A Procedure for the Rapid, Large-Scale Purification of *Escherichia coli* DNA-Dependent RNA Polymerase Involving Polymin P Precipitation and DNA-Cellulose Chromatography†

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ABSTRACT: An improved method is described for the purification of the DNA-dependent RNA polymerase [ribonucleosidetriphosphate:RNA nucleotidyltransferase, EC 2.7.7.6] from *Escherichia coli*. The method involves lysozyme–sodium deoxycholate lysis, low-speed centrifugation, precipitation with Polymin P, elution from the Polymin P precipitate, ammonium sulfate precipitation, and chromatography on DNA-cellulose and Bio-Gel A 5m. RNA polymerase is purified to electrophoretic homogeneity in 2 days with a recovery of 45%, resulting in a yield of 250 mg of holoenzyme from 500 g of cells.

The DNA-dependent RNA polymerase from *Escherichia coli* is widely used in the study of transcription. It is being purified in many laboratories by a variety of methods which vary in ease, speed, yield, reproducibility, and final purity (Babinet, 1967, Burgess, 1969, Zillig et al., 1970, Burgess and Travers, 1971, Berg et al., 1971, Nüsslein and Heyden, 1972, Mukai and Iida, 1973, Humphries et al., 1973, Yarbrough and Hurwitz, 1974). We present here a procedure which is very rapid and reproducible, requires no dialysis, ultracentrifugation, or expensive special equipment, and gives high yields of highly purified enzyme. Cell disruption is accomplished by lysozyme–sodium deoxycholate treatment instead of grinding with glass beads. Viscosity due to released DNA is reduced by shearing in a blender, rather than by DNase treatment. Nucleic acid and much protein are removed by precipitation with Polymin P and elution from the Polymin P pellet as introduced by Zillig et al. (1970) but simplified and detailed here. Final purification involves DNA-cellulose chromatography, introduced by Alberts and Herrick (1971) and successfully utilized by Bautz and Dunn (1971) and by Humphries et al. (1973), and Bio-Gel A 5m gel filtration chromatography.

A new method for preparing core polymerase is described which uses Bio-Rex 70 chromatography in place of phosphocellulose chromatography.

Experimental Procedures

Materials. Bio-Rex 70 (100–200 mesh), Bio-Gel A 5m, and electrophoresis grade acrylamide, *N,N'*-methylenebisacrylamide, Temed,[1] and ammonium persulfate were obtained from Bio-Rad; calf thymus DNA and lysozyme were from Worthington; glycerol, 99.1% pure, was from Fisher; sodium deoxycholate, Coomassie Brilliant Blue R-250, and ammonium sulfate (enzyme grade) were from Schwarz/Mann; unlabeled nucleoside triphosphates were from P-L Biochemicals; [^{14}C]ATP was from New England Nuclear; phenylmethanesulfonyl fluoride ($PhCH_2SO_2F$) and dithiothreitol were from Calbiochem; 2-mercaptoethanol and Trizma base were from Sigma. *E. coli* K12 bacterial cells were either purchased from Grain Processing, Muscatine, Iowa (¾ log, enriched medium) or strain PR7 (Reiner, 1969) grown on yeast–tryptone medium at 37°C to ¾ log phase.

Buffers. All buffers are prepared from double distilled water and the highest grade chemicals available. Stock solutions of 2.0 *M* Tris-HCl (pH 7.9) at 25°C, 0.20 *M* Na_2EDTA (pH 7.9), and 4.0 *M* NaCl, all Millipore filtered, and 0.1 *M* dithiothreitol were diluted to prepare the following buffers. Grinding Buffer: 0.05 *M* Tris, 5% (v/v) glycerol, 2 m*M* EDTA, 0.1 m*M* dithiothreitol, 1 m*M* 2-mercaptoethanol, 0.233 *M* NaCl, 130 μg/ml of lysozyme, and 23 μg/ml of $PhCH_2SO_2F$. The basic buffer used throughout the rest of the preparation was TGED: 0.01 *M* Tris (pH 7.9), 5% (v/v) glycerol, 0.1 m*M* EDTA, and 0.1 m*M* dithiothreitol, which contained NaCl at the final concentration noted. Storage buffer: same as TGED + 0.1 *M* NaCl except 50% (v/v) glycerol instead of 5%.

Polymin P was the kind gift of BASF, WHOZ Hauptlaboratorium B9, Hochschullieferungen, 6700 Ludwigshafen/Rhein, Germany. A 10% (v/v) solution titrated with concentrated HCl to pH 7.9 was prepared and clarified by filtration through Miracloth (Calbiochem) or by low-speed centrifugation. More details on Polymin P properties, protein determination, and conductivity measurement are found in the accompanying paper (Jendrisak and Burgess, 1975).

RNA Polymerase Assay. RNA polymerase was assayed as described previously (Burgess, 1969), except that trichloroacetic acid precipitates were collected on Whatman GF/C filters and counted by liquid scintillation counting.

DNA-Cellulose Column. DNA-cellulose was prepared as described by Alberts and Herrick (1971). Washed and dried Whatman CF-11 cellulose (160 g) was mixed with 500 ml of 2 mg/ml of calf thymus DNA in 0.01 *M* Tris-HCl (pH 7.9)–0.001 *M* EDTA. The paste was dried at

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[1] Abbreviations used are: $PhCH_2SO_2F$, phenylmethanesulfonyl fluoride; Temed, *N,N,N',N'*-tetramethylethylenediamine; TGED, 0.01 *M* Tris (pH 7.9), 5% (v/v) glycerol, 0.1 m*M* EDTA, and 0.1 m*M* dithiothreitol.

50°C for 3 days, lyophilized overnight, suspended and washed as described (Alberts and Herrick, 1971), and packed into a column. The column contained 0.43 mg of DNA/ml of packed column volume.

Bio-Rex 70 Column. Bio-Rex 70 (100–200 mesh) was resuspended in 0.5 *N* NaOH, mixed 20 min, collected on a Büchner funnel, washed with double distilled water to about pH 8, resuspended in 0.5 *N* HCl, mixed, washed to pH 4, resuspended in 0.05 *M* Tris-HCl (pH 7.9), titrated with 1 *N* NaOH to pH 7.9, diluted with TGED, poured into a column, and stored at 4°C.

Polyacrylamide Gel Electrophoresis. Stacking dodecyl sulfate polyacrylamide gels were run as described by Laemmli (1970) with the following modifications: glass tubes (with 5 mm i.d.) were siliconized for 5 min in 2% (v/v) dimethyldichlorosilane (Matheson Coleman and Bell) in carbon tetrachloride, drained, and heated 30 min at 120°C just before use. Water-saturated 2-butanol (2-methyl-1-propanol, reagent grade, J.T. Baker Chem. Co.) was layered on top of the gel solutions in the tubes prior to polymerization. Since 2-butanol is immiscible with water it can be layered on quickly without permanently disturbing the surface and as a result very flat upper gel surfaces can be obtained easily. The 2-butanol is rinsed off with distilled water after polymerization. Gels are stained 6 hr in 0.05% Coomassie Brilliant Blue R-250 in 95% ethanol–glacial acidic acid–water (5:1:5) and diffusion destained in 7.5% (v/v) acetic acid for several hours at 50°C and then at room temperature until the background is free of stain. Ethanol works as well as methanol which is normally used in staining and undue exposure to toxic methanol is avoided.

Quantitation of $\beta'\beta$ Subunits on Gels. Samples of various stages of the purification were diluted 1:10 with the dodecyl sulfate sample buffer of Laemmli (1970) (3% (w/v) dodecyl sulfate, 5% (v/v) 2-mercaptoethanol, 10% (v/v) glycerol, and 0.063 *M* Tris-HCl (pH 6.8)) and heated to 90°C for 2 min. Several different amounts of each dilution were subjected to electrophoresis on dodecyl sulfate–8.75% polyacrylamide gels, 12-cm long, for 10 hr at 1 mA/tube as described above, stained for 6 hr, and destained overnight. The $\beta'\beta$ region of the gel was scanned in an Beckman ACTA III spectrophotometer at 550 nm at a rate of 1.5 cm/min with an 0.1-mm slit width. The absorbance was recorded continually and the area under the $\beta'\beta$ peak determined by cutting out the peak and weighing it. A standard curve is prepared by electrophoresis of 0.25, 0.625, 1.25, and 2.50 μg of holoenzyme on four different gels which are then stained, destained, and scanned along with samples being analyzed for $\beta'\beta$. Protein concentration of the standard enzyme is determined using an extinction coefficient for holoenzyme of $E_{280}(1\%)$ 6.5 (Richardson, 1966).

Purification Procedure

All steps were carried out at 4°C unless otherwise noted.

A. Cell Disruption. E. coli K12 cells (500 g), frozen at −70°C, are broken into pieces 2 cm or less in diameter and placed in a 1-gal Waring Blendor with 1500 ml of grinding buffer. The cells are blended at low speed for 2–3 min until they are completely resuspended and the temperature has increased to 2–5°C. After 20 min, 25 ml of 4% (w/v) sodium deoxycholate was added with stirring to give a final concentration of 0.05% and the mixture blended for 30 sec at low speed. After 20 min at 8–12°C the mixture was blended 30 sec at high speed to shear the DNA, 2000 ml of TGED + 0.2 *M* NaCl was added, and the mixture was sheared again at high speed for 30 sec. This yielded 4000 ml of cell extract.

The cell extract was centrifuged in 500-ml polycarbonate bottles in a G-3 rotor of a Sorvall RC-2B centrifuge at 4°C for 45 min at 8000 rpm. The clear amber somewhat viscous supernatant was collected, yielding 3460 ml of low-speed supernatant.

B. Polymin P Fractionation. The low-speed supernatant was placed in the blender and a 10% (v/v) solution of Polymin P (pH 7.9) was added slowly with stirring to a final concentration of approximately 0.35% (3.5 ml of polymin P solution per 100 ml of supernatant). After continuing to stir for 5 min, the mixture was centrifuged for 15 min at 6000 rpm and the supernatant poured off and discarded. The drained pellet was scraped into the blender and resuspended in 2.0 l. of TGED + 0.5 *M* NaCl with gentle stirring to avoid foaming for 5 min. Any particles remaining can quickly be resuspended in a 100-ml Dounce homogenizer. The mixture was centrifuged for 15 min at 6000 rpm and the supernatant, the 0.5 *M* NaCl wash, discarded. The washed pellet was again scraped into the blender and resuspended in 2.0 l. of TGED + 1 *M* NaCl with gentle stirring for 5 min. Again any remaining particles were resuspended in a homogenizer. The mixture was centrifuged for 30 min at 6000 rpm and the supernatant collected, yielding 1970 ml of 1 *M* NaCl eluate. Solid ammonium sulfate was added with stirring to the 1 *M* NaCl eluate (35 g/100 ml) to give 50% saturation. After the mixture was stirred for 20 min, it was centrifuged for 45 min at 8000 rpm and the supernatant discarded. The drained pellet was dissolved in TGED and diluted with TGED until the conductivity is equal to that of TGED + 0.15 *M* NaCl (requires 1000–1500 ml of TGED).

C. DNA-Cellulose Chromatography. A 500-ml (4 × 40 cm) calf thymus DNA-cellulose column was prepared as described in Experimental Procedures and freshly equilibrated with 1000 ml of TGED + 0.15 *M* NaCl. The dissolved and diluted sample above was pumped onto the column at 100 ml/hr, washed with 500 ml of TGED + 0.15 *M* NaCl, and eluted with a 1000-ml linear salt gradient from 0.15 *M* NaCl to 1.0 *M* NaCl. The enzyme activity elutes between 0.3 and 0.45 *M* NaCl as seen in Figure 1. Peak fractions were pooled and concentrated (precipitated with 35 g of ammonium sulfate/100 ml, stirred gently for 15 min at 0°C, centrifuged 30 min at 8000 rpm, and dissolved in TGED to 25 ml).

D. High Salt Bio-Gel A 5m Chromatography. The above sample was applied to a 2.5 × 100 cm column of Bio-Gel A 5m equilibrated with TGED + 0.5 *M* NaCl. The column was eluted as 25 ml/hr. The void volume was fraction 15 and the RNA polymerase elutes at about 1.8 times the void volume as seen in Figure 2. The peak fractions (24–29) were pooled and dialyzed against 1000 ml of storage buffer for 1 day. Dialysis against storage buffer results in an approximately threefold concentration. Final protein concentration is determined from an absorption spectrum corrected for light scattering and the extinction coefficient of $E_{280}(1\%)$ 6.5 (Richardson, 1966).

The dialyzed enzyme was aliquoted into screw-capped plastic tubes, and stored at −70°C. Once a sample is removed from −70°C and thawed it is stored at −20°C and used as needed without refreezing.

E. Bio-Rex 70 Chromatography (only used for purification of core RNA polymerase). If core enzyme is desired then the holoenzyme prepared above is chromatographed

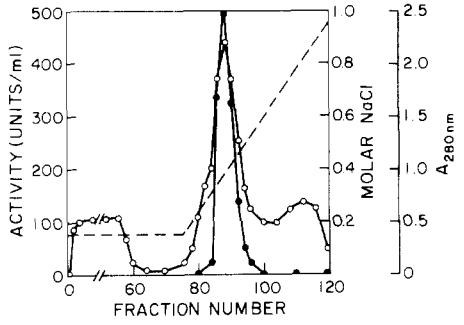

FIGURE 1: DNA-cellulose chromatography. The ammonium sulfate precipitated 1 *M* NaCl eluate was dissolved in TGED to 1000 ml and applied to a 500-ml calf thymus DNA-cellulose column as described in the text; 25-ml fractions were collected and assayed for absorbance at 280 nm (O—O), and RNA polymerase activity (●—●) and NaCl concentration by conductivity measurements (- - -).

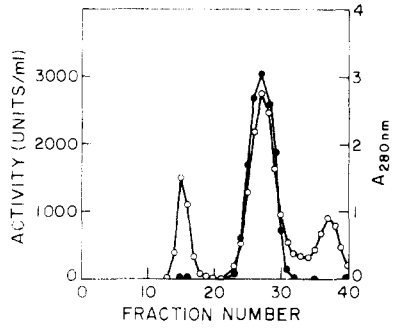

FIGURE 2: High salt Bio-Gel A 5m chromatography. Fractions 85–94 from the DNA-cellulose column shown in Figure 1 were pooled, concentrated by ammonium sulfate precipitation, dissolved in TGED to 25 ml, and applied to a Bio-Gel A 5m column as described in the text; 12.5-ml fractions were collected and assayed for absorbance at 280 nm (O—O) and RNA polymerase activity (●—●).

on a column containing Bio-Rex 70 freshly equilibrated with TGED + 0.15 *M* NaCl. Holoenzyme from the A 5m column peak is diluted with TGED to 0.15 *M* NaCl final concentration and 100 mg applied to a Bio-Rex 70 column (2 cm × 15 cm; about 1-ml column for 2 mg of holoenzyme) at a flow rate of 50 ml/hr. The column is washed with 100 ml of TGED + 0.15 *M* NaCl and eluted with a 400-ml linear salt gradient from 0.15 *M* NaCl to 1.0 *M* NaCl. The σ subunit comes through in the flow through and the core polymerase elutes at about 0.37 *M* NaCl. To obtain pure core enzyme the core eluting from the Bio-Rex 70 column should be diluted to 0.15 *M* NaCl with TGED and rerun as before. This removes traces of contaminating σ. The core is stored at −20 to −70°C after dialysis against storage buffer. To obtain pure σ the flow through from the Bio-Rex 70 column is pooled, diluted with TGED to 0.1 *M* NaCl, and concentrated by applying it to a 1-ml DEAE-cellulose column as described by Berg et al. (1971). The column is washed with TGED + 0.1 *M* NaCl and eluted with TED + 0.4 *M* NaCl (TGED without glycerol). The eluted σ is layered on a sucrose gradient (0.5 ml of σ on a 12.0-ml 5–20% (w/v) sucrose gradient containing TED + 0.5 *M* NaCl) and centrifuged for 30 hr at 40,000 rpm at 4°C in a SW41 rotor. The σ, sedimenting about half-way down the gradient, is pooled and stored at −20 or −70°C after dialysis against storage buffer.

Comments on the Purification Steps

A. Cell Disruption. $PhCH_2SO_2F$ is added to help prevent proteolytic degradation in the extract (Goldberg, 1971). Lysozyme is added to produce spheroplasts (Guthrie and Sinsheimer, 1963) which lyse upon addition of the nonionic detergent, sodium deoxycholate. The DNase used previously in RNA polymerase purification (Burgess, 1969) to decrease viscosity was not used since it itself can become an unwanted contaminant. In addition, DNase contains significant amounts of chymotrypsin and can lead to protein degradation (Price et al., 1969, Engbaek and Burgess, unpublished observation). Blending the extract briefly at high-speed shears the DNA and decreases viscosity adequately. Lipids solubilized by the detergent can cause problems of nonsedimentable turbidity if ammonium sulfate precipitation of the extract is attempted before the Polymin P precipitation. Other methods of cell disruption can be used in place of the lysozyme–sodium deoxycholate lysis.

B. Polymin P Fractionation. A Polymin P precipitation curve was made on the low-speed supernatant. The results are seen in Figures 3A and 4A. It is clear that the RNA polymerase is completely precipitated by 0.35% Polymin P while only 25–30% of the protein is precipitated.

From Figures 3B and 4B it is clear that washing the 0.35% Polymin P pellet with 0.5 *M* NaCl removes 50% of the extractable protein and no RNA polymerase while eluting with 1.0 *M* NaCl efficiently elutes the rna polymerase.

Precipitation of the 1.0 *M* NaCl eluate with 50% saturated ammonium sulfate concentrates the enzyme, removes a small amount of protein, and removes the residual Polymin P which would interfere with the subsequent DNA-cellulose column chromatography.

The Polymin P precipitation and elution results in a 15-fold purification of RNA polymerase with 90% recovery as measured by the amount of β' and β subunits on dodecyl sulfate gels. It also removes most of the nucleic acid since the A_{280}/A_{260} of the sample applied to the DNA-cellulose column is 1.66 when corrected for scattering by the method of Leach and Scheraga (1960).

It should be stressed that the amount of Polymin P needed to precipitate the RNA polymerase is a function of both the salt concentration and the nucleic acid and protein content of the low-speed supernatant. It is strongly suggested that a precipitation curve be made initially to obtain the optimal conditions for a particular batch of cells and Polymin P, and if variations are made in the method of cell disruption or dilution of the extract. Once conditions are optimized the method is completely reproducible. It is difficult to assay for RNA polymerase in the presence of Polymin P although it can be done if 2 μl of supernatant is assayed in a reaction containing ten times the normal specific activity of ATP. A much easier and perhaps more reliable way of determining the correct amount of Polymin P to add to the low-speed supernatant to quantitatively precipitate the RNA polymerase is the following: a precipitation curve such as described in Figure 3A is prepared. The supernatant will be slightly turbid when 0–0.30 ml of Polymin P is added per 10 ml of low-speed supernatant; 0.35 ml and greater amounts of Polymin P result in clear supernatants. We always find that RNA polymerase is completely precipitated by that amount of Polymin P which just gives a clear supernatant. It should be noted that various batches and various strains of *E. coli* vary in their nucleic acid content and ease of lysis. As a result we have used from 0.25 ml to 0.60 ml of 10% Polymin P to just precipitate RNA polymerase on various occasions. Also if the extract is diluted five-

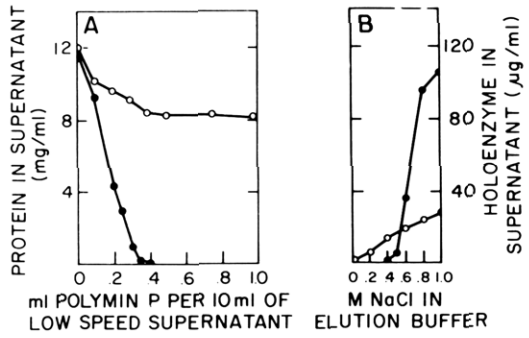

FIGURE 3: Polymin P precipitation curve and NaCl elution curve. (A) To 2-ml aliquots of the low-speed supernatant, 10% (v/v) Polymin P was added in various amounts. The tubes were rapidly mixed, incubated 5 min at 0°C, mixed again, and centrifuged for 5 min in a clinical centrifuge at top speed. The supernatants were assayed for total protein (O—O) and for $\beta'\beta$ subunits on the dodecyl sulfate polyacrylamide gels shown in Figure 4a (●—●). (B) An elution curve was prepared by adding 0.35 ml of 10% Polymin P per 10 ml of low-speed supernatant, mixing, dividing into 2-ml aliquots centrifuging as described above, and discarding the supernatant. The identical pellets were resuspended for 10 min in 2 ml of TGED buffer containing various NaCl concentrations, and the tubes were centrifuged again. The supernatants were assayed for total protein (O—O) and for $\beta'\beta$ subunits on the dodecyl sulfate polyacrylamide gels shown in Figure 4b (●—●).

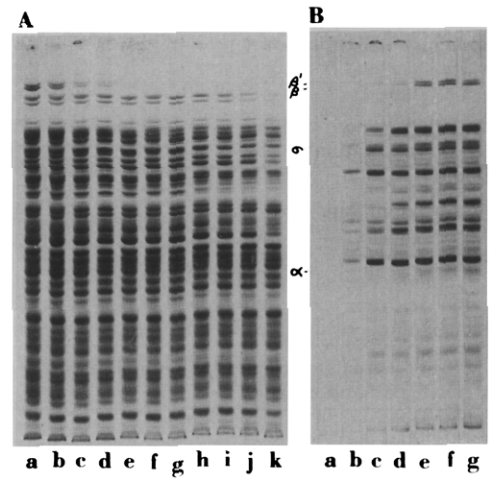

FIGURE 4: Dodecyl sulfate polyacrylamide gel electrophoresis of supernatants from the Polymin P precipitation curve and the NaCl elution curve. Aliquots of the supernatants in Figure 3a and b were diluted tenfold with dodecyl sulfate sample buffer and heated for 2 min at 90°C, and 100 μl was electrophoresed on polyacrylamide gels containing 0.1% dodecyl sulfate and 8.75% acrylamide as described in Experimental Procedures. (A) Aliquots of supernatants of a Polymin P precipitation curve where, per 10 ml of low-speed supernatant, 10% Polymin P was added, in the following volumes: (a) 0.0 ml, (b) 0.1 ml, (c) 0.2 ml, (d) 0.25 ml (e) 0.3 ml, (f) 0.35 ml, (g) 0.4 ml, (h) 0.5 ml, (i) 0.75 ml, (j) 1.0 ml. (B) Aliquots of supernatants of a NaCl elution curve where 0.35% Polymin P pellets were eluted with TGED containing the following concentrations of NaCl: (a) 0.0 *M*, (b) 0.2 *M*, (c) 0.4 *M*, (d) 0.6 *M*, (e) 0.8 *M*, (f) 1.0 *M*. The positions of the RNA polymerase subunits β', β, σ, and α (with molecular weights of 165,000, 155,000, 87,000, and 39,000, respectively) are indicated.

fold, then approximately fivefold less Polymin P is added per 10 ml to just precipitate RNA polymerase.

C. DNA-Cellulose Chromatography. The DNA-cellulose column can be reused repeatedly if it is washed with TGED + 1.0 *M* NaCl and reequilibrated to TGED + 0.15 *M* NaCl immediately after and again immediately before each use. The DNA will come off the column slowly at 4°C (Alberts and Herrick, 1971) but we have used columns for over 9 months with good results. Any Polymin P present in the sample and binding to the DNA-cellulose can be eluted off with TGED + 2 *M* NaCl.

Occasionally some turbidity will elute off of the column just before and just after the RNA polymerase. This appears to be lipid and any which contaminates the polymerase peak is completely removed by the agarose column. Although one could elute the column with a 0.15–0.75 *M* salt step as utilized by Humphries et al. (1973) it is relatively easy to use a steep gradient and remove several impurities.

D. High Salt Bio-Gel A 5m Chromatography. The high salt agarose column very effectively removes the last traces of impurities. The column can be reused indefinitely as long as it is stored in TGED + 0.5 *M* NaCl (magnesium has been removed from all buffers, both to prevent DNase action on the DNA-cellulose column, but also to prevent growth of mold in the agarose column). The material eluting in the void peak has a A_{280}/A_{260} ratio of about 1.0, contains almost no protein, and appears to be lipid. The material eluting after the RNA polymerase consists of several proteins which contaminate the DNA-cellulose purified enzyme. A Bio-Gel A 1.5m column could also be utilized in place of the A 5m to move the RNA polymerase nearer the void peak and even further from these contaminating proteins.

E. Bio-Rex 70 Chromatography. We have often had difficulty in obtaining reproducible results using phosphocellulose chromatography to prepare core RNA polymerase. These difficulties include the slow kinetics of binding of RNA polymerase to phosphocellulose (which often results in significant amounts of RNA polymerase present in the flow-through) and the slow release of the σ subunit into the flow-through (resulting in very dilute and unstable solutions of σ). We have found that Bio-Rex 70 when prepared and run as described here gives much more satisfactory and reproducible results. The σ subunit is quickly released and flows straight through the column without delay. The core enzyme elutes off in a sharp peak at 0.34–0.40 *M* NaCl and accelerated flow rates can be used.

F. Yield and Purity. The purification of *E. coli* K12 RNA polymerase from 500 g of cells is summarized in Table I and Figure 5; 245 mg of enzyme is obtained with an overall yield of 45% based on the amount of $\beta'\beta$ in various fractions. This higher yield not only reflects more effective lysis than was obtained earlier by grinding with glass beads (Burgess, 1969) but also less loss due to the reduced number of purification steps. The activity is not an accurate measure of the purity until the last step due to inhibitors and RNase present in earlier stages. However, the final specific activity is similar to that obtained earlier (Burgess and Travers, 1971). No detectable impurity bands can be observed when 10 μg of A 5m column purified RNA polymerase is applied to the dodecyl sulfate polyacrylamide gels. The σ content seems to be high. We estimate that greater than 80% of the enzyme contains σ factor (Ahrens and Burgess, in preparation). When holoenzyme with saturating amounts of σ are needed, we usually run $\frac{1}{3}$ of the pooled peak from the DNA-cellulose column through the Bio-Rex column, pool the σ-containing flow through with the other $\frac{2}{3}$, ammonium sulfate precipitate, and run the concentrated material on the agarose column as described. Another method is to chromatograph A 5m column purified enzyme on a phosphocellulose column in TED + 50% (v/v) glycerol + 0.1 *M* NaCl and elute with a gradient of 0.1–1.0 *M* NaCl in TED + 50% glycerol. This general method was discovered by Gonzalez, N. and Chamberlin, M., unpublished

Table I: Summary of Purification.[a]

Stage of Purification	Volume (ml)	Total Protein[b] (mg)	Total Activity (units)	Activity (units/mg)	Total Polymerase[c] (mg)	Yield[d] (%)
Cell Extract	4000	59,000	58,000	1.0	540	100
Low Speed Sup.	3460	41,900	37,400	0.9	410	75
1 *M* NaCl Eluate	1970	2,800	72,000	26	370	67
DNA-cellulose column pooled peak	250	410	75,200	183	320	59
Bio-Gel A-5m column pooled peak	75	245	198,500	810	245	45

[a] From 500 g of cells. [b] Determined by method of Lowry et al. (1951). [c] Determined by quantitation of $\beta'\beta$ subunits on gels. [d] Calculated from total polymerase, not activity.

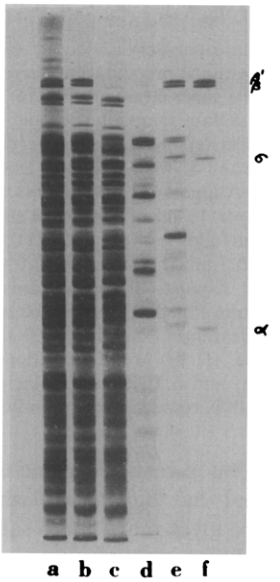

FIGURE 5: Dodecyl sulfate polyacrylamide gels of various fractions of the purification. The following fractions were diluted with dodecyl sulfate sample buffer and heated 2 min at 90°C, and aliquots were electrophoresed as described above: (a) cell extract (150 μg of total protein), (b) low-speed supernatant (120 μg), (c) 0.35% Polymin P supernatant (90 μg), (d) 0.5 *M* NaCl wash (14 μg), (e) 1.0 *M* NaCl eluate (7 μg), (f) Bio-Gel A 5m pooled peak (1.25 μg). Fractions a–e each represent 1/400,000 of the total preparation; fraction f, 1/200,000. The positions of the β', β, σ, and α subunits are indicated.

results. Under these conditions σ is not released and pure saturated holoenzyme elutes at 0.21 *M* NaCl, followed by core enzyme at 0.32 *M* NaCl.

We have occasionally noted slight RNase I contamination eluting just before the polymerase peak from the DNA-cellulose column and just after the polymerase peak from the A 5m column. Although by pooling away from those regions as has been done in this paper one can obtain polymerase essentially free of RNase I, we strongly recommend that enzyme be purified from an RNase I strain of *E. coli* such as MRE 600 (Cammack and Wade, 1965) or PR7 (Reiner, 1969) when the size of synthesized RNA is being studied.

Acknowledgment

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