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Purification of RNA Polymerase from Actinomycin Producing and Nonproducing Cells of *Streptomyces antibioticus*

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DNA-dependent RNA polymerase has been purified approximately 700-fold from 12-h-old cells of *Streptomyces antibioticus* and 400-fold from 48-h cells. Both enzymes appear nearly homogeneous as judged by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Both enzymes possess subunits corresponding to the β, β', and α subunits of *Escherichia coli* RNA polymerase but no band corresponding to the σ subunit was observed on polyacrylamide gels. Moreover, neither enzyme appears to have σ activity as judged by the rifampicin and heparin challenge assays using T4 DNA as template. In addition to the β, β', and α subunits, electrophoresis of the polymerase from 12-h cells reveals a 45,000 M_r protein which is present at a level of 0.40 mol/mol of $\beta + \beta'$. The polymerases from 12- and 48-h *S. antibioticus* cells differ slightly in their template specificity, with the 48-h polymerase showing a slightly greater preference for calf thymus DNA as compared with several other native DNAs which were tested. Further, the polymerase from 48-h cells was slightly more active with poly (dA-dT) (relative to calf thymus DNA) than was the polymerase from 12-h cells. Neither polymerase was capable of catalyzing actinomycin-resistant transcription.

Streptomyces antibioticus is a gram-positive actinomycete which produces the antibiotic actinomycin (1). Since actinomycin is a potent inhibitor of DNA-dependent RNA synthesis (2), it is of some biochemical interest to examine the effects of the antibiotic on the producing organism. In previous reports from this laboratory, it was shown that RNA synthesis catalyzed by crude extracts of actinomycin producing *S. antibioticus* cells was less sensitive to inhibition by actinomycin than was synthesis catalyzed by extracts of nonproducing *S. antibioticus* cells or *Escherichia coli* cells (3, 4). It was further shown that a partially purified RNA polymerase preparation from actinomycin producing *S. antibioticus* cells was capable of catalyzing transcription in the presence of actinomycin concentrations which completely inhibited transcription by *E. coli* RNA polymerase (4). Since the *S. antibioticus* polymerase was not purified to homogeneity, the possibility remained that the ability of the enzyme to catalyze actinomycin-resistant transcription resulted from its association with some accessory factors rather than from some intrinsic differences in the structure of the enzyme as compared with polymerases from other procaryotes. It also seemed possible that differences in the RNA polymerase from actinomycin producing and nonproducing cells might exist.

Some properties of RNA polymerase from actinomycin producing cells have been reported recently in a preliminary communication from this laboratory (5). In the present report, the details of the purification of RNA polymerase from actinomycin producing and nonproducing cells are presented. These enzymes are compared in terms of subunit structure and stoichiometry and template specificity. The *S. antibioticus* polymerases are further compared with *E. coli* RNA polymerase in terms of subunit composition and actinomycin sensitivity.

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MATERIALS AND METHODS

Materials

S. antibioticus cells were grown as described previously (4), and washed with 1 M KCl in Buffer A[1] (see below) prior to polymerase purification. Calf thymus DNA, salmon sperm DNA, *E. coli* DNA, rifampicin, actinomycin D, and phenylmethylsulfonylfluoride were obtained from Sigma. Bacteriophage T4 DNA, poly-(dA-dT) and frozen cells of *E. coli* K-12 were from Miles. Polymin P was from BDH Chemicals, while cellulose powder CF 11 was from Whatman. DNA-cellulose was prepared according to Alberts and Herrick (6). *S. antibioticus* DNA was prepared as described previously (4). [^{3}H]UTP (45–50 Ci/mmol) was from Amersham. T7 DNA was generously donated by Dr. Michael Chamberlin, University of California, Berkeley.

Buffers

The basic buffer used throughout the purification contained 10 mM Tris–HCl, pH 7.8, 0.1 mM potassium-EDTA, 0.1 mM dithiothreitol, 1 mM phenylmethylsulfonylfluoride (Buffer A), generally also containing 5% glycerol (Buffer A5).

Miscellaneous Methods

The RNA polymerase assay was performed as previously described (4) except that each of the four nucleoside triphosphates was present at 0.2 mM. Calf thymus DNA was the template generally used in the polymerase assay. One enzyme unit represents the incorporation of 1 nmol of [^{3}H]UMP into an acid-insoluble form after 10 min of incubation at 30°C. Assays for RNase and DNase were as previously described (4). Protein was determined by the method of Lowry *et al.* (7). Sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis was performed according to Laemmli (8) and urea–SDS–polyacrylamide gel electrophoresis by the method of Wu and Breuning (9) as described by Halling *et al.* (10). Gels were stained with Coomassie brilliant blue, destained, and scanned at 550 nm using a Gilford Model 6510-S gel scanner attached to a Model 240 spectrophotometer. A 0.05-mm slit was used to resolve closely spaced protein bands. The relative amounts of the protein present in the gel bands was determined by cutting out and weighing the appropriate peaks from the scanner tracings. Gels containing 2–10 μg of purified polymerase protein were scanned and it was established that within this range, the areas of the subunit peaks were proportional to the amounts of protein applied.

[1] Abbreviations used: Buffer A, 10 mM Tris–HCl, pH 7.8, 0.1 mM potassium-EDTA, 0.1 mM dithiothreitol, 1 mM phenylmethylsulfonylfluoride; Buffer A5, Buffer A + 5% glycerol; SDS, sodium dodecyl sulfate.

Purification of RNA Polymerase

The purification procedures are similar to those described by Burgess and Jendrisak (11) for *E. coli* RNA polymerase. All steps were carried out at 0–4°C.

Step 1. Generally, 50 g of 12- or 48-h *S. antibioticus* cells was disrupted by grinding with an equal weight of glass beads in an Omnimixer homogenizer. Cell disruption was accomplished in 100–150 ml of Buffer A5 containing 0.3 M KCl, with homogenization for 4 min in 2-min bursts. The homogenizer cup was immersed in an ice–salt bath during homogenization. The homogenate was then centrifuged for 10 min at 13,000*g*. The supernatant was decanted and the pellet was then homogenized and centrifuged twice more as above using 100–150 ml of buffer each time. The supernatants were combined and denoted "crude extract."

Step 2. RNA polymerase was recovered from the crude extract by precipitation with polyethyleneimine (Polymin P). Polymin P was added from a 10% stock solution (11) to give a final concentration of 0.33%. The extract was vigorously stirred during Polymin P addition and stirring was continued for 5 min thereafter. The resulting suspension was then centrifuged for 10 min at 13,000*g* and the supernatant discarded. The precipitate was extracted with 200 ml of Buffer A5 containing 0.3 M KCl by homogenizing briefly with a motor-driven Teflon–glass homogenizer. The suspension was centrifuged as above and the supernatant again discarded. RNA polymerase was extracted from the resulting pellet by homogenization in 200 ml of Buffer A5 containing 1.0 M KCl. After centrifugation as above, the pellet was discarded.

Step 3. The 1.0 M KCl eluate was brought to 50% saturation with solid ammonium sulfate and the resulting suspension was stirred for 30 min in the cold. The precipitated protein was collected by centrifugation for 20 min at 20,000*g*. A white, flocculent substance precipitated along with the enzyme at this step, but its presence affected neither the activity of the enzyme nor the subsequent purification steps. The entire precipitate was dissolved in about 100 ml of Buffer A5 and dialyzed overnight against the same buffer.

Step 4. The dialyzed enzyme was applied to a 2 × 20-cm column of DNA-cellulose equilibrated with Buffer A5 containing 0.05 M KCl. The column was washed with Buffer A5 containing 0.10 or 0.15 M KCl until the A_{280} of the effluent was less than 0.2 and then connected to a 300-ml linear gradient of 0.10–1.10 M KCl in Buffer A5 for the purification of enzyme from 12-h cells, or to a 350-ml linear gradient of 0.15–1.15 M KCl for the purification of enzyme from 48-h cells. The columns were eluted at a flow rate of 50 ml/h. Every third fraction was assayed for polymerase activity. Results of typical columns are shown in Fig. 1. The enzyme-containing fractions (40–60 ml) were pooled and the enzyme was recovered by the addition of solid ammonium sulfate (50 g/100 ml) or by dialysis of the

pooled fractions against ammonium sulfate (50 g/100 ml in Buffer A5) without stirring as described by Schrier *et al.* (12). Ammonium sulfate precipitates were collected as described above and redissolved in a minimal volume of Buffer A5 containing 0.5 M KCl.

Step 5. The concentrated enzyme solution was applied to a 1.6 × 95-cm column of Bio-Gel A1.5m equilibrated with Buffer A5 containing 0.5 M KCl. Enzyme-containing fractions were pooled and dialyzed overnight against Buffer A containing 50% glycerol and 0.05 M KCl.

RNA polymerase holoenzyme was prepared from frozen cells of *E. coli* K-12 by Polymin P precipitation and DNA-cellulose chromatography and core enzyme was prepared by chromatography on Bio-Rex 70 as described by Burgess and Jendrisak (11). Spectrophotometric scanning showed that, relative to α, the *E. coli* holoenzyme was about 33% saturated with σ factor (see Fig. 2c).

RESULTS

Purification of RNA Polymerase

Results of a typical purification of the *S. antibioticus* RNA polymerases are summarized in Table I. The enzyme was generally purified about 700-fold from 12-h cells relative to the crude extract, and the yield of enzyme activity varied between 30 and 40% of that assayed in the crude extract. Approximately 400-fold purification of enzyme from 48-h cells was routinely obtained

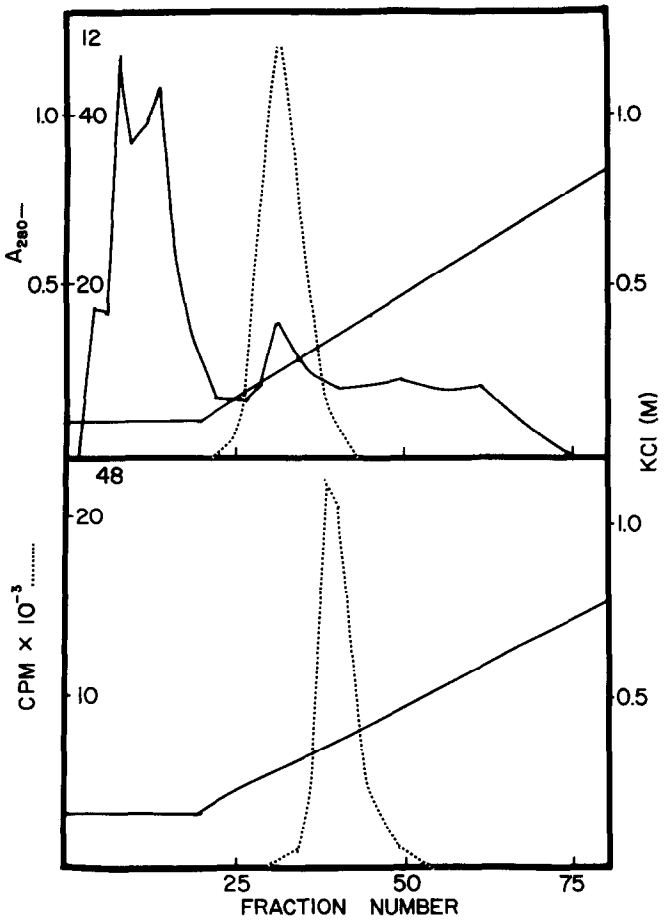

FIG. 1. DNA-cellulose chromatography of *S. antibioticus* RNA polymerase. Dialyzed step 3 enzyme was applied to a 2 × 20-cm column of DNA-cellulose. The column was eluted with 0.10 or 0.15 M KCl in Buffer A5 at 50 ml/h and 6.4-ml fractions were collected to fraction 20. The column was then attached to a 300-ml linear gradient of 0.10–1.10 M KCl in Buffer A5 for the 12-h enzyme or 0.15–1.15 M KCl in 350 ml for the 48-h enzyme, and fractions of 2.7 ml were collected. Fractions 26–40 (12 h) and 35–47 (48 h) were pooled and processed for further purification.

TABLE I

PURIFICATION OF *S. antibioticus* RNA POLYMERASE[a]

Step	Volume (ml)		Protein (mg)		Units		Specific activity[b]		Purification		Percentage yield	
	12 h	48 h	12 h	48 h	12 h	48h	12 h	48 h	12 h	48 h	12 h	48h
1 Crude extract	405	287	5800	3067	138	145	0.02	0.05	—	—	100	100
2 KCl eluate	200	168	584	530	104	84	0.18	0.16	9	3.2	75	58
3 Ammonium sulfate fraction	105	93	316	262	256	240	0.81	0.92	41	18.4	186	166
4 DNA-cellulose[c]	2	2.3	6.5	18	70	168	10.8	9.3	540	186	51	116
5 BioGel A 1.5 m[c]	5.6	16.6	2.9	6.8	44	133	15.2	19.6	760	392	32	92

[a] Starting from 50 g of 12- or 48-h cells.
[b] Units/mg protein.
[c] For these fractions activity was measured on concentrated enzyme.

with 85–100% recovery as compared with crude extracts (5). As reported previously (4, 5), purification of the enzyme was generally accompanied by an increase in activity at the intermediate steps (e.g., step 3 of Table I). The yield of enzyme protein varied between 6 and 15 mg/100 g of 12- or 48-h cells which is considerably lower than the 50 mg/100 g obtained for *E. coli* (11). In addition, the specific activity of the *S. antibioticus* enzymes is considerably lower than that reported for *E. coli* polymerase (11), in contrast to earlier studies with partially purified *S. antibioticus* RNA polymerase (4). The reason for this discrepancy is unclear, although the stock culture used to obtain cells for the present experiments was not the same as that used for the studies reported previously. The specific activity was not considerably increased by the use of DNAs from other sources as templates (see below). It should be noted that Watanabe and Tanaka (13) also found that the RNA polymerase from a rifampicin producing strain of *S. mediterranei* had a specific activity much lower than that of *E. coli* polymerase. Further, it should be noted that the specific activity of the *E. coli* RNA polymerase purified for the studies reported herein was only four- to fivefold greater than the specific activities of the *S. antibioticus* polymerase (67 units/mg protein for the *E. coli* enzyme versus 15–20 units/mg protein (Table I) for the *S. antibioticus* enzymes) under the assay conditions described under Materials and Methods. When poly(dA-dT) was used as template the specific activities were 37.2 units/mg (12-h enzyme), 57.6 units/mg (48-h enzyme), and 191 units/mg (*E. coli* enzyme). Thus, with this synthetic template, the *E. coli* enzyme was still no more than three- to fivefold more active than the *S. antibioticus* enzymes. It should be noted, however, that the assay conditions used in the present study are not identical to those employed by Burgess and Jendrisak (11). The purified *S. antibioticus* enzymes were not contaminated with RNase or DNase activities, although DNase was occasionally detected eluting after the polymerase peak from the Bio-Gel column.

Since this report represents the first

TABLE II

DEPENDENCE OF [^{3}H]UMP INCORPORATION ON SUBSTRATES FOR RNA SYNTHESIS

System	nmol [^{3}H]UMP incorporated by polymerase from	
	12-h cells	48-h cells
Complete[a]	0.275	0.302
−ATP	0.006	0.008
−GTP	0.010	0.012
−CTP	0.016	0.018
−DNA	0.004	0.004
+RNase (10 μg)	0.008	0.012

[a] The complete system was prepared as described under Materials and Methods. Incubation was for 10 min at 30°. Components were added or omitted as indicated in the table.

thorough examination of the properties of purified *S. antibioticus* RNA polymerase, it was essential to establish that the enzymes had properties similar to those of other procaryotes. As shown in Table II, incorporation of [^{3}H]UMP into an acid-insoluble form was dependent on the presence of DNA and all four nucleoside triphosphates in reaction mixtures and was abolished by the inclusion of ribonuclease. Magnesium stimulated [^{3}H]UMP incorporation at concentrations of 1–15 mM and potassium stimulated incorporation at concentrations of 50–150 mM. No difference in cation dependence was observed when polymerases from 12- and 48-h *S. antibioticus* cells were compared.

Subunit Structure and Stoichiometry of the S. antibioticus RNA polymerases

The subunit structure of the purified polymerases was analyzed by SDS–polyacrylamide gel electrophoresis with or without urea. Figure 2, gels a and b, shows the electrophoretic patterns for the step 5 enzyme from 12- and 48-h cells. Some minor bands were visible in gels a and b of Fig. 2, but these were not always observed in other polymerase preparations.

The major bands observable in gels a and b suggest that the *S. antibioticus* RNA polymerase has subunits corresponding to the β, β', and α subunits of *E. coli* RNA polymerase, but no band corresponding to the σ subunit was observed on the SDS–gels. Further, the β and β' subunits of the *S. antibioticus* RNA polymerase could not be resolved by the Laemmli gel system, whereas the corresponding *E. coli* subunits could be resolved (Fig. 2, gel c). Some separation of the β and β' *S. antibioticus* polymerase subunits was obtained in the urea–SDS system of Wu and Breuning ((9, 10), Fig. 2, gels d and e). Since the molecular weights of the β and β' subunits have not been conserved in evolution (10), a positive identity has not been assigned to the two protein bands separated on urea–SDS gels. Thus, these two subunits of *S. antibioticus* RNA polymerase will be referred to collectively below as $\beta + \beta'$.

Figure 2, gel a, also shows a 45,000 M_r protein (Band Y) which is associated with

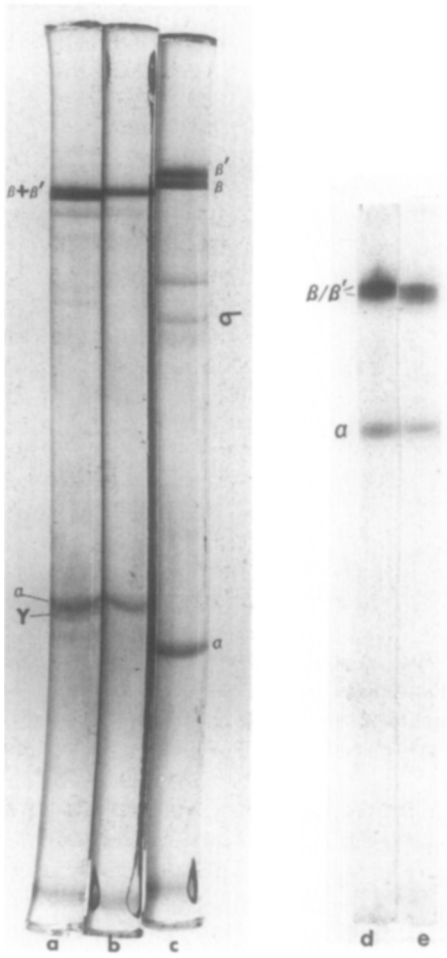

FIG. 2. Polyacrylamide gel electrophoresis of purified polymerases. Gels a and d represent 12-h enzyme (7.5 and 12 μg, respectively) and gels b and e, 48-h enzyme (4 and 10 μg, respectively). Gel c depicts *E. coli* holoenzyme (5 μg). The acrylamide concentration was 8.7% in gels a–c which were prepared as described by Laemmli (8). Gels d and e were prepared by the method of Wu and Breuning (9) as described by Halling *et al.* (10) and the acrylamide concentration was 5%. The following proteins were used as molecular weight markers: the β (155,000), β' (165,000), and α (39,000) subunits of *E. coli* polymerase, β-galactosidase (135,000), bovine serum albumin (68,000), ovalbumin (44,000), DNase I (30,000), immunoglobulin light chain (25,000), cytochrome *c* (13,000).

the 12-h polymerase only. A minor band (called X in Ref. (5)) of 145,000 M_r is probably an artifact of proteolysis and is not always observed in purified enzyme preparations. Figure 3 represents spectrophotometric

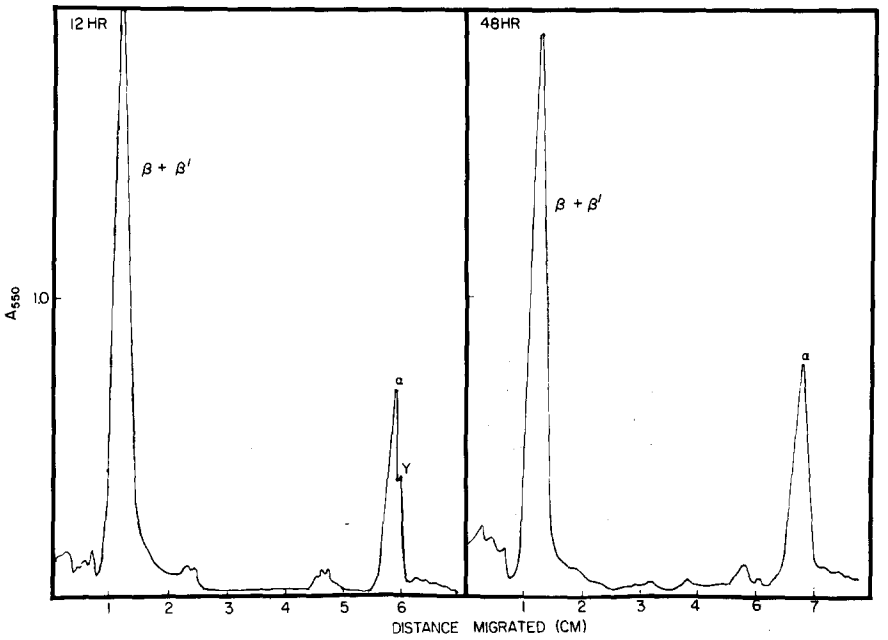

FIG. 3. Densitometric scan of step 5 *S. antibioticus* RNA polymerase. Gels similar to a and b of Fig. 2 were stained, destained, and scanned as described under Materials and Methods. Scanning was performed using a 0.05-mm slit to allow resolution of the α and Y bands by the scanning apparatus.

scans of SDS–gels of the polymerases from 12- and 48-h cells. It was estimated from these tracings that at least 90% of the Coomassie brilliant blue reactive materials on these gels represents the $\beta + \beta'$ and α bands. β and β' have molecular weights of about 150,000 while α has a molecular weight of about 50,000. Figure 2 clearly shows that the α subunit of the *S. antibioticus* RNA polymerases is larger than the corresponding subunit from *E. coli* polymerase. The corresponding molecular weights for *E. coli* RNA polymerase (11) are 165,000 (β'), 155,000 (β), and 39,000 (α).

An estimate of the stoichiometry of the *S. antibioticus* subunits was made by comparing the areas of the peaks in Fig. 3. To obtain better separation of the α and Y bands, polymerase from 12-h cells was also electrophoresed on 12.5% acrylamide gels. Results of a typical analysis are shown in Table III. It can be seen that the molar ratio of β or β' to α (or $\beta + \beta'$ to 2α) is 1:1 for both polymerases, while the ratio of Y to $\beta + \beta'$ is 0.40:1. These calculations serve to confirm that the α subunit is the 50,000 M_r protein in gel a of Fig. 2 and not the 45,000 M_r band, since the expected molar ratio of $\beta + \beta'$ to 2α is 1:1 (14).

Functional Comparisons of RNA Polymerase from 12- and 48-h S. antibioticus cells

It was somewhat surprising to find that neither the polymerase from 12- nor 48-h *S. antibioticus* cells possessed a subunit corresponding to *E. coli* σ, since the purification procedure employed has been reported to yield holoenzyme (11). However, procaryotic polymerase subunits which differ in size but are equivalent in function to *E. coli* σ have been reported (15). It thus seemed possible that both the 12- and 48-h polymerases might possess some σ activity. This hypothesis was first tested using the rifampicin challenge assay of Mangel and Chamberlin (16) as described by Amemiya *et al.* (17). In these experiments, *S. antibioticus* and *E. coli* RNA polymerases were allowed to form binary complexes with T4 DNA at 37°C in the absence of nucleoside triphos-

phates. Nucleoside triphosphates were then added to reaction mixtures alone or simultaneously with rifampicin. Incubation was continued for 10 min at 37°C and RNA synthesis was assayed as described above. Since σ activity is required for complex formation (16, 18), any RNA synthesis observed in the presence of rifampicin should be due to σ induced binding of the RNA polymerase to DNA. Table IV shows that the level of RNA synthesis in the presence of rifampicin was 25% of that observed in its absence when *E. coli* holoenzyme was the polymerase source. In contrast, essentially no rifampicin-resistant RNA synthesis was observed with *E. coli* core enzyme or either *S. antibioticus* polymerase. The KCl concentration was 50 mM, in these experiments. When the KCl concentration was increased to 150 mM, the percentage of rifampicin-resistant complexes formed in the presence of *E. coli* holoenzyme was decreased, but the level of rifampicin-resistant RNA synthesis catalyzed by the *S. antibioticus* polymerases was not affected (data not shown). These results suggest that rifampicin-resistant transcription of the T4 DNA is σ dependent under the conditions described, and that the *S. antibioticus* polymerases are present as core rather than holoenzymes. Virtually identical results were obtained with phage T7 DNA except that all the polymerases were more active with this template than with T4 DNA (see Table V, for example). The levels of rifampicin-resistant transcription of T7 DNA were: 12-h enzyme (7.2%); 48-h enzyme (8.7%); *E. coli* holoenzyme (42.8%); *E. coli* core enzyme (7.2%).

TABLE III

RELATIVE MOLAR CONCENTRATIONS OF RNA POLYMERASE SUBUNITS AND ASSOCIATED POLYPEPTIDES[a]

Polymerase subunit or polypeptide	Relative M_r		Relative amount		Relative molar concentration	
	12 h	48 h	12 h	48 h	12 h	48 h
$\beta + \beta'$	3.0	3.0	2.82	2.93	0.94	0.98
α	1.0	1.0	1.0	1.0	1.0	1.0
Y	0.92	—	0.38	—	0.41	—

[a] The relative amounts of each protein were determined by scanning SDS–polyacrylamide gels as described in the legend to Fig. 3. Molecular weights were assigned using the standards listed in the legend to Fig. 3. Subunit proportions were calculated relative to α.

TABLE IV

EFFECTS OF RIFAMPICIN AND HEPARIN ON DNA–RNA POLYMERASE COMPLEX FORMATION[a]

Enzyme used	nmol [^{3}H]UMP incorporated		
	No inhibitor	Plus rifampicin	Plus heparin
12 h	0.100	0.003 (3.0)	0.005 (5.0)
48 h	0.048	0.0008 (1.6)	0.003 (5.6)
E. coli holoenzyme	0.202	0.051 (25)	0.096 (48)
E. coli core	0.120	0.004 (3.3)	0.007 (5.9)
48 h step 3	0.032	0.023 (71)	0.003 (8)

[a] RNA polymerase (15–20 μg) was incubated for 10 min at 37°C with 10 μg of T4 DNA. Rifampicin (1 μg) or heparin (10 μg) was then added to selected reaction mixtures. Nucleoside triphosphates were then added and incubation was continued for 10 min at 37°C. RNA synthesis was measured as described under Materials and Methods. Values in parentheses represent percentage residual RNA synthesis relative to incubation mixtures lacking rifampicin or heparin.

TABLE V

TEMPLATE SPECIFICITIES OF *S. antibioticus* RNA POLYMERASES[a]

Template	nmol [^{3}H]UMP incorporated by polymerase from	
	12-h cells	48-h cells
Calf thymus DNA	0.233 (100)	0.252 (100)
S. antibioticus DNA	0.042 (18)	0.042 (17)
E. coli DNA	0.107 (46)	0.098 (39)
Micrococcus lysodeikticus DNA	0.032 (14)	0.024 (10)
T4 DNA	0.143 (61)	0.120 (48)
poly(dA-dt)	0.570 (245)	0.740 (294)
T7 DNA	0.379 (163)	0.259 (103)

[a] Reaction mixtures contained 10 μg of template and 10 μg of RNA polymerase in 100 μl. Values in parentheses represent template activity relative to calf thymus DNA, set arbitrarily at 100% activity.

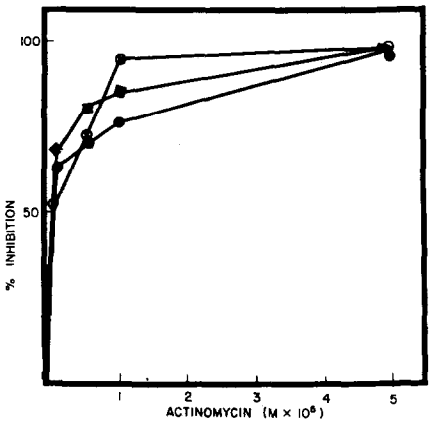

FIG. 4. Effects of actinomycin on transcription of *S. antibioticus* DNA by *E. coli* and *S. antibioticus* RNA polymerases. Reaction mixtures contained about 15 μg of RNA polymerase. Results are expressed as percentage inhibition of [^{3}H]UMP incorporation by actinomycin using 0.15 mg of DNA as template. The filled circles represent transcription by 12- or 48-hr *S. antibioticus* polymerases both of which showed an essentially identical response to actinomycin. The squares represent transcription by *E. coli* holoenzyme and the open circles represent transcription by *E. coli* core enzyme.

These results were supported by similar experiments (again at 50 mM KCl) in which polymerase–DNA complexes were challenged with heparin. Significant levels of heparin-resistant RNA synthesis were catalyzed by *E. coli* holoenzyme but not by core enzyme or *S. antibioticus* polymerase (Table IV). Table IV also shows that step 3 polymerase from 48-h *S. antibioticus* cells catalyzed RNA synthesis which was quite resistant to rifampicin but which was sensitive to heparin. This result was consistently observed with T4 DNA.

As shown in Table V, the enzymes from 12- and 48-h *S. antibioticus* cells also differed in their template specificities. Relative to enzyme from 12-h cells, the enzyme from 48-h *S. antibioticus* cells showed a slight preference for calf thymus DNA as compared with several other native DNAs which were tested. Table V also shows that the polymerase from 48-h *S. antibioticus* cells was slightly more active with poly(dA-dT) as template (relative to calf thymus DNA) than was the polymerase from 12-h cells.

Actinomycin Sensitivity of Transcription by Purified Polymerases

As reported previously (4), a partially purified *S. antibioticus* RNA polymerase preparation catalyzed transcription at actinomycin concentrations which inhibited transcription by *E. coli* RNA polymerase. One goal of the studies presented in the present report was to determine whether highly purified *S. antibioticus* polymerase retained this property. In Fig. 4, the effects of actinomycin on transcription of *S. antibioticus* DNA by *S. antibioticus* and *E. coli* polymerases are shown. In contrast to the results obtained with crude *S. antibioticus* extracts and partially purified RNA polymerase, there was little difference in the actinomycin sensitivity of transcription catalyzed by *S. antibioticus* polymerase as compared with the *E. coli* enzyme (Fig. 4). With both enzymes, a given concentration of actinomycin inhibited transcription of *S. antibioticus* DNA to a somewhat greater extent than transcription of calf thymus DNA (data not shown). The data of Fig. 4 further show that the pattern of actinomycin inhibition was essentially the same whether enzyme from 12- or 48-h *S. antibioticus* cells was employed. These data suggest that the previous observation of actinomycin-resistant transcription by crude extracts and by partially purified *S. antibioticus* RNA polymerase was not attributable to an intrinsic property of the enzyme itself.

DISCUSSION

The results presented in this paper and the earlier preliminary communication (5) represent the first reports of the purification of RNA polymerase from *Streptomyces antibioticus*. These studies show that the *S. antibioticus* polymerases have properties which are similar to those of other gram-positive and gram-negative bacteria. Like the polymerases of certain *Bacilli* (10, 19, 20), the β′ and β subunits of the *S. antibioticus* polymerase have very similar molecular weights and cannot easily be resolved on SDS–gels. The *S. antibioticus* polymerases appear to be unlike both the *Bacillus* and *E. coli* polymerases, however, in the size of the α subunit. Figure 2 above

clearly shows that the α subunit of the *S. antibioticus* polymerases is considerably larger than the corresponding subunit from *E. coli*.

It is noteworthy that the *S. antibioticus* RNA polymerases do not possess a subunit which corresponds to *E. coli* σ, and some RNA polymerases have been shown to possess a σ factor which has a molecular weight between 40 and 50,000 (15). Although the polymerase from 12-h cells is associated with a 45,000 M_r protein it seems unlikely that this protein represents *S. antibioticus* σ since polymerase from 12-h cells showed no σ dependent synthetic activity in the rifampicin or heparin challenge assays with T4 DNA as template (Table IV). Indeed, the *S. antibioticus* polymerase was no more active than *E. coli* core polymerase in these assays. It might be argued that the 45,000 M_r protein is actually the α subunit of *S. antibioticus* RNA polymerase. However, when 12- and 48-h polymerases were electrophoresed together, the 50,000 and 45,000 M_r bands were still resolved and the staining intensity of the 50,000 M_r band increased while that of the 45,000 M_r band was the same as when 12-h polymerase alone was subjected to electrophoresis. Thus the 45,000 M_r band does not seem to correspond to either the σ or the α proteins of *E. coli*. In summary, then, the structural and functional studies suggest, but certainly do not prove, that the *S. antibioticus* enzymes represent core polymerases. With reference to the challenge assays, however, it must be kept in mind that neither T4 nor T7 DNAs are homologous templates for *S. antibioticus* polymerases. It is possible that the use of homologous phage templates might reveal the presence of σ activity in the purified polymerase preparations. To date, however, such templates have not been available for activity studies.

The question thus remains, what is the nature of *S. antibioticus* σ factor? Although the purification procedure employed in this study can be used to prepare holoenzyme from *E. coli*, only enzymes apparently representing core polymerase have been obtained from *S. antibioticus* cells. No evidence for multiple polymerase forms has ever been observed on DNA-cellulose or Bio-Gel chromatograms. Thus, the nature of the *S. antibioticus* σ factor cannot be definitively established at this time, but the situation could be analogous to that observed in sporulating *B. subtilis*. In those cells, it can be shown that σ activity, though present, is prevented from associating with the core polymerase. Thus, the polymerase purified from these cells is nearly devoid of σ activity even though normal amounts of the protein are present in the cells (20). Since σ can function catalytically (18), it seems possible that the *S. antibioticus* polymerase might associate with small amounts of σ protein "*in vivo*" to permit gene transcription to take place. The results of the rifampicin challenge experiments with 48-h step 3 enzyme (Table IV) at least suggest that active σ does exist in *S. antibioticus* cells.

The data of Fig. 4 indicate that step 5 *S. antibioticus* RNA polymerase has lost the ability to catalyze actinomycin-resistant transcription which was observed with crude cell extracts and partially purified enzyme (4). This finding suggests that some substances are removed during the purification which are responsible for this property. If this interpretation is correct, one would predict that the sensitivity of transcription to actinomycin inhibition would increase as more highly purified polymerase preparations were used. This is, in fact, what is observed. In a previous report, for example, it was shown that 50 μM actinomycin inhibited RNA synthesis catalyzed by a crude extract of 48-h *S. antibioticus* cells by 15% and synthesis catalyzed by a 110-fold purified enzyme by about 50%, with *S. antibioticus* DNA as template (4). In the present studies, this actinomycin concentration inhibited [^{3}H]UMP incorporation by 12% when crude extract was the polymerase source and *S. antibioticus* DNA the template and by 43% when step 3 enzyme was the polymerase source (data not shown). As can be seen in Fig. 4, transcription of *S. antibioticus* DNA by highly purified *S. antibioticus* RNA polymerase was inhibited by greater than 95% at 50 μM actinomycin. Thus, the ability to catalyze actinomycin-resistant transcription is certainly not an intrinsic property of the *S. antibioticus* core poly-

merase. Experiments are in progress to isolate substances conferring actinomycin resistance from *S. antibioticus* cell extracts. To date, no evidence for enzymes in *S. antibioticus* cells which degrade actinomycin has been found.

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