EXCISION OF γ-RAY DAMAGED THYMINE BY E. COLI EXTRACTS IS DUE TO THE 5′→3′ EXONUCLEASE ASSOCIATED WITH DNA POLYMERASE I

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SUMMARY

Extracts of E. coli polAexl which contains a temperature sensitive 5′→3′ exonuclease function of polymerase I accomplish the selective excision of products of the 5,6-dihydroxy-dihydrothymine type from γ-irradiated DNA and OsO_4-oxidized polyd(A-T) at the permissive temperature (30°) but not at the nonpermissive temperature (42°). The 5′→3′ exonuclease activity of polymerase I, therefore, acts as a repair exonuclease in γ-ray excision repair.

INTRODUCTION

Exposure of cells to ionizing radiation leads to extensive damage of the DNA bases. Products of the 5,6-dihydroxy-dihydrothymine type (t') represent a major class of γ-ray lesions formed from thymine (1,2). Ring saturation products of the t'-type are removed from the DNA during postirradiation incubation of mammalian (3,4) and bacterial cells (5). It was shown that crude extracts of E. coli endoI$^-$ and E. coli endoI$^-$ uvrA6$^-$ possess the capacity to remove t' from γ-irradiated bacteriophage DNA (4,6). In this paper we demonstrate that the second step in the excision repair of t', namely the exonucleolytic degradation of the damaged DNA region, is accomplished by the 5′→3′ exonuclease associated with polymerase I of E. coli.

EXPERIMENTAL PROCEDURES

The removal of products of the 5,6-dihydroxy-dihydrothymine type (t') from γ-irradiated PM-2 DNA and OsO_4-oxidized polyd(A-T) was investigated using crude extracts of E. coli K12 strains, E. coli endoI$^-$ (strain MRW25 from Dr. R. McMacken referred to below as wild type), E. coli endoI$^-$ polA$_1$ (strain D110 originally from Dr. C. C. Richardson), and E. coli polAexl (strain RS5052 from Dr. E. B. Konrad). The preparation of the crude extracts, the incubation conditions, the determination of the disappearance of t' from the acid precipitable DNA substrate, and the determination of the acid soluble material released from the substrate have been described previously (6). The incubation temperature was 37°, except for the experiments with extracts of the temperature-

sensitive mutant E. coli polAexl, where the experiments were carried out in parallel at 30° (permissive temperature) and 42° (non-permissive temperature). Pseudomonas phage PM-2 DNA labeled in thymine-methyl (^{3}H) was prepared by a combination of the procedures of Franklin et al (7) and Yamamoto et al (8), while polyd(A-T) thymine-methyl (^{3}H) was obtained from commercial sources (Miles Laboratories, Elkhart, Indiana; General Biochemicals (GIBCO), Grand Island, New York). PM-2 DNA was further purified on hydroxylapatite before each experiment. The DNA was irradiated under aerobic, non-protective conditions with a dose of 15 - 25 krads γ-rays. After irradiation the sample was evaporated to dryness to remove tritiated water (1), resuspended in phosphate buffer (5mM) and freed from low molecular weight material by Sephadex G 50 chromatography. The oxidation of polyd(A-T) with OsO_4 was carried out by a modification of the procedure of Beer et al (9), as described in reference (6). OsO_4-oxidation of polyd(A-T) mostly introduced 5,6-dihydroxy-dihydrothymine and a small number of apyrimidinic sites, but in contrast to γ-rays no adenine damage or strand breakage. The t'-content of the γ-irradiated PM-2 DNA and the OsO_4-oxidized polyd(A-T) in our experiments varied from 0.25 to 0.4%, corresponding to approximately 1.2 to 2% total thymine ring destruction (6).

RESULTS AND DISCUSSION

Before discussing our results, we would like to recall that polymerase I from the mutant E. coli $polA_1$ was found to be deficient in the polymerization and 3'→5' exonuclease activities, but not the 5'→3' exonuclease activity associated with the enzyme (10). The conditional lethal mutation, polAexl on the other hand, is deficient at the non-permissive temperature (42°) in the 5'→3' exonuclease activity, but not the polymerizing activity of polymerase I (11).

The radiosensitivity to γ-rays of E. coli endoI$^-$, E. coli $polA_1$, and E. coli polAexl was determined following standard procedures (12). After irradiation with γ-rays under aerobic, non-protective conditions, the cultures were divided into two portions and incubated at 30° or 42° for one hour in complete growth medium before plating and incubation for colony formation at 30°. The radiosensitivity of all three strains proved to be independent of the pre-incubation temperature. Both E. coli endoI$^-$ $polA_1$ (see references 13,14) and E. coli polAexl were found to be substantially more sensitive to γ-rays than the wild type strain. E. coli endoI$^-$ $polA_1$ was slightly more sensitive than E. coli polAexl. Figures 1 and 2 contain the results of our experiments on the removal of t' and undamaged thymine from γ-irradiated PM-2 DNA and OsO_4-oxidized polyd(A-T) by extracts of wild type E. coli and the two polymerase I mutants $polA_1$ and polAexl. The disappearance of t' from

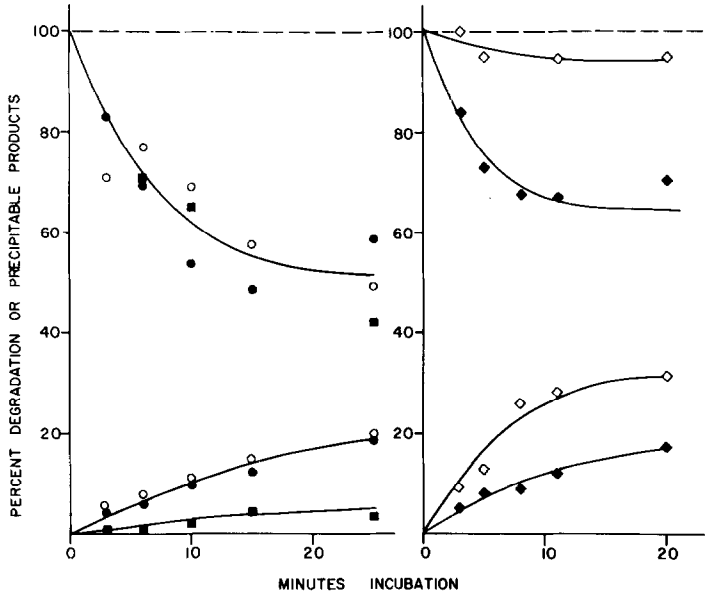

Figure 1. Excision of products of 5,6-dihydroxy-dihydrothymine (t') from γ-irradiated PM-2 DNA by extracts from three E. coli strains: E. coli endoI⁻, E. coli endoI⁻ polA$_1$ and E. coli polAexl. The amount of total thymine label released as a function of incubation time was determined from the acid soluble fraction. The ratio of t' in the acid precipitable fraction over the total thymine in the acid precipitable and acid soluble material was computed for each time point and expressed as the percent precipitable products of the non-incubated control sample. For experimental details see under "Experimental Procedures" and in reference 6.

Top: percent precipitable products, t'.
Bottom: percent acid soluble thymine label.
E. coli endoI⁻: ○——○, 42°; ●——●, 30°
E. coli endo I⁻ polA1: ■——■, 37°. E. coli polAexl: ◇——◇, 42°; ◆——◆, 30°.

the acid precipitable polydeoxynucleotide substrate and the appearance of acid soluble thymine label are plotted as a function of incubation time. As shown on the left side of Figure 1, extracts of E. coli endoI⁻ and E. coli endoI⁻ polA$_1$ were equally efficient in the excision of t' from γ-irradiated PM-2 DNA while unspecific polymer degradation was considerably higher for the wild type extracts. Comparable results were obtained for incubation at 30° and 42° (for E. coli endoI⁻) and 37° (for E. coli endoI⁻ polA$_1$). The right half of Figure 1 shows the results obtained with extracts from E. coli polAexl incubated with γ-irradiated PM-2 DNA at the permissive temperature (30°) and the non-permissive temperature (42°). It is evident that efficient, selective excision of approximately 35% of t' only occurred at 30°. Only 5% of t' was removed at 42°. Acid solubilization of the DNA substrate was 17% at 30° and

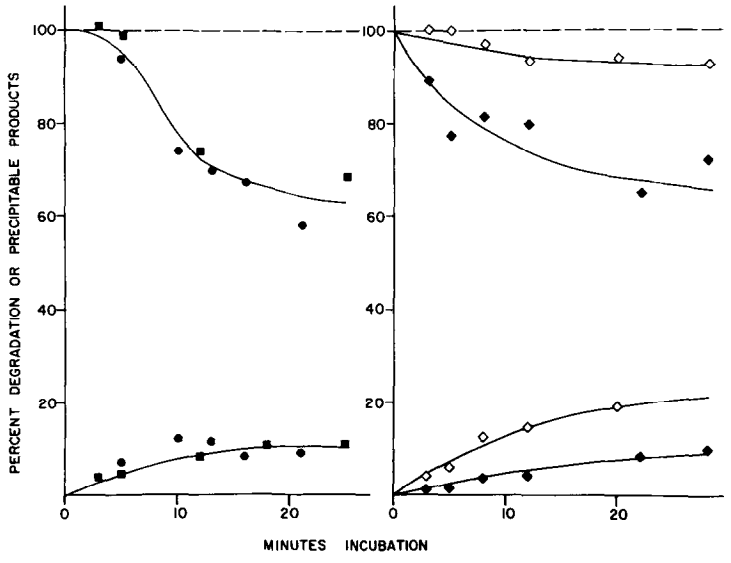

Figure 2. Excision of 5,6-dihydroxy-dihydrothymine (t') from OsO_4-oxidized polyd(A-T) by extracts from three E. coli strains: E. coli endoI$^-$, E. coli endoI$^-$ polA1, and E. coli polAexl. For experimental details see legend to Figure 1 and reference 6.

Top: percent precipitable products t'
Bottom: percent acid soluble thymine label
E. coli endoI$^-$:
E. coli endoI$^-$ polA1:
E. coli endoI$^-$ polAexl: , 30°; , 42°

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approximately twice this value at 42° within 20 minutes incubation.

Similar results were obtained with OsO_4-oxidized polyd(A-T) as polydeoxynucleotide substrate, and the data is given in Figure 2. Selective excision of t' was accomplished by extracts of E. coli endoI$^-$, E. coli endoI$^-$ $polA_1$ at 37°, and E. coli endoI$^-$ polAexl at 30°. Approximately 35% of t' was removed from the polymer within 20 minutes of incubation. Only 20% and 7% of t' were removed by the extracts of E. coli polAexl at 37° (not shown) and 42°, respectively. As observed for the γ-irradiated substrate acid solubilization of the polymer by the polAexl extracts was approximately twice as high at 42° than 30°.

Our results indicate that the 5′→3′ exonuclease associated with polymerase I is responsible for the selective excision by E. coli extracts of ring-saturated thymine residues of the 5,6-dihydroxy-dihydrothymine type from both γ-irradiated phage DNA and OsO_4-oxidized synthetic polyd(A-T). Since OsO_4-oxidized polyd(A-T) does not contain radiation-induced strand breakage it follows that radiation-induced single strand breaks are not required for

product excision. It appears unlikely,therefore, that radiation-induced strand breaks in PM-2 DNA are used as starting points by the 5′→3′ exonuclease for local DNA degradation leading to product removal. In agreement with the double strand specificity of the 5′→3′ exonuclease of polymerase I (15) we were unable to detect product excision from γ-irradiated single stranded ϕX174-DNA. The results obtained with extracts of E. coli endoI⁻ $polA_1$ show that the 3′→5′ exonuclease and polymerization activity of polymerase I are not required for the removal of ring-saturated thymine products in vitro.

From results obtained with extracts of E. coli polAexl, it appears that the 5′→3′ exonuclease of polymerase I is also involved in the excision of cyclobutane-type photodimers (Friedberg and Lehman, cited in Reference 11). In vitro removal of thymine photodimers from polydA·dT by purified polymerase I has been observed earlier by Kelley et al (15). Excision of thymine dimers by proteolytic (16) and amber fragments (17) of E. coli DNA polymerase I which retain only 5′→3′ exonuclease activity have been reported. Inefficient removal of photodimers was also observed in E. coli $polA_{107}$ which lacks the 5′→3′ exonuclease activity of polymerase I (18). It appears, therefore, that the major difference between the excision of monofunctional lesions of the t'-type which are characteristic for ionizing radiation damage and of difunctional cyclobutane-type dimers induced in DNA by ultraviolet light lies in the endonucleolytic incision rather than the later steps of excision repair in E. coli.

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REFERENCES

1. Roti Roti, J., Stein, G., and Cerutti, P. (1974) Biochemistry 13, 1900-1904.
2. Cerutti, P. A. (1974) Naturwissenschaften 61, 51-59.
3. Mattern, M., Hariharan, P. V., Dunlap, B. and Cerutti, P. A. (1973) Nature New Biol. 245, 230-232.
4. Hariharan, P. V., Remsen, J. R. and Cerutti, P. A. (1974, in press) in "Molecular Mechanisms for the Repair of DNA", (eds. P. C. Hanawalt and R. B. Setlow) Plenum Press, New York.
5. Hariharan, P. V. and Cerutti, P. A. (1972) J. Mol. Biol. 66, 65-81.
6. Hariharan, P. V. and Cerutti, P. A. (1974) Proc. Natl. Acad. Sci. 71, 3532-3536.
7. Franklin, R. M., Salditt, M. and Silbert, J. A. (1969) Virology 38, 627-640.
8. Yamamoto, K., Alberts, B., Benzinger, R., Lawhorne, L. and Treiber, G. (1970) Virology 40, 734-744.

9. Beer, M., Stern, S., Carmalt, D. and Mohlhenrich, K. (1966) Biochemistry 5, 2283-2288.
10. Lehman, I. R. and Chien, J. (1973) J. Biol. Chem. 248, 7717-7722.
11. Konrad, E. B. and Lehman, I. R. (1974) Proc. Natl. Acad. Sci. 71, 2048-2051.
12. Tomlin, N. V. (1974) Molec. Gen. Genet. 129, 97-103.
13. Boyle, J. M., Paterson, M. C. and Setlow, R. B. (1970) Nature 226, 708-710.
14. Town, C. D., Smith, K. C. and Kaplan, H. S. (1971) Science 172, 851-853.
15. Kelley, R. B., Atkinson, M. R., Huberman, T. A. and Kornberg, A. (1969) Nature 224, 495-501.
16. Setlow, P. and Kornberg, A. (1972) J. Biol. Chem. 247, 232-240.
17. Friedberg, E. C. and Lehman, I. R. (1974) Biochem. Biophys. Res. Commun. 58, 132-139.
18. Glickman, B. (1974, in press) in "Molecular Mechanisms for the Repair of DNA", (eds. P. C. Hanawalt and R. B. Setlow) Plenum Press, New York.