REPAIR DEFICIENCY IN A BACTERIAL MUTANT DEFECTIVE IN DNA POLYMERASE

Lee Kanner* and Philip Hanawalt
Herrin Laboratories of Biological Sciences
Stanford University, Stanford, California 94305

Received February 25, 1970

SUMMARY

The effect of ultraviolet irradiation (UV) has been studied in Escherichia coli K12 strain W3110 pol+, and in its derivative, W3110 polA1, which lacks appreciable DNA-polymerase activity in extracts. The "polymeraseless" derivative appears to be deficient in some step in the excision-repair process, although not totally lacking in repair capacity, since: (1) It is more UV sensitive than the parent strain, yet less UV sensitive than an excision-deficient K12 strain, AB2500; (2) It exhibits a lower single-strand DNA molecular weight than the parent strain during the growth period following UV irradiation.

The simplest model for the enzymatic dark repair of damaged DNA in Escherichia coli involves a three-step process: (a) Single-strand incision near the damage by an endonuclease that recognizes structural distortions in DNA (1,2); (b) Repair replication, with concurrent excision of the damaged strand using the undamaged strand as template (3,4); (c) Rejoining of the repaired segment to the contiguous parental DNA strand by the polynucleotide ligase (5,6,7).

The DNA polymerase from Escherichia coli exhibits properties in vitro (4) which identify it as the enzyme which probably performs function (b) above, in vivo. It shows a 5'-exonucleolytic activity which results in the release of ultraviolet (UV)-induced pyrimidine dimers, and a concurrent polymerization of new nucleotides to fill the resultant gap. If this enzyme does perform repair replication in vivo, it would be predicted that a mutant strain,

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*Present address: Department of Pharmacology, Stanford Medical Center.

defective in this enzyme, would be sensitive to UV and would exhibit abnormalities in the excision-repair process. A mutant with an altered DNA polymerase has in fact, been isolated and partially characterized by de Lucia and Cairns, who showed that its extracts contain only 0.5 to 1% of the DNA polymerase activity of the parent and that it is UV sensitive (8). Genetic studies by Gross and Gross have shown that it is an amber (9). In the present study we have looked for abnormalities in the repair process in this mutant by assaying the final rejoining step as an indication of successful completion of repair.

METHODS

Strains W3110 pol+ and W3110 polA1 (obtained through the courtesy of John Cairns) were cultured aerobically in a glucose salts synthetic medium previously described (10,11), supplemented by 2 μg/ml thymine, 20 μg/ml vitamin B_1, and 1 mg/ml vitamin-free casamino acids. The same medium was used for growth of AB2500, except that it was supplemented, additionally, with proline, arginine, histidine, leucine and threonine, all at 20 μg/ml. A solution of the salts of this medium lacking glucose or the above supplements is referred to below as "tris-salts".

Media transfers were accomplished by harvesting cells on a 9 cm Schleicher and Schuell type B-6 filter, rinsing with tris-salts, and resuspending the cells by shaking the filter in the new medium.

To obtain survival curves, strains W3110 pol+, W3110 polA1, and AB2500 were grown to an optical density (O.D.) of 0.43 (Bausch and Lomb Spectronic 20, 450 mμ, using a tube of diameter 2.3 cm), which corresponds to about 6×10^7 colony-forming cells/ml for W3110 pol+ and W3110 polA1, and to 1.3×10^7 colony-forming cells/ml for AB2500. The cultures were then resuspended at twice their former volume in tris-salts at 0°, and subjected to UV from a Pen-Ray Quartz Lamp at 4.5 ergs/mm^2/sec, with stirring by aeration. Small aliquots were removed at various times, and diluted in 0° tris-salts as

required for plating. Colony formation was determined on glucose-salts agar, supplemented with thymine only (2 μg/ml) in the case of W3110 pol+ and W3110 polA1, and with proline, arginine, histidine, leucine, threonine, and B_1 at 20 μg/ml, in addition, for AB2500. Colonies were counted two and one-half days after plating and incubation at 37°. To prevent photoreactivation, cells were kept in the dark after irradiation.

To determine molecular weights of DNA after UV, the culture media for W3110 pol+ and W3110 polA1 were as described above, but the former contained H^3-thymine at 5μc/μg, and the latter contained C^{14}-thymine at 0.17 μc/μg. 12 ml of each were grown to an O.D. of 0.3; each was then resuspended in the same volume of unlabeled, but otherwise identical medium, and growth was continued to an O.D. of 0.4. Cells were then resuspended in 12 ml of tris-salts at 0°. Each cell suspension received 270 ergs/mm^2 incident UV dose. One-fourth of each suspension was immediately combined in 50 ml of EDTA buffer (0.1 M NaCl, 0.01 M EDTA, 0.01 M tris, pH 8.0) at 0° which is referred to below as NET. The remaining three-fourths were returned to 37°. After one minute pre-warming, with aeration, the cell suspensions were then provided with glucose and supplements. One-fourth volume aliquots were combined in NET in the same way as for the zero-time sample after 30, 60 and 90 minutes. Each combined sample was filtered, washed with cold NET, and resuspended in 0.15 ml of a 1/10 dilution of NET. The cells were transferred to Beckman SW39 tubes, and lysed by the addition of 15 μl of lysozyme at 4 mg/ml, followed by a 10 min incubation period at 37°. 15 μl of 2.5% Sarkosyl and 15 μl of pronase at 4 mg/ml were then added, and the lysates were again incubated for 10 min at 37°. Sucrose gradients were then layered under the lysates, using a Polystaltic Pump (Buchler Instruments). The layers added, in order of underlayering, were: (1) 0.3 ml 2% sucrose in water; (2) 0.3 ml 4% sucrose in 0.4 M NaOH; (3) a linear 5-20% sucrose gradient in 0.9 M NaCl, 0.01 M NaOH adjusted to pH 12. Gradients were centrifuged at 20°, 35,000 rpm, for 1 hour. Three drop fractions were

collected, precipitated in cold 5% trichloroacetic acid, filtered on Millipore (HA) filters, and rinsed with water. The filters were dried and counted in 5 ml of toluene-based scintillation cocktail in a Packard Tri-Carb liquid scintillation spectrometer.

RESULTS AND DISCUSSION

Our data indicate that some dark repair is completed in the polymeraseless mutant. Fig. 1 shows that although the mutant is more sensitive to UV than the parent strain, it is more resistant than AB2500, a uvrA6 mutant which cannot excise thymine dimers (1,2) and therefore can perform no excision-repair (12).

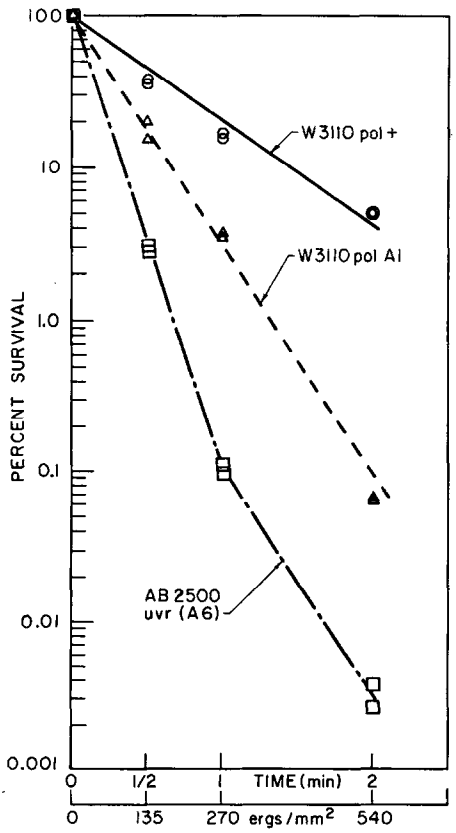

Fig. 1 UV survival curves for exponentially growing cultures, as shown.

To determine whether the amount of completed dark repair is reduced, we compared the single-strand molecular weights of DNA from the parent and mutant strains at times from 0 to 90 minutes after UV. Fig. 2 shows that except at 0 minutes, at which time excision has not yet occurred, the average single-strand molecular weight of the DNA from the mutant is always less than that of the parent. If we assume that this reduction in number of rejoined DNA fragments correlates with a reduction in completed repair, then Fig. 2 shows that dark-repair is at least slower in the mutant than in the parent; perhaps the final rejoining step in some cases is never completed.

Loss of assayable DNA polymerase activity and UV sensitivity have been

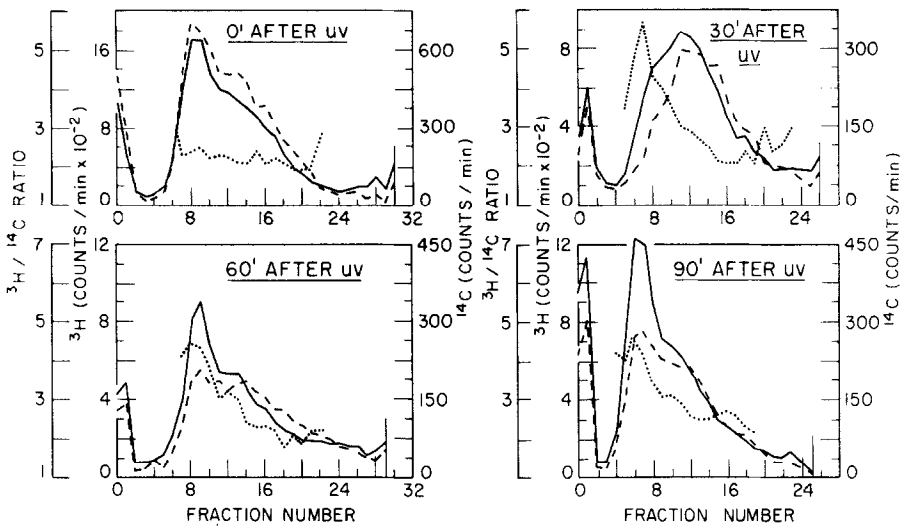

Fig. 2 Alkaline sucrose gradients showing molecular weight distribution of DNA after UV irradiation and post-irradiation growth. Fractions are numbered from bottom to top of the gradients and fraction "0" is the residue left in the centrifuge tube after collecting the gradient. Graphs consist of line segments joining the data points obtained for each fraction.

——— H^3-thymine incorporated into trichloroacetic acid-precipitable material during prelabeling period by W3110 pol+.

------- C^{14}-thymine incorporated into trichloracetic acid-precipitable material during prelabeling period by W3110 polA1.

..... H^3 cpm/C^{14}cpm.

shown to result from the same mutational step (9). Based on the above data, the UV sensitivity seems attributable to a deficiency in dark repair, so that loss of assayable polymerase activity and of dark repair capability can be said to result from the same mutational step. The loss of polymerase activity in extracts from the mutant can mean that either the number of active polymerase molecules present in the cell is very low, or else that the molecules are altered in structure in a way which makes them labile upon extraction, though functioning in vivo. The first possibility poses two problems; (a) since the mutant is unaffected in its ability to synthesize DNA under normal growth conditions this amounts to acceptance of the hypothesis that DNA polymerase is not the enzyme used in normal synthesis or else that very little of it is needed for this function; (b) DNA polymerase has now been shown to excise dimers and repolymerize bases on a single-strand template in a manner entirely consistent with the requirements for the repair enzyme (4). Assuming, as seems likely, that it is the repair enzyme, then reduction of the enzyme level should correspondingly reduce repair replication in the mutant. Preliminary experiments in this laboratory, however, indicate that the level of repair replication in the UV irradiated mutant is similar to that in the parent strain (12). If one assumes, instead that DNA polymerase is actually present and active in vivo, but altered in structure, one must further assume that some step in dark repair occuring after repolymerization is blocked by this alteration. For example, this might be the joining together of the repaired DNA fragments. It has been shown that UV sensitivity results when the joining process is defective in ligase-deficient mutants of Escherichia coli (13) or bacteriophage T4 (14). A currently-favored model for the excision-repair scheme involves the displacement of the repair polymerase from the DNA by the ligase after repolymerization. In W3110 polA1, the altered DNA polymerase may bind too tightly to the DNA to be displaced. Alternatively, it may hinder recognition of single-strand breaks or prevent access to the site by the joining enzyme.

ACKNOWLEDGEMENT

This research was supported by a contract AT(04-3)326-7 with the U.S. Atomic Energy Commission and a grant GM 09901 from the U.S. Public Health Service.

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