DISAPPEARANCE OF THYMINE PHOTODIMER IN ULTRAVIOLET IRRADIATED DNA UPON TREATMENT WITH A PHOTOREACTIVATING ENZYME FROM BAKER'S YEAST

Daniel L. Wulff* and Claud S. Rupert†

Gates and Crellin Laboratories of Chemistry,‡ California Institute of Technology, Pasadena, California, and Department of Biochemistry, The Johns Hopkins University School of Hygiene and Public Health, Johns Hopkins University, Baltimore 5, Maryland

Received March 7, 1962

A dimer of thymine is formed by ultraviolet irradiation of frozen aqueous solutions of thymine (Beukers and Berends, 1960 and 1961, Wang, 1961, and Wulff and Fraenkel, 1961).

Thymine dimer has been isolated by hydrolysis of ultraviolet irradiated DNA (Beukers, Ijlstra and Berends, 1960 and Wacker, Dellweg and Weinblum, 1960). This suggests the interesting hypothesis that formation of thymine dimer is the, or one of the, significant chemical events in ultraviolet damage of microorganisms. Furthermore, the discovery that short wavelength ultraviolet irradiation of dilute aqueous solutions of thymine dimer causes reconversion to thymine (Beukers, Ijlstra and Berends, 1959) leads to the speculation that "photoreactivation" of 254 mμ ultraviolet damage to living microorganisms by light of wavelengths centering around 370 mμ might be due to a similar reconversion of thymine dimer to thymine. This hypothesis is supported by the present work in which it is shown that thymine dimer formed in irradiated DNA *in vitro* can be eliminated by illuminating the DNA in the presence of a photo-

---

* NSF Predoctoral Fellow

† Current address: University Institute of Microbiology, Copenhagen, Denmark

‡ Contribution No. 2801

reactivating enzyme from baker's yeast which repairs ultraviolet damage to bacterial transforming DNA (Rupert, 1960). (It may be recalled that irradiation of aqueous solutions of thymine dimer with light around 3700 Å in the absence of enzyme does not cause reconversion to thymine (Wang, 1960).)

Preparation and irradiation of H^3-thymine DNA. H^3-thymine DNA (1.2 μc/μg obtained by growing E. coli 15 $A^-T^-U^-$ in a medium containing methyl-H^3 thymidine) was purified by cesium chloride centrifugation (Marmur, 1961) and exposed to 3100 ergs/mm^2 of 254 mμ ultraviolet light in an apparatus described by Johns et al. (in press).

Treatment of H^3-thymine DNA with an enzyme from baker's yeast. Incubation mixtures, containing 2.5 μg/ml DNA and 1250 μg/ml of a partially purified (ammonium sulfate) preparation of yeast photoreactivating enzyme were warmed to 37° for 30 minutes either in the dark or illuminated with 2000 μ watts/cm^2 of 340-400 mμ light from suitably filtered "blacklight" fluorescent bulbs. On the basis of previous experience with transforming DNA, this would be expected to give maximum repair of the biologically significant damage. The mixtures were then deproteinized with 1/3 volume 6 M NaCl and chloroform-octanol. Heat inactivated enzyme was prepared by warming a 2500 μg/ml enzyme solution to 65°C. for 10 minutes. Photoreactivating enzyme is reduced to 5% activity in 2 minutes at 65°C. (Rupert, 1961 and in press).

Analysis for thymine and thymine dimer. DNA was subjected to formic acid hydrolysis (.03 to .3 μg of DNA in 25 μl of formic acid) in evacuated tubes at 175°C. for 30 minutes (Wyatt and Cohen, 1953). The yield of thymine dimer from irradiated DNA was independent of hydrolysis time over a range of 15 to 60 minutes at 175°C.

DNA hydrolysates, to which were added 10 μg carrier thymine dimer (prepared from u.v. irradiated frozen thymine solutions) and 10 μg carrier thymine, were paper chromatographed in isopropanol : conc HCl : water (68 : 15.5 : 16.5). Thymine dimer was further purified by paper

chromatography in saturated ammonium sulfate : 1 N sodium acetate : isopropanol (40 : 9 : 1) (Wacker, 1960).

Tritium was counted in a liquid scintillation spectrometer, using the dioxane-water (15 ml : 1 ml) system of Butler (1961). The paper and salts from chromatography did not interfere with counting. The thymine activity constituted more than 98% of the total radioactivity in unirradiated DNA hydrolysates. The recovery of carrier thymine dimer (considerably less than 100%) was assayed spectrophotometrically after u.v. induced reconversion of thymine. (This estimation procedure was standardized with 10 μg carrier thymine dimer.)

Results and Discussion

In the second and third columns of Table I are tabulated the net radioactivities observed for thymine and thymine dimer in the various

Table I

Sample	Thymine cpm	Observed Dimer cpm	% Dimer Recovery	Corrected Dimer cpm	Fraction of Thymine Present as Dimer
Unirradiated DNA	48834	8	91	-	-
	49671	6	66	-	-
u.v.'d DNA	50199	447	84	533	.011
	41971	418	86	483	.011
u.v.'d DNA + Enzyme in Dark	14124	81	54	151	.011
	15439	97	64	150	.010
u.v.'d DNA + Heated Enzyme in Light	10676	107	91	118	.011
	8109	84	88	95	.012
u.v.'d DNA + Enzyme in Light	9307	4	91	4	<.001
	8497	0	91	0	<.001

Results of duplicate hydrolyses and analyses of a single sample are listed. Columns explained in text.

hydrolysates. In the fourth column the recovery of carrier thymine dimer is tabulated and in the fifth column the appropriate correction is made for the loss of H^3-thymine dimer upon chromatography. The fraction of thymine present as dimer is shown in the last column.

The important result is that, whereas samples of irradiated DNA incubated with photoreactivating enzyme in the dark and samples incubated with heat inactivated enzyme in the light both show the same amount of thymine dimer as is present in the untreated irradiated DNA, incubation of irradiated DNA with enzyme plus light destroys over 90 percent of the dimer present.

A similar result was obtained independently by Wacker (1961), using a crude yeast extract which would be expected to contain photoreactivating enzyme. Both findings permit the interpretation that the enzyme causes the dimer in DNA to disappear upon incubation with light, presumably by converting it back to thymine.

Acknowledgements. One of us (D.W.) would like to thank Professors N. Davidson and M. Delbrück for their interest, advice and encouragement. This work was supported by U.S.P.H.S. Grant No. A-3907(CI), and U.S.P.H.S. Senior Research Fellowship SF-312.

REFERENCES

R. Beukers and W. Berends, Biochem. Biophys. Acta, 41, 550 (1960)
R. Beukers and W. Berends, Biochem. Biophys. Acta, 49, 181 (1961)
R. Beukers, J. Ijlstra and W. Berends, Rec. Trav. Chim., 78, 883 (1959)
R. Beukers, J. Ijlstra and W. Berends, Rec. Trav. Chim., 79, 101 (1960)
F. E. Butler, Anal. Chem., 33, 409 (1961)
H. Johns, S. Rappaport, and M. Delbrück, in press
J. Marmur, J. Mol. Biol., 3, 208 (1961)
C. S. Rupert, J. Gen. Physiol., 43, 573 (1960)
C. S. Rupert, J. Cell & Comp. Physiol., 58, Suppl. 1, 57 (1961)
C. S. Rupert, J. Gen. Physiol., in press
A. Wacker, H. Dellweg and D. Weinblum, Naturwissenschaften, 47, 477 (1960)
A. Wacker, J. Chimie Physique, 58, 1041 (1961)
S. Y. Wang, Nature, 188, 846 (1960)
S. Y. Wang, Nature, 190, 690 (1961)
D. L. Wulff and G. Fraenkel, Biochem. Biophys. Acta, 51, 332 (1961)
G. R. Wyatt and S. S. Cohen, Biochem. J., 55, 774 (1953)