The Hydrogen Bonding Properties of Cytosine: A Computational Study of Cytosine Complexed with Hydrogen Fluoride, Water, and Ammonia

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Density functional theory is used to study the hydrogen bonding pattern in cytosine, which does not contain alternating proton donor and acceptor sites and therefore is unique compared with the other pyrimidines. Complexes between various small molecules (HF, H_2O, and NH_3) and four main binding sites in (neutral and (N1) anionic) cytosine are considered. Two complexes (O2(N1) and N3(N4)) involve neighboring cytosine proton acceptor and donor sites, which leads to cooperative interactions and bidendate hydrogen bonds. The third (less stable) complex (N4) involves a single cytosine donor. The final (O2−N3) complex involves two cytosine proton acceptors, which leads to an anticooperative hydrogen bonding pattern for H_2O and NH_3. On the neutral surface, the anticooperative O2−N3 complex is less stable than those involving bidentate hydrogen bonds, and the H_2O complex cannot be characterized when diffuse functions are included in the (6-31G(d,p)) basis set. On the contrary, the anionic O2−N3 structure is the most stable complex, while the HF and H_2O N3(N4) complexes cannot be characterized with diffuse functions. B3LYP and MP2 potential energy surface scans are used to consider the relationship between the water N3(N4) and O2−N3 complexes. These calculations reveal that diffuse functions reduce the conversion barrier between the two complexes on both the neutral and anionic surfaces, where the reduction leads to a (O2−N3) energy plateau on the neutral surface and complete (N3(N4)) complex destabilization on the anionic surface. From these complexes, the effects of hydrogen bonds on the (N1) acidity of cytosine are determined, and it is found that the trends in the effects of hydrogen bonds on the (N1) acidity are similar for all pyrimidines.

Introduction

DNA (deoxyribonucleic acid) has a unique double helical structure that is formed when complementary nucleobases on neighboring strands interact through hydrogen bonding. In addition to aiding the formation of the DNA double helix, hydrogen bonding interactions with DNA nucleobases play important roles in DNA replication, gene expression, and DNA repair. For example, enzymes that replicate or repair DNA often rely on hydrogen bonding interactions between protein amino acid residues and DNA nucleobases.

Although understanding hydrogen bonding interactions between DNA components and other molecules is vital to the understanding of the properties of DNA polymers and the mechanisms of biological processes, it is difficult to identify the role of individual nucleobase interactions from experimental data. For this reason, an abundance of computational studies have appeared in the literature that consider hydrogen bonding interactions involving DNA residues.[1−3] From these computational studies, the structures of hydrogen bonded complexes are isolated and the relative importance of various interactions are characterized.

In our group, we have been interested in the effects of hydrogen bonds on the properties of DNA components.[4−6] In particular, due to the proposed formation of nucleobase anions in the base excision DNA repair pathway,[7] our group has focused on the effects of hydrogen bonds on the acidity of natural and damaged nucleobases. In our preliminary studies,[4−6] we have considered interactions between the nucleobases and small molecules (hydrogen fluoride, water, and ammonia) in order to gain insight into the effect their properties have on hydrogen bonding interactions.

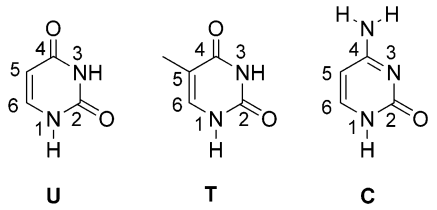

Figure 1. Chemical structure and numbering of the pyrimidines: uracil (U), thymine (T), and cytosine (C).

In the present study, we extend upon our previous computational work by focusing on the effects of complexation with small molecules on the properties of cytosine. Although cytosine is a natural DNA nucleobase that may not be directly involved in DNA repair processes, we are interested in the effects of hydrogen bonds on the acidity of this pyrimidine due to its unique hydrogen bonding pattern compared with thymine and uracil (Figure 1). In particular, thymine and uracil contain alternating hydrogen bond donor and acceptor groups. However, in cytosine, one of the thymine (uracil) carbonyl groups is replaced with an amino group, which directly results in the loss of a hydrogen at the N3 position and disrupts the consecutive pattern of alternating hydrogen bond donors and acceptors.

Although the hydrogen bonding properties of cytosine have not been as well studied as the other pyrimidines, several computational investigations have considered hydrogen bonding interactions with this nucleobase.[8−21] Due to the importance of water in biological systems and the numerous tautomeric forms

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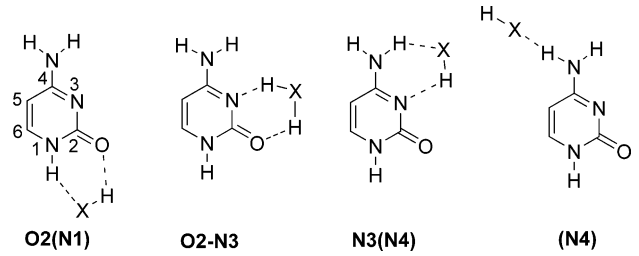

Figure 2. Cytosine complexes considered in the present study.

of cytosine, most studies have been interested in the interactions between cytosine,[8−21] or its tautomers,[13−20] and water. Some studies have also considered interactions with more than one water molecule,[9,10,12,14,16,21] where up to fourteen water molecules have been considered,[21] in hopes to gain an understanding of the solvation pattern of cytosine through a supermolecular approach. The hydrogen bonding interactions in base pairs involving cytosine,[22−24] as well as interactions between cytosine and amino acid fragments,[25−29] have also been investigated.

In the present study, we consider cytosine complexed with hydrogen fluoride, water, or ammonia at four main binding sites (Figure 2). Although the canonical cytosine tautomer is not the lowest energy structure in the gas phase, we focus on this form due to its biological significance where this tautomer is forced upon glycosylation at N1 and is the most stable upon inclusion of solvent effects. Similar levels of theory to those implemented in our work on other DNA nucleobases are applied, which will allow comparison of the pyrimidines. Although complexes between water and biomolecules have obvious implications for understanding interactions in biological systems, we believe that it is also important to understand interactions with other small molecules. Studying a range of molecules that differ in their hydrogen bonding abilities is especially important due to the range in the properties of amino acid residues that interact with nucleobases during important biological processes. From this study, a greater understanding of hydrogen bonding interactions involving DNA components will be obtained.

Computational Details

All calculations were performed using the GAUSSIAN 03 program.[30] The B3LYP functional was used in conjunction with the 6-31+G(d,p) and 6-31G(d,p) basis sets for the optimization of complexes between cytosine and HF, H_2O, or NH_3. Polarization functions were included since these have been shown to be essential for optimizations of the DNA bases.[31] The MP2 method (with both 6-31+G(d,p) and 6-31G(d,p)) was also used for cytosine−water complexes. Higher level single-point calculations were performed on B3LYP optimized geometries using the 6-311+G(2d,p) basis set to obtain improved binding strengths and acidities. All relative energies, binding strengths and acidities include scaled (0.9806 for B3LYP and 0.9608 for MP2) zero-point vibrational energy (ZPVE) corrections and basis set superposition error (BSSE) corrections, which were calculated according to the Boys and Bernardi approach.[32] The B3LYP/6-311+G(2d,p)//B3LYP/6-31+G(d,p) method has been previously employed to study similar hydrogen bonded complexes between hydrogen fluoride, water, or ammonia and uracil,[4] adenine,[6] guanine,[6] or various derivatives of these nucleobases.[5,6] Furthermore, good agreement between our calculations and previous studies is obtained for all complexes.

Results and Discussion

Neutral Cytosine Complexes. *(i) Complexes with HF, H_2O, or NH_3.* As mentioned in the Introduction, four main complexes

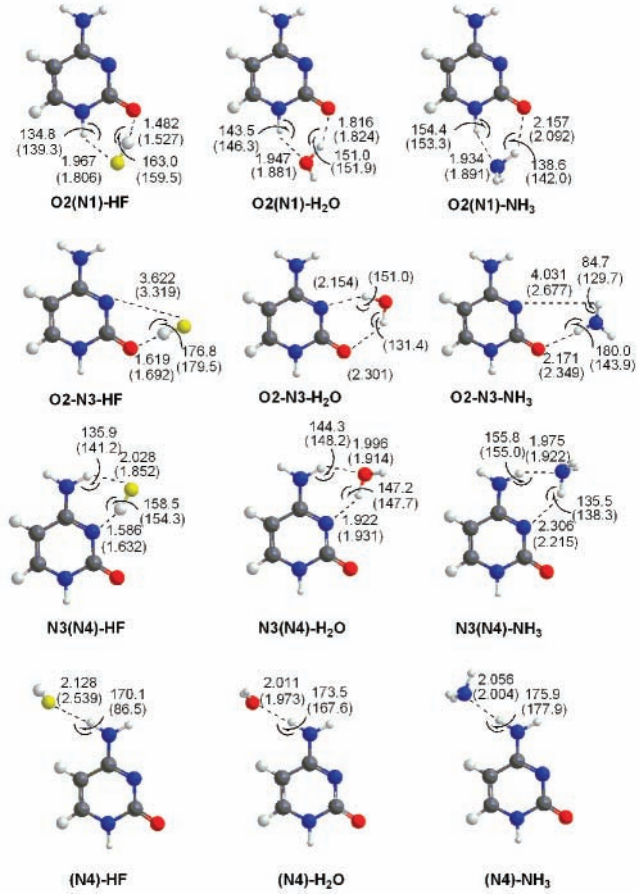

Figure 3. Selected B3LYP/6-31+G(d,p) bond lengths (Å) and angles (deg) in (neutral) cytosine complexes with hydrogen fluoride, water, or ammonia (B3LYP/6-31G(d,p) values in parentheses).

between cytosine and HF, H_2O, or NH_3 were investigated (Figure 2). In our nomenclature, the bracketed sites are cytosine proton donors, while the remaining sites are cytosine proton acceptors. The O2(N1) and N3(N4) complexes involve interactions between the small molecule (XH) and both a cytosine proton acceptor and a cytosine proton donor. The O2−N3 complex involves two cytosine proton acceptors (indicated by the lack of brackets in our notation), while the (N4) complex involves interactions with a single cytosine proton donor.

Select B3LYP/6-31+G(d,p) geometrical parameters for the optimized complexes are displayed in Figure 3. Among the cytosine−water complexes (Figure 2), O2−N3 was not found to be a stable minimum with B3LYP/6-31+G(d,p), which has been previously noted in the literature for calculations performed with comparable basis sets.[11−15,18] The remaining water complexes have C1 symmetry due to the puckering of the cytosine amino group and the out-of-plane position of the water hydrogen that does not directly interact with cytosine.[33] The amino group puckering is largest in the O2(N1) complex (up to 9°), while the puckering is reduced in the N3(N4) complex (by up to 7.5°) and the amino group is planar in (N4). The planar versus pyramidal shape of amino groups within the nucleobases described by computational methods has been discussed in the literature.[31,34] Another interesting geometrical feature, which has been previously noted in the literature,[11,12] is the shorter hydrogen bond length between the water hydrogen and the cytosine proton acceptor compared with the distance between the water oxygen and the cytosine proton donor for the O2(N1) (by 0.13 Å) and N3(N4) (by 0.07 Å) complexes (Figure 3). Our geometries are in good agreement with previously

TABLE 1: B3LYP Relative Energies and Binding Strengths for Cytosine Complexed with Hydrogen Fluoride, Water, or Ammonia[a,b]

	6-31+G(d,p)		6-31G(d,p)	
site	ΔE	D_e	ΔE	D_e
		HF		
O2(N1)	0.0	59.3	0.0	54.9
O2−N3	12.8	46.5	15.1	39.8
N3(N4)	4.0	55.3	3.2	51.7
(N4)	51.1	8.2	48.2	6.7
		H_2O		
O2(N1)	0.0	37.6	0.0	36.5
O2−N3	−	−	15.3	21.2
N3(N4)	3.0	34.5	1.9	34.6
(N4)	21.9	15.6	17.8	18.7
		NH_3		
O2(N1)	0.0	33.3	0.0	36.5
O2−N3	24.5	8.8	30.1	6.4
N3(N4)	5.5	27.8	3.9	32.6
(N4)	14.3	18.9	11.9	24.6

[a] Relative energies include zero-point vibrational energy and basis set superposition error corrections. [b] See Figures 1 and 2 for the chemical structure, numbering, and nomenclature of cytosine complexes.

reported water and cytosine[11−14,19] (or 1-methylcytosine)[15] complexes that were optimized at comparable levels of theory.

Structures similar to the cytosine−water complexes discussed above were found between cytosine and hydrogen fluoride or ammonia (Figure 3). However, the hydrogen fluoride complexes have considerably shorter hydrogen bond distances to the cytosine proton acceptor, while the reverse trend is found for ammonia complexes. These differences occur due to the properties of the small molecules, where hydrogen fluoride is a strong acid and ammonia a strong base in the gas phase.[35]

Interestingly, the majority of the hydrogen fluoride and ammonia complexes contain nearly planar amino groups. The exception to this is the O2−N3 ammonia complex where the amino hydrogens are out of the cytosine molecular plane by up to 11°. In this complex, ammonia is also located out of the cytosine molecular plane (by approximately 20°) and is more closely coordinated to O2 compared with N3 (by 1.26 Å). The O2−N3 HF complex was also found, where hydrogen fluoride is also primarily coordinated to O2 (Figure 3). However, hydrogen fluoride is located in the molecular plane and the cytosine amino group is planar.

Table 1 contains the B3LYP/6-31+G(d,p) relative energies and binding strengths for the cytosine-XH complexes. Our calculated relative energies of the cytosine−water complexes are in good agreement with previous studies performed at comparable levels of theory.[11−14,19] The O2(N1) complex is found to have the largest binding strength for all small molecules, where the binding energy decreases as HF (59.3 kJ mol^{-1}) $\gg$ H_2O (37.6 kJ mol^{-1}) > NH_3 (33.3 kJ mol^{-1}). This trend in the binding strengths with respect to the small molecule is similar to that reported for other pyrimidines[4,5] and purines,[6] and generally follows the acidity of the small molecule.[35] Furthermore, water and ammonia tend to produce similar binding strengths at bidendate binding sites due to a balance between their proton accepting and donating abilities.

N3(N4) is the next lowest energy complex for all three small molecules, where the energy difference from O2(N1) is roughly 4 kJ mol^{-1} for HF, 3 kJ mol^{-1} for H_2O, and 6 kJ mol^{-1} for NH_3. The N3(N4) binding strengths are slightly smaller than those for the O2(N1) complexes due to the decreased acidity of the cytosine donor (N1 acidity is 1444 kJ mol^{-1}, while N4 (N3 side) acidity is 1481−1482 kJ mol^{-1}).[11,19,36] Once again the trend in the binding strengths at this site is dominated by the large proton affinity of the cytosine acceptor (N3) (955 kJ mol^{-1}).[11,19,36]

The cooperative hydrogen bonding within O2(N1) and N3(N4) contributes to the stability of these complexes compared with (N4) (Table 1), which involves only one cytosine proton donor. The binding strengths for the ammonia and water (N4) complexes are approximately 19 and 16 kJ mol^{-1}, respectively. The larger binding strength of the ammonia complex is expected due to the larger proton affinity of ammonia. The hydrogen fluoride (N4) complex has a smaller (8.2 kJ mol^{-1}) binding strength than the corresponding water and ammonia complexes since HF is a weak proton acceptor.

The relative stability of the O2−N3 and (N4) complexes depends on the properties of the small molecule bound to cytosine. Due to the large proton affinity of ammonia, the O2−N3 ammonia complex is 10.2 kJ mol^{-1} less stable than (N4). Alternatively, the O2−N3 hydrogen fluoride complex is 38 kJ mol^{-1} more stable than the corresponding (N4) complex due to the large acidity of hydrogen fluoride. Although a similar comparison cannot be made for water complexes, it is anticipated that the O2−N3 complex is higher in energy than the other complexes since, as previously noted in the literature,[11,12,15,18] we find that this structure falls to the N3(N4) complex during the course of geometry optimizations.

It is perplexing that the O2−N3 complex can be characterized for (acidic) hydrogen fluoride and (basic) ammonia, but not for water, since the proton donating ability of water falls between those of the other two small molecules.[35] Interestingly, however, the O2−N3 cytosine−water complex has been isolated using basis sets void of diffuse functions.[9,10,15,17,18] Therefore, a parallel study of the cytosine−XH complexes was conducted using the 6-31G(d,p) basis set to determine the basis set effects on cytosine−XH complexes.

The general geometrical features of the cytosine−XH complexes discussed above are preserved upon removal of diffuse functions from the basis set (Figure 3). The puckering of the cytosine amino group does not change significantly, while the non-hydrogen bonding water hydrogen is located slightly further out of the cytosine molecular plane for the N3(N4) complex.[37] More significant changes in the hydrogen bond lengths are found, where water and hydrogen fluoride generally move closer to the cytosine proton donor and further from the proton acceptor, while both hydrogen bond distances generally decrease in ammonia complexes. Additionally, in the water and ammonia (N4) complexes, the small molecule moves closer to the C5 position (due to O···H−N4 angle bending in the case of H_2O[12] and significant shortening of the N4−H···N distance for NH_3), which may stabilize this structure through additional (weak) hydrogen bonding interactions. The most notable geometrical changes found upon removal of diffuse functions from the basis set occur in the (N4) hydrogen fluoride complex, and the O2−N3 water and ammonia complexes, which will now be discussed in more detail.

Upon removal of diffuse functions from the basis set, HF in the (N4) complex rotates to lie above the cytosine molecular plane such that both F−H···N4 and F···H−N4 interactions are present. This is represented by the large F···H−N4 hydrogen bond distance and small corresponding hydrogen bond angle in Figure 3. When this structure is used as a starting geometry for an optimization with a basis set that includes diffuse functions, hydrogen fluoride rotates to 50° out of the molecular plane (residing closer to C5 than N3) and only one F−H···N4 interaction remains (Figure 4a). A similar water complex has been previously reported by Smets et al.[15,38] The present study finds that this water complex (Figure 4a), where the water

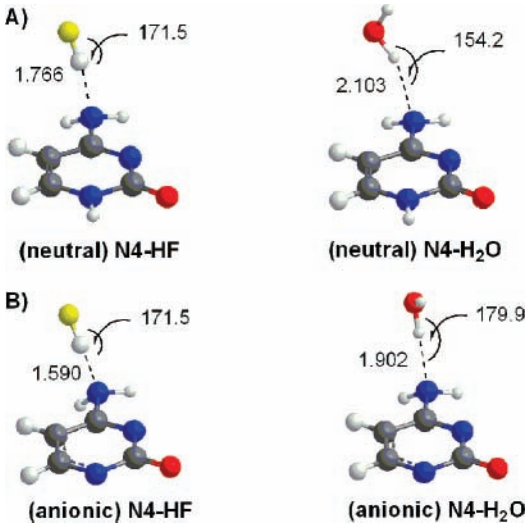

Figure 4. Selected B3LYP/6-31+G(d,p) bond lengths (Å) and angles (deg) in the N4 cytosine complexes with hydrogen fluoride or water.

oxygen is 30° out of the molecular plane, is approximately 10 kJ mol^{-1} higher in energy than the (N4) complex (at the B3LYP/6-31+G(d,p) level of theory). Furthermore, the (B3LYP/6-31+G(d,p)) HF complex is only approximately 2 kJ mol^{-1} more stable than the corresponding (N4) complex. Therefore, the (neutral) complexes involving single X−H···N4 interactions are not further considered in the present work.

As mentioned previously, the geometry of the O2−N3 cytosine−ammonia complex changes significantly upon removal of diffuse functions, where the ammonia nitrogen is only slightly out of the cytosine molecular plane (by approximately 2° compared with 20° when diffuse functions are included). Furthermore, the N3···H−N hydrogen bond length is significantly shorter (by 1.354 Å), while the O2···H−N hydrogen bond length increases by 0.177 Å. The O2···H−N bond length remains the shortest hydrogen bond distance since the O2 site has a slightly (1.3 kJ mol^{-1}) larger proton affinity than the N3 site.[11,19,36] This bonding orientation may lead to strain within ammonia where the internal bond angle is decreased to 101.8° from 105.8° in isolated ammonia (optimized at the same level of theory).

As reported previously in the literature,[9,10,15,17,18] the O2−N3 water complex can be isolated when the basis set does not include diffuse functions (Figure 3). In the optimized structure, water lies in the cytosine molecular plane and, contrary to ammonia, is more closely coordinated to N3 compared with O2 by 0.147 Å. As noted previously,[12,18] the hydrogen bond angles in the O2−N3 water complex deviate significantly from 180° (by 30−50°), and the bond angle within water decreases significantly (to 99.5°) upon complexation.

The relative energies and binding strengths of the cytosine and hydrogen fluoride or ammonia complexes calculated with and without diffuse functions in the basis set are within 5−7 kJ mol^{-1} (Table 1), and the trends in the data are the same. In general, the binding strengths for HF complexes decrease upon exclusion of diffuse functions, while those for ammonia complexes increase. The O2−N3 complex on both surfaces becomes slightly destabilized relative to O2(N1) upon exclusion of diffuse functions, where the destabilization is slightly larger for NH_3 (5.6 kJ mol^{-1}) compared with HF (2.3 kJ mol^{-1}).

The water binding strengths and relative energies for the three complexes characterized on both the 6-31G(d,p) and 6-31+G(d,p) surfaces are within approximately 5 kJ mol^{-1}, which suggests that the surfaces are similar in these regions. On the

TABLE 2: Comparison of B3LYP and MP2 Relative Energies and Binding Strengths for Cytosine−Water Complexes[a]

	B3LYP				MP2			
	6-31+G(d,p)		6-31G(d,p)		6-31+G(d,p)		6-31G(d,p)	
	ΔE^b	$\Delta E_{ZP+BE}{}^c$	ΔE^b	$\Delta E_{ZP+BE}{}^c$	ΔE^b	$\Delta E_{ZP+BE}{}^c$	ΔE^b	$\Delta E_{ZP+BE}{}^c$
O2(N1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
O2−N3			27.0	15.3			22.2	10.9
N3(N4)	3.0	3.0	4.4	1.9	2.9	1.8	4.1	0.9
(N4)	25.2	21.9	34.4	17.8	23.6	18.2	32.1	12.2
	$D_e{}^b$	$D_{e,ZP+BE}{}^c$	$D_e{}^b$	$D_{e,ZP+BE}{}^c$	$D_e{}^b$	$D_{e,ZP+BE}{}^c$	$D_e{}^b$	$D_{e,ZP+BE}{}^c$
O2(N1)	51.2	37.6	67.3	36.5	54.5	34.5	64.3	31.7
O2−N3			40.3	21.2			42.1	20.8
N3(N4)	48.2	34.5	62.9	34.6	51.6	32.7	60.3	30.8
(N4)	26.0	15.6	32.9	18.7	30.9	16.3	32.2	19.5

[a] See Figures 1 and 2 for chemical structure, numbering and notation of cytosine complexes. [b] Relative energy (ΔE) and binding strength (D_e) without zero-point vibrational energy and basis set superposition error corrections. [c] Relative energy (ΔE_{ZP+BE}) and binding strength ($D_{e,ZP+BE}$) including zero-point vibrational energy and basis set superposition error corrections.

B3LYP/6-31G(d,p) surface, the O2−N3 complex is found to fall 15.3 kJ mol^{-1} higher in energy than the O2(N1) complex, and 2.5 kJ mol^{-1} lower in energy than the (N4) complex.

Due to the discrepancies in the results from different basis sets along with differences in the way density functional and ab initio techniques characterize hydrogen bonding interactions, the cytosine−water complexes were investigated using MP2 and both basis sets. The geometries of the B3LYP (Figure 3) and MP2 (Figure S1, Supporting Information) complexes are similar. Furthermore, the B3LYP relative energies and binding strengths are generally within 5−7 kJ mol^{-1} of the MP2 values (Table 2), regardless of whether zero-point and basis set superposition errors are included. Most importantly, the O2−N3 complex can be characterized with MP2/6-31G(d,p), but not with MP2/6-31+G(d,p). Thus, since there is good agreement between all data and trends, we gain further confidence in our computational approach.

It has been previously hypothesized that the O2−N3 cytosine−water complex does not exist due to the anticooperative hydrogen bonding interactions within this complex where cytosine behaves as a proton acceptor in both hydrogen bonds.[11,18] However, it is interesting to note that the O2−N3 complex is more stable than the (N4) complex at the B3LYP/6-31G(d,p) level of theory and the (N4) complex was characterized on the B3LYP/6-31+G(d,p) surface. Furthermore, similar anticooperative interactions between water at the O2−N3 site for other cytosine derivatives have been found using Pople's double-ζ basis set with diffuse functions.[38] An anticooperative O6−N7 guanine−water complex has also been identified with diffuse functions,[6,36] although the hydrogen-bond angles are more linear compared with the probable cytosine−water complex. These facts lead us to question the apparent absence of the O2−N3 minimum on the B3LYP/6-31+G(d,p) cytosine−water surface. Since the O2−N3 complex falls to the N3(N4) minimum during geometry optimizations, we more closely consider the relationship between the O2−N3 and N3(N4) structures.

(ii) Closer Investigation of Complexes with Water. To monitor the migration of water between the O2−N3 and N3(N4) binding sites of cytosine, optimizations were performed where the distance between the amino hydrogen and the water oxygen was fixed at values ranging from 1.6 to 6.0 Å. Increments of 0.2 Å were typically utilized; however, in some instances,

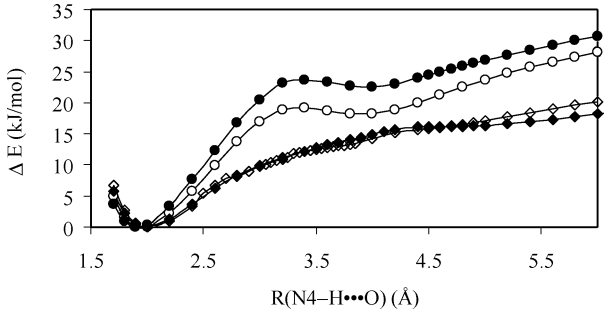

Figure 5. B3LYP (closed symbols) and MP2 (open symbols) relative energies (kJ mol^{-1}) calculated with the 6-31G(d,p) (circles) and 6-31+G(d,p) (diamonds) basis sets for fixed optimizations of the (neutral) cytosine−water complexes as a function of the N4−H···O_{water} hydrogen bond length.

smaller increments (0.05 or 0.1 Å) were implemented in order to ensure that a proper description of the surface was obtained.

Figure 5 plots the B3LYP relative energy as a function of the N4−H···O distance for the 6-31G(d,p) and 6-31+G(d,p) basis sets. Two distinct minima can be extracted from the 6-31G-(d,p) relative energies, where the O2−N3 minimum (at ∼4.0 Å) is approximately 22.5 kJ mol^{-1} higher in energy than the N3(N4) minimum, which is in agreement with the relative energies reported in Table 2 (ΔE). This value decreases to approximately 13 kJ mol^{-1} when zero-point vibrational and BSSE energy corrections are included (ΔE_{ZP+BE}, Table 2). From Figure 3, it can be seen that the O2−N3 structure is contained within a very shallow (1.0 kJ mol^{-1}) energy well on the 6-31G-(d,p) surface compared with the N3(N4) complex.

Inclusion of diffuse functions (the 6-31+G(d,p) basis set) significantly changes the potential energy surface connecting the N3(N4) and O2−N3 complexes (Figure 5). First, the activation barrier for conversion between N3(N4) and O2−N3 significantly decreases, which flattens the surface to the extent that the defined well at 4.0 Å corresponding to the O2−N3 complex is eliminated. Second, an energy plateau centered on a N4−H···O distance of approximately 4.7 Å is generated. The points along the plateau differ in energy by only 0.3 kJ mol^{-1} and fall approximately 16 kJ mol^{-1} above the N3(N4) minimum.

It has been established that B3LYP is a suitable method to study interactions between the DNA nucleobases and water,[4−6,11,13,18,19] and B3LYP has been shown in the present work to yield similar structures and relative energies as MP2 for cytosine−water complexes (Table 2). Nevertheless, it is known that density functional theory often flattens potential energy surfaces and sometimes yields different hydrogen bonded complexes compared with higher level ab initio methods. Therefore, we have reinvestigated the shape of the surface connecting the O2−N3 and N3(N4) complexes using MP2 and Pople's 6-31G(d,p) basis set with and without diffuse functions (Figure 5).

The MP2 and B3LYP surfaces obtained with the 6-31G(d,p) basis set are similar (Figure 5). The most notable differences are the slight (approximately 4 kJ mol^{-1}) reduction in both the barrier for conversion between O2−N3 and N3(N4) and the stability of the O2−N3 complex. Despite these energy reductions, the O2−N3 complex is found to exist within a shallow (1 kJ mol^{-1}) energy well on both the B3LYP and MP2 (6-31G-(d,p)) surfaces. Upon inclusion of diffuse functions, the MP2 conversion barrier decreases as previously discussed for B3LYP. This leads to flattening of the surface, where a slightly larger energy difference between the points along the plateau region exists on the MP2 surface (1 kJ mol^{-1}) compared with B3LYP (0.3 kJ mol^{-1}).

From this comparison, we conclude that B3LYP and MP2 provide similar descriptions of the surface connecting the O2−N3 and N3(N4) cytosine−water hydrogen bonded complexes. Furthermore, when small (double-ζ) basis sets are implemented without diffuse functions, the O2−N3 minimum exists within a shallow energy well. The depth of this well decreases, to the point where only a plateau region is observed, as the basis set is expanded to include diffuse functions.

Upon closer examination of the complex at the center of the B3LYP/6-31+G(d,p) energy plateau, it is noted that the O−H···O2 distance is within hydrogen bonding distance (1.94 Å), while the N3···H−O distance is significantly longer (3.04 Å). The O−H···O2 hydrogen bond angle (167.9°) and the internal water angle (103.1°) have significantly increased compared with those in the B3LYP/6-31G(d,p) optimized structures (131.4° and 99.5°, respectively), which reduces the strain in the complex. The calculated increase in the bond distance to N3 and decrease in the bond distance to O2 in the O2−N3 water complex upon addition of diffuse functions are similar to the phenomenon previously noted for the cytosine−ammonia O2−N3 complex (Figure 3). Thus, both the fully optimized ammonia complex and the water complex centered on the B3LYP plateau could be considered ring-opened structures that involve only one (strong) hydrogen bond. Experimental evidence for a ring-opened complex between 1-methylcytosine and water has been provided by Smets et al.[15]

Cytosine Anion Complexes. *(i) Complexes with HF, H_2O, or NH_3.* As mentioned in the Introduction, we have previously investigated the effects of hydrogen bonds on the (N1) acidity of uracil,[4] thymine,[5] and other pyrimidine derivatives.[5] To compare with these previous studies, we continue the present discussion of the hydrogen bonding properties of cytosine by considering complexes between the (N1) cytosine anion and hydrogen fluoride, water, or ammonia. Since we are interested in the N1 acidity, the anionic O2(N1) cytosine complex cannot be considered. Furthermore, we find that the (N4) anionic complexes are not stable minima on the potential energy surfaces since the weak proton donating ability of the cytosine anion leads to migration of the small molecule to a position over the cytosine molecular ring. The corresponding neutral complexes could not be characterized, and therefore these anionic complexes are not further considered in the present work. This leaves the N3(N4) and O2−N3 anionic complexes to be considered. The B3LYP/6-31+G(d,p) optimized structures of these anionic complexes are displayed in Figure 6. To the best of our knowledge, this represents the first report of complexes between small molecules and the cytosine (N1) anion.

As previously discussed for uracil[4] and thymine,[5] the small molecules move away from the cytosine donor and closer to the cytosine acceptor sites upon anion formation. Another notable feature is increased puckering of the cytosine amino group compared with the neutral complexes, and the isolated cytosine (N1) anion. Specifically, the amino hydrogens are up to 10° out of the molecular plane in the isolated cytosine anion and the neutral complexes, while the amino hydrogens are up to 35° out of the molecular plane in the anionic complexes.

Despite the fact that the O2−N3 complex between (neutral) cytosine and water is difficult to characterize, O2−N3 is the only complex isolated between the cytosine (N1) anion and water with B3LYP/6-31+G(d,p) due to stronger interactions with the cytosine acceptor sites upon anion formation. As previously discussed for the neutral complex, the O2−N3 cytosine−water anionic complex is likely strained by a nonlinear (155.7°) O−H···O2 hydrogen bond and small water bond angle (97.4°). The hydrogen bond distance between water and O2 is shorter than the distance to N3 (by 0.46 Å). The O2−N3

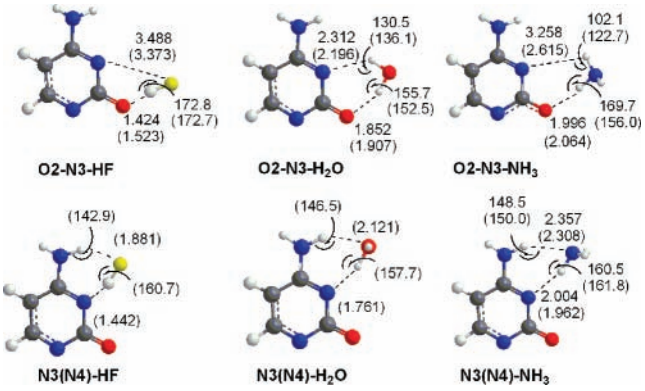

Figure 6. Selected B3LYP/6-31+G(d,p) bond lengths (Å) and angles (deg) in cytosine (N1) anionic complexes with hydrogen fluoride, water, or ammonia (B3LYP/6-31G(d,p) values in parentheses).

TABLE 3: B3LYP Relative Energies and Binding Strengths for the (N1) Cytosine Anion Complexed with Hydrogen Fluoride, Water, or Ammonia[a,b]

	6-31+G(d,p)		6-31G(d,p)	
site	ΔE	D_e	ΔE	D_e
		HF		
O2−N3	0.0	103.4	4.7	89.0
N3(N4)			0.0	93.8
		H_2O		
O2−N3	0.0	60.6	0.0	57.1
N3(N4)			6.0	51.1
		NH_3		
O2−N3	0.0	29.8	1.8	27.6
N3(N4)	0.7	29.1	0.0	29.4

[a] Relative energies include zero-point vibrational energy and basis set superposition error corrections. [b] See Figures 1 and 2 for the chemical structure, numbering and nomenclature of cytosine complexes.

complex between the cytosine anion and hydrogen fluoride is also the only stable minimum characterized on this surface.[39] The hydrogen-bond distance between O2 and hydrogen fluoride is extremely short (1.424 Å) and the hydrogen-bond angle is nearly linear (172.8°). These geometrical features suggest that the binding strength of this HF complex is large.

The anionic O2−N3 cytosine−ammonia complex was also characterized and, similar to previously discussed for the (neutral) cytosine complex, ammonia is located out of the molecular plane, where the ammonia nitrogen falls ∼10−15° above the cytosine ring. Similar to the water complex, a shorter hydrogen bond distance between ammonia and O2 compared with N3 (by the 1.262 Å) suggests that interactions with the cytosine anion are strongest at the carbonyl group. The O2−N3 binding strength (Table 3) is strongest for HF (∼100 kJ mol^{-1}) and weakest for NH_3 (∼30 kJ mol^{-1}), while the water value falls between (∼60 kJ mol^{-1}). Thus, the binding strength of the anionic O2−N3 complex significantly increases with the acidity of the small molecule bound to cytosine.

The N3(N4) complex was found on the potential energy surface for the cytosine anion and ammonia complex. This complex involves a shorter (2.004 Å) hydrogen bond distance to the cytosine acceptor than the donor (2.357 Å). This structure was likely characterized for ammonia, but not water or hydrogen fluoride, due to the larger proton affinity of ammonia, which permits N4−H···N interactions. Due to the puckered cytosine amino group, ammonia is located out of the molecular plane (by approximately 8°) in this complex. The N3(N4) binding strength for the ammonia complex is less than 1 kJ mol^{-1} smaller than the corresponding O2−N3 value.

It should be noted that the N4 complexes discussed for (neutral) cytosine (Figure 4a) can also be found between the cytosine anion and water or hydrogen fluoride (Figure 4b). The hydrogen fluoride position is similar to that in the neutral complex with the exception of a shorter F−H···N4 distance. A binding strength of 40 kJ mol^{-1} indicates that this complex is considerably more stable in the anionic form compared with the neutral structure, which has a binding strength of 11 kJ mol^{-1}. Nevertheless, the anionic N4 complex is found to be approximately 64 kJ mol^{-1} higher in energy than the corresponding O2−N3 complex. In the N4 water−cytosine−anionic complex (Figure 4b), water is 40° out of the molecular plane and forms a more linear hydrogen bond ($\angle$(O−H···N4) = 179.9°) than the equivalent neutral counterpart. With a binding strength of 35 kJ mol^{-1}, this water complex falls 26 kJ mol^{-1} above the N3(N4) complex.

Since unique complexes between (neutral) cytosine and water were isolated upon removal of diffuse functions from the 6-31+G(d,p) basis set, the 6-31G(d,p) surfaces were investigated for the anionic complexes. The geometries of the 6-31G(d,p) optimized complexes (Figure 6) are very similar to those obtained when diffuse functions are included in the basis set. In the O2−N3 complexes, the small molecules move away from the cytosine O2 carbonyl by up to 0.1 Å. This is accompanied by migration toward N3, which is more predominant (0.64 Å) for ammonia compared with hydrogen fluoride and water (0.12 Å). Despite the migration toward N3, the distance to O2 remains considerably shorter for all three small molecules.

Unlike the 6-31+G(d,p) results, the N3(N4) complexes were isolated for all three small molecules with 6-31G(d,p). The N3(N4) geometries (Figure 6) are reminiscent of the neutral complexes (Figure 3). An exception is that the small molecules migrate closer to the cytosine proton acceptor in the anionic complexes. Additionally, the water hydrogen not interacting with cytosine in the anionic complex is significantly out of the plane compared with the neutral complex. These trends are similar to those previously reported for other anionic-pyrimidine complexes.[4,5]

The 6-31G(d,p) binding energies (Table 3) decrease for all three small molecules compared with 6-31+G(d,p) for the O2−N3 complex (by 14.4 kJ mol^{-1} for HF, 3.5 kJ mol^{-1} for H_2O, and 2.2 kJ mol^{-1} for NH_3). However, the binding energy increases slightly for the N3(N4) ammonia complex. These energy changes upon inclusion of diffuse functions result in reordering of the lowest energy minimum. O2−N3 is the global minimum for all three small molecules when diffuse functions are employed. However, without diffuse functions, N3(N4) is the global minimum for hydrogen fluoride and ammonia, while the O2−N3 complex is the global minimum for water. The energy differences between O2−N3 and N3(N4) are under 6 kJ mol^{-1}, where the largest difference occurs for H_2O.

In summary, the N3(N4) anionic cytosine−water complex cannot be found with diffuse functions, but is only 6 kJ mol^{-1} higher in energy than O2−N3 without diffuse functions. Since scans of the surface connecting the O2−N3 and N3(N4) complexes provide useful information about the neutral cytosine−water surface, we further consider the relationship between the anionic N3(N4) and O2−N3 water complexes in the next section.

(ii) Closer Investigation of Complexes with Water. Scans similar to those discussed for the (neutral) cytosine−water complex (Figure 5) were conducted to assess the relationship between the anionic N3(N4) and O2−N3 minima (Figure 7). Contrary to the neutral surface scans, where the O2−N3 minimum is over 20 kJ mol^{-1} higher in energy than the N3(N4) complex, the anionic O2−N3 minimum is more stable than

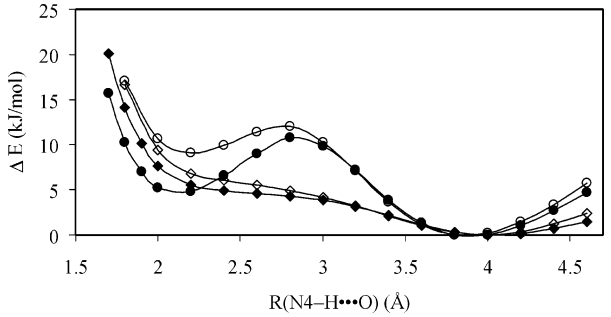

Figure 7. B3LYP (closed symbols) and MP2 (open symbols) relative energies (kJ mol^{-1}) calculated with the 6-31G(d,p) (circles) and 6-31+G(d,p) (diamonds) basis sets for fixed optimizations of the (anionic) cytosine−water complexes as a function of the N4−H•••O$_{water}$ hydrogen bond length.

the N3(N4) counterpart by 5 kJ mol^{-1} on the B3LYP/6-31G-(d,p) surface. A minimum corresponding to the N3(N4) complex is located at a N4−H•••O distance equal to approximately 2.1 Å, while the O2−N3 minimum is found at approximately 4.0 Å. Both minima are located in deeper (6−7 kJ mol^{-1}) energy wells compared with the neutral surface.

The major effect of including diffuse functions in Pople's 6-31G(d,p) basis set is a decrease in the activation barrier that connects the N3(N4) and O2−N3 minima (Figure 7), as previously discussed for the neutral cytosine−water complex. However, flattening of the transition barrier on the anionic surface leads to complete destabilization of the N3(N4) complex, where the energy steadily decreases from a N4−H•••O distance of 2 to 4 Å, which corresponds to the O2−N3 minimum.

As discussed for the neutral cytosine−water complexes, the MP2 method was also employed to study the potential energy surface (Figure 7). Comparison of the MP2 and B3LYP scans indicates that the method does not significantly affect the potential energy surface. A notable effect is an increase in the energy difference between the N3(N4) and O2−N3 minima to approximately 9.1 kJ mol^{-1} (from about 5 kJ mol^{-1} for B3LYP) on the 6-31G(d,p) surfaces. Additionally, the 6-31G(d,p) O2−N3 energy well decreases to 2.9 kJ mol^{-1} from roughly 6 kJ mol^{-1} for the B3LYP surface. Perhaps most importantly, as seen for B3LYP, the N3(N4) minimum is completely destabilized on the MP2/6-31+G(d,p) surface, where the slope to the O2−N3 minimum is slightly steeper than that calculated with B3LYP.

Thus, including diffuse functions in Pople's double-ζ basis set, leads to similar results on the neutral and anionic surfaces, namely a reduction in the conversion barrier between the O2−N3 and N3(N4) complexes. However, the higher energy (N3-(N4)) anionic cytosine−water complex is completely destabilized upon inclusion of diffuse functions in the basis set at both the MP2 and B3LYP levels of theory. This is contrary to the effects of diffuse functions on the neutral surface, where the local minimum energy (O2−N3) well is replaced with an energy plateau.

Effects of Hydrogen Bonding on the Acidity of Cytosine. We extend upon our previous work[4−6] by investigating the effects of hydrogen bonds on the (N1) acidity of cytosine. The B3LYP/6-311+G(2d,p) acidities calculated using B3LYP geometries optimized with the 6-31+G(d,p) and 6-31G(d,p) basis sets are compared in Table 4. It should be noted that the acidities are reported as deprotonation enthalpies, where a smaller deprotonation enthalpy represents a larger acidity. The effect of hydrogen bonding interactions on the acidity (Δ, Table 4) is calculated as the difference between the acidity of the cytosine complex and the acidity of isolated cytosine, where a positive value indicates that the hydrogen bonding interactions increase the acidity.

TABLE 4: B3LYP/6-311+G(2d,p) (N1) Acidity of Cytosine Complexed with Hydrogen Fluoride, Water, or Ammonia, the Effects of Hydrogen Bonding on the Acidity (Δ) and the Corresponding Binding Strengths for Neutral and Anionic Complexes (kJ mol^{-1})[a,b]

	6-31+G(d,p)				6-31G(d,p)			
	acidity	Δ[c]	$D_{e,neutral}$	$D_{e,anion}$	acidity	Δ[c]	$D_{e,neutral}$	$D_{e,anion}$
				HF				
O2−N3	1387.4	55.3	44.4	99.7	1389.0	53.6	44.6	98.1
N3(N4)					1394.0	48.6	50.1	98.7
				H_2O				
O2−N3	1405.3[d]	37.4[d]	20.4[d]	57.8	1406.1	36.4	20.5	57.0
N3(N4)					1422.2	20.4	28.2	48.5
				NH_3				
O2−N3	1422.3	20.4	7.8	28.2	1422.5	20.1	4.7	24.8
N3(N4)	1441.2	1.5	25.0	26.5	1441.2	1.3	24.5	25.9

[a] Relative energies include ZPVE and BSSE corrections and were calculated using B3LYP geometries optimized with 6-31+G(d,p) or 6-31G(d,p). [b] See Figures 1 and 2 for chemical structure, numbering and nomenclature of cytosine complexes. [c] The difference between the acidity of the cytosine complex and the acidity of isolated cytosine calculated at the same level of theory (1442.7 and 1442.6 kJ mol^{-1} for geometries optimized with 6-31+G(d,p) and 6-31G(d,p), respectively), where a positive Δ indicates an increase in acidity upon complexation. [d] Calculated using the neutral geometry estimated from the center of the plateau in the 6-31+G(d,p) surface scan R(N4−H•••O) (Figure 5).

The calculated (N1) acidities of cytosine optimized using the 6-31+G(d,p) and 6-31G(d,p) basis sets are 1442.7 and 1442.6 kJ mol^{-1}, respectively, which indicates that any geometrical changes due to the use of diffuse functions are minimal. A similar conclusion can be drawn for the cytosine complexes. In particular, the effect of diffuse functions in the optimizations on the calculated acidity of the cytosine−ammonia complexes is small (Table 4). This is slightly surprising for the ammonia O2−N3 complex due to the difference in the 6-31G(d,p) and 6-31+G(d,p) optimized geometries (Figures 3 and 6), where the latter involves only one significant hydrogen bonding interaction. The acidity of the O2−N3 cytosine−hydrogen fluoride complex also does not significantly depend on the level of theory implemented in the optimizations (Table 4).[40]

The similarity of the acidities calculated using geometries optimized with and without diffuse functions suggests that good approximations of the effects of water on the cytosine (N1) acidity can be obtained from 6-31G(d,p) optimized structures. Further verification comes from the acidity of the O2−N3 cytosine−water complex calculated by estimating the geometry of the neutral complex from the center of the plateau on the B3LYP/6-31+G(d,p) surface (Figure 5). In particular, the acidity calculated with the 6-31+G(d,p) estimated neutral O2−N3 geometry (R(N4−H•••O) = 4.7 Å) and fully optimized anion (1405.3 kJ mol^{-1}) is only 0.8 kJ mol^{-1} larger than that calculated using the fully optimized B3LYP/6-31G(d,p) structures. Therefore, accurate estimates of the effects of hydrogen bonding within the N3(N4) region of cytosine can be obtained using the 6-31G(d,p) geometries.

The acidity of cytosine is greatly affected by the properties of the small molecules bound, where complexes with hydrogen fluoride have the largest acidities, while complexes with ammonia yield the smallest acidities. Specifically, hydrogen fluoride generally increases the acidity by up to approximately 55 kJ mol^{-1}, while ammonia can lead to a very small (1−2 kJ mol^{-1}) increase.

TABLE 5: B3LYP/6-311+G(2d,p) (N1) Acidity of Cytosine, Thymine, and Uracil Complexed with Hydrogen Fluoride, Water, or Ammonia, the Effects of Hydrogen Bonding on the Acidity (Δ), and the Corresponding Binding Strengths for Neutral and Anionic Complexes (kJ mol^{-1})[a]

binding site	acidity			Δ[b]		
	HF	H_2O	NH_3	HF	H_2O	NH_3
			cytosine			
O2−N3	1387.4	1405.3	1422.3	55.3	37.4	20.4
N3(N4)	1394.0[c]	1422.2[c]	1441.2	48.6[c]	20.4[c]	1.5
			thymine[d]			
O2(N3)	1325.3	1376.2	1399.1	51.2	20.4	−2.6
O4(N3)	1354.2	1380.5	1401.4	42.3	16.0	−4.9
			uracil[e]			
O2(N3)	1338.0	1369.2	1392.6	51.4	20.2	−3.2
O4(N3)	1346.7	1373.7	1394.8	42.7	15.7	−5.4

[a] Relative energies include ZPVE and BSSE corrections and were calculated using B3LYP geometries optimized with 6-31+G(d,p). See Figures 1 and 2 for chemical structure, numbering and nomenclature of cytosine complexes. [b] The difference between the acidity of the pyrimidine complex and the acidity of isolated pyrimidine calculated at the same level of theory (1442.6 kJ mol^{-1} for geometries optimized 6-31G(d,p) for cytosine, 1396.5 kJ mol^{-1} and 1389.4 kJ mol^{-1} for thymine and uracil respectively at 6-31G(d,p)), where a positive Δ indicates an increase in acidity upon complexation. [c] Values estimated using 6-31G(d,p) geometries. [d] Reference 5. [e] Reference 4.

For all small molecules, the O2−N3 complex has a larger acidity than N3(N4). There is a significant difference in the effect on the acidity of binding at O2−N3 and N3(N4) for water (16.0 kJ mol^{-1}) and ammonia (18.8 kJ mol^{-1}) due to the extra stabilization provided by double proton donation from the small molecule to two cytosine acceptors in O2−N3. Furthermore, anion formation reduces the proton donating ability of cytosine sites, which reduces the N3(N4) binding strength, but does not affect that for O2−N3 (since this complex lacks a proton donor site).

Since HF contains only one proton donor, there is a smaller (5 kJ mol^{-1}) difference between the effects of hydrogen bonds at the O2−N3 and N3(N4) sites for this small molecule. Interestingly, hydrogen fluoride in the N4 complex (Figure 4) increases the acidity by 50.2 kJ mol^{-1}, which falls between the enhancements provided by binding at the other two sites.

Comparison with Other Pyrimidines. Isolated cytosine has a smaller (N1) acidity than uracil or thymine (by approximately 45−55 kJ mol^{-1}).[11,19,36] Therefore, it is not surprising that the cytosine−XH complexes have smaller (N1) acidities than the corresponding uracil[4] or thymine[5] complexes (Table 5). More interesting trends become prevalent when the magnitude of the effect of hydrogen bonding interactions on the N1 acidities of the pyrimidines are compared.

Since cytosine has a different hydrogen bonding pattern compared with uracil and thymine, it is difficult to directly compare the effects of the binding site. Nevertheless, both the cytosine N3(N4) and the thymine (uracil) O4(N3) complexes contain a proton donor and a proton acceptor, and therefore the effects of hydrogen bonding in this region of the pyrimidines are expected to be similar. Indeed, we find that the effects on the cytosine acidity are only 5 kJ mol^{-1} larger than that for thymine (or uracil), which is likely due to the larger proton affinity of the cytosine proton acceptor.[11,19,36] More significant differences between the effects on the acidity occur for binding within the O2−N3 region of the pyrimidines. The hydrogen bonding effects are larger for cytosine (by up to 25 kJ mol^{-1}) since the cytosine O2−N3 anionic complex involves interactions with two proton acceptors, which provides greater stabilization than interactions with a single proton acceptor in the thymine (uracil) O2(N3) complex.

All pyrimidines show analogous trends in the effects of hydrogen bonding on the (N1) acidity with respect to the small molecule bound. In particular, the effect is largest when (acidic) HF binds to the pyrimidine, while (basic) NH_3 produces the smallest changes in acidity. Since HF primarily coordinates with the pyrimidines through one hydrogen bond, the effects of HF are similar among the pyrimidines (with a range of 2−5 kJ mol^{-1}). Conversely, water and ammonia coordinate with the pyrimidine through two hydrogen bonds, and therefore there are larger differences (16−25 kJ mol^{-1}) in the magnitude of these effects among the pyrimidines. Interestingly, ammonia increases the acidity of cytosine, but decreases the acidity of thymine (uracil).

In summary, despite the fact that the alternating pattern of proton acceptor and donor sites common to most pyrimidines is disrupted in cytosine, similar trends in the effects of hydrogen-bonding interactions with various small molecules on the acidity are obtained for all pyrimidines.

Conclusions

The present study investigates the effects of hydrogen bonds on the properties of cytosine. Cytosine is of interest since it lacks the alternating proton donor and acceptor pattern found in other pyrimidines (thymine, uracil), and therefore has unique hydrogen bonding possibilities. Complexes between HF, H_2O, or NH_3 and four main binding sites in (neutral and anionic) cytosine were considered. Two binding positions involve neighboring cytosine proton donor and acceptor sites, which permits the formation of bidentate hydrogen bonded complexes observed for other pyrimidines. However, one binding site (O2−N3) involves two cytosine proton acceptors, which thereby prohibits a (cooperative) bidentate hydrogen bonded complex from being formed. The final complex (N4) involves one cytosine donor.

The (neutral) cytosine−water (O2−N3) complex that involves anticooperative hydrogen bonding interactions can be isolated with both B3LYP and MP2 using the 6-31G(d,p) basis set. However, this complex appears to be unstable on the corresponding 6-31+G(d,p) surfaces. For the first time, the reason for this phenomenon is elucidated. Specifically, potential energy surface scans reveal this (O2−N3) complex resides in a shallow local minimum energy well on the 6-31G(d,p) surface, which disappears upon inclusion of diffuse functions. However, a plateau exists on the 6-31+G(d,p) surface that corresponds to a O2−N3 complex with an elongated O−H···N3 hydrogen bond distance. A similar problem is observed on the corresponding anionic surfaces, where the N3(N4) minimum is found on the 6-31G(d,p) surfaces, but disappears upon inclusion of diffuse functions.

Despite the different hydrogen bonding sites available in cytosine compared with the other pyrimidines, the trends in geometries, binding strengths and (N1) acidities of complexes with small molecules are similar for all pyrimidines. The properties of the small molecule bound to cytosine affect the change in acidity more than the binding position, where hydrogen fluoride leads to the greatest increase. The effects of hydrogen bonds on the (N1) acidity is similar for corresponding binding sites in all pyrimidines, but differ more significantly when the hydrogen bonding pattern changes.

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Supporting Information Available: MP2 geometries optimized with 6-31+G(d,p) and 6-31G(d,p) for (neutral) cytosine−water complexes. This information is available free of charge via the Internet at http://pubs.acs.org.

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