Photodimerization and van der Waals Stacking of Dimethylthymine in Water

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THE photodimerization reactions of natural 2,4-dioxopyrimidines: uracil, thymine, orotic acid, and some of their monomer derivatives, in dilute solutions are commonly interpreted[1] as bimolecular, diffusion-controlled triplet-state processes, based on evidence from triplet quenching studies with oxygen and olefins. Because it is soluble in most solvents, 1,3-dimethylthymine (DMT) is a better model for dioxopyrimidine dimerization. Its photodimerization[2,3] in several solvents has been interpreted[3] as occurring from both singlet and triplet states, to yield a mixture of four possible cyclobutane dimer isomers[4] from either excited state, with the product composition depending on the natures of the excited-state precursor and of the solvent. Only a small reduction in the dimer yield in concentrated (0·1M) acetonitrile solution containing a triplet quencher (*cis*-penta-1,3-diene) suggested[3] a predominantly singlet pathway for the reaction and the possibility of singlet excimer intermediate involvement. We now present evidence that the reaction in water over a broad concentration range may be interpreted exclusively in terms of a singlet excimer state formed upon light absorption by ground state van der Waals stacked complexes.

Degassed water solutions 3×10^{-3}—4×10^{-1}M in 1,3-dimethylthymine, thermostatted at various temperatures (26—80°), were irradiated at 297, 302, and 313 nm. The dimeric products were determined by further irradiation of diluted solutions[5] at 254 nm. and by u.v. spectrophotometry [the initial quantum yields of dimer formation ϕ_{df} ($=1/2\ \phi_{md}$, the quantum yield of monomer disappearance) measured by means of uranyl oxalate actinometry[6]].

The reaction exhibited marked temperature dependence and $1/\phi_{df}$ against 1/[DMT] plots, linear over the entire concentration range, converged at a common intercept corresponding to the limiting $\phi_{df} = 0{\cdot}125 \mp 0{\cdot}015$ at infinite DMT concentration (Figure). We were unable to suppress the reaction by use of efficient triplet quenchers such as molecular oxygen and I^- and Br^- (up to 4M-Br^-) although in control experiments the triplet-state dimerization of orotic acid,[1a] 10^{-4}M, was almost completely quenched even at 10^{-2}M-Br^-. Quenching with *cis*-penta-1,3-diene in non-aqueous solutions of DMT,[2,3] may be due in part to side reactions, since in 0·01M-benzene solution, 0·1M in *cis*-penta-1,3-diene, at 40% DMT conversion the yield of dimeric products amounted to only some 10%. The photodimerization in water is thus apparently a singlet-state reaction. Its temperature and concentration dependence may be then analysed in terms of either (a) photoassociation between an excited singlet molecule and one in the ground state or, (b) ground state van der Waals' stacking, known to exist in aqueous solutions of pyrimidines.[7]

If photodimerization involves step (a), leading to a transient excimer precursor of the cyclobutane dimer, ϕ_{df} should be equal to the product of the quantum yield of photoassociation[8] $\phi_{DM} = k_{DM}\tau_M \times [DMT]/1 + k_{DM}\tau_M \times [DMT]$ (k_{DM} is the bimolecular rate constant and τ_M the lifetime of the lowest monomer singlet state) and the probability that the excimer intermediate will proceed on to a stable dimer $P_d^e = \phi_{df} \times [DMT]_\infty$. Since DMT is non-fluorescent in solution, τ_M^0 must be $\leqslant 10^{-12}$ sec.; k_{DM} may be equated[8] to the diffusion-limited rate constant $k_d \sim 7{\cdot}5 \times 10^9$ l.mole^{-1} sec.$^{-1}$ in water at 25°. Photoassociation is thus a very inefficient process ($\phi_{DM} \leqslant 10^{-3}$ at 0·1M-DMT) and calculated ϕ_{df} values are about 3 powers of ton lower than those found experimentally. Also the marked solvent dependence of the rate of photodimerization[3] disagrees with this mechanism since photoassociation equilibrium constants are apparently solvent independent.[8] The second alternative (b), may then be considered, *i.e.* a singlet excimer precursor is formed upon light absorption by van der Waals' complexes and ϕ_{df} is the product of P_d^e and the fraction of light absorbed by DMT molecules contained

therein. If we limit ourselves for the sake of simplicity to the bimolecular step of what is actually a multistep stacking association[7] (K_a = association constant), this fraction is approximately $2K_a$ [DMT]/1 + 2 K_a [DMT] and $1/\phi_{df} = 1/P_d^e\{1 + 1/2\ K_a$ [DMT]$\}/\ K_a = 0{\cdot}62$ l.mole^{-1}, obtained at 26° from the slope of the experimental $1/\phi_{df}$ against 1/[DMT] plot (Figure), is in excellent agreement with $K_a^{25°}$ values of 0·71 and 0·9 derived from thermal osmometry data for water solutions of uridine and deoxythymidine,[7] respectively. This same holds for the enthalpy of association $\Delta H° = 2{\cdot}4$ kcal./mole, calculated from the temperature dependence of K_a, and other thermodynamic parameters.

The product distribution between various cyclobutane dimer isomers strongly supports involvement of stacked complexes, for both *cis*-isomers (*syn* and *anti*) comprise some 80% of the photoproduct in water and in organic solvents.[3] The *cis,syn*-isomer is formed exclusively in DMT crystals with $\phi_{df} = 0{\cdot}165$,[9] very close to the solution value of 0·125 at infinite DMT concentration. The solid-state dimerization of DMT is interpreted[9] according to the singlet excimer model,[10] and similarity of quantum yields in both phases points to similar configurations and lifetimes of DMT singlet excimers in solution and in crystals as has been observed for pyrene.[11] Finally, much lower quantum yields of dimerization in non-aqueous solutions[3] are readily explained in the light of the proposed mechanism by the sensitivity of hydrophobic association to the presence of organic solvents disrupting pyrimidine stacks.

The mechanism of dioxopyrimidine photodimerization in water solutions involving ground state van der Waals' stacking association followed by an excimer formation may be a more general phenomenon in sufficiently concentrated solutions, as it is suggested for the intermolecular dimerization in the dinucleotide thymidyl-3′,5′-thymidine,[12] pyrimidine polynucleotides,[13] and DNA.[14]

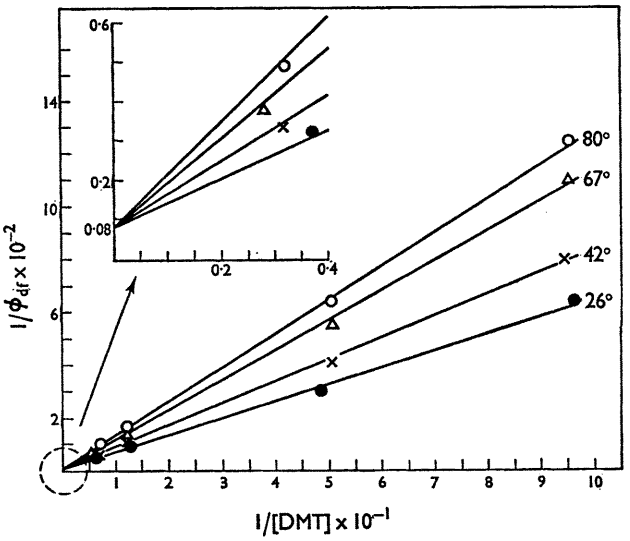

FIGURE. *Concentration and temperature dependence of quantum yield of 1,3-dimethylthymine photodimerization* (ϕ_{df}) *in water*: $\phi_{df[DMT]_\infty} = 0{\cdot}125 \pm 0{\cdot}015$.

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[1] (a) E. Sztumpf-Kulikowska, D. Shugar, and J. W. Boag, *Photochem. Photobiol.*, 1967, **6**, 41; (b) A. A. Lamola and J. P. Mittal, *Science*, 1966, **154**, 1560; (c) C. L. Greenstock, I. H. Brown, J. W. Hunt, and H. E. Johns, *Biochem. Biophys. Res. Comm.*, 1967, **27**, 431; (d) P. J. Wagner and D. J. Bucheck, *J. Amer. Chem. Soc.*, 1968, **90**, 6530.
[2] H. Morrison, A. Feeley, and R. Kleopfer, *Chem. Comm.*, 1968, 358.
[3] H. Morrison and R. Kleopfer, *J. Amer. Chem. Soc.*, 1968, **90**, 5037.
[4] D. L. Wulff and G. Fraenkel, *Biochem. Biophys. Acta*, 1961, **51**, 332.
[5] D. Shugar and A. D. McLaren, "Photochemistry of Proteins and Nucleic Acids," Pergamon Press, Oxford, 1964.
[6] W. G. Leighton and G. S. Forbes, *J. Amer. Chem. Soc.*, 1930, **52**, 3139.
[7] T. N. Solie and J. A. Schellman, *J. Mol. Biol.*, 1968, **33**, 61, and references cited.
[8] B. Stevens in "Energetics and Mechanisms in Radiation Biology," ed. G. O. Phillips, Academic Press, 1968, p. 253.
[9] R. Lisewski and K. L. Wierzchowski, *Photochem. Photobiol.*, 1969, in the press.
[10] A. A. Lamola, *Photochem. Photobiol.*, 1968, **7**, 619.
[11] J. B. Birks, A. A. Kazzaz, and T. A. King, *Proc. Roy. Soc.*, 1966, *A*, **291**, 556.
[12] J. Eisinger and A. A. Lamola, *Biochem. Biophys. Res. Comm.*, 1967, **28**, 558.
[13] Z. Tramer, K. L. Wierzchowski, and D. Shugar, *Acta Biochim. Polon.*, 1969, **16**, 83.
[14] J. Eisinger, *Photochem. Photobiol.*, 1968, **7**, 597.