SENSITIVITY OF TRANSCRIPTION BY PURIFIED STREPTOMYCES ANTIBIOTICUS RNA POLYMERASE TO ACTINOMYCIN

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SUMMARY

DNA-dependent RNA polymerase has been purified 400-fold from 48 hr old, actinomycin producing cells of Streptomyces antibioticus. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of the purified S. antibioticus polymerase reveals the presence of bands corresponding to the \( \beta, \beta', \) and \( \alpha \) subunits of Escherichia coli polymerase, but no \( \sigma \) subunit. The purified S. antibioticus polymerase is also associated with a protein component of 145,000 daltons and it is suggested that this protein is produced by limited proteolysis of either the \( \beta \) or \( \beta' \) subunits. "In vitro" studies have shown that transcription catalyzed by either S. antibioticus or E. coli RNA polymerase is inhibited to about the same extent by actinomycin.

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 E. coli cells (1, 2). 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 (2). 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 prokaryotes. To examine this possibility, RNA polymerase has been purified 400-fold from 48 hour old, actinomycin producing cells of S. antibioticus. The
purified enzyme has been compared with *E. coli* RNA polymerase in terms of subunit structure, and sensitivity to actinomycin.

**MATERIALS AND METHODS**

*S. antibioticus* cells were grown for 48 hr as described previously (2). The RNA polymerase assay was performed as previously described (2), except that each of the four nucleoside triphosphates was present at 0.1 mM. One enzyme unit represents the incorporation of 1 nmole of [3H]-UMP into an acid insoluble form after 10 min of incubation at 30°.

The sedimentation coefficient of the purified *S. antibioticus* RNA polymerase was determined in the Beckman model E analytical ultracentrifuge using ultraviolet optics. Measurements were made using 1.0 A280 unit of purified RNA polymerase which corresponded to about 0.7 mg protein. Centrifugation was at 56,000 rpm and photographs were taken at 4 minute intervals after reaching speed. Photographic negatives were scanned with a Joyce-Loebl densitometer and the resulting tracings were utilized to calculate S. Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis was performed according to Laemmli (3). Gels were stained with Coomassie Brilliant Blue.

The RNA polymerase purification procedure is similar to that described by Burgess and Jendrisak for *E. coli* RNA polymerase (4), and will be reported in detail in a subsequent publication. Briefly, the purification involved polyethyleneimine precipitation of the polymerase from crude cell extracts, ammonium sulfate extraction, DNA-cellulose chromatography and gel filtration.

**RESULTS AND DISCUSSION**

**Purification of RNA polymerase** - Results of a typical purification of *S. antibioticus* RNA polymerase are summarized in Table I. The enzyme was generally purified about 400-fold relative to the crude extract, and the yield of enzyme activity varied between 85-100% of that assayed in the crude extract.

The purified enzyme was not contaminated with RNase or DNase activities, although DNase was detected eluting after the polymerase peak from Biogel A 1.5m columns.

**Subunit structure and size of the *S. antibioticus* RNA polymerase** - The subunit structure of the purified polymerase was analyzed by SDS-polyacrylamide gel electrophoresis. Fig. 1 shows the electrophoretic pattern for the step 5 enzyme.

Some minor bands are visible in the gel of Fig. 1, but these were not always observed in other polymerase preparations. The major bands observable in the gel suggest that the *S. antibioticus* RNA polymerase has a subunit structure similar to that reported for *E. coli* except that the pattern for the *S. antibioticus* polymerase lacks a band corresponding to the σ factor of the *E. coli*
<table>
<thead>
<tr>
<th>STEP</th>
<th>VOLUME (ml)</th>
<th>PROTEIN (mg)</th>
<th>UNITS</th>
<th>SPEC. ACT. (^b)</th>
<th>PURIFICATION</th>
<th>%YIELD</th>
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</thead>
<tbody>
<tr>
<td>1. Crude extract</td>
<td>287</td>
<td>3067</td>
<td>145</td>
<td>0.05</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>2. KCl eluate</td>
<td>168</td>
<td>530</td>
<td>84.4</td>
<td>0.16</td>
<td>3.2</td>
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<tr>
<td>3. Ammonium sulfate fraction</td>
<td>93</td>
<td>262</td>
<td>240</td>
<td>0.92</td>
<td>18.4</td>
<td>166</td>
</tr>
<tr>
<td>4. DNA-cellulose (^c)</td>
<td>2.3</td>
<td>18</td>
<td>168</td>
<td>9.3</td>
<td>186</td>
<td>116</td>
</tr>
<tr>
<td>5. Biogel A 1.5m (^c)</td>
<td>16.6</td>
<td>6.8</td>
<td>133</td>
<td>19.6</td>
<td>392</td>
<td>92</td>
</tr>
</tbody>
</table>

\(^a\)Starting from 50 g of 48 hour cells  \(^b\)Units/mg protein  \(^c\)For these fractions activity was measured on concentrated enzyme
Fig. 1 - SDS-polyacrylamide gel electrophoresis of 12 μg of purified S. anti-
bioticus RNA polymerase. The β/β' and Χ bands can be clearly seen in this
gel. Molecular weights of electrophoretic bands were determined using the
following standards run on parallel gels: β', β, σ and α subunits of E. coli
RNA polymerase, bovine serum albumin, ovalbumin, DNase I, immunoglobulin light
chain and cytochrome c. The acrylamide monomer concentration was 8.7%.

In addition, the β and β' bands were not resolved by SDS-polyacrylamide
gel electrophoresis, even at acrylamide concentrations as low as 7%. However,
these two subunit bands could be resolved by electrophoresis in the presence
of urea and SDS (manuscript in preparation). It should also be noted that the
protease inhibitor, phenylmethylsulfonylfluoride, was included in all buffers
used in the purification and storage of the polymerase. When this inhibitor
was omitted from buffers, several bands in addition to those shown in Fig. 1
were observed upon electrophoresis of the purified enzyme. By analogy with
E. coli polymerase, the largest subunits are referred to as β and β' and
have molecular weights of about 155,000 as determined by gel electrophoresis.
Fig. 1 also shows that the S. antibioticus polymerase possesses a subunit with
mobility corresponding to the a (50,000 molecular weight) subunit of the E. coli polymerase. The corresponding molecular weights reported by Burgess and Jendrisak for E. coli polymerase (3) were 165,000 (β'), 155,000 (β), 87,000 (σ) and 39,000 (α).

In addition to these subunit bands, the gel pattern of step 5 enzyme also revealed the presence of a band of molecular weight 145,000 (Band X of Fig. 1).

This band was always observed, even in the most highly purified enzyme preparations and can be seen from Fig. 1 to be distinct from the β' and β bands. The source of this protein band is not known at this time but it may have been produced by limited proteolysis of the β or β' polymerase subunits. Limited proteolysis of the β' subunit of Bacillus subtilis RNA polymerase has been reported (5). Alternatively, this band may represent the σ factor of the S. antibioticus polymerase.

When step 5 enzyme was analyzed by analytical ultracentrifugation in 0.5 M KCl, a sedimentation coefficient of 14S was obtained. This value is somewhat lower than that reported for E. coli RNA polymerase (15S, reference 6).

### Actinomycin sensitivity of transcription by purified polymerases

As reported previously (2), a partially purified S. antibioticus RNA polymerase preparation catalyzed transcription at actinomycin concentrations which inhibited transcription by E. coli RNA polymerase. The major goal of the studies presented in this report was to determine whether highly purified S. antibioticus polymerase retained this property. In Fig. 2, the effects of actinomycin on transcription of calf thymus and S. antibioticus DNA's 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 RNA polymerase as compared with the E. coli enzyme. With both purified enzymes, a given concentration of actinomycin inhibited transcription of S. antibioticus DNA to a somewhat greater extent than transcription of calf thymus DNA. These data suggest that the previous observation of actinomycin
Fig. 2 - Effects of actinomycin on transcription of calf thymus and S. antibioticus DNA's by E. coli and S. antibioticus RNA and 4 μg of RNA polymerase. Reaction mixtures (100 μl) contained 0.15 mg of DNA and 4 μg of RNA polymerase. Results are expressed as percentage inhibition of 3H-UMP incorporation by actinomycin. The absolute levels of incorporation with calf thymus or S. antibioticus DNA's were similar with a given polymerase. Solid line, transcription by E. coli RNA polymerase; dashed line, transcription by S. antibioticus polymerase.

Resistant transcription by crude extracts and by partially purified S. antibioticus RNA polymerase was due to the association of accessory factors with the polymerase and not to an intrinsic property of the enzyme itself.

The data of Fig. 2 thus 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 (2). This finding suggests that some substances removed during the purification 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 are used. This is, in fact, what is observed. In a previous report, for example, it was shown that 5 x 10^{-5} M actinomycin inhibited RNA synthesis catalyzed by a crude extract of 48 hour S. antibioticus cells by 15% and synthesis catalyzed by 110-fold purified enzyme by about 50%, with S. antibioticus DNA as template (2). 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. 2, transcription of *S. antibioticus* DNA by highly purified *S. antibioticus* RNA polymerase was inhibited by 94% at $5 \times 10^{-5}$ actinomycin. Thus, the ability to catalyze actinomycin resistant transcription does not seem to be an intrinsic property of the *S. antibioticus* RNA polymerase.

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REFERENCES