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DFT Study of the [2 + 2] Cycloreversion of Uracil Dimer Anion Radical: Waters Matter

Nicolas J. Saettel and Olaf Wiest*

Department of Chemistry and Biochemistry
University of Notre Dame
Notre Dame, Indiana 46556-5670

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UV irradiation induces a [2 + 2] cycloaddition between adjacent thymine bases in DNA to form cyclobutane-like *cis,syn*-thymine dimer T⟨⟩T as the major product (Figure 1). This lesion is the principal cause for skin cancer as it blocks cell replication and transcription. The enzyme DNA photolyase is capable of reversing this process using visible light. The accepted pathway is a photoinduced electron transfer from a cofactor $FADH_2$ to T⟨⟩T via a light-harvesting.[1] Less known is the mechanism of the splitting of the thymine dimer radical anion. The cycloreversion of thymine or uracil dimer has been studied computationally in an attempt to reproduce the thermodynamics of the reaction, as well as getting insight into the mechanism.[2] However, these studies were performed in the gas phase, and their applicability to reactions in solution or the enzyme is not clear. This is particularly true in light of experimental[3−5] and computational[6] studies of the electron attachment to uracil. These studies indicate a dipole-bound state with an electron affinity (EA) of 1.24 kcal/mol, whereas the valence-bound state is not stable.[7] This behavior should be even more pronounced in the case of the uracil dimer as the stabilization of the dipole-bound state increases with the dipole moment. The valence-bound state of pyrimidines can be stabilized by hydrogen bonding with water[8−10] or between base pairs.[11] It can therefore be expected that the gas-phase calculations will not give an adequate representation of the reaction. We have therefore investigated the electron affinity of uracil and the cycloreversion of the uracil dimer U⟨⟩U, which is also a substrate for DNA photolyase.[12] Three water molecules are introduced to mimic the specific interactions in aqueous solution and in the enzyme DNA photolyase, where Trp^{384} is hydrogen-bonded to the C4 carbonyl of the U⟨⟩U substrate.[13]

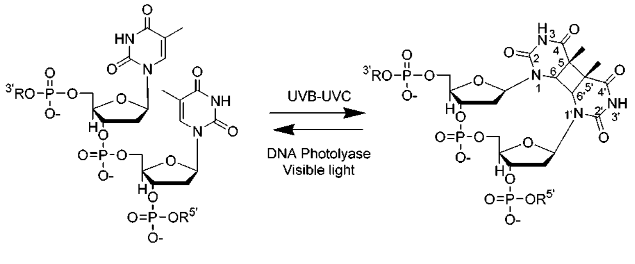

Figure 1. Formation and cycloreversion of the *cis,syn*-thymine dimer.

Table 1. Vertical (VEA) and Adiabatic (AEA) Electron Affinities of Uracil and Uracil Dimer with Different Number of Water (in kcal/mol)

base/ number of H_2O molecules	VEA calc.[a]	VEA exp.	AEA calc.[a,d]	AEA exp.
U/0 H_2O	−8.18	+ 6.91[b] + 7.60[c]	+ 4.24	+18.44 +17.28[e]
U/1 H_2O	−4.59	+11.52[b]	+11.85	+23.04[b]
U/2 H_2O	−7.39		+14.97	
U/3 H_2O	−6.78	N/A	+15.35	N/A
U⟨⟩U/1 H_2O	−5.23		+28.10	
U⟨⟩U/3 H_2O	+3.98		+31.81	

[a] B3LYP/6-31+G*. [b] Reference 9, photoelectron spectroscopic data of U/1 H_2O, assuming a 0.2 eV hydration energy. [c] Electron beam.[15] [d] Zero-point corrected. [e] Scaling of half-wave reduction potentials.[16,17]

Table 1 summarizes our results obtained at the B3LYP/6-31+G* level of theory using the Gaussian98[14] series of programs. On the basis of the gas-phase calculations of electron affinities, these results demonstrate how incorporation of hydrogen-bonded water molecules stabilizes the uracil anion valence state and provide a reasonable model for the study of the subsequent reaction. UHF or MP2/6-31G* calculations give a negative EA of about −28 kcal/mol;[2d] introduction of one bridging H_2O across the C4 carbonyl groups of U⟨⟩U increases the EA to +28 kcal/mol. Three water molecules do not change the adiabatic EA significantly but give a positive vertical EA. We thus decided to investigate the mechanism of hydrogen-bonded uracil dimer anion radical splitting.

Previous work calculated a stepwise pathway in which the C5−C5′ bond breaks with a small activation energy at the UHF level of theory but becomes barrierless at the MP2[2d] and B3LYP[2f] level of theory.

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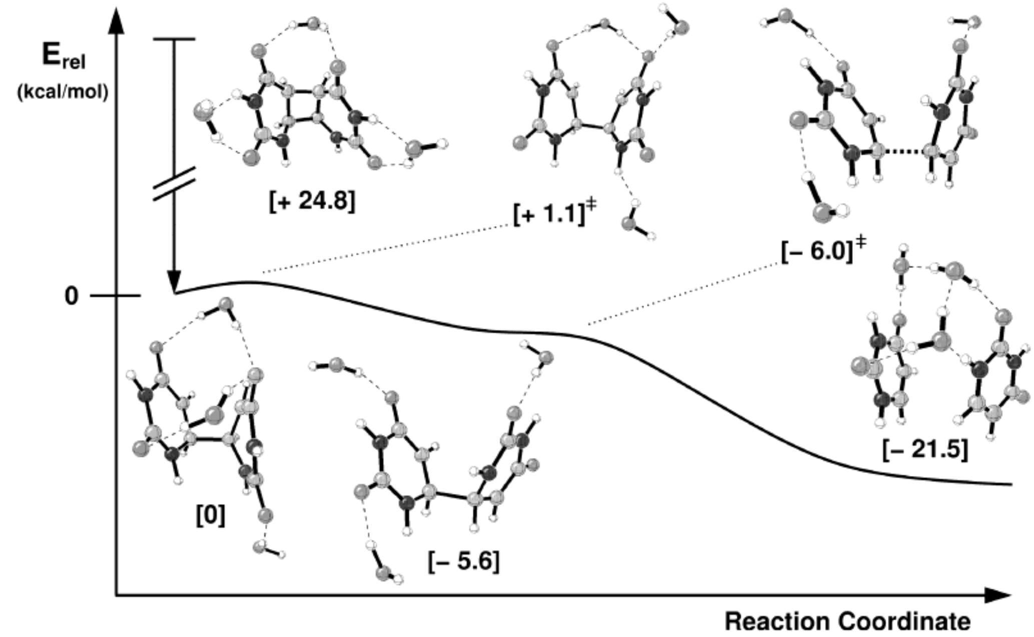

Figure 2. Potential energy surface of the three-water system. Energies are B3LYP/6-311++G**// B3LYP/6-31G* and zero-point corrected.

We found a barrier for C6−C6′ cleavage of 14.1 kcal/mol at the UHF/6-31G* level[18] and an endothermicity of 13.2kcal/mol, in accordance with results by Rösch.[2d] Eriksson reported a lower activation energy (2.3 kcal/mol) using B3LYP/6-311++G**// B3LYP/6-31+G* calculations and a small exothermicity of 2.4 kcal/mol for this bond breakage.[2f]

However, when a water molecule is hydrogen-bonded to uracil dimer, the anionic cycloreversion becomes downhill by 15.3 kcal/mol and essentially barrierless. The first transition state for the C5−C5′ bond opening was not found, and the second transition state corresponding to the C6−C6′ bond breaking, 0.28 kcal/mol above the intermediate, drops 0.12 below upon zero-point correction. This explain the fact that, although a concerted mechanism in Woodward−Hoffmann terms is forbidden,[19] no intermediate can be found by trapping through a cyclopropyl carbinyl rearrangement[20] or can be observed by low-temperature EPR.[21] It also enlightens the kinetic isotope effect results, which concluded[12,22] a concerted pathway although further evidence showed the difficulties of such a study; in particular the isotope effects of the electron-transfer step have to be considered.[23]

During the U〈〉U radical anion cycloreversion, the cyclobutane framework undergoes distinctive changes. Upon ionization, the C5−C5′ bond is considerably increased, going from 1.6 to 2.5 Å. This can be rationalized in terms of delocalization of the singly occupied C4−O π^* orbital into the C5−C5′ σ^* orbital.[2,19] Then the dihedral C5−C6−C6′−C5′ changes from 30° in the anion to −71° in the intermediate. On the way to the final complex, the two uracil molecules can either orient in a π-stacking fashion (shown in Figure 2) or align their dipole moments in opposite directions.

The quasi-concertedness of this reaction is now rationalized in terms of localization of spin and charge through the hydrogen-bonded water molecule, on the uracil−uracil anion complex, and not through a destabilization of the gas-phase intermediate by the geometry-constraining enzyme.[2d] The one-water system does not reproduce the overall thermodynamic of the reaction. Photothermal beam deflection calorimetry[24] for the neutral reaction, along with fluorescence quenching experiments,[25] gives a value of 21 kcal/mol. The value of 15.34 kcal/mol is thus underestimated when only one water is considered.

The problem inherent to the one-water system is that the water molecule has to "choose" between two uracils in the final complex, and that costly process can be remedied by the augmentation of the solvation shell. Figure 2 shows the potential energy surface of a three-water system. It should be pointed out at that stage that we are not looking at the dynamics of the reaction but rather at low-energy "snapshots" of three *generic* water molecules.[26] Through the introduction of three water molecules and single-point B3LYP/6-311++G** calculations, we find a 21.5 kcal/mol exothermic cycloreversion, proceeding in a non-synchronous quasi-concerted mechanism, first by breaking the C5−C5′ bond with an activation energy of 1.1 kcal/mol, and consecutively the C6−C6′ bond barrierless.

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Supporting Information Available: All coordinates, energies, zero-point corrections of the species discussed, along with negative frequencies of transition states (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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