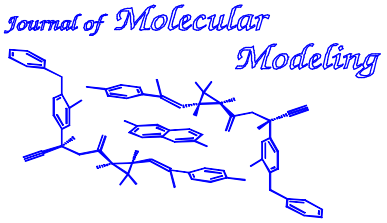
J. Mol. Model. **1995**, *1*, 18–21.



A PM3 Study of the Effect of Conformation on the Enthalpy of Splitting of Cyclobutane Type Pyrimidine Dimers.

Paul F. Heelis

Faculty of Science, Health and Medical Studies, Mold Rd, North East Wales Institute, Wrexham, Clwyd, LL11 2AW, United Kingdom (heelisp@newi.ac.uk)

Received: 15 November 1994 / Accepted: 2 January 1995

Abstract

Cyclobutane type pyrimidine dimers are the most common product of UV irradiation of DNA. This potentially lethal damage is reversed by photolyase enzymes, which cleave the cyclobutane ring of the pyrimidine dimer by electron transfer from the excited state of the flavin cofactor of the enzyme to the dimer.
Several studies have suggested that the energy-wasting reverse electron transfer process may be kinetically competitive with ring-opening. One of the principal factors governing the rate of the splitting reaction is the degree of strain in the cyclobutane ring, which is directly reflected in the enthalpy of the splitting process. Hence, the present work utilizes the MNDO-PM3 method to examine the influence of base composition and stereochemistry on the enthalpy of cleavage of the cyclobutane ring of various pyrimidine dimers.

Keywords: DNA, Pyrimidine cyclobutane dimers, PM3

Introduction

Solar Ultraviolet light (290-400 nm) is known to have mutagenic, carcinogenic, and lethal effects[1]. The main targets of UV are the purine and pyrimidine DNA bases, with cyclobutane type pyrimidine dimers (Pyr<>Pyr) being by far the most common product[2]. Fortunately, this potentially lethal damage is reversed by a number of enzymes[3], of which the photolyases are the most interesting because they utzlise the energy of a photon of light (300-500nm) to cleave the cyclobutane ring of the pyrimidine dimer[4] directly without excision of the damaged base.

Picosecond flash photolysis, time resolved esr and thermodynamic considerations suggest that the photoenzymic pathway proceeds by electron transfer from the excited state of the flavin cofactor of the enzyme to the dimer[5]. The thus formed dimer anion radical then splits to form a monomer and monomer anion radical (eq.1). In principle both a pericyclic, i.e.concerted process or a totally non-concerted splitting proceeding by an initial cleavage of the 5-5' bond of the cyclobutane ring are possible.

The rate of the forward electron transfer (k_{et}, eq.1) from the flavin cofactor to the pyrimidine cyclobutane dimer has been measured directly as $6x10^9$ s^{-1} and occurs over a distance of 14 Å [5].

$$\text{Donor} + \text{Dimer} \underset{k_{bet}}{\overset{k_{et}}{\rightleftharpoons}} \text{Dimer}^{\bullet-} \xrightarrow{k_{spl}} \text{Monomer} + \text{Monomer}^{\bullet-} \qquad (1)$$

Recently, the splitting rate (k_{spl}, eq.1) of cis-syn thymine-thymine dimers in a model system[6] using dimethylaniline as electron donor was determined to be 10^6 s^{-1}. This relatively slow rate of cyclobutane monomer formation suggests that the back electron transfer process (k_{bet}, eq.1, i.e return of the electron to the donor without splitting) could be competitive with ring opening.

Thus a structural dependence of the rate of dimer splitting might thus explain the wide variations observed[4] for different pyrimidine dimers in the overall efficiencies of monomerisation, both in the enzyme catalyzed and model compound photocatalyzed reactions[5].

One of the principal factors governing the splitting reaction is the degree of strain in the cyclobutane ring, which would be expected to be reflected in the enthalpy of the splitting process. Hence, the present work utilizes the MNDO-PM3 method to examine the influence of base composition and stereochemistry on the enthalpy of cleavage of the cyclobutane ring of various pyrimidine dimers.

Calculations

The calculations were performed using MOPAC version 6.49 in the April 1994 release of Chem-X (Chemical Design Ltd). All geometric parameters were optimized unless otherwise stated. The keywords PRECISE and MMOK were included to increase precision 100 fold and to increase the rotational barrier in the peptide linkage, respectively. RHF calculations were performed on the neutral molecules and UHF calculations on the anion radicals using CHARGE=-1.

The enthalpy of splitting (ΔH_{spl}) was calculated as follows,

$$(\Delta H_{spl}) = \Delta H_f \text{ (monomer(s))} - \Delta H_f \text{ (dimer)}$$

Results and Discussion

Enthalpies of splitting (ΔH_{spl}) calculated using the MNDO/PM3 Hamiltonian are listed in Table 1.

As can be seen, splitting of the cis-syn uracil dimer (**1a**, ΔH_{spl}= 4 $kJ \cdot mol^{-1}$) is considerably more exothermic than the splitting of cyclobutane itself (**5**) (calculated = +154, experimental 75 $kJ \cdot mol^{-1}$). This undoubtedly reflects the large degree of strain involved in this configuration due to the interaction of the pyrimidine rings. Although the trans-syn stereoisomer of uracil is 18.7 $kJ \cdot mol^{-1}$ more stable than the cis-syn isomer because of the reduced interactions of the pyrimidine rings, there is still a difference of ~ 131.4 $kJ \cdot mol^{-1}$ compared to cyclobutane. As would be expected, the introduction of a methyl group at C(5),C(5') as in thymine (**1b**, **2b**), increases the exothermicity of splitting for both the cis-syn and trans-syn isomers.

Table 1. *Enthalpy of splitting of pyrimidine cyclobutane type dimers.*

Compound	R_1	R_1'	R_2	R_2'	**ΔH_{spl}($kJ \cdot mol^{-1}$)**	
					RHF calc. on Neutral molecule	**UHF calc. on anion radical**
1a	H	H	H	H	4.0	-16.4
1b	H	H	Me	Me	-27.1	-36.1
2a	H	H	H	H	22.7	-2.9
2b	H	H	Me	Me	-12.1	-26.1
3	-	-	-	-	-23.0	
4	-	-	H	H	-26.0	
5	-	-	-	-	154.1	
6	-	-	-	-	76.3	

The differences in ΔH_{spl} between cis-syn and trans-syn stereoisomers correlates quite well with the experimental observation[7] that X-ray irradiation at 77K of cis-syn N(1),N(1'),N(3),N(3') tetramethyluracil dimer results in splitting too fast to measure by esr detection (i.e. radiolysis formed the monomer radical anions directly). In marked contrast, the trans-syn isomer gave a novel isotropic 19G epr doublet, assigned to an asymetric dimer radical anion, which was stable for several minutes. In fact, if a small increase in strain enhances the splitting rate, then this has important biological implications as the cis-syn dimer in DNA has been shown to be puckered due to the deformation of the double helix[4].

Compound **6** was recently shown to resist splitting in a model system involving dimethylaniline as electron donor, even though one-electron reduction had almost certainly taken place[8]. The calculated value of ΔH_{spl} suggests that the strain is indeed much lower in this case due to the presence of only one pyrimidine ring. Hence back electron transfer from the dimer anion radical to the electron donor cation radical would be more likely to predominate over cyclobutane ring cleavage.

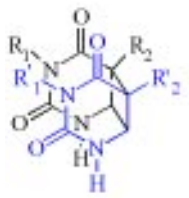
Experimental values of ΔH_{spl} are available for only one cyclobutane type pyrimidine dimer[9] from a calorimetric study of the heats of combustion of an N(3)-N(3') propane bridged compound **4**. A value of ΔH_{spl} = -109 kJ·mol^{-1} was obtained. The authors suggested that the greater exothermicity of splitting of the pyrimidine dimer relative to cyclobutane itself, involves a contribution from the extra delocalisation of the alkene formed in the former case, in addition to the pyrimidine ring interactions. While this indeed would be expected to be the case, the calculated value of ΔH_{spl} = -26 kJ·mol^{-1} for compound **4** suggests that at least some of the increase in exothermicity of splitting is due to constraints imposed by the N(3)-N(3') bridge rather than solely the cyclobutane ring.

As reported by Stewart[10], the PM3 method performs poorly in calculating the geometry of the cyclobutane ring, generally producing a planar ring unlike the puckered ring observed experimentally (torsion angle = 27^{o}). In this work, cyclobutane torsion angles of 0.13-6.51^{o} where obtained. In addition, a search of the Cambridge structural database revealed a number of pyrimidine cis-syn cyclobutane dimers. Cyclobutane torsional angles varying from 2.6^{o} [11] to 19.6^{o} [12] were observed. This inability of the PM3 method to model the cyclobutane ring correctly is probably the main reason for the discepency between the calculated and observed values for ΔH_{spl} noted above. In fact, if ΔH_{spl} is calculated with the ring torsion angle constrained to 24.5^{o}, but with all other geometric parameters optimized the value of ΔH_{spl} of both the N(3)-N(3') bridged structure **1c** and cyclobutane itself approaches the experimental values observed.

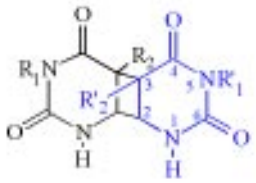
In an NOE study[13] of a decamer containing the cis-syn thymine dimer, Taylor et al., reported that the two thymine rings are twisted in a right handed fashion as are the bases of the B-form of DNA. In contrast, the crystal structure of the thymidine cis-syn dimer[14] is twisted in the opposite fashion. Taken together with the general variation of the cyclobutane torsion angle as revealed by the search of the Cambridge database, it is clear that cyclobutane ring is quite flexible. It follows that the extra strain imposed on it by the helical structure of DNA would be reflected in a greater ΔH_{spl}. This could result in a more efficient splitting of the dimer and hence a greater efficiency of repair. In fact, the quantum efficiency of the reverse reaction, (the photochemical formation of thymine dimers in DNA) is known to be highly dependent upon the DNA conformation[15].

As dimer splitting in both the enzymatic and many of the model systems studied actually proceeds by a one-electron reduction of the dimer to form the anion radical, the calculations were repeated for this state using the UHF formalism and the results are shown in table 1. Values of ΔH_f obtained using the RHF and UHF methods are not comparable due to the treatment of spin polarization effects by the latter. However, values of ΔH_{spl} are more comparable as such effects may cancel out to some extent as the ΔH_f of both the product and reactant would be altered. Most importantly, the general dependence of ΔH_{spl} on the structure i.e cis-syn>trans-syn and thymine>uracil is maintained in the radical state.

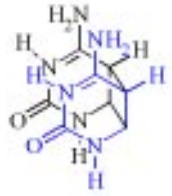
Cyclobutane Pyrimidine dimers included in this study



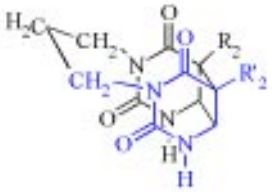
1 (cis-syn)



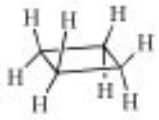
2 (trans-syn)



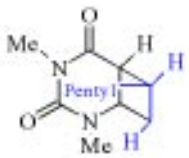
3 (cis-syn)



4



5



6

Acknowledgement: The work was supported in part by a grant from the Wellcome Trust (grant 036648)

References

1. Jagger, J. in *Solar UV -Actions on Living Cells*, Preager, N.Y.1985.
2. Patrick, M.H.; Rahn, R.O. in *Photochemistry and Photobiology of Nucleic Acids*, Wang,S.Y. (ed.) 35-95, Vol. 2 , Academic Press, N.Y. 1976.
3. Sancar, A.; Sancar, G.B. , *Annu. Rev. Biochem.* **1988**, *57*, 29.
4. Sancar, A. in *Advances in Electron Transfer Chemistry* (ed Mariano, P.E.), 215-272 Vol.2, JAI Press, London 1992.
5. Heelis, P.F.; Kim, S.-T.; Okamura, T.; Sancar, A. *J. Photochem. Photobiol.* **1993**, *17*, 219.
6. Yeh, S.-R.; Falvey, D.E. *J.Am.Chem.Soc.* **1991**, *113*, 8557.
7. Podmore, I.D.; Heelis; .F.; Symons M.C.R.; Pezeshk, A. *Chemical Communications*, **1994**, 1005.
8. Yang, D-Y.; Begley, T.P. *Tetrahed., Lett.* **1993**, *34*, 1709.
9. Diogo, H.P.; Dias, A.R.; Dhalla, A.; Minas da Piedade, M.E.; Begley, T.P. *J.Org.Chem.* **1991**, *56*,7340.
10. Stewart, J.J.P. *J.Comp.Aided.Mol.Design.* **1990**, *4*, 1.
11. D.Burdi,T.P.Begley *J.Am.Chem.Soc.* **1991**, *113*, 7768.
12. Camerman, N.; Camerman, A. *J.Am.Chem.Soc.* **1970**, *92*, 2523.
13. Taylor, J.S.; Garrettt, D.S.; Brockie, I.R.; Svoboda, D.L.; Telser, J. *Biochemistry* **1990**, *29*, 8858.
14. Cadet, J.; Voituriez, L.; Hruska, F.E.; Grand, A. *Biopolymers* **1985**, *24*, 897.
15. Fisher, G.J.; Johns, H.E. in *Photochemistry and Photobiology of Nucleic Acids*, Wang,S.Y. (ed.) 35-95, Vol. 2 , Academic Press, N.Y. 1976.