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DNA-STRAND SCISSION AND LOSS OF VIABILITY AFTER X IRRADIATION OF NORMAL AND SENSITIZED BACTERIAL CELLS*

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Although radiochemical lesions in DNA appear to be responsible for the loss of viability in X-irradiated cells,^{1, 2} the nature of such lesions has not been established. Recently, Freifelder³ correlated the inactivation of X-irradiated coliphage T7 with the yield of double-strand scissions in DNA. In alkaline sucrose gradients, McGrath and Williams⁴ observed a decreased sedimentation rate, attributed to single-strand scission, in alkali-denatured DNA of X-irradiated *E. coli*; reincubation of irradiated cells of a radioresistant strain (B/r) restored the sedimentation rate essentially to the preirradiated level, whereas reincubation of a radiosensitive strain (B_{s-1}) had no effect. In the studies reported here, alkaline and neutral pH sucrose gradients were used to study the effect of X rays on the sedimentation behavior of DNA from normal and sensitized cultures of *E. coli*.

Materials and Methods.—The bacterium employed was a mutant of *E. coli* K12, substrain JE-850, kindly provided by Dr. Y. Hirota, Department of Biology, University of Osaka, Osaka, Japan, who has characterized it as F⁻, Thy⁻, Try⁻, Pur⁻, Lac₈₅⁻, Xyl₂⁻, Ara₂⁻, Mtl⁻, Gal₂⁻, Sm^r, Phos⁻, Tb^r, λ_{ind}⁻, Rhamnose₂⁻. The culture media, incubation conditions, irradiation procedures, and viability determinations have been previously described.²⁻⁴ Cultures were supplemented during log phase growth with 5 μc/ml of tritiated thymidine (H³-TdR) to label the bacterial DNA. The pyrimidine analogue 5-bromodeoxyuridine (BUdR) was purchased from California Corporation for Biochemical Research, Los Angeles.

The procedure of McGrath and Williams⁴ was used for sedimentation analysis of alkali-denatured DNA on 5–20 per cent sucrose gradients, pH ~13. The procedure for undenatured (double-stranded) DNA was similar, except that the sucrose gradients were adjusted to pH 7, and cells were layered on the sucrose gradient immediately after resuspension in Tris-EDTA-lysozyme mixture (0.17 ml), incubated at 23°C for 3 min and then lysed with 0.18 ml Duponol, 1 per cent, with gentle mixing. Centrifugation time at pH 7 was 35–45 min at 30,000 rpm. After centrifugation, 4–6-drop fractions were collected on filter-paper disks, washed with 10 per cent trichloroacetic acid, 95 per cent ethanol, and acetone, dried, immersed in 5 ml of toluene scintillation mixture,⁵ and counted in a scintillation counter. Aliquots (0.01–0.03 ml) of each original cell-lysozyme mixture were counted to determine total radioactivity. Data are plotted as per cent total radioactivity against fraction number (meniscus ~ fraction 40). The first moment of each curve about an arbitrary ordinate (0 = fraction 20), calculated as

$$\frac{\sum x_i y_i}{\sum y_i},$$

was used as a convenient index of sedimentation behavior.

Results.—(1) *Effect of irradiation on sedimentation behavior of alkali-denatured DNA from control cultures:* In alkaline sucrose gradients, DNA from cells grown for 90 min in complete medium and then irradiated with doses in the 5–25 krad range exhibited a sharply decreased sedimentation rate, which was restored almost to the preirradiation level when the cells were reincubated for 40 min at 37°C after irradiation (Fig. 1). These observations confirm those reported for *E. coli* B/r by

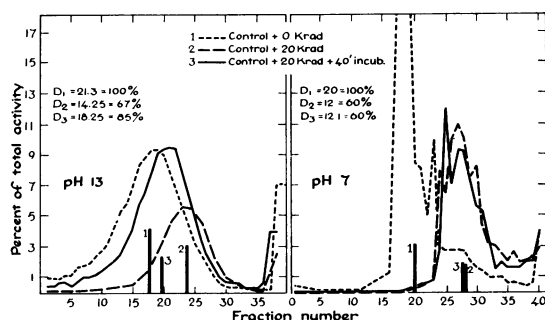


FIG. 1.—Sedimentation patterns of DNA from irradiated control cells. In pH 13 sucrose gradients, there is a sharp decrease in sedimentation rate of alkali-denatured DNA after 20 krad, with restoration almost to normal during 40 min of reincubation at 37°C. A marked change in sedimentation behavior at pH 7 is also seen with undenatured DNA after 20 krad, but there is no evidence of repair during reincubation.

McGrath and Williams,⁴ who concluded that the increase in sedimentation rate during postirradiation reincubation “reflects a repair process that rejoins broken pieces of DNA with alkali-stable bonds.” The highly radiosensitive mutant, *E. coli* B_{s-1}, apparently lacks such a repair system: irradiation of this organism caused a similar decrease in sedimentation rate of its alkali-denatured DNA, but there was no increase during reincubation.⁴

(2) *Effect of irradiation on sedimentation behavior of undenatured DNA from control cultures:* In neutral sucrose gradients, the sedimentation behavior of replicate samples of DNA from unirradiated control cultures was quite reproducible when cell lysis was carried out directly on the gradient to minimize shear. In addition to the sharply defined major peak, two small satellite peaks were observed which sedi-

mented less rapidly. After irradiation with doses of 5–10 krad, there was a sharp decrease (at higher doses, complete disappearance) of radioactive material sedimenting at the position of the major peak; instead, increased amounts of radioactive material appeared in a broad, heterogeneous, slowly sedimenting peak which was unaffected by reincubation at 37°C for 40 min after irradiation (Fig. 1). The altered sedimentation behavior of irradiated undenatured DNA indicates an increased proportion of smaller molecular weight DNA fragments,^{6, 7} presumably as a consequence of double-strand scission, for which these cells appear to have no repair system.

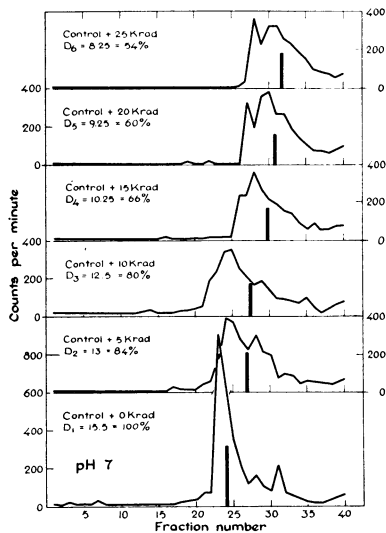


FIG. 2.—There is a progressive decrease of sedimentation rate of undenatured DNA from control cells with increasing X-ray dose. The position of the first moment of each curve is indicated by the vertical bar, and the sedimentation distance, D , from the meniscus (fraction 40) is expressed as a percentage of the value for the DNA of unirradiated cells.

Both single- and double-strand scission, manifested as decreased sedimentation rates on alkaline and neutral sucrose gradients, respectively, were observed when cells were incubated for 90 min in thymidine-free medium containing 20 $\mu\text{g}/\text{ml}$ of BUdR, and then exposed to an X-ray dose of 7.5 krad. This dose produced about the same degree of change in undenatured BUdR-DNA as 20 krad in undenatured control DNA. Reincubation at 37°C for 40 min after irradiation partially restored the sedimentation rate of alkali-denatured DNA, but there was again no evidence of repair of double-strand scissions (Fig. 3).

Aliquots of 90-min BUdR cultures were exposed to increasing doses of X rays. Samples were plated for determination of viability, and other samples exposed to the corresponding X-ray doses were lysed on neutral sucrose gradients. There was a progressive decrease, with increasing X-ray dose, of the sedimentation rate of undenatured BUdR-DNA (Fig. 4), similar to that seen in control DNA, but of much greater extent for any given dose. The sedimentation distances from meniscus to

Reincubation also failed to restore the viability of irradiated cells. When aliquots of the same control culture were exposed to increasing doses of X rays, there was a progressive decrease in sedimentation rate of their undenatured DNA (Fig. 2). The sedimentation distance, D , from the meniscus to the first moment of each curve, expressed as a fraction of the unirradiated control sedimentation distance, decreased exponentially with increasing radiation dose (Fig. 5b).

(3) *Effect of BUdR incorporation:* Previous studies have established that 5-bromouracil and its deoxyriboside (BUdR) become extensively incorporated in place of thymine in the DNA of thymine-requiring bacterial mutants,^{8, 9} and that such incorporation is associated with a two- to threefold increase in X-ray sensitivity.¹⁰ It was therefore of interest to investigate whether the sedimentation behavior of DNA from BUdR-grown cells exhibits a similarly augmented response to irradiation.

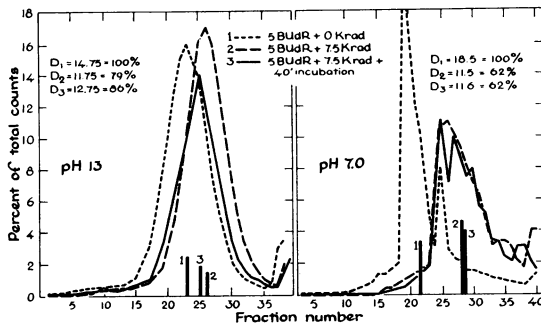


FIG. 3.—Similar to Fig. 1, except that the cells were grown for 90 min at 37°C in thymidine-free medium containing 20 $\mu\text{g}/\text{ml}$ of BUdR. Repair during reincubation is again evident, though perhaps slightly reduced, in the single-strand scissions (pH 13); there is again no repair of double-strand scissions. Note that the relative sedimentation distance at pH 7 is about the same for BUdR-DNA after 7.5 krad as for control DNA after 20 krad (Fig. 1).

first moment of each curve again decreased exponentially with increasing X-ray dose (Fig. 5b), with a slope about threefold greater than that for control DNA, corresponding closely to the threefold increase in slope of the dose-survival curve for BUdR-grown cells (Fig. 5a). It appears, therefore, that BUdR incorporation sensitizes cells of substrain JE-850 to the lethal effect of X rays by increasing the yield per unit dose of a nonreparable DNA lesion, viz., double-strand scission.

Discussion.—An impressive degree of double-strand scission occurs after X-ray doses in the range of the D_{37} for substrain JE-850 (about 15 krad). Neither double-strand scissions nor viability exhibit repair during reincubation. These correlations suggest that double-strand scissions are the major radiochemical lesion leading to loss of viability in X-irradiated cells of this strain. This conclusion is bolstered by the observation that a pyrimidine analogue radio-sensitizing agent, BUdR, increases the yield of double-strand scissions per unit dose to an extent proportional to its effect on radiation-induced lethality. The mechanism whereby the incorporation of BUdR into DNA elicits such a striking increase in double-strand scission by X rays remains to be elucidated. Other experiments, to be published separately, indicate that the radiosensitization which occurs in cells starved for thymidine¹¹ or natural purine¹² or exposed to purine analogues¹³ is probably due to the summation of single-strand scissions which develop during starvation and those randomly induced by radiation at or near the complementary position on the opposite strand, thus producing additional double-strand scissions, the increased yield of which is again of about the same magnitude as the effect on viability.

Single-strand scissions induced by X rays are repairable in two strains of *E. coli*, B/r and JE-850, but not in strain B_{s-1}. Calculations⁴ indicate that single-strand scissions could account quantitatively for lethality in the highly radiosensitive strain B_{s-1}, although double-strand scissions, produced in lesser yield, would also be

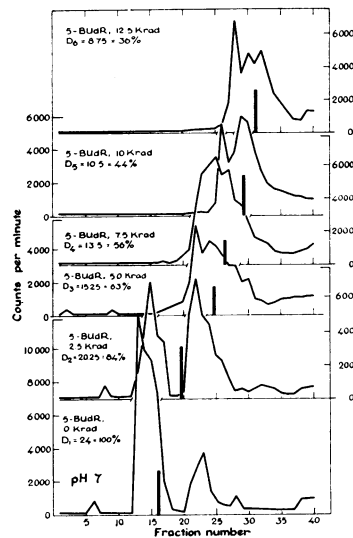


FIG. 4.—Similar to Fig. 2, for undenatured DNA from BUdR-grown cells. There is again a progressive decrease of sedimentation rate with increasing dose, but of obviously greater magnitude for any given dose.

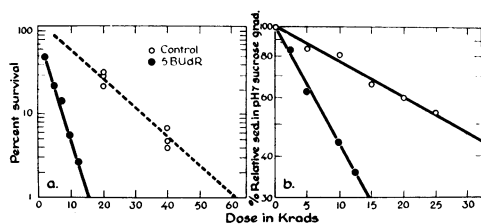


FIG. 5.—(a) Dose survival curves for control and BUdR-grown cells reveal a threefold increase in slope after BUdR incorporation into DNA. (b) A semilog plot of relative pH 7 sedimentation distance vs. X-ray dose (data of Figs. 2 and 4) yields exponential curves with a similar threefold increase in slope for BUdR-DNA vs. that from control cells.

double-strand scissions to the same extent (threefold) as it increased radiation lethality. It is concluded that double-strand scissions in DNA are the radiochemical lesions principally responsible for the lethal effect of ionizing radiation in *E. coli*. However, single-strand scissions are probably important in radiosensitive mutants lacking the repair system, and perhaps also in cells grown under conditions in which repair is inhibited.

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expected to contribute to some extent. Under normal conditions, the reparability of these lesions in the other strains makes it unlikely that they would be lethal.

Summary.—Irradiation of *E. coli* induced a decrease in sedimentation rate of alkali-denatured and native DNA, attributable to single- and double-strand scission, respectively. Single-strand scissions were repaired during reincubation of the irradiated cells, whereas double-strand scissions were not. BUdR increased the yield of