KINETICS OF THYMINE PHOTODIMERIZATION IN DNA

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ABSTRACT The kinetics of thymine photodimerization in *E. coli* DNA have been measured at various wavelengths of ultraviolet light. The initial quantum yield is not strongly dependent on wavelength. The ratio of thymine dimer to thymine in the photostationary state is much more dependent on wavelength. At the 235 mμ photosteady state 1.7 per cent of the thymine is present as dimer. This shifts to 6.5 per cent at 254 mμ and to 20 per cent of 275 mμ. While the change in position of the photosteady state with wavelength fails to fit a simple model, the data do indicate that not all thymines are capable of participation in dimer formation.

1. INTRODUCTION

Ultraviolet irradiation of frozen aqueous solutions of thymine induces the formation of a covalently bonded dimer of thymine (Beukers and Berends, 1960, 1961; Wang, 1961; Wulff and Fraenkel, 1961). Moreover, ultraviolet irradiation of the thawed solution causes this dimer of thymine to revert back to thymine (Beukers, Ijlstra, and Berends, 1959). On the basis of several lines of evidence, it would seem that photodimerization in frozen solution is made possible by the existence of suitably oriented microcrystals of thymine (Wang, 1961; Wulff, 1962). Since both the forward and back reactions are produced photochemically, a photosteady state is postulated to exist between dimer and monomer, the position of which is strongly influenced by the relative orientations of the monomer thymine molecules. It has been found that in compounds where the thymine moieties are held close to one another, such as thymidylyl-(5′-3′)-thymidine (TpT) and DNA, some dimerization will occur upon irradiation with 254 mμ ultraviolet light (Beukers, Ijlstra, and Berends, 1960; Wacker, Dellweg, and Weinblum, 1960; Beukers and Berends, 1961; Wacker, 1961; Wacker, Dellweg, and Lodemann, 1961).

Measurements of the quantum yield for the monomer to dimer reaction and the dimer to monomer reaction in TpT have shown that the quantum yield is essentially constant with wavelength (Johns, Rappaport, and Delbrück, 1962). However, the

ratio of the absorption coefficients of dimer to monomer varies by a factor of about 10^3 between 235 mμ and 285 mμ and hence the position of the photosteady state in TpT can be dramatically altered by varying the wavelength of irradiation. At 235 mμ, where the extinction coefficients of dimer and monomer are about the same, the photosteady state lies almost entirely on the side of the monomer, since the monomer to dimer reaction has a much lower quantum yield (about 0.01) than the quantum yield for the reverse reaction (about 1). However, at 285 mμ, where the extinction coefficient of the monomer is about 10^3 times higher than the extinction coefficient of the dimer, the photosteady state lies almost entirely on the side of the dimer (Johns *et al.*, 1962).

In DNA one might expect the same qualitative behavior, except that the maximum fraction of dimerization obtainable might be considerably lower, because one would expect that a considerable proportion of thymine moieties in the DNA would not be close enough to each other in order to dimerize. The object of the work reported here was to measure the wavelength dependence of thymine dimerization in DNA and to analyze the observed wavelength dependence in terms of plausible models.

2. MATERIALS AND METHODS

E. coli DNA, labeled with thymine-H^3 was prepared as described previously (Wulff and Rupert, 1962; Wulff, 1962). DNA solutions (0.3 μg/ml in 0.015 M NaCl, 0.0015 M sodium citrate, pH 7) were irradiated as described by Johns *et al.* (1962) and Wulff (1962). H^3-thymine and H^3-thymine dimer were assayed by liquid scintillation counting, following acid hydrolysis of the DNA and paper chromatography, as described previously (Wulff & Rupert, 1962; Wulff, 1962).

3. RESULTS

The rates of formation of thymine dimer in *E. coli* DNA at various wavelengths are shown in Figs. 1, 2, and 3. It can be seen that the fraction of thymine present as dimer increases linearly with dose and then reaches a plateau which is characteristic of the wavelength of light used. It is seen that the plateau characteristic of 275 mμ light may be converted to the 250 mμ plateau with 250 mμ light (Fig. 2) and then nearly back to the 275 mμ plateau with 275 mμ light (Fig. 3). The experimental points of the figures indicate that the position of the plateau slowly decreases with very high doses of ultraviolet light; the initially formed plateaus may therefore lie a little higher than those drawn in Fig. 1. The value obtained here for the photosteady state at 254 mμ is in good agreement with that of Dellweg and Wacker (1962).

4. DISCUSSION

The Quantum Yield for the Forward Reaction. The quantum yield for thymine dimerization, expressed a the fraction of thymine present as dimer per quantum absorbed by a nucleotide, may be calculated from the initial slopes of the

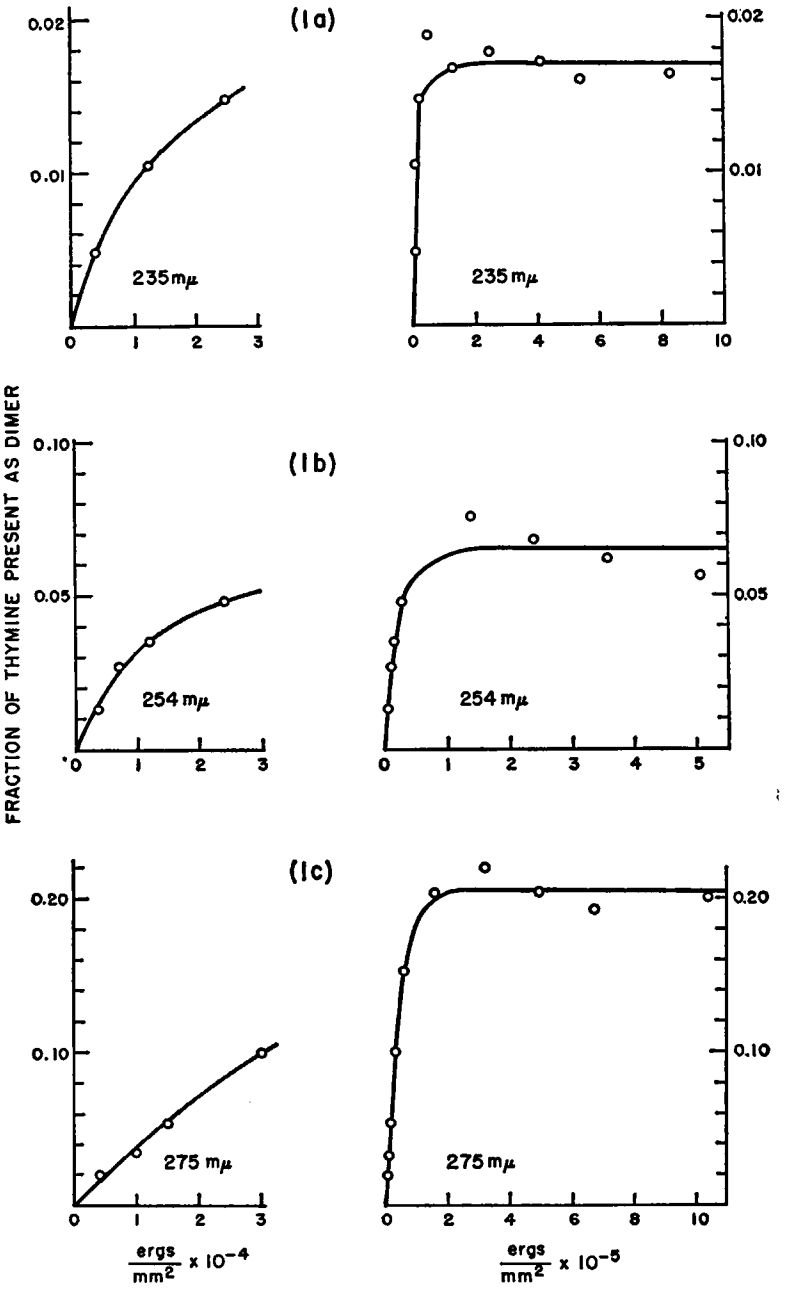

FIGURE 1 (1*a*) Fraction of thymine present as dimer plotted against dose at 235 mμ. (1*b*) Fraction of thymine present as dimer plotted against dose at 254 mμ. (1*c*) Fraction of thymine present as dimer plotted against dose at 275 mμ.

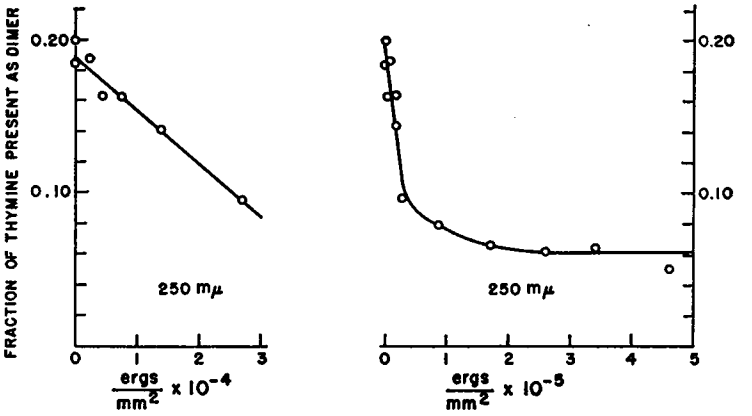

FIGURE 2 Fraction of thymine present as dimer plotted against dose at 250 mμ for a sample which had previously been given 10^6 ergs/mm² of 275 mμ irradiation as shown in Fig. 1c.

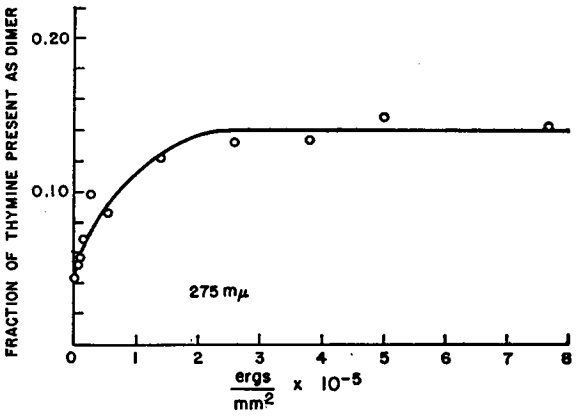

FIGURE 3 Fraction of thymine present as dimer plotted against dose at 275 mμ for a sample which had first been given 10^6 ergs/mm² of 275 mμ light (Fig. 1c) and then 5×10^5 ergs/mm² of 250 mμ light (Fig. 2).

curves of Fig. 1 and the ultraviolet extinction coefficients of DNA (Table I). The initial quantum yields (Table II) increase slightly with increasing wavelength.

If energy transfer processes are negligible in DNA and if thymine dimerization occurs between adjacent pairs of thymines, one can express the quantum yield in the more conventional terms of number of reactions per quanta absorbed by a TT pair. The extinction coefficient of DNA is 0.6 times the sum of the extinction coefficients of its constituent nucleotides (Beaven, Holiday, and Johnson, 1955). The extinction coefficient of a TT pair in DNA is shown in Table I, assuming that it is 0.6 times twice the extinction coefficient of thymidine. Assuming a thymine-thymine

TABLE I

EXTINCTION COEFFICIENTS FOR DNA PER MOLE OF PHOSPHORUS, FOR TT PAIRS IN DNA AND FOR THYMINE DIMER

λ	DNA*	TT IN DNA‡	Thymine dimer§
mμ	ϵ/P	ϵ/TT	ϵ
235	3000	2800	1570
254	6400	8200	285
275	4500	10200	14.8

* Courtesy Mr. R. Jensen.

‡ This number is 0.6 times twice the extinction coefficient of thymidine, as described in the text. Thymidine extinction coefficients are from Beaven, Holiday, and Johnson (1955).

§ Courtesy Dr. H. Johns.

TABLE II

QUANTUM YIELD FOR DIMERIZATION

λ	Q_a	Q_b
mμ		
235	0.009	0.036
254	0.012	0.033
275	0.016	0.023

Q_a = Fraction of thymine dimerized per quantum absorbed per nucleotide.

Q_b = Number of reactions per quantum absorbed by a TT pair, assuming no energy transfer as described in the text.

nearest neighbor frequency of 0.3 (Josse, Kaiser, and Kornberg, 1961), the fraction of TT pairs dimerized per unit dose is 1/0.3 of the total fraction of thymine dimerized per unit dose. The initial quantum yields, calculated from 1/0.3 times the initial slopes of the curves of Fig. 1 and the assumed extinction coefficients for a TT pair in DNA (Table I), decrease slightly with increasing wavelength (Table II).

Thus, the results, as analyzed in Table II, do not clearly favor either the energy transfer or the no energy transfer hypothesis.

Ideal Statistics of Dimer Formation in a Polynucleotide. Before further analyzing dimer formation, it is necessary to present a model for thymine dimerization in DNA and to explore some of its predictions. This model is as follows: Photodimerization can take place between any two thymines which are adjacent to each other along a polynucleotide chain. The probability, X_m, that a quantum incident upon the sample will cause a photodimerization of any given adjacent pair of thymines is a constant independent of ultraviolet dose and independent of the identity of the surrounding nucleotides. (This assumption may not be a good one, for it is quite conceivable that the rate of dimerization will change as the DNA molecule is distorted by the formation of photoproducts. In addition, there is no

a priori reason to expect the rate of the forward reaction to be independent of the identity of surrounding nucleotides.) Conversely, the probability that an incident quantum will cause a photodimer to revert, X_d, is also a constant independent of dose and the identity of surrounding nucleotides. The probability that two adjacent thymine molecules, bounded by nucleotides different from thymine, will exist as dimer at the photosteady state is then simply X_m/X_d.

Assuming that all thymine-thymine pairs are bounded by nucleotides different from thymine and using the experimentally determined thymine-thymine nearest neighbor frequency of 0.3 (Josse, Kaiser, and Kornberg, 1961), the fraction of thymine present as dimer has been plotted against X_m/X_d in Fig. 4 ($n = 2$). Of course not all thymine-thymine pairs will be bounded by nucleotides different from thymine. For any given number of consecutive thymines bounded by non-thymines, it is possible to calculate the fraction of thymine present as dimer as a function of X_m/X_d (Wulff, 1962). Again using the experimentally determined nearest neighbor

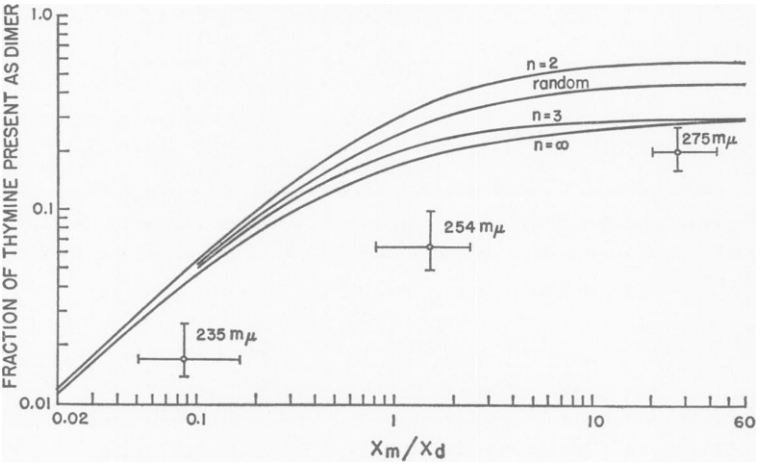

FIGURE 4 The solid lines signify the fraction of thymine present as dimer at the photosteady state (for $X_m/X_d = 0.02$ to $X_m/X_d = 60$) for various modifications of the model:

$n = 2$ Thymine-thymine nearest neighbor frequency 0.3; all thymines occur in runs of 1 and 2 consecutive thymines.

$n = 3$ Thymine-thymine nearest neighbor frequency 0.3; all thymines occur in runs of 1 and 3 consecutive thymines.

$n = \infty$ Thymine-thymine nearest neighbor frequency 0.3; all thymine-thymine nearest neighbors occur in long chains of thymines.

Random Thymine-thymine nearest neighbor frequency 0.3; distribution of thymine-thymine nearest neighbors is random along the polynucleotide chain.

The observed photostationary states for 235 mμ, 254 mμ, and 275 mμ are plotted against the X_m/X_d values for these three wavelengths. The X_m/X_d values were calculated from the initial measured rates of dimerization, the extinction coefficients for thymine dimer, and an assumed quantum yield of 0.6 for the back reaction, as explained in the text.

frequency of 0.3, the fraction of thymine present as dimer has been plotted in Fig. 4 against X_m/X_d for several different assumptions concerning the distribution of thymine-thymine nearest neighbors (not thymines!) in the polynucleotide chain. These curves serve to define a range within which the experimentally determined values must fall if the model is correct.

Comparison of the Observed Photosteady States with those Predicted by the Model. In order to compare the photosteady state observed at a given wavelength with that predicted by the model, it is necessary to estimate the X_m/X_d characteristic of any wavelength.

The parameter X_m may be calculated as follows: The initial slopes of the curves in Fig. 1 give the fraction of total thymine dimerized per unit dose. Taking the thymine-thymine nearest neighbor frequency as 0.3, the fraction of thymine-thymine nearest neighbor pairs dimerized per unit dose, X_m, is then 1/0.3 of the fraction of total thymines dimerized per unit dose.

The parameter X_d may be calculated by either of the following two methods: (*a*) One can multiply the extinction coefficients of thymine dimer in aqueous solution (Table I) by 0.6, the quantum yield for reversion of thymine dimer in aqueous solution (Johns *et al.*, 1962; Setlow, 1961). (*b*) One can measure the initial slope of Fig. 2, where thymine dimer formed at 275 mμ is converted back to thymine by 250 mμ light. Method (*b*) has the disadvantage that the initial slope of Fig. 2 can only be roughly estimated. While the data required for method (*a*) have been measured with much greater accuracy, it is not at all obvious that the ultraviolet cross-section for reversion of thymine dimer in DNA will be the same as the ultraviolet cross-section for reversion of thymine dimer in aqueous solution, especially since the possibility exists that energy absorbed by other nucleotides in the DNA can be transferred to thymine dimer molecules and cause them to revert to thymine molecules. Assuming that thymine dimer in DNA has the same extinction coefficient as thymine dimer in solution, the slope drawn in Fig. 2 gives a quantum yield of 0.9 for the reversion of thymine dimer in DNA, compared to 0.6 for thymine dimer in aqueous solution. Therefore energy transfer processes, if they exist at all, are not of sufficient magnitude to alter grossly the rate of the reverse reaction.

In Fig. 4 the experimentally measured fraction of thymine present as dimer at the plateau is plotted against the X_m/X_d characteristic of that particular wavelength, where X_d was calculated by multiplying the extinction coefficients of thymine dimer in aqueous solution by 0.6 (method (*a*), preceding paragraph). Calculation of X_d by method (*b*) would give an X_m/X_d at 254 mμ which is 30 per cent lower than that obtained by method (*a*).

In spite of the uncertainties involved in estimating X_m/X_d and in experimentally measuring the position of the photosteady state, it is quite clear that the fraction of thymine present as dimer at the photosteady state is significantly lower than what one would predict on the basis of the initial rate of dimerization, regardless of what

assumptions are made about the distribution of thymine-thymine nearest neighbors in the polynucleotide chain. This conclusion is in agreement with the work of Setlow and Carrier (1963), who made a spectrophotometric analysis of irradiated DNA. There are at least two possible explanations of this apparent decrease in the rate of the forward reaction: First, it is possible that photochemically induced changes in the DNA distort the DNA in such a way that further dimerization occurs with greater difficulty. The factors responsible for the slowing down of the forward reaction could be thymine dimerization itself as well as other photoproducts which are formed in irradiated DNA (Dellweg and Wacker, 1962). Secondly, it is possible that the rate of the forward reaction is not independent of the identity of the surrounding nucleotides. We cannot decide between these two alternatives.

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