P.C. Hanawalt, E.C. Friedberg and C.F. Fox, ed.
Academic Press, N.Y. (1978)

B30

PHOTOREACTIVATION OF *Escherichia coli* IRRADIATED WITH IONIZING RADIATION[1]

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ABSTRACT By comparing the results for a *uvrA recA* and a *uvrA uvrB recA phr* strain, we conclude that the photoreactivation after ionizing irradiation involves the same enzyme that is responsible for the photoreactivation of UV-induced cyclobutadipyrimidines. A comparison of the photoreactivable sectors in a *uvrA recA* strain after UV and ^{137}Cs-γ-irradiation indicates that 10 krads of γ-radiation produces about 0.07 Jm^{-2}-equivalents of 254 nm-induced photoreactivable damage. After 400 krads, an acid hydrolysate of the isolated DNA revealed the presence of thymine-containing cyclobutadipyrimidines, as evidenced by their chromatographic properties and their photochemical reversibility. Thus, the photoreactivation observed after ionizing radiation in strains that are blocked in excision repair and in postreplication repair is due to the production of trace amounts of cyclobutadipyrimidines.

INTRODUCTION

Recently, Myasnik and Morozov (1) demonstrated that photoreactivation (PR) after γ-irradiation can be observed in certain strains of *Escherichia coli*, e.g., *uvrA recA* of K-12 and B_{s-1} (*uvrB lexA*). In the same work, the authors described that this PR is not produced by preillumination and is mediated by a temperature-dependent process, suggesting that it acts through photoenzymatic repair rather than by indirect photoreactivation (2) or the direct photochemical reversal of the damage (3). The only known photoenzymatic repair involves the use of photoreactivating enzyme (PRE), which specifically monomerizes cyclobutadipyrimidines in the presence of light

[1]This work was supported by U.S.P.H.S. research grant CA-06437, and research program project grant CA-10372 from the National Cancer Institute, DHEW.

ISBN 0-12-3226-50-3

(310-480 nm). (For reviews see 4-6). Therefore, two major questions arose: 1) does the observed PR after ionizing irradiation involve the same PRE as after UV irradiation, and 2) what is the γ-ray-induced lethal damage that is removed after exposure to light? The present work presents evidence that cyclobutadipyrimidines are produced by ionizing radiation, and that the observed PR after ionizing irradiation involves the same PRE as after UV irradiation.

Photoreactivating Enzyme is Required for Photoreactivation After Ionizing Irradiation. We have compared K-12 strains AB2480 (*uvrA recA*) and SR362 (*uvrA uvrB recA phr*) for their ability to be photoreactivated after γ-irradiation.

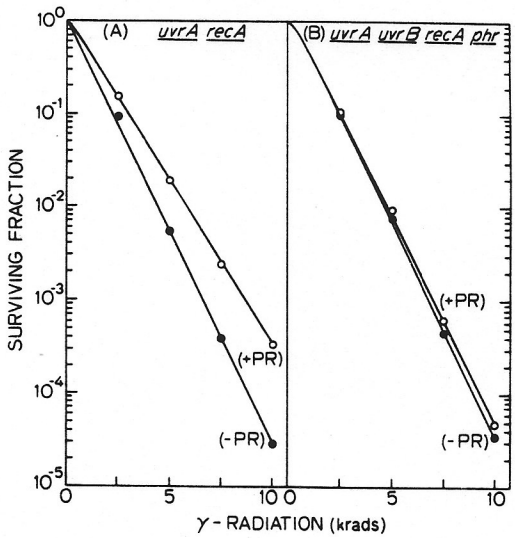

FIGURE 1. The effect of post-illumination with near-UV radiation on the survival of AB2480 (*uvrA recA*) and SR362 (*uvrA uvrB recA phr*) after γ-irradiation. Stationary phase cells grown in YEP (1) were suspended in DTM buffer (9) at 1 x 10^8 cells/ml, and irradiated with ^{137}Cs-γ-rays at room temperature without aeration. Samples were either kept in the dark (-PR), or post-illuminated (+PR) with 15-W Daylight fluorescent lamps for 3 min at a distance of 3 cm above the light source with a glass plate in between.

As shown in Fig. 1, Phr^+ AB2480 showed PR after γ-irradiation, as reported by others (1,7). In contrast, Phr^- SR362, which showed no detectable PR after UV irradiation, failed to show any detectable PR after γ-irradiation. This indicates that the same PRE is responsible for the observed PR after ionizing irradiation as well as after UV irradiation. A comparison of the photoreactivable sectors in strain AB2480 after UV irradiation (data not shown) and after γ-irradiation (Fig. 1) reveals that 10 krads of ^{137}Cs-γ-radiation produced about 0.07 J m^{-2}-equivalents of 254 nm-radiation-induced photoreactivable damage.

Production of Dimers by γ-Irradiation. Since the only substrate known for the PRE is cyclobutadipyrimidines (see 6), and there is material chromatographing similar to pyrimidine dimers after ionizing irradiation (8), we examined the possibility of dimer production by ionizing radiation. Strain SR73, a *thy* derivative of AB2480, was labeled in DTM-glucose medium (9) in the presence of 100 μCi/ml of ^3H-thymidine, and was γ-irradiated at 4°C in DTM buffer with 400 krads. The DNA was extracted, hydrolyzed with trifluroacetic acid at 175°C for 75 min, and chromatographed on Whatman #1 paper using: (A) *n*-butanol: acetic acid: H_2O (40:6:15), (B) *n*-butanol: H_2O (43:7), (C) isopropanol: NH_4OH: H_2O (7:1:2), and (D) *sec*-butanol saturated with H_2O. Authentic T<>T and U<>T (via deamination during acid-hydrolysis of C<>T) were isolated from UV-irradiated ^{14}C-labeled SR73 and used in parallel runs to locate the regions corresponding to T<>T and U<>T. In assays for T<>T, radioactivity eluted from the T<>T region in solvent (A) was rechromatographed in the same solvent to eliminate the streaking of thymine in the T<>T region. Of the isolated material, about 25% could be reversed to thymine by irradiation at 254 nm. In the subsequent development with solvent (B), material having Rf 0.2 can be separated from the T<>T region (Rf 0.11). Using solvent (C), material isolated from the T<>T region (Rf ∿0.57) can be further separated from other contaminating materials (Rfs ∿0.31 and 0.42, respectively). The isolated material that chromatographs identically with T<>T in solvents (A), (B), and (C), also chromatographs identically with T<>T in solvent (D), and is completely reversed to thymine upon exposure to UV radiation (data not shown). This material, therefore, can be confidently identified as T<>T.

In assays for U<>T, material corresponding to the U<>T region from solvent (A) was similarly chromatographed with solvents (B) and (C), as was done for T<>T. There is radioactive material that chromatographs as U<>T in solvents (B) and (C), but the separation of U<>T from other contaminating

materials is not satisfactory, and its yield can't be quantitated with confidence.

The purified T<>T after 400 krads represents 7.4 x 10^{-5} of the total ^{3}H-thymine radioactivity. Therefore, 400 krads of ^{137}Cs-γ-radiation is equivalent to 2.6-3.7 J m^{-2} of 254 nm-radiation in producing cyclobutadipyrimidines, if we take the initial rate of production of T<>T in the DNA of UV-irradiated cells as 2.0-2.75 x 10^{-5} radioactivity in T<>T/total radioactivity as T per J m^{-2} (10,11). This result is in good agreement with our *in vivo* estimate based upon survival curves. (10 krads=0.07 J m^{-2} of 254 nm radiation).

It is understandable why PR after ionizing radiation can only be maximally detected in *E. coli* K-12 *uvrA recA* and B_{s-1}; these strains show no shoulder in their UV-inactivation curves. Likewise, it is to be anticipated that no detectable PR can be demonstrated in *uvrB* or wild-type cells, because the γ-radiation-dose used for detecting PR has an equivalent UV-effect that corresponds to the shoulder region of the UV-inactivation curves in these strains.

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