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Investigation of Proton Transport Tautomerism in Clusters of Protonated Nucleic Acid Bases (Cytosine, Uracil, Thymine, and Adenine) and Ammonia by High-Pressure Mass Spectrometry and Ab Initio Calculations

Ronghu Wu and Terry B. McMahon*

Contribution from the Department of Chemistry, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada

Received July 17, 2006; E-mail: mcmahon@uwaterloo.ca

Abstract: The energetics of the ion−molecule interactions and structures of the clusters formed between protonated nucleic acid bases (cytosine, uracil, thymine, and adenine) and ammonia have been studied by pulsed ionization high-pressure mass spectrometry (HPMS) and ab initio calculations. For protonated cytosine, uracil, thymine, and adenine with ammonia, the measured enthalpies of association with ammonia are −21.7, −27.9, −22.1, and −17.5 kcal mol^{-1}, respectively. Different isomers of the neutral and protonated nucleic acid bases as well as their clusters with ammonia have been investigated at the B3LYP/6-31+G-(d,p) level of theory, and the corresponding binding energetics have also been obtained. The potential energy surfaces for proton transfer and interconversion of the clusters of protonated thymine and uracil with ammonia have been constructed. For cytosine, the experimental binding energy is in agreement with the computed binding energy for the most stable isomer, **CN01-01**, which is derived from the enol form of protonated cytosine, **CH01**, and ammonia. Although adenine has a proton affinity similar to that of cytosine, the binding energy of protonated adenine to ammonia is much lower than that for protonated cytosine. This is shown to be due to the differing types of hydrogen bonds being formed. Similarly, although uracil and thymine have similar structures and proton affinities, the binding energies between the protonated species and ammonia are different. Strikingly, the addition of a single methyl group, in going from uracil to thymine, results in a significant structural change for the most stable isomers, **UN01-01** and **TN03-01**, respectively. This then leads to the difference in their measured binding energies with ammonia. Because thymine is found only in DNA while uracil is found in RNA, this provides some potential insight into the difference between uracil and thymine, especially their interactions with other molecules.

1. Introduction

DNA is among the largest of the naturally occurring biological macromolecules, containing many thousands of nucleic acid bases, and it is of prime significance in genetic determination.[1] The five nucleic acid bases, cytosine, thymine, uracil, adenine, and guanine, found in DNA and RNA govern the replication of DNA, store information required to synthesize proteins, and translate this information to the protein.

Tautomerism is a well-known phenomenon occurring in nucleic acid bases,[2−16] in which proton transfer from the heterocyclic ring nitrogen to an exocyclic oxo- or imino- group leads to the formation of either an −OH or an −NH_2 functionality. These processes are keto−enol or imino−amino tautomerism, respectively. In addition, in the protonated nucleic acid bases, the mobility of the proton can result in different isomers. Tautomerism thus makes the ion−molecule behavior of these molecules complex because there can be several different isomers for each species that can potentially coexist.

The surrounding environment also plays a determinant role in proton transfer and the tautomerism of nucleic acid bases. The interactions between nucleic acid bases and molecules or ions in the gas phase have been the subject of numerous studies.[17−23] There are many reported studies of the clusters

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between nucleic acid bases and water.[17,21,24−31] Water molecules have significant effects on the structures, energetics, and kinetics of neutral and ionized nucleic acid bases, and the impact of hydration can be studied on a molecular level by investigating how the property of a molecule changes as it is hydrated by stepwise addition of the individual water molecule.[32,33] The interactions between nucleic acid bases and acetic acid,[34] alcohols,[35] and other species have also been reported. The interaction of metal ions with nucleic acid bases is important in biological processes, and several metal cations (such as Li^+, K^+, Na^+, Ca^{2+}, Mg^{2+}, etc.) have been studied extensively.[36−45] The bond energies, attachment sites, and conformational changes of nucleic acid bases all have been investigated carefully. In comparison to studies involving water and metal ions, the interactions with ammonia or organic ions have seldom been studied.[45−49] Electrospray ionization mass spectrometry has been used to study nucleic acid bases.[44,50] Alkali and ammonium cations significantly increase self-aggregation of the nucleic acid bases and lead to the formation of stable magic number clusters.[44] The addition of ammonium chloride to adenine and cytosine does not result in adduct formation. However, the ammonium cation does form adducts with monomer, dimers, trimers, and tetramers of thymine, and clusters with five thymine or uracil molecules to generate a magic number cluster.[44] Brodbelt et al.[48] investigated the H/D exchange of adenine, cytosine, and uracil with CH_3OD and ND_3. These species exchange all of their labile hydrogens plus the added proton when interacting with ND_3. However, when protonated cytosine reacts with CH_3OD, it undergoes only two H/D exchanges because of the lower gas-phase basicity of CH_3OD relative to the basicity of ND_3.

Protonation of nucleic acid bases plays a crucial role in many biochemical reactions such as, for example, enzymatic reactions, stabilization of triplex and higher order structures, and mutagenic process.[51] There have been many studies of the protonation of nucleic acid bases using both experimental and theoretical approaches. The earliest theoretical study of the protonation process of nucleic acid bases was performed using a molecular electrostatic potential derived from ab initio wave functions in the 1970s.[52] This study provided indications of protonation sites but did not yield proton affinity values. A recent systematic theoretical study on the determination of proton affinities of nucleic acid bases has been performed by Russo et al. using density functional theory.[53] Comprehensive post-Hartree−Fock calculations have also been performed to study protonation of nucleic acid bases.[54] In addition, Wollken et al. investigated the protonation of uracil by many computational methods ranging from density functional theory through Moller−Plesset theory up to quadratic configuration interaction.[55] Greco et al.[56] determined proton affinities (PA) experimentally from the kinetics of the gas-phase unimolecular dissociations of their proton-bound hetero-complexes with amines of known proton affinity. These clusters were formed by fast atom bombardment and the unimolecular dissociations followed by tandem mass spectrometry. High-pressure mass spectrometry (HPMS) has also been used to measure the proton affinities of cytosine, thymine, and adenine.[57] Thus, it is clear that an investigation of the interactions between protonated nucleic acid bases and small solvent molecules will certainly provide valuable insights into nucleic acid base behavior.

HPMS is well known as a powerful technique for the study of gas-phase ion thermochemistry. Equilibrium constants for many processes can be determined by following the temporal profiles of charged species of interest, formed via pulsed ionization, and using the long time steady-state ratios of the ion intensities involved in equilibrium reactions. Important thermochemical data can be obtained, such as proton affinities, gas-phase acidities, ionization energies, electron affinities, hydrogen-bond energies, Lewis affinities, and metal cation affinities.[58] HPMS is therefore an ideal tool to study ion−molecule association reactions leading to cluster formation[59−63] and for the investigation of solvation of ionic species[64−68] by

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one or more solvent molecules. Quantitative enthalpy and entropy changes for these processes can then be obtained directly by measuring equilibrium constants as a function of temperature. The combination of such experiments with ab initio calculations leads to a detailed understanding of both the energetics of the potential energy surface for the reaction and the structural information for all of the key species on the potential energy surface.

In the present study, the enthalpy and entropy changes for the formation of clusters of protonated nucleic acid bases (cytosine, uracil, thymine, and adenine) with ammonia have been measured by HPMS. Using ab initio calculations, the structures for the different isomers of the neutral and protonated cytosine, uracil, thymine, and adenine, as well as their clusters with ammonia, have been optimized at the B3LYP/6-31+G(d,p) level of theory. At the same time, the potential energy surfaces for proton transfer within the protonated nucleobases and the interconversion of the various isomers of their clusters with ammonia have also been obtained. The ab initio calculated binding energetics may also be compared to the experimental values and used to infer the structures of the clusters formed experimentally. In addition, the interaction between neutral nucleic acid bases (cytosine, uracil, and thymine) and ammonium ion has also been investigated and compared to their interactions with Na^+. The understanding gained of the interaction between protonated nucleic acid bases and various small solvent molecules, such as ammonia, will inevitably lead to a deeper insight into the structures, reactions, and properties of DNA and RNA and their interactions with the surrounding medium.

2. Experimental and Computational Methods

2.1. Experiment. All experiments were carried out on a high-pressure mass spectrometer constructed at the University of Waterloo and whose general design has been described in detail elsewhere.[69−71] 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 performed in positive ion mode with an ion energy of 4 keV.

Gas mixtures were prepared in a 2.54 L reservoir using methane as bath gas at a pressure of 500−1200 Torr. The partial pressure of ammonia was typically in the range of 0.1−10 Torr, depending on the ions desired in the equilibrium to be investigated. A small amount of CCl_4 was typically also added to the reservoir to promote ion pair diffusion as the dominant diffusion mode, effectively leading to an increase in ion source residence times by slowing the rate of ion diffusion to the source walls. Gas mixtures were flowed into the ion source to a total pressure of 4−8 Torr. A solid sample of the nucleic acid base of interest was introduced directly inside the high-pressure source such that, when the high-pressure source is heated, gaseous nucleic acid base is present at its equilibrium vapor pressure. Ionization is initiated by a beam of energetic (2 kV) 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 the high-pressure source. The difference in proton affinities between ammonia and nucleic acid bases is sufficiently large that no NH_4^+ signal is detectable. The cluster ions of ammonia with the protonated species of interest are formed in the high-pressure source, and the equilibrium ion intensities are attained relatively early in the ion temporal profiles at ion source pressures of several Torr. Mass selected ion temporal profiles were monitored using a PC-based multichannel scaler data acquisition system, typically configured between 10 and 30 μs per channel. A total of 1024 channels were acquired, and more than 3000 electron gun pulses were accumulated.

For the association reaction, eq 1, the corresponding equilibrium constant is given by eq 2. The equilibrium constants can be calculated from the relative ionic abundance ($I_{B(NH_3)H^+}/I_{BH^+}$) and partial pressure of ammonia. $I_{B(NH_3)H^+}/I_{BH^+}$ may be measured directly from the HPMS steady-state relative ionic abundances. The partial pressure of ammonia in the ion source 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.

$$BH^+ + NH_3 \rightleftarrows B(NH_3)H^+ \qquad B = \text{Cyt, Ura, Thy, or Ade} \tag{1}$$

$$K_{eq} = \frac{I_{B(NH_3)H^+}}{I_{BH^+}} \cdot \frac{1}{p_{NH_3}} \tag{2}$$

As the temperature is changed, K_{eq} can be determined at each temperature. Next, 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 K_{eq} versus reciprocal temperature.

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

2.2. Ab Initio Calculations. Ab initio calculations have been carried out with the Gaussian 03 program package.[72] The structures of the neutral and protonated nucleic acid bases and their clusters with ammonia were calculated at the density functional theory (DFT) level, employing the B3LYP exchange-correlation functional and the 6-31+G-(d,p) basis set. The potential energy surfaces for proton transfer and the interconversion of the ammonia clusters have also been obtained at the same level of theory. Vibrational frequencies were calculated for all structures to verify that no negative frequencies were present for local and global minimum structures on the potential energy surface. For transition states, 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 reactants and products. To obtain more accurate interaction energetics, single point energies have been calculated using a MP2(full)/6-311++G(2d,2p)// B3LYP/6-31+G(d,p) protocol. Zero-point energy and thermal energy corrections at 298 K were also included. Basis set superposition error (BSSE) was computed following geometry optimization using the counterpoise correction method at the MP2(full)/6-311++G(2d,2p) level.[73] The entropies of the association reaction were obtained from B3LYP/6-31+G(d,p) geometries and harmonic vibrational frequencies.

Gas-phase proton affinity (PA) is defined as the negative of the enthalpy change for the addition of a gaseous proton to a gaseous neutral molecule. For example, the protonation of cytosine, eq 4, leads to a

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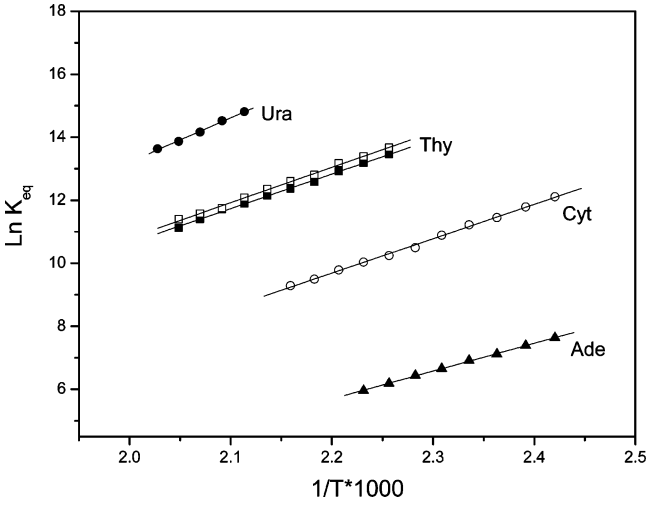

Figure 1. van't Hoff plots for the association reactions, $BH^+ + NH_3 \rightleftarrows B(NH_3)H^+$, B = Cyt, Thy, Ura, Ade.

proton affinity of cytosine given by eq 5,

$$\mathrm{Cyt} + \mathrm{H}^+ \rightarrow \mathrm{CytH}^+ \quad (4)$$

$$\mathrm{PA(Cyt)} = -\Delta H_{298}(4) = -(H_{298}(\mathrm{CytH}^+) - H_{298}(\mathrm{Cyt})) + {}^5/_2 RT \quad (5)$$

where the enthalpy functions, H_{298}, include the electronic energy and zero-point energy and temperature corrections to the vibrational enthalpy of the reactant Cyt and product $CytH^+$ at 298 K. The ${}^5/_2RT$ term is the sum of the transitional energy of the proton and the work term, ΔnRT, for the addition of a proton.

Similarly, the enthalpy change for the association reaction, for example, eq 1, is given by eq 6. Here, the enthalpy change is simply the difference in the enthalpy functions of product and reactants because the work term is inherently included in the enthalpy terms.

$$\Delta H_{298} = H_{298}(\mathrm{Cyt(NH_3)H^+}) - (H_{298}(\mathrm{CytH^+}) + H_{298}(\mathrm{NH_3})) \quad (6)$$

In the present work, the binding enthalpy of the clusters denotes the enthalpy changes calculated using the single point energy determinations. For entropies and the potential energy surfaces, the values obtained at B3LYP/6-31+G(d,p) are employed. In addition, for many species, several possible rotatamers exist, and, in these cases, only the value for the most stable rotatamer is reported.

3. Results

3.1. Equilibrium Measurements. The experimental van't Hoff plots for the association reactions investigated are shown in Figure 1. The thermochemical data extracted from these van't Hoff plots are summarized in Table 1. For the association reaction of protonated cytosine and ammonia, the measured enthalpy and entropy changes are -21.7 kcal mol^{-1} and -28.4 cal mol^{-1} K^{-1}, respectively. The calculated enthalpy change for the formation of the most stable isomer, **CN01-01** (shown in Figure 2), is -24.6 kcal mol^{-1} at the B3LYP/6-31+G(d,p) level, which is a little greater than the measured value. When the enthalpy change is taken from the single point energies determined at the MP2(full)/6-311++G(2d,2p)//B3LYP/6-31+G(d,p) level, this value is reduced to -23.4 kcal mol^{-1}. When BSSE is taken into account, the calculated enthalpy change of -20.8 kcal mol^{-1} is slightly lower than the experimental value. It is well known that the BSSE correction often underestimates the binding energies when the basis set used is not sufficiently large and when the structure computed is sufficiently loose such as those previously reported.[74,75] The calculated entropy change of **CN01-01** is -27.3 cal mol^{-1} K^{-1}, in excellent agreement with the experimentally determined value. For thymine, uracil, and adenine, the results of the MP2-(full) calculations are reported without BSSE correction.

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

	ΔH (kcal mol^{-1})[a]	ΔS (cal mol^{-1} K^{-1})[b]
$CytH^+ + NH_3 \leftrightarrows Cyt(NH_3)H^+$	-21.7	-28.4
$UraH^+ + NH_3 \leftrightarrows Ura(NH_3)H^+$	-27.9	-29.6
$ThyH^+ + NH_3 \leftrightarrows Thy(NH_3)H^+$	-22.3	-23.1
	-21.8	-22.5
$AdeH^+ + NH_3 \leftrightarrows Ade(NH_3)H^+$	-17.5	-27.2

[a] Maximum uncertainty in ΔH values is ± 1.0 kcal mol^{-1}. This uncertainty is greater than that which is obtained from the van't Hoff plot due to assumed additional maximum uncertainties in temperature and pressure measurements. [b] Maximum uncertainty in ΔS values is ± 3.0 cal mol^{-1} K^{-1}. This uncertainty is greater than that which is obtained from the van't Hoff plot due to assumed additional maximum uncertainties in temperature and pressure measurements.

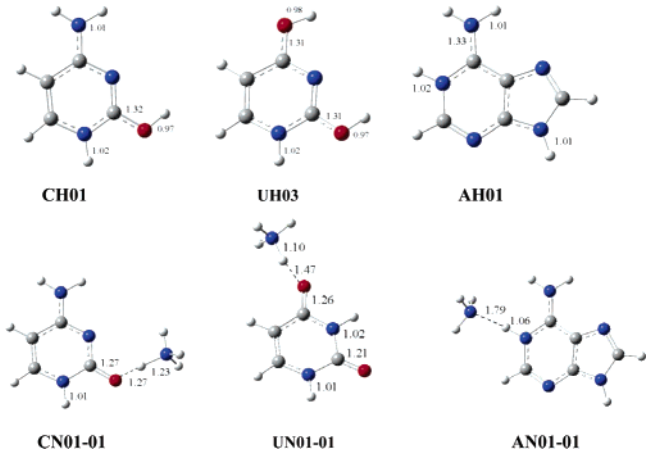

Figure 2. The structures of the most stable isomers of the protonated cytosine (**CH01**), uracil (**UH01**), and adenine (**AH01**) and their clusters with ammonia (**CN01-01**, **UN01-01**, and **AN01-01**) calculated by B3LYP/6-31+G(d,p).

The measured enthalpy and entropy changes for the clustering reaction of protonated uracil with ammonia are -27.9 kcal mol^{-1} and -29.6 cal mol^{-1} K^{-1}, respectively. The corresponding computed enthalpy change for the most stable isomer, **UN01-01**, is -29.2 kcal mol^{-1}, which is close to the experimental value.

For the association reaction of protonated thymine and ammonia, the experimentally measured enthalpy and entropy values are -22.3 kcal mol^{-1} and -23.1 cal mol^{-1} K^{-1}, respectively. The enthalpy change can be seen to be substantially different from that of uracil, and, to confirm this, replicate determinations were carried out, with the most different value obtained being -21.8 kcal mol^{-1} for the enthalpy change. The average enthalpy value obtained is -22.1 kcal mol^{-1} with a corresponding entropy change of -22.8 cal mol^{-1} K^{-1}. Thus, although uracil and thymine have similar structures and proton affinities, the binding energies of the protonated species with

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ammonia are quite different. This difference is discussed in some detail below.

The measured enthalpy and entropy changes for the association reaction of protonated adenine with ammonia are −17.5 kcal mol^{-1} and −27.2 cal mol^{-1} K^{-1}, respectively. These two values agree well with the computed enthalpy and entropy changes of −18.3 kcal mol^{-1} and −27.1 cal mol^{-1} K^{-1}, respectively, for the most stable isomer, **AN01-01**.

Kebarle[76,77] and Meot-Ner[78] et al. have previously carried out numerous investigations of proton-bound dimers of the form $BH^+ \cdot A$ and have demonstrated an excellent qualitative trend between the binding energy and the proton affinity difference, ΔPA, between the A and B species. This trend shows that binding energy decreases as the difference between the proton affinities of A and B increases. This may be understood in terms of the hydrogen-bond formation in $BH^+ \cdot A$ being viewed as partial proton transfer from BH^+ to A in the association complex. Partial proton transfer is facilitated either when BH^+ becomes a more efficient proton donor, that is, when the proton affinity of neutral B decreases, or when A becomes a more efficient proton acceptor, that is, when the proton affinity of A increases. This trend has also been confirmed in many studies.[79−82] Desmeules et al.[83] have analyzed this relationship between the binding energy and the difference of proton affinities computationally and have verified the occurrence of partial proton transfer in the proton-bound dimers as measured by the elongation of the $B-H^+$ bond.

The proton affinities, taken from the NIST database, of cytosine, uracil, thymine, and adenine are 227.0, 208.6, 210.5, and 225.3 kcal mol^{-1}, respectively, while that of ammonia is 204.0 kcal mol^{-1}.[84] Thus, according to the relationship between the binding energy and the PA difference, the binding energy of protonated cytosine to ammonia should be the lowest and the binding energies of protonated uracil and thymine to ammonia should be similar. Contrary to this expectation, however, the experimental binding energy of protonated adenine to ammonia is the lowest of the four association reactions, those of cytosine and thymine are similar, and that of uracil is much greater than that of thymine. Several possibilities exist to explain these inconsistencies, most notably potential differences in the nature of the functionalities participating in hydrogen-bond formation as well as isomerization of the clusters. These possibilities are discussed below in light of more detailed computational investigations.

3.2. Computational Energetics and Structures. 3.2.1. Cytosine. There are extensive computational calculation and experimental data concerning the tautomers of neutral and protonated cytosine and their relative stabilities.[3,4,12−14,85−87] Based on these previous results, the various possible stable tautomers of both neutral and protonated cytosine were explored by B3LYP/6-31+G(d,p), and the most stable tautomers are summarized in Figure S1, with their calculated relative energetics summarized in Table S1 in the Supporting Information. The most stable tautomer is **C01** at the B3LYP/6-31+G(d,p) level, in which a hydrogen is located at the N1 position giving a cyclic secondary amine functionality. The corresponding enol forms, **C02**, with a hydroxyl hydrogen cis to N1, and **C03**, with a hydroxyl hydrogen trans to N1, are the second and third most stable forms. Protonated cytosine has been generally assumed to occur only in a keto−amino tautomeric form. According to diffraction data[86] and ab initio calculations (HF/STO-3G),[87] cytosine protonation should occur at the N3 position giving rise to structure **CH02**. In the present study, **CH01**, the enol−amino form, is found to be the most stable isomer. **CH02**, the keto−amino form, is only 0.3 kcal mol^{-1} higher in energy than that of **CH01**, and the energy of **CH03** is 8.5 kcal mol^{-1} higher. However, the single point energy calculation gives **CH01** as 1.5 kcal mol^{-1} more stable than **CH02**. These findings are consistent with the literature data,[54,84,88] but it still must be considered to be undetermined which of the isomers **CH01** or **CH02** is actually the more stable. The calculated proton affinity, based on cytosine protonation of **C02** at N1 leading to **CH01**, is 225.7 kcal mol^{-1}, which is in good agreement with the value from the NIST database of 227.0 kcal mol^{-1}.

Because the energy of **CH03** is sufficiently higher than that of either **CH01** or **CH02**, only clusters of protonated cytosine and ammonia derived from **CH01** and **CH02** have been considered. The four most stable isomers obtained from each of **CH01** and **CH02** are displayed in Figure S1. Among these isomers, **CN01-01** is the most stable (also shown in Figure 2), in which ammonia forms a hydrogen bond with the hydroxyl proton at O2 and partial proton transfer occurs from O2 to ammonia with N−H and O−H distances of 1.23 and 1.27 Å, respectively. The calculated enthalpy change is −23.4 kcal mol^{-1}, and −20.8 kcal mol^{-1} when BSSE is considered. In **CN01-02**, ammonia forms a hydrogen bond with the ring nitrogen, N1. For **CN01-03** and **CN01-04**, ammonia binds to one or the other of the two exocyclic amino hydrogens of **CH01**. In comparison to **CN01-01**, the binding energies of these other three isomers are about 4 kcal mol^{-1} lower at 298 K, and their H-bond lengths of 1.68, 1.85, and 1.80 Å are obviously longer than that in **CN01-01**. From the order of the H-bond strengths, the order of acidities of the different sites in **CH01** may be inferred with the acidity of the −OH group as the strongest, and the amino hydrogens weaker than that at the aromatic nitrogen.

From **CH02**, the other four most stable isomers obtained for adduct formation with ammonia are shown in Figure S1, among which **CN02-01** is the most stable. The calculated binding energy obtained is 19.8 kcal mol^{-1}, which decreases to 17.9 kcal mol^{-1} with the inclusion of the BSSE correction. However the relative energy is still 5.6 kcal mol^{-1} higher than that of **CN01-01** at the B3LYP/6-31+G(d,p) level of theory and 2.9 kcal mol^{-1} at single point energy calculation.

The experimental enthalpy value for this association energy is −21.7 kcal mol^{-1}, which agrees best with the computed binding energy of **CN01-01**, clearly higher than the binding energy of **CN02-01**. Thus, the dominant species should be the

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Table 2. Calculated Values of Relative Energies, Enthalpy (298 K), and Entropy Changes of the Different Isomers of the Clusters of Protonated Cytosine and Ammonia (**CN**), Protonated Uracil and Ammonia (**UN**), and Protonated Adenine and Ammonia (**AN**)

		B3LYP/6-31+G(d,p)		MP2(full)/6-311++G(2d,2p)// B3LYP/6-31+G(d,p)	
	relative energy[a] (kcal mol^{-1})	ΔH (kcal mol^{-1})	ΔS (cal mol^{-1} K^{-1})	ΔH (kcal mol^{-1})	ΔH with BSSE[b] (kcal mol^{-1})
CN01-01	0	−24.6	−27.3	−23.4	−20.8
CN01-02	5.8	−18.8	−27.7	−19.2	−17.4
CN01-03	10.3	−14.3	−25.7	−14.3	−12.9
CN01-04	11.0	−13.6	−25.7	−13.6	−12.2
CN02-01	5.6	−19.3	−29.1	−19.8	−17.9
CN02-02	6.3	−18.7	−28.1	−18.6	−16.7
CN02-03	7.6	−17.3	−24.8	−17.2	−15.8
CN02-04	8.3	−16.3	−26.5	−16.5	−15.1
UN01-01	0	−29.7	−24.9	−29.2	
UN01-02	7.7	−21.9	−28.8	−22.4	
UN01-03	9.1	−20.6	−28.6	−20.6	
UN03-01	1.2	−27.1	−26.3	−26.4	
UN03-02	4.0	−24.3	−23.7	−23.2	
UN03-03	7.6	−20.8	−28.0	−21.2	
AN01-01	0	−17.9	−27.1	−18.3	
AN01-02	1.7	−16.2	−26.4	−16.7	
AN01-03	1.7	−16.2	−30.9	−16.6	
AN01-04	2.3	−15.6	−26.1	−15.5	
AN04-01	−0.6	−18.4	−27.4	−19.2	
AN04-02	1.4	−16.5	−26.0	−17.2	
AN04-03	4.0	−13.9	−26.1	−14.0	

[a] With ZPE and thermal energy correction at 298 K. [b] BSSE calculated by MP2(full)/6-311++G(2d,2p).

most stable isomer, **CN01-01**, under the experimental conditions used here. The experimental entropy change is −28.4 cal mol^{-1} K^{-1}, which also corresponds well with the calculated value for **CN01-01** of −27.3 cal mol^{-1} K^{-1}.

3.2.2. Uracil. In agreement with several previous studies,[53−55] the most stable form of neutral uracil, **U01**, is found to arise from the structure with two hydrogen atoms bound to the two ring nitrogens, as shown in Figure S2. The energy of each of the other possible isomers is at least 10 kcal mol^{-1} higher than that of **U01**. With such large energy differences, the abundance of any of these isomers will be much less than that of **U01** within the experimental temperature range.

The four most stable tautomers of protonated uracil can be considered to be derived from protonation of **U01**−**U04** at their respective most basic sites. These structures are shown in Figure S2, and their relative energies are given in Table S2. Alternatively, **UH01** and **UH02** could be considered to be derived from **U01** by protonation at either of the two carbonyl oxygens, with **UH01** seen to be more stable than **UH02**. Even though **UH03** is the most stable isomer, it cannot form directly from **U01**, the only tautomer expected to be present in any abundance. The calculated barrier for intramolecular proton transfer from **UH01** to **UH03** is in excess of 35 kcal mol^{-1} at 298 K, which is too high to lead to any appreciable formation of this most stable tautomer. It has therefore previously been concluded that under thermal conditions in the gas phase **UH03** will not be formed[55] even though it is the most stable protonated species.

Because **UH02** and **UH04** are high in energy, the only clusters of protonated uracil and ammonia considered in the present work were based on association of ammonia with **UH01** and **UH03**. The three most stable isomers derived from **UH01** obtained, designated **UN01-01**, **UN01-02**, and **UN01-03**, are shown in Figure S2, and their relative energetics are given in Table 2. In **UN01-01**, it can be seen that an endothermic proton transfer has effectively occurred from protonated uracil to ammonia, giving a hydrogen-bond length from an ammonium ion hydrogen to a carbonyl oxygen of 1.47 Å. The enthalpy change thus calculated for formation of this adduct is −29.2 kcal mol^{-1}, which is nearly 7 kcal mol^{-1} more favorable than that for formation of the next most stable species, **UN01-02**. Three more stable isomers, **UN03-01**, **UN03-02**, and **UN03-03**, derived from **UH03**, are also displayed in Figure S2 and have binding energies of 26.4, 23.2, and 21.2 kcal mol^{-1}, respectively. Comparison of the relative energetics of all six of these isomers would seemingly give rise to the prediction that the most stable isomer, **UN01-01**, is the dominant species formed under the experimental conditions. The calculated binding energy of 29.2 kcal mol^{-1} is in reasonably good agreement with the experimentally measured value of −27.9 kcal mol^{-1}.

3.2.3. Thymine. The structure of thymine is similar to that of uracil with only one more methyl group adjacent to the ring carbonyl group in thymine. By analogy to uracil, the most stable neutral isomer has two hydrogen atoms bound to the two ring nitrogen atoms. This species, **T01**, is more than 10 kcal mol^{-1} lower in energy than any other possible tautomer and is thus predicted to be the only species present in measurable abundance under the experimental conditions here. The structures and relative energetics are given in Figure 3 and Table 3, respectively.

The four most stable protonated thymine species have also been determined. Again, by analogy to uracil, the most stable tautomer is **TH03** with an energy 2.7 kcal mol^{-1} lower than that of **TH01**. The energies of **TH02** and **TH04** are more than 10 kcal mol^{-1} higher than that of **TH03**, the most stable isomer.

The three most stable adduct species with ammonia derived from **TH01**, shown in Figure 3, are **TN01-01**, **TN01-02**, and **TN01-03**. The relative binding energetics given in Table 3 show that **TN01-01** is the most stable of these with a binding energy of 27.6 kcal mol^{-1}. This is sufficiently greater than that of the other two structures that it should be the dominant species possible to form from **TH01**. From the most stable, but

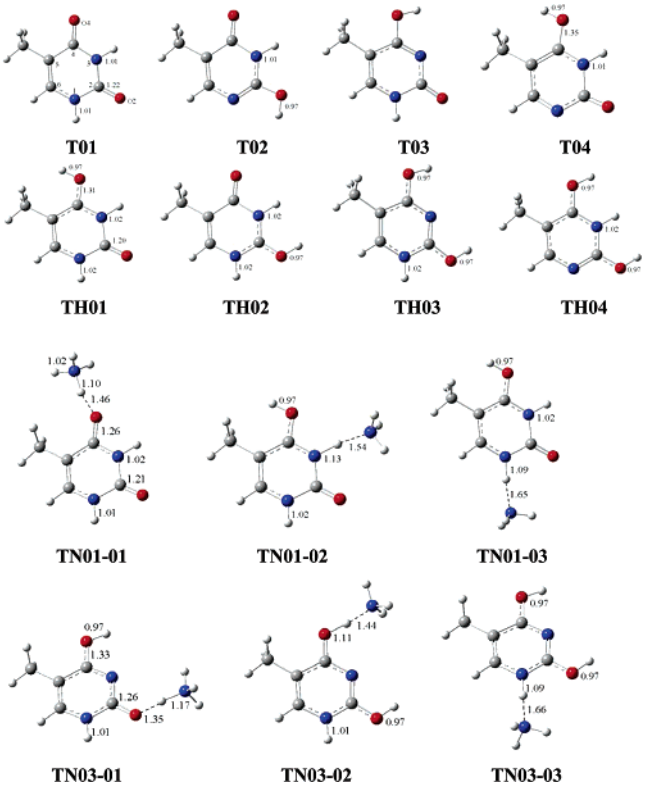

Figure 3. The structures of the different isomers of neutral (**T**) and protonated thymine (**TH**) and the cluster of protonated thymine and ammonia (**TN**) obtained by B3LYP/6-31+G(d,p).

apparently inaccessible, protonated thymine, **TH03**, three other species, **TN03-01**, **TN03-02**, and **TN03-03**, may be derived. These are also shown in Figure 3 with their relative energetics also given in Table 3. From these relative energetics for all six possible species, **TN03-01** is seen to be the most stable isomer with an energy 0.8 kcal mol^{-1} lower than that for **TN01-01**. However, the enthalpy change for formation of **TN03-01** from **TH03** of −24.8 kcal mol^{-1} is smaller than that for formation of **TN01-01** from **TH01**, and thus might be predicted not to be present in any appreciable amount due to the failure to generate **TH03**. In **TN03-01**, as in **TN01-01**, an endothermic proton transfer can also be seen to occur from protonated thymine to ammonia. As compared to the corresponding isomer of uracil, the hydrogen-bond length is slightly shorter at 1.35 Å for **TN03-01** relative to 1.38 Å for **UN03-01**. This is most easily rationalized by the relative proton affinities of **U03** and **T03** where the lower proton affinity of uracil facilitates a slightly more complete proton transfer to ammonia than is the case for the more basic thymine.

Among the isomers of the cluster of protonated uracil with ammonia, the most stable isomer is **UN01-01**, with an energy 1.2 kcal mol^{-1} lower than that of the second most stable isomer, **UN03-01**. In contrast, in the corresponding isomers of thymine, the relative energy of **TN03-01** is 0.8 kcal mol^{-1} lower than that of **TN01-01**. This may be the result of the smaller difference in proton affinities of **U01** relative to **U03** as compared to that of **T01** relative to **T03**, and/or there may be a contribution from a methyl group steric effect depressing the interaction energy with ammonia in **TN01-01**.

According to the calculated values in Tables 2 and 3, the strongest binding energies are similar for the clusters of uracil

Table 3. Calculated Values of Relative Energies, Enthalpy (298 K), and Entropy Changes of the Different Isomers of the Neutral, Protonated Thymine, and Its Clusters with Ammonia

	B3LYP/6-31+G(d,p)			MP2(full)/6-311++G(2d,2p)//B3LYP/6-31+G(d,p)
	relative energy[a] (kcal/mol)	ΔH (kcal/mol)	ΔS (cal/mol·K)	ΔH (kcal/mol)
T01	0		0	
T02	10.7		−0.7	
T03	12.6		1.0	
T04	22.5		−0.6	
TH01	2.7	−207.1	0.3	−205.4
TH02	10.0	−199.8	1.3	−199.4
TH03	0	−222.4	0.1	−220.8
TH04	22.5	−198.0	3.2	−196.9
TN01-01	0.8	−27.6	−24.5	−27.6
TN01-02	7.5	−20.9	−29.1	−21.5
TN01-03	8.7	−19.7	−28.6	−19.7
TN03-01	0	−25.7	−27.0	−24.8
TN03-02	2.9	−22.8	−27.6	−21.9
TN03-03	5.8	−19.9	−25.9	−20.5

[a] With ZPE and thermal energy correction at 298 K.

(29.2 kcal mol^{-1}) and thymine (27.6 kcal mol^{-1}), with a difference is only 1.6 kcal mol^{-1}. Such a slight difference could normally readily be rationalized on the basis of the small difference (1.8 kcal mol^{-1}) in the proton affinities of what are expected to be the only species present. However, the experimental values for the energetics of ammonia adduct formation are markedly more dissimilar with measured values of −27.9 kcal mol^{-1} for uracil and only −22.1 kcal mol^{-1} for thymine for a difference of 5.8 kcal mol^{-1}. As noted above, the measured binding enthalpy between protonated uracil with ammonia is in good agreement with the largest calculated value leading to **UN01-01**. However, the experimentally measured value for the cluster of protonated thymine and ammonia is 5.5 kcal mol^{-1} less than that calculated for the formation of what was considered to be the only possible species, **TN01-01**. This difference is much too large to be due to experimental uncertainties. This thus leads to the possibility that consideration must be given to formation of ionic species in the present experiments other than those which can be derived straightforwardly from the presumed dominant neutral tautomer in the gas phase. This possibility is considered in more detail below.

3.2.4. Adenine. Adenine is one of the two purine nucleobases used in forming nucleotides of the nucleic acids DNA and RNA. In DNA, adenine binds to thymine to assist in stabilizing the nucleic acid structures, and binds to uracil in RNA. Four different tautomers of neutral adenine have been calculated in the present work, as illustrated in Figure S3, and their relative energetics are summarized in Table S3. It is clearly evident that **A01** is the most stable tautomer, while **A02** and **A03** have the same energy, 8.2 kcal mol^{-1} higher than that of **A01**.

Three different protonated adenine species, **AH01**, **AH02**, and **AH03**, can be considered to be derived from simple protonation of **A01** at different sites. The energy of **AH02** is only 1.5 kcal mol^{-1} higher than that of the most stable tautomer, **AH01**. In addition, **AH04**, which can be thought of as being derived from protonation of either **A02** or **A03**, has nearly the same energy as **AH01** determined at the B3LYP/6-31+G(d,p) level of theory, but it is 1 kcal mol^{-1} higher in energy than **AH01** when single point energies are calculated. The calculated proton affinity of adenine based on **A01** giving **AH01** is 222.8

kcal mol^{-1}, a value which is similar to that given in the NIST tables (225.3 kcal mol^{-1}).

The four most stable clusters derived from association of **AH01** with ammonia as well as a further three clusters obtained from association of **AH04** with ammonia are shown in Figure S3. From the corresponding energetic data given in Table 2, it is evident that those structures involving hydrogen bonding of the nitrogen of ammonia to a hydrogen of the exocylic amino group are inherently less strongly bound than those species in which ammonia binds to a hydrogen bound to one of the ring imino functions. For example, both **AN01-03** and **AN01-04** are less favorable than either **AN01-01** or **AN01-02**, and **AN04-03** is less favorable than either **AN04-01** or **AN04-02**. In the former case, this is true even though there are two hydrogen bonds formed in **AN01-03**. The most stable adduct isomer found is **AN04-01** at the B3LYP/6-31+G(d,p) level of theory; however, **AN01-01** is 0.6 kcal mol^{-1} more stable than **AN04-01** at the MP2(full)/6-311++G(2d,2p)//B3LYP/6-31+G(d,p) level. If the binding energy to ammonia for **AN01-01** is calculated from **AH01** and that for **AN04-01** is calculated from **AH04**, the final binding energy to ammonia for **AN04-01** of 19.2 kcal mol^{-1} is found to be a little higher than the corresponding value for **AN01-01** of 18.3 kcal mol^{-1}. The experimental value of 17.5 kcal mol^{-1} for the binding of ammonia to protonated adenine is in good agreement with this latter calculated binding energy for **AN01-01**.

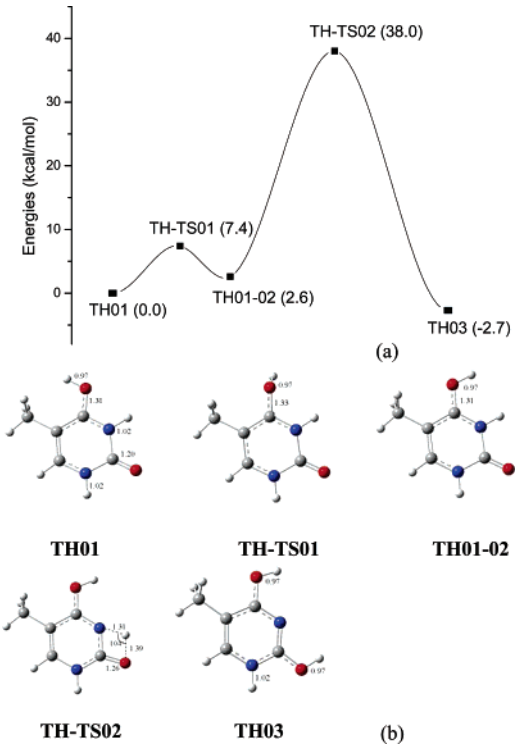

Figure 4. (a) Potential energy surface for unimolecular proton transfer in protonated thymine including ZPC and thermal energies at 298 K; the relative enthalpies of each stationary point are given in the associated parentheses; and (b) the structures of each stationary point.

4. Discussion

4.1. Potential Energy Surface Intramolecular Proton Transfer in Protonated Thymine. The most stable isomer of protonated thymine is **TH03**, which can, in principle, be formed either by direct protonation of the neutral tautomer **T03** or, alternatively, from **TH01** via an intramolecular proton-transfer reaction. Because **T03** has a much higher energy than **T01**, the only species of significant abundance present in the gas phase in these experiments should be **T01**, leading to the conclusion that in the present experiments **TH03** cannot be formed by direct protonation. This leads to the question of whether **TH03** might be formed from **TH01** via a unimolecular proton-transfer process. The calculated unimolecular potential energy surface for transformation of **TH01** to **TH03** is shown in Figure 4. The lowest energy pathway found involves a two-step process in which first a rotation occurs about the exocyclic C−OH bond, followed by a 1,3 proton transfer from a ring nitrogen to the second exocyclic oxygen. The C−OH rotation occurs via a relatively low lying transition state, **TH-TS01**, to give another local minimum, **TH01-02**, whose energy is 2.6 kcal mol^{-1} higher than that of **TH01**. The subsequent proton transfer from the ring nitrogen in **TH01-02** to the second carbonyl oxygen to yield **TH03** must pass through a transition structure, **TH-TS02**, lying 38.0 kcal mol^{-1} above **TH01** in energy. The four-center transition state, in which a hydrogen atom bridges the nitrogen and oxygen atoms, is thus clearly inaccessible energetically under the experimental conditions involved. Therefore, **TH03** cannot be formed from **TH01** via this pathway. A similar argument applies to protonated uracil where the more stable **UH03** cannot be readily accessed from the dominant tautomer **U01** either by direct proton transfer or by unimolecular isomerization of **UH01**. The situation for uracil is analogous to that of thymine, although **UH03** is the most stable tautomer. In support of this finding, it can be noted that the measured proton affinity of uracil is consistent with the formation of **UH01**.[55]

4.2. Ammonia-Catalyzed Proton Transport Isomerization in Protonated Thymine. The measured binding energy of protonated thymine to ammonia is close to the value calculated on the basis of formation of the most stable isomer, **TN03-01**, from **TH03** and ammonia. However, because, as demonstrated above, **TH03** cannot be generated by either direct protonation or unimolecular isomerization of **TH01**, thus it might have been expected that the experimental value should actually correspond to that calculated for the addition of ammonia to **TH01**. Because the experimental data differ so dramatically from this preconception, it became necessary to seek other explanations whereby **TH03** and **TH03-01** might be formed and the possibility of proton transport isomerization suggested itself. A potential energy surface for isomerization from **TN01-01** to **TN03-01** in the presence of ammonia was thus explored with the results shown in Figure 5. Figure 5a represents the electronic potential energy surface at 0 K with no zero-point energy correction, while Figure 5b includes both zero-point energy and thermal energy corrections at 298 K, which more closely resembles true experimental conditions. Interestingly, while the electronic potential energy surface shows actual minima and transition states, when the thermal and zero-point energy corrections are added some energies of the transition states become lower than the energies of the nearby local minima. Significantly, all minima and transition states are much lower in energy than the separated reactants **TH01** and NH_3, indicating that the tautomerization to **TH03** can occur unimpeded under thermal energy conditions. The strong hydrogen bonding present in the ammonia adduct thus allows for this catalyzed tautomerization to occur. The first step involves an effective rotation about the

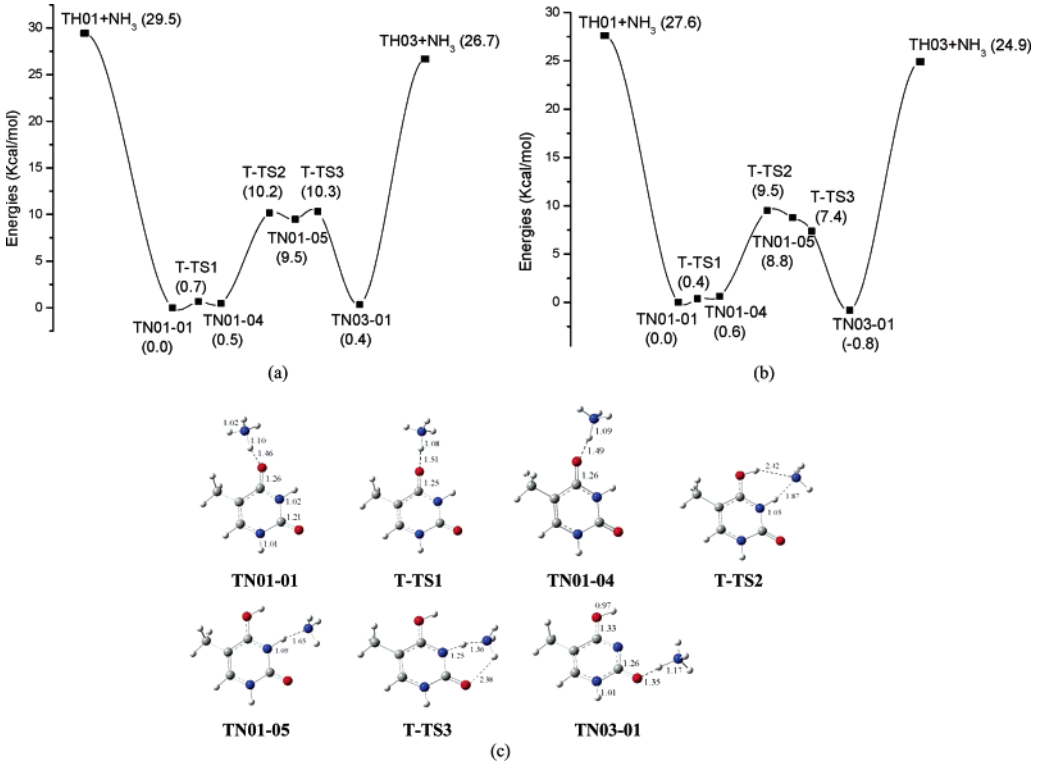

Figure 5. (a) Vibrationless potential energy surface at 0 K for the isomerization of protonated thymine and ammonia with the relative energies of each stationary point given in the associated parentheses; (b) potential energy surface including ZPC and thermal energies at 298 K; and (c) the structures of each stationary point.

C−O bond via **T-TS1** to yield a second minimum only slightly higher in energy, **TN01-04**. Proton transfer from ammonium ion back to the carbonyl oxygen then occurs accompanied by translation of the NH_3 moiety via a bifurcated transition state, **T-TS2**, to form a new hydrogen-bonded adduct between ammonia and an imino hydrogen, **TN01-05**. This species then passes via another bifurcated hydrogen-bonding transition state, **T-TS3**, to give the global minimum on the adduct surface, **TN03-01**. It can then smoothly dissociate to yield the lower energy protonated thymine, **TH03**, and ammonia. This bimolecular association process leads overall to the exothermic tautomerization, which was not possible on a unimolecular surface. The fact that this isomerization is so facile would then dictate that the measured equilibrium for the association reaction observed actually involves **TH03** and a statistical mixture of **TN01-01** and **TN03-01**, which are close in free energy throughout the experimental temperature range. The calculated association enthalpy is still greater than the experimentally observed value by ~2.5 kcal mol^{-1}; however, it should be noted that calculated binding energies are often overestimated when the basis set size is insufficient.

4.3. Ammonia-Catalyzed Proton Transport Isomerization in Protonated Uracil. In view of the structural similarity of uracil and thymine, an analogous exploration of the potential energy surface for interaction of protonated uracil and ammonia was undertaken. The resulting surface, shown in Figure 6, exhibits a shape similar to that of thymine with analogous minima and transition states as depicted in Figure 5. The net result is once again that the interaction of **UH01**, as the only species initially formed by protonation of the dominant neutral, **U01**, with ammonia can lead to the exothermic tautomerization to **UH03** via proton transport. The major difference between the uracil and thymine surfaces is that in the case of uracil the most stable ammonia adduct corresponds to the interaction of **UH01** with ammonia. Thus, the dominant ionic species participating in this equilibrium will be **UH03** and **UN01-01**. The calculated value for the enthalpy change for this association reaction is then −27.9 kcal mol^{-1}, in exact agreement with experiment. It should be noted, however, that significant amounts of both **UH01** and **UN03-01** will also be present in a statistical mixture at the experimental temperatures.

4.4. Correlation of the Strength of Interactions between Protonated Nucleic Acid Bases and Ammonia with Proton Affinity. Given the structural similarities of uracil and thymine and the general observation that within a homologous series of compounds the binding energies of the protonated species with a given reference base should be proportional to the difference in proton affinities of the parent compound and the reference base, it might have been expected that protonated uracil and thymine would exhibit similar binding energies with ammonia. However, as noted above, experimental values are markedly different.

For uracil, the experimentally measured enthalpy of association corresponds to the interaction of the most stable protonated isomer, **UH03**, with ammonia to yield the most stable adduct, **UN01-01**. Although the most stable protonated isomer is **UH03**, it differs in energy by only 1.3 kcal mol^{-1} from **UH01** in the gas phase. All previous experiments used to determine the proton affinity of uracil will have effectively probed the enthalpy change for addition of a proton to **U01** to yield **UH01**.[55] The dominant cluster observed in the present work, **UN01-01**, thus involves a proton shared between **U01** and ammonia, and the similar proton affinities of **U01** and ammonia give rise to the fairly strong enthalpy of interaction of 27.9 kcal mol^{-1}.

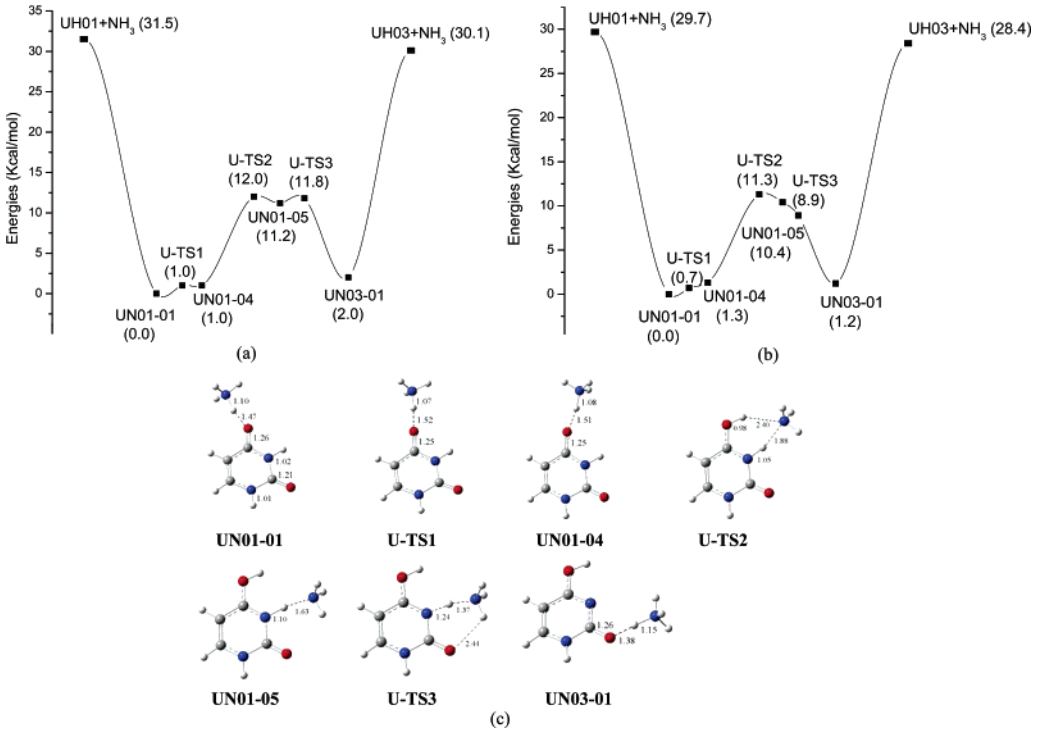

Figure 6. (a) Vibrationless potential energy surface at 0 K for the isomerization of protonated uracil and ammonia with the relative energies of each stationary point given in the associated parentheses; (b) potential energy surface including ZPC and thermal energies at 298 K; and (c) the structures of each stationary point.

For thymine, as for uracil, experimental determinations of the proton affinity will have resulted in exclusive formation of **TH01**, and thus the reported proton affinity corresponds to the enthalpy change for addition of a proton to **T01** to yield **TH01**.[54] However, for the cluster of protonated thymine with ammonia, the experimental binding energy is the value of the enthalpy change for the formation of the most stable isomer, **TN03-01**, from **TH03** and ammonia. Therefore, in this cluster, a proton is actually being shared between **T03** and ammonia, and the binding energy of ammonia to **TH03** will actually be related to the proton affinity difference between **T03** and ammonia. As shown from the calculations reported in Table 3, the proton affinity of **T03** is 220.8 kcal mol^{-1}, and the proton affinity difference between the two bases participating in this cluster is thus substantially larger than that in the corresponding proton-bound dimer of uracil and ammonia. This then gives rise to the significantly lower enthalpy of interaction experimentally observed here of 22.0 kcal mol^{-1}.

In addition, it should be noted that the most stable structures for the interactions of protonated cytosine, uracil, and thymine with ammonia all involve a hydrogen bond between a protonated carbonyl oxygen and the nitrogen of ammonia. As such, it might again be expected that the correlation of hydrogen-bond strength with proton affinity difference for the two bases participating in the hydrogen bond should apply. The measured proton affinity of cytosine corresponds to addition of a proton to **C01** leading to **CH01**, and, as shown by the calculations presented here, these should be the dominant, although not exclusive, species present in the gas phase experimentally. The calculations also predict that the only adduct present in significant abundance, **CN01-01**, is a proton-bound dimer of **C01** and ammonia. The calculated proton affinity of **C01** of 225.7 kcal mol^{-1} is substantially greater than that of **U01** and somewhat greater than that of **T03**. Therefore, it would be predicted that the binding energy of protonated cytosine to ammonia should be the lowest of these three nucleic acid bases, and this is indeed observed with the measured enthalpy of interaction between protonated cytosine and ammonia of 21.7 kcal mol^{-1}.

Finally, it should be noted that the enthalpy of interaction between protonated adenine and ammonia is the lowest of those for the four nucleic acid bases examined here, even though the measured and calculated proton affinities of **A01** are intermediate between those of cytosine and thymine. This can be readily understood, however, from the fact that a different type of hydrogen bond is formed because it involves a NH$^+$···N linkage between two nitrogen atoms rather than the C═OH$^+$···N linkage formed in the proton-bound dimers involving the other three nucleic acid bases. Meot−Ner[78] has previously proposed an empirical correlation for proton-bound dimer bond strengths based on difference in proton affinity, ΔPA, for both NH$^+$···N and OH$^+$···N species, as given in eq 7,

$$\Delta H = a - b\cdot\Delta\mathrm{PA} \qquad (7)$$

where the constants a and b are 23.2 and 0.25, respectively, for the NH$^+$···N bond and 28.3 and 0.23, respectively, for the OH$^+$···N bond. Using these constants and the proton affinity values calculated in the present work allows a prediction of the enthalpy of interaction between protonated adenine and ammonia of 18.5 kcal mol^{-1} and values of 23.3, 24.4, and 28.0 kcal mol^{-1} for cytosine, thymine, and uracil, respectively. These four empirically predicted values are in fair quantitative agreement with the present experimental data and exactly reproduce the qualitative order of binding energies observed.

4.5. Interactions between Neutral Nucleic Acid Bases and NH_4^+ and Comparison with Na^+. Noncovalent interactions

Table 4. Comparison of the Binding Energy between NH_4^+ and Na^+ with Cytosine, Thymine, and Uracil

	NH_4^+		Na^+
	exp. (kcal mol^{-1})	cal.[a] (kcal mol^{-1})	exp. (kcal mol^{-1})
cytosine	44.7	44.8	42.3[b]
thymine	28.6	28.9	34.4;[b] 32.3[c]
uracil	32.8	29.2	33.7;[b] 32.2[c]

[a] Calculated by MP2(full)/6-311++G(2d,2p)//B3LYP/6-31+G(d,p) with ZPC and thermal energy correction by B3LYP/6-31+G(d,p). [b] From ref 36. [c] From ref 42.

between neutral nucleic acid bases and organic or inorganic ions are important in biological systems. To date, although there has been a limited number of reports concerning interaction with metal ions in the gas phase,[36−42,89−91] the interaction with organic ions has almost completely absent. Protonated amino groups are ubiquitous in biological systems, such as protonated amino acids, peptides, proteins, as well as DNA and RNA. The simplest ammonium ion, NH_4^+, may thus serve as a model for the interaction between nucleic acid bases and organic ions, leading to a more fundamental understanding of the characteristics and strengths of these kinds of interactions.

In the clusters of the protonated uracil and thymine with ammonia, an examination of the O−H and N−H bond distances involved in the strong hydrogen bond reveals that, in effect, a proton transfer has occurred from either the protonated uracil or the thymine to ammonia, even though the proton affinities of both uracil and thymine are greater than that of ammonia (204 kcal mol^{-1}). A similar examination of the corresponding hydrogen-bond distances in the clusters involving cytosine and adenine shows that no proton transfer occurs in the cluster of protonated adenine with ammonia, whereas a partial proton transfers from the protonated cytosine to ammonia in the most stable isomer, **CN01-01**, where the O−H and N−H bond distances are close at 1.27 and 1.23 Å, respectively, even though the proton affinity of cytosine is greater than that of adenine. The difference between the extent of proton transfer involving adenine relative to that for the other three nucleic acid bases is likely due to the electrostatic interactions possible between the resulting ammonium ion and these three neutral carbonyl-containing species, which will have appreciable local dipole moments, thus favoring the endothermic proton transfer.

Because, in the cases of uracil and thymine, and to a lesser extent cytosine, the proton-bound dimer species can be regarded as interactions of the neutral nucleic acid bases with NH_4^+, it is of interest to compare these interactions with those of these nucleic acid bases with the sodium ion, Na^+. Using the experimentally measured enthalpy changes from the present work, as well as the proton affinity differences of 23, 6.5, and 4.9 kcal mol^{-1} for cytosine, thymine, and uracil, respectively, from the NIST database,[84] values for the interaction energies of the neutral nucleic acid bases with NH_4^+ can be obtained as 44.7, 28.6, and 32.8 kcal mol^{-1}, respectively. These data are summarized in Table 4 together with the corresponding computational values as well as experimental literature data for the binding energies of Na^+ to these same species. The computations are carried out at the same level as those described above and are based on the most stable isomers, **CN01-01**, **TN03-01**, and **UN01-01**, dissociating to give NH_4^+ and the most stable neutral nucleic acid bases, **C01**, **T01**, and **U01**. The enthalpy changes for the dissociation reactions thus obtained are 44.8, 28.9, and 29.2 kcal mol^{-1}, respectively. It can be seen that the experimental values are in reasonable agreement with the calculated values, particularly for cytosine and thymine. If these binding energies to NH_4^+ are compared to the corresponding values determined for Na^+, the interaction energies with cytosine and uracil are indeed similar, whereas the interaction energy of NH_4^+ with thymine is found to be somewhat lower than that between Na^+ and thymine. This is discussed below in light of the fact that the structures of their most stable isomers are different.

Although the binding energies between nucleic acid bases with NH_4^+ and Na^+ are similar, the NH_4^+ adduct structures exhibit some subtle but significant differences relative to those of the Na^+ clusters as described by Russo et al.[39] For cytosine, the Na^+ interacts not only with O2 of cytosine, but also with N3, where the distances between the metal ion and O2 and N3 are close, 2.208 and 2.472 Å, respectively, at the B3LYP/6-311+G(2df,2p) level of theory.[39] However, in **CN01-01**, NH_4^+ forms a strong H-bond with O2, with a hydrogen-bond distance of only 1.27 Å, and the distance between the two heavy atoms of 2.50 Å. In contrast to the situation for Na^+, the NH_4^+ forms a weak H-bond with N3, with the distance between N3 and a proton in NH_4^+ of 3.20 Å. In the most stable structures of the clusters of uracil with NH_4^+ and Na^+, the ions each bind to the same position, O4 of uracil; however, the $Na^+\cdots O{=}C$ bond is linear, whereas the corresponding $NH\cdots O{=}C$ bond angle in the NH_4^+ adduct is 143°. For thymine, the two ions exhibit different interaction energies, and this has been explained above. A comparison of their most stable structures reveals substantial differences with the most stable structure of the Na^+ adduct being comparable to that for uracil where Na^+ interacts with O4 of thymine. However, the corresponding NH_4^+ adduct isomer, **TN01-01**, is the second most stable structure. In the most stable isomer, **TN03-01**, NH_4^+ forms a hydrogen bond with O2 of thymine with a H-bond length of 1.35 Å, which is much shorter than that in **TN01-01** (1.46 Å). These differences may be attributed to a fundamental difference between NH_4^+ and Na^+ interactions with nucleic acid bases, where, for example, the interaction between Na^+ and cytosine is mainly electrostatic, while the interaction between NH_4^+ and cytosine is via hydrogen bond.

5. Conclusions

High-pressure mass spectrometry (HPMS) and ab initio calculation have been used to probe the interaction and structure of the various possible clusters formed between ammonia and the protonated nucleic acid bases, cytosine, uracil, thymine, and adenine. Various isomers of both the neutral and the protonated nucleic acid species as well as their clusters with ammonia have been computed by B3LYP/6-31+G(d,p), and the binding energies have also been obtained. The potential energy surfaces for proton transfer and proton transport tautomerism in protonated thymine and in the clusters of protonated thymine and uracil with ammonia have been investigated.

For the cluster between protonated cytosine and ammonia, the experimental binding energy of 21.7 kcal mol^{-1} is in excellent agreement with the computed binding energy for the most stable isomer, **CN01-01**, which can be formed directly

(89) Russo, N.; Toscano, M.; Grand, A. *J. Phys. Chem. A* **2003**, *107*, 11533.
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(91) Sychrovsky, V.; Sponer, J.; Hobza, P. *J. Am. Chem. Soc.* **2004**, *122*, 663.

from addition of ammonia to the most stable protonated species, **CH01**. From the calculated energetics, it is clear that this will be the only cytosine adduct species present in any significant amount under the experimental conditions employed. The computational data also suggest that there will be two protonated cytosine species present in comparable amounts; however, each of these can easily lead to **CN01-01** without barrier in reaction with ammonia.

For uracil and thymine, the most stable protonated isomers, **UH03** and **TH03**, cannot be readily formed from protonation of the most stable neutral species, **U01** and **T01**. The experimentally measured proton affinities are presumably those derived from protonation of **U01** and **T01** to give the second most stable tautomers, **UH01** and **TH01**, respectively.[54,55] For the adduct formed between protonated uracil and ammonia, the experimental enthalpy data correspond well with the calculated binding energy leading to the most stable isomer, **UN01-01**. In contrast, the experimental value obtained for the cluster of protonated thymine and ammonia is not in good agreement with the computational data calculated presuming that **TH01** adds ammonia to yield **TN01-01**. The experimental data do agree, however, with a value calculated, presuming that **TH03** reacts with ammonia to yield **TN03-01**. In the absence of ammonia, the barrier for intramolecular proton transfer isomerization from **TH01** to the most stable protonated isomer, **TH03**, is 38.0 kcal mol^{-1}. In the presence of ammonia, however, it can be demonstrated that the barrier from **TH01-01** to the most stable isomer, **TH03-01**, is only 9.5 kcal mol^{-1} at 298 K. This is well below the energy of the separated reactants, and thus the transformation of different isomers of protonated thymine becomes facile through the proton transport isomerization action of the added ammonia. Interestingly, despite their structural similarity, the most stable clusters of uracil and thymine, **UN01-01** and **TN03-01**, are different.

Although uracil and thymine have similar structures and proton affinities, the binding energies between the protonated species with ammonia are quite different. On the surface, this would appear to be a contradiction to the generally expected trend within a homologous series of compounds of decreasing binding energy with increasing difference in proton affinity of the two species participating in proton-bound dimer formation. This has been demonstrated to be due to the fact that the reported proton affinity of thymine does not correspond to formation of the more stable protonated thymine isomer formed by proton transport isomerization in the present work. Thus, the literature data for the proton affinity of thymine and the measured binding energy of protonated thymine to ammonia in the present work involve different isomers. The reported proton affinity is that leading to **TH01**, while the measured binding energy of the cluster with ammonia involves **TH03**.

Adenine also has a proton affinity similar to that of cytosine; however, the relative values of the binding energies of their protonated species to ammonia are contrary to that expected on the basis of the order of their proton affinities. This is due to the formation of two distinctly different kinds of H-bond. In the case of adenine, a proton-bound dimer is formed exhibiting an $N-H^+\cdots N$ moiety, while in cytosine, the hydrogen bond involves an $O-H^+\cdots N$ linkage. This demonstrates the inherently weaker nature of $N-H^+\cdots N$ hydrogen bonds relative to their $O-H^+\cdots N$ counterparts.

For the clusters of protonated cytosine, uracil, and thymine with ammonia, the most stable isomers each closely resemble the structure of a neutral nucleic acid base interacting with NH_4^+, even though the proton affinities of cytosine, uracil, and thymine are each higher than that of ammonia. Using the experimentally measured enthalpy changes and the proton affinity differences, the interaction energies of the neutral nucleic acid bases with NH_4^+ can be obtained as 44.7, 28.6, and 32.8 kcal mol^{-1}, respectively. These experimental values are in reasonable agreement with the calculated values of 44.8, 28.9, and 29.2 kcal mol^{-1}. Although the binding energies of the nucleic acid bases with NH_4^+ and Na^+ are similar, the NH_4^+ adduct structures exhibit some subtle but significant differences relative to those of the Na^+ clusters as described by Russo et al.[39] These differences may be attributed to a fundamental difference between NH_4^+ and Na^+ interactions with nucleic acid bases. For example, the interaction between Na^+ and cytosine is mainly electrostatic, while the interaction with NH_4^+ is via hydrogen bonding.

Significantly, the present work demonstrates that measurement of the energetics of clustering of protonated nucleic acid bases with ammonia can be used as a probe to investigate the structure of the protonated nucleic acid base. At the same time, ammonia may also serve as an important model compound to study the interaction of important functional groups with protonated nucleic acid bases. In particular, the study of isomerization on the potential energy surface for these important species may serve as an aid to the understanding of biological structure and function. Finally, if a single ammonia molecule is taken as a model for solvent, it can be seen that solvent-assisted proton transfer is a facile process. Thus, in polar solvent solutions, solvent assistance can lead to the transformation of structure where the most stable protonated species does not necessarily correspond to the structure of the most stable neutral tautomer.

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Note Added after ASAP Publication. Due to a production error, panels a and b were incorrect in Figure 5 in the version of this paper published ASAP on December 29, 2006. The corrected version was published ASAP on January 5, 2007.

Supporting Information Available: Complete version of ref 72, as well as the structures of the different isomers of neutral and protonated cytosine, uracil, and adenine and their clusters with ammonia, together with their relative energetics, and the binding energies and entropy changes associated with formation of the ammonia clusters. This material is available free of charge via the Internet at http://pubs.acs.org.

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