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On the Mechanism of the Mutagenic Action of 5-Bromouracil: A DFT Study of Uracil and 5-Bromouracil in a Water Cluster

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Density functional theory calculations on the canonical (keto) and rare (enol) tautomeric forms of uracil and 5-bromouracil in a cluster consisting of 50 water molecules are presented. The keto form of uracil is favored over the enol tautomer in both the gas phase and solution. However, the presence of the water cluster reverses the tautomeric preference of 5-bromouracil, rendering the rare tautomeric form to be preferred over the canonical form in aqueous solution. This effect is, to a large extent, due to the more favorable water–water interactions in the cluster around 5-bromouracil and can therefore only be obtained by including explicit water–water interactions in the calculations.

The mechanism of the mutagenic activity of 5-bromouridine has been under some debate over the last few decades. The canonical keto form of 5-bromouracil (5BrU) is complementary to adenine. However, this tautomeric form cannot bind with guanine. Mechanisms that have been brought forward to explain the pairing of 5BrU with guanine include keto \rightarrow enol tautomerization of 5BrU, ionization (deprotonation) of 5BrU, and the involvement of “wobble” hydrogen bonds (see, for example, ref 1). Additionally, a mutagenic mechanism involving triplet excited states of U and 5BrU has been suggested.² A mechanism involving base pairing between the ionic form of BrU and guanine is not very likely. The reason for this is that, at interbase distances normally occurring in DNA duplex structures, such base pairs prefer nonplanar structures, exhibiting very large propeller twist angles due to the unfavorable arrangement of the oxygen atoms. The nonplanarity of these structures hinders their incorporation into DNA.³ The possible formation of wobble hydrogen bonds should not depend on the presence or absence of bromine and can therefore also be discarded as a likely cause for the mutagenicity of 5-bromouridine. The involvement of excited states is very unlikely as the mutation reaction is a dark reaction, that is, no light or other type of radiation is required to induce the mutations. The only remaining mutagenic mechanism, the tautomeric model of the mutagenic activity of 5BrU, has also been discarded by several authors.^{1,2} The rejection of the tautomeric model was based on quantum chemical calculations that showed that solvation in water does not change the known gas-phase preference for the canonical form of 5BrU. As a result, the tautomeric model is

currently not considered as a viable candidate to explain the mutagenic activity of 5BrU. However, these calculations employed continuum solvation models to describe the bulk water, which lack explicit interactions between individual water molecules and may therefore not be accurate enough to reliably obtain the tautomeric preferences of solvated 5BrU. In addition, it should be noted that Katritzky and Waring⁴ showed that a bromine in the five position considerably increases the proportion of the enol form present in 1-methyluracil. In this Letter, we show that consideration of explicit water molecules dramatically alters the tautomeric preference of 5BrU. Our results therefore reinstate the tautomeric mechanism as a possible explanation of the mutagenic properties of 5BrU.

To reliably predict the tautomeric preferences of U and 5BrU, one needs to treat the water–water and water–base interactions at the molecular level by quantum chemistry methods. In addition to model bulk water, a sufficiently large cluster of water molecules needs to be employed. Previous calculations on uracil and thymine complexes comprising 11 water molecules⁵ and cytosine and adenine complexes comprising 14 and 16 water molecules, respectively,⁶ showed that structures with a clustering of the water molecules were preferred over structures with the water molecules distributed around the central base. This is due to the increased attractive interaction between the water molecules in the clustered complexes. The results indicate that the limited-size water clusters used in these calculations do not accurately represent bulk water; in the clustered structures, the water only interacts with one face of the central base, whereas in the distributed structures, the attractive water–water interaction is underestimated as compared to the clustered structures and, consequently, also to bulk water. It was recently shown that a RNA base pair in a sufficiently large nanodroplet (water cluster) mimics the behavior of a base pair in bulk water.⁷ For the RNA base pairs, the stability observed in bulk water was

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TABLE 1: The Formation Energy and Its Components of the Keto and Enol Tautomeric Forms of the U–W50 and 5BrU–W50 Systems Computed at the B3LYP/6-31G(d,p) and M05-2X/6-31G(d,p) Levels (kJ/mol)

method	complex	$\Delta E_{\text{BW50}}^{\text{CP}}$	$\Delta E_{\text{B}-(\text{W50})}^{\text{CP}}$	$\Delta E_{\text{W50}}^{\text{CP}}$
B3LYP/6-31G(d,p)	U(keto)–W50	–2355.2	–146.5	–2208.8
	U(enol)–W50	–2338.9	–186.0	–2152.0
	5BrU(keto)–W50	–2245.2	–142.7	–2102.5
	5BrU(enol)–W50	–2300.9	–156.7	–2144.2
M05-2X/6-31G(d,p) ^a	U(keto)–W50	–2595.3	–228.4	–2366.9
	U(enol)–W50	–2561.8	–252.9	–2308.9
	5BrU(keto)–W50	–2517.8	–239.5	–2278.3
	5BrU(enol)–W50	–2529.5	–237.4	–2292.0

^a Computed using the B3LYP/6-31G(d,p) optimized geometries.

reproduced as the number of water molecules increased above 100. For the single bases studied in the current Letter, the number of water molecules required to mimic bulk water is expected to be much smaller, and 50 water molecules should suffice to approach the bulk water limit. Thus, we have fully optimized the structures of U and 5BrU solvated in a cluster consisting of 50 water molecules at the B3LYP/6-31G(d,p) level of theory. These complexes are denoted as U–W50 and 5BrU–W50 from this point onward. In a subsequent paper, we will present results containing even larger water clusters (comprising 100 water molecules).⁸ To the best of our knowledge, these are the first calculations on the tautomerization of uracil that employed a large number of water molecules explicitly using electronic structure theory.

The geometry optimizations were started from structures with favorable (for example, H-bonded) orientations of the water molecules in the complexes. These structures were obtained from Monte Carlo (MC) simulations in the canonical (NVT) ensemble using Metropolis sampling.⁹ Simulations were performed employing the physical cluster theory^{10,11} at 298 K. Each complex under investigation (base + water) included 400 water molecules. We selected the 50 water molecules closest to the center-of-mass of the base. In these simulations, we used the refined semiempirical potential functions suggested by Poltev and his colleagues.^{12–14} In our calculations, the statistical error (dispersion value) was calculated with a precision of ± 0.005 .

The formation energies $\Delta E_{\text{BW50}}^{\text{CP}}$, computed with respect to the isolated base and water molecules, were computed with B3LYP/6-31G(d,p) and M05-2X/6-31G(d,p). M05-2X¹⁵ is a metahybrid density functional designed to yield broad applicability in chemistry and has consistently been shown to give much improved binding energies for weak noncovalent as well as dispersion-dominated interactions.^{16–20} The formation energies were corrected for basis set superposition error (BSSE) using the counterpoise (CP) procedure.²¹ Monomer deformation energies were taken into account. As in our previous work,^{5,6} the formation energy was divided into base–water ($\Delta E_{\text{B}-(\text{W50})}^{\text{CP}}$) and water–water ($\Delta E_{\text{W50}}^{\text{CP}}$) components.

The tautomerization energies were computed as the difference between the CP-corrected formation energies of the enol and keto forms of U–W50 or 5BrU–W50. For comparison, the tautomerization energies were also computed as the difference between the energies of the enol and keto forms of the isolated bases (U or 5BrU), that is, in the absence of the water environment.

All density functional theory calculations were done with the Gaussian 03²² program package.

Table 1 shows that in a water cluster, the formation of the keto form of U is more preferable than that of the rare enol tautomer. In contrast, for 5BrU, the formation of the rare

TABLE 2: Tautomerization Energies $\Delta E(\text{keto} \rightarrow \text{enol})$ for the Isolated Bases and for the Complexes of the Bases Interacting with 50 Water Molecules (kJ/mol)

method	counterpoise	$\Delta E(\text{keto} \rightarrow \text{enol})$	
		U	5BrU
Isolated Bases			
B3LYP/6-31G(d,p)	n/a	49.9	48.5
M05-2X/6-31G(d,p) ^a	n/a	44.7	47.6
Hydrated Bases			
B3LYP/6-31G(d,p)	no	38.5	0.2
	yes	16.3	-55.7
M05-2X/6-31G(d,p) ^a	no	51.5	35.9
	yes	33.6	-11.7

^a Computed using the B3LYP/6-31G(d,p) optimized geometries.

tautomer is more favorable. For both U–W50 and BrU–W50, the water–base interaction favors the formation of the rare tautomeric form. However, the water–water interaction favors the formation of the rare enol form of BrU, thereby making this more favorable than the canonical form. Thus, the preference of the formation of the rare tautomeric form of 5BrU is mainly determined by the water–water interaction. Clearly, the favorable tautomeric shift from U to 5BrU is determined by the water structure around the base. The general conclusions are the same for both levels of theory applied, even though there are differences in the individual energy terms. The M05-2X base–water and water–water interaction energies and, consequently, also the formation energies are larger than the corresponding B3LYP values. This is likely due to the better description of the intermolecular interactions, including dispersion, by the M05-2X functional. This is corroborated by the fact that the base–water interaction energies, which are expected to be more affected by dispersion-type interactions than the water–water interactions, are most affected by the change in the density functional; the base–water interaction energies increase by roughly 25–35% upon going from B3LYP to M05-2X, as compared to increases of 6–8% for the water–water interaction.

It should be noted that the MC results obtained by us and by Orozco et al.¹ predict the keto form of 5BrU to be more preferable than the enol form, in variance with the B3LYP and M05-2X results. The disagreement between the MC and B3LYP/M05-2X results may be due to the use of semiempirical potential functions in the MC simulations, which do not account for effects like polarization and water monomer deformation during geometry optimization of the total system.

The preference for the rare tautomeric form of 5BrU in an aqueous environment can also be seen from the tautomerization energies listed in Table 2. In the gas phase, both U and BrU favor the canonical form, and the tautomerization energies of the two bases are very similar at both levels of theory employed. The gas-phase tautomerization energy computed by M05-2X/6-31G(d,p) is remarkably close to the CCSD(T) value of 44.4 kJ/mol (10.6 kcal/mol) of Hobza et al.,²³ confirming the excellent performance of this density functional. In contrast to the gas-phase results, bromination of uracil favors the rare tautomeric form when the bases are hydrated by 50 water molecules. It can be seen that counterpoise correction is essential to obtain the correct description of the tautomerism of U and 5BrU.

Inspection of the distribution of the water molecules around the two tautomers of 5BrU reveals that, in general, the water molecules are more closely packed around the rare tautomer. Although the density of the water molecules differs in the different layers around the two bases, the outer hydration shell

contains more water molecules in 5BrU(enol)–W50 than in 5BrU(keto)–W50. As the outer shell contains a larger number of water molecules than layers closer to the central base, its contribution to the water–water interaction energy is more important. In addition, the hydration shell extends further out for the keto tautomer (up to 7.8 Å from the center-of-mass of the base) than for the enol tautomer (up to 7.4 Å), further confirming the denser water shell around the enol tautomer. This denser water shell is responsible for the increase in the water–water interaction from 5BrU(keto)–W50 to 5BrU(enol)–W50, thereby favoring the formation of the rare tautomer of 5BrU in a hydrated environment.

In conclusion, the tautomeric preference of 5BrU is greatly influenced by aqueous solvation. This effect is largely due to the more favorable water–water interactions in the cluster around 5BrU and can therefore only be obtained by including explicit water–water interactions in the calculations. In a subsequent paper,⁸ we will present more detailed results and will show that the use of a larger cluster, consisting of 100 water molecules, does not change the conclusions obtained in the current paper. Although entropy and relativistic effects may have some effect on the results, we do not expect these to affect the main conclusion of this work. This is supported by results by Hobza et al., who found that entropy does not significantly affect the relative stability of U²³ and 5BrU² tautomers. Our results show that, at present, the tautomeric mechanism cannot be ruled out as an explanation of the mutagenic properties of 5BrU.

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