# A Quantum Chemical Study of Photoinduced DNA Repair: On the Splitting of Pyrimidine Model Dimers Initiated by Electron Transfer

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**Abstract:** As a first step toward modeling the photoinduced repair of DNA, electronic structure calculations on the cleavage reaction of various pyrimidine dimers (uracil, thymine, and cytosine) as well as of their anion and cation radicals have been carried out using the AM1 UHF method. Two different paths of the splitting reaction have been studied by locating all stationary points. Along the first path, the opening of the cyclobutane ring is initiated by breaking the C5−C5′ bond which leads to the formation of an intermediate, followed by the cleavage of the C6−C6′ bond; along the second path, the two C−C′ bonds are broken in reverse order. The results for the dimer anion radical favor a cleavage reaction along the first path while the second path is preferred for the cation radicals. Electron transfer to the dimers does not appreciably influence the enthalpy of the reaction for cycloreversion in the uracil and thymine dimers; however, it causes a dramatic reduction of the activation barrier for the cleavage reaction. In contrast, the reactivity of the cytosine dimer is only weakly affected by this electron uptake. Differences in the various reaction profiles are rationalized by invoking an energetic stabilization associated with the charge delocalization between fragments in the corresponding transition states. The calculated solvent effects evaluated by a dielectric continuum model show that the splitting reaction is sensitive to a polar environment. The reaction barriers of the splitting reaction are found to increase with the polarity of the medium, rationalizing the experimentally observed solvent effects on the dimer cleavage.

## 1. Introduction

Absorption of ultraviolet light may cause neighboring pyrimidine bases (Pyr) in a DNA strand to form cyclobutane-type dimers (see Figure 1). Such lesions have mutagenic effects and block the replication and the translation of DNA. Cells have various mechanisms to remove these pyrimidine dimers, one of which is enzymatic photoreactivation.[1−5] The enzyme DNA photolyase is able to repair this damage by splitting the pyrimidine dimers by utilizing the energy of an absorbed photon of near-UV light. Photolyases are monomeric proteins of about 60 kDa and contain two noncovalently bound chromophores (cofactors). One cofactor, the photoantenna MTHF (5,10-methenyltetrahydrofolate), absorbs light and transmits the excitation energy to another chromophore, the catalytic cofactor FAD (flavin adenine dinucleotide). From the analysis of various biochemical,[1,2] chemical,[1,3,4] and spectroscopic investigations,[1,5,6] supported by the recently resolved crystal structure of DNA photolyase,[7] the following steps of the photorepair mechanism have been established: (1) light-independent binding of the substrate to the enzyme, (2) light absorption and energy transfer from MTHF to FAD, (3) electron transfer activated splitting of the pyrimidine dimer, and (4) dissociation of the enzyme−substrate complex. While the overall efficiency of the photo-

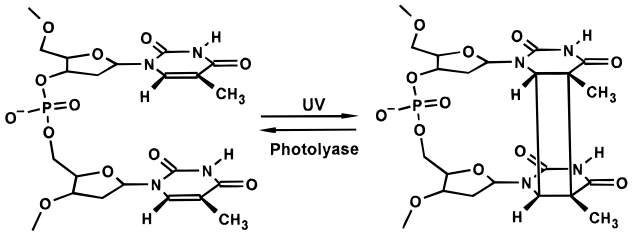


**Figure 1.** Schematic representation of the UV-induced damage and the enzymatic photorepair of DNA.

repair reaction is due to a proper tuning of each step, it is useful to consider the various steps separately. Central to the enzymatic reactivation of the dimer is the cycloreversion step (3). The photoexcited state of the catalytic cofactor FAD initiates the splitting of the pyrimidine dimer by a highly efficient transfer of a single electron over a short distance between the substrate and the chromophore.[1,5,7] In the course of the reaction, the C5−C5′ and C6−C6′ bonds of the pyrimidine dimer anion radical are cleaved and ultimately back electron transfer occurs. At the end of the photorepair process the substrate is forced out of the pocket of the enzyme which then dissociates from the DNA strand, and the catalytic cycle is complete.

Many important questions concerning details of the photocatalytic process have yet to be answered,[1,5,7] despite a series of experimental studies. At present, it is unclear whether the splitting reaction proceeds in a concerted fashion or via a two-step mechanism. In the latter case it is important to clarify what determines the order in which the two bonds between the pyrimidine units are broken and how large the activation barriers of these bond-breaking steps are. It is also of interest to know what effects the substituent of the pyrimidine and the enzyme environment exert on the course of the dimer splitting reaction and on its dynamics. Furthermore, it is uncertain whether the

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(1) Sancar, A. In *Advances in Electron Transfer Chemistry*; Mariano, P. E. Ed.; JAI Press: London, 1992; Vol. 2, p 215.
(2) Hearst, J. E. *Science* **1995**, *268*, 1858.
(3) Heelis, P. F.; Hartman, R. F.; Rose, S. D. *Chem. Soc. Rev.* **1995**, *24*, 289.
(4) Begley, T. *Acc. Chem. Res.* **1994**, *27*, 394.
(5) Heelis, P. F.; Kim, S.-T.; Okamura, T.; Sancar, A. *J. Photochem. Photobiol., B* **1993**, *17*, 219.
(6) Kim, S.-T.; Volk, M.; Rousseau, G.; Heelis, P. F.; Sancar, A.; Michel-Beyerle, M.-E. *J. Am. Chem. Soc.* **1994**, *116*, 3115.
(7) Park, H. W.; Kim, S.-T.; Sancar, A.; Deisenhofer, J. *Science* **1995**, *268*, 1866.

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back electron transfer occurs before or after the breaking of the second bond (i.e., whether the last step in the sequence of events is an electron transfer from the monomer $Pyr^-$ or a bond breaking in a neutral intermediate held together by one C−C′ inter-ring bond). Quantum chemical calculations can contribute to the understanding of the dimer cleavage reaction by a determination of the structures and energetics of the reactants and the intermediates as well as those of the transition states. Indeed, an analysis of Hückel-based orbital correlation diagrams provided an explanation for the activation of the pyrimidine dimer splitting by electron transfer.[8] Some energetic aspects of the dimer splitting reaction have very recently been the subject of a quantum chemical investigation.[9]

It was the goal of the present computational electronic structure investigations on a series of dimer models to consider in detail various aspects of the pyrimidine dimer fragmentation reaction activated by electron transfer. For comparison, cleavage of a neutral pyrimidine dimer as well as electron transfer both to and from the dimer have been considered.

## 2. Models

Quantum chemical calculations on systems of biological importance require a particularly careful choice of a proper model system. To rationalize the construction of the dimer models employed in our study, it is useful to discuss some aspects of the DNA photorepair.

The DNA backbone plays a very important role for the binding of the substrate (step 1) which may also involve van der Waals contacts between the dimer and the catalytic cofactor. This may be deduced from the fact that the quantum yield of repair is about the same for a thymine dimer in DNA as for a dinucleotide T<>T, but the equilibrium binding constant of the dinucleotide is about $10^4$ times lower than that of T<>T in DNA.[10] Nevertheless, photolyase is able to cleave T<>T at the base level. Therefore, it should be possible in a first model study on the dimer splitting to neglect factors which determine the formation of the enzyme−substrate complex (e.g., electrostatic contacts, hydrophobic interactions, hydrogen bonds, and a deformation of the DNA backbone). However, it cannot be excluded that the cleavage reaction is influenced by a possible strain on the dimer due to the formation of the enzyme−substrate complex. The dimer can only bind to the enzyme by flipping out of the helix into the pocket of the enzyme,[7] thereby weakening the hydrogen bonds between the two bases in the dimer and their partners in the DNA helix. This may indicate that hydrogen bonds do not play a major role in the cleavage (step 3) of the repair reaction. Thus, "simple" dimers of nucleic bases may be expected to provide reasonable models of the substrate as far as the ring-opening reaction is concerned.

In the present study, three different dimers have been investigated: U<>U, T<>T, and C<>C (formed by the dimerization of uracil (U), thymine (T), and cytosine (C), see Figure 2). In this way, we are able both to model the effects of substituents near one of the cyclobutane bonds and to study the influence of varying electronic effects of pyrimidine rings. While pyrimidine dimers may exist in six isomeric forms, only the cis−syn isomer is significant for the photorepair reaction[1,3,4] and has therefore been considered in the present investigation. To further elaborate on possible effects of a pyrimidine ring substitution, we have also calculated the mixed dimers U<>T and U<>C. However, the results did not yield any new aspects

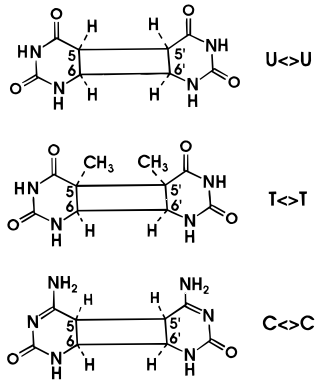


**Figure 2.** Various pyrimidine model dimers considered in the present study: U uracil, T thymine, and C cytosine.

beyond those expected from the results of the homodimers, and we therefore refrain from reporting them.

The dipole moment of pyrimidine bases plays a role in the stability of their gas phase molecular complexes. The dissociation energy of neutral pairs Pyr···Pyr is only about 5 kcal/mol, while pyrimidines undergo a rather strong interaction of about 15−20 kcal/mol in ion−molecule complexes.[11] This ion−molecule interaction substantially depends on the relative spatial arrangement of the pyrimidine bases. In DNA, they are linked to the sugar−phosphate backbone and may therefore be subject to geometrical constraints. The most favorable dimer structures, which correspond to complexes of free nucleic bases, may be disfavored in DNA since they might require considerable deformations of the backbone. Unfortunately, structural data for the enzyme−substrate complex are not yet available. Therefore, at present it would be rather difficult to construct realistic quantum chemical models that include DNA backbone deformations in photolyase−DNA complexes. Thus, as a first approximation, we decided to restrict our investigations to dimers of the pyrimidine bases, and we located stationary points on the potential surface of the corresponding model systems. The strategy of these model studies implies the assumption that once an electron has been added to (or subtracted from) a dimer, ring cleavage will take place via the same mechanism whether the dimer is bound to the enzyme or not. In the present investigation, possible effects of the environment have been included only in a rather approximate fashion via a polarizable continuum model. Thus, any extrapolation of the results obtained for model systems to dimer splitting "in vivo" has to be undertaken with due caution.

## 3. Method

The semiempirical AM1 method[12] was used to describe the cleavage of pyrimidine dimers. To evaluate the role of the electron transfer in the activation of the cycloreversion reaction, results for neutral as well as for negatively and positively charge systems will be compared. Correlation effects are known to be important for an accurate quantum chemical description of cycloaddition reactions.[13−17] Therefore, a proper

(8) Hartman, R. F.; Van Camp, J. R.; Rose, S. D. *J. Org. Chem.* **1987**, *52*, 2684.
(9) Heelis, P. F. *J. Mol. Model. Electronic Publication* **1995**, *1*, 18.
(10) Kim. S.-T.; Sancar, A. *Biochemistry* **1991**, *30*, 8623.
(11) Keesee, R. G.; Castleman, A. W. *J. Phys. Chem. Ref. Data* **1986**, *15*, 1011.
(12) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.
(13) Bernardi, F.; Bottoni, A.; Robb, M. A.; Schlegel, H. B.; Tonachini, G. *J. Am. Chem. Soc.* **1985**, *107*, 2260.
(14) Bernardi, F.; Bottoni, A.; P; Olivucci, M.; Venturini, A. P.; Robb, M. A. *J. Chem. Soc., Faraday Trans.* **1994**, *90*, 1617.
(15) Bernardi, F.; Celani, P.; Olivucci, M.; Robb, M. A.; Suzzi-Valli, G. *J. Am. Chem. Soc.* **1995**, *117*, 10531.
(16) Alvarez-Idaboy, J. R.; Eriksson, L. A.; Fängström, T.; Lunell, S. *J. Phys. Chem.* **1993**, *97*, 12737.
(17) Jungwirths, P.; Carsky, P.; Bally, P. *J. Am. Chem. Soc.* **1993**, *115*, 5776.

treatment of nondynamic and dynamic correlation effects is essential for obtaining reliable estimates of reaction energies. The AM1 method (as well as other semiempirical approaches) includes dynamic correlation in an averaged manner due to the special treatment of the electron−electron repulsion and due to the specific parametrization procedure based on experimental data.

The reaction between two pyrimidine bases resulting in a dimer is a cycloaddition reaction similar to that of two ethylene molecules. According to the Woodward−Hoffmann rules,[18] the splitting of the cyclobutane ring is a thermally forbidden reaction and is characterized by an orbital crossover in the region of the transition state. Thus, a quantum chemical description has to take nondynamic correlations into account in order to properly describe the bond splitting. As a less demanding alternative to multiconfigurational (MC) calculations, the spin-unrestricted Hartree−Fock method (UHF) may be used to estimate nondynamic electron correlation energy in such systems. UHF is especially appropriate at the semiempirical level, because the dynamic correlation has already been included in the effective Hamiltonian. For the systems studied in present work, the UHF wave functions were found not suffer from any significant contamination by higher spin components.

A comparison of AM1 results with those of ab initio calculations at the MP2 correlated level for the ethylene−ethylene radical cation addition[16] revealed that AM1 yields similar geometries and relative energies. Nevertheless, it should be noted that four-membered rings are consistently predicted to be too stable by AM1 and, consequently, the method can overestimate the reaction energy for cyclobutane ring cleavage (while the AM1 errors for molecules containing four-membered rings and for crowded molecules are less than the errors for related semiempirical MNDO method[12]). To evaluate the reliability of AM1 in predicting reaction energies for pyrimidine dimers, we carried out ab initio calculation using the program GAUSSIAN 92.[19] The geometries of the uracil monomer and dimer were fully optimized using the 6-31G(d) basis set. The Hartree−Fock reaction energy for the U<>U splitting is −6.9 kcal/mol, while MP2 calculations predict a reaction energy of 4.9 kcal/mol. Taking into account that MP2 often overcorrects the Hartree−Fock energy, one can conclude that the AM1 reaction energy of 1.0 kcal/mol seems to be reliable, and therefore, the AM1 method can be used to estimate relative energies of stationary points on the potential surface for the cleavage of pyrimidine dimers.

No constraints were applied in the AM1 geometry optimizations. Transition state structures were identified by minimizing the gradient norm[20] or/and by the eigenmode-following method[21] using geometries obtained by the reaction coordinate method as starting points. All stationary points were checked by a vibrational analysis, showing that only one negative eigenvalue of the Hessian matrix existed for transition states and that all eigenvalues were positive for the minima found. Molecular entropies were calculated using unscaled frequencies and standard statistical thermodynamics formulas.[22] Free energies at standard temperature ($T = 298.15$ K) were estimated as $\Delta G = \Delta H - T\Delta S$.

The self-consistent reaction field approach[23] was employed to described solvation effects at the level of a polarizable dielectric continuum. The cavity scheme of Miertus, Scrocco, and Tomasi was applied to the model reactions in solution using cavities defined in terms of intersecting spheres around the atoms of the solute molecule.[24,25] The parameters of this solvent approach are the radii $R_A$ of the various atomic spheres and the dielectric permittivity $\epsilon$. The sphere radii are taken to be equal to the van der Waals radii of the corresponding atoms ($R_H = 1.20$ Å, $R_C = 1.70$ Å, $R_N = 1.55$ Å, $R_O = 1.52$ Å).[26] Two types of solvents were studied: one exhibiting a low value of the dielectric permittivity (hexane: $\epsilon = 1.89$) and the other one (dimethylformamide, DMF: $\epsilon = 32.0$) with a medium dielectric constant which might be more typical for the situation in the enzyme−substrate complex. The program SIBIQ[27] was used in a modified form to perform the AM1 calculations.

**Table 1.** Enthalpy of Formation ($\Delta H_f$), Molecular Entropy ($S$), and Electron Affinity (EA) of Various Pyrimidine Monomers and Dimers Calculated by the AM1 method

| | $\Delta H_f$ (kcal/mol) | | $S$ (cal/(K mol)) | | |
|---|---|---|---|---|---|
| system | neutral | anion | neutral | anion | EA (eV) |
| uracil | −53.9 | −80.1 | 79.2 | 81.5 | 1.14 |
| uracil dimer | −108.7 | −131.7 | 112.4 | 111.5 | 1.00 |
| thymine | −61.1 | −87.8 | 88.9 | 91.1 | 1.16 |
| thymine dimer | −114.0 | −136.3 | 126.7 | 124.8 | 0.97 |
| cytosine | 2.7 | −24.6 | 78.3 | 79.4 | 1.18 |
| cytosine dimer | 12.2 | −26.8 | 106.2 | 107.9 | 1.69 |

**Table 2.** Gas Phase Reaction Enthalpy ($\Delta H_r$) and Free Energy ($\Delta G_r$) (in kcal/mol) for the Splitting of Various Pyrimidine Dimers Calculated by the AM1 Method

| reaction | $\Delta H_r$ | $\Delta G_r$ |
|---|---|---|
| U<>U → 2U | 1.0 | −12.7 |
| U<>U$^-$ → U + U$^-$ | −2.3 | −16.9 |
| T<>T → 2T | −8.2 | −23.4 |
| T<>T$^-$ → T + T$^-$ | −12.6 | −25.6 |
| C<>C → 2C | −6.8 | −21.8 |
| C<>C$^-$ → C + C$^-$ | 4.9 | −9.9 |

## 4. Thermochemistry

The calculated gas phase heats of formation and molecular entropies for various pyrimidine monomers and dimers are given in Table 1. The resulting enthalpy and free energy values for the dimer splitting reaction are listed in Table 2. Different from the cyclobutane cleavage which is known to be clearly endothermic (the experimental reaction enthalpy is 18.2 kcal/mol[28]), the enthalpy of the splitting reaction for the various pyrimidine dimers found by AM1 is close to zero for U<>U or even negative for T<>T and C<>C. This change in the heat of reaction reflects a relative destabilization of the four-membered ring by substitution.

On the basis of the calculated values of the splitting enthalpy (Table 2), one expects the cleavage reaction of anionic dimers to be exothermic (with a possible exception for the C<>C anion radical). The substitution of H atoms in U<>U by methyl groups in T<>T destabilizes the dimer by about 10 kcal/mol due to steric crowding at the C5−C5′ bond. The enthalpy for the splitting of the neutral dimer changes from 1.0 kcal/mol for U<>T to −8.2 kcal/mol for T<>T. Experimentally, the heat of the reaction for a model photodimer with a *N*3,*N*3′-trimethylene linker[29] was found to be −26.4 kcal/mol. This value is more negative than the one calculated for the U<>U dimer possibly because of the additional strain within this model dimer introduced by the linker. Ab initio calculations yield −6.9 kcal/mol at the HF/6-31G(d) level and 4.9 kcal/mol at the MP2 level; the AM1 value is 1.0 kcal/mol. To estimate the strain contribution, we have compared the calculated splitting enthal-

(18) Woodward, R. B.; Hoffmann, R. *The Conservation of Orbital Symmetry*; Verlag Chemie and Academic Press: Weinheim, 1970.
(19) Frisch, M. J.; Trucks, G. W.; Head-Gordon, M.; Gill, P. M.; Wong, M. W.; Foresman, J. B.; Johnson, B. G.; Schlegel, H. B.; Robb, M. A.; Replogle, E. S.; Gomperts, R.; Andres, J. L.; Raghavachari, K.; Binkley, J. S.; Gonzalez, C.; Martin, R. L.; Fox, D. J.; Defrees, D. J.; Baker, J.; Stewart, J. J. P.; Pople, J. A. *Gaussian 92*, Revision G.4; Gaussian, Inc.: Pittsburgh, PA, 1992.
(20) McIver, J. W.; Komornicki, A. *J. Am. Chem. Soc.* **1972**, *94*, 2625.
(21) Wales, D. *J. Mol. Phys.* **1991**, *74*, 1.
(22) Moore, W. J. *Physical Chemistry*, 4th ed.; Prentice Hall International, Inc.: London, 1972.
(23) Tomasi, J.; Persico, M. *Chem. Rev.* **1994**, *94*, 2027.
(24) Miertus, S.; Scrocco, E.; Tomasi, J. *J. Chem. Phys.* **1981**, *55*, 117.
(25) Miertus, S.; Tomasi, J. *J. Chem. Phys.* **1982**, *65*, 239.
(26) Bondi, A. *J. Phys. Chem.* **1964**, *68*, 441.
(27) Voityuk A. *SIBIQ 2.4. A program for semiempirical calculations*, 1995.
(28) Pedley, J. B.; Naylor, R. D.; Kirby, S. P. *Thermochemical data of organic molecules*, 2nd ed.; Chapman and Hall: London, 1986.
(29) Diogo, H.; Dias, A. R.; Dhalla, A.; Minas de Piedade, M.; Begley, T. *J. Org. Chem.* **1991**, *56*, 7340.

pies for U<>U (1.0 kcal/mol) and for the U<>U dimer linked by a trimethylene bridge (−1.7 kcal/mol). This difference of about 3 kcal/mol shows that the linker has only a small effect on the reaction enthalpy. In any case, both experimental and calculated results find the gas phase free energies of the splitting for the neutral and anionic dimers to be negative, which provides the thermodynamic driving force for the reaction.

A considerable contribution to the driving force of the reaction in the gas phase is due to the entropy term ($-T\Delta S$) which is about −14 kcal/mol for both neutral species and for the related anion radicals. However, one may expect the absolute value of the entropy term for biologically relevant systems to be substantially smaller than that for the model reaction. In the DNA−enzyme complex there are no translational and rotational contributions to the reaction free energy and the term $T\Delta S$ is due to the change in the vibrational entropy which should be relatively small. To estimate the free energy change ($\Delta G$) for the reaction in the bound system, we calculated the cleavage of the U<>U and U<>U$^-$ dimers bridged by a trimethylene group. The reaction entropy is found to be 10.8 and 8.8 cal/(K mol) for the neutral and anionic species, respectively. The entropy term of 10 cal/(K mol) might be considered as the upper bound to the reaction entropy, which corresponds to a contribution of −3 kcal/mol to the reaction free energy. Thus, the driving force of the reaction is determined mainly by the reaction enthalpy ($\Delta H_r$). The calculated absolute values of $\Delta H_r$ are relatively small (see Table 2), therefore one can expect that the reactants and products should be in equilibrium, which is shifted to the monomers for U<>U$^-$ and T<>T$^-$. Since the reaction entropy is only slightly dependent on the composition and on the charge of a pyrimidine dimer, the corresponding values of $\Delta G$ are close to those of the enthalpy. Thus, in the following discussion we will focus mainly on the reaction enthalpy values.

It has been suggested[9] that the large difference in the enthalpy of the splitting reaction for cyclobutane and for the pyrimidine dimers is due to the strain within the dimers. However, a closer analysis reveals that other factors may contribute, as well. In fact, there are two effects which destabilize the dimers: (1) the loss of $\pi$ delocalization energy resulting from the conversion of two $\pi$ bonds in the monomers into two $\sigma$ bonds in the dimers and (2) the electrostatic and steric repulsion between the pyrimidine rings. To evaluate the first factor, we note that the enthalpy contribution of a specific carbon−carbon double bond may be estimated as the difference between the hydrogenation enthalpy of the C=C bond in the molecule under consideration and the corresponding value of a reference system (e.g., the C=C bond in ethylene). The experimental value for the latter is −32.6 kcal/mol,[28] and the AM1 value is rather close, −28.7 kcal/mol. The calculated heat of reaction for the hydrogenation of the C5−C6 bonds in uracil, thymine, and cytosine is −15.9, −13.0, and −10.1 kcal/mol, respectively. The difference between the reaction enthalpies of uracil and thymine, 2.9 kcal/mol, may be attributed to steric repulsion of a methyl group in 5,6-dihydrothymine. By comparing uracil and cytosine with ethylene, we estimate the loss of the delocalization energy for the nucleic bases to be 12.8 and 18.6 kcal/mol, respectively. The delocalization energy for thymine should be rather close to that of uracil. In the dimers, this energy is twice as large, 25.6 kcal/mol for U<>U and T<>T and 37.2 kcal/mol for C<>C. Thus, the decrasing reaction enthalpy for the splitting of pyrimidine dimers relative to the cleavage of cyclobutane (−33.0, −42.3, and −40.8 kcal/mol for U<>U, T<>T, and C<>C, respectively) is determined to a large extent by the loss of the $\pi$ delocalization energy of the monomers. The remainder (−7.4, −16.7, and −3.6 kcal/mol) may be assigned to steric and electrostatic repulsions between the pyrimidine rings in the dimers.

Electron transfer to U<>U and T<>T does not strongly affect the reaction enthalpy according to the AM1 calculations. The cleavage of the dimer anion radicals is calculated to be slightly more exothermic than that of the neutral dimers (Table 2), the differences in the heat of reaction being only 3−4 kcal/mol. This small effect of the electron transfer on the fragmentation enthalpy for U<>U and T<>T is connected to similarly small differences calculated for the electron affinities (EA) of U and T and of their dimers (0.15−0.2 eV). On the other hand, the EA values of C and C<>C are found to differ appreciably, by 0.5 eV. This difference is reflected in the change of the reaction enthalpy: electron transfer to the dimer renders the cleavage of C<>C less exothermic by about 12 kcal/mol, at variance with the findings for U<>U and T<>T.

Recently, conformation and base composition effects on the splitting enthalpy of pyrimidine dimers and of some model systems have been calculated employing the semiempirical PM3 method.[9] It was concluded that the trend of the cleavage enthalpy for a series of neutral dimers is maintained for the corresponding anion radicals. This observation seems to be of limited validity. The present AM1 calculations confirm the previously noted trend for U<>U and T<>T, but are at variance with the aforementioned results for the comparison of C<>C and U<>U. Unlike the splitting of neutral dimers, the reaction enthalpy of the U<>U$^-$ anion radical was calculated to be more exothermic than that of C<>C$^-$. Clearly, when discussing enthalpy trends in a seris of neutral and anion radical dimers, one also has to take variations of the electron affinities into account.

### 5. Cleavage Mechanisms

Two types of mechanisms are conceivable for the splitting of pyrimidine dimers: a concerted cleavage of both interpyrimidine C−C bonds, C5−C5′ and C6−C6′, which takes place in one step via a single transition state as well as a two-step process via a stable intermediate that is separated by transition states from both the reactant and the products. The pyrimidine dimer splitting bears some similarity to the unimolecular cycloreversion of cyclobutane. For the latter molecule, a concerted cleavage reaction is forbidden according to the Woodward−Hoffmann rules[18] and thus is expected to have a very high activation energy. A thorough analysis of nonpolar cycloaddition based on MCSCF calculations leads to the conclusion that the reaction proceeds in a stepwise fashion only, involving a biradical intermediate and two transition states.[12−16] The corresponding reaction barriers remain rather high, about 40−60 kcal/mol.

Experimentally, pyrimidine dimers, both as cation and anion radicals, are highly reactive species which undergo a facile cleavage reaction in a stepwise fashion.[1,4,5] The dimer splitting in the photolyase active site might thus be activated by transferring an electron either to or from the substrate. A thermodynamical estimate shows[5] that the catalytic cofactor FAD in its excited state is able to transfer an electron to, but not from, a pyrimidine dimer. Therefore, the cationic mechanism has been excluded for the photorepair reaction. Nevertheless, we have included its investigation in the present work for comparison with the cleavage of the neutral dimer and of the corresponding anion radical.

Along the two-step reaction path, the following two pathways may be compared (see Scheme 1).

Path 1 is initiated by breaking the C5−C5′ bond which leads to an intermediate of two fragments covalently bound by the

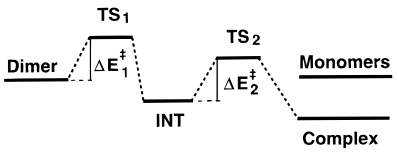


**Figure 3.** Sketch of the energy profile of the two-step splitting rection; the shown relative energies correspond to the cleavage of dimer anion radicals.

**Scheme 1**

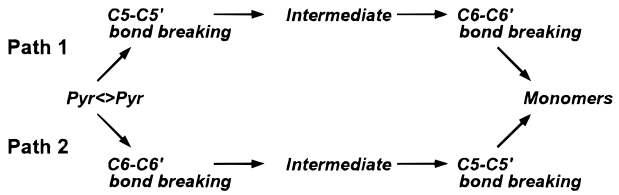


**Table 3.** Energies of the Stationary Points for the Gas Phase Cleavage of Neutral Pyrimidine Dimers and of the Corresponding Anion Radicals[a]

| dimer | path | $TS_1$ ($\Delta E^{\ddagger}_1$) | INT | $TS_2$ | $\Delta E^{\ddagger}_2$ | complex |
|---|---|---|---|---|---|---|
| U<>U | 1 | 36.6 | 31.9 | 39.0 | 7.1 | −6.9 |
| | 2 | 20.9 | 9.5 | 28.3 | 18.8 | −6.9 |
| T<>T | 1 | 28.2 | 19.0 | 26.5 | 7.5 | −12.8 |
| | 2 | 23.0 | 15.6 | 26.0 | 10.4 | −12.8 |
| C<>C | 1 | 34.7 | 27.7 | 33.8 | 6.1 | −12.8 |
| | 2 | 20.9 | 8.8 | 25.0 | 16.2 | −12.8 |
| U<>$U^-$ | 1 | 3.9 | −7.5 | −1.7 | 5.8 | −21.1 |
| | 2 | 20.9 | 6.2 | 8.3 | 2.1 | −21.1 |
| T<>$T^-$ | 1 | 4.7 | −18.5 | −13.2 | 5.3 | −30.6 |
| | 2 | 22.6 | 10.8 | 13.4 | 2.6 | −30.6 |
| C<>$C^-$ | 1 | 16.2 | 4.7 | 10.0 | 5.3 | −13.9 |
| | 2 | 20.4 | 5.3 | 25.3 | 13.9 | −13.9 |

[a] First transition state $TS_1$, intermediate INT, second transition state $TS_2$, and final product complex. (Relative energies with respect to the reactants are in kcal/mol). The barrier heights $\Delta E^{\ddagger}_i$ for both steps are also indicated.

C6−C6′ bond. This latter bond is split during the second step, and either a dipole−dipole complex (if the reactant is a neutral dimer) or an ion−dipole complex (in the case of an anion radical) is formed. Along path 2, the order in which the two bonds are broken is reversed: first C6−C6′ and then C5−C5′. We have examined both pathways of the splitting reaction for the neutral dimers as for their anion and cation radicals, focusing on the location of stationary points on the potential energy surface.

We begin by discussing the results obtained for the neutral dimers U<>U, T<>T, and C<>C. For the concerted process, the stationary points found along the reaction coordinate are of second order (i.e., the Hessian matrix has two negative eigenvalues). Thus, we were unable to locate a "true" transition state in the region of the highest activation energy. This finding is in line with the results of the MCSCF studies on the cycloaddition of two ethylene molecules.[13−15] Because the stationary points of the concerted process have no chemical significance, they will not be discussed here.

To characterize the energetics of the two-step splitting reaction (Figure 3) of the neutral dimers, the calculated gas phase energies of various stationary points as well as the barrier heights for each activation step have been collected in Table 3. Along both pathways, breaking the first dimer bond requires the highest activation energy which therefore determines the reaction rate. In all cases considered, the first barrier $\Delta E^{\ddagger}_1$ is lower for the splitting of the C6−C6′ bond than that for the C5−C5′ bond. The difference between the two values of $\Delta E^{\ddagger}_1$ is about 15 kcal/mol for U<>U and C<>C and 5 kcal/mol for T<>T. Thus, for the splitting of the neutral dimers, pathway 2 is favored over pathway 1. Overall, the energetic characteristics of the dimers U<>U and C<>C turned out to be quite similar. Comparing the results for T<>T and U<>U, one notes that the activation energy for the C5−C5′ bond cleavage ($\Delta E^{\ddagger}_1$ on path 1, $\Delta E^{\ddagger}_2$ on path 2) is reduced substantially (by about 8 kcal/mol according to AM1) in T<>T compared to that in U<>U, while the barriers for the C6−C6′ bond splitting ($\Delta E^{\ddagger}_2$ on path 1, $\Delta E^{\ddagger}_1$ on path 2) are rather similar for both dimers. As one might have expected, this effect of the steric repulsion of methyl groups is similar to that found for the splitting enthalpy (Table 2). The high values of the reaction barriers calculated for all neutral dimers rationalize their kinetic stability, rendering them rather inert toward cleavage despite the overall exothermicity of this reaction. In fact, pyrimidine dimers are experimentally found to be rather stable (e.g., they can be heated to 200 °C without decomposition).[4]

For normal closed-shell molecules, the singlet triplet energy difference $\Delta E_{ST}$ is related to the energy of a HOMO−LUMO excitation (i.e., it is on the order of several electron volts), whereas diradicals feature very small values of $\Delta E_{ST}$.[30] An analysis of the spin density and of the energy difference $\Delta E_{ST}$ for the various reaction species revealed that the transition states and the intermediates during the splitting of the neutral dimers exhibit an electronic structure of biradical nature. As an example, let us consider the dimer U<>U and its cleavage. The calculated value of $\Delta E_{ST}$ for U<>U is 3.14 eV. However, for the intermediates along both pathways 1 and 2, $\Delta E_{ST}$ values of less than 0.04 eV are obtained; therefore, the intermediates clearly are biradicals. The four related transition states have $\Delta E_{ST}$ values of about 0.5 eV and thus also correspond to biradicaloid structures.

The uptake of an electron by the dimers U<>U and T<>T dramatically changes their reactivity. The calculated characteristics of the energy profile (see Figure 3) for the dimer anion radical splitting as well as the barrier heights for each activation step are also displayed in Table 3. Different from the situation of the neutral dimers, the fragmentation of anion radicals is expected to occur via path 1 since the first transition state along path 2 lies at a considerably higher energy. The activation energy $\Delta E^{\ddagger}_1$ of path 1 is quite low, only about 4 kcal/mol for U<>U and 5 kcal/mol for T<>T. The activation barriers for the second reaction step are also rather low, never exceeding 6 kcal/mol. Therefore, these low activation barriers found by AM1 calculations lead to the prediction that T<>T and U<>U anions are highly reactive species and that the corresponding anion radicals undergo a facile stepwise splitting reaction. Thus, while electron transfer to U<>U and T<>T does not have a large effect on the enthalpy of the cleavage reaction (Table 2), the reactivity of the dimers is changed dramatically (Table 3).

On the other hand, the calculated activation energies along the alternative path 2 are substantially larger, more than 20 kcal/mol, rendering this reaction route for the splitting of T<>$T^-$ and U<>$U^-$ rather unlikely. Comparing the activation barriers for the radical anions T<>$T^-$ and U<>$U^-$, one does not notice any significant influence of the methyl groups on the reactivity of the dimer anion radicals. However, there is a noticeable effect on the energy of the intermediate relative to the reactant, in agreement with the previously observed steric substituent effects. The amount of (relative) stabilization of the T<>T intermediate by 11 kcal/mol compared to that of U<>U essentially equals the difference of the splitting enthalpy for the anion radicals T<>$T^-$ and U<>$U^-$, 10.5 kcal/mol.

(30) Yamaguchi, K. *Int. J. Quantum Chem.* **1982**, *22*, 459.

Different from U<>U and T<>T, the reactivity of C<>C is less affected by an electron transfer to the dimer. In C<>C, the values $\Delta E^{\ddagger}_1$ for the first reaction barrier are 16.2 and 20.4 kcal/mol for path 1 and path 2, respectively. Thus, one expects C<>C$^-$ to be relatively inert with respect to a cleavage. This result agrees with the experimental observation[10] that the splitting efficiency of C<>C$^-$ is considerably less than that of T<>T$^-$ and U<>U$^-$.

For the two-step mechanism, there is a special aspect to consider, namely the question of when the back electron transfer from the substrate to the chromophore occurs, before or after the splitting of the intermediate.[1,5] First, one may consider the calculated reaction barriers $\Delta E^{\ddagger}_2$ for the second bond cleavage of the neutral dimer and their anion radicals (Table 3). Despite the fact that the high activation energy $\Delta E^{\ddagger}_1$ of the first bond breaking along path 1 is tremendously reduced through the electron transfer (by 32.7 and 23.5 kcal/mol for U<>U and T<>T, respectively), there is hardly any change in the second activation energy $\Delta E^{\ddagger}_2$ of this path. Thus, the reactivity of the intermediate does not provide a clue to whether the back transfer of the electron to the chromophore occurs at the intermediate or the product stage. However, a comparison of the calculated electron affinities of the *neutral* intermediates and products may be helpful: the lower the ionization potential of an anion (i.e., the smaller the electron affinity of the corresponding neutral molecule), the smaller the energy expense for an back electron transfer from the anion to chromophores. According to the AM1 results, the adiabatic electron affinity of the nucleic bases U, T, and C is almost 1.2 eV (Table 1). For the intermediate formed along path 1, the electron affinity was calculated to be 2.7, 2.6, and 2.7 eV for U<>U, T<>T, and C<>C, respectively. This sharp increase of the electron affinity along the reaction path may be rationalized by the instability of the neutral biradical intermediate. Thus, judging from the relatively high electron affinities of the reaction intermediates, it seems likely that the back electron transfer to the chromophore proceeds after the splitting of the dimer is complete, but not at the intermediate stage of the anion radical.

Since an electron transfer *to* the dimers changes the activation of the fragmentation reaction in such a profound way, it seemed interesting to consider possible effects associated with an electron transfer *from* the dimers as well. Despite a thorough analysis of the potential energy surface of the pyrimidine cation radicals, we were unable to locate any stationary points for the dimer structure containing a cyclobutane-like ring. In other words, removing an electron from the dimer immediately induces the cleavage of one of the interpyrimidine bonds. In fact, the C6−C6′ bond breaks without activation and a structure is found with the two nucleic bases held together by the C5−C5′ bond. This structure is similar to that of the intermediate resulting during the anion radical splitting along path 2. According to unrestricted MP2/6-31G(d) calculations,[17] the optimized geometry of the dimethylcyclobutane cation radical corresponds to a trapeze with one very elongated C−C bond (1.996 Å) while the other three C−C bonds fall in the usual length range. This result is in agreement with our findings that the cyclobutane ring of pyrimidine dimer cation radicals is unstable with respect to a transformation to an open structure. Recently, photochemically induced dynamic nuclear polarization (photo CIDNP) experiments have been performed to study the pyrimidine dimer splitting via a cation radical intermediate.[31] Nonbridged dimer cations were found to be very unstable with a lifetime of less than $10^{-10}$ s. In contrast, 1,1′-trimethylene-bridged dimer cations have a relatively long lifetime. To consider a possible stabilization of the cation radicals due to the bridge, 1,1′-trimethylene-bridged U<>U$^+$ was calculated. AM1 predicts a $C_s$ structure for the cation radical which corresponds to a dimer with a C5−C5′ bond of the usual length (1.551 Å) and a very long C6−C6′ bond of 2.166 Å. Thus, the trimethylene bridge prevents a mutual rotation of pyrimidine bases along the C5−C5′ bond which occurs spontaneously in the nonbridged dimer cation radicals.

The activation energy for the cleavage of the remaining interpyrimidine bond in the cation radicals was found to be rather insensitive to the reactant composition; the calculated AM1 values are 15.2, 14.1, and 14.4 kcal/mol for uracil, thymine, and cytosine cation radicals, respectively. These values are large enough so that one might be able to detect these cationic reaction intermediates experimentally. Thus, different from the dimer anion radicals, the three dimer cation radicals seem to be of comparable reactivity.

In summary, electron transfer to or from a pyrimidine dimer leads to a dramatic reactivity increase of the cycloreversion. The preferred reaction path depends on the direction of the electron transfer. Electron transfer from the chromophore to the dimer is associated with a significant reduction of the C5−C5′ bond strength while the removal of an electron from the dimer leads to a spontaneous C6−C6′ bond breaking.

## 6. Geometries

The gas phase geometry for uracil was obtained by electron diffraction.[32] A comparison of experimental and calculated data for the molecule shows that the deviations are within the experimental errors, except for the C−O bond length and the N1−C2−O bond angle (AM1 overestimates this bond length by 0.03 Å and the bond angle by 3.6°). There are no structural data for dimers in the gas phase; however, the crystal structures of the cis−syn dimer of uracil[33] and of the photodimer of 1,3-dimethylthymine[34] have been investigated. An important structural feature of the pyrimidine dimer is the four-membered cyclobutane-like ring. The AM1 method used in the present study (as do MNDO and PM3) predicts a planar ring ($D_{4h}$) for cyclobutane, different from the puckered ring structure ($D_{2d}$) observed experimentally[35] and found in ab initio calculations.[17] This effect is due to the fact that the cyclobutane ring in AM1 is described as more rigid than found experimentally or with ab initio calculations. However, the energy difference between the planar and puckered structures is quite small, about 2 kcal/mol, according to a MP2/6-31G* calculation[17] and 1.4 kcal/mol according to experiment.[35] Similar to the findings for cyclobutane, AM1 yields a structure with a planar four-membered ring also for the pyrimidine dimers, at variance with the puckered ring observed in the crystal structures of the uracil dimer[33] and of the dimethylthymine dimer[34] which exhibit dihedral angles of 25° and 27°, respectively. In a Hartree−Fock geometry optimization employing the 6-31G(d) basis set, a dihedral angle of 20.5° was calculated. This raises the question of how important the structural deviation obtained with AM1 is for calculated energies and geometries of the dimers. To estimate this influence of ring planarity, a hypothetical structure with a planar four-membered ring was optimized at HF/6-31G(d) level assuming $C_s$ symmetry of the dimer. The

(31) Powels, P.; Hartman, R.; Rose, S.; Kaptein, R. *Photochem. Photobiol.* **1995**, *61*, 563.

(32) Ferenczy, G.; Harsanyi, L.; Rozsondai, B.; Hargittai, I. *J. Mol. Struct.* **1986**, *140*, 71.

(33) Adman, E.; Gordon, M. P.; Jensen, L. H. *J. Chem. Soc., Chem. Commun.* **1968**, 1019.

(34) Camerman, N.; Camerman, A. *J. Am. Chem. Soc.* **1970**, *92*, 2523.

(35) Egava, T.; Fukuyama, T.; Takabayashi, F.; Kambara, H.; Ueda, T.; Kuchitsu, K. *J. Chem. Phys.* **1987**, *86*, 601.

**Table 4.** Structural Changes of the Various Pyrimidine Dimers (Neutral and Anion Radical) along Reaction Path 1 Exemplified by the C5−C5′ and C6−C6′ Distances (Å)

| | U<>U | | T<>T | | C<>C | |
|---|---|---|---|---|---|---|
| | C5−C5′ | C6−C6′ | C5−C5′ | C6−C6′ | C5−C5′ | C6−C6′ |
| | | | neutral Dimer | | | |
| reactant | 1.550 | 1.597 | 1.569 | 1.592 | 1.548 | 1.595 |
| $TS_1$ | 2.229 | 1.592 | 2.179 | 1.586 | 2.174 | 1.593 |
| INT | 2.889 | 1.589 | 3.054 | 1.593 | 2.951 | 1.591 |
| $TS_2$ | 2.981 | 1.889 | 3.157 | 1.913 | 3.018 | 1.882 |
| product complex | 6.292 | 6.506 | 5.082 | 5.550 | 6.085 | 7.413 |
| | | | anion Radical | | | |
| reactant | 1.557 | 1.594 | 1.572 | 1.592 | 1.555 | 1.594 |
| $TS_1$ | 1.758 | 1.589 | 1.763 | 1.585 | 1.985 | 1.587 |
| INT | 2.941 | 1.603 | 3.116 | 1.607 | 2.964 | 1.602 |
| $TS_2$ | 3.079 | 1.921 | 3.263 | 1.917 | 3.171 | 1.902 |
| product complex | 4.585 | 4.712 | 4.637 | 4.707 | 5.815 | 7.321 |

bond lengths and bond angles are quite similar in the optimized and the planar dimer structures. At the MP2 level, the energy of the U<>U dimer with a planar ring geometry was calculated 6.9 kcal/mol above that of the optimized dimer. Furthermore, the AM1 energies of the planar structure of cyclobutane, U<>U, and U<>$U^-$ are 5.2, 4.6, and 3.3 kcal/mol, respectively, below the corresponding puckered structures (with the dihedral angle of 20.5° calculated by the HF method in U<>U). A comparison of these energy differences leads to the conclusion that the alignment effect is 0.6 kcal/mol in U<>U and 1.9 kcal/mol in U<>$U^-$. Thus, the aligned polar groups in dimers do not seem to exert any substantial destabilizing effect.

According to the present AM1 calculations, the closest interatomic contact between the methyl groups in T<>T and in the corresponding dimer anion radical is about 2.9 Å. The corresponding distance in the crystal structure is about 3.0 Å.[31] Since a normal van der Waals contact between methyl groups is nearly 4 Å, the observed overcrowding results in an additional strain of about 10 kcal/mol which is reflected in the change of the reaction enthalpy (see Table 2).

While electron transfer to the dimers significantly increases their reactivity with respect to C5−C5′ bond cleavage, it hardly affects the bond distances. According to AM1 (see Table 4), the C5−C5′ bond length is 1.569 Å in the neutral T<>T dimer and 1.572 Å in the dimer anion radical. Very similar bond distances are calculated for U<>U and C<>C as well as for their anions. Therefore, comparing the C5−C5′ bond lengths in the neutral dimers and in the corresponding anion radicals, one would hardly expect such a dramatic bond activation due to the reduction of the dimer.

Finally, we consider the change of the dimer geometry along the pathway of the cleavage reaction (see Figure 4 and Table 4). In the neutral dimers, the transition state structure $TS_1$ on the way to the C5−C5′ bond splitting lies very high in energy and a considerable elongation of the corresponding bond distance is required to reach the transition state. The difference in the C5−C5′ bond length from the dimer ground state to $TS_1$ is about 0.5−0.6 Å. On the other hand, in the U<>$U^-$ and T<>$T^-$ anion radicals, the formation of the transition state structure is associated with a moderate increase of the bond length by only about 0.2 Å, while for the less reactive C<>C anion radical the difference is about 0.4 Å. In intermediates, the C5−C5′ distance is 2.9−3.1 Å and is only slightly affected by the electron transfer. For the intermediate, the two pyrimidine residues are rotated with respect to each other; the dihedral angle N1−C6−C6−N1 is about 70°. Since one assumes an angular displacement of 36° for adjacent nucleic bases in DNA,[36] there seem to be several constraints for the intermediate structure in the substrates of the actual photorepair process, resulting in an increased relative energy of the intermediate. In the transition state $TS_2$, the C6−C6′ bond length amounts to 1.9 Å in both neutral and anionic species.

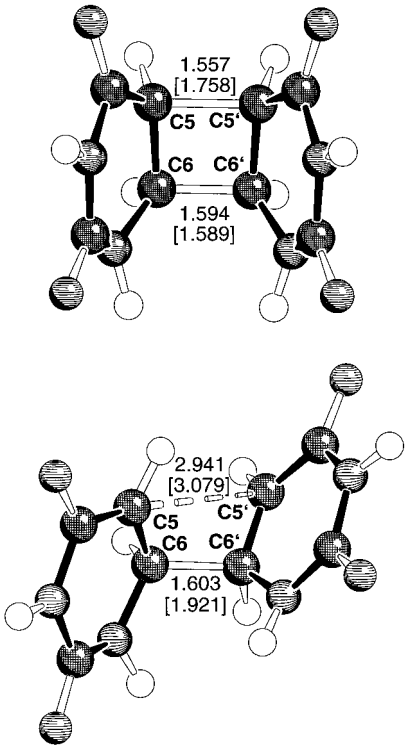


**Figure 4.** SCHAKAL representations[38] of the calculated structures of stationary points on the potential energy surface for the cleavage of the uracil dimer anion radical along path 1. Upper panel: reactant and, in brackets, transition state $TS_1$ corresponding to the splitting of the C5−C5′ bond. Lower panel: intermediate and, in brackets, transition state $TS_2$ corresponding to the splitting of the C6−C6′ bond. Bond lengths are in angstroms.

## 7. Charge and Spin Delocalization

So far, this account focused on locating stationary points of the pyrimidine dimer cleavage reaction and on estimating reaction barriers in order to identify preferential reaction pathways. For our understanding of the photoinduced DNA repair, it is also important to rationalize the chemical reactivity and its dependence on the electronic structure of the various molecules. In particular, it seems desirable to explain why electron transfer to the dimer facilitates the C5−C5′ bond breaking while removing an electron from the dimer leads to a cleavage of the C6−C6′ bond.

We start with a qualitative consideration of the anionic and cationic mechanisms (Figure 5). Addition of an electron to the dimer places a negative charge and an unpaired electron on the C4═O fragment. The spin densities are $\rho$(C4) = 0.56 and $\rho$(O) = 0.31. The spin density at C4 induces $\pi$ bonding with C5 and a concomitant cleavage of the C5−C5′ bond. In the resulting intermediate, the unpaired electron localizes on C5′ of the second ring, giving $\rho$(C5′) = 0.98 and, in turn, induces the formation of a conjugated double bond C5′═C6′ with a subsequent breaking of the C6−C6′ $\sigma$ bond. A neutral pyrimidine base and a resonance stabilized anion radical are produced.

(36) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984.

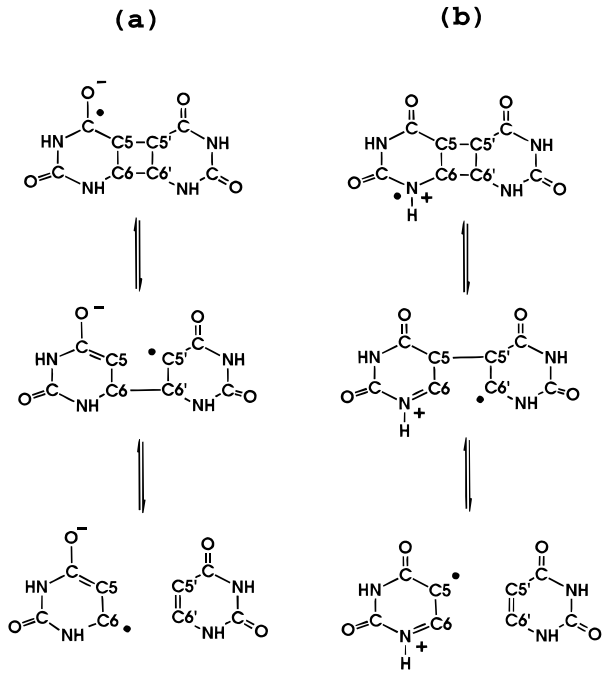


**Figure 5.** Sketch indicating the localization of the unpaired electron (a) in the anion dimer radicals and (b) in the cation dimer radicals during the cleavage reaction.

We now turn to the dimer cation radicals (see Figure 5). When U<>U is ionized, an electron is removed from one of the N1 lone pairs. The spin density localized mainly on the atom N1, giving $\rho(N1) = 0.59$, induces $\pi$ bonding to C6 and results in the cleavage of the C6−C6′ bond. In the resulting intermediate, the unpaired electron localized on C6′, giving $\rho(C6') = 0.89$, facilitates the cleavage of the bond C5−C5′ which finally leads to the products.

Thus, the order of the bond cleavage is determined by the spin density localization in the reactant, and the splitting of the $\sigma$ bond occurs at the center adjacent to that atom where the unpaired electron will be (to some extent) localized. The localization of the unpaired electron on the C4═O group in the dimer anion radical correlates with the splitting of the C5−C5′ bond. On the other hand, the more the spin density localizes on N1 in the dimer cation radical, the easier the initial C6−C6′ bond cleavage. How facile the second $\sigma$ bond splits in the intermediate is determined by the localization of the unpaired electron on the C5′ (or C6′) atom of the "uncharged" fragment in the vicinity of the bond to break.

In the first and second reaction steps (i.e., when going from the reactant to the intermediate and from the intermediate to the monomers), the unpaired electron moves between the two nucleic bases (see Figure 5). Thus, in the transition states separating these stationary states, the spin density (and the charge) should be delocalized over both subunits; this expectation is confirmed by the AM1 results. As these pyrimidine dimers are constructed from two similar moieties, we may quantify the charge delocalization in a simple fashion by the difference between the charges on each pyrimidine subunit: the smaller the difference, the larger and charge delocalization within the system. In Table 5 the fragment charges are listed for various stationary points along the path 1 of the dimer cleavage reaction, comparing U<>U$^-$ and C<>C$^-$. We refrain from presenting the results for thymine since the introduction of two methyl groups in U<>U$^-$ has no major effect on the charge distribution in the reaction species.

In the reactants (e.g., in the U<>U anion radical), one observes a clear localization of the ionic charge on one of two

**Table 5.** Charges $q$ (au) of the Pyrimidine Fragmetns in Dimer Anion Radicals at Various Stationary Points along the Reaction Path 1

| | U<>U$^-$ | | C<>C$^-$ | |
|---|---|---|---|---|
| stationary point | $q$ | $q'$ | $q$ | $q'$ |
| reactant | −0.811 | −0.189 | −0.832 | −0.168 |
| $TS_1$ | −0.512 | −0.488 | −0.619 | −0.381 |
| INT | −0.794 | −0.206 | −0.823 | −0.177 |
| $TS_2$ | −0.526 | −0.474 | −0.607 | −0.393 |
| product complex | −0.984 | −0.016 | −0.980 | −0.020 |

the fragments: $q(U) = -0.81$ and $q(U') = 0.19$. This difference in the fragment charge distribution diminishes almost completely in the transition state $TS_1$: $q(U) = -0.51$ and $q(U') = -0.49$. Thus, a substantial charge delocalization occurs on the way to the barrier. In the intermediate (INT), the charge delocalization is relatively small ($q(U) = -0.79$ and $q(U') = -0.21$), but increases again in the second transition state ($TS_2$) ($q(U) = -0.53$ and $q(U') = -0.47$). The cleavage reaction terminates in a product complex where the charge is essentially localized on one pyrimidine fragment.

Comparing the charge separation on the fragments for two different pyrimidine dimer anion radicals, U<>U$^-$ and C<>C$^-$ (Table 5), we note that for C<>C$^-$ the charge delocalization in both transition states $TS_1$ and $TS_2$ is still present but is less pronounced than in U<>U$^-$. This finding on the charge separation correlates with higher values of the activation barriers for the cleavage reaction.

The amino acid residues in the reaction pocket of the photolyase were found to be ideally suited for accepting a pyrimidine dimer.[7] On the side which interacts with the hydrophobic cyclobutane ring of the substrate, the residues are hydrophobic, and on the other side, they are polar and suited to interact with the nitrogen and oxygen atoms of the nucleic bases. Since the active site of the enzyme is designed to initially bind a neutral pyrimidine dimer, one may speculate that dimer structures corresponding to anion radicals with a delocalized charge might be bound better than those with a localized ion charge. In this way, the enzyme hole is expected to additionally stabilize transition state structures relative to the dimer anion radical, thus possibly leading to even lower activation barriers for the cleavage reaction.

It is interesting to extend the charge distribution analysis to the pyrimidine dimer cation radicals. Since the cyclobutane ring of the cation radicals is not stable with respect to a breaking of the C6−C6′ bond (see section 5), two characteristic "model" structures were chosen along reaction path 2. In the model for the reactants, the C6−C6′ bond length was fixed at 1.56 Å, and in the model for the "transition state" $TS_1$, at 1.80 Å. All other structural parameters were optimized. While the fragment charges differ considerably for the reactant U<>U$^+$ ($q(U) = +0.67$ and $q(U') = +0.33$), they are evenly balanced in the assumed "transition structure" $TS_1$ ($q(U) = +0.50$ and $q(U') = +0.50$). Thus, for both the dimer cation and anion radicals, the reaction path preference is associated with a delocalization of the ionic charge over both nucleic bases in the structures modeling the transition states of the cleavage reaction.

## 8. Solvent Effects

Kim and Rose found that the pyrimidine dimer splitting which includes the formation of the anion radicals dramatically depends on the dielectric constant of the solvent.[37] In nonpolar solvents,

(37) Kim, S.-T.; Rose, S. D. *J. Photochem. Photobiol., B* **1992**, *12*, 179.
(38) Keller, E. *Program SCHAKAL92*; Kristallographisches Institut der Universität Freiburg: Germany, 1992.

**Table 6.** Calculated Free Energy $\Delta G$ of Solvation (kcal/mol) in Hexane and Dimethylformamide (DMF) for Various Pyrimidine Dimer Anion Radicals at the Stationary Points along Path 1 of the Cleavage Reaction

| | U<>U$^-$ | | T<>T$^-$ | | C<>C$^-$ | |
|---|---|---|---|---|---|---|
| | hexane | DMF | hexane | DMF | hexane | DMF |
| reactant | −35.8 | −84.2 | −34.8 | −83.0 | −35.5 | −85.5 |
| $TS_1$ | −32.1 | −76.8 | −33.6 | −81.2 | −34.0 | −81.5 |
| INT | −31.7 | −73.4 | −30.6 | −71.1 | −35.6 | −84.3 |
| $TS_2$ | −30.3 | −69.7 | −29.4 | −67.9 | −34.0 | −80.5 |
| monomers | −38.3 | −88.0 | −37.0 | −85.0 | −34.4 | −82.6 |

the cleavage reaction is much more efficient than in polar solvents, reaching a maximum for the least polar solvent investigated. It has been suggested that this observation may be rationalized by the fact that the back electron transfer from the dimer to the chromophore is slowed in nonpolar solvents, thus increasing the efficiency of the cleavage reaction.

To contribute to a discussion of solvent effects on the splitting reaction itself, we have carried out self-consistent reaction field model calculations on the energy profiles for cleavage reactions that take place in the solvents hexane and DMF. The corresponding free energies of solvation are listed in Table 6. One notes that the absolute values of the solvation energies increase with the dielectric constant, $\epsilon$(hexane) < $\epsilon$(DMF). The contribution of a small element of the solute surface to the solvation energy is proportional to the square of the charge of this surface element.[23−25] Thus, for systems of similar spatial extension one expects the solvation energy to decrease when the charge becomes delocalized. Another factor influencing the solvation energy is the solute surface available to the solvent, which in turn depends on the size and the shape of the solute molecule. Both factors vary along the reaction coordinate and therefore affect the relative energies of the stationary points. As noted previously, the charge delocalization is larger in the transition states than those in the reactants, intermediates, and products. Therefore, when going from a reactant dimer to the transition state $TS_1$, the solvation energy decreases due to the fact that the charge delocalizes over both fragments of the anion radical (Table 5). However, while the charge is again more localized in the intermediate than in the preceding transition state $TS_1$, the observed lowering of the solvation energy is caused by a decreasing solute surface. The reduction of the solvation energy in the transition state $TS_2$ following the intermediate is again due to the charge delocalization because both stationary states exhibit a similar molecular structure. Since the solvation energy of the dimer radicals U<>U$^-$ and T<>T$^-$ is smaller than that of the corresponding products, the exothermicity of the cleavage is found to increase with the polarity of solvents. On the other hand, a polar medium slightly reduces the exothermicity of the splitting reaction for C<>C$^-$.

The resulting solvent effects on the reaction barriers for the dimer anion radical splitting in various solvents are collected in Table 7. One notes that the free energies of activation $\Delta G^{\ddagger}_1$ and $\Delta G^{\ddagger}_2$ increase with the dielectric constant of the solvents, and one expects the reactivity of dimer anion radicals to be lower in polar solvents than in nonpolar solvents or in the gas phase. Therefore, higher activation barriers for the cyclobutane ring cleavage reaction may be invoked to rationalize the experimentally observed reduced efficiency of the dimer splitting in polar media, in addition to the medium effect on the back electron transfer from the dimer to the chromophore.[37]

**Table 7.** Comparison of Calculated Free Energies $\Delta G^{\ddagger}$ of Activation (kcal/mol) along Both Reaction Pathways of the DImer Cleavage for Various Pyrimidine Dimer Anion Radicals in the Gas Phase and in the Solvents Hexane and Dimethylformamide (DMF)

| | | path 1 | | | path 2 | | |
|---|---|---|---|---|---|---|---|
| dimer | | gas phase | hexane | DMF | gas phase | hexane | DMF |
| U<>U$^-$ | $\Delta G^{\ddagger}_1$ | 4.9 | 8.6 | 12.3 | 22.9 | 23.5 | 23.7 |
| | $\Delta G^{\ddagger}_2$ | 5.9 | 7.3 | 9.6 | 1.7 | 3.0 | 4.9 |
| T<>T$^-$ | $\Delta G^{\ddagger}_1$ | 7.8 | 9.0 | 9.6 | 22.5 | 23.1 | 24.0 |
| | $\Delta G^{\ddagger}_2$ | 5.6 | 6.8 | 8.8 | 3.8 | 7.2 | 12.5 |
| C<>C$^-$ | $\Delta G^{\ddagger}_1$ | 16.3 | 17.8 | 20.3 | 19.8 | 20.5 | 21.5 |
| | $\Delta G^{\ddagger}_2$ | 5.4 | 7.0 | 9.2 | 14.3 | 18.0 | 19.8 |

## 9. Conclusions

Using AM1 UHF calculations, we have located stationary points on the potential energy surface for the cleavage reaction of uracil, thymine, and cytosine dimers. From these results a concerted mechanism seems unlikely. The splitting of the neutral dimers occurs via a biradical structure formed after opening the C6−C6′ bond. The activation barriers for the first bond breaking exceeds 20 kcal/mol, and the activation barrier of the breaking of the second bond is significantly lower. For U<>U and T<>T, the transfer of a single electron to or from the pyrimidine dimers leads to a considerable activation of the cleavage reaction. The sequence of the bond breaking in the dimer anion radicals is found to be opposite to that in cation radicals. While the C5−C5′ bond splits first in the cleavage reaction of the anion radicals, the ring opening of the cation radical is predicted to start by breaking the C6−C6′ bond. The cleavage of the first bond in the anion radicals U<>U$^-$ and T<>T$^-$ is associated with an activation barrier of about 5 kcal/mol, while the activation energy for C<>C$^-$ is considerably larger (by about 16 kcal/mol). Thus, the splitting efficiency is found to significantly depend on the nucleic base (T<>T$^-$ ≅ U<>U$^-$ ≫ C<>C$^-$), in agreement with experiment.[10] According to the AM1 results, electron transfer to the uracil and thymine dimers renders the cleavage reaction somewhat more exothermic but leads to a reduction of the exothermicity for the cleavage of C<>C$^-$. Due to the high calculated ionization energies of the intermediate anion radicals, the back electron transfer in the DNA photorepair from the substrate to the cofactor is not expected to occur after the splitting of the first dimer bond but only as the last step after completion of the ring cleavage. The analysis of calculated solvent effects shows that while the splitting of the U<>U and T<>T anion radicals becomes more exothermic in a polar medium, the activation barriers also become higher than those in a nonpolar medium, reducing the splitting efficiency of the cleavage reaction. It was found that the introduction of methyl groups in U<>U to form T<>T causes only small changes in the dimer reactivity. On the other hand, the dimer C<>C is significantly different from both U<>U and T<>T with respect to many characteristics of the dimer cleavage reaction.

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