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Purification and Partial Characterization of the DNA-Dependent RNA Polymerase from *Rickettsia prowazekii*

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The DNA-dependent RNA polymerase was purified from *Rickettsia prowazekii*, an obligate intracellular bacterial parasite. Because of limitation of available rickettsiae, the classical methods for isolation of the enzyme from other procaryotes were modified to purify RNA polymerase from small quantities of cells (25 mg of protein). The subunit composition of the rickettsial RNA polymerase was typical of a eubacterial RNA polymerase. *R. prowazekii* had β′ (148,000 daltons), β (142,000 daltons), σ (85,000 daltons), and α (34,500 daltons) subunits as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The appropriate subunits of the rickettsial RNA polymerase bound to polyclonal antisera against *Escherichia coli* core polymerase and *E. coli* σ^{70} subunit in Western blots (immunoblots). The enzyme activity was dependent on all four ribonucleoside triphosphates, Mg^{2+}, and a DNA template. Optimal activity occurred in the presence of 10 mM $MgCl_2$ and 50 mM NaCl. Interestingly, in striking contrast to *E. coli*, approximately 74% of the rickettsial RNA polymerase activity was associated with the rickettsial cell membrane at a low salt concentration (50 mM NaCl) and dissociated from the membrane at a high salt concentration (600 mM NaCl).

Rickettsia prowazekii, the causative agent of epidemic typhus, morphologically resembles a typical gram-negative bacterium. However, like all members of the genus *Rickettsia*, it is an obligate intracellular parasite. *R. prowazekii* has a generation time of about 8 h (21). This appears to be a slow growth rate for a procaryotic organism in cytoplasm—what most of us would consider a very rich medium. Probably this slow growth rate has evolved to maximize the yield of the parasite from the host cell by minimizing damage to the host cell. Mechanistically, it is not known how rickettsiae grow so slowly and, more specifically, how their RNA polymerase has adapted in their unique environment. To begin to answer these questions, the enzymatic components of macromolecular synthesis must be understood.

A typical procaryotic RNA polymerase consists of four polypeptide chains, β′, β, α, and σ. It can take two forms, a holoenzyme ($\beta'\beta\alpha_2\sigma$) or a core polymerase that lacks a σ subunit. The capacity to synthesize RNA resides in the core polymerase. The role of the σ subunit is to direct initiation to the promoter (4, 8).

The primary purpose of the present study was to purify the RNA polymerase from *R. prowazekii* and to investigate the general properties of the enzyme. No information is available in the literature at this time. This work is the beginning of a broader effort to completely characterize the rickettsial RNA polymerase, the mechanisms that control the process of transcription, and the rate-limiting step in the slow growth of the rickettsiae. This information is essential for understanding the signals that rickettsiae sense in their intracytoplasmic environment and how they appropriately adjust gene expression to cope with this unusual environment.

MATERIALS AND METHODS

Rickettsial preparation and growth. *R. prowazekii* Madrid E was cultivated in the yolk sacs of antibiotic-free, embryonated hen eggs and purified as described elsewhere (22). Further purification included a variation of Renografin density gradient centrifugation (10, 13) to remove contaminating yolk sac mitochondria and other host cell components. The rickettsiae were layered onto 25% Renografin (E. R. Squibb, & Sons, Princeton, N.J.) with SPG (218 mM sucrose, 3.76 mM KH_2PO_4, 7.1 mM K_2HPO_4, 5 mM potassium glutamate, pH 7.0) as the diluent. Centrifugation was done at 15,000 rpm for 30 min at 4°C in an SA-600 rotor (Dupont Instruments, Newtown, Conn.). Rickettsiae sedimented in this manner are referred to herein as Renografin-purified rickettsiae.

Purification of RNA polymerase. The method for purification of the *R. prowazekii* RNA polymerase was based on the procedures developed by Burgess and Jendrisak (5) combined with heparin-agarose chromatography (7, 18). Renografin-purified rickettsial cells (typically 25 mg of protein) were suspended in 1.5 ml of buffer A (10 mM Tris hydrochloride [pH 8.0], 10 mM $MgCl_2$, 1 mM EDTA, 0.3 mM dithiothreitol, 7.5% [vol/vol] glycerol, 50 mM NaCl, 0.5 mM phenylmethylsulfonyl fluoride). The cell suspension was lysed by three passes through a French pressure cell at 20,000 lb/in^2, and the cell lysate was clarified at 10,000 rpm in a Beckman JA-20 rotor for 10 min. The clarified cell lysate was applied to a heparin-agarose column (2 volumes of matrix per volume of lysate) previously equilibrated with buffer A. After adsorption of the sample for 60 min at 4°C, the column was washed with 15 ml of buffer A, followed by elution with 10 ml of buffer B (consisting of buffer A containing 600 mM NaCl instead of 50 mM NaCl). A flow rate of approximately 15 ml/h was maintained. Fractions of 1 ml were collected and assayed for RNA polymerase activity. The fractions with RNA polymerase activity were pooled (HA fraction), the proteins in the HA fraction were precipitated by adding ammonium sulfate to 60% saturation, and this precipitate was suspended in 0.5 ml of buffer C (10 mM Tris hydrochloride [pH 7.9], 0.1 mM EDTA, 0.1 mM dithiothreitol, 5% [vol/vol] glycerol, 500 mM NaCl). The sample was applied to a column of Bio-Gel A-5m (1 by 38 cm) equilibrated with buffer C and eluted at 25 ml/h. The frac-

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tions with the enzyme activity were pooled, precipitated by adding ammonium sulfate to 60% saturation, suspended in 1 ml of buffer D (same as buffer C except with 150 mM NaCl instead of 500 mM NaCl), and dialyzed against two changes of buffer D overnight at 4°C. The sample was applied to a 1.5-ml double-stranded DNA-cellulose column equilibrated with buffer D. After adsorption of the sample for 60 min at 4°C, the column was washed with 15 ml of buffer D, and the RNA polymerase was eluted with the same buffer containing 750 mM NaCl instead of 150 mM NaCl. Fractions (0.5 ml) were collected and assayed for RNA polymerase activity. The fractions containing the peak of enzyme activity were pooled and used immediately or dialyzed overnight against storage buffer (10 mM Tris hydrochloride [pH 7.9], 0.1 mM EDTA, 0.1 mM dithiothreitol, 50% [vol/vol] glycerol, 100 mM NaCl) and frozen at −80°C.

Escherichia coli RNA polymerase was prepared from strain JM101 by the same protocol described for the rickettsial RNA polymerase or was purchased from Sigma Chemical Co. (St. Louis, Mo.) as a σ^{70}-enriched product.

RNA polymerase assay. RNA polymerase activity was assayed in the following mixture (final volume of 50 μl): 40 mM Tris hydrochloride (pH 7.9), 10 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM K_2HPO_4 (pH 7.0), 50 mM NaCl, 100 μg of bovine serum albumin per ml, 0.8 mM CTP, GTP, and UTP, 0.2 mM ATP, 3 μCi of [α-^{32}P]ATP, and either 2.5 μg of poly[d(A-T) · d(A-T)] or plasmids (pMW264 and pMW150) containing the rickettsial citrate synthase gene (24, 25). Samples were incubated at 37°C for 15 min and quenched with 3 ml of cold 5% trichloroacetic acid on ice. After 30 min, each sample was filtered onto a Whatman GF/C filter, washed, and dried, and the radioactivity was determined in a liquid scintillation counter (Pharmacia LKB Biotechnology, Inc., Piscataway, N.J.). Values were the average of duplicate assays.

Protein determination. Protein concentration was estimated with the bicinchoninic acid protein assay reagent (Pierce Chemical Co., Rockford, Ill.) with bovine serum albumin as the standard.

SDS-PAGE. Proteins were analyzed by electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate (SDS-PAGE) (Phast Gel, gradient of 10 to 15% polyacrylamide or homogeneous 7.5% polyacrylamide), followed by silver staining according to the procedures provided by the manufacturer (Pharmacia).

Western blot (immunoblot) of RNA polymerase preparations. *R. prowazekii* RNA polymerase was subjected to SDS-PAGE (see above), and the proteins were electroblotted (1 h at 140 mA) to a sheet of Immobilon PVDF transfer membrane (Millipore Corp., Bedford, Mass.). The membrane blot was soaked in a solution containing 5% powdered nonfat milk (wt/vol) and TBS (20 mM Tris hydrochloride [pH 7.4], 15 mM NaCl) for 1 h at 37°C with gentle agitation. The blot was rinsed four times with washing solution (1 mg of bovine serum albumin per ml in TBS) and then was incubated for 2 h at room temperature with anti-*E. coli* σ^{70} or anti-*E. coli* core polymerase serum that had been diluted 1:1,000 in antibody incubation solution (10 mg of bovine serum albumin per ml, 0.05% Tween 20 [vol/vol] in TBS). The blot was washed again four times with washing solution and then incubated for 2 h at room temperature with alkaline phosphatase-conjugated anti-rabbit immunoglobulin G that had been diluted 1:500 in antibody incubation solution. The blot was washed four times before the colored reaction product was developed by the 5-bromo-4-chloro-3-indolylphosphate/nitroblue tetrazolium substrate system (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Md.).

Sedimentation of RNA polymerase. A clarified lysate of rickettsiae, prepared as above, was sedimented by ultracentrifugation at 85,000 rpm for 40 min at 4°C in a TLA-100.3 rotor (Beckman Instruments, Fullerton, Calif.). Both the supernatant (S85) and the suspended pellet (P85) were subjected to heparin-agarose chromatography. RNA polymerase in the resulting HA fractions was assayed by the standard method.

To determine whether the RNA polymerase in the pellet was associated with either chromosomal DNA or polysomes, we used DNase digestion or low-magnesium buffer A. In the DNase digestion experiments, DNase I was added to the rickettsial lysate at a final concentration of 20 μg/ml. The lysate was incubated at room temperature for 15 min and then for 45 min on ice. The subsequent steps, ultracentrifugation and heparin-agarose chromatography, were performed as described above. In the low-magnesium experiments, the lysate was incubated in buffer A with 0.1 mM $MgCl_2$ instead of 10 mM $MgCl_2$.

Separation of membranes from ribosomes. A sample (0.2 ml) of the clarified lysate was layered onto 2 ml of a 5 to 20% (wt/vol) linear sucrose gradient in buffer A without glycerol. After centrifugation at 55,000 rpm for 1 h at 5°C in a Beckman TLA-55 rotor, fractions were collected and their A_{260} was measured. The pellet was suspended in buffer A. The fractions composing the more dense peak (P1) were pooled, diluted with buffer A, and centrifuged at 85,000 rpm for 40 min in a Beckman TLA-100.3 rotor, and the resulting pellet was resuspended in buffer A. The fractions in peak P2 were close to the supernatant fraction at the top of gradient; therefore, they were pooled, diluted with buffer A, and centrifuged at 85,000 rpm for 40 min. This pellet, suspended in buffer A, and the supernatant were referred to as P2P and P2S, respectively. The four preparations (pellet, P1, P2P, and P2S) were assayed for RNA polymerase activity and analyzed by SDS-PAGE.

Chemicals. [α-^{32}P]ATP (25 Ci/mmol) was obtained from ICN Biomedicals, Inc. (Irvine, Calif.). Bio-Gel A-5m resin was purchased from Bio-Rad Laboratories (Richmond, Calif.). Antisera to *E. coli* σ^{70} subunit and core polymerase were kindly provided by C. Gross (University of Wisconsin, Madison) and M. Chamberlin (University of California, Berkeley), respectively. Other biochemicals were purchased from Sigma Chemical Co.

RESULTS

Purification of *R. prowazekii* RNA polymerase. The DNA-dependent RNA polymerase was purified from *R. prowazekii* (Table 1; Fig. 1) by the procedures developed by combining chromatography on heparin-agarose, Bio-Gel A-5m, and DNA-cellulose columns (1, 5, 7, 18). Because of the limited quantities of available rickettsial cells, the chromatography was markedly scaled down from the original procedures.

The heparin-agarose chromatography was not only a purification step but also was very effective in removing RNA polymerase inhibitors present in the cell lysate, as indicated by the fact that up to 270% of RNA polymerase activity was recovered compared with the cell lysate (Table 1). It was also noted that Bio-Gel A-5m filtration removed the RNA polymerase inhibitor(s) which remained in the fractions after heparin-agarose chromatography, since the protein fractions that eluted right before and/or after the peak RNA polymerase fractions, when concentrated, were able to completely

TABLE 1. Purification of RNA polymerase from *R. prowazekii*

Stage of purification	Vol (ml)	Total protein (mg)	Total activity (U)[a]	Sp act (U/mg)	Purification (fold)
Cell lysate	2.5	25.0	37	1.5	1
HA fraction	9.0	5.6	100	18.0	12
Bio-Gel A-5m peak	18.0	2.2	88	40.0	27
DNA-cellulose peak	13.5	0.2	53	252.0	168

[a] One unit represents the incorporation of 1 nmol of AMP into the acid-insoluble material in 15 min at 37°C with poly[d(A-T) · d(A-T)] as the template.

inhibit RNA polymerase activity (data not shown). However, neither DNase nor RNase activity could be demonstrated in these fractions.

Affinity chromatography on a DNA-cellulose column yielded a nearly homogeneous enzyme preparation (Fig. 1). In our experiments, unlike those described by Burgess et al. (5, 11) to purify RNA polymerase from *E. coli*, a good recovery of the enzyme was not obtained unless the sample was absorbed for 60 min at 4°C on the DNA-cellulose column.

Subunit composition of rickettsial RNA polymerase. To determine the polypeptide composition of the rickettsial RNA polymerase, fractions obtained from different stages of the purification were electrophoresed on 10 to 15% gradient and on 7.5% homogenous SDS-polyacrylamide gels and the gels were stained with silver. The gel pattern of the DNA-cellulose peak (Fig. 1) demonstrates that the rickettsial RNA polymerase had a subunit structure typical of the eubacteria, with four major subunits (β′, β, σ, α) (4). As judged by Western blots, the β′, β, and α subunits cross-reacted with antisera to *E. coli* core polymerase, and the σ subunit cross-reacted with antisera to *E. coli* σ^{70} subunit (Fig. 2).

The density of the σ subunit band was much less than that of other subunit bands (Fig. 1), which suggested that the enzyme preparation had less than stoichiometric amounts of σ. Similar results have been obtained by most methods for purification of RNA polymerase from *E. coli* (4). The approximate molecular weights of the subunits were established from SDS-PAGE by comparison with protein standards including *E. coli* RNA polymerase subunits (4, 9). The values for the four major polypeptides in the rickettsial RNA polymerase preparation were 148,000 (β′), 142,000 (β), 85,000 (σ), and 34,500 (α). It was difficult to separate the β′ and β subunits by 10 to 15% SDS-PAGE. However, they did have slightly different mobilities when they were run on low-concentration (7.5% homogeneous) polyacrylamide gels (Fig. 1).

From the SDS-PAGE, the apparent molecular weight of the rickettsial σ subunit was 85,000. Although the rickettsial σ subunit was smaller than the *E. coli* σ^{70} subunit (87,000 molecular weight), it was similar in size to those from a wide range of other organisms (4). It has been noted that the σ^{70} subunit of *E. coli* migrates on SDS-PAGE much slower than expected from its molecular mass calculated from sequence data (70,263 daltons) (6, 9).

Properties of rickettsial RNA polymerase. Incorporation of AMP into RNA by *R. prowazekii* RNA polymerase is shown

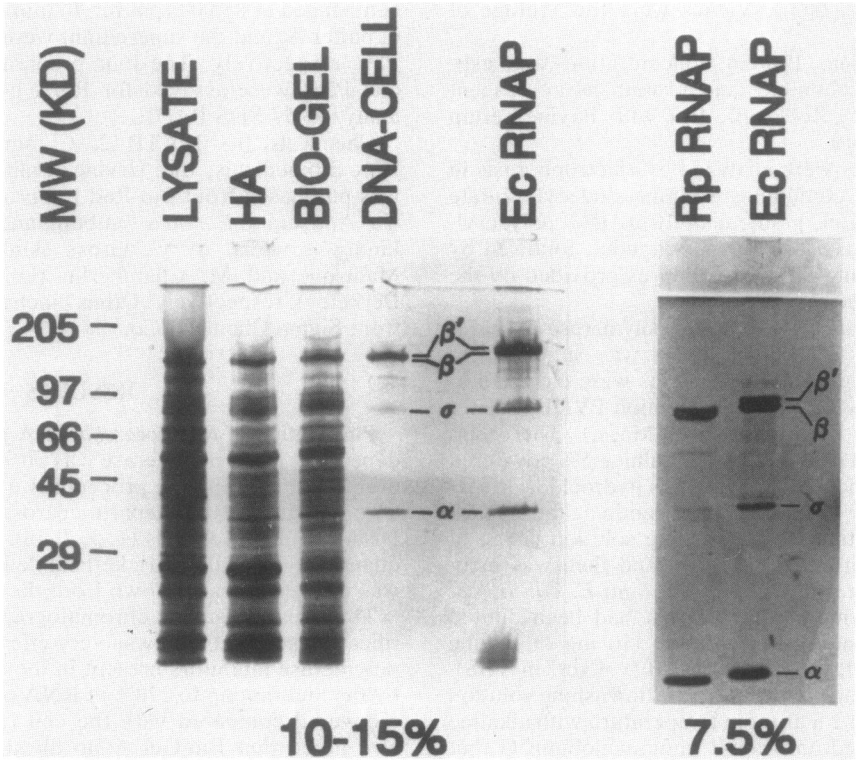

FIG. 1. Gel profiles of the rickettsial RNA polymerase fractions. Samples were separated on SDS-polyacrylamide (10 to 15% and 7.5%) gels and stained with silver. Lanes: HA, fractions from heparin-agarose chromatography; BIO-GEL, fractions from Bio-Gel A-5m chromatography; DNA-CELL, fractions from DNA-cellulose chromatography. *E. coli* RNA polymerase (Ec RNAP) with enriched σ^{70} subunit was purchased from Sigma. Rp RNAP, *R. prowazekii* RNA polymerase. Numbers on left show molecular mass (MW) in kilodaltons (KD).

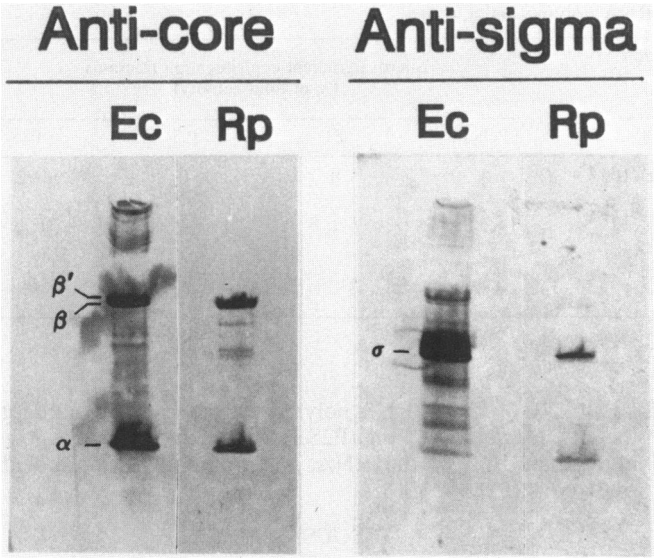

FIG. 2. Western blot analysis of the rickettsial and *E. coli* RNA polymerase with antisera to *E. coli* core polymerase and to σ^{70} subunit. Lanes: Ec, *E. coli* RNA polymerase with enriched σ^{70} subunit purchased from Sigma; Rp, DNA-cellulose-purified RNA polymerase of *R. prowazekii*.

in Fig. 3. The enzyme activity was totally dependent on exogenous DNA template (Fig. 3A). Both poly[d(A-T) · d(A-T)] and plasmids containing the rickettsial citrate synthase gene served as templates, although the synthetic double-stranded polynucleotide poly[d(A-T) · d(A-T)] was transcribed about 7 (7±4) times more efficiently than the plasmid DNA. The optimal concentration of $MgCl_2$ was approximately 10 mM (Fig. 3B). No rickettsial RNA polymerase activity was observed in the absence of Mg^{2+}, and the rate of RNA synthesis decreased at higher Mg^{2+} concentrations (Fig. 3B). The effect of monovalent ionic strength on the enzyme activity was significantly different between the poly[d(A-T) · d(A-T)] and plasmid assay systems (Fig. 3C). In the poly[d(A-T) · d(A-T)] system, a high transcription rate was observed with 5 mM NaCl and 25 mM NaCl was required to obtain maximal enzyme activity. In the plasmid system, however, the transcription rate was very low with 5 mM NaCl and 50 mM NaCl was the optimal concentration. In both systems, the RNA polymerase activity was significantly inhibited at higher NaCl concentrations.

The activity of the rickettsial RNA polymerase was completely inhibited by rifampin at a concentration of 25 μg/ml, and this finding was consistent with the previous in vivo observation (23). Sham rickettsial preparations from uninfected yolk sacs prepared by the same method were unable to synthesize RNA in the same assay (data not shown).

Sedimentation of *R. prowazekii* RNA polymerase. The majority of the RNA polymerase (an average of 74% of total enzyme activity) from *R. prowazekii* was associated with the pellet (P85) after ultracentrifugation of the French press cell lysate in the presence of 50 mM NaCl (Table 2). In contrast, only about 19% of the *E. coli* RNA polymerase activity was found in the P85 fraction in low-salt buffer. At a higher salt concentration (600 mM NaCl), however, 91% of the rickettsial enzyme activity was found in the supernatant (S85) under the same conditions (85,000 rpm, 40 min, Beckman TLA-100.3 rotor). To be sure that this activity was truly membrane associated and was not simply lost or inactivated in the low-salt soluble fraction, the rickettsial preparation was divided equally and one half was processed to membrane and soluble fractions in low salt and the other half was processed in high salt (Fig. 4). The total recovered activity calculated by summing the measured activities in the soluble and membrane fractions was very similar in both low- and high-salt protocols. Furthermore, in Fig. 4 it can be seen that the antigenic masses detected in the ββ' bands of the various fractions on Western blots corresponded to the measured

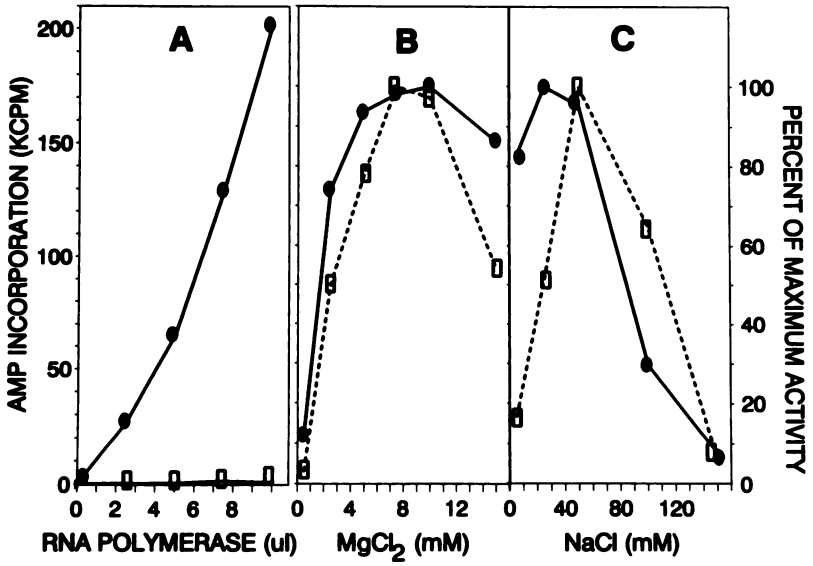

FIG. 3. Characterization of RNA polymerase activity. (A) Template dependence of RNA synthesis of the rickettsial RNA polymerase. Standard assay conditions were used in the presence (●) and absence (□) of poly[d(A-T) · d(A-T)]. (B) Divalent cation requirement for the rickettsial RNA polymerase activity. Standard assay conditions were used with various $MgCl_2$ concentrations on both poly[d(A-T) · d(A-T)] (●) and pMW150 (□). (C) Effect of NaCl concentration on the rickettsial RNA polymerase activity with poly[d(A-T) · d(A-T)] (●) or pMW150 (□) as a template.

TABLE 2. Distribution of RNA polymerase activity

Organism	Treatment	Differential centrifugation fractions (% of total activity)[a]		Sucrose gradient centrifugation fractions (% of total activity)			
		P85	S85	Pellet	P1	P2P	P2S
E. coli	50 mM NaCl	19 ± 16	81 ± 16	0	8	0	92
R. prowazekii	50 mM NaCl	74 ± 12	26 ± 12	15 ± 7	7 ± 5	47 ± 7	31 ± 4
R. prowazekii	600 mM NaCl	9 ± 4	91 ± 4				
R. prowazekii	DNase digestion	66 ± 3	34 ± 3				
R. prowazekii	0.1 mM MgCl	60 ± 1	40 ± 1				

[a] Each value represents the mean ± standard deviation for at least three experiments.

enzyme activities in these fractions and supported the membrane association of the rickettsial RNA polymerase.

The sedimentation of rickettsial RNA polymerase by ultracentrifugation might be due to its binding to (i) chromosomal DNA, (ii) polysomes, (iii) membranes, or (iv) ribosomes. However, neither treatment of the French press cell lysate with DNase nor disruption of polysomes by a low magnesium concentration (0.1 mM) changed the distribution pattern of the enzyme activity (Table 2). These findings indicated that the association of the rickettsial RNA polymerase with the P85 fraction was independent of chromosomal DNA and polysomes.

To identify with which component of the P85 fraction (now either membranes or ribosomes) the RNA polymerase was associated, we separated the membranes and ribosomes by centrifugation of the French press cell lysate through a 5 to 20% sucrose gradient. The outer membranes and some inner membranes were found in the pellet, the ribosomes were in the fractions close to the bottom (P1), and inner membranes and cytoplasmic proteins were in the fractions close to the top of gradient (P2). The inner membranes and cytoplasmic proteins were further separated by ultracentrifugation (P2P and P2S). The gel profiles of rickettsial outer (pellet) and inner (P2P) membranes were consistent with the data of Smith and Winkler (17), and the rickettsial ribosome fraction (P1) showed a gel profile similar to that of *E. coli* 70S ribosomes (Fig. 5). As judged by the gel profiles, there was little cross contamination between the membrane and ribosome fractions. Also, the P1 fraction contained at least 95% of the labeled rRNA when the *E. coli* lysate was applied to the same gradient system (data not shown).

Enzyme assays of the fractions showed that up to 62% of the total rickettsial RNA polymerase activity was in the membrane fractions (pellet plus P2P, Table 2). In contrast, about 92% of *E. coli* RNA polymerase activity was found in the cytoplasmic fraction (P2S). With neither species was more than 10% of the RNA polymerase associated with ribosomes (P1).

DISCUSSION

We purified the DNA-dependent RNA polymerase from *R. prowazekii*, an obligate intracellular parasitic bacterium. Modifications of the published methods (1, 5, 7, 18) were adopted to successfully isolate and purify the RNA polymerase from small quantities of rickettsial cells. The fact that the enzyme was of rickettsial origin and was not due to contaminating host cell or mitochondrial RNA polymerase was indicated by the facts that (i) the enzyme was very sensitive to rifampin, a potent inhibitor or procaryotic RNA polymerase (12), (ii) its component polypeptides were different from those of eucaryotic or mitochondrial RNA polymerases (3, 16, 19), (iii) proteins purified from sham preparations of rickettsiae from uninfected yolk sacs showed no activity, and (iv) the activity was not removed by further purifications of the rickettsiae by Renografin gradients (data not shown).

Several lines of evidence indicated that the rickettsial RNA polymerase had a typical procaryotic holoenzyme composition (β′, β, σ, and α). SDS-PAGE profiles demonstrated that the subunit composition of the purified rickettsial RNA polymerase was very similar to that of the *E. coli* enzyme and that the molecular weights of the rickettsial RNA polymerase subunits (148,000 [β′], 142,000 [β], 85,000 [σ], and 34,500 [α]) were very close to those of the *E. coli* counterparts. The four subunits of the rickettsial RNA polymerase cross-reacted with antiserum to *E. coli* polymerase, and the transcription activity of the rickettsial enzyme could be partially inhibited by antiserum to *E. coli* core polymerase (data not shown).

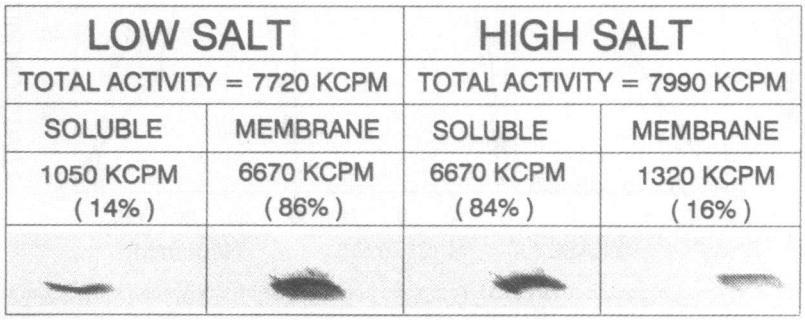

FIG. 4. Recovery of rickettsial RNA polymerase. Soluble and membrane fractions were prepared in low and high salt from equal portions of a rickettsial preparation. These fractions were passed over a heparin-agarose column, and the eluates were assayed with poly(dA-T) as a template. The ββ′ bands of Western blots of this crude material are shown.

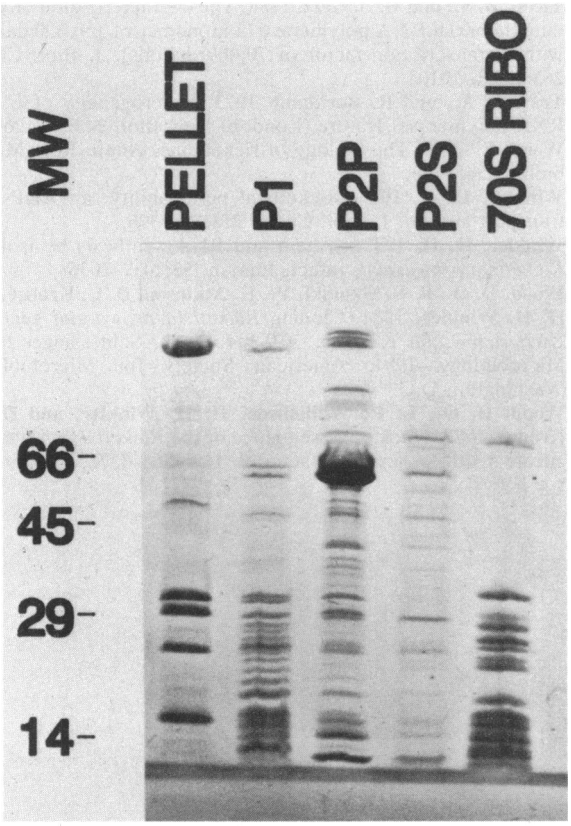

FIG. 5. Gel profile of sucrose gradient fractions of the rickettsial lysate. Samples were separated by SDS-PAGE and stained with silver. Abbreviations are defined in the text. 70S RIBO, *E. coli* 70S ribosomes. Numbers on left show molecular weight (10^3).

The activity of the rickettsial enzyme preparation was totally DNA dependent. The enzyme could utilize both plasmids containing a rickettsial gene and poly[d(A-T) · d(A-T)], although poly[d(A-T) · d(A-T)] served as the most active template. Probably, poly[d(A-T) · d(A-T)] could be transcribed by the core polymerase and was only slightly affected by the σ subunit (2), but the plasmids could only be transcribed by the holoenzyme.

The ability of the rickettsial RNA polymerase to be sedimented by ultracentrifugation in low-salt buffer is strikingly dissimilar to the *E. coli* RNA polymerase. Under the same experimental conditions, up to 74% of total rickettsial enzyme activity was in the pellet fraction (P85) after ultracentrifugation of the French press cell lysate, while only about 19% of the total *E. coli* enzyme activity was associated with the pellet fraction. Furthermore, up to 62% of the rickettsial RNA polymerase activity cosedimented with the membranes on a 5 to 20% sucrose gradient, while almost all the *E. coli* RNA polymerase remained in the cytoplasmic fraction (P2S) after the centrifugation.

This association was salt dependent, so that under high salt conditions (600 mM NaCl), the polymerase was dissociated from the membranes and could be purified by column chromatography. The ratio of membrane-bound and cytoplasmic RNA polymerase was variable; the amount of cytoplasmic RNA polymerase varied from 10 to 40% of the total enzyme activity in different rickettsial preparations. It has been noted that RNA polymerase is interconvertible between various states through physical interaction with a number of accessory proteins (termed transcription factors [15]), nucleotides such as ppGpp, and specific tRNA (20). It is generally thought that protein factors with regulatory functions might form complexes with RNA polymerase under certain conditions, and attempts have been made to isolate and characterize proteins that copurify along with RNA polymerase under mild conditions (14). The role of the association of the rickettsial RNA polymerase with the bacterial membrane in transcription still remains to be established.

The availability of purified rickettsial RNA polymerase and knowledge of its properties provides a means to identify components which participate in the transcriptional process including potential promoter sites in rickettsial genes. Such studies will contribute to an understanding of transcription in rickettsiae and its role in the regulation of rickettsial growth.

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