The Acidity of Uracil from the Gas Phase to Solution: The Coalescence of the N1 and N3 Sites and Implications for Biological Glycosylation

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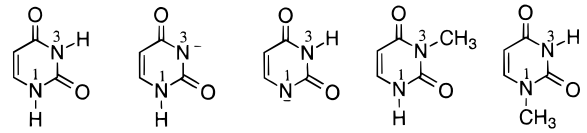
Abstract: The gas-phase acidities of the N1 and N3 sites of uracil have been bracketed to provide an understanding of the intrinsic reactivity of this nucleic base. The experiments indicate that in the gas phase, the N3 site is far less acidic ($\Delta H_{acid} = 347 \pm 4$ kcal mol^{-1}) than the N1 site ($\Delta H_{acid} = 333 \pm 4$ kcal mol^{-1}), in direct contrast to in solution, where the two sites are so close in acidity as to be unresolvable. This intrinsic difference and the coalescence in solution is interpreted through gas-phase and dielectric-medium calculations. The results point to a possible chemical reason that N1 is the preferred glycosylation site in nature: nature may simply take advantage of the differential N1 and N3 acidities in a nonpolar environment to achieve selectivity.

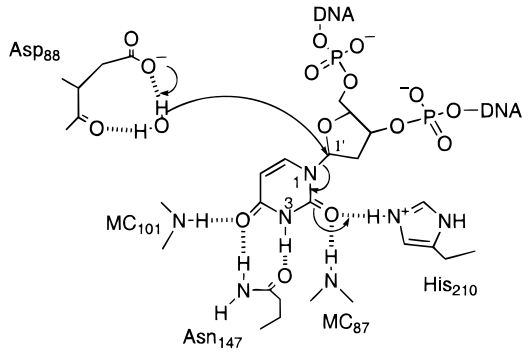
The acidity of the two NH bonds in uracil (**1**) impinges on issues ranging from the biological to the chemical. Biologically, the NH bonds affect aspects from hydrogen bonding capabilities to the activity of enzymes for which uracil is a substrate.[1,2]



1 **2** **3** **4** **5**

While the gas-phase basicity of uracil's carbonyl groups has been studied experimentally and computationally, there are no experimental data on the *gas phase acidity* of the two NH bonds in uracil, and only recently have acidity calculations been conducted.[3−8] The intrinsic, gas-phase acidities are of interest for purely chemical reasons, but also could be of importance for biological reasons, since biological environs are often relatively nonpolar in nature.[9] Furthermore, hydrogen bonding modulates recognition of DNA and RNA bases, and the interaction energy between two complementary nucleobases that are held together by NH−O and NH−N hydrogen bonds is dependent on the intrinsic basicity of the acceptor atoms as well as on the acidity of the NH donor groups.[3,10] Gas-phase acidities of the bases are unknown and comparison of those acidities to solution values will yield valuable information on intrinsic base reactivity and the role of solvent in affecting base reactivity. In essence, gas-phase experiments can provide the link between calculations and condensed-phase data.

Scheme 1



Our interest in the acidity of uracil is also related to the mechanism of uracil-DNA glycosylase (UDGase).[1,2] UDGase cleaves uracil from DNA in an essential genome-protecting reaction.[11−14] Uracil in place of thymine in DNA can significantly disrupt specific protein binding and must be removed.[1] The proposed mechanism for uracil excision by UDGase involves nucleophilic attack by some form of activated water at C1′ (Scheme 1). This prompts an immediate question: How good a leaving group is uracil N1$^-$? Kimura et al. have explored the possibility of activating uracil to facilitate departure,[15] and

(1) Savva, R.; McAuley-Hecht, K.; Brown, T.; Pearl, L. *Nature* **1995**, *373*, 487−493.
(2) Dianov, G.; Sedgwick, B.; Daly, G.; Olsson, M.; Lovett, S.; Lindahl, T. *Nucleic Acids Res.* **1994**, *22*, 993−998.
(3) Nguyen, M. T.; Chandra, A. K.; Zeegers-Huyskens, T. *J. Chem. Soc., Faraday Trans.* **1998**, *94*, 1277−1280.
(4) Meot-Ner (Mautner), M. *J. Am. Chem. Soc.* **1979**, *101*, 2396−2403.
(5) Greco, F.; Liguori, A.; Sindona, G.; Uccella, N. *J. Am. Chem. Soc.* **1990**, *112*, 9092−9096.
(6) Green-Church, K. B.; Limbach, P. A. *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 24−32.
(7) Wilson, M. S.; McCloskey, J. A. *J. Am. Chem. Soc.* **1975**, *97*, 3436−3444.
(8) *NIST Chemistry WebBook, NIST Standard Reference Database Number 69, November 1998*; Mallard, W. G., Linstrom, P. J., Eds.; National Institute of Standards and Technology: Gaithersburg, MD, 1998; Vol. http://webbook.nist.gov.
(9) Simonson, T.; Brooks, C. L., III *J. Am. Chem. Soc.* **1996**, *118*, 8452−8458.
(10) Chandra, A. K.; Nguyen, M. T.; Uchimaru, T.; Zeegers-Huyskens, T. *J. Phys. Chem. A* **1999**, *103*, 8853−8860.
(11) Shroyer, M. J. N.; Bennett, S. E.; Putnam, C. D.; Tainer, J. A.; Mosbaugh, D. W. *Biochemistry* **1999**, *38*, 4834−4845.
(12) Drohat, A. C.; Xiao, G.; Tordova, M.; Jagadeesh, J.; Pankiewicz, K. W.; Watanabe, K. A.; Gilliland, G. L.; Stivers, J. T. *Biochemistry* **1999**, *38*, 11876−11886.
(13) Luo, N.; Mehler, E.; Osman, R. *Biochemistry* **1999**, *38*, 9209−9220.
(14) Drohat, A. C.; Jagadeesh, J.; Ferguson, E.; Stivers, J. T. *Biochemistry* **1999**, *38*, 11866−11875.

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we have conducted computational studies of different activation mechanisms.[16] These studies led to a more fundamental question having to do with uracil N1 and N3 acidities: Why does nature choose N1 for glycosylation? The condensed phase pK_a values of the N1 and N3 sites are not differentiable; Nakanishi et al. have shown that uracil deprotonates with a pK_a value of 9.5 to form the N3$^-$ (**2**) species, which is in equilibrium with the N1$^-$ (**3**) species in a 1:1 ratio. (Throughout this paper, we will refer to uracil deprotonated at N1 as the N1$^-$ ion and uracil deprotonated at N3 as the N3$^-$ ion.) 3-Methyluracil (**4**) has a higher pK_a (10.0) than 1-methyluracil (**5**) (9.8), implying that the N3 site in uracil might be slightly more acidic. Why then is the N1 site the glycosylated position?[17,18] The N3 and N1 sites are both readily alkylated in chemical reactions, but what happens in an enzyme active site?[15] We thus became interested in pursuing the intrinsic acidity of uracil in the ultimate nonpolar environment, the gas phase. The experimental gas-phase acidity of uracil has until now not been determined. Intriguingly, despite the proximity of pK_a values for the uracil N1 and N3 sites in solution, our calculations, as well as those from others, predict that the gas-phase N1 and N3 acidities should be separated by more than 10 kcal mol^{-1}. In this paper, we describe the experimental determination of the acidity of the N1 and N3 sites of uracil and the biological and chemical implications of our results.

Experimental Section

All experiments were conducted on a dual-cell Finnigan 2001 Fourier transform mass spectrometer (FTMS). Each side of the 2 in. cubic dual cell is pumped down to a baseline pressure of less than 1×10^{-9} Torr. The dual cell is positioned collinearly with the magnetic field produced by a 3.3 T superconducting magnet.

Neutral samples were introduced into the FTMS using a Finnigan heated batch inlet system, a home-built heated batch inlet system, via a pulsed valve system, or a heated solids probe. All chemicals were available commercially and were used as received. Most ions were produced by proton transfer to hydroxide. Hydroxide was generated by pulsing water into the cell and sending an electron beam (typically 6 eV, 8 μA, beam time 5 ms) through the center of the cell. A trapping potential of -2 V was applied to the cell walls perpendicular to the magnetic field at all times except when ions were transferred from one cell to another. Transfer is accomplished by temporarily grounding (50−150 μs) the conductance limit plate, the trapping plate separating the two cells. The ions then can pass through a 2 mm hole in the center of the conductance limit plate. Transferred ions were cooled with argon.[19,20]

Acidity bracketing was utilized to measure the gas-phase acidities. Species of known acidities are allowed to react with the anion of unknown acidity. The ability of the anion of unknown acidity to deprotonate relatively stronger acids and the inability of the anion to deprotonate weaker acids (stronger bases) allow one to bracket the acidity of the unknown. Also, for the N1 site of uracil and the N3 site of 1-methyluracil, the conjugate bases of the reference acids were used to deprotonate the unknown.[8,21]

Calculations were conducted at B3LYP/6-31+G* using Gaussian94 and Gaussian98.[22,23] Medium effects were calculated using the SCI-PCM method.[22]

Table 1. Calculated ΔH_{acid} Values of the N1 and N3 Sites of Uracil, the N3 Site of 1-Methyluracil, and the N1 Site of 3-Methyluracil, in kcal mol^{-1}

method	N1, uracil	N3, uracil	N1, 3-methyl-uracil	N3, 1-methyl-uracil
B3LYP/6-31+G* [a]	329	343	332	344
B3LYP/6-31++G** [b]	332	346		

[a] From this work. Calculations are at 0 K and include zero-point vibrational energies. [b] From ref 3.

Table 2. Summary of Results of Proton Transfer from Reference Acids and Bases to Uracil N1

reference compd	ΔH_{acid}[a]	proton transfer[b]	
		ref acid	conjugate base
HCOOH	345.3 ± 2.2	−	+
$CH_3COCH_2COCH_3$	343.8 ± 2.1	−	+
$CH_3CH_2OCH_2COOH$	342.0 ± 2.2	−	+
m-CF_3PhOH	339.3 ± 2.1	−	+
$CH_3CHClCOOH$	337.0 ± 2.1	−	+
$CH_3COCOOH$	333.5 ± 2.9	+	+
HCl	333.4 ± 0.1	+	+
CHF_2COOH	331.0 ± 2.1	+	−
$CHCl_2COOH$	328.4 ± 2.1	+	−

[a] Acidities are in kcal mol^{-1} and come from ref 8. [b] A "+" indicates the occurrence and a "−" denotes the absence of proton transfer.

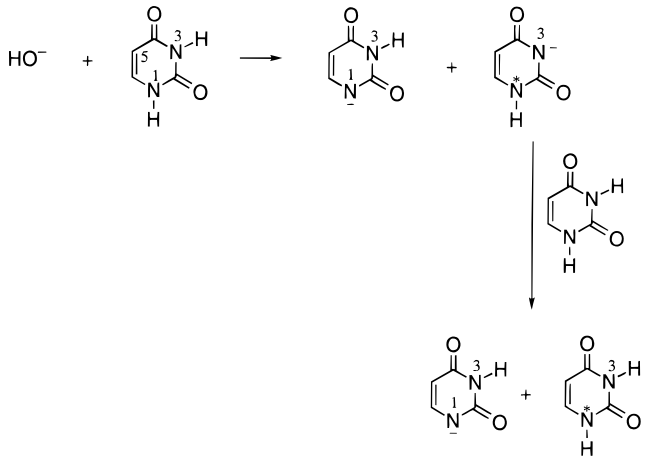
Results

The results of our acidity calculations at B3LYP/6-31+G* for the N1 and N3 sites of uracil, for 3-methyluracil, and for 1-methyluracil are shown in Table 1. Also listed are previous calculations at B3LYP/6-31++G** conducted by Zeegers-Huyskens *et al.*[3] The acidity values for a given site on uracil at each level are in agreement to within 3 kcal mol^{-1}. Also, the difference between the N1 and N3 sites is the same at both levels: the N3 site is predicted to be less acidic than the N1 by 14 kcal mol^{-1}. The acidity of 3-methyluracil, which by dint of its structure (**4**) can only deprotonate at N1, is predicted to be within 3 kcal mol^{-1} of the N1 site of uracil, and the acidity of 1-methyluracil (**5**) is calculated to be within 1 kcal mol^{-1} of the predicted acidity of the N3 site of uracil.

Our experimental results in bracketing the proton affinity of the uracil N1$^-$ ion are shown in Table 2. We find that Cl$^-$ (ΔH_{acid}(HCl) = 333.3 kcal mol^{-1}) and pyruvate (ΔH_{acid}(pyruvic acid, $C_3H_4O_3$) = 333.5 kcal mol^{-1}) both deprotonate uracil, while 1,1-difluoroacetate (ΔH_{acid}(difluoroacetic acid, $C_2H_2F_2O_2$) = 331.0 kcal mol^{-1}) does not. Furthermore, the uracil N1$^-$ ion also deprotonates pyruvic acid and hydrochloric acid, implying close-to-thermoneutral reactions, since the reactions proceed in

(15) Kimura, E.; Kitamura, H.; Koike, T.; Shiro, M. *J. Am. Chem. Soc.* **1997**, *119*, 10909−10919.
(16) Kurinovich, M. A.; Lee, J. K. Unpublished results.
(17) Jones, M. E. *Annu. Rev. Biochem.* **1980**, *49*, 253−79.
(18) Voet, D.; Voet, J. G. *Biochemistry*, 2nd ed.; John Wiley & Sons: New York, 1995.
(19) Amster, I. J. *J. Mass. Spectrom.* **1996**, *31*, 1325−1337 and references therein.
(20) Marshall, A. G.; Grosshans, P. B. *Anal. Chem.* **1991**, *63*, 215A−229A and references therein.
(21) *Advances in Gas-Phase Ion Chemistry*; JAI Press Inc.: Greenwich, CT, 1992; Vol. 1.
(22) GAUSSIAN94, Revision E.2; Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. Gaussian, Inc.: Pittsburgh, PA, 1995.
(23) GAUSSIAN98; 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.; 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.; Gonzalez, C.; 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, Inc.: Pittsburgh, PA, 1998.

Scheme 2



both directions. We therefore bracket the ΔH_{acid} of the N1 position of uracil to be 333 ± 4 kcal mol^{-1}.

The N1$^-$ ion of uracil was formed via the reaction of hydroxide with neutral uracil. Hydroxide has a proton affinity of 390.7 kcal mol^{-1};[8] our calculations place the ΔH_{acid} values for the N1 and N3 sites of uracil well below that value, and therefore hydroxide should be basic enough to deprotonate both the N1 and N3 sites. We believe, however, that the reaction of hydroxide with uracil under our standard conditions results in solely N1$^-$ ion. First, we unambiguously bracket *one* ΔH_{acid} at 333 kcal mol^{-1}, which is in agreement with the ΔH_{acid} predicted by calculations (329 kcal mol^{-1} at B3LYP/6-31+G* and 332 kcal mol^{-1} at B3LYP/6-31++G**). Second, we believe that any N3$^-$ formed will undergo uracil-catalyzed isomerization to yield N1$^-$ ion (Scheme 2). This type of isomerization between sites of differing acidity on the same molecule has precedence in the reaction of hydroxide with acetic acid in the gas phase.[24] Like uracil, acetic acid has two sites of differing acidity, the carbon and the oxygen sites. Formation of both the enolate and acetate ions in the gas phase in the presence of neutral acetic acid results in fast acid-catalyzed isomerization to acetate. Likewise, we believe that neutral uracil catalyzes the isomerization of any present N3$^-$ ion to N1$^-$ ion under our normal experimental conditions.

Toward the end of establishing the presence of the isomerization and of bracketing the acidity of the N3 site, we performed the deprotonation of uracil under conditions that would allow the N3$^-$ to be sustained: that is, we remove the N1$^-$/N3$^-$ mixture from the neutral uracil environment as quickly as possible. First, we allow hydroxide to deprotonate uracil, presumably at N1 *and* at N3; then we immediately transfer the ions to our second cell, which is free of uracil neutral. We then allowed the M − 1 of uracil (*m*/*z* 111) to react with reference acids. Our results are summarized in Table 3. We find that while the N3$^-$ ion of uracil appears to deprotonate formic acid (ΔH_{acid}-(CH_2O_2) = 345.3 kcal mol^{-1}), the N3$^-$ does not deprotonate acetic acid (ΔH_{acid}($C_2H_4O_2$)= 348.1 kcal mol^{-1}). We therefore estimate the ΔH_{acid} of the N3 site of uracil to be 347 ± 4 kcal mol^{-1}. It should also be noted that bracketing a less acidic site in a molecule with two acidic sites can be tricky and our estimate is a lower limit. The difficulty arises from the fact that the conjugate base of the reference acid can deprotonate the more acidic N1 site (pathway B, Scheme 3). Thus, the lack of the presence of the conjugate base of the reference acid may be a result of how ion−molecule complex **6** partitions, not of whether proton transfer has occurred.

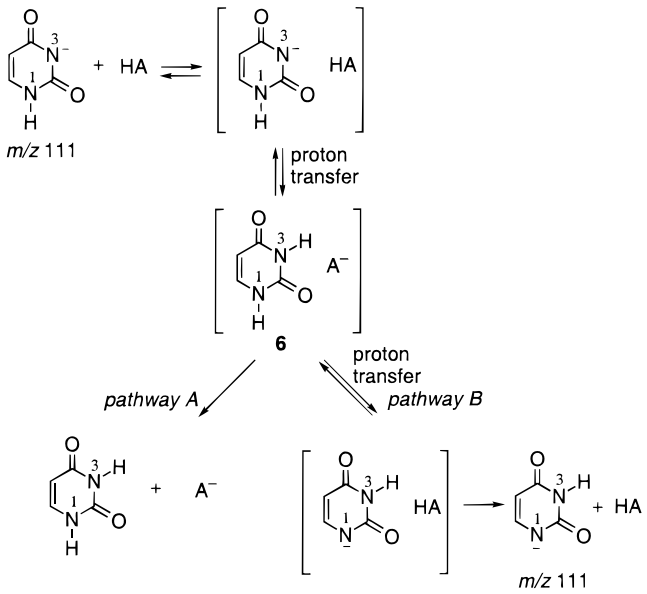
(24) Grabowski, J. J.; Cheng, X. *J. Am. Chem. Soc.* **1989**, *111*, 3106−3108.

Table 3. Summary of Results of Proton Transfer from Reference Acids and Bases to Uracil N3

reference compd	ΔH_{acid}[a]	proton transfer[b] ref acid
$CH_3CHCHCHO$	354.7 ± 2.1	−
m-CH_3PhOH	349.6 ± 2.1	−
CH_3COOH	348.1 ± 2.2	−
HCOOH	345.3 ± 2.9	+
$CH_3COCH_2COCH_3$	343.8 ± 2.1	+
m-CF_3PhOH	339.3 ± 2.1	+

[a] Acidities are in kcal mol^{-1} and come from ref 8. [b] A "+" indicates the occurrence and a "−" denotes the absence of proton transfer.

Scheme 3

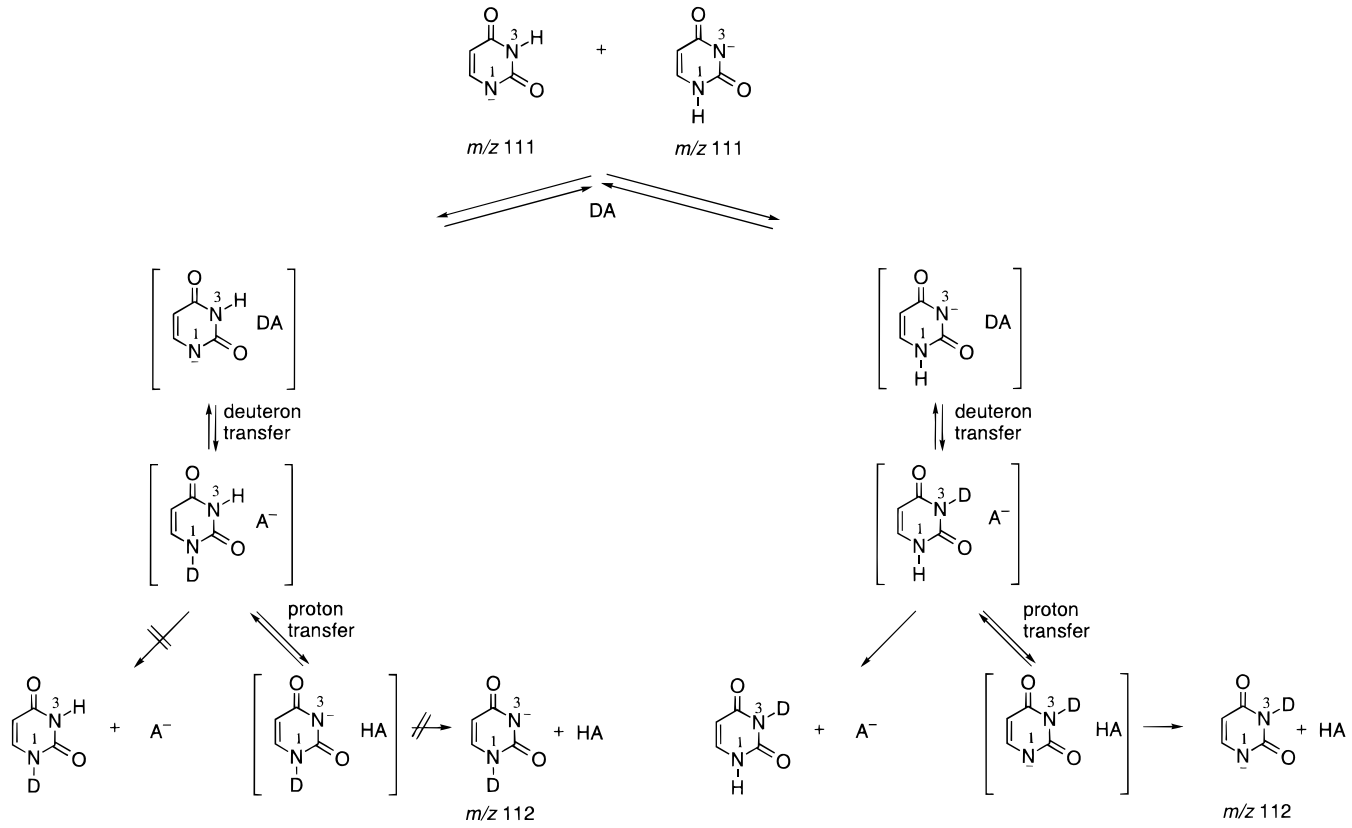


In an effort to establish further that we are producing and bracketing the N3$^-$ ion of uracil, we also conducted the acidity bracketing experiments described in Table 3 under conditions in which we expect N1$^-$ only. We find that under these conditions, the M − 1 of uracil, *m*/*z* 111, is unable to deprotonate those acids listed in Table 3.

Our final experiment toward establishing the presence of the more reactive N3$^-$ ion was to allow the N1$^-$/N3$^-$ ion mixture to react with DCOOD (ΔH_{acid} ∼ 345 kcal mol^{-1}). The scheme by which uracil N1$^-$ and N3$^-$ ions react with deuterated formic acid is shown in Scheme 4. Because formic acid has an acidity between that of the N1−H and the N3−H of uracil, one can expect the reaction of N3$^-$ with the deuterated acid to result not only in deprotonation but also in exchange (to form *m*/*z* 112). Because of the high acidity of the N1−H, the N1$^-$ ion of uracil will *not* produce *m*/*z* 112. Reaction of DCOOD indeed results in the appearance of *m*/*z* 112, as well as a peak at *m*/*z* 46 (DCOO$^-$), indicating proton transfer. Therefore, this experiment indicates the presence of a more reactive anion than N1$^-$.

We have also conducted studies on 1-methyluracil (**5**). Unlike in uracil, deprotonation can occur only at N3. Our calculations indicate that the ΔH_{acid} of this site should be near that of the N3 site of the parent uracil, at 344 kcal mol^{-1}. We find that the N3$^-$ ion of 1-methyluracil deprotonates acetic acid (ΔH_{acid}-($C_2H_4O_2$) = 348.1 ± 2.2 kcal mol^{-1}) in a fast reaction; acetate also deprotonates 1-methyluracil in a similarly fast reaction. Formate (ΔH_{acid}(CH_2O_2) = 345.3 ± 2.9 kcal mol^{-1}) deprotonates 1-methyluracil very slowly, while proton transfer between

Scheme 4



the $N3^-$ ion of 1-methyluracil and formic acid is fast. The $N3^-$ ion of 1-methyluracil does not deprotonate 4-trifluoroaniline ($\Delta H_{acid}(C_7H_6NF_3)$ = 353.3 kcal mol^{-1}). These results are consistent with the N3 site of 1-methyluracil having an acidity around that of formic and acetic acids, in the 342−350 kcal mol^{-1} range, which is consistent with the bracketed acidity of the N3 site of uracil.

Discussion

Our results indicate that the ΔH_{acid} of the N1 site of uracil is 333 kcal mol^{-1} while the N3 site has a ΔH_{acid} of 347 kcal mol^{-1}. These measurements are closest to the values predicted by Zeegers-Huyskens *et al.* at B3LYP/6-31++G** (332 and 346 kcal mol^{-1}, respectively), thus validating that computational method and level.

Recently, Gronert and co-workers bracketed the ΔH_{acid} of the C6 vinylic site of 1,3-dimethyluracil, at a surprisingly acidic 369.9 kcal mol^{-1}. Calculations by the same authors also estimate the C5 vinylic site to have a ΔH_{acid} of 378 kcal mol^{-1}.[25−27] Under our conditions, we do not appear to bracket the C5 or the C6 anion, since we do not see proton transfer between the $[M - 1]^-$ ion of uracil and any acid less acidic than formic acid. However, as noted earlier and as depicted in Scheme 3, the more reactive sites can be tricky to bracket because of the manner in which complex **6** partitions. There are two main possibilities regarding why we do not see $C5^-$ or $C6^-$ reactivity. First, under our conditions, it is possible that the C5 and C6 vinylic ions are isomerizing to $N3^-$ and $N1^-$ before transfer. The second possibility is that $N3^-$ reacts mostly via pathway A in Scheme 3, whereas the $C5^-$ and $C6^-$ ions react primarily via pathway B and therefore cannot be bracketed. One possible reason for this difference in behavior is that after the $N3^-$ accepts a proton, the resultant conjugate base may not be mobile enough to move easily past the 2-carbonyl and around the ring and deprotonate the N1 site (that is, pathway B could be somewhat suppressed). This mode of behavior has been observed in the reaction of deuterated reagents with *p*-difluorophenyl anions.[28] The presence of both $DCOO^-$ (*m*/*z* 46) and deuterated, deprotonated uracil (*m*/*z* 112) in our $N3^-$/DCOOD experiments indicates that, at least for $DCOO^-$, the ion is mobile enough such that both pathways A *and* B are allowed. The C5 and C6 sites, however, would not have any mobility issue in terms of deprotonating at N1, and therefore, pathway B might *prevail* and bracketing would be impossible for those sites. Given these limitations, we can conclude that we have bracketed a site with an acidity close to 347 kcal mol^{-1}, which must be the N3 site.

Why do the N1 and N3 sites have such different relative acidities in the gas phase and in solution? In an effort to "draw a line" from solution to the gas phase, we conducted dielectric medium calculations on the N1 and N3 acidities to ascertain how acidities change with medium dielectric (Table 4). We find that while the $N1^-$ ion is stabilized by 34.4 kcal mol^{-1} by a change in dielectric from the gas phase (ϵ = 1) to water (ϵ = 78), the $N3^-$ ion gets stabilized by a greater amount: by 42.2 kcal mol^{-1}. Therefore, although the N1 and N3 sites are intrinsically quite different in acidity, the preferential solvation of the $N3^-$ site results in the two acidities coalescing in solution.

If one considers the benzenoid resonance structure of uracil (Scheme 5), one can rationalize that the N1−H, which is proximal to only one negatively charged oxygen, will be more

(25) Gronert, S. Personal communication.

(26) Feng, W. Y.; Austin, T. J.; Chew, F.; Gronert, S.; Wu, W. *Biochemistry* **2000**, *39*, 1778−1783.

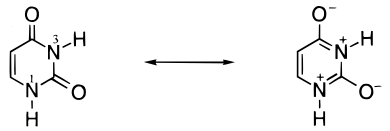
(27) Gronert, S.; Feng, W. Y.; Chew, F.; Wu, W. *Int. J. Mass Spectrom. Ion Proc.* **2000**, in press.

(28) Kato, S.; DePuy, C. H.; Gronert, S.; Bierbaum, V. M. *J. Am. Soc. Mass Spectrom.* **2000**, *10*, 840−847.

Table 4. Calculated Acidities of the N1 and N3 Sites of Uracil at B3LYP/6-31+G*, in kcal mol^{-1}

	ε = 1	ε = 4	ε = 10	ε = 78.54
N(1)-H	329.2	302.7	297.6	294.8
	Δ = 34.4 kcal/mol			
N(3)-H	342.6	311.2	304.4	300.4
	Δ = 42.2 kcal/mol			

Scheme 5



benzenoid resonance structure

easily removed than the N3−H, which is proximal to two negatively charged oxygens. This effect would be mitigated in solution, which explains the coalescence of the N3 and N1 acidities.

What are the implications of our results in terms of recognition and catalysis? As the environment becomes more nonpolar, the N3 site becomes relatively less acidic than the N1 site. This may be a reason that glycosylation occurs at N1: in a nonpolar environment, such as that within the enzyme active site, deprotonation is more facile at N1. Furthermore, deglycosylation would be favorable at N1 to produce the relatively more stable N1$^-$ ion. Therefore, our results point to a possible chemical reason that the N1 site is glycosylated. Perhaps nature simply takes advantage of the differential N1 and N3 acidities in a nonpolar environment to achieve selectivity. While the enzyme may still bind uracil in such a way that the N1 site is favored for reaction, we have shown a chemical favorability that nature may well take advantage of. Furthermore, recent NMR data reveal that when bound to uracil-DNA glycosylase, uracil appears to be anionic; the uracil N1 pK_a is also found to be unusually low.[29] This low pK_a is attributed to possible hydrogen bonding of a histidine to the O2. Our results are consistent with these NMR data; we predict that selectivity-wise, this necessary lowering of pK_a in a nonpolar environment is more effective at the intrinsically more acidic N1 site.

Conclusion

In summary, our measurements and calculations of the N1 and N3 acidities of uracil have established that (1) the N1 site is intrinsically more acidic than the N3 site, by 14 kcal mol^{-1}, (2) the dual cell FTMS can be used to bracket lower acidity sites in multiple-acidic-site-molecules, (3) B3LYP/6-31++G** is a relatively inexpensive and very reliable method for predicting these acidities, and (4) the coalescence of uracil acidities in solution is probably due to the mitigation of the Coulombic effects in the benzenoid resonance structure of uracil. Our results also point to a possible chemical reason for the prevalence of biological alkylation at the N1 site. We are continuing studies of other nucleic bases and nucleotides to ascertain the generality of our conclusion, in terms of how acidity and proton affinity change as the dielectric changes and the biological implications of those chemical changes.

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Supporting Information Available: Energies (in hartrees) and Cartesian coordinates for the optimized structures of **1**−**5**, deprotonated **4**, and deprotonated **5** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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