Communications to the Editor

Photoenzymic Repair of the DNA 6−4 Photoproduct−A Density Functional Theory and Semiempirical Study

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Solar ultraviolet light (290−400 nm) is known to have mutagenic, carcinogenic, and lethal effects.[1] The main targets of UVA and UVB radiation are the pyrimidine DNA bases, with cyclobutane type pyrimidine dimers (Pyr<>Pyr) and the 6−4 photoadduct (pyrimidine (6−4) pyrimidones) the most common products.[2] In the case of the pyrimidine dimers, one enzyme system in particular has been the subject of much study, namely the photolyases.[3] DNA photolyases are of especial interest as they utilize the energy of a photon of light (λ = 300−500 nm) to cleave the cyclobutane ring of the pyrimidine dimer directly without excision of the damaged base.

More recently, evidence[4] has been presented that the 6−4 photoproduct is more mutagenic that the pyrimidine dimer lesion. Of particular interest, therefore, is that the 6−4 photoproduct can also be removed enzymatically, in this case by the action of a new type of photolyase. Such 6−4 photolyases have been discovered in organisms such as *Drosophila melanogaster*[5] and *Xenopus laevis*.[6] Of even greater interest is that recent evidence suggests that the cyclobutane and 6−4 type photolyases are structurally related to each other and in turn to the plant blue light photoreceptor protein.[7]

A mechanism has been proposed for the action of 6−4 photolyases that is analogous to that for pyrimidine dimer photolyases. The mechanism involves electron transfer from the excited states of the enzyme to the damaged site resulting in restoration of the original components.[8] However, unlike cyclobutane dimers, the actual primary photoproduct of 2 + 2 addition, the oxetane species, is not actually observed, but undergoes rapid rearrangement at temperatures above −80 °C, leading to the formation of the 6−4 product[9] (Figure 1). Kim et al.[8] rejected the possibility that electron transfer to the 6−4 product could result in the reformation of the original DNA bases. Instead they proposed an ingenious mechanism in which the equilibrium between the 6−4 product and oxetane is shifted toward the latter upon enzyme binding. Electron transfer to the oxetane could thus lead to oxetane splitting by an analogous mechanism to that proposed for cyclobutane splitting.[3] An implicit assumption in this mechanism is that the binding energy of the 6−4 photolyase to the 6−4 product is sufficient to perturb the 6−4/oxetane equilibrium in favor of the latter. However, no experimental data are available for the necessary thermodynamic parameters needed to assess the viability of this key proposal. Hence in this work we employ high-level ab initio and density functional theory plus semiempirical molecular orbital calculations to calculate the 6−4/oxetane energy difference.

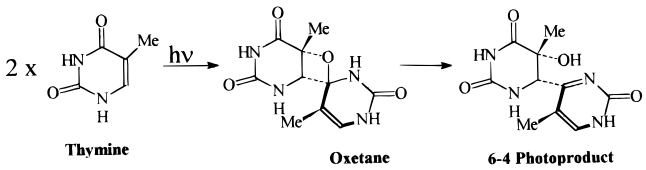

Figure 1. Formation of the 6−4 photoproduct from thymine via the hypothetical oxetane intermediate.

The 6−4 and oxetane species formed between two thymine bases were optimized by the restricted Hartree−Fock self-consistent-field method using the standard 6-31G++(p,d) basis set. Subsequently, single point calculations were carried out for both structures using the DFT method including both local and nonlocal exchange and correlation energy density functionals. Combinations of local exchange and correlation energy functionals are SVWN and XαVWN, and those of the nonlocal exchange and correlation energy functionals are BLYP, BP86, BPW91, B3LYP, B8P86, and B3PW91. For comparison, RHF and MP2 calculations were also performed. Calculations were carried out using both the Gaussian 94[10] and DMol[11] packages.

The results are summarized in Table 1. It can be seen that both the Gaussian 94 and DMol packages give almost the same energies, even though different strategies of implementing DFT calculations are utilized by each method. It was also found that, while the nonlocal methods predict a larger energy gap, at about 11 kcal/mol on average, local DFT methods tend to give a smaller energy gap between the oxetane and the 6−4 product, averaging about 4 kcal/mol (data not shown). It is generally accepted that the B3 method for the exchange energy functional and PW91 for the correlation energy functional reproduce experimental values very closely. Thus, taking into consideration this point, the best estimate of the energy difference by the nonlocal DFT method would be 9−11 kcal/mol. Including the zero point energy increased the energy difference by 2 kcal/mol. Other methods, including the restricted Hartree−Fock method and the second-order Møller−Plesset perturbation method (MP2), yielded even larger energy differences between the oxetane and the 6−4 product.

To verify that the methods employed can be considered reliable, the heat of formation of oxetane itself (i.e., C_3H_6O) was calculated by the B3LYP/6-31G** method. This was

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Table 1. Energy Difference between Oxetane and the 6−4 Product in Terms of Different Theoretical Methods

	energy difference (oxetane) − (6−4) (kcal/mol)	
method	Gaussian 94	DMol
	DFT Nonlocal Methods	
BLYP	13.90	14.60
BP86	10.69	
BPW91	10.46	10.89
B3LYP	11.49	
B3P86	9.59	
B3PW91	9.79	
	Other Methods	
RHF	12.98	
MP2	14.34	

calculated from the heat of reaction of ethene + methanal → oxetane and using the experimental heats of formation of ethene and methanal.[12] This yielded a ΔH_f of −17.9 kcal/mol compared with an experimental value of −19.2 kcal/mol.[12]

Several other effects need to be considered before a realistic estimate of the 6-4/oxetane free energy difference relevant to the biological situation can be derived. For this, the semiempirical AM1 method was used, which yields a 6−4/oxetane enthalpy difference of 19 kcal/mol. In this case, the AM1 method is being used to evaluate relative differences rather than absolute values. Entropy effects are likely to increase the 6−4/oxetane energy difference due to the greater degree of freedom in the 6−4 species. This was confirmed using the AM1 Hamiltonion, which predicts that entropy effects increase the 6−4/oxetane energy gap by approximately 2 kcal/mol. In addition, solvation effects were estimated using the AMSOL (SM1) method. This predicts that the 6−4/oxetane energy gap would be increased by a further 1.5 kcal/mol compared to the gas phase.

The 6−4/oxetane energy gap was found to be not greatly influenced by the base structure, with comparable values (±2 kcal/mol) found for thymine-thymine; thymine-cytosine, and uracil-uracil.

Several studies have been carried out with sulfur analogues of the 6−4 adduct. In this case, in contrast to the oxetane ring, thietane derivatives are stable at room temperature.[13] Correspondingly, the 6−4/thietane energy gap for the thymine-2-thiothymine compound was found to be 5 kcal/mol (cf. 19 kcal/mol for the oxetane).

In conclusion, the best estimate for the free energy difference between the hydrated 6−4 and oxetane species of thymine-thymine would be approximately 14.5−16.5 kcal/mol (DFT enthalpy difference + AM1 entropy and solvation effects). This strongly suggests that perturbation of the 6−4/oxetane equilibrium is unlikely to be a feature of the photoenzymic repair mechanism as the computed value exceeds the likely difference in binding energy between the two species.

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