Kinetics of Thymine Dimerization

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On the basis of computer simulation a mechanism for photosensitized thymine dimerization involving association between thymine and photosensitizer is proposed.

Thymine dimerization is the main photochemically induced lesion occurring in u.v. irradiated DNA.[1—3] Dimerization in DNA may either be a primary photochemical effect or a secondary effect resulting from the action of endogenous photosensitizers.[2,4] An understanding of the mechanisms of both direct and photosensitized dimerization is therefore biologically significant. The work described here involved attempts to establish the kinetics and mechanisms of acetone, acetophenone, and benzophenone photosensitized dimerization. This was done by comparing experimental results with results obtained by computer simulation.

A water-cooled pyrex cell (25 ± 1 °C) containing solutions

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of thymine (10^{-4}—10^{-2} M) and acetophenone (10^{-3} M) was irradiated for six hours using a Xenon lamp. Quantitative detection of dimer was achieved using h.p.l.c. techniques based on those described by Cadet *et al.*[5] A programme designed to solve differential rate equations was used for the computer simulation of reaction mechanisms.[6,7]

Previous workers have assumed a diffusion controlled mechanism for photosensitized thymine dimerization,[8—10] although Kornhauser *et al.*[11,12] did propose the formation of a free radical intermediate between thymine and excited photosensitizer, their mechanism is still basically diffusion controlled. This assumption was confirmed for acetone photosensitization over the entire range of thymine concentration considered (10^{-4}—10^{-2} mol dm^{-3}). The mechanism involves diffusion controlled triplet energy transfer from photosensitizer to thymine followed by bimolecular collision of a ground and an excited state thymine monomer to give dimer. This mechanism was also proposed for acetophenone and benzophenone by Charlier and Helene,[10] and was found to hold at low thymine concentrations. At higher concentrations ($\geqslant 10^{-3}$ mol dm^{-3}) however, far lower yields were predicted than those observed in this work. In an attempt to resolve this disagreement the effect of thymine base stacking was considered, but as thymine momomer would be present in far greater concentrations than associomer ($K \sim 1$ for base stacking)[13—15] the monomer could compete much more efficiently for photosensitizer. Hence the inclusion of this step in a mechanistic analysis did not significantly increase the dimer yield. In order to increase the simulated yield of dimerization (*i.e.* effective quantum yield) it was found necessary to postulate association between ground state photosensitizer and thymine. Experiments in low temperature aqueous glasses (77 K, $MgCl_2$) indicate that the yield of thymine dimer obtained from acetophenone/thymine irradiations (λ >300 nm) are much higher than those which would be expected on the basis of a model which allows each thymine six randomly chosen nearest neighbours and requires a thymine molecule and an acetophenone molecule to be in the nearest neighbour shell for dimerization to occur. This provides supporting evidence for the proposed association,[16] however direct confirmation for the association is still required. Good agreement between experimental and computed data was found with equilibrium constants of 130 and 50 dm^3 mol^{-1} for thymine association with acetophenone and benzophenone, respectively. Although these constants are not insignificant they are not unreasonable.

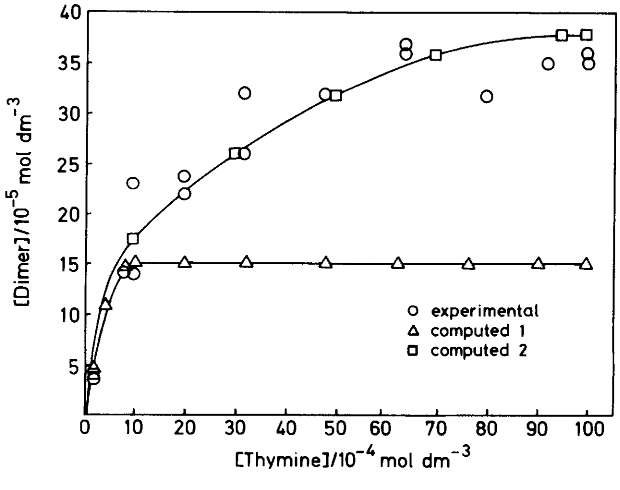

Figure 1. Comparison of experimental data for the effect of initial thymine concentration on acetophenone [10^{-3} M] photosensitized dimerization with computed data obtained using the combined diffusion controlled and acetophenone–thymine association mechanism.

The mechanism is a combination of the diffusion controlled triplet transfer mechanism[10] (steps 7—15, Table 1), and an association mechanism (steps 1—6). Step 3 was included to account for the experimentally observed independence of the dimer yield on thymine concentration at high monomer concentrations. Stable association of pyrimidines in aqueous solution is a well established phenomenon[17] and the formation of a base-stacked thymine pair *via* the bimolecular collision process which step 3 describes seems a reasonable supposition. Steps 1,3,5, and 6 were taken as approximately diffusion controlled and the final values for these elementary processes and step 2 were obtained by fitting the computer simulation to the experimental data by trial and error. Step 6 describes dimerization *via* a thymine (T)–associomer (AcpT^3) interaction in which triplet energy initially residing in the acetophenone moiety is transferred to thymine. Step 4 is the light absorption step by the thymine–acetophenone associomer and it is assumed that this associomer absorbs energy with the same characteristics as the acetophenone monomer (see step 7). The computer programme CAKE determines the rate of reaction for each step by multiplying the given rate constant by the concentrations of the reacting species. For the light absorbing steps $k_a = I_a/[\mathrm{Acp}]$ was used as a convenient fiction in the computer model and therefore the units of k_a are s^{-1} and I_a is the intensity absorbed per unit volume. Other rate constants are either taken from or recalculated from the references indicated.

Figure 1 shows the failure of a simple diffusion controlled mechanism (computed 1) to predict the observed dimer yields. In contrast it shows the agreement obtained when photosensitizer–thymine association was included (computed 2) in the dimerization mechanism. The latter mechanism was also tested by comparing the experimental and computed variation in dimer yield with time. In both cases the relationship was linear and the gradients, as calculated by linear regression, agreed fairly well (see Table 2).

Table 1. Rate constants for thymine dimerization.

Step		Rate constants[a]	Reference
1	$\mathrm{Acp + T \rightarrow AcpT}$	1.3×10^9	
2	$\mathrm{AcpT \rightarrow Acp + T}$	1×10^7	
3	$\mathrm{AcpT + T \rightarrow Acp + 2T}$	2×10^9	
4	$\mathrm{AcpT \xrightarrow{u.v.} AcpT^3}$	3.6×10^{-5}	
5	$\mathrm{AcpT^3 + Acp \rightarrow 2Acp + T}$	5×10^8	
6	$\mathrm{AcpT^3 + T \rightarrow Dimer + Acp}$	2×10^9	
7	$\mathrm{Acp \xrightarrow{u.v.} Acp^1}$	3.6×10^{-5}	
8	$\mathrm{Acp^1 \rightarrow Acp^3}$	6×10^{10}	18
9	$\mathrm{Acp^3 \rightarrow Acp}$	5×10^2	10,19
10	$\mathrm{Acp^3 + Acp \rightarrow 2Acp}$	1.5×10^8	19
11	$\mathrm{Acp^3 + T \rightarrow T^3 + Acp}$	2×10^9	10
12	$\mathrm{Acp^3 + T \rightarrow Product}$	6×10^7	10
13	$\mathrm{T^3 \rightarrow T}$	8×10^3	20
14	$\mathrm{T^3 + T \rightarrow Dimer}$	4.7×10^8	20
15	$\mathrm{T^3 + T \rightarrow 2T}$	1.8×10^9	20

[a] Rate constant units are dm^3 mol^{-1} s^{-1}, or s^{-1} as appropriate.

Table 2. Dimer yield *vs.* time. Gradient/mol dm^{-3} s^{-1}.

	Acetophenone	Benzophenone
Experimental	1.3×10^{-8}	0.8×10^{-8}
Computed	1.8×10^{-8}	1.2×10^{-8}

Future work will involve further testing of this mechanism by both kinetic studies and by direct experimental investigations of the nature of photosensitizer–thymine interactions. All results and detailed kinetic analyses of the proposed mechanisms will be presented in a later paper. It is worthy of note that this mechanism suggests that macromolecules which act as photosensitizers in cellular systems may damage DNA to a greater extent than that envisaged *via* diffusion controlled collisional processes.

We thank Professor P. F. Ridler (University of Zimbabwe) for the turbo-pascal version of CAKE. Research funding from A.E.C.I. (RSA) and URF (University of Natal) is also acknowledged. V. K. thanks the C.S.I.R. for a Master's bursary.

Received, 11th August 1987;‡ Com. 1186

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‡ Received in revised form, 18th February 1988.