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Pyrimidine Dimers: Effect of Temperature on Photoinduction

Abstract. *Ultraviolet-induced pyrimidine dimer formation in DNA and polyuridylic acid was inhibited on irradiation at 77°K. Enhancement of thymine dimer formation in solutions of DNA occurred upon addition of ethylene glycol. Low temperature measurements of absorbance of polyuridylic acid at low temperature showed that no significant alteration of the residues occurred after irradiation at 77°K and before thawing.*

Bacteriophage (*1*) and bacteria (*2, 3*) are much more sensitive to inactivation by ultraviolet light when irradiated in the frozen state than when irradiated at room temperature. There is considerable evidence (*4*) that DNA is the ultraviolet-sensitive target and that pyrimidine dimers in the DNA can account for much of the biological inactivation at room temperature. Therefore, we compared the light-induced information of pyrimidine dimers at room temperature and at 77°K in *Escherichia coli* DNA and in polyuridylic acid (polyU). That many fewer dimers were formed at the lower temperature in both those systems suggests that factors other than enhanced pyrimidine dimer formation are responsible for the increased sensitivity of bacteria and phage in the frozen state.

Escherichia coli DNA labeled with ^{3}H-thymidine was dissolved in a mixture of ethylene glycol and water (1:1); this mixture forms an optically clear glass at 77°K. A *p*H of 7, measured at room temperature, was maintained with 0.005*M* phosphate. Irradiations were carried out on samples (0.65 μg/ml) in quartz electron-spin-resonance (ESR) tubes which were held in a fingertip, quartz Dewar. The Dewar was rotated during irradiation to ensure even irradiation of the sample. Monochromatic ultraviolet radiation (±50 Å) was obtained from a Hilger quartz-prism monochromator and a 500-watt Phillips high-pressure mercury lamp. The results presented here were obtained with 2800-Å irradiation at about 50 erg mm^{-2}sec^{-1}. After irradiation, the samples were subjected to hydrolysis by formic acid, and the products of hydrolysis were chromatographed on paper with a solvent mixture of butanol, acetic acid, and water to separate the thymine photoproducts from thymine (*5*). The distribution of radioactivity was measured and used to calculate the percentage of thymine photoproduct.

From the large difference in the initial slopes (Fig. 1), thymine photoproducts are formed at a much slower rate at 77°K. Although no $\overline{T}\overline{T}$ ($R_F = 0.31$) or $\overline{U}\overline{T}$ ($R_F = 0.22$) (dimers of thymine and of uracil and thymine, respectively) formation was observed at 77°K, there was some indication of a thymine photoproduct with $R_F = 0.40$, corresponding to that obtained for irradiated dry DNA or in spores (*3, 6*).

Inhibition of thymine dimer formation at 77°K was also observed in ice. As indicated in Table 1, for an incident dose of 3 × 10^4 erg/mm^2 the percentage of thymine photoproduct formed at 77°K was about the same in water as in the mixture of ethylene glycol and water, between 1 and 2 percent. The effective radiation dose in ice was less than that in the ethylene glycol and water because of light-scattering by ice crystals. A rough estimate of the amount of light lost by scattering in ice, based on measurements by Füchtbauer and Mazur (*7*), is about 50 percent. The reduction of thymine photoproduct in ice, then, seems only partially due to scattering.

The formation of thymine photoproduct was also measured for heat-denatured DNA both in water and in ethylene glycol and water at 77°K. Although there was no significant difference in yields between native and denatured DNA in water at 77°K, the formation of thymine photoproducts, mostly dimer, was slightly greater for denatured DNA irradiated in the ethylene glycol and water mixture.

The loss in transforming activity of *Hemophilus influenzae* DNA (*4*) upon 2800-Å irradiation at 77°K was also measured. The persistence of activity (survival) both in water and in ethylene glycol and water at room temperature and at 77°K is given in Table 1. For a dose of 3 × 10^4 erg/mm^2, the percentage of activity remaining at 77°K was several orders of magnitude greater than at room temperature, consistent with the absence of dimer formation at 77°K in *Escherichia coli* DNA. Furthermore, the activity persisting at room temperature was less in the presence of glycol (0.02 percent) than in water (0.12 percent). This result is in accord with the increased rate of dimer formation found for *E. coli* DNA irradiated in the mixture of ethylene glycol and water. Freezing alone has no effect on the transforming activity.

Polyuridylic acid (Miles) was irradiated in quartz ESR tubes as described for DNA except that a 2650-Å interference filter (Baird Atomic) with a 150-Å bandpass was used in place of the monochromator. Samples were dissolved in the mixture of ethylene glycol and water, and no salt was added. The formation of photoproduct was followed by measuring the corresponding changes in the optical absorbance, the initial absorbance at 2600 Å being around 0.50. The absorbance measurements were

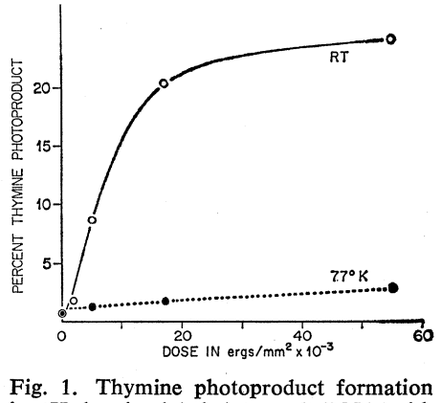

Fig. 1. Thymine photoproduct formation in ^{3}H-thymine-labeled *E. coli* DNA with 2800-Å radiation at room temperature and at 77°K. Solvent was ethylene glycol and water (1:1), 0.005*M* phosphate, *p*H 7. The percentage of thymine photoproduct was calculated from radioactivity in the $\overline{T}\overline{T}$ and $\overline{U}\overline{T}$ regions of the chromatograms (R_F between 0.16 and 0.40) relative to the total radioactivity.

Table 1. Thymine photoproduct formation in *E. coli* DNA and persisting ability of *H. influenzae* transforming DNA to transform cells to cathomycin resistance after 3×10^4 erg/mm^2 at 2800 Å.

DNA	Solvent*	Temp. (°K)	Thymine photoproduct (%)	Persistence of transforming DNA (%)
Native	W	298	8.2	0.12
Native	W	77	1.7	13.7
Native	EG:W	298	18.9	0.02
Native	EG:W	77	1.2	18.5
Denat.†	W	77	1.9	
Denat.	EG:W	77	5.2	

* Either W, water; or EG:W, a mixture of ethylene glycol and water (1:1). † Denat., denatured.

made at room temperature in the same quartz tubes used for irradiation. The use of quartz ESR tubes as optical cells proved satisfactory provided that the sample tubes were centered in the light beam. Since changes in the absorption spectrum of polyU upon irradiation reflect both dimer and hydrate formation (*8*), a rough estimate of dimer of total photoproduct was determined by short-wavelength radiation with a 2250-Å filter to reverse the dimer.

Dimer formation in polyU is much less extensive for irradiation at 77°K. The change in absorbance at 2600 Å is given in Fig. 2 for irradiation at room temperature and 77°K. Two different curves were obtained for irradiation at 77°K. The curve labeled "uninterrupted" was obtained by irradiating a series of samples for successively longer intervals and measuring the absorbance after they were thawed. After a small initial decrease, there was no change in absorbance, even upon prolonged irradiation. The saturation dose of approximately 15,000 erg/mm^2 decreased the absorbance by roughly 5 percent.

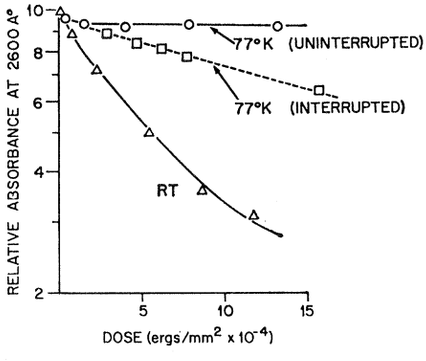

Fig. 2. Loss of absorbance at 2600 Å for polyU in ethylene glycol and water (1:1); irradiated 2650 Å. The points on the curve labeled "uninterrupted" are for separate samples brought to 77°K and given the indicated dose without intermediate warming to room temperature. The points on the dotted curve labeled "interrupted" were obtained by irradiating a single sample at 77°K, thawing it, measuring the absorbance at room temperature, and then repeating the freezing, irradiation, and so forth.

Irradiation at 77°K, interrupted by successive thawings gave higher conversion to dimer than did continuous irradiation. A single sample of polyU was subjected to successive cycles of freezing, irradiation with 15,000 erg/mm^2, thawing, and measurement of absorbance at room temperature (Fig. 2, curve labeled "interrupted"). Polyuridylic acid irradiated in this way was again irradiated at room temperature with short-wavelength ultraviolet light to dissociate any dimers present. The relative absorbance increased from 0.56 to a limiting value of 0.65, indicating some dimer formation at 77°K.

Herskovits (*9*) has shown that high concentrations of ethylene glycol (more than 50 percent) denature DNA, as indicated by the accompanying absorbance changes. But at the concentration of ethylene glycol used here (50 percent), DNA shows no alteration in absorbance. However, even in the absence of absorbance changes, sufficient alteration of the forces stabilizing the double-strand conformation do occur, as shown by the lowering of the melting temperature of DNA in 50 percent ethylene glycol (*10*). Since the rate of dimer formation has been found to be about twice as fast for denatured DNA as for native DNA (*11*), it appears that as far as dimer formation is concerned, 50 percent ethylene glycol is equivalent to denaturation. These results are in conflict with those obtained by Dellweg and Wacker (*12*), who found no increase in dimer formation in glycol.

The fact that pyrimidine dimers are not readily formed between adjacent bases in DNA and polyU at 77°K is explained by a lack of motional freedom necessary for adjacent pyrimidines to attain the proper orientation. According to Nagata *et al.* (*13*), the most probable thymine dimer isomer in DNA requires that the adjacent thymine moieties be rotated through an angle of 36°.

The change in absorbance of polyU was measured at 77°K after irradiation and before thawing to check the possibility that the saturation effect in polyU at 77°K was due to the formation of some altered form of the residues, which, upon thawing, could either revert to normal uracil or form stable photoproduct. It was assumed that the alteration of a base would change its absorbance and that a dose of 15,000 erg/mm^2 was sufficient to obtain a maximum concentration of altered residues. Upon comparison with the unirradiated sample, no change in absorbance was observed (within the experimental error of 5 percent) even after a dose of 200,000 erg/mm^2. Thus, unless the altered residues have the same absorbance as the unaltered bases, there is no evidence for more than a small number (5 percent) of the bases being altered at 77°K. Therefore it seems likely that uracil dimers and hydrates are formed directly from optically excited singlet or triplet states. At 77°K only a small percentage (5 percent) of the bases must be in the right position to form irreversible damage. Upon thawing and refreezing, another group of bases becomes suitably arranged to form photoproducts.

It is concluded then, that the increase in sensitivity to ultraviolet light previously found for bacteria (*2*) and phage (*1*) at low temperatures is not due to an increase in thymine dimer formation since, as shown here, pyrimidine dimer formation is strongly inhibited at 77°K. Furthermore, the increased resistance of transforming DNA to ultraviolet light at 77°K can also be explained by an inhibition of dimer formation at the reduced temperature.

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