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Properties of a Deoxyribonucleic Acid Ligase Mutant of *Escherichia coli*: X-Ray Sensitivity

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A deoxyribonucleic acid ligase-deficient mutant is X-ray sensitive relative to the parent strain, suggesting that deoxyribonucleic acid ligase functions in repair of X-ray-induced, single-strand scissions.

One of the primary consequences of X-irradiation to bacterial cells is the breakage of phosphodiester bonds in the deoxyribonucleic acid (DNA) backbone, resulting in single-strand scissions (3, 7). There is evidence that these single-strand scissions can be repaired (1, 5, 7). An enzyme, DNA ligase, has been described which catalyzes the formation of covalent bonds between polynucleotides (2, 8); possibly this enzyme functions in the repair of X-ray-induced, single-strand scissions. Should this be true, one would expect a ligase-deficient mutant to be X-ray sensitive. In our laboratory, we isolated a temperature-sensitive, ultraviolet (UV) radiation-sensitive mutant of *Escherichia coli* TAU-bar, ts-7 (4), which is deficient in DNA ligase (9, 10). Accordingly, we have investigated ts-7 for X-ray sensitivity.

Cultures were grown aerobically at 25 C in a glucose-salts medium with appropriate supplements (4, 6) to early log phase (approximately 2×10^8 cells/ml), harvested by centrifugation, and suspended in the salts medium without glucose or supplements. The cells were placed in a covered, plastic petri dish and were continuously stirred during irradiation. X rays were produced at a rate of 1 kr/min by a Westinghouse Quadrocondex X-ray tube, operating at 250 kVp and 15 ma at a target distance of 22 cm. Dosimetry was made with a Victoreen dosimeter. At appropriate intervals samples were taken, and viable cell counts were determined by the agar overlay method by using the glucose-salts medium with added supplements and 15 g of agar per liter.

Data for survival of TAU-bar and ts-7 are presented in Fig. 1. From these data, ts-7 appears to be X-ray sensitive. At a dose of 20 kr, survival of TAU-bar is 0.07%, whereas that of ts-7 is 0.001%. The TAU-bar survival curve has a small shoulder at low doses; this shoulder is absent for

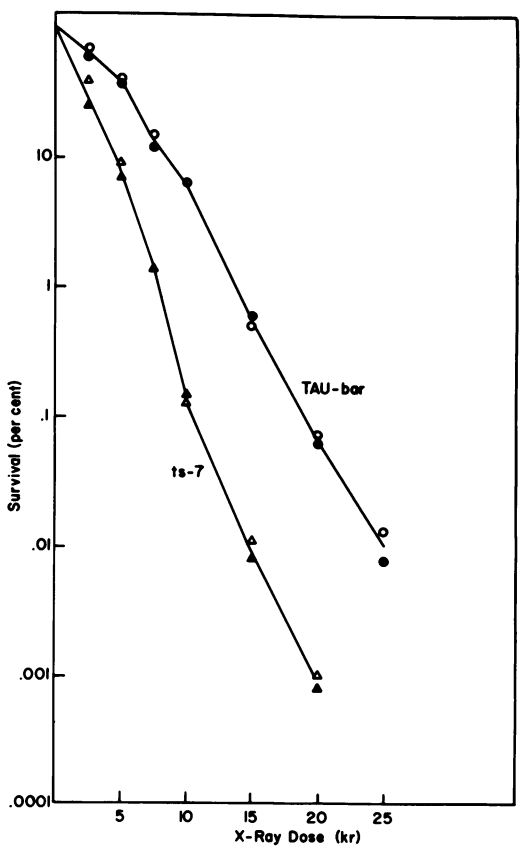

FIG. 1. *Survival of TAU-bar and ts-7 after X-irradiation. Cells growing exponentially at 25 C were irradiated for the doses indicated, and viable cell counts determined. The open symbols represent plates incubated at 25 C; the closed symbols, plates incubated for 2 hr at 40 C immediately after plating and then transferred to 25 C. The circles represent TAU-bar; the triangles, ts-7.*

ts-7, and the slope of the curve is steeper. With UV irradiation, TAU-bar shows a slight temperature effect; survival of this strain when it is incubated for 2 hr at 40 C before incubation at 25 C is greater than survival when incubated only at 25 C (9, 10). This temperature effect is not expressed with X-irradiation. With UV irradiation, survival of ts-7 is markedly reduced when incubated for 2 hr at 40 C followed by incubation at 25 C, compared to survival when incubated only at 25 C (9, 10). In contrast to this result, ts-7 does not appear to be temperature-sensitive with respect to X-ray sensitivity. This behavior may be due to the observation that in vitro DNA ligase activity of ts-7 is not temperature-sensitive but is reduced at either temperature in comparison to TAU-bar activity (9).

We have shown that ts-7 is X-ray sensitive in comparison to the parent strain TAU-bar. Three independently isolated revertants of ts-7 are identical to TAU-bar with respect to temperature sensitivity, UV sensitivity, kinetics of accumulation of single-stranded fragments of newly replicated DNA (C. Pauling and L. Hamm, Proc. Nat. Acad. Sci. U.S.A., **64:** 1195–1202), and X-ray sensitivity; we conclude that the phenotype of ts-7 is the consequence of a single revertible mutation. These results suggest that a common DNA ligase functions in the repair of X-ray-induced, single-strand scissions and the dark repair of UV radiation-induced lesions, and confirm our prediction that a DNA ligase-deficient mutant would be X-ray sensitive.

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