The Effect of Lysogenic Induction with Mitomycin C on the
Deoxyribonucleic Acid Polymerase of Escherichia coli K12λ

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Infection by the virulent bacteriophages of the T series is known to cause the formation of a number of new enzymatic activities in Escherichia coli (1). One of the virus-induced enzymes which appears in large amounts after T2 infection of E. coli is a new DNA polymerase which differs markedly from the DNA polymerase of normal E. coli (2). In addition, it is known that the DNA of the T phages is quite different from the DNA of E. coli in both base composition and nearest neighbor sequences (3). In contrast, the DNA of the temperate phage λ, as shown by base composition, and nearest neighbor sequence studies, is very similar to that of its host cell, E. coli (3). The close similarity of the deoxyribonucleic acids of λ and its host cell may relate to the ability of such a temperate phage to establish a lysogenic relationship (4).

We have examined E. coli K12A cells, which have been lysogenically induced to form phage λ, to see if a new DNA polymerase is synthesized, as is the case when E. coli B is infected with T2. This paper will show that a DNA polymerase present in E. coli K12A after lysogenic induction with mitomycin C exhibits the same properties as the DNA polymerase that is present in normal (uninduced) cells of E. coli K12A. The DNA polymerase, from both normal and induced cells, has been purified about 7400-fold by similar procedures. Both enzymes fractionate in a parallel fashion during the purification. The purified DNA polymerases, obtained from either normal or induced cells, have similar pH curves, Mg++, requirements, responses to p-hydroxymercuribenzoate, rates of heat inactivation, and activities with various DNA primers. A preliminary communication dealing with part of this work has appeared (5).

The similarity of the DNA polymerases found in normal and induced cells of E. coli K12A is therefore in contrast to the effects seen after T2 infection of E. coli B. It is clear, now, that the enzymological changes associated with λ formation are not the same as those associated with T2 formation. It has also been shown that the new deoxyribonuclease formed after λ infection of E. coli K12A is not seen when these cells are infected with the phage T4 (6).

EXPERIMENTAL PROCEDURE

Materials

Bacterial strains were obtained from Dr. Francois Jacob of the Institut Pasteur, Paris, France. Mitomycin C was obtained from the Cancer Chemotherapy National Service Center, National Cancer Institute. Deoxyribonucleoside triphosphates were purchased from the California Corporation for Biochemical Research, and tritiated as described below. Ribonuclease (twice crystallized) was obtained from the Worthington Biochemical Corporation. Tryptone and glycerol were obtained from Brown and Company. Hydroxylapatite (Hypatite C) was purchased from Clarkson Chemical Company. Amberlite XE-64 was a product of Rohm and Haas, and was further treated by the procedure of Hirs (7). Sephadex G-200 was obtained from Pharmacia, Inc.; ammonium sulfate (special enzyme grade) was a product of Mann Research Laboratories. Fiberfilm T-20A-60 was obtained from Kopp Scientific Company.

DNA was prepared from E. coli cells by the procedure of Lehman (8). DNA from λ phage was prepared by phenol extraction (9) of λ phage which had been purified by banding in CsCl (10). After treatment of the phage with phenol, the DNA was precipitated with 2 volumes of ethanol, dissolved in 0.02 M Tris-chloride, pH 7.4, 0.02 M NaCl, and dialyzed against the same buffer to remove any remaining phenol. The DNA concentrations referred to in this paper are expressed as nucleotide phosphorus, as determined by a method described by Ames and Dubin (11).

Growth and Harvest of Cells—E. coli K12A was grown in tryptone broth (12) at 37° with vigorous aeration. Cultures were harvested when the population density was 4 x 10^8 per ml by quickly cooling the culture to 0° in a stainless steel container immersed in a -30° bath. The cells were collected by centrifugation at 700 x g and were washed once with water.

Lysogenically induced cells were prepared by the addition of mitomycin C at a final concentration of 10 μg per ml, to E. coli K12A (2 x 10^8 cells per ml) growing as described above. The cells were collected by centrifugation 60 minutes after the addition of mitomycin C. Under these conditions, 100% induction occurs within 15 to 20 minutes and lysis starts in 90 to 105 minutes after addition of mitomycin C (12). Approximately 100 λ phages are obtained per cell.

Methods

Preparation of Heated Sonicate—E. coli K12A, 13 g, was suspended in 52 ml of 0.05 M Tris buffer, pH 7.4, and placed in a Raytheon 10-kc sonic oscillator for 20 minutes. The resulting sonicate was then heated at 75° for 5 minutes, cooled to 0°, and centrifuged at 25,000 x g for 15 minutes. The supernatant fluid obtained was used as a primer for DNA polymerase.

Enzymatic Assays—The preparation of T K12A DNA and the DNase assay have been described (13). The DNA polymerase assay was a modification of the procedure of Lehman et al. (14) with tritiated deoxyribonucleoside triphosphates as the substrates. Deoxyribonucleoside triphosphates were subjected to tritiation by the Wilzbach technique at the New England Nuclear Corpora-
tion. Twenty-five milligrams of each of dATP, dCTP, dGTP, and dTTP were mixed and exposed to 3 curies of H2 for 2 weeks at 0°.
Two successive 5-ml portions of water were added and removed by lyophilization. The mixed deoxynucleoside triphosphates containing 30 mc of 3H were chromatographed on Dowex 1 as described by Lehman et al. (14). The concentration of each nucleotide peak was determined by spectrophotometry, and the solution was brought to pH 2 with 1 N HCl. To each pooled peak added 1 ml of 55 volume % acid-washed Norit A (Pfanstiehl) per 10 μmoles of nucleotide. After 15 minutes at 0°, the charcoal was separated by centrifugation and the adsorption step was repeated with the supernatant fluid. The charcoal precipitates were combined and suspended in 15 times their volume of 0.05 N NH4OH in 50% ethanol. After 15 minutes at 0°, the charcoal was removed by centrifugation and re-extracted with NH4OH-ethanol. The combined extracts were centrifuged in a stream of air. Recoveries averaged about 50% of the nucleotide present in the Dowex peaks. Specific activities of the different nucleotides ranged from 3 to 40 PC per pmole of deoxynucleoside triphosphate. The tritiated dATP, dGTP, dCTP, and dTTP so obtained were combined to give a solution containing approximately 2 μmoles of total nucleotide per ml. This mixed 3H-deoxynucleoside triphosphate solution was the substrate in the DNA polymerase assay described below.

DNA Polymerase Assay—Incubation mixtures (0.30 ml) contained 1 μmole of MgCl2, 0.3 μmole of 2-mercaptoethanol, 0.25 μmole of EDTA, 50 μmoles of Tris-ethanolamine buffer (pH 8.75), 0.05 ml of heated crude sonicate as primer, 12.5 mpmoles of mixed 3H-deoxynucleoside triphosphates containing 3.6 X 106 c.p.m. and enzyme as indicated. Incubations were performed at 37° for 20 or 30 minutes. At the end of the incubation period, the tubes were chilled at 0° and 0.20 ml of salmon sperm DNA (2.5 mg per ml), 1.0 ml of H2O, and 4 μmoles of ATP were added. After mixing, the tubes were heated at 100° for 2 minutes, and cooled to 0°. Perchloric acid (0.50 ml), 6%, was added with mixing, and after a few minutes the tubes were shaken or scratched to induce flocculation. The suspension was filtered through Fiberglas T-20A-60 Teflon-coated glass-fiber filter paper (1 inch in diameter) and washed successively with 10 ml of cold 0.2 N perchloric acid and 10 ml of ethanol. The filter paper was placed in a vial with 0.50 ml of H2SO4 and 10 ml of scintillation fluid (containing 2 g of 2,5-diphenyloxazole, and 100 mg of 1,4-bis-2(5’-phenyloxazolyl)-benzene per 473 ml of toluene). The vials were heated in a 57° water bath for 10 minutes and then counted in a liquid scintillation spectrometer.

Proteins were measured by the method of Lowry, Rosebrough, Farr, and Randall (15). For samples obtained from Amberlite XE-64 column chromatography, which were low in protein and relatively high in phosphate, the above method was modified as follows: Reagent A, 15% anhydrous Na2CO3 in 1.5 N NaOH; Reagent B, 2% CuSO4·5H2O in water; Reagent C, 4% sodium tartrate in water; Reagent D, undiluted Polin-Coolite phenol reagent (Fisher Scientific Company); Reagent E, 0.4 ml each of Reagents B and C were mixed and diluted to 20 ml with Reagent A.

To 0.5 ml of sample (2 to 20 μg of protein) was added 0.5 ml of Reagent E. After 10 minutes at room temperature, 0.2 ml of Reagent D was introduced rapidly and mixed after each addition. Ten minutes later, the sample was centrifuged for 5 minutes at 20,000 RCF. The absorbance of the supernatant fluid was read against water in a Beckman model DU spectrophotometer at 750 mp. Care was taken to make readings immediately after centrifugation, since the solutions soon became turbid again. Blank values were taken from a curve (linear) and were obtained by substitution of the appropriate phosphate buffer (25 to 200 μmoles) for protein.

In the range 4 to 20 μg of bovine plasma albumin, the absorbance-concentration curve was nearly linear. Four micrograms of bovine plasma albumin gave a reading of 0.100 by this method. NaCl, up to 600 μmoles, did not affect the readings, and as much as 200 μmoles of phosphate buffer, in the pH range 6 to 8, could be present.

Purification of DNA Polymerase from E. coli K12 Cells—Centrifugation and chromatography were conducted at 2°. Fractions I, II, and III were stored at -18°, all others at 0-2°.

Fraction I. Preparation of Extract—Packed cells of E. coli K12, 75 g, were suspended in 0.05 M Tris-chloride buffer (pH 7.4), 1 X 10-4 M EDTA (Tris-EDTA), 4 ml per g of frozen cells, and were subjected to a 20-minute treatment in the Raytheon 10-kc sonicator. The volume of the extract so obtained was 350 ml. Cell debris was not removed at this time (Fraction I).

Fraction II. RNase Treatment—A 4-ml aliquot of the sonicate was treated with 20 μg of RNase and incubated for 5 hours at 37°. Samples were withdrawn at 30-minute intervals and stored frozen until all had been collected. The thawed samples were centrifuged and the supernatant solutions were assayed for polymerase activity. Maximum DNA polymerase activity was found after 1/2 hours of RNase treatment. Accordingly, to 340 ml of Fraction I were added 6.8 ml of RNase solution (1 mg per ml), and the mixture was incubated at 37° for 4½ hours. After cooling, the precipitate was removed by centrifugation and washed by resuspension in 75 ml of Tris-EDTA solution, followed by centrifugation. The volume of the combined supernatant fluids was 365 ml (Fraction II).

Fraction III. Ammonium Sulfate Concentration I—To 365 ml of Fraction II were added 35 ml of m potassium phosphate buffer, pH 7.5, and 205 g of ammonium sulfate. After 10 minutes at 0°, the precipitate was collected by centrifugation and dissolved with 0.05 m potassium phosphate buffer, pH 7.4, in 1 X 10-3 M EDTA, to a volume of 113% of the applied activity. These were pooled and their combined volume was 345 ml (Fraction IV).

Fraction IV. Sephadex Fractionation—Fraction III (103 ml) was applied to a column of Sephadex G-200 (24 cm 3 X 30 cm) which had previously been equilibrated with 0.02 m potassium phosphate buffer, pH 7.4, in 1 X 10-3 M EDTA. Elution was with the equilibrating mixture, at a flow rate of 0.6 to 1.0 ml per minute. Fractions of 50 ml were collected. Tubes 5 to 11 contained 113% of the applied activity. These were pooled and their combined volume was 345 ml (Fraction IV).

In anticipation of long storage at 0°, Fraction IV was concentrated by ammonium sulfate precipitation. Molar potassium phosphate buffer, pH 7.4 (55 ml), and 200 g of ammonium sulfate were added to Fraction IV, and the precipitate was collected by centrifugation after 10 minutes at 0°. The precipitate was dissolved to 108 ml with phosphate-EDTA buffer as in Step III. The recovered activity was 87% of Fraction IV, with no change in specific activity.

For our reagents, the blank correction was

\[ 0.102 - \left( 0.054 \frac{\text{μmoles phosphate}}{200} \right) \]
Fraction V. Incubation in Ammonium Sulfate Solution—The purpose of this step was to detach most of the remaining nucleic acid so that it could be separated during subsequent DEAE-cellulose fractionation. Fraction IV (100 ml) was diluted with 1 x 10^-3 M EDTA (in water) to 250 ml. An equal volume of 0.7 saturated ammonium sulfate (diluted from a solution saturated at 20°C in 1 x 10^-4 M EDTA, which had been adjusted to pH 7.5 with KOH, was added. The resultant protein concentration was 4.6 mg per ml. After incubation for 45 minutes at 37°C, a flocculent precipitate was separated by centrifugation and discarded. To the supernatant solution (495 ml) were added 181 g of ammonium sulfate (final saturation about 0.9). After 10 minutes at 0°C, the precipitate was collected by centrifugation, dissolved, and diluted to a volume of 110 ml with 0.05 M potassium phosphate buffer, pH 7.4, in 1 x 10^-3 M EDTA (Fraction V).

Fraction VI. DEAE-cellulose Fractionation—Fraction V (110 ml) was dialyzed for 72 hours in 3 liters of 0.02 M potassium phosphate buffer, pH 7.4, in 1 x 10^-4 M EDTA, and was applied to a DEAE-cellulose column (10.5 cm x 19 cm), previously equilibrated with the dialysis mixture. Elution was by linear gradient from 0 to 0.5 M NaCl in 0.02 M phosphate buffer, pH 7.0-1 x 10^-4 M EDTA. The total reservoir volume was 1000 ml. The flow rate was 4 ml per minute. and 18- to 20-ml fractions were collected. Tubes 20 to 29 (0.18 to 0.24 M NaCl), containing 82% of the applied activity, were pooled to yield a volume of 154 ml. The ratio of absorbance at 260 μm to that at 290 μm was 1.75. This represents a content of about 0.04% nucleic acid (16) (Fraction VI).

Fraction VII. Hydroxylapatite Fractionation—Fraction VI (183 ml) was dialyzed for 7 hours in three changes (1000 ml each) of 0.01 M potassium phosphate buffer, pH 6.9, in 1 x 10^-4 M EDTA, and was applied to a column of hydroxylapatite (5.6 cm x 18 cm) which had been previously equilibrated with 0.01 M potassium phosphate buffer, pH 6.5, in 1 x 10^-4 M EDTA. Elution was by linear gradient with potassium phosphate buffer, pH 6.5, in 1 x 10^-4 M EDTA, the limiting concentrations being 0.01 M and 0.5 M. The total volume of eluting fluid was 1000 ml. Fractions of 18 to 19 ml were collected at a flow rate of 1.6 ml per minute. The pooled fractions, tubes 15 to 25 (0.14 to 0.23 M phosphate), had a combined volume of 202 ml and contained 83% of the applied activity (Fraction VII).

Fraction VIII. Fractionation on Amberlite XE-64—Fraction VII (201 ml) was dialyzed for 8 hours in two changes (1700 ml each) of 0.10 M potassium phosphate buffer, pH 6.5, containing 1 x 10^-4 M EDTA, and was applied to a column (6.3 cm x 12.5 cm) of Amberlite XE-64 which had been equilibrated with the dialysis solution. Linear gradient elution was carried out in the range 0 to 0.8 M NaCl in 0.1 M potassium phosphate buffer, pH 6.5-1 x 10^-4 M EDTA. The total volume of eluting fluid was 1000 ml. Fractions of 20 to 21 ml were collected at a flow rate of 2.5 ml per minute. The specific activity of the polymerase in tube 15 was 83,800, representing a 9,750-fold increase over that of the crude extract; the total activity of this one fraction was 40% of that in the crude extract. Tubes 12 to 18 (0.2 to 0.28 M NaCl) contained 72% of the applied activity, and these were pooled to give a combined volume of 146 ml (Fraction VIII).

Fraction IX. Ammonium Sulfate Concentration—Solid, crystalline bovine plasma albumin, 2 mg per ml, and 520 mg per ml of ammonium sulfate were added to Fraction VIII. After 10 minutes at 0°C, the precipitate was collected by centrifugation, and dissolved to 17 ml with 0.05 M potassium phosphate buffer, pH 7.0, in 1 x 10^-3 M EDTA (Fraction IX).

Fraction I. Preparation of Extract—Mitomycin C-induced K12α cells—The steps used were identical, except where indicated, to those used in purification of the polymerase from normal E. coli K12α cells.

Fraction II. RNase Treatment—The sonicate (255 ml) was treated with 8 ml of RNase (1 mg per ml) and incubated at 37°C for 3/4 hours. The volume of combined supernatants was 282 ml (Fraction II).

Fraction III. Ammonium Sulfate Concentration—Phosphate buffer, 20 ml, and 155 g of ammonium sulfate were added to 280 ml of Fraction II. The precipitate was dissolved to 102 ml (Fraction III).

Fraction IV. Sephadex Fractionation—Fraction III (100 ml) was applied to the same regenerated Sephadex column used in the purification of the normal polymerase. Tubes 4 to 10 were pooled (350 ml) and found to contain 81% of the applied activity. In this case, the pooled Sephadex fractions were not concentrated with ammonium sulfate (Fraction IV).

Fraction V. Incubation in Ammonium Sulfate Solution—To 345 ml of Fraction IV were added 15 ml of 0.1 M potassium phosphate buffer, pH 7.4, and 234 ml of saturated (at 25°C) ammonium sulfate solution, containing 1 x 10^-3 M EDTA and previously adjusted to pH 7.5 with KOH. The resultant protein concentration was 3.2 mg per ml. After incubation at 37°C for 1 hour, the heavy, flocculent precipitate was separated by centrifugation. This was washed by resuspension in 25 ml of 0.33 saturated ammonium sulfate, (final saturation, 0.78), and the precipitate was collected and dissolved, as before, to a volume of 90 ml (Fraction V).

The increased specific activity obtained in this step, compared with no increase in the similar step for the normal extract may have been due to the greater initial and the lower final concentrations of ammonium sulfate used here.

Fraction VI. DEAE-cellulose Fractionation—Fraction V (90 ml) was dialyzed for 11 hours in 4 liters of phosphate buffer-EDTA, and was applied to a DEAE-cellulose column (10.5 cm x 14.3 cm). The combined activities of Fractions 12 to 20 (0.12 to 0.2 M NaCl) represented 90% of the activity applied. The combined volume of these was 176 ml (Fraction VI).

Fraction VI was concentrated with ammonium sulfate as follows. To 176 ml of Fraction VI were added 19 ml of 0.1 M potassium phosphate buffer, pH 7.4, and 109 g of ammonium sulfate. The precipitate was collected, dissolved, and diluted to 38 ml, as in Step III. The ratio of absorbance at 280 μm to that at 290 μm was 1.72.

Fraction VII. Hydroxylapatite Fractionation—The ammonium sulfate concentrate of Fraction VI (38 ml) was dialyzed for 7 hours in three changes (300 ml each) of phosphate buffer-EDTA, and applied to a column of hydroxylapatite (3.87 cm x 14 cm). Elution was as described for the normal enzyme, except that a total volume of 600 ml of eluting fluid were used and 16-ml fractions were collected at a flow rate of 0.8 ml per minute. Tubes 12 to 20 (0.14 to 0.24 M phosphate) contained 83% of the
TABLE I

Purification of polymerase

The details of each purification step are described in the text. One unit of enzyme is defined as that amount which will incorporate 1 mpmole of substrate per hour under the conditions described in Fig. 1 for the DNA polymerase assay.

<table>
<thead>
<tr>
<th>Fraction No. and step</th>
<th>Normal</th>
<th>Mitomycin C-Induced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Units</td>
<td>Protein</td>
</tr>
<tr>
<td></td>
<td>Per ml</td>
<td>Total</td>
</tr>
<tr>
<td>I. Sonic extraction</td>
<td>258</td>
<td>87,500</td>
</tr>
<tr>
<td>II. RNase treatment</td>
<td>1,185</td>
<td>432,000</td>
</tr>
<tr>
<td>III. Ammonium sulfate concentration</td>
<td>5,380</td>
<td>553,000</td>
</tr>
<tr>
<td>IV. Sephadex</td>
<td>1,085</td>
<td>585,000</td>
</tr>
<tr>
<td>V. Ammonium sulfate digestion</td>
<td>4,185</td>
<td>454,000</td>
</tr>
<tr>
<td>VI. DEAE-cellulose</td>
<td>2,039</td>
<td>514,000</td>
</tr>
<tr>
<td>VII. Hydroxylapatite</td>
<td>1,142</td>
<td>231,000</td>
</tr>
<tr>
<td>VIII. Amberlite XE-64</td>
<td>1,135</td>
<td>160,000</td>
</tr>
<tr>
<td>IX. Ammonium sulfate concentration</td>
<td>9,680</td>
<td>168,000</td>
</tr>
</tbody>
</table>

FIG. 1. Chromatography of DNA polymerase on Amberlite XE-64. DNA polymerase, obtained from induced cells of E. coli K12λ, was chromatographed on XE-64 as described in "Experimental Procedure." The medium for the DNA polymerase assay contained 30 pmol of Tris-ethanolamine buffer, pH 8.75, 1 μmol of MgCl₂, 0.25 μmol of EDTA, 0.3 μmol of mercaptopethanol, 12 μmol of 5'-deoxyribonucleoside triphosphates containing 3.5 × 10⁴ c.p.m., 0.06 ml of heated crude sonicate as primer and 0.005 ml of each fraction in a final volume of 0.30 ml. Incubations were performed for 20 minutes at 37°C. The medium for the DNase assay contained 0.5 pmole of MgCl₂, 1 μmol of mercaptopethanol, 20 μmol of Tris-ethanolamine buffer, pH 8.5, and 0.02 ml of each fraction in a final volume of 0.25 ml. 5'-K12λ DNA (0.10 absorbance unit containing 1 × 10⁶ c.p.m.) was the substrate, and incubations were for 30 minutes at 37°C.

Applied activity. The combined volume was 141 ml (Fraction VII).

Fraction VIII. Fractionation on Amberlite XE-64—Fraction VII (140.5 ml) was dialyzed for 9 hours in two changes of buffer (1000 ml each) and applied to a 3.87 cm x 13 cm column of Amberlite XE-64. Elution was as described for the normal polymerase, except that a total volume of 600 ml of buffer was used in the linear gradient and 6- to 7-ml aliquots were collected at the rate of 1 ml per minute.

FIG. 2. pH Curve of DNA polymerase. The incubation mixtures are as described in Fig. 1 with the following changes: 50 μmol of the appropriate Tris-ethanolamine buffer were used and 25 μmol of λ DNA was the primer. Incubations were for 30 minutes at 37°C with Fraction IX of normal or induced DNA polymerase.

The polymerase in tube 23 represented 11% of the activity in the crude extract and its specific activity was 166,600, a 10,160-fold increase over that of the crude extract. Tubes 22 to 26 (0.2 to 0.24 M NaCl) were pooled. The combined volume was 32 ml, and the activity was 30% of that applied (Fraction VIII).

Fraction IX. Ammonium Sulfate Concentration—Fraction VIII (32 ml) was concentrated as described for the normal polymerase. The concentrated solution had a volume of 3.15 ml (Fraction IX).

RESULTS

Lysogenic induction of E. coli K12λ with mitomycin C causes a small rise (about 2-fold) in DNA polymerase activity of crude extracts after 60 minutes. This is shown in Table I along with a
August 1964  W. E. Pricer, Jr., and A. Weissbach
2611

summary of the recovery and purification of DNA polymerase during the fractionation procedure; 75 g of normal cells and 57 g of mitomycin C-induced cells were the starting materials in this table. The enzyme obtained from both normal and induced cells of K12\(\alpha\) behaves much the same throughout the fractionation procedure, although unexplained minor differences were noted in some of the early purification steps (i.e. RNase treatment), as shown in Table I. In the later purification steps the DNA polymerase from either normal or induced cells of K12X behaves much the same throughout the fractionation procedure, although unexplained minor differences were noted in some of the early purification steps (i.e. RNase treatment), as shown in Table I. In the later purification steps the DNA polymerase from either normal or induced cells was found to be eluted by the same salt concentrations during DEAE-cellulose, hydroxylapatite, and XE-64 chromatography (cf. "Experimental Procedure").

Fig. 1 shows in detail the final Amberlite XE-64 column chromatography step of the purification of DNA polymerase from extracts of induced cells. The peak tube (tube 23) of DNA polymerase shows a purification of 10,100-fold over the crude extract. This degree of purification was also found when the enzyme from normal cells was purified on XE-64 since the corresponding peak tube was enriched 9,759-fold. For the studies reported in this paper, the fractions containing the bulk of polymerase activity from the XE-64 chromatography were pooled, concentrated with ammonium sulfate, and then used. The pooled XE-64 fractions from normal and induced K12\(\alpha\) had a

**Table II**

<table>
<thead>
<tr>
<th>p-Chloromercuribenzoate</th>
<th>Deoxynucleotide incorporated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal enzyme</td>
</tr>
<tr>
<td>( \mu \times 10^4 )</td>
<td>m(^{\text{moles}})</td>
</tr>
<tr>
<td>0</td>
<td>1.48 (100%)</td>
</tr>
<tr>
<td>1.34</td>
<td>1.31 (88%)</td>
</tr>
<tr>
<td>3.3</td>
<td>1.21 (81%)</td>
</tr>
</tbody>
</table>

Fig. 2. Mg\(^{++}\) requirement of DNA polymerase. Incubation mixtures (0.30 ml) were as described in Fig. 1 with 0.1 \(\mu\) mole of K12\(\alpha\) DNA in place of heated crude sonicate as primer. Reactions were carried out for 30 minutes at 37\(^\circ\). Fraction IX of the DNA polymerase purification was used in both cases.

**Table III**

Relative priming ability of native and heated DNA

Incubation mixtures were set up as in Fig. 1 with 10 or 100 m\(^{\text{moles}}\) of the appropriate DNA as primer. About 20 units of Fraction IX of either induced or uninduced DNA polymerase were used, and the reaction was carried out for 30 minutes at 37\(^\circ\). "Heated" DNA refers to DNA, at a concentration of 1 \(\mu\) mole per ml, heated at 100\(^\circ\) for 5 minutes in 0.02 \(M\) Tris buffer (pH 7.4)-0.02 \(M\) NaCl, and quickly cooled to 0\(^\circ\).

<table>
<thead>
<tr>
<th>Source of DNA polymerase</th>
<th>C.p.m. incorporated with native DNA</th>
<th>C.p.m. incorporated with heated DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>K12(\alpha) DNA primer</td>
<td>10 (\mu) moles 100 (\mu) moles</td>
<td>10 (\mu) moles 100 (\mu) moles</td>
</tr>
<tr>
<td>(\lambda) DNA primer</td>
<td>10 (\mu) moles 100 (\mu) moles</td>
<td>10 (\mu) moles 100 (\mu) moles</td>
</tr>
<tr>
<td>Mitomycin C-DNA primer</td>
<td>10 (\mu) moles 100 (\mu) moles</td>
<td>10 (\mu) moles 100 (\mu) moles</td>
</tr>
</tbody>
</table>

Uninduced cells............ 0.64 4.45 1.08 2.06 0.74 3.08

Induced cells.............. 0.65 2.46 1.19 1.41 0.65 1.52

Fig. 3. Utilization of DNA primers by DNA polymerase. Reaction mixtures were as described in Fig. 1 with the various primers substituted for the heated crude sonicate. About 25 units of either normal or induced polymerase (Fraction IX) were used in the incubations. The mitomycin C-DNA (MC-DNA) was obtained from cells of *E. coli* K12\(\alpha\) exposed to 1 \(\mu\)g per ml of mitomycin C for 15 minutes. •, normal polymerase; O---O, induced polymerase.

Fig. 4. Heat inactivation of DNA polymerase. DNA polymerase incubation mixtures were the same as in Fig. 1. The enzymes (Fraction IX) were preincubated for various times in a water bath at 57\(^\circ\), and aliquots were removed and assayed as described in Fig. 1.
relative purification of 7,300- and 7,480-fold, respectively, over the crude sonicate. Fig. 1 also shows that there is a peak of DNase activity which chromatographs with the DNA polymerase activity. This DNase is presumably similar to the exonuclease which accompanies the highly purified DNA polymerase of *E. coli* B (17, 18). The occurrence of this DNase in *E. coli* K12 has previously been suggested (13). The other nuclease peak shown in Fig. 1 at tube 14 was not identified.

Comparison of the pH curves, Mg++ requirements, and heat inactivation curves of the DNA polymerase (7300- to 7400-fold purified) obtained from normal or induced cells of *E. coli* K12 are shown in Figs. 2, 3, and 4. Both polymerases show a pH optimum of about 9 and require Mg++. The optimum concentration of the latter being in the range of 1.5 to 3 mM. The normal and induced polymerases are stable at 45°C for 30 minutes but are rapidly inactivated at 57°C (Fig. 4). Hence, the two polymerases seem very similar by these criteria. Table II shows the effect of p-hydroxymercuribenzoate on the normal or induced polymerase to be essentially the same. In addition, neither polymerase is stimulated or inhibited by mercaptoethanol at levels up to 2 \times 10^{-3} \text{M}.

The normal and induced DNA polymerases have been tested for their abilities to utilize various DNA primers. Fig. 5 shows the ability of the 7300- to 7400-fold purified DNA polymerase from normal or induced *E. coli* K12 to utilize DNA obtained from λ phage, *E. coli* K12λ, and from *E. coli* K12λ exposed to 1 μg per ml of mitomycin for 15 minutes (Mc-15 DNA). As previously reported (5), the purified DNA polymerases from normal or induced cells of *E. coli* K12 utilize various DNA primers at the same relative rates. The inability of the DNA obtained from induced cells to prime DNA polymerase has been previously discussed (5) and is under investigation. On a relative basis, the best primer thus far tested for K12λ DNA polymerase is λ DNA. Various preparations of λ DNA have ranged from 2 to 4 time better than K12λ DNA in their ability to prime for DNA polymerase.

A further similarity between normal and induced polymerases is shown in Table III, where the relative abilities of heated and native deoxyribonucleic acids to serve as primer are compared. When an excess of primer was used, denatured DNA was less efficient than native DNA in its priming behavior for both enzymes. However, with limiting levels of primer, the rates (with both polymerases) with heat-denatured DNA was equal to or greater than that obtained with native DNA.

**DISCUSSION**

*E. coli* K12λ, when exposed to mitomycin C at concentrations of 1 μg per ml under the conditions employed in these studies, will lyse with optimal production of λ phage in 90 to 105 minutes (12). One would therefore expect that 60 minutes after exposure of *E. coli* K12λ to mitomycin C, the synthesis of phage-directed enzymes should have been started. This is illustrated by studies on the kinetics of formation of the λ-associated exonuclease during lysogenic induction (19) and of endolysin synthesis during λ reproduction (20). By all the criteria used in the present study, a DNA polymerase purified from *E. coli* K12λ, 60 minutes after induction with mitomycin C, is the same as the DNA polymerase found in normal cells of *E. coli* K12λ. It would seem therefore, that synthesis of λ phage DNA does not require a new DNA polymerase in apparent contradiction to the synthesis of phage T2 DNA in *E. coli* B. This finding emphasizes the question of why the *E. coli* K12λ cell, after lysogenic induction, will preferentially synthesize λ DNA rather than its own DNA. A possible explanation is suggested by the data in Fig. 5 where it is shown that bacterial DNA obtained from mitomycin C-induced cells of *E. coli* K12λ is apparently a poor primer for DNA polymerase and is much less effective than λ DNA. A previous communication (5) has examined in more detail the inability of DNA obtained from mitomycin C-treated cells, to serve as primer for DNA polymerase. The ability of the normal *E. coli* DNA polymerase to synthesize λ phage DNA in vivo is not surprising in view of the close similarities of λ DNA and that of *E. coli* K12λ (3).

**SUMMARY**

1. DNA polymerase has been purified 7300 to 7400-fold from normal and lysogenically induced cells of *Escherichia coli* K12λ.

2. By the criteria used in these studies, a DNA polymerase obtained from cells of *E. coli* K12λ, which have been induced to form the phage λ, is the same enzyme as that found in normal, uninduced *E. coli* K12λ.

**REFERENCES**


The Effect of Lysogenic Induction with Mitomycin C on the Deoxyribonucleic Acid Polymerase of *Escherichia coli* K12λ

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