THE EXCISION OF THYMINE DIMER FROM THE DNA OF UV-IRRADIATED E. COLI 15 $T^-A^-U^-$ DURING THYMINE DEPRIVATION

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Received June 1, 1964

Irradiation of DNA with ultraviolet light causes the formation of dimers between adjacent thymine molecules (Wacker et al, 1960). The presence of these dimers in DNA is largely responsible for the adverse biological effects produced by UV light (Setlow and Setlow, 1962, 1963). Bacteria possess two known mechanisms whereby thymine dimers are eliminated from the DNA. One of these is photoreactivation, whereby, thymine dimers are reconverted to thymine *in situ* in the presence of light and an enzyme fraction isolated from yeast and *E. coli* (Rupert, 1960; Wulff and Rupert, 1962). This splitting of thymine dimers has been correlated with a return of biological activity of UV-irradiated transforming DNA (Setlow and Setlow, 1963). In addition, there is also a dark reactivation process which involves the excision of intact thymine dimers from the DNA (Boyce and Howard-Flanders, 1964; Setlow and Carrier, 1964). It has been proposed that DNA synthesis in UV-resistant strains resumes only after the dimers have been removed (Setlow and Carrier, 1964).

It has recently been found that partial dark repair of UV injury, as measured by the subsequent recovery of the ability of bacteria to synthesize DNA, can occur in the absence of thymine (Shuster, 1964). The purpose of this investigation was to determine whether thymine dimers are also excised from DNA under conditions of thymine deprivation.

Growth and Labeling of Cells

E. coli 15 $T^-A^-U^-$, a multiple auxotroph requiring thymine, arginine and uracil was grown to late log phase in 12 ml of M9* supplemented with 2.5 mg/ml of casamino acids, 20 μg/ml of uracil and 2.8 μg/ml or methyl-H^3 thymidine (H^3-TdR) of specific activity 4.2 c/mM. The bacteria were washed three times in M9 and suspended in 10 ml of M9 supplemented only with 1.8 μg/ml of H^3-TdR of specific activity 6.65 c/mM. They were incubated at 37° with aeration for 90 minutes in order to develop immunity to subsequent withdrawal of thymine (Maaløe and Hanawalt, 1961). They were washed three times, suspended in 150 ml of M9 and divided into five 30 ml samples which were treated as follows:

1) No UV followed by 60 minutes incubation in M9 containing no thymine, supplemented with arginine and uracil (-T+AU).
2) UV, but no incubation.
3) UV, followed by 60 minutes in -T+AU.
4) UV, followed by 60 minutes in -T-AU.
5) UV, followed by 60 minutes in -T-AU and then an additional incubation for 2 hours in +T+AU.

Irradiations were carried out in 14 cm petri dishes on a rotary shaker with a low pressure mercury germicidal lamp. The average dose to the cells was calculated to be 1,040 ergs/mm^2. All incubations were carried out in the dark after which the samples were centrifuged and treated with cold 5% TCA. Acid soluble and insoluble fractions were separated and hydrolyzed in concentrated trifluoroacetic acid, chromatographed on Whatman #1 paper and scanned for radioactivity, as previously described (Boyce and Howard-Flanders, 1964). Thymine dimer was identified by its characteristic Rf value and by its reversion to thymine after elution and UV-irradiation in aqueous solution.

* Contains per liter of water: NH_4Cl, 1 gm; $NaH_2PO_4 \cdot 7\ H_2O$, 11 gm; NaH_2PO_4, 3 gm; NaCl, 5 gm; $MgSO_4$, 120 mg; glucose, 4 gm.

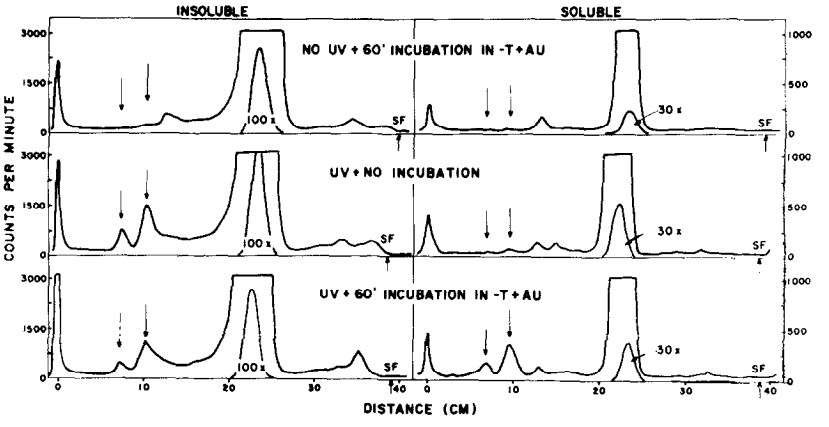

Fig. 1 Radiochromatograms showing distribution of radioactivity in the acid insoluble and soluble fraction of irradiated and unirradiated E. coli 15 $T^-A^-U^-$. Arginine and uracil, but not thymine (-T+AU) were present during post irradiation incubation. Incubation in -T-AU, not presented in the figure, gave similar results as shown in Table I. Cells were labeled with H^3-TdR, irradiated with a dose of 1,040 ergs/mm^2, incubated, extracted with cold acid and chromatographed. Solvent fronts are marked by arrows. The two photoproducts containing labeled thymine appear at approximately 8 and 10 cms from the origin.

Results

Figure 1 shows typical radiochromatograms of the hydrolyzed acid soluble and insoluble fractions of cells which had been treated as described. In the irradiated, unincubated samples, two photoproducts were present in the acid soluble fraction, but were absent in the acid soluble. After 1 hour incubation in -T+AU, the photoproducts appeared in the acid soluble fraction of the cells. The two photoproducts were eluted together and UV-irradiated in aqueous solution. After rechromatography, they appeared as a single entity with the Rf value of thymine. In other experiments, each photoproduct was tested separately for reversion to thymine after UV-irradiation in aqueous solution (Boyce and Howard-Flanders, 1964). The resulting products each chromatographed like thymine. It is, therefore, believed that both photoproducts are dimers of thymine, the larger one being characteristic of thymine-thymine dimer with an Rf value of 0.27.

TABLE I

RADIOACTIVITY OF THYMINE AND THYMINE DIMER IN THE ACID INSOLUBLE AND SOLUBLE FRACTIONS OF *E. COLI* 15 $T^-A^-U^-$

Insoluble

Treatment	$\hat{TT}$* c.p.m.	Thymine c.p.m.	$\hat{TT}$/T%
UV - no incubation	3.2×10^3	1.04×10^6	0.31%
No UV 60 -T+AU	< 150	9.06×10^5	0.02%
1040 ergs/mm^2 60 -T+AU	2.18×10^3	8.11×10^5	0.27%
1040 ergs/mm^2 60 -T-AU	2.27×10^3	8.11×10^5	0.28%
1040 ergs/mm^2 60 -T-AU 120 +TAU	1.67×10^3	6.06×10^5	0.28%

Soluble

Treatment	$\hat{TT}$ c.p.m.	Thymine c.p.m.	$\hat{TT}$/T%
UV - no incubation	< 50	4.68×10^4	0.10%
No UV 60 -T+AU	< 50	2.25×10^4	0.22%
1040 ergs/mm^2 60 -T+AU	1.16×10^3	3.27×10^4	3.5 %
1040 ergs/mm^2 60 -T-AU	8.5×10^2	3.27×10^4	2.6 %
1040 ergs/mm^2 60 -T-AU 120 +TAU	8.0×10^2	1.74×10^4	4.6 %

* Thymine dimer

Table I gives the numerical results of these experiments. Radioactivity of thymine dimer includes counts per minute of both photoproducts.

These figures have been used to compute the ratio of thymine dimer to thymine in the various fractions. It can be seen that this ratio decreased after incubation under various conditions by about 10% in the acid insoluble fraction. In the acid soluble fraction, the ratio increased on an average of 10-fold over the insoluble fraction of the irradiated, unincubated control. For the samples incubated for 1 hour in -T+AU or in -T-AU, the sum of thymine dimer in the acid soluble and insoluble fractions agreed closely with the thymine dimer in the irradiated, unincubated control. Thus, for cells incubated in -T+AU, total thymine dimer was 3.34×10^3 cpm while for -T-AU, it was 3.12×10^3 as compared to the unincubated control of 3.20×10^3. For cells incubated in -T-AU followed by incubation for two hours in +T+AU, total thymine dimer decreased to a value of 2.47×10^3. Since the growth medium was not examined for radioactive components, loss of thymine dimer could have resulted from cell lysis or passage through the cell wall. However, in experiments with another strain of E. coli, no thymine dimer was detected in the incubation medium after 1500 ergs/mm^2 followed by incubation for 2 hours (Boyce, unpublished results).

Contrary to the data for thymine dimer, there is an apparent decrease in total thymine activity resulting from post irradiation. incubation. This is shown by the fact that the sum of thymine activity in the acid soluble and insoluble fractions in the incubated samples does not equal that in the irradiated, unincubated sample. This is probably largely due to the loss of thymine into the incubation medium (Boyce, unpublished results), although this was not measured in these experiments.

Discussion

The excision of thymine dimers from acid precipitable DNA of UV-irradiated bacteria that occurs in strains carrying the wild type uvr gene when incubated in the dark (Boyce and Howard-Flanders, 1964; Setlow and Carrier, 1964), is probably an essential part of the process of dark recovery from UV injury. The uvr mutants that have been isolated and tested in this laboratory (some 20 in all) are deficient both in this mechanism (Boyce, unpublished results)

and in the ability to repair UV-irradiated DNA (Howard-Flanders et al, submitted for publication).

After incubation, photoproducts appeared in the acid soluble fractions resulting in thymine dimer to thymine ratios greater than that found in the insoluble fraction of the irradiated, unincubated control. These increased ratios could be due to the retention of thymine dimer inside the cell since they are excised with an intact phosphodiester sugar linkage which is resistant to nuclease attack (Setlow and Carrier, 1964). This may explain the failure of the dimers to pass through the cell membrane while thymine is extruded into the incubation medium. The sum of activity in the insoluble and soluble fractions after incubation adds up to 60-80% of that in the irradiated, unincubated control. Twenty to 40% of the thymine radioactivity has presumably been lost into the medium. Correcting the thymine dimer to thymine ratio in the soluble fraction of the sample incubated in -T+AU so as to include the thymine which has been lost, still gives a two-fold increase over the insoluble fraction of the irradiated, unincubated samples. These results indicate that the breakdown of DNA in irradiated *E. coli* 15 $T^-A^-U^-$ may be initiated by the excision of fragments containing thymine dimers and that the excision can occur during thymine deprivation and, therefore, in the absence of net DNA synthesis. It appears that the factors responsible for the removal of thymine dimers during incubation in the dark are not subject to the same regulatory mechanisms that control DNA synthesis.

ACKNOWLEDGEMENT

We wish to thank Professor Paul Howard-Flanders for his helpful comments.

This work was supported by U. S. Public Health Service Research Grant No. CA 06519-02 from the National Cancer Institute.

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