Photochemistry and Photobiology, 1996, 64(5): 764–768

Model Studies of DNA Photorepair: Enthalpy of Cleavage of a Pyrimidine Dimer Measured by Photothermal Beam Deflection Calorimetry

Michael P. Scannell, Syun-Ru Yeh and Daniel E. Falvey*
Department of Chemistry and Biochemistry, University of Maryland, College Park, MD, USA

Received 8 April, 1996; accepted 9 July 1996

ABSTRACT

The enzyme DNA photolyase mediates the repair of pyrimidine dimers. This repair step, a net retro [2+2] reaction, proceeds through either the cation or anion radical of the pyrimidine dimer. In order to understand how electron transfer makes the repair process possible, its energetics have been examined by photothermal beam deflection calorimetry, fluorescence quenching and quantum yield studies. The enthalpy for the cleavage reaction of *cis-syn* 1,3-dimethylthymine dimer itself was found to be −19 kcal/mol. In addition, from the redox potentials, the enthalpies for the cleavage reactions of the dimer cation radical and the anion radical were determined to be −19 kcal/mol and −28 kcal/mol, respectively.

INTRODUCTION

Sunlight-induced damage to DNA is becoming more prevalent with the depletion of the ozone layer (1). The most lethal type of damage is pyrimidine dimerization, and the most prevalent of the pyrimidine dimers are thymine–thymine dimers. These are formed by a formal [2+2] cycloaddition between the 5,6 double bonds of adjacent thymines. In single-celled organisms this damage is reversed by exposure to near UV/visible light, and is catalyzed by the enzyme DNA photolyase (2–5). The initial step in the repair pathway is proposed to be a photoinduced single electron transfer from the enzyme to the dimer (6–10). The splitting of the dimer anion radical is very rapid, having a lifetime of 500 ns (11). Electron transfer from the dimer to the enzyme has also been suggested as a possible pathway (12–14). Although the lifetime of the dimer cation radical is not known, high quantum efficiencies observed in model systems suggest that it could occur on a similar timescale (15–16). In either case, the rate of photorepair is accelerated by electron transfer.

To understand the rate acceleration produced by electron transfer, the energetics of the bond-cleaving step have been examined. Reported herein are cleavage enthalpies for 1,3-dimethylthymine dimer (DMT><DMT)†, its anion radical and its cation radical. In each case, the cleavage was found to be exothermic.

Given the reduction potentials of the dimer and the monomer, along with the enthalpy of cleavage of the dimer ($\Delta H_{neutral}$), the enthalpy of cleavage of the anion radical of the dimer (ΔH_{anion}) can be calculated. The reduction potential for the dimer (−2.6 V) and the monomer (−2.2 V) have been determined from fluorescence quenching experiments (17). The enthalpy change for the cleavage of the cation radical (ΔH_{cation}) can also be determined in the same way, using the oxidation potentials for the monomer and dimer (12). These have been determined to be +1450 mV for both monomer and dimer. The only unknown quantity is $\Delta H_{neutral}$ (18).

Diogo *et al.* have used combustion calorimetry to determine the corresponding value for a uracil photodimer (19). Their compound contains a methylene bridge linking the N3 positions of the two monomer units. The $\Delta H_{neutral}$ of this compound was found to be −26 kcal/mol, but because the redox potentials for this derivative were not measured it is not possible to determine the enthalpy of the ion radical cleavage. Others have estimated that for unlinked systems $\Delta H_{neutral}$ should be as low as −12 kcal/mol (18).

MATERIALS AND METHODS

Synthesis. 1,3-Dimethylthymine (DMT) was synthesized as previously described (20). The DMT< >DMT was obtained by irradiating a 1 m*M* frozen aqueous DMT solution with a medium-pressure Hg lamp filtered with vycor glass. The *cis-syn* dimer was isolated from the reaction mixture by flash chromatography on silica gel eluted with 60:40 ethyl acetate : hexane. The dimer was recrystallized from methanol. The structure was confirmed by ^1H nuclear magnetic resonance (NMR) (21). Trimethylbenzidine (TMB) was used from Aldrich without further purification. CH_3CN was used from Aldrich and purified by distillation.

Quantum yield. Photolysis of TMB in the presence of DMT< >DMT was carried out using 313 nm light from a 200 W Hg lamp. Concentrations of DMT< >DMT were varied from 5 m*M* to 30 m*M*. Concentrations of TMB were kept at $>10^{-3}$ *M* to avoid self-quenching. The input energy was measured by sampling 10%

---

*To whom correspondence should be addressed at: Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742, USA. Fax: 301-314-9656;
e-mail: df37@umail.umd.edu.

†*Abbreviations:* BET, back electron transfer; DMT, 1,3-dimethylthymine; DMT< >DMT, 1,3-dimethylthymine dimer; HBP, 2-hydroxybenzophenone; PBD, photothermal beam deflection calorimetry; SET, single electron transfer; TMB, tetramethylbenzidine.

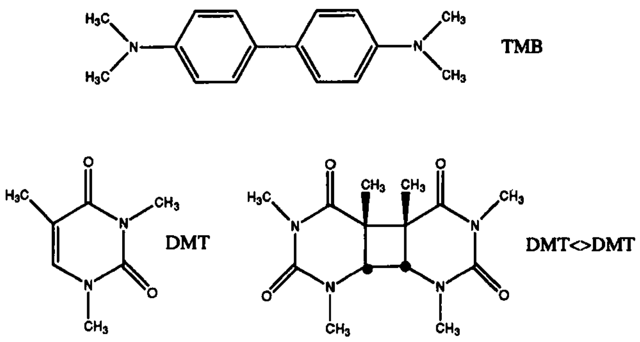

Figure 1. Tetramethylbenzidine (TMB), *cis-syn* 1,3-dimethylthymine (DMT) and 1,3 dimethylthymine dimer (DMT< >DMT). The $E^*_{ox}$for TMB is −3.17 eV and the reduction potentials are −2.2 V (DMT) and −2.6 V (DMT< >DMT).

of the incident light with a photodiode. The light intensity and the response of the photodiode were measured using a standard actinometer system, potassium ferrioxalate (22). The progress of the reaction was monitored by high-performance liquid chromatography (HPLC), which detects for the appearance of monomer. The quantum yields (Φ_T) were obtained from the slope of a plot of monomer yield versus number of photons absorbed.

Photothermal beam deflection calorimetry (PBD) apparatus. The pump beam is the output of a XeCl excimer laser (308 nm, 10 ns pulse width). The probe beam is a cw He-Ne laser with 0.5 mW nominal output. The pump and probe beam are counterpropagated through the center of the sample cell. A solution filter (2-hydroxybenzophenone [HBP]) is used to adjust the pump beam intensity. The excimer output power (excitation energy) is measured by a joulemeter (1–6 mJ). The alignment is maximized before each set of experiments by using a test sample of HBP in CH_3CN. The signal is detected by the deflection of the probe beam onto a split photodiode. This causes a current difference between the two halves of the photodiode. This current difference is amplified and transferred to a wideband digital oscilloscope. The signal is averaged and transferred to a microcomputer for further analysis. All samples are purged with nitrogen to remove dissolved oxygen.

RESULTS AND DISCUSSION

DMT< >DMT is used as a model system for pyrimidine dimers (Py< >Py). Tetramethylbenzidine sensitizes the cleavage of these dimers via a photoinduced electron transfer reaction. The excited singlet state of TMB donates an electron, which initiates the splitting reaction (Fig. 2). Irradiation of TMB in the presence of DMT< >DMT has been shown to result in clean splitting of the latter into DMT with no detectable side products (23).

In contrast to its photochemical liability, DMT≤DMT is found to be thermally stable. To test this DMT≤DMT solutions were heated for 60 days at 100°C, which caused less than 0.1% conversion to DMT. This gives an extrapolated lifetime of >4100 years under these conditions.

To determine the $\Delta H_{neutral}$ for the cleavage of DMT≤DMT, the quantum efficiency ($\phi_{cleavage}$) for cleavage must be first calculated. According to Fig. 2, the overall cleavage quantum yield (Φ_T) depends on the rate of cleavage (k_{split}) and the cleavage efficiency ($\phi_{cleavage}$). This can be described by:

$$\Phi_T = \frac{k_{split}(Py<>Py)}{k_{et}(Py<>Py) + k_f + k_{nr}} \times \phi_{cleavage}. \qquad (1)$$

Equation 1 can be simplified to:

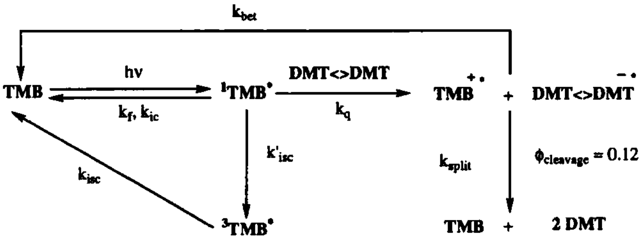

Figure 2. A scheme of possible reactions of TMB with DMT< >DMT.

$$\frac{1}{\Phi_T} = \frac{1}{\phi_{cleavage}} + \frac{1}{\phi_{cleavage}k_{split}\tau} \times \frac{1}{(Py><Py)} \qquad (2)$$

where τ is the lifetime of the excited state sensitizer (TMB). The overall quantum yield (Φ_T) can be determined from a plot of monomer yield versus photons absorbed. A plot of $1/\Phi_T$ versus 1/(Py< >Py) is shown in Fig. 3. The reciprocal of the intercept gives $\phi_{cleavage}$, which equals 0.12.

Knowing the cleavage efficiency of DMT< >DMT, the ΔH of breaking of the cyclobutane ring was determined. This was accomplished using time-resolved PBD. In brief, this technique involves a short, focused pulse of laser light (308 nm) that is used to initiate a photochemical reaction. The heat produced from the reaction creates a local hot zone in the sample, which in turn creates a change in the index of refraction. This change deflects a probe beam from an He-Ne laser, which is focused antiparallel to the excitation beam. The angle by which the probe beam is deflected is related to the energy released. The deflection of the probe beam is detected using a position sensitive photodiode (24,25).

We define S as the slope of the plot of signal intensity (mJ) *vs* excitation energy (mV). This is compared to a plot obtained from HBP obtained under identical conditions of solvent, sample absorbance and so on. The HBP rapidly converts 100% of its excitation energy into heat through internal conversion and therefore acts as a standard (26). The nonradiative efficiency can be calculated from Eq. 3:

$$\alpha = \frac{(E_{exc} - E_s\phi_f)}{E_{exc}} \qquad (3)$$

where α is the nonradiative efficiency and E_{exc} is the excitation energy (92.8 kcal/mol). E_s and ϕ_f are the single energy and the fluorescence quantum yield. These values for TMB

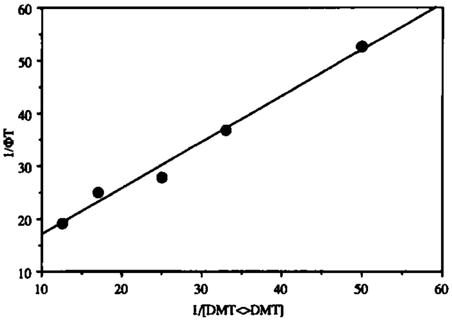

Figure 3. A double reciprocal plot of the cleavage quantum yield of DMT< >DMT. The reaction is sensitized by TMB in CH_3CN. The Φ_T is determined from a plot of cleavage of dimer versus photons absorbed.

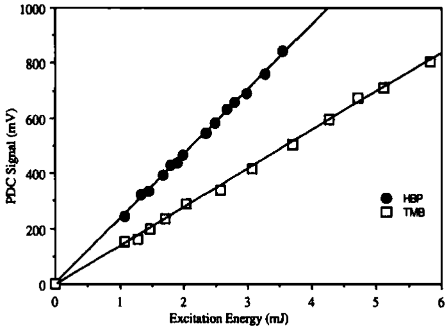

Figure 4. PDC signals for 2-HBP (solid circles) and TMB (open squares) as a function of excitation energy. In both cases the solvent is CH_3CN and the sample optical density at 308 nm is 0.3.

are 3.60 eV and 0.38 (22). The $\alpha_{TMB}/\alpha_{HBP}$ calculated from Eq. 3 is 0.66.

PBD measurements were carried out on optically matched solutions of TMB and HBP. The results are shown in Fig. 4.

$$\frac{S_{TMB}}{S_{HBP}} = \frac{\alpha_{TMB}}{\alpha_{HBP}} \qquad (4)$$

The ratio of the slopes from Fig. 4 is 0.62. This means, from the PBD analysis, that 62% of the photonic energy absorbed is given off as heat. This can be compared to the nonradiative efficiency (α) calculated from Eq. 3. Thus, $\alpha_{TMB}/\alpha_{HBP}$ obtained from PBD analysis is in good agreement with that calculated from the literature quantum yield in Eq. 3.

Next, we investigated the behavior of TMB in the presence of DMT. DMT quenches the fluorescence of TMB via an electron transfer mechanism. There is rapid single electron transfer (SET) followed by a subsequent back electron transfer (BET). There is no net photochemistry, so as the quenching efficiency (ϕ_q) approaches unity, α approaches 1.0. Figure 5 shows the increase in the slope (S) with increasing [DMT]. This increase is caused because of the electron transfer from TMB to DMT, which reduces the amount of fluorescence. The quenching efficiency of DMT at given concentration of quencher, ϕ_q, can be calculated from the PBD data from Eq. 5:

$$\phi_q = \frac{S^0_{TMB} - S_{TMB}}{S_{HBP} - S_{TMB}} \qquad (5)$$

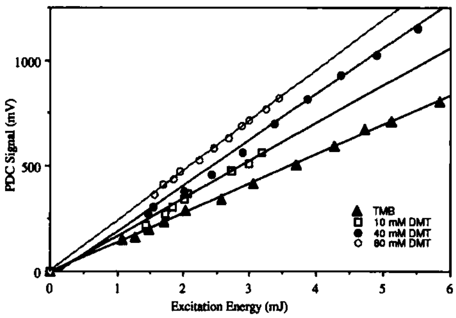

Figure 5. PDC signal amplitude as a function of excitation energy for TMB and various concentrations of DMT (10, 40, 80 m*M*). All samples are purged with nitrogen to avoid oxygen quenching.

Table 1. Quenching efficiencies (ϕ_q) of DMT and DMT Ⓝ DMT measured for both PBD and fluorescence quenching

Quencher (m*M*)	ϕ_q DMT fluorescence	ϕ_q DMT PDC	ϕ_q DMT Ⓝ DMT fluorescence	ϕ_q DMT Ⓝ DMT PDC
0	0.00	0.00	0.00	0.00
10	0.52	0.50	0.37	0.44
40	0.81	0.79	0.70	0.77
80	0.89	0.87	0.82	0.89

where S^0_{TMB} is the slope of TMB at a given concentration of DMT. DMT does not absorb light at 308 nm. Thus, its direct absorption does not contribute to the signal. The quenching efficiency can also be independently calculated from fluorescence quenching experiments by Eq. 6:

$$\phi_q = \frac{k_q\tau[DMT]}{k_q[DMT] + 1} \qquad (6)$$

The Stern-Volmer quenching constants ($k_q\tau$) measured from fluorescence quenching experiments for the monomer and dimer are 110 M^{-1} and 59 M^{-1}, respectively. A comparison of the ϕ_q calculated from Eq. 6 and those equations measured by PBD are given in Table 1 for both the monomer and dimer. The quenching efficiency measured from PBD and fluorescence quenching are in agreement.

Finally, PBD was used to analyze the splitting of DMT<>DMT. The waveform, shown in Fig. 6, shows TMB with various concentrations of DMT<>DMT. The waveform obtained in the absence of DMT<>DMT shows prompt heat release, followed by a slow heat release. The fast component is assigned to the nonradiative relaxation of ^{1}TMB via internal conversion and intersystem crossing processes. The slow component is assigned to the decay of ^{3}TMB. A lifetime of 9 μs was obtained for ^{3}TMB by fitting the slow component to a first order exponential growth function.

Three important features are observed in these waveforms. First, the slow heat release from ^{3}TMB decreases with increasing dimer concentrations. This is because ^{1}TMB is

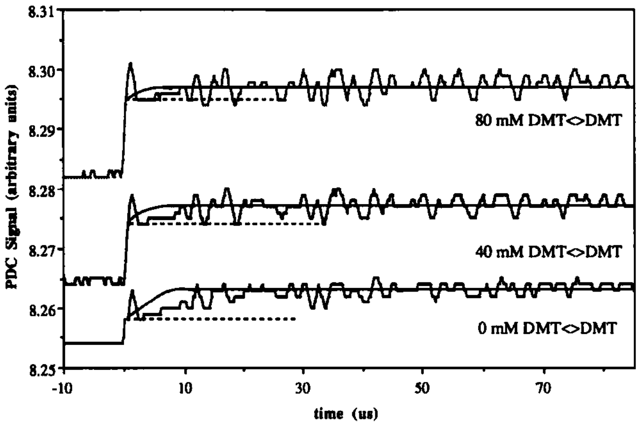

Figure 6. Photothermal beam deflection waveforms from XeCl laser (308 nm, 10 ns, 3.2 mJ, 1 mm diameter) irradiation in the absence and presence of dimethylthymine dimers (DMT<>DMT) in Ch_3CN. Deflection was measured using a 0.5 mW, He-Ne laser beam. The initial spike is due to an acoustic pressure wave, which is accompanied by the excitation of the sample.

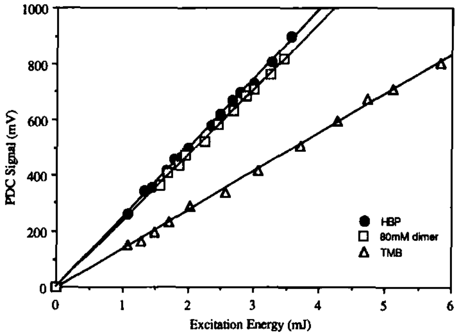

Figure 7. PDC signal versus excitation energy for HBP, TMB and 80 m*M* DMT< >DMT.

quenched by DMT< >DMT, therefore less ^{3}TMB is formed in the presence of DMT< >DMT. Second, the lifetime of ^{3}TMB is independent of dimer concentration. DMT< >DMT does not quench ^{3}TMB, therefore the triplet lifetime is unchanged. And finally, the total heat released increases with increasing dimer concentration. This observation is consistent with Fig. 2 because DMT< >DMT quenches ^{1}TMB and consequently decreases the fluorescence from ^{1}TMB, therefore more nonradiative relaxation is observed. Finally, the chemical reaction, splitting of DMT< >DMT, also contributes to the signal.

The total energy was measured at various dimer concentrations (Fig. 7). If ϕ_q is substituted into Eq. 5 for the various concentrations of quencher, then the contribution of the heat evolved from the splitting of DMT< >DMT can be calculated. For example, the slope of HBP, which gives back all photonic energy as heat, is 92.8 kcal/mol. The slope for 80 m*M* DMT< >DMT, when compared to HBP, is 88.1 kcal/mol. If the ϕ_q for 80 m*M* DMT< >DMT from fluorescence quenching is inserted into Eq. 5, then E_{total} equals 85 kcal/mol (Fig. 8). The average ΔH_{obs} for splitting of the dimer to form the monomer is −3.0 kcal/mol. This number is divided by the quantum efficiency of cleavage of splitting, $\phi_{cleavage}$, to give $\Delta H = -25$ kcal/mol. It should be noted that this value corresponds to the overall conversion (*i.e.* neutral DMT< >DMT to neutral DMT). Because ΔH_{obs} is a thermodynamic quantity, it is independent of the mechanism that connects the products and reactants. Therefore, it gives no direct information about the intermediate ion radicals.

To obtain an accurate $\Delta H_{neutral}$, the contribution of the reaction volume change must be calculated. There are two possible contributions to the volume change in a photoinduced reaction in solution. First is the expansion of the medium upon the release of heat by a radiationless process. Second, there is a structural volume change (ΔV_{st}), which is the difference in molar volume between the products and the reactants. The molar volume change for the breaking of two C-C bonds is typically 20 mL/mol (27). This was taken as a reasonable estimate for the ΔV_{st} of DMT< >DMT. The ΔH for neutral dimer splitting can be calculated from Eq. 7:

$$\Delta H_{neutral} = \Delta H_{obs} - \frac{\Delta V_{st}\beta}{\rho C_p} = -19 \text{ kcal/mol} \qquad (7)$$

where β is the cubic expansion coefficient, C_p is the specific heat capacity and ρ is the density (28). This volume contribution is subtracted from the ΔH_{obs} leaving $\Delta H_{neutral} = -19$ kcal/mol for the neutral dimer splitting. From the thermodynamic cycle in Fig. 9, ΔH_{anion} is −28 kcal/mol and ΔH_{cation} is −19 kcal/mol.

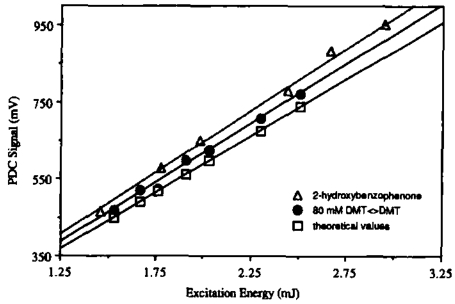

Figure 8. Amount of stored energy in cyclobutyl bonds in 1,3-dimethylthymine (DMT). Theoretical slope for 80 m*M* DMT< >DMT was calculated using ϕ_q for 80 m*M* DMT.

Photothermal beam deflection calorimetry is a useful technique for measuring microscopic real-time heat release in solution. The ΔH for neutral DMT< >DMT → 2 DMT was found to be −19 kcal/mol. This is in reasonable agreement with data from Begley's dimer (−26 kcal/mol). It should be noted that Begley's dimer has a linking group connecting the N3 position of each uracil residue. The differences in the enthalpies may be a result of the additional ring strain from this linker group. This value is also comparable to the estimate of Heelis *et al.* (18).

The fragmentation of cyclobutane is endothermic (+18 kcal/mol) in contrast to the exothermic fragmentation of DMT< >DMT (−19 kcal/mol). The exothermicity of DMT< >DMT fragmentation reflects both the loss of strain and the formation of the conjugated 5,6 double bond. This suggests that the reaction is effectively irreversible. The neutral-neutral reaction is only slightly less exothermic than that of the anion radical, by 9 kcal/mol. This value suggests that

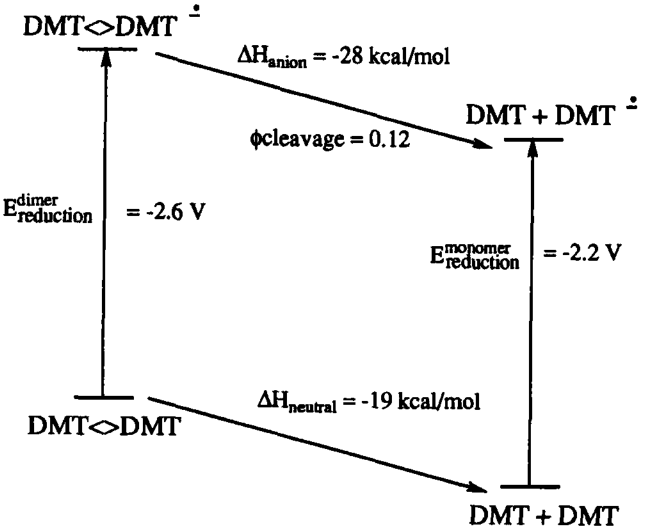

Figure 9. Thermodynamic cycle for the cleavage reaction. The reduction potentials for the *cis,syn* dimer (E^{dimer}_{red}) and the monomer ($E^{monomer}_{red}$) of 1,3-dimethylthymine were determined by fluorescence quenching rates (16). Analysis for the radical cation cleavage is analogous, in this case the oxidation potentials for the dimer and monomer are +1450 mV (16), making $\Delta H_{cation} = \Delta H_{neutral} = -19$ kcal/mol.

the increase is not the only reason for facile cleavage. Other factors, such as localization of the odd electron density, near the cyclobutyl ring, weaken the C-C bond of the cyclobutyl ring.

Acknowledgements—This work was supported by the National Institutes of Health (GM45856-01A1). We would like to thank Gautam Prakash for his help in preparing this manuscript.

REFERENCES

1. Sancar, A. and G. B. Sancar (1988) DNA repair enzymes. *Ann. Rev. Biochem.* **57**, 29-67.
2. Wang, S. Y. (1976) Pyrimidine bimolecular photoproducts. In *Photochemistry and Photobiology of Nucleic Acids* (Edited by S. Y. Wang), pp. 219–263. Academic Press, New York.
3. Sancar, A. (1994) Structure and function of DNA photolyase. *Biochemistry* **33**, 2-9.
4. Freidberg, E. C. (1985) *DNA Repair.* W. H. Freeman, New York.
5. Park, H.-W., S.-T. Kim, A. Sancar and J. Diesenhofer (1995) Crystal structure of DNA photolyase from *Escherichia coli. Science (Wash. DC)* **268**, 1866-1872.
6. Hartzfeld, D. G. and S. D. Rose (1993) Efficient pyrimidine dimer radical anion splitting in low polarity solvents. *J. Am. Chem. Soc.* **115**, 850–854.
7. Whitmer, M. R., E. Altmann, H. Young and T. P. Begley (1989) Mechanistic studies on DNA photolyase. 1. Secondary deuterium isotope effects on the cleavage of 2′-deoxyuridine dinucleotide photodimers. *J. Am. Chem. Soc.* **111**, 9264–9265.
8. Kim, S.-T., P. F. Heelis, T. Okamura, Y. Hirata, N. Mataga and A. Sancar (1991) Determination of rates and yields of interchromophore (folate → flavin) energy transfer and intermolecular (flavin → DNA) electron transfer in *Escherichia coli* photolyase by time-resolved fluorescence and absorption spectroscopy. *Biochemistry* **30**, 11262–11270.
9. Young, T., S.-T. Kim, J. R. v. Camp, R. F. Hartman and S. D. Rose (1988) Transient intermediates in intramolecularly photosensitized pyrimidine dimer splitting by indole derivatives. *Photochem. Photobiol.* **48**, 635–641.
10. Charlier, M. and C. Hélène (1975) Photosensitized splitting of pyrimidine dimers in DNA by indole derivatives and tryptophan-containing peptides. *Photochem. Photobiol.* **21**, 31–37.
11. Yeh, S.-R. and D. E. Falvey (1991) Model studies of DNA photorepair: radical anion cleavage of thymine dimers as probed by nanosecond laser spectroscopy. *J. Am. Chem. Soc.* **113**, 8557–8558.
12. Pac, C., K. Miyake, Y. Masaki, S. Yanagida, T. Ohno and A. Yoshimura (1992) Flavin-photosensitized monomerization of dimethylthymine cyclobutane dimer: remarkable effects of perchloric acid and participation of excited-singlet, triplet, and chain-reaction pathways. *J. Am. Chem. Soc.* **114**, 10756–10762.
13. Ben-Hur, E. and I. Rosenthal (1970) Photosensitized splitting of pyrimidine dimers. *Photochem. Photobiol.* **11**, 163–168.
14. Burdi, D. and T. P. Begley (1991) Mechanistic studies on DNA photolyase. 3. The trapping of the one-bond-cleaved intermediate from a photodimer radical cation model system. *J. Am. Chem. Soc.* **113**, 7768–7770.
15. Pouwels, P. J., R. F. Hartman, S. D. Rose and R. Kaptein (1995) Photo-CIDNP study of pyrimidine dimer splitting I: reactions involving pyrimidine radical cation intermediates. *Photochem. Photobiol.* **61**, 563–574.
16. Roth, H. D. and A. A. Lamola (1972) Cleavage of thymine dimers sensitized by quinones. Chemically induced dynamic nuclear polarization in radical ions. *J. Am. Chem. Soc.* **94**, 1013–1014.
17. Yeh, S.-R. and D. E. Falvey (1992) Model studies of DNA photorepair: energetic requirements for the radical anion mechanisms determined by fluorescence quenching. *J. Am. Chem. Soc.* **114**, 7313–7314.
18. Heelis, P. F., R. T. Hartman and S. D. Rose (1995) Photoenzymic repair of UV-damaged DNA: a chemist's perspective. *Chem. Soc. Rev.* **24**, 289–297.
19. Diogo, H. P., A. R. Dias, A. Dhalla, M. E. Minas de Piedade and T. P. Begley (1991) Mechanistic studies on DNA photolyase. 4. The enthalpy of cleavage of a model photodimer. *J. Org. Chem.* **56**, 7340–7341.
20. Kloepfer, R. and H. Morrison (1972) Organic photochemistry. XVII. The solution phase dimerization of dimethylthymine. *J. Am. Chem. Soc.* **94**, 255–264.
21. Wulff, D. L. and G. Fraenkel (1961) On the nature of the thymine photoproduct. *Biochim. Biophys. Acta* **51**, 332–339.
22. Murov, S. L., I. Carmichael and G. L. Hug (1993) *Handbook of Photochemistry.* Marcel Dekker, New York.
23. Fenick, D. J., H. S. Carr and D. E. Falvey (1995) Synthesis and photochemical cleavage of *cis-syn* pyrimidine cyclobutane dimer analogs. *J. Org. Chem.* **60**, 624–631.
24. Aamodt, L. C. and J. C. Murphy (1983) Thermal effects in photothermal spectroscopy and photothermal imaging. *J. Appl. Physics.* **54**, 581–591.
25. Laman, D. M. and D. E. Falvey (1995) A photothermal beam deflection apparatus for the time-resolved kinetic study of fast photophysical and photochemical processes. *Rev. Sci. Instrum.* (In press)
26. Burkey, T. J., M. Majewski and D. Griller (1986) Heats of formation of radicals and molecules by a photoacoustic technique. *J. Am. Chem. Soc.* **108**, 2218–2221.
27. Asano, T. and J. leNoble (1978) Activation and reaction volumes in solution. *Chem. Rev.* **78**, 407–489.
28. Braslavasky, S. E. and K. Heinoff (1989) Photothermal methods. In *Handbook of Organic Photochemistry* (Edited by J. C. Scaiano), pp. 327–355. CRC Press, Boca Raton, FL.