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Rapid Micromethod for the Purification of *Escherichia coli* Ribonucleic Acid Polymerase and the Preparation of Bacterial Extracts Active in Ribonucleic Acid Synthesis

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A rapid micromethod is described for the preparation of nucleic acid-free extracts from *Escherichia coli* that involves precipitation with polyethylene glycol. Extracts can be prepared from growing cells in 75 min by three short, low-speed centrifugations. The extract did not inhibit added purified ribonucleic acid (RNA) polymerase, suggesting that major inhibitors of RNA synthesis had been removed. This extract should be ideal for assessing the properties of mutant RNA polymerases. The rapid chromatography of the extracts with step elution from deoxyribonucleic acid- and diethylaminoethyl-cellulose columns resulted in high yields of substantially pure RNA polymerase. We used this technique to purify ^{35}S-labeled RNA polymerase. This system should find application for the purification of small quantities of other bacterial RNA polymerases that share the general chromatographic properties of *E. coli* RNA polymerase.

Many procedures have been described for the purification of ribonucleic acid (RNA) polymerase (EC 2.7.7.6). Their purpose, in general, has been to produce large amounts of enzyme for physical and biochemical characterization (for example, see reference 6). Several methods have been reported for the purification of RNA polymerase from small quantities of cells (3, 9, 10, 13, 16). In general, these methods involve scaling down large-scale preparative procedures and do not provide speed or simplicity in handling small amounts of cells.

In the present communication, we describe a method for the rapid purification of RNA polymerase from small quantities of cells. This procedure is designed to serve two purposes. The first is to prepare partially purified extracts suitable for assaying polymerase activity, particularly from cells with mutations affecting RNA synthesis. The second is to prepare purified enzyme for a more-detailed characterization of mutant enzyme or radioactively labeled wild-type RNA polymerase. In our procedure lysis is accomplished by a modification of the detergent lysis procedure reported by Stonington and Pettijohn (18). Initial purification steps take advantage of the fact that RNA polymerase is bound to deoxyribonucleic acid (DNA) at low ionic strength. When DNA is precipitated by polyethylene glycol 6000 (PEG) in low salt, RNA polymerase is co-precipitated with the DNA, although most proteins remain in the supernatant. RNA polymerase is then eluted from the PEG precipitate by high salt under conditions where nucleic acid remains predominantly in the pellet. This generates a DNA-free crude extract in which RNA polymerase activity can be reproducibly assayed. Further purification is effected by rapid chromatography on diethylaminoethyl (DEAE)- and DNA-cellulose columns.

This method can be used to purify polymerase from a number of independent samples simultaneously. The procedure results in a 20 to 50% yield from milliliter quantities of cells with a final purity of 80 to 90% and takes several hours from start to finish. We anticipate that the method will find a wide application for the purification of radioactive RNA polymerase and for the purification and assay of altered (temperature sensitive and bacteriophage modified) RNA polymerase.

MATERIALS AND METHODS

Materials. Ribonucleoside triphosphates (adenosine triphosphate [ATP], guanosine triphosphate [GTP], cytidine triphosphate [CTP], and uridine triphosphate [UTP]) were purchased from P-L Biochemicals, and [^{3}H]UTP (26.9 Ci/mmol) and $H_2{}^{35}SO_4$ (NEX-042) were purchased from New England Nuclear. $H_2{}^{35}SO_4$ was used without neutralization. Calf thymus DNA and lysozyme (salt free) were purchased from Worthington; tris(hydroxylmethyl)aminomethane (Tris) base, ethylenediaminetetraacetic acid (EDTA), morpholinopro-

pane sulfonic acid (MOPS), glycerol (spectroanalyzed), and N-tris(hydroxylmethyl)methylglycine (Tricine were purchased from Sigma; dithiothreitol (DTT) was purchased from Calbiochem; Coomassie brilliant blue R-250, sodium deoxycholate, and PEG were purchased from Schwarz/Mann; tryptone and yeast extract were purchased from Difco; DEAE-cellulose (DE 52) and CF 11 cellulose were purchased from Whatman; Soluene-100 was purchased from Packard; bovine serum albumin (BSA) was purchased from Miles; and sodium dodecyl sulfate (SDS) was purchased from Bio-Rad. PEG was recrystallized as described by Albertsson (2) prior to use.

Buffers and solutions. Double-distilled water and reagent grade chemicals were used for all buffers and solutions. All buffers used in column chromatographic steps contained 0.05 M Tris-hydrochloride, pH 7.9, at 25°C, 0.5 mM EDTA, 25% (vol/vol) glycerol, and 1 mM DTT (TEGD buffer). DTT was added just prior to use from a frozen stock stolution (0.1 M).

The following solutions were used in preparation of the extract: (solution A) 0.01 M Tris-hydrochloride, pH 7.9, 25% (wt/vol) sucrose, and 0.1 M NaCl; (solution B) 0.3 M Tris-hydrochloride, pH 7.9, 0.1 M EDTA, and 4 mg of egg white lysozyme per ml added just before use; (solution C) 1.0 M NaCl, 0.02 M EDTA, pH 7.0, and 0.08% (wt/vol) deoxycholate; (solution D) 17% (wt/vol) PEG and 0.157 M NaCl, add DTT to 0.01 M just before use; (solution E) 5% (wt/vol) PEG, 2.0 M NaCl, and 0.01 M Tris-hydrochloride, pH 7.9, add 0.01 M DTT just prior to use.

All components except for lysozyme and DTT were sterilized prior to use and stored at 4°C.

Sample buffer for gel electrophoresis contained 3% (wt/vol) SDS, 5% (vol/vol) 2-mercaptoethanol, 10% (vol/vol) glycerol, and 0.063 M Tris-hydrochloride, pH 6.8.

Column materials. DEAE-cellulose was prepared according to Burgess (4). DNA-cellulose was prepared by the method of Alberts and Herrick (1) using calf thymus DNA and washed Whatman CF11 cellulose as described by Burgess and Jendrisak (6).

DNA. Deoxyadenylate-deoxythymidylate copolymer {poly[d(A-T)]} was a kind gift of Robert Wells. T4 DNA was extracted from CsCl-banded T4 phage by 0.5% SDS lysis at 65°C followed by precipitation of SDS by 0.5 M KCl at 0°C. The DNA was dialyzed against several changes of 1 mM EDTA-10 mM Tris (pH 7.9) buffer and refrigerated until use.

Assay of RNA polymerase activity. RNA polymerase activity was assayed in the following mixture (final volume of 0.1 ml): 25 mM Tris, pH 7.9, 10 mM $MgCl_2$, 1 mM EDTA, 0.2 mM CTP, 0.2 mM GTP, 0.2 mM ATP, 0.05 mM UTP, 1 μCi of [^{3}H]UTP, 1.1 μg of poly[d(A-T)], 1 mM K_2HPO_4 (pH 7.0), 1 mM DTT, and 50 μg of BSA. Final NaCl concentration was adjusted to 200 mM for each assay. Samples were incubated at 37°C for 10 min (unless otherwise indicated in the figure legends) and quenched with 3 ml of cold 5% trichloroacetic acid on ice. Each sample was filtered onto Whatman GF/C filters, washed, dried, and incubated overnight in 0.5 ml of Soluene-100, and the radioactivity was determined in a scintillation counter. Values are averages of duplicate assays.

Preparation of rifampin-resistant RNA polymerases. RNA polymerase was prepared by the method of Burgess (4). The rifampin-resistant strain was a spontaneous mutant, selected on broth plates containing 100 μg of rifampin per ml.

Media and growth of cells. *Escherichia coli* K-12 (E9000) was grown on a New Brunswick gyrotory shaker at 30°C with a doubling time of about 40 min in either standard broth (1% tryptone [Difco], 0.5% yeast extract, 0.5% NaCl and 2 ml per ml of trace metals = concentrated "O" as described by Neidhardt et al. [15] or the MOPS-Tricine-buffered medium supplemented with a complete mixture of amino acids as described by Neidhardt et al. (15) (herein called MOPS medium).

Preparation of radioactively labeled cells. We obtained labeling of growing cells with $^{35}SO_4$ in MOPS medium. Cells were grown overnight in the MOPS medium described above except that the final sulfate concentration was 9.2 μM and cysteine was omitted from the amino acid mixture. Prior to the start of labeling, cells were centrifuged and suspended in 100 ml of MOPS medium containing 1.8 μM sulfate, 0.2% glucose, and all amino acids except for cysteine. When the optical density at 450 nm (OD_{450}) became 0.1, labeling was commenced by the addition of 10 mCi of unneutralized $^{35}SO_4$. Under these conditions, inorganic sulfur was exhausted when OD_{450} = 0.4. However, the cells continued to grow using MOPS as a sulfur source. Cells were harvested when OD_{450} = 0.6. Between 30 and 70% of the $^{35}SO_4$ was incorporated into trichloroacetic acid precipitated counts. The specific activity obtained was about 5 μCi/μg of protein.

Quantitation of the recovery of RNA polymerase during purification. The amount of RNA polymerase at each step in the purification was monitored by determining the amount of [^{35}S]cysteine present in the β' subunit of core polymerase using extracts made from the radioactively labeled cells described above. Owing to contamination of β by chi protein (7, 10) in our gel system, only β' was measured. The amount of radioactivity present in the β' subunit is a convenient measure of total polymerase because one dimensional electrophoresis on 6% polyacrylamide −0.1% SDS gels is sufficient to resolve the β' subunit from all other proteins in the cell. Samples to be analyzed were dissolved in sample buffer, heated at 80°C for 2 min, and subjected to electrophoresis on 6% polyacrylamide gels, containing 0.1% SDS, as described by Laemmli and Favre (12) with the modifications described by Burgess and Jendrisak (6). A 1.25-μg sample of RNA polymerase was added as a marker for each gel. The cylindrical gels (5 mm in diameter and 12 cm long) were subjected to electrophoresis at 2 mA per tube for about 6 h until the marker dye was off the gel. They were stained in 0.05% Coomassie brilliant blue R-250 in ethanol-acetic acid-water (5:1:5) at room temperature for 1.5 h with diffusion destained in 7.5% acetic acid overnight. The band containing the β' subunit was cut out and dissolved in 0.2 ml of 30% peroxide by overnight incubation at 45°C in a tightly capped scintil-

lation vial (14). The dissolved gel slice was resuspended in 10 ml of scintisol (Isolabs) and counted in a liquid scintillation counter. Background radioactivity was determined for each gel by cutting a slice 1/3 of the way between the top of the gel and the β' band and counting as described above.

The distribution of total [^{35}S]cysteine at each stage was determined on parallel, 8.0% acrylamide gels. After staining and destaining, these gels were sliced longitudinally, and one of the centerpieces was dried on filter paper under a vacuum (8). The dried gel was autoradiographed for 24 h using Kodak No Screen X-ray film. Purified RNA polymerase was not added to this set of gels, since overloading a band causes spreading of radioactivity in that band.

Determination of β-galactosidase. The extent of cell lysis was determined by measuring the release of β-galactosidase from the cells. Cells were labeled with $^{35}SO_4$ as above, except that 1 mM isopropyl-β-D-thiogalactoside was added to the medium to induce β-galactosidase. The step (ii) extract described below was centrifuged to obtain a pellet fraction containing unbroken cells and cell debris and a supernatant fraction. The β-galactosidase present in these two fractions was determined by gel electrophoresis as described above for β', since it also falls in a region of the SDS gel devoid of significant amounts of other polypeptides.

RESULTS

Summary of purification. A summary is given below of our standard protocol for the preparation of purified RNA polymerase from 60 OD_{450} units of cells (100 ml of cells at OD_{450} = 0.6).

(i) Quickly cool cells in a dry ice-ethanol bath and harvest by centrifugation for 10 min at 10,000 rpm in an SS-34 rotor of a Sorvall centrifuge.

(ii) Lyse the cellular pellet by the sequential addition of 1.0 ml of solution A (see Materials and Methods) (0°C for 15 min), 0.25 ml of solution B (0°C for 5 min), and 1.25 ml of solution C (10°C for 10 min). This is referred to as the step (ii) extract.

(iii) Precipitate the RNA polymerase with PEG by adding 3.5 ml of solution D (0°C for 10 min). Thorough mixing is important at this step. Centrifuge at 7,000 rpm for 10 min and decant the supernatant.

(iv) Extract the polymerase from the PEG pellet by adding 0.5 ml of solution E to the precipitate, dispersing the pellet, and centrifuging at 10,000 rpm for 10 min. The supernatant is saved for assay and purification and is referred to as the step (iv) extract.

(v) Dilute 0.5 ml of step (iv) extract to 0.15 M NaCl with 6.15 ml of TEGD and pass through a 1.5-ml packed volume DNA-cellulose column previously equilibrated with TEGD + 0.15 M NaCl. Wash the column with 10 ml of TEGD + 0.15 M NaCl and elute with TEGD + 0.75 M NaCl. To obtain efficient elution in a small volume, it is necessary to add approximately 0.5-column volume of TEGD + 0.75 M NaCl, wait 15 min, and then add an additional 2.0 ml of TEGD + 0.75 M NaCl.

(vi) Dilute the 2.0-ml DNA cellulose eluate to 0.11 M NaCl by adding 12.0 ml of TEGD and apply to a 1.0-ml DEAE-cellulose column, previously equilibrated with TEGD + 0.11 M NaCl. Wash the column with 10 ml of TEGD + 0.11 M NaCl and elute with 1.5 ml of TEGD + 0.25 M NaCl.

A summary of the yield and purification of RNA polymerase at each step in the procedure is presented in Table 1, and an autoradiogram of dried SDS gel at various steps in the purification is presented in Fig. 1. It can be seen that the preparation appears to consist essentially only of RNA polymerase following the two-column steps.

Comments on the purification procedure. (i) Lysis of cells. Lysis was between 95 to 100% as determined by the partition of endogenous radioactive β-galactosidase between the pellet and the supernatant fraction as described above. In some strains, addition of a freeze-thaw step is necessary to obtain complete lysis.

(ii) PEG precipitation of RNA polymerase. The partition of RNA polymerase between PEG

TABLE 1. *Summary of purification*

Step	Yield of RNA polymerase (%)[a]	Purity of RNA polymerase
1. Lysed cells [step (ii) extract]	100	1.6[b]
2. Low-salt PEG precipitate (10% PEG-0.3 M NaCl)	83	4.5[b]
3. High-salt PEG supernatant [step (iv) extract]	60	9.6[b]
4. DNA-cellulose chromatography (0.75 M NaCl step)	31	42[b]
5. DEAE-cellulose chromatography (0.25 M NaCl step)	20	84[c]

[a] The amount of β' present in each fraction was determined as described in Materials and Methods. Results are expressed as the percentage of β' in lysed cell extract.

[b] These values are based on the counts in the β' bands and the stoichiometry determined in the step 5 sample. Results are expressed as percentage of total applied counts present in RNA polymerase.

[c] This value represents the fraction of the total counts present in the gel that are found in β', β, σ, and α bands. From the counts in each band and the cysteine content per polypeptide chain of β', β, σ, and α (see accompanying paper [5]), we calculate a subunit stoichiometry of $1\beta':1\beta:2\alpha:0.3\sigma$.

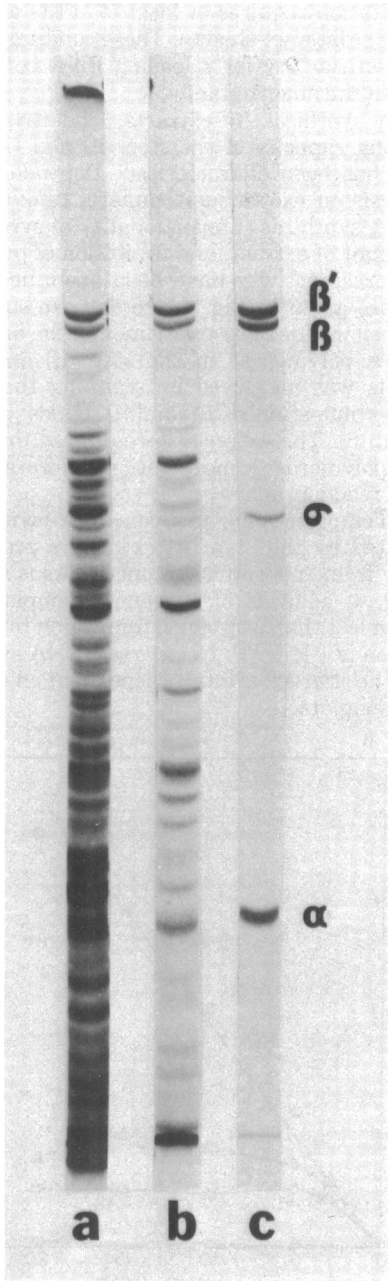

FIG. 1. *Autoradiograms of samples from various stages of purification. Samples a, b, and c, corresponding to steps 1, 3, and 5 listed in Table 1, were analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography as described in Materials and Methods. The following amounts of material were applied to the gel in 200 μl of sample buffer: (a) 4 μl of step 1 containing 48 × 10⁵ cpm of [³⁵S]cysteine out of a total of 4.8 ml; (b) 10 μl of step 3 containing 9.0 × 10⁵ cpm out of a total of 6.7 ml; (c) 10 μl of step 5 containing 2.1 × 10⁵ cpm out of a total of 1.0 ml. Migration is from top to bottom. The polymerase subunits are indicated at the right side.*

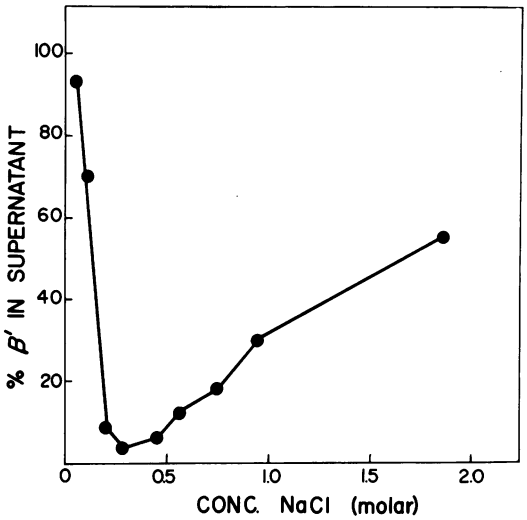

FIG. 2. *Precipitation of RNA polymerase by PEG as a function of NaCl concentration. A 40-μl portion of a ³⁵S-labeled step (ii) extract was distributed to each of a series of tubes containing 10% PEG and 0.5 to 2.0 M NaCl (final concentration) in a volume of 500 μl. Each sample was incubated for 10 min on ice and centrifuged at 7,000 rpm in a Sorvall centrifuge. A 40-μl sample was removed from each tube prior to centrifugation for determination of total β' present by gel analysis. A 40-μl sample was removed for each tube after centrifugation for determination of the amount of β' present in the supernatant fluid by gel analysis. Quantitation of β' on 6% gels was as described in Materials and Methods. Results are reported as percentage of total β' that is present in the supernatant fluid.*

precipitate and supernatant was determined for various concentrations of NaCl. The results of such an experiment are shown in Fig. 2, where it can be seen that precipitation of RNA polymerase was maximal (about 95%) at about 0.3 M NaCl. Although the PEG precipitation reported in Fig. 2 was performed at a protein concentration corresponding to 10 OD_{450} units of cells per ml, precipitation was equally effective over a range of 1.2 to 48 OD_{450} units per ml. Below 1.2 OD_{450} units per ml, precipitation efficiency decreased. Under these conditions, 1 OD unit is about 0.7 mg (wet weight) of cells, or about 85 μg of protein.

It may be noted that the precipitation curve for NaCl concentrations less than or equal to 0.3 M is similar to that observed for the precipitation of DNA (1), reinforcing our belief that RNA polymerase is precipitating as a conse-

quence of binding to endogenous DNA. Indeed, we have shown that RNA polymerase is not precipitated by 10% PEG between 0.1 and 2.0 M NaCl in the absence of DNA (data not shown).

(iii) Elution of RNA polymerase from the PEG precipitate. Figure 3 shows that RNA polymerase was eluted from the PEG precipitate as the salt concentration was raised. The extent of elution of RNA polymerase varied between 45 and 75%, depending on the particular experiment. RNA polymerase that remained in the pellet may represent the fraction actively transcribing DNA at the time of harvest. This point has not been pursued further.

The extract must be substantially free of nucleic acids to be useful in assays and further purification steps. We have therefore investigated which conditions of elution minimize nucleic acid contamination of the extract by following the partition of endogenous radioactively labeled DNA and RNA and OD_{260}/OD_{280} ratio. We have found that optimal nucleic acid precipitation requires at least 5% PEG and 2.0 M NaCl (data not shown). These conditions are quite satisfactory for releasing RNA polymerase into the supernatant.

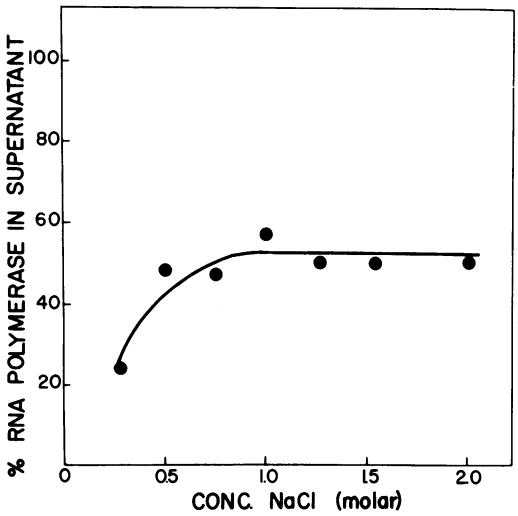

FIG. 3. *Elution of RNA polymerase from the PEG-precipitated pellet. A 40-μl portion of an ^{35}S-labeled step (ii) extract was distributed to a series of tubes containing a final concentration of 10% PEG and 0.3 M NaCl in 500 μl. Each sample was incubated for 10 min on ice and centrifuged at 7,000 rpm. The supernatant fluid was decanted, and the pellets were resuspended in 500 μl of a solution containing 5% PEG and 0.1 to 2.0 M NaCl. A 20-μl sample of the resuspended pellet was taken into 1.0 ml of sample buffer for determination of total β' present in the sample. Samples were centrifuged at 10,000 rpm, and 20-μl sample of each supernatant fluid was taken into 1.0 ml of sample buffer for determination of β' in the supernatant fluid. Results are expressed as the percentage of total β' present in the supernatant fluid.*

RNA synthesis by extracts. The RNA-synthesizing capacity of the step (ii) and (iv) extracts has been characterized. Dependence of synthesis on exogenous template, time course of RNA synthesis, proportionality of synthesis to amount of extract, and inhibition of purified polymerase activity have been examined. Results are given in Fig. 4 to 6 and are summarized below. For these experiments, the amount of RNA polymerase in the step (ii) and (iv) extracts was measured by scanning the Coomassie blue-stained β' and β bands as described (6). The extracts were diluted to equal RNA polymerase concentration before adding to the reaction mix.

(i) Template dependence. RNA synthesis exhibited by the step (ii) extract is predominantly from endogenous template. As is shown in Fig. 4, addition of exogenous template results in less than a twofold stimulation in incorporation of [^{3}H]UTP. In contrast, RNA synthesis in the step (iv) is totally dependent on exogenous template.

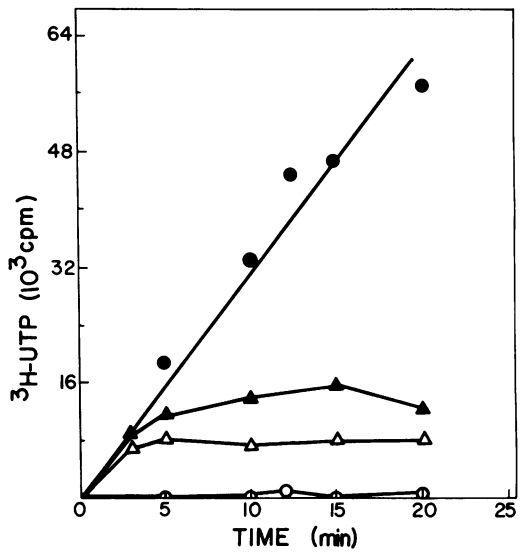

FIG. 4. *Time course of RNA synthesis of step (ii) and step (iv) extracts in the presence and absence of exogenous DNA template. A 10-μl portion of extract containing 0.2 μg of RNA polymerase was assayed in the presence and absence of poly[d(A-T) as described in Materials and Methods for the indicated times. Symbols: (△, ▲) step (ii) extract; (○, ●) step (iv) extract. Open symbols indicate the absence of added poly[d(A-T)], and closed symbols indicate the presence of poly[d(A-T)].*

(ii) Linearity of RNA synthesis. RNA synthesis carried out by the step (ii) extract is not proportional either to the amount of extract added to the reaction mixture or to the time of assay (Fig. 4 and 5). Both long times and higher amounts of extract give less synthesis than predicted. The step (iv) extract does carry out synthesis that is proportional both to time of synthesis and amount of extract added. This is the expected result if the step (iv) extract is free of major inhibitors of RNA synthesis.

(iii) Inhibition of exogeneous polymerase. The effect of step (ii) and step (iv) extracts on the amount of RNA synthesized by purified RNA polymerase was examined. In these experiments, rifampin-resistant purified enzyme was added to extracts made from rifampin-sensitive cells. RNA synthesis was carried out in the presence of rifampin and increasing concentrations of cellular extracts from steps (ii) and (iv). The results are presented in Fig. 6. It can be seen that increasing the concentration of step (ii) extract results in substantial inhibition of the RNA synthesis of the purified rifampin-resistant enzyme. These same concentrations of step (iv) extract do not inhibit the activity of exogenous rifampin-resistant RNA polymerase. This is a further indication that the step (iv) extract contains few, if any, inhibitors of RNA synthesis.

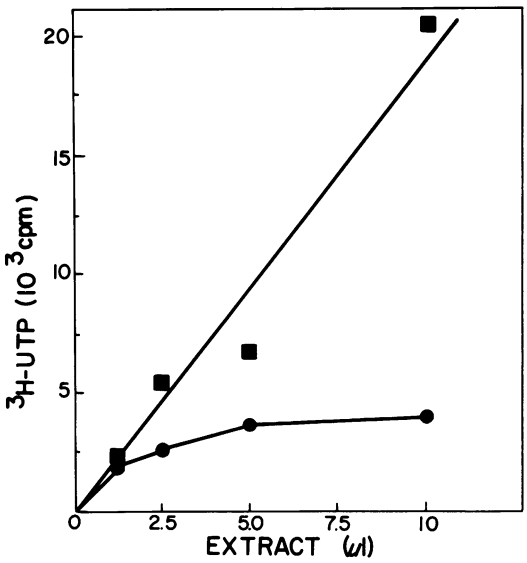

FIG. 5. *Synthesis of RNA by increasing concentrations of step (ii)) and (iv) extracts. Increasing volumes of extract containing 20 µg of RNA polymerase per ml were assayed as described in Materials and Methods. Symbols: ●, step (ii) extract; ■, step (iv) extract.*

DISCUSSION

The method for purification of RNA polymerase presented in this article has several advantages over previously reported micromethods for enzyme preparation. The initial purification steps are very rapid. After three low-speed centrifugation steps, RNA polymerase is about 10% pure and separated from most major inhibitors. Furthermore, initial steps are performed on concentrated lysates, in the presence of PEG, which has been reported to exert a protective effect on some macromolecular species (17). All of these factors operate to minimize losses of material and enzyme activity and contribute to the stability exhibited by RNA polymerase in this procedure.

The RNA polymerase is substantially purified by this technique, as can be seen in Fig. 1. In our final step, 84% of the total radioactivity is in RNA polymerase. The rest of the radioactivity is not in several major species of proteins, since the RNA polymerase appears to be the only major protein on the SDS gel autoradiogram. It is likely that the impurities are small amounts of many proteins. It can be calculated

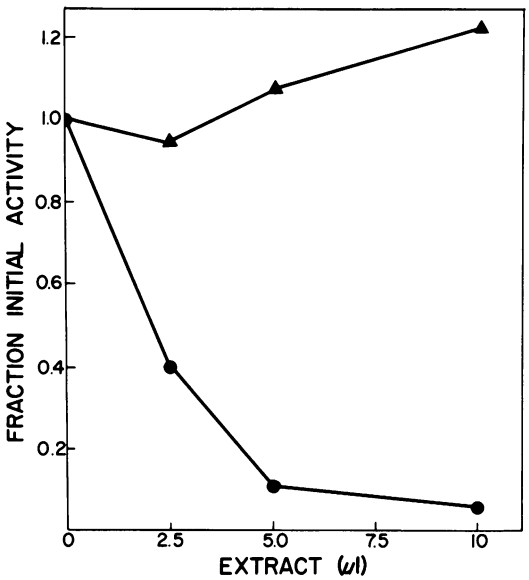

FIG. 6. *Effect of step (ii) and step (iv) extracts on RNA synthesis by rifampin-resistant RNA polymerase. Increasing volumes of extract containing 140 µg of endogenous RNA polymerase per ml were assayed as described in Materials and Methods in the presence of 0.5 µg of rifampin and in the presence and absence of 1.4 µg of rifampin-resistant RNA polymerase. Synthesis by the extract only was subtracted from that exhibited by extract in the presence of added rifampin-resistant RNA polymerase. Symbols: ●, step (ii) extract; ▲, step (iv) extract.*

from the initial and final total radioactivity that the presence of as little as 0.05% of each protein initially present in the extract would be sufficient to account for the observed impurities. The addition of a sizing step, gradient elution from the columns, or an increased number of washes at earlier stages would probably increase the purity of the enzyme in the final step. The sigma content we obtain is consistent with that reported to be present in the cell (7, 10).

The procedure as it has been developed yields enzyme of sufficient purity for many applications. We have already made use of this micromethod of purification to examine purified polymerase subunits. In addition to the experiments reported in the accompanying paper (5), we have used the method to aid in the identification of the α subunit of RNA polymerase on the λ-spectinomycin transducing phage (11) and to show that the σ subunit of *E. coli* and *S. typhimurium* run with identical mobilities on SDS gels (unpublished data).

We compared the RNA polymerase activity in our step (iv) extract with that of a crude cell lysate [step (ii) extract]. Although our step (iv) extract is dependent on exogenous template, only about 40% of the synthesis of the step (ii) extract exhibits this requirement. Furthermore, although the amount of RNA made by our step (iv) extract is proportional to the amount of extract added and the time of incubation, that made by the step (ii) extract does not exhibit linearity over the same range. The crucial step accounting for this difference appears to be the fractionation of proteins introduced by precipitation in low salt and subsequent elution in high salt. The initial precipitation step can be eliminated by adding solution E directly to the step (ii) extract, centrifuging, and using the supernatant for assay. The RNA synthesis exhibited by such an extract is dependent on exogenous template but behaves in other respects like the crude extract (unpublished data). Thus, the ability of our standard partially purified extract [step (iv)] to carry out RNA synthesis proportional to the amount of extract and the time of incubation is dependent on the initial low-salt-precipitation step in our procedure.

The RNA synthetic characteristics of our high-salt eluate [step (iv)] make it ideal as a first step in a screen for mutant polymerases. The extracts are stable for about 1 week at 4°C and many months at −70°C; thus, a series of assays can be carried out on any one extract. The absence of major inhibitors means that the reliable measurements of enzyme activity can be made at this stage in the purification procedure. Dependence on exogenous template means that mutant enzymes can be assayed on many different templates. We have used these high-salt extracts to characterize the temperature lability of hundreds of mutants of *E. coli* that are temperature sensitive for growth and have found it possible to identify extracts containing thermolabile RNA polymerase (manuscript in preparation).

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