

DNA SYNTHESIS KINETICS, CELL DIVISION DELAY, AND POST-REPLICATION REPAIR AFTER UV IRRADIATION OF FROZEN CELLS OF *E. COLI* B/r

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Abstract—Previous studies have shown that the relative yields of photoproducts produced in the DNA of *Escherichia coli* cells UV irradiated at -79°C differ from those produced at $+21^{\circ}\text{C}$; the yield of DNA-protein cross-links was markedly enhanced at -79°C while the yield of thymine dimers was reduced. In the present studies, cells of *E. coli* B/r *thy* were frozen at -79°C , and then UV irradiated (254 nm) while frozen (4.7 J m^{-2}), or after thawing (22 J m^{-2}). Essentially the same survival, cell division delay, and DNA synthesis kinetics were observed for these two samples after irradiation, even though the UV fluence differed by a factor of ~ 5 . This supports previous observations that a correlation exists between the magnitude of the effects of UV radiation upon DNA synthesis kinetics and on cell survival. The weight average molecular weight of the pulse labeled DNA in the sample irradiated at $+21^{\circ}\text{C}$ was one-half that of the sample irradiated at -79°C , and complete repair of daughter-strand gaps was observed in both cases. Thus, UV-induced lesions produced in cells at -79°C (i.e. DNA-protein cross-links) appear to be amenable to post-replicative repair. While the overall DNA synthesis kinetics were the same for the two irradiation procedures, the apparent number of lesions produced per unit length of DNA was not. This suggests that each of the lesions produced in frozen cells, although apparently fewer in number, must cause a longer local delay in DNA synthesis than those lesions produced at $+21^{\circ}\text{C}$.

INTRODUCTION

Previous studies have shown that cells of *E. coli* B/r are about 5 times more sensitive to killing by UV radiation (254 nm) while frozen than they are at room temperature (Ashwood-Smith *et al.*, 1965; Smith and O'Leary, 1967). In addition, frozen cells showed a much reduced yield of cyclobutane-type thymine dimers, and a correspondingly reduced amount of photoreactivation (Smith and O'Leary, 1967). Thus, the increased UV sensitivity of the frozen cells did not correlate with the production of cyclobutane-type thymine dimers. A photochemical lesion that showed a positive correlation with the enhanced sensitivity of the frozen cells to UV-induced killing was the yield of DNA-protein cross-links (Bridges *et al.*, 1967; Smith and O'Leary, 1967).

The present study was undertaken to determine if, at the same survival level, the DNA synthesis kinetics and cell division delays are comparable for cells irradiated under the two conditions. In addition, we wished to determine if the principal lesions produced in DNA by the UV irradiation of frozen cells (i.e. DNA-protein cross-links) are accommodated by the post-replicative repair process (Rupp and Howard-Flanders, 1968), a process that has been shown to be controlled by the *recA* (Smith and Meun, 1970), *exrA*, *uvrD*, and *recB* (Youngs and Smith, 1976a) and *recF* (Rothman *et al.*, 1975) gene products.

MATERIALS AND METHODS

Survival and DNA synthesis experiments. Cells of *E. coli* B/r *thy* (obtained from D. Freifelder) were grown overnight in a salts-glucose medium (Kaplan *et al.*, 1962) supplemented with thymine-2- ^{14}C ($2\text{ }\mu\text{g/m}^{\ell}$; 31.6 Ci/mol , Schwartz). The culture was then diluted 1:50 with the same medium and grown in log phase to a density of $\sim 10^8$ cells/ m^{ℓ} , harvested by centrifugation, washed with and suspended in 0.1 M PO_4 buffer (pH 6.8) at $\sim 2 \times 10^7$ cells/ m^{ℓ} . The cells were frozen for 30 min at -70°C and irradiated (254 nm) while frozen or after thawing (Smith and O'Leary, 1967). Controls were frozen for 30 min but were not irradiated.

For DNA synthesis and survival studies, the cell samples were harvested on membrane filters, washed with medium minus glucose and thymine, and then suspended in twice the original volume of complete growth medium containing thymine-2- ^{14}C as used above. The cells were grown in a Gyrotory water bath (New Brunswick Scientific Co.) at 37°C . Aliquots taken for viability were diluted with buffer and plated on agar plates made with salts-glucose medium supplemented with $10\text{ }\mu\text{g/m}^{\ell}$ thymine.

Aliquots taken for measuring radioactivity content were diluted 1:2 with 4% formaldehyde to stop metabolism; 0.1 m^{ℓ} samples were then pipeted onto 2.3 cm Whatman 3 MM discs mounted on pins, and the samples were dried under heat lamps. The discs were washed twice with 5% trichloroacetic acid, twice with 95% ETOH, once with acetone, dried, and counted in a liquid scintillation counter with 5 m^{ℓ} of a solution composed of 4 g PPO and 0.1 g POPOP per liter of toluene.

The survival of the control cells after freezing was 50-60%. This value is somewhat lower than observed previously for stationary phase cells of *E. coli* B/r (Smith and

O'Leary, 1967). We have observed that *E. coli* K-12 strains are more easily killed by freezing than is *E. coli* B/r (data not shown).

Pulse label experiments. Log phase *E. coli* B/r *thy* were harvested at 2×10^8 cells/ml, washed and resuspended in 0.1 M PO_4 buffer (pH 6.8). Samples were frozen for 30 min at -79°C and irradiated (254 nm) while frozen or after thawing. Thawing was accomplished by incubation for ~ 5 min at 37°C with intermittent mixing. Controls were frozen for 30 min but were not irradiated. Samples were diluted to twice their volume with double-strength growth medium devoid of thymine. Thymidine-methyl- ^3H (New England Nuclear; 58.7 Ci/mmol , $4.0 \mu\text{g/ml}$) was added to each culture to a final concentration of $250 \mu\text{Ci/ml}$ and $1.0 \mu\text{g}$ of thymidine per ml, and incubation was continued at 37°C for 10 min. The samples were harvested by membrane filtration, washed twice with and resuspended at their original volume in non-radioactive complete growth medium. Samples were then treated with lysozyme and EDTA, and $\sim 2 \times 10^6$ cells in 0.02 ml were placed on top of alkaline sucrose gradients (Smith and Meun, 1970) immediately after the pulse labeling, or after further incubation at 37°C in growth medium for 90 min.

The gradients were centrifuged at 20°C for 16 h at 10,000 rpm in a SW 50.1 rotor in a Beckman Model L2 or L2-65B ultracentrifuge. Phage T2 DNA, labeled with [^{14}C]-thymine, was used in each centrifugation run as a molecular weight marker. The procedures for processing the gradients and analyzing the data have been described (Youngs and Smith, 1976b).

RESULTS AND DISCUSSION

In confirmation of previous results (Smith and O'Leary, 1967), cells of *E. coli* B/r are about 5 times more sensitive to killing by UV radiation (254 nm)

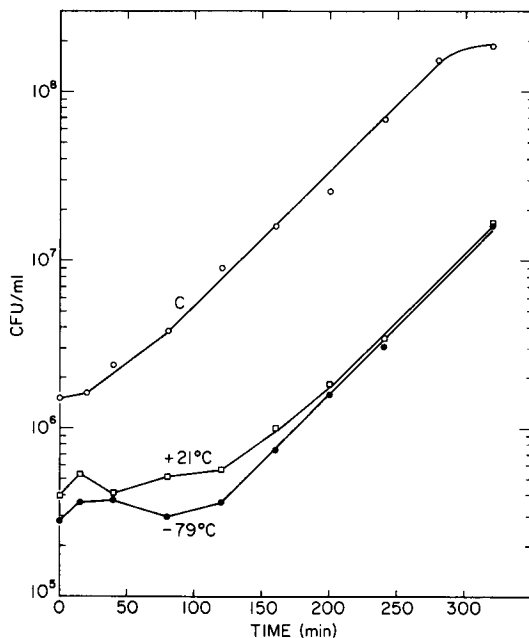


Figure 1. Survival and cell division kinetics after UV irradiation. *E. coli* B/r *thy* were UV irradiated (254 nm) while frozen at -79°C (4.7 J m^{-2}) or at $+21^\circ\text{C}$ (22.0 J m^{-2}) after thawing, and then returned to 37°C and sampled for viability as a function of time. The control cells (C) were frozen but not irradiated.

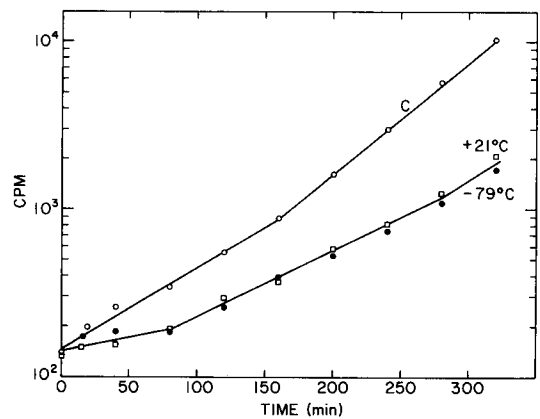


Figure 2. DNA synthesis kinetics after UV irradiation. The cultures (prelabeled with ^{14}C -thymine) used in the experiment described in Fig. 1 were assayed for the incorporation of ^{14}C -thymine as a function of time at 37°C in the control culture (C) or in the cultures that had been UV irradiated at $+21^\circ\text{C}$ (22.0 J m^{-2}) after thawing, or at -79°C (4.7 J m^{-2}). See the text for further details. The values on the ordinate are counts per minute (CPM) for acid-insoluble ^{14}C -thymine per 0.05 ml of culture.

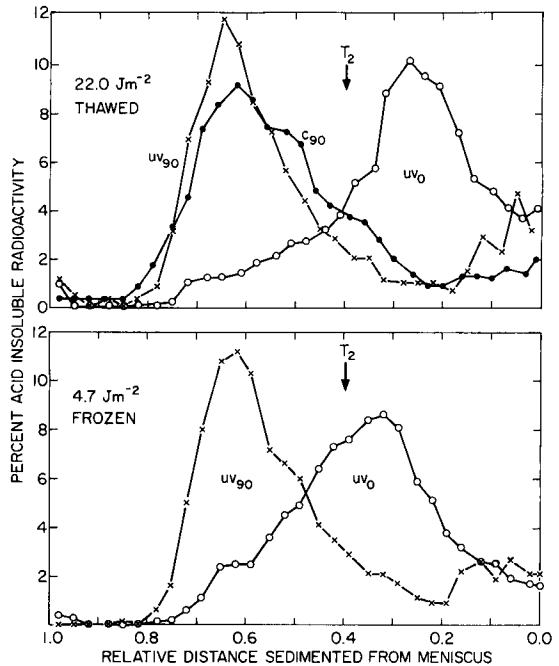


Figure 3. Post-replicative repair after UV irradiation. *E. coli* B/r *thy* cells were UV irradiated (254 nm) while frozen at -79°C (4.7 J m^{-2}) or at $+21^\circ\text{C}$ (22.0 J m^{-2}) after thawing. The cells were warmed to 37°C and pulse labeled for 10 min with ^3H -thymidine. Aliquots were either treated with lysozyme for 5 min at 0°C and then placed on alkaline sucrose gradients (C_0 , UV_0), or were resuspended in non-radioactive medium and incubated for 90 min at 37°C (C_{90} , UV_{90}) before the lysozyme treatment. The average weight average molecular weights of the DNA from three such experiments with their standard deviations are: (weight average molecular weights $\times 10^{-8}$) C_0 , 1.25 ± 0.09 ; $\text{UV}_0(-79^\circ\text{C}, 4.7 \text{ J m}^{-2})$, 0.59 ± 0.07 ; $\text{UV}_0(+21^\circ\text{C}, 22 \text{ J m}^{-2})$, 0.30 ± 0.06 ; C_{90} , 1.39 ± 0.13 ; $\text{UV}_{90}(-79^\circ\text{C}, 4.7 \text{ J m}^{-2})$, 1.68 ± 0.20 ; $\text{UV}_{90}(+21^\circ\text{C}, 22 \text{ J m}^{-2})$, 1.62 ± 0.15 .

while frozen than they are at room temperature (Fig. 1). Furthermore, cell division delay (Fig. 1), and DNA synthesis kinetics (Fig. 2) showed the same 5-fold difference in sensitivity under the two irradiation conditions. Thus, as has been pointed out previously for different radiation-sensitive mutants of *E. coli* (i.e. B, B_{s-1}, B/r), there appears to be a good correlation between the magnitude of the effect of UV radiation upon DNA synthesis kinetics and on cell survival (Smith, 1969). A similar conclusion was reached by Rude and Doudney (1973) when studying the effect of amino acid prestarvation on survival and DNA synthesis kinetics in UV-irradiated *E. coli* B/r.

The gradient results (Fig. 3) indicate that lesions produced in DNA, when cells are UV irradiated while frozen or thawed, are bypassed during DNA synthesis, producing short pieces of newly-synthesized DNA. Upon subsequent incubation, the newly-synthesized DNA reached the sedimentation characteristics of the control DNA in both cultures (Fig. 3). The weight average molecular weight of the DNA synthesized during a 10 min pulse in the cells that were UV irradiated (4.7 J m^{-2}) while frozen was about twice as large as that synthesized by the cells irradiated (22 J m^{-2}) after thawing (Fig. 3), although the UV fluence differed by a factor of ~ 5 .

Although the DNA synthesis kinetics (Fig. 2), cell division delay and survival (Fig. 1) are comparable, the total number of lesions that cause discontinuous DNA synthesis in cells UV irradiated with 4.7 J m^{-2} while frozen appears to be about half of that produced by 22.0 J m^{-2} in the thawed cells, based upon the relative size of the newly-synthesized DNA (Fig.

3). This suggests that each of the lesions produced in frozen cells, although apparently fewer in number, must cause a longer local delay in DNA synthesis than those lesions produced at $+21^\circ\text{C}$. This is consistent with our hypothesis that a bulky DNA-protein cross-link, which is produced with greater efficiency in frozen cells, should be more disruptive of DNA synthesis than would a pyrimidine dimer. Similarly, in interpreting the effect of freezing on the UV sensitivity of various repair deficient mutants of *E. coli*, Bridges *et al.* (1967) concluded that the DNA lesions produced in frozen cells appear to be more difficult to repair.

Since repair proficient wild-type cells are sensitized to killing by UV irradiation to a much greater extent by freezing than are repair deficient mutants (*uvr*, *rec*, *exr*) (Bridges *et al.*, 1967), this suggests to us that the lesions produced at -79°C may be repaired by the wild-type cells with less accuracy than those produced at $+21^\circ\text{C}$. This hypothesis is consistent with the higher rate of mutagenesis observed for cells UV irradiated at -79°C , as compared with room temperature (Bridges *et al.*, 1967).

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