the difference between the neutral and zwitterionic glycine as 20 kcal mol^{-1},[75] which is in excellent agreement with our calculated value of the deformation energy. As the proton affinity of the amino acid increases the deformation energy correspondingly decreases such that, for proline, the deformation energy is only 14.7 kcal mol^{-1}, which is 5.5 kcal mol^{-1} less than that of glycine. For the amino acids with a higher proton affinity, the zwitterionic form becomes increasingly more stable as proton transfer from the carboxylic acid group to the amino function becomes more favorable.

The deformation energies of the dimethylammonium ion are nearly the same in each of the clusters at about 1.5 kcal mol^{-1}. The net binding energies can then be obtained from the difference in the salt bridge interaction energies of the clusters and the sum of deformation energies of the zwitterionic amino acids and the dimethylammonium ion. For the most stable zwitterionic isomers, the net binding energies of glycine, alanine, valine, serine, and proline are the 26.4, 28.3, 30.0, 30.4, and 35.5 kcal mol^{-1}, respectively.

(75) Locke, M. J.; Mclver, R. T. *J. Am. Chem. Soc.* **1983**, *105*, 4226.

Whether the zwitterionic structure is the most stable isomer is related not only to its net binding energy but also to the net binding energy of the most stable non-zwitterionic isomer. For the most stable non-zwitterionic isomer, the interaction energies between the amino acid and dimethylaminium ion are 30.8, 31.7, 32.4, 33.1, and 33.0 kcal mol^{-1}, respectively, for glycine, alanine, valine, serine, and proline. These values are about 18 kcal mol^{-1} lower than those of their corresponding zwitterionic isomers. The interaction energy also increases slightly (2.2 kcal mol^{-1}) from glycine to valine, but the magnitude is lower than that of the zwitterionic isomers (3.9 kcal mol^{-1}). The deformation energies of amino acids in the charge solvated clusters are much less than those of the zwitterionic isomers, at only 1.4, 1.5, 1.3, 2.3 and 1.1 kcal mol^{-1} for glycine to proline, respectively. Further, the deformation energy of the dimethylammonium ion is about 0.8 kcal mol^{-1} for each cluster. In contrast, the net binding energies obtained from a similar analysis for the charge solvated clusters are found to be 28.7, 29.4, 30.3, 30.0, and 31.1 kcal mol^{-1}, respectively, for glycine, alanine, valine, serine, and proline. A comparison of the net binding energies in the two different types of clusters then reveals which of the two isomeric forms of the clusters should be formed. In this way, it can be predicted that the zwitterionic forms of serine and proline should be dominant.

In addition, the effect of several ammonium ions (NH_4^+, $CH_3NH_3^+$, $(CH_3)_2NH_2^+$, $(CH_3)_3NH^+$) on the formation of the zwitterionic structure of alanine has also been investigated, as shown in Figure 10. In proceeding from NH_4^+ to $(CH_3)_3NH^+$, the salt bridge interaction energies between the ammonium ions and zwitterionic alanine decrease gradually. For example, the salt bridge interaction energy between NH_4^+ and alanine of 66.2 kcal mol^{-1} drops to corresponding values of 52.3, 49.1, and 47.3 kcal mol^{-1} for $CH_3NH_3^+$, $(CH_3)_2NH_2^+$, and $(CH_3)_3NH^+$, respectively. The strong interaction between alanine and NH_4^+ also causes the deformation energy for NH_4^+ (11.0 kcal mol^{-1}) to be notably higher than that of other ions (~2 kcal mol^{-1}).

Despite this higher deformation energy for NH_4^+, the final net binding energy of the zwitterionic isomer is still greater than that of the non-zwitterionic isomer. An examination of Mulliken population analyses reveals that when zwitterionic adducts of NH_4^+ and $CH_3NH_3^+$ are formed, a considerable transfer of electron density takes place from the carboxylate group to the ammonium moieties.

Conclusions

Gas-phase ion−molecule interactions involving several amino acids and ammonia or amines have been studied by high-pressure mass spectrometry and ab initio calculations. The enthalpy changes for the association reactions of protonated glycine, alanine, valine, leucine, isoleucine, serine, and proline with ammonia have been measured as −23.2, −21.9, −21.0, −20.8, −20.6, −22.6, and −20.4 kcal mol^{-1}, respectively. There is a very good linear relationship between the enthalpy changes and their proton affinities, except for serine, where the hydroxyl group substituent forms an extra hydrogen bond with ammonia. The measured values are in very good agreement with the corresponding calculated data for the most stable isomers at the MP2(full)/6-311++G(2d,2p)//B3LYP/6-311+G(d,p) level of theory.

For the interaction between protonated proline and methylamine, the measured enthalpy and entropy changes are −26.6 kcal mol^{-1} and −30.1 cal mol^{-1} K^{-1}, respectively. These are consistent with the corresponding calculated values of the most stable isomer, **Pma1**, in which proline is a zwitterionic structure. In addition, the measured enthalpy change is obviously higher than the calculated value 22.9 kcal mol^{-1} for the most stable non-zwitterionic isomer (**Pma2)**. The potential energy surface for the interconversion of the isomers of the protonated proline clusters with methylamine has been constructed at the B3LYP/6-311+G(d,p) level of theory. Calculations of the potential energy surface for this system show that the highest barrier on the PES lies 19.2 kcal mol^{-1} above the global minimum energy isomer (**Pma1**). Given that the exothermicity of formation of the minimum energy structure is 8 kcal mol^{-1} greater than the barrier, the entire surface, involving 7 stable minima and 6 transition states, can be fully sampled during the association-dissociation equilibrium process. Moreover, the most stable isomer on this surface, **Pma1**, involves $CH_3NH_3^+$ bound to proline in a zwitterionic form, and the binding energy is much higher than any other isomer. Considering the calculated difference in binding energies of the various isomers, the zwitterionic isomer is essentially the exclusive species under our experimental conditions. The experimental and calculated results confirm that the zwitterionic structure of proline may be well stabilized by $CH_3NH_3^+$.

The interaction strengths between neutral amino acids and NH_4^+ have also been obtained. Obviously the interaction between amino acids and Na^+ are much stronger than the corresponding values for NH_4^+ by ∼7 kcal mol^{-1}. This may be attributed to a fundamental difference between NH_4^+ and Na^+ interactions with amino acids where, for example, the interactions between Na^+ and amino acids are mainly electrostatic while the interactions with NH_4^+ are *via* hydrogen bonds. The difference in the nature of the interactions also results in the structural distinction between the complexes of amino acids with Na^+ or NH_4^+.

Stabilization of the zwitterionic structure of a series of amino acids (Gly, Ala, Val, Ser, and Pro) by various organic ammonium ions (NH_4^+, $CH_3NH_3^+$, $(CH_3)_2NH_2^+$, $(CH_3)_3NH^+$) has been investigated systematically. Energy decomposition analysis has been performed so that the salt bridge interaction strengths between zwitterionic amino acids and ammonium ions could be obtained. Some generalizations with respect to the relative stability of zwitterionic structures of amino acids may be drawn. First, as the PA of the amino acid increases, within the series Gly, Ala, Val, the zwitterionic structure becomes more energetically favorable relative to the non-zwitterionic isomer. Second, as the PA of the amine increases, the zwitterionic structure of a given amino acid within the complex becomes gradually less favorable. Third, compared to the other amino acids, Pro, the only secondary amine among the 20 naturally occurring amino acids, has a much more pronounced tendency to form the zwitterionic structure, which has been confirmed by the experimental results. Finally, substitutents on the amino acid backbone that may participate in additional hydrogen bond interactions in the non-zwitterionic isomer may render it as being more stable, as in the case for Ser.

Organic ammonium ions are very common and also exist extensively as groups in protonated amino acids and nucleic acid bases and other biomolecules, as well as in the zwitterions of neutral amino acids and peptides. Investigation of the stabilization of zwitterionic structures of a series of amino acids by various organic ammonium ions aids the further understanding of the structure, property, and function of amino acids, peptides, and proteins.

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Supporting Information Available: Figure S1−S9, Table S1, and complete ref 45. This material is available free of charge via the Internet at http://pubs.acs.org.

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