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Purification and Properties of
Ribonucleic Acid Polymerase from
Escherichia Coli

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Purification and Properties of
Ribonucleic Acid Polymerase from
Escherichia Coli

by

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ABSTRACT

A procedure for purification of RNA polymerase from Escherichia coli is described. This method allows processing of several times as many cells at once as do previous procedures. Investigations of contaminating enzyme activities and attempts to further purify the enzyme reveal that it is essentially a homogeneous protein. The amino acid composition of the enzyme was determined. The purified enzyme has an $S_{20,w}$ value of 24.5 and a molecular weight of about 1.0×10^6 . In 0.5 M KCl, the enzyme sediments about half as fast as in low ionic-strength buffers.

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INTRODUCTION

Several procedures for the purification of ribonucleic acid (RNA) polymerase (nucleoside triphosphate: RNA nucleotidyl transferase, Enzyme Commission No. 2.7.7.6) from Escherichia coli have been described (1-5). These procedures utilize high speed centrifugation to remove ribosomes in one of the early purification steps. Since limited ultracentrifuge capacity often restricted to a few hundred grams of cells the amount of material that could be processed at once, we have devised a procedure in which the cell extract is concentrated by an ethanol precipitation step prior to ultracentrifugation. This method allows processing of several times as much E. coli for a given ultracentrifuge capacity. This report describes our purification scheme, the enzyme purity obtained, and some catalytic and physical properties, including amino acid composition. Some catalytic properties of these enzyme preparations have already been described (6, 7).

EXPERIMENTAL PROCEDURE

Materials

Frozen cells of E. coli B were obtained from the Grain Processing Company of Muscatine, Iowa, and stored at -20°C until used. Glass beads (average diameter 200 μ) were purchased from Aloe Scientific. ¹⁴C-labeled ribonucleoside triphosphates were obtained from Schwarz BioResearch, Inc.,

and $^{32}\text{P}_i$ was supplied by Squibb, Inc. Unlabeled ribonucleoside triphosphates, DEAE-cellulose, crystallized bovine plasma albumin, Bio-Gel P-200, and salmon sperm DNA were obtained from Calbiochem. Streptomycin sulfate was a product of Eli Lilly and Company. Amberlite CG-50 (100 to 200 mesh) was obtained from Mallinckrodt Chemical Works. Calf thymus DNA was purchased from Worthington Biochemical Corporation. Hydroxylapatite was prepared by the method of Tiselius et al. (8). The d(pT)_6^* and d(pT)_{13} oligodeoxynucleotides were prepared in our Laboratory using the method of Khorana and Vizsolyi (9). Chinese hamster ovary RNA labeled with ^3H -uracil was obtained from M. D. Enger of this group.

All reagents were prepared using deionized glass-distilled water. Buffer A consisted of 0.01 M Tris-HCl, pH 7.9; 0.01 M MgCl_2 ; 0.01 M 2-mercaptoethanol; and 0.1 mM EDTA. Ammonium sulfate solutions were prepared in 0.05 M potassium phosphate, and the pH was adjusted with 1 N ammonium hydroxide at 5°C. All buffers used in the purification of the enzyme contained 0.01 M 2-mercaptoethanol unless otherwise noted.

Methods

The reaction mixture for assay of RNA polymerase and the definition of unit of enzyme activity have been described previously (1). The reaction was stopped by placing the mixture on a disk of glass-fiber filter paper which was soaked in a mixture of 5% trichloroacetic acid-0.01 M sodium pyrophosphate followed by several washings with 95% ethanol.

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*The abbreviation used is d(pT)_n , oligodeoxythymidylate with a 5'-phosphate end, where the subscript refers to the chain length in monomer units.

After the disk had been dried in an oven, the acid-insoluble radioactivity was measured in a Packard Model 3324 liquid scintillation spectrometer.

During purification of the enzyme, protein was determined by the method of Gornall et al. (10) through 30% ammonium sulfate extraction and by the method of Warburg and Christian (11) for the concentrated CG-50 fraction. In several experiments designed to check homogeneity of purified RNA polymerase, protein was determined by the method of Lowry et al. (12). Other methods are described in conjunction with the appropriate procedures.

RESULTS

Purification of Polymerase

This section describes the procedure for processing 1.36 kg of cell paste. Table 1 gives the data obtained from the average of eleven 1.36-kg preparations. This procedure is highly reproducible, the final specific activity being within 10% of the average value in all cases. The described purification procedure has occasionally been scaled up to handle 4.54 kg of E. coli.

All operations were carried out at 0 to 5°C. Unless otherwise indicated, all centrifugations were at 15,000 x g for 30 min. The extraction and streptomycin precipitation procedure of Chamberlin and Berg (1) was adopted because of its suitability for isolation of RNA and DNA polymerases from a single preparation.

Fraction I. Partially thawed cells (1.36 kg) were mixed with 1.36 liters of buffer A in a 2-gal Lourdes Volu Mix

Table 1. Purification of RNA Polymerase

Fraction	Purification Step	Total Activity	Protein	Specific Activity
		(units x 10 ⁻⁵)	(mg/ml)	(units/mg protein)
I	Extract	17.70	24.5	19
II	Streptomycin Supernatant	9.15	11.8	17
III	Ethanol Precipitate	6.54	39.6	26
IV	4-Hr 30,000-rpm Supernatant	5.73	32.7	26
V	(NH ₄) ₂ SO ₄ 0.5 Saturation	7.89	29.6	77
VI	Concentrated Hydroxylapatite Fractions	5.40	11.9	178
VII	Pooled DEAE-Cellulose Fractions	4.77	1.0	370
VIII	(NH ₄) ₂ SO ₄ 0.3 Saturation Extract	4.29	11.8	840
IX	Concentrated Amberlite CG-50 Eluate	2.85	10.8	2237

homogenizer at 5°C. The mixture was blended at low speed (setting 30) for several minutes, and 3.4 kg of glass beads were added to the suspension. The suspension was then blended for 15 min at high speed (setting 70). An additional 1.36 liters of buffer A were added, and the mixture was blended for 5 min. After the mixture settled, the supernatant liquid was decanted, and the residue was extracted with 1.36 liters more of buffer A by blending for 5 min. The two supernatant fractions were combined and stirred for 5 min before centrifuging. The supernatant liquid obtained after centrifuging is Fraction I.

Fraction II. The protein concentration of Fraction I was determined, and 1 g of streptomycin sulfate as a 10% (w/v) solution in buffer A was added with stirring for each 2.4 g of protein. The solution was stirred for 20 min and centrifuged. The precipitate was suspended in 0.05 M potassium phosphate, pH 7.4 (one-tenth volume of extract), and stored at -20°C until several kilograms of cell paste had been processed to this point. This suspension was then used for isolation of DNA polymerase (13). The supernatant fluid from the streptomycin precipitation (Fraction II) was used for isolation of RNA polymerase.

When DNA polymerase was not to be purified from the same preparation, the procedure was altered somewhat; a DNase digestion of the DNA in Fraction I was done instead of precipitating DNA with streptomycin sulfate. DNase digestion produced a much better overall yield of enzyme than did streptomycin precipitation of DNA, which carried a considerable fraction of RNA polymerase with it (Table 1). Large amounts of DNase interfered with assay of the enzyme in the steps following (until after DEAE-cellulose chromatography), but the overall yield was increased by at least 50%. The

final specific activity was the same in both cases. For DNase digestion of Fraction I, CaCl_2 was added to 0.01 M, the pH was adjusted to 7.2, and DNase was added to 1 mg/liter of Fraction I. The mixture was stirred for 3 to 4 hr, 3 mg DNase/liter of Fraction I were added, and the mixture was stirred overnight.

Fraction III. Fraction II was concentrated by adding 95% ethanol (chilled to -20°C) to a concentration of 30%. The solution was stirred for 30 min, and the precipitate was collected by centrifugation. The supernatant liquid was discarded, and the precipitate was dissolved in 0.05 M ammonium citrate-0.05 M EDTA, pH 8.0, to a final volume of 600 ml (Fraction III).

Fraction IV. The dissolved ethanol precipitate was centrifuged in a Beckman Spinco Model L preparative ultracentrifuge for 4 hr at 30,000 rpm in the No. 30 rotor. The supernatant fluid (Fraction IV) was decanted from the pellet and fractionated with ammonium sulfate.

Fraction V. Solid $(\text{NH}_4)_2\text{SO}_4$ was added to Fraction IV to a concentration of 40% saturation. The solution was stirred for 20 min, and the precipitate was removed by centrifugation. The 40% saturated supernatant fraction was brought to 50% saturation with ammonium sulfate and centrifuged after being stirred for 20 min. The precipitate was dissolved in 0.05 M potassium phosphate, pH 7.5 (Fraction V). The protein concentration was determined, and the solution was diluted to 5 mg protein/ml of solution using the same buffer.

Fraction VI. The diluted solution was added to hydroxylapatite (950 ml settled volume equilibrated with 0.05 M potassium phosphate, pH 7.5) in a 4-liter beaker. The protein solution and hydroxylapatite were stirred for 30 min. The suspension was poured into a 69-cm² x 30-cm column and allowed to run until all of the diluted protein solution had passed through. The column was washed with 200 ml of 0.05 M phosphate buffer, and the enzyme was eluted with a linear gradient of 0 to 1 M ammonium sulfate, pH 7.5 (total volume 4 liters). All ammonium sulfate solutions used in this procedure were prepared in 0.05 M potassium phosphate as described under "Materials." Fractions which contained most of the RNA polymerase activity were combined, and the enzyme was precipitated by bringing the solution to 80% saturation with solid ammonium sulfate. After being stirred for 20 min the solution was centrifuged, and the precipitate was dissolved in 0.05 M K₂HPO₄ (Fraction VI) and diluted to 1 mg of protein/ml of solution with the same buffer.

Fraction VII. The diluted protein was adsorbed onto a DEAE-cellulose column using 30 mg of cellulose/mg of protein. The DEAE-cellulose (chloride form) had been equilibrated with 0.05 M K₂HPO₄. The enzyme was eluted with a linear gradient of 0 to 1 M ammonium sulfate, pH 8.4 (total volume 4 liters). The flow rate was adjusted to 1 ml/min, and the eluate was collected in 15-ml fractions. The fractions containing most of the enzyme activity were combined (Fraction VII), and polymerase was concentrated as before by bringing the solution to 80% saturation with solid ammonium sulfate.

Fraction VIII. The precipitate from Fraction VII was

collected by centrifugation and washed twice with 150 ml of 60% saturated ammonium sulfate, pH 7.8, by suspension and centrifugation. The washed precipitate was fractionated with ammonium sulfate by extraction with 50 ml of 40% saturated ammonium sulfate, pH 7.8. The solution was centrifuged, and the precipitate was extracted with 50 ml of 30% saturated ammonium sulfate, pH 7.8. The 30% saturation extract (Fraction VIII) was dialyzed overnight against 50 volumes of 0.05 M K_2HPO_4 using dialysis tubing which had been boiled in 0.05 M ammonium citrate-0.05 M EDTA, pH 8.0.

Fraction IX. Amberlite CG-50, prepared according to the procedure of Hirs et al. (14), was equilibrated with 0.05 M K_2HPO_4 and adjusted to pH 7.5 with 1 N KOH over a 2-hr period. A column of 250 mg resin/mg protein was prepared, and the dialyzed solution was passed through it. A gradient of 0 to 1 M ammonium sulfate, pH 8.4 (total volume 2 liters), was used to elute the RNA polymerase at 1 ml/min. Tubes which contained the peak of enzyme activity were combined and concentrated with 80% saturated ammonium sulfate. The precipitate was dissolved in 0.05 M potassium phosphate, pH 7.5 (no 2-mercaptoethanol added to this buffer), to a final protein concentration of 10 to 15 mg/ml (Fraction IX).

When scaling up this procedure, it is advisable to process at one time only as much solution as can be eluted from the CG-50 column in 6 to 8 hr. Although rather large amounts of enzyme activity are lost in this step, it has been found very useful in removing contaminating nuclease activity.

Other Considerations. Fraction IX enzyme has occasionally been passed through a Bio-Gel P-200 column to remove residual ammonium sulfate and any smaller proteins, including nucleases,

which might be contaminants. This is especially useful when the procedure is scaled up.

RNA polymerase is obtained after 120-fold purification with 16% overall yield and has an A_{280}/A_{260} of 1.70.

Fraction IX was routinely stored at -196°C at concentrations ranging from 1 to 15 mg/ml and retained nearly all of its activity for over a year. When thawed, aliquots of enzyme were usually diluted to a solution containing 50% glycerol. These solutions slowly lost activity over a period of several weeks but when incubated at 37°C retained activity for several hours. Dialysis produced no loss in activity if the dialysis tubing had been boiled in a solution containing EDTA.

Homogeneity of the Purified Enzyme

Contaminating Enzymatic Activities. Fraction IX polymerase was tested for the presence of DNA polymerase, polynucleotide phosphorylase, RNase, and DNase.

DNA polymerase was assayed by the method of Richardson et al. (15) with dCTP-2- ^{14}C as substrate and "activated" calf thymus DNA as primer. The reaction was stopped and processed prior to determination of radioactivity as described for RNA polymerase assays. In a series of experiments in which 15 to 150 μg of Fraction IX enzyme were used, no DNA polymerase activity was observed.

Polynucleotide phosphorylase activity was measured by the exchange reaction between a nucleoside diphosphate and $^{32}\text{P}_i$ as described by Ochoa et al. (16). A series of experiments using up to 150 μg of enzyme showed no exchange of $^{32}\text{P}_i$.

Chinese hamster ovary RNA with a specific activity of 1.3×10^5 counts/min-optical density unit at 260 m μ was used

for ribonuclease assays. Purified RNA polymerase was incubated with 0.2 optical density unit of ^3H -RNA under conditions of RNA synthesis except that no DNA was present. Labeled RNA was heat-denatured before use. After incubation at 37°C for 1 hr, the acid-insoluble material was precipitated and processed for counting as described for RNA polymerase assays. No hydrolysis of labeled RNA could be detected when it was incubated with 80 μg of Fraction IX polymerase. The stability of the enzymatic RNA product was also an indication of a low RNase activity in purified preparations. Thus, in experiments similar to curve 2 in Fig. 1, carried out for over 24 hr, no hydrolysis of poly A was observed.

DNase activity was measured in a manner similar to that used for RNase determinations. Polydeoxynucleotides in which all four nucleotides were labeled with ^{14}C were synthesized using terminal deoxynucleotidyl transferase from calf thymus (17,18). Incubations of this material with over 300 μg of purified RNA polymerase showed no evident hydrolysis. The sensitivities of this test and that for RNase are such that a small contamination of the enzyme with nucleases might not be detected. Indeed, when purified polymerase was incubated with Hemophilus influenzae DNA, some decrease in transforming activity of the DNA could be detected, indicating that some DNase was present in the enzyme preparation.

The fact that all of the labeled ATP added to a reaction mixture can be polymerized (Fig. 1) indicates a low level of ATPase activity.

Attempts at Further Purification. As a check on homogeneity and an attempt to further purify the polymerase, Fraction IX enzyme was rechromatographed on DEAE-cellulose using a KCl gradient as eluting agent. A single protein peak containing all of the RNA polymerase activity was obtained.

Fig. 1. Kinetics of RNA polymerase reactions using various templates.

The reaction mixtures contained, in 1.0 ml, 30 μ moles Tris-HCl, pH 7.9; 3.0 μ moles $MgCl_2$; 0.75 μ mole $MnCl_2$; 10 μ moles 2-mercaptoethanol; and the following templates: 0.03 μ mole d(pT)₆ (curve 1), 0.03 μ mole d(pT)₁₃ (curve 2), 0.45 μ mole (as phosphorus) heat-denatured calf thymus DNA (curve 3), and 0.45 μ mole native calf thymus DNA (curve 4). Reactions 1, 2, and 3 contained 0.7 μ mole ATP-8-¹⁴C (2.65×10^5 counts/min- μ mole) and no other nucleotides. Reaction 4 contained 0.6 μ -mole each ATP, GTP, UTP, and CTP-2-¹⁴C (2.15×10^5 counts/min- μ mole). Each reaction mixture contained 85 μ g of enzyme. The reactions were incubated at 37°C, and 0.1 ml was removed for each assay during the first 1-1/2 hr; then 0.05 ml was removed for the last three assays.

Since RNA polymerase as isolated by us and others (4, 19,20) is a very large enzyme, any contaminating proteins probably are smaller. If any such proteins were not bound to the polymerase, they should be retarded relative to RNA polymerase in molecular sieve chromatography. When Fraction IX polymerase was passed through a Bio-Gel P-200 column and the eluate was assayed for protein and polymerase activity, no contaminating proteins were found.

A further check on homogeneity of the enzyme was accomplished by density gradient centrifugation. As noted in the following section and by Richardson (20), in high ionic-strength buffers RNA polymerase dissociated into subunits which sedimented about half as fast as in low ionic-strength buffers. Fraction IX enzyme was sedimented in glycerol gradients of low- and high-ionic strengths. Fractions from these gradient runs were assayed for protein and for RNA polymerase activity. In both cases a single peak of protein and activity was obtained, although the enzyme sedimented about twice as fast in low ionic-strength buffers.

Catalytic and Physical Properties of the Enzyme

Catalytic Properties. Catalytic properties of RNA polymerase isolated by this procedure were similar to those reported previously for *E. coli* RNA polymerase (1-3). With the exception noted previously (6,7), incorporation of labeled nucleotides required the presence of a template. A divalent cation was required, and optimal concentrations were found to be similar to those previously reported (1-3).

Some catalytic properties of the enzyme are summarized in Fig. 1. In accord with previous observations (21), $d(pT)_6$ does not serve as a template for poly A formation at 37°C,

while d(pT)₁₃ is very effective. As shown by Chamberlin and Berg (22), heat-denatured calf thymus DNA also is an excellent template for poly A formation when ATP-8-¹⁴C is the only nucleotide substrate. With d(pT)₁₃ and denatured calf thymus DNA, all of the added ATP is polymerized in about 40 min under the conditions used. Curve 4 shows the time-course of the reaction when native calf thymus DNA was the template. The substrate was CTP-2-¹⁴C, and incorporation of label was corrected so that the result is expressed in terms of total nucleotide polymerized.

Ultracentrifugal Analysis. Fraction IX enzyme in 0.15 M potassium phosphate, pH 7.4, was centrifuged in a Beckman Model E analytical centrifuge (Fig. 2). Movement of the protein was followed by schlieren optics using a phase plate. When an aliquot of the same protein solution was dialyzed against 0.1 M KCl-0.005 M potassium phosphate, pH 7.5, and sedimentation was followed through use of ultraviolet optics, an S_{20,w} value of 24.5 was found. This is similar to the value of 24S reported by Fuchs et al. (4) and Colvill et al. (19) and is slightly higher than the value of 21S reported by Richardson (20). Some possible reasons for these discrepancies were discussed by Richardson.

Amino Acid Composition. The amino acid composition of the purified enzyme was determined (Table 2). Comparison of the values with those for DNA polymerase (15) shows that the amino acid compositions of the two enzymes are remarkably similar; however, DNA polymerase appears to be a much smaller protein (15). The amino acid composition is also quite similar to that of total protein of E. coli (23).

Krakov has presented evidence that sulfhydryl groups

Fig. 2. Sedimentation of RNA polymerase.

Fraction IX polymerase (7.6 mg/ml) in 0.15 M potassium phosphate buffer, pH 7.4, was sedimented at 35,600 rpm at 25°C. Photographs were taken soon after full speed was reached and 12 min later using a 70° phase-plate angle.

Table 2. Amino Acid Composition of RNA Polymerase

Amino Acid	Molar Content Relative to Histidine
Lysine	3.9
Histidine	1.0
Arginine	4.6
Threonine	3.7
Aspartic Acid	7.0
Serine	3.7
Glutamic Acid	9.2
Proline	2.9
Glycine	5.2
Alanine	5.2
Valine	4.8
Methionine	1.6
Isoleucine	3.6
Leucine	6.7
Tyrosine	1.7
Phenylalanine	1.7

A sample of Fraction IX polymerase was hydrolyzed in sealed tubes with 6.0 N HCl at 110°C for 22 hr. Amino acid analysis was carried out with a Beckman Model 120B automatic amino acid analyzer. The values for threonine and serine were corrected for losses during hydrolysis of 5 and 10%, respectively. Tryptophan, cysteine, and cystine were not determined and not included in relative amino acid content.

are involved in the catalytic reaction of Azotobacter vinelandii RNA polymerase (24). Experiments in our Laboratory also showed that RNA synthesis was inhibited by p-chloromercuribenzoate, indicating that sulfhydryl groups are involved in the active center and/or structure of the enzyme. When RNA polymerase was sedimented in glycerol gradients containing 10^{-4} M p-chloromercuribenzoate, there was no significant change in sedimentation properties compared to controls.

Molecular Weight. The molecular weight of the enzyme was determined using the approach to equilibrium method (25). The enzyme was in 0.1 M KCl-0.005 M potassium phosphate, pH 7.5. The partial specific volume was 0.732 cc/g as calculated from the amino acid composition (26), neglecting tryptophan, cysteine, and cystine, which were not determined. The molecular weight as calculated from two experiments using different enzyme preparations was 1.0×10^6 . Richardson found a value of $8.8 \pm 1.6 \times 10^5$ using sedimentation equilibrium techniques (20).

Effect of Increasing Ionic Strength on the Enzyme. When RNA polymerase was added to solutions containing KCl, the enzyme activity first increased and then decreased as KCl concentration was increased (Table 3). Thus, it was possible to assay the enzyme in fairly high salt concentrations. Other experiments were done in which the enzyme solution was made 0.5 M in KCl. Aliquots of this solution were removed at various times and assayed in reaction mixtures which were 0.5 M in KCl. At the times tested (up to 1-1/2 hr), enzyme activity was only slightly lower than in control reactions when salmon sperm DNA was the template but rapidly decreased

Table 3. Effect of KCl Concentration on RNA Polymerase Activity

KCl Concentration (<u>M</u>)	AMP-8- ¹⁴ C Polymerized (nmoles)
0.00	4.44
0.10	5.93
0.20	7.60
0.30	7.95
0.40	6.57
0.50	3.70
0.75	0.52
1.00	0.14
1.50	0.14

The reaction mixtures, 0.4 ml, contained 10 μ moles Tris-HCl, pH 7.9; 1.0 μ mole $MgCl_2$; 0.25 μ mole $MnCl_2$; 0.1 μ mole CTP, GTP, UTP, and ATP-8-¹⁴C (4.2×10^5 counts/min- μ mole); and 110 μ g salmon sperm DNA. KCl was added in amounts such that, when the enzyme (30 μ g) was added, the final KCl concentration would be as indicated. The reaction was incubated 30 min at 37°C and assayed as described in "Experimental Procedure."

to about 0.1% of the control activity when d(pT)₁₃ was the template.

Millette et al. (27) have also found that the activity of 24S RNA polymerase is increased by raising the ionic strength of the enzyme; however, others have found decreasing enzyme activity as ionic strength is raised (28,29). The reason for these differing results is not immediately apparent.

Richardson has recently shown that 21S RNA polymerase dissociates into subunits of one-half the original molecular weight when ionic strength is raised (20). Experiments in which RNA polymerase was subjected to density gradient centrifugation through glycerol gradients also indicated that when the ionic strength was raised the enzyme dissociated (Fig. 3). Thus, when sedimented in a glycerol gradient containing 0.5 M KCl, RNA polymerase sedimented about half as fast as in the absence of KCl. This was a reversible dissociation as shown by the fact that when the enzyme was placed in 0.5 M KCl and then diluted and layered onto a low ionic-strength gradient it sedimented as did the untreated enzyme.

It is not clear from these experiments which form of the enzyme was enzymatically active or whether both forms had activity. In the assay of fractions obtained from the glycerol gradient that contained KCl (Fig. 3), the final KCl concentration was 0.2 M. Thus, the enzyme may have re-associated under those conditions. However, in a similar experiment, aliquots of fractions from the glycerol gradient that contained 0.5 M KCl were assayed in reaction mixtures which were also 0.5 M in KCl. In these reactions, enzyme activity was about one-fifth that of aliquots assayed in the usual way. Thus, it is clear that the enzyme can be assayed

Fig. 3. Density gradient centrifugation of RNA polymerase.

Fraction IX RNA polymerase (94 μ g) in a volume of 0.1 ml was layered over 4.8 ml of linear gradients of 15 to 35% glycerol. One gradient and the polymerase solution layered over it were also 0.5 M in KCl. Common to both gradients were 0.01 M NaCl; 0.05 M Tris-HCl, pH 7.5; 1.0 mM EDTA; and 0.01 M 2-mercaptoethanol. The mixtures were centrifuged in a SW-39 rotor at 35,000 rpm for 12 hr at 4°C. After centrifugation the tubes were punctured, and the gradient liquid was displaced by pumping a solution of 50% sucrose into the tubes. Fractions were assayed for RNA polymerase activity as described in "Experimental Procedure" with ATP-8- ^{14} C (3.5×10^5 counts/min- μ mole) as the radioactive substrate. The solid line shows results obtained in the gradient without KCl, while the dashed line shows results obtained in the gradient containing 0.5 M KCl.

under conditions which favor formation of subunits. It does not prove, of course, that the subunits were enzymatically active, since DNA and/or some other component of the reaction mixture may have caused reassociation.

DISCUSSION

This procedure for purification of RNA polymerase allows processing several times as much E. coli at once as do previous procedures. We have found it highly reproducible, and the purified enzyme has catalytic properties similar to those of RNA polymerase obtained by other methods. Enzymatic and physical criteria indicate that the purified polymerase is essentially a homogeneous protein.

There is disagreement regarding the size of the RNA polymerase particle as isolated from E. coli. Chamberlin reported that the enzyme sedimented at an $S_{20,w}$ value of 9.6 in 0.01 M Tris, pH 8.0; 0.01 M $MgCl_2$; 0.01 M 2-mercaptoethanol; and 10^{-4} M EDTA (30). Maitra found that the enzyme sedimented at 11S to 12S in 0.1 M KCl; 0.05 M Tris, pH 7.5; 0.01 M 2-mercaptoethanol; and 0.01 M EDTA (31). Fuchs et al. (4) reported that the native enzyme had an $S_{20,w}$ value of 23.7, although enzymatic activity could be demonstrated in particles that sedimented both slower and faster than 24S. Colvill et al. (19) reported that the enzyme sedimented at about 24S, and Richardson (20) found that it sedimented at 21S in low ionic-strength buffers and at 13S in high ionic-strength buffers. We have found a sedimentation coefficient of 24.5S for purified polymerase in low ionic-strength buffers. Richardson has discussed some possible reasons for these discrepancies (20). Investigations of the size of the active RNA polymerase particle are continuing in this Laboratory.

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