A Simple and Rapid Purification Method for Escherichia coli DNA Polymerase I

(Received for publication, March 29, 1979, and in revised form, June 6, 1979)

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We report a simple, three-step method for the purification of Escherichia coli DNA polymerase I. Its advantages over other procedures are ease and rapidity, the absence of an autolysis or any high speed centrifugation step, and applicability to large quantities of material. In addition, RNA polymerase can be isolated as a by-product.

We have applied this method to purify DNA polymerase both from wild type E. coli cells and from cells bearing a λ prophage carrying the polA gene (Kelley, W. S., Chalmers, K., and Murray, N. E. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5632–5636). This latter source amplifies the amount of DNA polymerase in the cells by at least 10-fold.

Several procedures have been published for the purification of DNA polymerase I (1–6). Most employ phosphocellulose or DNA-cellulose for the main purification step. A major problem with these methods is the initial removal of ribosomes and nucleic acids from the cell extracts so that the enzyme will bind to subsequent columns. Richardson et al. (1) accomplished this by a prolonged autolysis step. Disadvantages of this procedure are the high degree of variability, the frequent loss of substantial activity, and the proteolytic modification of the enzyme. Other methods (3–5) utilize high speed centrifugation which severely limits the amount of extract which can be processed conveniently.

Several methods for the purification of RNA polymerase employ polym P, a basic polymer (polyethyleneimine or aziridine homopolymer), in order to precipitate the enzyme (7, 8). We have found that the DNA polymerase activity remains in solution at concentrations of polym P which completely precipitate RNA polymerase. After precipitation of the DNA polymerase with ammonium sulfate and desalting, the enzyme is loaded onto a phosphocellulose column. This provides a simple alternative to the autolysis, centrifugation, DEAE-cellulose chromatography, or phase partition steps to remove the nucleic acids.

EXPERIMENTAL PROCEDURES

Materials—Polym P was obtained from Fluka. A 15% (v/v) solution was made, titrated to pH 8.0 with concentrated HCl, and then diluted to 10% (v/v). Sodium deoxycholate was purchased from Merck. Calf thymus DNA was obtained from Sigma and activated by the procedure of Aposhian and Kornberg (9).

Whatman phosphocellulose (P-11) was washed with 1 N NaOH, water, 1 N HCl, and water until neutral. It was equilibrated with PC04 buffer (see below) before use. DNA-Sepharose was prepared as described by Arndt-Jovin et al. (10) and had 50 nmol of single-stranded salmon sperm DNA/mg of dry Sepharose. It was washed with 3 M KCl after use and was employed repeatedly.

Growth of Bacteria—Escherichia coli strain W3110 carrying the temperature-inducible prophage NM 857 polA Qam73 Sam7 att V red was the kind gift of Dr. N. Murray. One liter of L broth (11) was inoculated with an overnight culture to an A500 of 0.7 and grown at 39–39°C to an A500 of 4. The phase was induced by heating to 42°C for 10 min and growth was continued at 37°C to an A500 of 8 to 10 (about 5 h). The culture was then centrifuged and the cells were frozen. The increase in the specific activity of the DNA polymerase was approximately 20-fold upon induction when compared to wild type control strains. Wild type E. coli B MRE6000 cells grown to late log phase were obtained from Merck, Darmstadt.

Buffers—Grinding buffer (8) contains 50 mM Tris-HCl, pH 8.0, 2 mM EDTA, 0.1 mM dithiothreitol, 1 mM 2-mercaptoethanol, 0,233 M NaCl, 5% (v/v) glycerol, 0.10 mg/ml of lysozyme, and 29 μg/ml of phenylmethylsulfonyl fluoride. TGN buffer (TGED + 0.2 M NaCl (8)) is 10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA, 0.1 mM dithiothreitol, 0.2 M NaCl, and 5% (v/v) glycerol. PC04 buffer is 40 mM potassium phosphate, pH 6.9, 0.1 mM EDTA, 1 mM dithiothreitol (omitted during some purifications), and 5% (v/v) glycerol. PC3 buffer is the same but containing 300 mM potassium phosphate, pH 6.9. The basic buffer for DNA-Sepharose chromatography was DS buffer. This consists of 10 mM potassium phosphate, pH 6.9, 1 mM 2-mercaptoethanol (sometimes omitted), 0.1 mM EDTA, 5% (v/v) glycerol. KC1 was added to the DS buffer in the concentrations indicated. The sulfhydryl reagents in PC04, PC3, and DS buffers are not necessary for DNA polymerase purification (12) and may be omitted. We included them when assaying for other enzyme activities.

Methods—DNA polymerase was assayed by the method of Jovin et al. (2). Activated calf thymus DNA (9) was used in the early purification steps and poly[d(A-T)] later. A unit is defined as 10 nmol of nucleotide incorporated in 30 min at 37°C. Protein was measured by the method of Lowry et al. (13) using bovine serum albumin as the standard.

The enzyme was characterized by the following assays. The exonuclease activity associated with the pure DNA polymerase I was tested according to the method of Lehman and Richardson (14). 3H]Poly[d(A-T)] (7.4 cpm/pmol) was used as substrate. One unit is defined as the liberation of 10 pmol of nucleotides in 30 min at 37°C. For endonuclease activity, a test was used based on the conversion of supercoiled SV40 DNA Form I to open forms. ATPase activity was assayed in two buffers. The first was the standard buffer for the DNA polymerase assay: 75 mM potassium phosphate, pH 7.4, 7.5 mM MgCl2. We also used 40 mM Tris-HCl, pH 8.0, 10 mM 2-mercaptoethanol, 10 mM MgCl2, 0.1 mM EDTA. In both cases, [γ-32P]ATP was present at a concentration of 100 μM. The assay was started by addition of enzyme to a concentration of 1 μg/ml and was incubated 10 min at 37°C. EDTA (17 mM) and potassium phosphate, pH 6.9 (0.45 mM) were then added and 2 volumes of a 25% (v/v) suspension of activated charcoal. After 15 min at 0°C, the solution was centrifuged and the radioactivity in the supernatant was determined by Cerenkov counting.

Gel electrophoresis in the presence of sodium dodecyl sulfate was performed according to Laemmli (15). Slab gels (15.0 x 0.15 cm) were used under these conditions: 8% acrylamide, 0.13% bisacrylamide, 35 to 50 mA current and cooling by circulation with tap water.

RESULTS

Enzyme Purification

The following section describes a purification of 200 g of wild type E. coli cells and 25 g of the λpolA lysogen (Table

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TABLE I

| Purification of DNA polymerase I from 200 g of E. coli B cells |
|-----------------------------------------------------------|
| Fraction | Units* | Protein Specific activity | Yield |
|----------|---------|--------------------------|--------|
| 1. Extract | 78,000 | 10 | 5.7 (100) |
| 2. Polymin P supernatant | 50200 | 5.5 | 7 (64) |
| 3. Ammonium sulfate | 44,000 | 50 | 9 (56) |
| 4. Phosphocellulose | 29,000 | 0.17 | 680 (42) |
| 5. DNA-Sepharose | 27,100 | 0.5 | 4,500 45 |
|            | [19,000] | [29] | (100) |

* All data correspond to the assay with activated calf thymus DNA as template. The values in brackets (Fraction V) represent the data with optimally activated poly[d(A-T)].

TABLE II

| Purification of DNA polymerase I from 25 g of E. coli lysogenized with λpolA phage NM 857 |
|---------------------------------------------------------------------------------------------|
| Fraction | Units* | Protein Specific activity | Yield |
|----------|---------|--------------------------|--------|
| 1. Extract | 119,000 | 8 | 73 (100) |
| 2. Polymin P supernatant | 58,500 | 5 | 58 (49) |
| 3. Ammonium sulfate | 38,900 | 42 | 105 (52) |
| 4. Phosphocellulose | 29,000 | 0.07 | 3500 (24) |
| 5. DNA-Sepharose | 10,000 | 0.14 | 5000 10 |
|            | [19,400] | [29] | (100) |

* All data correspond to the assay with activated calf thymus DNA as template. The values in brackets (Fraction V) represent the data with optimally activated poly[d(A-T)].

** Phosphocellulose Chromatography**—The column was washed with 100 ml of PC04 buffer. The DNA polymerase activity was quite labile at this stage with half of the activity lost after 1 day. Rapid freezing of this fraction rendered it stable for at least 1/2 year.

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ml of DS + 0.1 M KCl, and eluted with a 400-ml gradient of DS + 0.1 M KCl to DS + 1.0 M KCl at a flow rate of 0.8 ml/min. The enzyme usually eluted about 0.4 to 0.5 M KCl. Fractions of similar specific activity were pooled (see Tables I and II). The enzyme was quite stable at 4°C at this stage with no observable loss of activity after several days.

**Concentration and Storage**—The pooled DNA-Sepharose fractions were dialyzed overnight against 20 mM potassium phosphate, pH 6.9, 0.1 mM EDTA and 5% (v/v) glycerol and then against the same buffer containing 50% (v/v) glycerol. This procedure resulted in a 3-fold concentration. The enzyme was stored at −20°C with no loss of activity after a year.

**Characterization of the Enzyme**

The specific activity with optimally activated poly[d(A-T)] was identical with that previously reported (1, 2). Specific assays for 5′ → 3′ and 3′ → 5′-exonuclease were not performed. When we used potassium phosphate buffer at pH 7.4 (optimal conditions for DNA polymerase I), the total exonuclease activity was about 5000 units/ml. Several enzyme purifications (including the data with the lysogenic phage) always showed the same ratio of polymerase to exonuclease activity of 5 to 6 under those conditions.

There was no evidence for endonuclease contamination as tested by incubating 5 units of DNA polymerase I with 1 μg of SV40 DNA Form I for 1 h at 37°C.

The assays for ATPase activity showed less than 3.6 pmol of phosphate released/min/mg of protein. This value was not increased by the addition of single or double-stranded DNA. Since its introduction by Zillig et al. (7), polymin P has been applied to cell masses ranging from 25 to 500 g. It can, in principle, be scaled to handle extremely large amounts of starting material. Table I presents the results of the general application for treatment of cellular extracts before column chromatography.

**DISCUSSION**

The above purification method for DNA polymerase is capable of yielding homogeneous enzyme in 3 to 4 days. We have applied it to cell masses ranging from 25 to 500 g. It can, in principle, be scaled to handle extremely large amounts of starting material. Table I presents the results of the general application for treatment of cellular extracts before column chromatography.

**Acknowledgments**—We are indebted to Dr. N. Murray for the lysogenic E. coli strain, Dr. W. S. Kellogg for unpublished information, Dr. P. Chambon for characterization of potential endonuclease contamination, and Ms. M. van der Ploeg for technical assistance.

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A simple and rapid purification method for Escherichia coli DNA polymerase I.
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*J. Biol. Chem.* 1979, 254:7465-7467.

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