Effects of Hydrogen Bonding on the Acidity of Uracil

Mariana Di Laudo, Sarah R. Whittleton, and Stacey D. Wetmore*

Department of Chemistry, Mount Allison University, 63C York Street, Sackville, New Brunswick E4L 1G8, Canada

Received: May 29, 2003; In Final Form: September 3, 2003

The present study uses density functional theory to investigate the effects of hydrogen bonding on the (N1) acidity of uracil. Uracil and uracil anion complexes with water, ammonia, and hydrogen fluoride at various uracil sites (O2(N3), O4(N3) and O4(C5)) are considered. The calculated geometries of the uracil anion complexes are significantly different from those of the (neutral) uracil counterparts, which leads to the significantly larger binding energies in the anionic complexes. The binding strength of each molecule to (neutral) uracil is largest at the O4(N3) position and at the O2(N3) position in the uracil (N1) anion. Our calculations reveal that hydrogen-bonding interactions with one molecule increase the (N1) acidity of uracil by up to approximately 50 kJ mol^{-1} and that the effect of two molecules is approximately equal to the sum of the individual effects. The acidity increase is largest when water and ammonia bind to the O4(C5) position and when hydrogen fluoride binds to the O2(N3) position. Our results lead to a greater fundamental understanding of hydrogen-bonding interactions involving uracil and have important implications for interactions in biological systems, such as those at the active site in uracil DNA glycosylase.

Introduction

Hydrogen bonds play fundamental roles in biological systems.[1] Intramolecular and intermolecular bonds are believed to be responsible for the binding between nucleotide (or nucleoside) bases, the formation of DNA double and triple helices, the structure of carbohydrates, and the folding pattern of proteins. They are also involved in the binding of many substrates to the active sites of enzymes. Therefore, hydrogen bonds between biomolecules, and the effects of hydrogen bonds on their properties, are of great interest.

Chemical calculations can provide information about the structure of hydrogen-bonded complexes and the strength of binding interactions. Due to this valuable information and the abundance of water in biosystems, the interactions between individual nucleobases and one or more water molecules have been extensively studied with computational techniques. Perhaps the most well studied water−nucleobase interactions involve uracil.[2−21] Uracil contains many consecutive hydrogen-bond-donor and -acceptor groups, which makes it ideal for studying hydrogen-bond interactions. Indeed, modified uracil−water complexes (such as those involving uracil hydroxy tautomers,[22] 5-substituted uracil derivatives,[23] thiouracils,[24] or amino derivatives of *N*,*N*′-dimethyluracil[25]) and the anions of uracil−water complexes[26,27] have also been investigated.

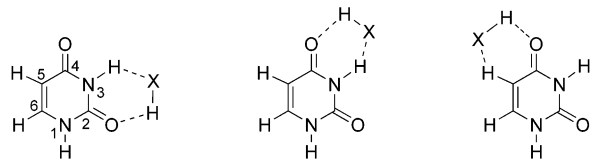
Although it is important to understand interactions between uracil and water, fundamental information about hydrogen-bonding interactions in biological systems that involve uracil can be obtained by investigating interactions between uracil and a variety of small molecules. Understanding the effects of hydrogen bonds on the molecular properties of uracil is also important. In particular, the (N1) acidity of uracil is of interest due to the mechanism of action of uracil DNA glycosylase, which is one of the enzymes responsible for removing uracil from DNA. The proposed mechanism of action of this enzyme involves nucleophilic attack of water at the sugar moiety and expulsion of uracil through an oxacarbenium ion−uracil anion intermediate.[28,29] Therefore, research has investigated the ability of uracil to act as a good leaving group, which is related to the (N1) acidity of this nucleobase.[30,31] To better understand the workings of uracil DNA glycosylase, we must understand the (N1) acidity of uracil and identify factors that influence this property, such as interactions with protein residues at the active site. Indeed, experimental evidence of a low N1 pK_a for uracil bound to uracil DNA glycosylase has been presented in the literature.[32] The presence of the N1 uracil anion in the proposed mechanism of action of uracil DNA glycosylase and the possible stabilization of this intermediate via hydrogen bonding with active site residues is one of the main driving forces of the present work.

In the present study, the binding properties of water, ammonia, and hydrogen fluoride to various positions in uracil (Schemes 1 and 2) are investigated. The three molecules chosen display a range in proton affinities and acidities and therefore have different hydrogen-bond-donating and -accepting abilities. The geometries and binding strengths in complexes between uracil (or the uracil anion) and each of these molecules, as well as the effects of interactions on the acidity of uracil, are considered. Previous studies have discussed, for example, the structure and binding energies of uracil−water complexes, as well as the relationship between the binding energy and the proton affinity or acidity of uracil sites.[2−21] To the best of our knowledge, there has only been one reported calculation on the effect of hydrogen bonding on a molecular property (deprotonation energy) of a uracil tautomer,[22] and few reports of hydrogen-bonding interactions with uracil[33,34] (or the uracil (radical) anion)[35−37] involving molecules other than water or other nucleobases. Because interactions with nucleobases play important roles in many biological processes, it is hoped that this study will enhance our understanding of possible interactions between uracil and other molecules present in biological

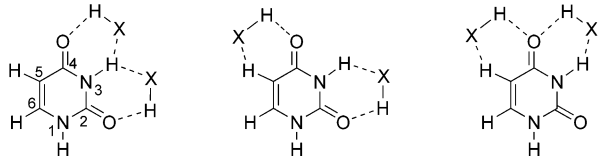
* Corresponding author. E-mail: swetmore@mta.ca.

10.1021/jp035496w CCC: $25.00
Published on Web 11/08/2003

SCHEME 1: Uracil Complexes Investigated in the Present Study (X = OH, NH_2, or F)



SCHEME 2: Uracil Complexes Investigated in the Present Study that Involve Simultaneous Binding of Two Molecules to Uracil (X = OH, NH_2, or F)



systems, and provide some insight into interactions important for the mechanism of action of uracil DNA glycosylase.

Computational Details

Geometries were obtained using the B3LYP functional in combination with the 6-31+G(d,p) basis set. Diffuse functions on heavy atoms and polarization functions on hydrogens were included in the basis set because it has been well established that these functions are required to properly describe hydrogen-bonded systems. No constraints were imposed on the molecular geometries of the complexes during the optimizations. Frequency calculations were performed at the same level of theory and all reported energies include scaled (0.9806) zero-point vibrational energy (ZPVE) corrections.

Acidities and binding energies were obtained from B3LYP/6-311+G(2d,p) single-point calculations. Binding energies calculated with this basis set are within 1 kJ mol^{-1} of those obtained using the larger 6-311+G(3df,2p) basis set for complexes between water, ammonia, or hydrogen fluoride and the **O2-(N3)** position in uracil.[38] All energies of the uracil complexes include basis set superposition error (BSSE) corrections, which were calculated according to the Boys and Bernardi counterpoise method.[39] Previous studies have considered the effect of including BSSE corrections during the optimization procedure for similar systems.[14,18] For a range of uracil−water complexes, it was concluded that the counterpoise correction changes the binding energy by approximately 3%.[14,18] BSSE corrections were not applied during the optimization procedure in the present study.

We note that the suitability of DFT methods to study hydrogen-bonded systems has been discussed in the literature. Although some reservations have been expressed,[40] DFT has been successfully used to study hydrogen-bonded complexes,[41,42] even the most weakly bound systems.[43] Furthermore, although DFT has been shown to strongly underestimate stabilization energies of stacked DNA base pairs,[44] it has been used successfully for the hydrogen-bonded complexes of nucleobases. Indeed, Zeegers-Huyskens et al.[11] have employed DFT (B3LYP) to study uracil−water complexes and found similar structures and energies as the more expensive MP2 method.[26a] We also note that we are mainly interested in trends in hydrogen-bond strengths, as well as trends in the effects of these interactions on other properties of uracil and that trends in our data are in agreement with previous studies.[14,15,27]

All calculations were performed with Gaussian 98.[56]

TABLE 1: Gas-Phase Proton Affinities and Acidities (kJ mol^{-1}) of Various Uracil Sites

	calcd[a]	expt
	Proton Affinity	
O2 (near N1)	812.1	840 ± 12[b]
O2 (near N3)	817.3	
O4 (near N3)	844.3	874 ± 12[b]
O4 (near C5)	855.6	
	Acidity	
N1	1389.4	1392 ± 16[c]
N3	1441.5	1450 ± 16[c]
C5	1580.3	1605 ± 12[d]
C6	1522.4	1517 ± 12[d,e]

[a] B3LYP/6-311+G(2d,p) single-point calculations performed on B3LYP/6-31+G(d,p) geometries. All energies include scaled (0.9806) ZPVE correction. [b] Reference 50. [c] Reference 30. [d] Bracketed values from studies of alkylated uracil derivatives (see ref 31). [e] Gronert et al. bracketed this value to be 1546 ± 13 kJ mol^{-1} (see ref 51).

Results and Discussion

Geometries. *(i) (Neutral) Uracil Complexes with H_2O, NH_3, and HF.* As mentioned in the Introduction, the structure of uracil offers many hydrogen-bond-donor and -acceptor sites. Protonation of uracil may occur at O2 or O4,[45] whereas deprotonation may occur at N1 or N3, or less likely at C5 or C6 (see Scheme 1 for the atomic numbering in uracil). Although the N1 and N3 positions in uracil could also accept a proton, this has previously been reported to be an unfavorable process.[46] Scheme 1 displays the complexes between uracil and water, ammonia, or hydrogen fluoride investigated in the present study that utilize these protonation and deprotonation sites, as well as the notation implemented throughout the paper. We note that it is also possible to consider hydrogen-bonded complexes where H_2O, NH_3, or HF donate a proton to O2 and accept a proton from N1 in uracil. These structures were not considered in the present study because we are mainly interested in the effects of hydrogen bonds on the uracil N1 acidity.[47]

The hydrogen-bond properties of molecules often correlate with the proton affinity (PA) and acidity of sites involved in the interactions. The protonation and deprotonation enthalpies for uracil sites have been documented in the literature.[2,11−12,30−31,46,48−51] Our calculated gas-phase proton affinities and acidities (Table 1) are in agreement with experiment[30,31,50,51] and previous calculations.[11,30,31,49] Among hydrogen-bond sites considered in the present study (Scheme 1), the data suggests that the O4 position will be most easily protonated and the N3 position most easily deprotonated.

Figure 1 displays selected geometrical parameters for the complexes between (neutral) uracil and water, ammonia, or hydrogen fluoride. Fully optimized structures for all complexes are provided in the Supporting Information to complement our discussion, which highlights the most important details. Upon hydrogen bonding with various small molecules, the structure of uracil remains relatively unchanged with the exception of the uracil sites involved in hydrogen-bonding interactions. The O2 and O4 carbonyl bonds stretch upon binding to small molecules by up to 0.018 and 0.021 Å, respectively. The N3−H and C5−H bond lengths increase by up to 0.025 and 0.003 Å, respectively.

Each molecule interacts with uracil through two hydrogen bonds and a cyclic structure is formed (Figure 1). This is in agreement with previous findings for complexes between uracil

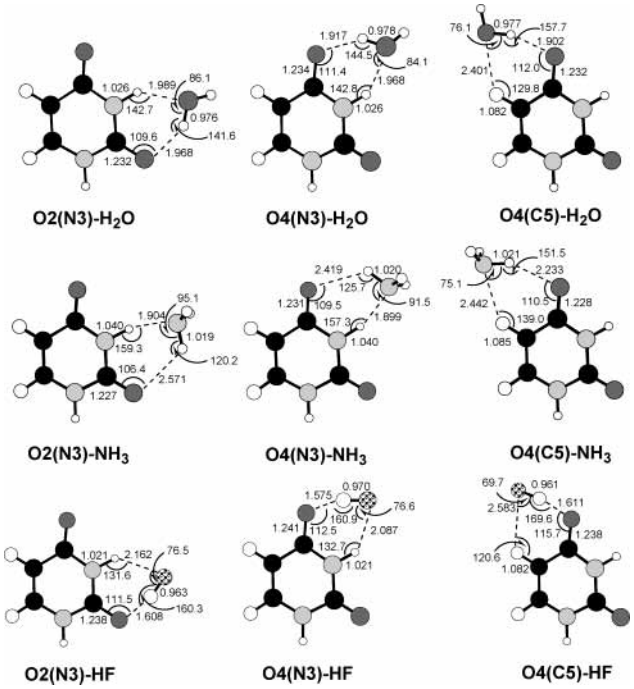


Figure 1. Selected bond lengths (Å) and angles (deg) in (neutral) uracil complexes with water, ammonia, and hydrogen fluoride.

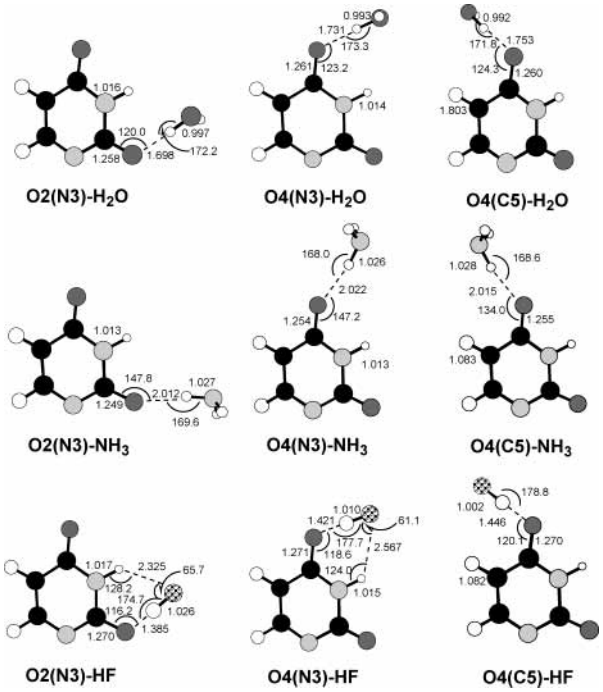


Figure 2. Selected bond lengths (Å) and angles (deg) in uracil anion complexes with water, ammonia, and hydrogen fluoride.

and water[11,14,15,20,26a,27] or hydrogen chloride.[33] Due to the formation of two intermolecular hydrogen bonds, the O···H−X and H···X−H hydrogen-bond angles deviate from linearity, ranging between approximately 120° and 170°.

It is noted that all geometrical parameters for the hydrogen-bond interactions in uracil−water complexes are in good agreement with those previously reported.[11,20,26a] We also note that the water hydrogen not interacting with uracil is found to be located out of the uracil molecular plane for the O2(N3) and O4(N3) complexes, and in the molecular plane for the O4(C5) complex. Although Ghomi et al.[20] found the free water hydrogen to be located out of the molecular plane for the O4(C5) complex, van Mourik et al.[14] determined that the potential energy surface for rotation about this hydrogen bond is very flat and there is no clear preference for the location of the free water hydrogen in this complex.

Geometrical parameters for the hydrogen-bonding interactions in the O2(N3), O4(N3) and O4(C5) complexes are very similar for each choice of X (Scheme 1). The most significant differences occur in the H···X−H and O···H−X interactions. For each value of X, the H···X-H distance increases (by approximately 0.5 Å) along the series O4(N3) < O2(N3) < O4(C5). We note that the calculated acidity at N3 is greater than the acidity at C5 (Table 1). The O···H−X hydrogen-bond distance increases as O4(C5) < O4(N3) < O2(N3) for water (by 0.09 Å) and ammonia (by 0.3 Å) complexes, which corresponds to a decrease in the PA of the uracil site (Table 1). However, the O···H−F hydrogen-bond distance in the HF complexes increases (by approximately 0.04 Å) according to O4(N3) < O2(N3) < O4(C5), despite the largest PA at the O4-(C5) position.

In attempts to gain additional insight into interactions between various molecules and uracil, we consider simultaneous binding of two molecules to uracil. All combinations of water, ammonia, and hydrogen fluoride at different uracil-binding sites are considered (Scheme 2). We note that the optimized geometries for the O2(N3)−O4(N3) complexes involve intermolecular hydrogen bonding between the two small molecules, and therefore these complexes more closely resemble interactions of a molecular dimer with uracil because each monomer does not form two hydrogen bonds with uracil. The instability of the O2(N3)−O4(N3) uracil−water complex depicted in Scheme 2 at the MP2 level has previously been reported in the literature.[18] We therefore focus our discussion on the O4(N3)−O4(C5) and O2(N3)−O4(C5) complexes, where some complexes with two water molecules bound to uracil have been previously studied in the literature.[18,20]

The optimized geometries of the O4(N3)−O4(C5) and O2-(N3)−O4(C5) uracil complexes are nearly superpositions of the geometries of the two uracil complexes from which they are composed (i.e., the structures displayed in Figure 1). Most hydrogen-bond distances change by less than 0.07 Å when two molecules interact with uracil compared with the individual hydrogen-bonded structures. The largest changes in the hydrogen-bond distances occur in the O4(N3)−O4(C5) complexes with NH_3 at O4(N3) and water or hydrogen fluoride at O4(C5) and the O2(N3)−O4(C5) complexes with NH_3 at O2(N3) and hydrogen fluoride at O4(C5). We note that the free water hydrogen remains in the uracil molecular plane in all complexes with water present at the O4(C5) position. The small differences in the complex geometries upon binding of the second molecule suggest that one molecule bound to uracil does not largely affect the hydrogen-bond-donor or -acceptor abilities of other uracil sites.

(ii) Uracil Anion Complexes with H_2O, NH_3, and HF. Because we are interested in the effects of hydrogen bonding on the acidity of uracil, we must also consider complexes formed between the uracil (N1) anion and water, ammonia, or hydrogen fluoride. Figure 2 displays selected geometrical parameters for the complex between the uracil anion and one molecule. As found for (neutral) uracil, the most significant changes in the uracil anion geometry upon complex formation occur at the sites involved in hydrogen bonding. The stretch of the carbonyl bond lengths in the uracil anion is larger than found for (neutral) uracil, where the O2 and O4 bond lengths increase by up to 0.026 and 0.021 Å, respectively. Alternatively, the changes in

the uracil anion N3−H and C5−H bond lengths are very small. It is noted that unlike the uracil radical anion, the uracil (N1) anion considered in the present study retains a planar geometry in all complexes.

The relative angular orientation of water, ammonia, or hydrogen fluoride with respect to uracil (Figure 1) changes upon deprotonation of uracil in all complexes (Figure 2), where the molecule moves further from the uracil hydrogen-bond donor and closer to the uracil hydrogen-bond acceptor. The O···H−X hydrogen-bond distances decrease by approximately 0.1−0.6 Å, whereas the H···X−H distances increase by approximately 0.3−2.2 Å, in the anionic complexes compared with corresponding neutral complexes. The largest changes in hydrogen-bond distances occur for the uracil−ammonia complexes. The significant increase in the H···X−H distances suggests that only one hydrogen bond exists in the anionic complexes. This statement is supported by the previously noted small changes in the uracil anion N3−H and C5−H bond lengths upon complex formation. Decreases in the O···H−X hydrogen-bond lengths suggest that the O2 and O4 sites are significantly more basic in the uracil anion compared with (neutral) uracil.

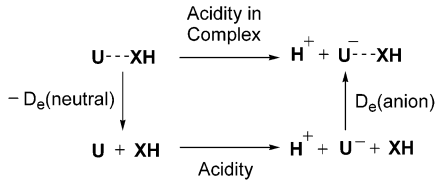
In addition to changes in hydrogen-bond lengths, the O···H−X hydrogen-bond angles are closer to 180° in the anionic complexes compared with the neutral counterparts, which is likely due to strengthening of this interaction. Furthermore, the free hydrogen moves out of the molecular plane in all complexes involving water when the anion is formed. Previous conclusions[14] that a small barrier exists for rotation about the corresponding hydrogen bond in neutral complexes likely extends to the uracil anion complexes considered in the present study.

The major structural changes noted here for the uracil (N1) anion complexes compared with the neutral uracil complexes are similar to those previously reported for conventional (radical) anions of uracil−water[26,27] and uracil−glycine[36] complexes. In conventional uracil−water anionic complexes, the H···OH_2 hydrogen bond breaks and the O···H−OH hydrogen bond shortens.[27] It has been suggested that three water molecules are connected by single hydrogen bonds to the uracil (radical) anion, which creates regions of high electron affinity to support the excess electron.[26b] Kryachko et al.[22] considered the anion formed through deprotonation of the O4-protonated uracil tautomer, which is equivalent to the O2(N3) anion in the present study and also noted significant changes in geometrical parameters upon deprotonation.

As found for complexes between neutral uracil and two molecules, the complexes between the uracil anion and two molecules represent superpositions of the individual hydrogen-bonded structures, where most bond lengths change by less than 0.2 Å. Changes in the H···X−H distances upon binding of two molecules are generally larger in the uracil anion complexes compared with (neutral) uracil complexes, whereas changes in the O···H−X hydrogen-bond distances are generally smaller. The most noteworthy geometrical change involves the dihedral angle of the free water hydrogen, which often shifts towards the molecular plane when two molecules are simultaneously bound to the uracil anion. However, it is once again noted that the barrier for rotation about this hydrogen bond is expected to be small.[14]

In summary, we find that changes in the geometry of complexes involving two molecules bound to uracil or the uracil anion relative to the geometry of the individual uracil complexes are insignificant. This suggests that interactions with one molecule do not largely affect the binding properties of other uracil sites. However, significant differences exist between the geometries of the uracil and uracil anion complexes. In particular, shorter hydrogen-bond distances to O2 and O4 exist in the anionic complexes, which may lead to stronger interactions at these positions in these complexes.

SCHEME 3: Thermodynamic Cycle for the Deprotonation of Uracil (U) Hydrogen-Bonded to Another Molecule (XH)



Binding Energies. The enthalpy for the deprotonation of uracil (U) that is hydrogen-bonded to a small molecule (XH) can be related to the enthalpy of deprotonation of isolated uracil via the thermodynamic cycle shown in Scheme 3, where U··· XH represents the uracil hydrogen-bonded complex and U^- represents the uracil anion. It is clear from Scheme 3 that the effect of hydrogen bonding on the acidity of uracil is directly related to the difference between the binding strengths of the small molecule to (neutral) uracil (D_e(neutral)) and the uracil anion (D_e(anion)). Additionally, the calculated variations in the geometries of the neutral and anionic complexes suggest that there exist significant differences in the binding strengths in these systems. Therefore, we consider the binding energies of the uracil and uracil anion complexes.

(i) (Neutral) Uracil Complexes with H_2O, NH_3, and HF. The calculated binding strengths (including scaled ZPVE and BSSE corrections) of water, ammonia, or hydrogen fluoride to various positions in (neutral) uracil (Scheme 1) range from 10.2 to 40.1 kJ mol^{-1} (Table 2). The hydrogen-bond strength at each site generally decreases according to HF > H_2O > NH_3. It is noted that the binding strengths of water or ammonia to uracil are very similar at O2(N3) and O4(N3) (Table 2) despite their distinct molecular properties.[52,53]

The binding strength also changes with the uracil-binding site. In agreement with previous calculations,[11,13,54] we find that the binding strength of both water and ammonia to uracil decrease as O4(N3) > O2(N3) > O4(C5). The binding strengths of the uracil−hydrogen fluoride complexes decrease according to O4-(N3) > O4(C5) > O2(N3). It is interesting to note that for each choice of X (Scheme 1) the binding is strongest at the uracil O4(N3) position. This correlates with the largest PA and acidity (Table 1) among uracil sites involved in hydrogen-bonded complexes considered in the present study (Scheme 1).

The binding strengths in complexes between uracil and two small molecules range between approximately 30−70 kJ mol^{-1} (Table 2). For the O2(N3)−O4(C5) and O4(N3)−O4(C5) complexes, which represent superpositions of the individual uracil complexes, the trends observed for one molecule interacting with uracil are still valid. For example, if NH_3 is located at O2(N3), then the binding energy increases according to HF > H_2O > NH_3 at O4(C5). Exceptions to the trend include the O4-(N3)−O4(C5) complexes with HF at O4(C5) and the O2(N3)−O4(C5) complex with water at O4(C5), where in both instances the binding strength increases according to HF > NH_3 > H_2O at the second position.

Table 2 contains a summary of the additive binding strengths for complexes considered in the present work (Scheme 2), as well as the difference between the calculated and additive binding strengths (Δ), where a positive value indicates that the

TABLE 2: Binding Energies (kJ mol^{-1}) in Uracil and Uracil (N1) Anion Complexes[a]

			neutral uracil complexes			uracil (N1) anion complexes		
O2(N3)	O4(N3)	O4(C5)		additive[b]	Δ[c]		additive[b]	Δ[c]
NH_3			22.5			19.3		
H_2O			22.4			42.6		
HF			35.6			87.1		
	NH_3		23.4			18.1		
	H_2O		24.9			40.6		
	HF		40.1			82.8		
		NH_3	10.2			19.3		
		H_2O	19.5			42.5		
		HF	37.3			84.2		
NH_3	NH_3		40.9	45.9	−5.0	35.5	37.4	−1.9
NH_3	H_2O		52.7	47.4	5.3	56.9	59.9	−3.0
NH_3	HF		72.0	62.6	9.4	99.7	102.1	−2.4
H_2O	NH_3		50.3	45.8	4.5	59.3	60.7	−1.4
H_2O	H_2O		55.6	47.3	8.3	78.5	83.2	−4.7
H_2O	HF		71.3	62.5	8.8	118.3	125.4	−7.1
HF	NH_3		68.6	59.0	9.6	104.1	105.2	−1.1
HF	H_2O		67.0	60.5	6.5	122.3	127.7	−5.4
HF	HF		64.0	75.7	−11.7	153.6	169.9	−16.3
NH_3		NH_3	32.7	32.7	0.0	35.5	38.6	−3.1
NH_3		H_2O	43.8	42.0	1.8	57.9	61.8	−3.9
NH_3		HF	54.8	59.8	−5.0	99.2	103.5	−4.3
H_2O		NH_3	33.0	32.6	0.4	60.4	61.9	−1.5
H_2O		H_2O	42.6	41.9	0.7	82.3	85.1	−2.8
H_2O		HF	61.3	59.7	1.6	122.2	126.8	−4.6
HF		NH_3	46.6	45.8	0.8	104.2	106.4	−2.2
HF		H_2O	54.8	55.1	−0.3	125.2	129.6	−4.4
HF		HF	70.2	72.9	−2.7	163.6	171.3	−7.7
	NH_3	NH_3	33.0	33.6	−0.6	34.3	37.4	−3.1
	NH_3	H_2O	42.9	42.9	0.0	56.7	60.6	−3.9
	NH_3	HF	65.7	60.7	5.0	97.1	102.3	−5.2
	H_2O	NH_3	35.4	35.1	0.3	56.2	59.9	−3.7
	H_2O	H_2O	42.8	44.4	−1.6	77.3	83.1	−5.8
	H_2O	HF	62.1	62.2	−0.1	115.5	124.8	−9.3
	HF	NH_3	49.7	50.3	−0.6	96.8	102.1	−5.3
	HF	H_2O	55.4	59.6	−4.2	113.9	125.3	−11.4
	HF	HF	69.0	77.4	−8.4	148.1	167.0	−18.9

[a] B3LYP/6-311+G(2d,p) single-point calculations were performed on the B3LYP/6-31+G(d,p) geometries. Scaled (0.9806) ZPVE and BSSE corrections included in all energies. See Schemes 1 and 2. [b] The sum of the binding strengths of the individual molecules to uracil. [c] The calculated minus the additive binding energies.

combined binding strength of the two molecules to uracil is greater than additive. For O2(N3)−O4(C5) and O4(N3)−O4(C5) complexes, the differences between calculated and additive bindings strengths (Table 3) is generally less than 8 kJ mol^{-1}, where the largest differences typically occur when HF is present.[55] This approximately represents a less than 10% difference and indicates that in most instances the binding strength is not strongly influenced by the presence of another molecule.

In summary, the hydrogen-bond strengths in (neutral) uracil complexes depend on the properties of the molecule bound to uracil and the uracil-binding sites. The hydrogen-bonding interactions with (neutral) uracil are strongest when hydrogen fluoride is present at O4(N3). The binding energies in the uracil anion complexes must be considered because the interactions of small molecules are expected to be stronger with anionic uracil, the geometries of the neutral and anionic complexes are significantly different, and the difference in the binding is directly related to the effect on the acidity.

(ii) Uracil Anion Complexes with H_2O, NH_3, and HF. The binding strength of water, ammonia, or hydrogen fluoride to the uracil anion ranges between 18.1 and 87.1 kJ mol^{-1} (Table 2). At each binding site, the binding strength decreases according to HF > H_2O > NH_3 as found for (neutral) uracil complexes.

TABLE 3: Calculated (N1) Acidity of Uracil (kJ mol^{-1}) in Complexes with Water, Ammonia, and Hydrogen Fluoride[a]

O2(N3)	O4(N3)	O4(C5)	acidity	Δ(acidity)[b]	additive[c]	Δ[d]
NH_3			1392.6	−3.2		
H_2O			1369.2	20.2		
HF			1338.0	51.4		
	NH_3		1394.8	−5.4		
	H_2O		1373.7	15.7		
	HF		1346.7	42.7		
		NH_3	1380.3	9.1		
		H_2O	1366.4	23.0		
		HF	1342.5	46.9		
NH_3	NH_3		1394.8	−5.4	−8.6	3.2
NH_3	H_2O		1385.2	4.2	12.5	−8.3
NH_3	HF		1361.8	27.6	39.5	−11.8
H_2O	NH_3		1380.4	9.0	14.8	−5.7
H_2O	H_2O		1366.5	22.9	35.9	−13.0
H_2O	HF		1342.4	47.0	62.9	−17.6
HF	NH_3		1354.0	35.4	46.0	−10.6
HF	H_2O		1334.1	55.3	67.1	−11.9
HF	HF		1299.8	89.6	94.1	−4.5
NH_3		NH_3	1386.6	2.8	5.9	−3.1
NH_3		H_2O	1375.3	14.1	19.8	−5.7
NH_3		HF	1345.0	44.4	43.6	0.8
H_2O		NH_3	1362.0	27.4	29.3	−1.9
H_2O		H_2O	1349.7	39.7	43.2	−3.5
H_2O		HF	1328.5	60.9	67.1	−6.2
HF		NH_3	1331.9	57.5	60.5	−3.0
HF		H_2O	1319.0	70.4	74.4	−4.0
HF		HF	1296.0	93.4	98.3	−4.9
	NH_3	NH_3	1388.1	1.3	3.7	−2.4
	NH_3	H_2O	1375.6	13.8	17.6	−3.8
	NH_3	HF	1358.0	31.4	41.5	−10.0
	H_2O	NH_3	1368.6	20.8	24.8	−4.0
	H_2O	H_2O	1354.9	34.5	38.7	−4.3
	H_2O	HF	1336.0	53.4	62.6	−9.2
	HF	NH_3	1342.3	47.1	51.8	−4.7
	HF	H_2O	1330.9	58.5	65.7	−7.2
	HF	HF	1310.3	79.1	89.6	−10.4

[a] B3LYP/6-311+G(2d,p) single-point calculations were performed on the B3LYP/6-31+G(d,p) geometries. Acidities include scaled (0.9806) ZPVE and BSSE corrections were added to the energy of the complexes. [b] The calculated acidity of isolated uracil (1389.4 kJ mol^{-1}) minus the calculated acidity of uracil complex. A positive value represents an increase in the acidity. [c] The sum of the effects of the individual molecules. [d] The difference between the additive and the calculated effect of two molecules, where a positive value indicates that the effects are greater than additive.

The magnitude of the decrease in the binding energy along this series ranges from 64.7 kJ mol^{-1} at O4(N3) to 67.8 kJ mol^{-1} at O2(N3), which is much larger than the decrease for the (neutral) uracil complexes along the same series. For each choice of X (Scheme 1), the binding energy decreases as O2(N3) > O4(C5) > O4(N3). The dependence of the binding energies on the binding site is very small (1.2−4.3 kJ mol^{-1}) for each molecule considered in the present study (X, Scheme 1). It should be noted that the calculated binding energies of ammonia to the O2(N3) and O4(C5) positions are equal and that the binding strengths of water at these sites vary by only 0.1 kJ mol^{-1}.

The interaction energies when two molecules bind to the uracil anion range from approximately 35 to 170 kJ mol^{-1}. Table 2 compares the calculated binding strengths to those predicted if the binding strengths are additive (Δ). The calculated binding strengths for all uracil anion complexes considered in the present study are smaller (by approximately 1−19 kJ mol^{-1}) than those predicted by additivity. The O4(N3)−O4(C5) complexes involving HF−H_2O or HF−HF show the largest deviations from additivity (11.4 and 18.9 kJ mol^{-1}, respectively). Nevertheless, these deviations represent differences from the calculated

binding energy of less than approximately 13%. Thus, our results indicate that the presence of one molecule leads to only slightly weaker binding of the second molecule to the uracil anion.

As expected, comparison of the binding strengths in Table 2 indicates that molecules bind to the uracil anion significantly stronger than to the corresponding site in (neutral) uracil. In general, the binding strength of water, ammonia or hydrogen fluoride to the uracil anion is greater than that to neutral uracil by a factor of 1.6−2.4. The largest differences occur for complexes involving hydrogen fluoride. The increases in the binding energies are similar to those previously reported for deprotonation of a uracil tautomer[22] and the formation of conventional anions.[27] In contrast, the binding energy of ammonia to the O2(N3) or O4(N3) positions is smaller in the uracil anion complexes (by 3.2 and 5.3 mol^{-1}, respectively). Simultaneous binding of two molecules is up to a factor of 2.3 stronger to the uracil anion than to (neutral) uracil. The smallest differences occur in complexes involving two ammonia molecules and the largest differences occur in complexes involving two hydrogen fluoride molecules. As discussed in the following section, the increased binding strength to the uracil anion leads to significant effects on the acidity of uracil.

Effects of Hydrogen Bonding on Uracil (N1) Acidity. Experimental and calculated gas-phase deprotonation enthalpies (Table 1) suggest that isolated uracil has a significant acidity at the N1 position. Because geometrical differences prevail when small molecules bind to the uracil anion compared with (neutral) uracil and the binding energies in these complexes are appreciably different, it is expected that hydrogen-bonding interactions at different uracil sites will significantly affect the (N1) acidity.

The B3LYP/6-311+G(2d,p) gas-phase acidity of uracil at N1 in the absence of hydrogen-bonding interactions is 1389.4 kJ mol^{-1} (Table 1). The calculated acidity changes significantly even when interactions with only one molecule are considered (Table 3). Hydrogen bonding with water, ammonia, or hydrogen fluoride changes the acidity to 1338.0−1394.8 kJ mol^{-1} depending on the molecule bound to uracil and the binding site. Interactions with a second molecule affect the acidity of uracil to an even greater extent, where the calculated acidity in these complexes ranges from 1296.0 kJ mol^{-1} (two hydrogen fluoride molecules) to 1394.8 kJ mol^{-1} (two ammonia molecules).

For a clear analysis of the effects of hydrogen bonds on the uracil (N1) acidity, Table 3 displays the change in the acidity due to the presence of hydrogen-bond interactions (Δ(Acidity)), where a positive value represents an increase in the acidity. Interactions of a single molecule at different uracil positions generally increase the acidity by 9.1−51.4 kJ mol^{-1}. However, binding of ammonia at the O2(N3) or O4(N3) position in uracil leads to a slight decrease in the acidity (by 3.2 and 5.4 kJ mol^{-1}, respectively).

The magnitude of the change in the uracil acidity decreases as HF > H_2O > NH_3 at each binding site. The variation in the acidity change along this series ranges from 37.8 kJ mol^{-1} at the O4(C5) position to 54.6 kJ mol^{-1} at O2(N3). The extent of the effect on the acidity also depends on the location of hydrogen-bonding interactions. The acidity decreases with binding of water or ammonia at O4(C5) > O2(N3) > O4(N3) and with binding of hydrogen fluoride at O2(N3) > O4(C5) > O4(N3). The smallest and largest acidities for binding H_2O, HF, and NH_3 at various uracil sites differ by 7.3, 8.7, and 14.5 kJ mol^{-1}, respectively.

Interactions between two molecules and uracil generally increase the acidity by up to 93 kJ mol^{-1}. The smallest increase (1.3 kJ mol^{-1}) occurs for the O4(N3)−O4(C5) complex with ammonia at both positions and the largest increase (93.4 kJ mol^{-1}) occurs for the O2(N3)−O4(C5) complex with hydrogen fluoride at both positions. The O2(N3)−O4(N3) complex with ammonia at both positions displays a slight decrease in the uracil acidity (by 6.9 and 0.2 kJ mol^{-1}, respectively).

Due to similarities in the geometries of many complexes regardless of the number of molecules bound to uracil, it is intriguing to consider whether the effects of two molecules on the uracil acidity are additive. For example, the effect of HF at the O2(N3) position on the uracil acidity is 51.4 kJ mol^{-1}, the effect of H_2O at O4(C5) is 23.0 kJ mol^{-1}, and therefore the additive effect of these interactions is 74.4 kJ mol^{-1}. Table 3 displays the additive effect on the acidity ("additive") and the difference between the additive effect of two molecules and the change in the acidity calculated from the optimized geometries of complexes involving both interactions (Δ), where a positive value indicates that the combined effects of two molecules are greater than additive.

For most O2(N3)−O4(C5) and O4(N3)−O4(C5) complexes, we find that the effect of two molecules is slightly less than the sum of the individual effects. Differences between the calculated effect of hydrogen-bonding interactions on the acidity and the additive effect range from 1.9 to 10.4 kJ mol^{-1}. The complex with ammonia at O2(N3) and HF at O4(C5) has a slightly greater than additive effect (by 0.8 kJ mol^{-1}). These results suggest that the change in the acidity depends only on individual interactions (additive) and that the presence of one molecule does not significantly increase or decrease the change imposed by interactions with another molecule.

In summary, our calculations indicate that hydrogen bonding to different uracil sites can significantly change the (N1) acidity. Water, ammonia, and hydrogen fluoride change the uracil acidity by varying degrees. The change in the acidity is also dependent on the molecular binding site. It should also be noted that solvation of the ions will lead to an even greater increase in the acidity of uracil, which is supported by previous calculations,[30] and may also enhance the effect of hydrogen bonding on this property.

Conclusions

Hydrogen bonds play important roles in biological systems and therefore we must strive to understand these interactions, as well as their effects on molecular properties. In the present study, we systematically consider the effects of hydrogen bonding on the (N1) acidity of uracil and the relationship between these effects and hydrogen-bond strengths in uracil complexes.

The uracil complexes considered in the present work involve water, ammonia, or hydrogen fluoride binding at O2(N3), O4(N3), and O4(C5), as well as all combinations of two molecules interacting with these uracil sites. Our calculations indicate that the geometries of complexes involving two molecules bound to uracil (or the uracil anion) represent superpositions of the individual geometries of complexes involving one molecule bound to uracil (or the uracil anion). These results indicate that the properties of uracil (or the uracil anion) do not change dramatically due to interactions with one molecule. This statement is supported by conclusions that the combined effect of two molecules on the uracil acidity and the combined binding energies of two molecules to uracil are only slightly less than the sum of the individual effects.

Our calculations indicate that the geometries of the uracil anion complexes are significantly different from the geometries

of the corresponding (neutral) uracil complexes. These variations lead to differences in the binding within the neutral and anionic complexes. Each molecule binds most strongly to uracil at the O4(N3) position, and to the uracil anion at O2(N3). As expected, the binding strength is greater in the uracil anion complex compared with the corresponding (neutral) uracil complex for all hydrogen-bonding interactions considered in the present study. The range in the binding strengths as the molecule bound to uracil varies is also greater for the anionic complexes compared with the neutral counterparts.

Although the calculated (N1) acidity of isolated uracil is significant (1389.4 kJ mol^{-1}), the difference in the binding energies of small molecules to (netural) uracil and the uracil anion leads to significant changes in the acidity of uracil. Our calculations suggest that hydrogen-bond interactions with water, ammonia, or hydrogen fluoride increase the acidity of uracil by up to 51 kJ mol^{-1} and that the effect of two molecules is approximately additive. Among the molecules considered in the present study, hydrogen fluoride leads to the largest increase in the acidity, whereas ammonia decreases the acidity when interacting with the O2(N3) and O4(N3) positions. The effects on the acidity change with the binding site, where the largest effect occurs when water or ammonia binds at the O4(C5) position or hydrogen fluoride at the O2(N3) position.

In addition to enhancing our understanding of general hydrogen-bonding interactions with natural nucleobases, the present study has implications for the mechanism of action of uracil DNA glycosylase, the enzyme responsible for removing uracil from DNA. In particular, our calculations indicate that the acidity of uracil can be significantly increased by hydrogen-bonding interactions with active site residues and provide insight into the relative importance of hydrogen bonding at different uracil sites. Additionally, our conclusion that binding of one molecule to uracil does not significantly affect the hydrogen-bonding properties of remaining uracil sites suggests that interactions between uracil (or the uracil anion) and more than one molecule at the active site may facilitate the reaction. The implications of the results from the present study to the mechanism of action of uracil DNA glycosylase will be considered in future work, which will examine larger models that more closely resemble the biochemical system.

Acknowledgment. We thank the Natural Sciences and Engineering Research Council of Canada (NSERC) for financial support. We also gratefully acknowledge the Advanced Computational Research Laboratory (ACRL) at the University of New Brunswick for grants of computer time. S.R.W. especially thanks the Chapman Fund for a summer undergraduate research grant. We thank an anonymous referee for valuable comments.

Supporting Information Available: Geometrical coordinates for the optimized (neutral) uracil and uracil anion complexes with water, ammonia and hydrogen fluoride (pdf). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) For a review of hydrogen bonding in biological systems, see: Jeffrey, G. A.; Saenger, W. *Hydrogen Bonding in Biological Structures*; Springer-Verlag: New York, 1994.

(2) Port, G. N. J.; Pullman, A. *FEBS Lett.* **1973**, *31*, 70−74.

(3) Pullman, B.; Miertus, Š.; Perahia, D.; *Theor. Chim. Acta* **1979**, *50*, 317.

(4) Del Bene, J. E. *J. Comput. Chem.* **1981**, *2*, 188.

(5) Scheiner, S. *Biopolymers* **1983**, *22*, 731.

(6) Del Bene, J. E. *J. Comput. Chem.* **1983**, *4*, 226.

(7) Sagarik, K.; Corongiu, G.; Clementi, E. *J. Mol. Struct. (THEOCHEM)* **1991**, *81*, 355.

(8) Rybak, S.; Szalewicz, K.; Jeziorski, B.; Corongiu, G. *Chem. Phys. Lett.* **1992**, *199*, 567−573.

(9) Ghomi, M.; Aamouche, A.; Cadioli, B.; Berthier, G.; Grajcar, L.; Baron, M. H. *J. Mol. Struct.* **1997**, *410*, 323−326.

(10) Aamouche, A.; Berthier, G.; Cadioli, B.; Gallinella, E.; Ghomi, M. *J. Mol. Struct. (THEOCHEM)* **1998**, *426*, 307−312.

(11) Nguyen, M. T.; Chandra, A. K.; Zeegers-Huyskens, T. *J. Chem. Soc., Faraday Trans.* **1998**, *94*, 1277−1280.

(12) Chandra, A. K.; Nguyen, M. T.; Zeegers-Huyskens, T. *J. Phys. Chem. A* **1998**, *102*, 6010−6016.

(13) Chandra, A. K.; Nguyen, M. T.; Uchimaru, T.; Zeegers-Huyskens, T. *J. Phys. Chem. A* **1999**, *103*, 8853−8860.

(14) van Mourik, T.; Price, S. L.; Clary, D. C. *J. Phys. Chem. A* **1999**, *103*, 1611−1618.

(15) Bencivenni, L.; Ramondo, F.; Pieretti, A.; Sanna, N. *J. Chem. Soc., Perkin Trans. 2* **2000**, 1685−1693.

(16) Gadre, S. R.; Babu, K.; Rendell, A. P. *J. Phys. Chem. A* **2000**, *104*, 8976−8982.

(17) van Mourik, T.; Benoit, D. M.; Price, S. L.; Clary, D. C. *Phys. Chem. Chem. Phys.* **2000**, *2*, 1281−1290.

(18) Shishkin, O. V.; Gorb, L.; Leszczynski, J. *Int. J. Mol. Sci.* **2000**, *1*, 17−27.

(19) van Mourik, T. *Phys. Chem. Chem. Phys.* **2001**, *3*, 2886−2892.

(20) Gaigeot, M.-P.; Kadri, C.; Ghomi, M. *J. Mol. Struct. (THEOCHEM)* **2001**, *565*−*566*, 469−473.

(21) Gaigeot, M. P.; Ghomi, M. *J. Phys. Chem. B* **2001**, *105*, 5007−5017.

(22) Kryachko, E.; Nguyen, M. T.; Zeegers-Huyskens, T. *J. Phys. Chem. A* **2001**, *105*, 1934−1943.

(23) Chandra, A. K.; Uchimaru, T.; Zeegers-Huyskens, T. *J. Mol. Struct.* **2002**, *605*, 213−220.

(24) Kryachko, E.; Nguyen, M. T.; Zeegers-Huyskens, T. *J. Phys. Chem. A* **2001**, *105*, 3379−3387.

(25) Shishkin, O. V.; Sukhanov, O. S.; Leszczynski, J. *J. Phys. Chem. A* **2002**, *106*, 7828−7833.

(26) (a) Smets, J.; McCarthy, W. J.; Adamowicz, L. *J. Phys. Chem.* **1996**, *100*, 14655−14660. (b) Smets, J.; Smith, D. M. A.; Elkadi, Y.; Adamowicz L. *J. Phys. Chem. A.* **1997**, *101*, 9152−9156.

(27) Dolgounitcheva, O.; Zakrzewski, V. G.; Ortiz, J. V. *J. Phys. Chem. A* **1999**, *103*, 7912−7917.

(28) For general reviews on the mechanism of action of DNA glycosylases, see, for example: (a) Dodson, M. L.; Michaels, M. L.; Lloyd, R. S. *J. Biol. Chem.* **1994**, *269*, 32709−32712. (b) McCullough, A. K.; Dodson, M. L.; Lloyd, R. S. *Annu. Rev. Biochem.* **1999**, *68*, 255−285. (c) Stivers, J. T.; Drohat, A. C. *Arch. Biochem. Biophys.* **2001**, *396*, 1−9.

(29) See, for example: (a) Savva, R.; McAuley-Hecht, K.; Brown, T.; Pearl, L. *Nature* **1995**, *373*, 487−493. (b) Kimura, E.; Kitamura, H.; Koike, T.; Shiro, M. *J. Am. Chem. Soc.* **1997**, *119*, 10909−10919. (c) Parikh, S. S.; Walcher, G.; Jones, G. D.; Slupphaug, G.; Krokan, H. E.; Blackburn, G. M.; Tainer, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 5083−5088. (d) Werner, R. M.; Jiang, Y. L.; Gordley, R. G.; Jagadeesh, G. J.; Ladner, J. E.; Xiao, G.; Tordova, M.; Gilliland, G. L.; Stivers, J. T. *Biochemistry* **2000**, *39*, 12585−12594. (e) Werner, R. M.; Stivers, J. T. *Biochemistry* **2000**, *39*, 14054−14064. (f) Dinner, A. R.; Blackburn, G. M.; Karplus, M. *Nature* **2001**, *412*, 752−755. (g) Jiang, Y. L.; Ichikawa, Y.; Stivers, J. T. *Biochemistry* **2002**, *41*, 7116−7124. (h) Jiang, Y. L.; Drohat, A. C.; Ichikawa, Y.; Stivers, J. T. *J. Biol. Chem.* **2002**, *277*, 15385−15392 and references therein.

(30) Kurinovich, M. A.; Lee, J. K. *J. Am. Chem. Soc.* **2000**, *122*, 6258−6262.

(31) Kurinovich, M. A.; Lee, J. K. *J. Am. Soc. Mass. Spectrom.* **2002**, *13*, 985−995.

(32) Drohat, A. C.; Stivers, J. T. *J. Am. Chem. Soc.* **2000**, *122*, 1840−1841.

(33) Latajka, Z.; Ratajczak, H.; Zeegers-Huyskens, T.; Scheiner, S. *J. Mol. Struct. (THEOCHEM)* **1991**, *235*, 409−415.

(34) (a) Dabkowska, I.; Rak, J.; Gutowski, M. *J. Phys. Chem. A* **2002**, *106*, 7423−7433. (b) Dabkowska, I.; Gutowski, M.; Rak, J. *Polish J. Chem.* **2002**, *76*, 1243−1247.

(35) Jalbout, A. F.; Hall-Black, C. S.; Adamowicz, L. *Chem. Phys. Lett.* **2002**, *354*, 128−133.

(36) Gutowski, M.; Dąbkowska, I.; Rak, J.; Xu, S.; Nilles, J. M.; Radisic, D.; Bowen, K. H., Jr. *Eur. Phys. J. D* **2002**, *20*, 431−439.

(37) Harańczyk, M.; Bachorz, R.; Rak, J.; Gutowski, M.; Radisic, D.; Stockes, S. T.; Nilles, J. M.; Bowen, K. M., Jr. *J. Phys. Chem. B* **2003**, *107*, 7889−7895.

(38) The B3LYP/6-311+G(3df,2p) binding strength including (scaled) ZPVE and BSSE is equal to 21.8 kJ mol^{-1} for uracil−NH_3, 21.6 kJ mol^{-1} for uracil−H_2O, and 35.1 kJ mol^{-1} for uracil−HF. The corresponding B3LYP/6-311+G(2d,p) values are 22.5, 22.4, and 35.6 kJ mol^{-1}, respectively.

(39) Boys, S. F.; Bernardi, F. *Mol. Phys.* **1970**, 553.

(40) See, for example: (a) Hobza, P.; Sponer, J.; Reschel, T. *J. Comput. Chem.* **1995**, *16*, 1315. (b) Del Bene, J. E.; Person, W. B.; Szczepaniak, K. *J. Phys. Chem.* **1995**, *99*, 10705. (c) Civalleri, B.; Garrone, E.; Ugliengo, P. *J. Mol. Struct. (THEOCHEM)* **1997**, *419*, 227. (d) Maerker, C.; von Ragué Schleyer, P.; Leidl, K. R.; Ha, T.-K.; Quack, M.; Suhm, M. A. *J. Comput. Chem.* **1997**, *18*, 1695. (e) Paizs, B.; Suhai, S. *J. Comput. Chem.* **1998**, *19*, 575. (f) Rappé, A. K.; Bernstein, E. R. *J. Phys. Chem. A* **2000**, *104*, 6117.

(41) See, for example: (a) Sim, F.; St-Amant, A.; Pápai, I.; Salahub, D. R. *J. Am. Chem. Soc.* **1992**, *114*, 4391. (b) Keininger, M.; Suhai, S. *Int. J. Quantum Chem.* **1994**, *52*, 465. (c) Kim, K.; Jordan, K. D. *J. Phys. Chem.* **1994**, *98*, 10089. (d) Latajka, Z.; Bouteiller, Y. *J. Chem. Phys.* **1994**, *101*, 9793. (e) Mele, F.; Mineva, T.; Russo, N. Toscano, M. *Theor. Chim. Acta* **1995**, *91*, 169. (f) Han, W.-G.; Suhai, *J. Phys. Chem.* **1996**, *100*, 3942. (g) Lozynski, M.; Rusinska-Roszak, D.; Mack, H.-G. *J. Phys. Chem. A* **1998**, *102*, 2899. (h) Lundell, J.; Latajka, Z. *J. Phys. Chem. A* **1997**, *101*, 5004. (i) Chandra, A. K.; Nguyen, M. T. *J. Chem. Res.* **1997**, 216. (j) Chandra, A. K.; Nguyen, M. T. *Chem. Phys.* **1998**, *232*, 299. (k) McAllister, M. A. *J. Mol. Struct. (THEOCHEM)* **1998**, *427*, 39. (l) Rablen, P. R.; Lockman, J. W.; Jorgensen, W. L. *J. Phys. Chem. A* **1998**, *102*, 3782. (m) Pan, Y.; McAllister, M. A. *J. Mol. Struct. (THEOCHEM)* **1998**, *427*, 221

(42) Lundell, J.; Latajka, Z. *J. Phys. Chem. A* **1997**, *101*, 5004.

(43) (a) Hartmann, M.; Radom, L. *J. Phys. Chem.* **2000**, *104*, 968. (b) Hartmann, M.; Wetmore, S. D.; Radom, L. *J. Phys. Chem. A* **2001**, *115*, 4470−4479. (c) Wetmore, S. D.; Schofield, R.; Smith, D. M.; Radom, L. *J. Phys. Chem. A* **2001**, *105*, 8718−8726.

(44) Sponer, J.; Leszczynski, J. *J. Phys. Chem.* **1996**, *100*, 1965.

(45) We note that calculations suggest a proton adds to the O2 or O4 position of uracil in the molecular plane and that the proton will align in one of two directions at each site (for example, addition to the O2 position can be directed towards N1 or N3).

(46) Podolyan, Y.; Gorb, L.; Leszczynski, J. *J. Phys. Chem. A* **2000**, *104*, 7346−7352.

(47) We also note that in DNA the hydrogen at N1 is replaced by a sugar moiety. Therefore, the O2(N1) complex is not possible if interactions with DNA, such as those at the active site of uracil DNA glycosylase, are being considered, which is one of the driving forces for the present study. Furthermore, hydrogen bonding at O2(N1) will likely greatly stabilize the uracil (N1) anion due to direct interactions with N1 and thus prevent a fair comparison of the effects of hydrogen bonding at different uracil sites.

(48) For earlier computational and experimental discussions, see, for example: (a) D'Albis, A.; Wickens, M. P.; Gratzer, W. B. *Biopolymers* **1975**, *14*, 1423. (b) Scheiner, S. *Biopolymers* **1983**, *22*, 731. (c) Falk, M.; Hartmann, K. A.; Lord, R. C. *J. Am. Chem. Soc.* **1983**, *85*, 387. (c) Spencer, J. N.; Barton, S. W.; Smith, W. S.; Wolbach, J. F.; Powell, J. F.; Kirshenbaum, M. R.; Firth, D. W.; Harris, E. M.; Judge, T. A. *Can. J. Chem.* **1983**, *61*, 2695. (d) Kasende, O.; Zeegers-Huyskens, T. *J. Phys. Chem.* **1984**, *88*, 2636. (e) Pranata, J.; Wierschke, S. G.; Jorgensen, W. J. *J. Am. Chem. Soc.* **1991**, *113*, 2810.

(49) Russo, N.; Toscano, M.; Grand, A.; Jolibois, F. *J. Comput. Chem.* **1998**, *19*, 989.

(50) Kurinovich, M. A.; Phillips, L. M.; Sharma, S.; Lee, J. K. *Chem. Commun.* **2002**, 2354−2355.

(51) Gronert, S.; Feng, W. Y.; Chew, F.; Wu, W. *Int. J. Mass Spectrom.* **2000**, *196*, 251−258.

(52) B3LYP/6-311+G(2d,p) acidities decrease according to HF (1606.8 kJ mol^{-1}) > H_2O (1678.2 kJ mol^{-1}) > NH_3 (1725.5 kJ mol^{-1}).

(53) B3LYP/6-311+G(2d,p) proton affinities decrease according to NH_3 (806.5 kJ mol^{-1}) > H_2O (630.4 kJ mol^{-1}) > HF (407.3 kJ mol^{-1}).

(54) We note that Zeegers-Huyskens et al. investigated the O2(N1) complex rather than the O4(C5) complex. It has been widely concluded that the strongest binding to uracil occurs at O2(N1).

(55) Because the O2(N3)−O4(N3) structures are not simply an overlay of the two complexes, the calculated interaction energy is generally greater than the additive effect (by 9−18%) due to extra intermolecular interactions. We note that the O2(N3)−O4(N3) complex with hydrogen fluoride at both positions represents a superposition of the individual structures and the calculated binding energy is slightly less than additive (by 11.7 kJ mol^{-1}), which is likely due to competing interactions with the N3 hydrogen.

(56) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Rega, N.; Salvador, P.; Dannenberg, J. J.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, revision A.11.3; Gaussian, Inc.: Pittsburgh, PA, 2002.