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DNA-dependent RNA polymerase from the chlorotetracycline producing strain of *Streptomyces aureofaciens*

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RNA polymerase from *Streptomyces aureofaciens* has been purified by polyethyleneimine precipitation followed by chromatography first on DEAE-cellulose, then heparin-Sepharose and finally on an aminooxybutylcellulose matrix containing immobilised *S. aureofaciens* DNA. The enzyme is composed of three subunits of ~145, 136 and 44 kDa that are in a ratio of approx. 1:1:2. In many isolations two additional subunits of ~68 and 39 kDa and some minor protein bands of ~110, 85 and 61 kDa are also present. Thus, the structure of this enzyme is very similar to other bacterial RNA polymerases, exhibiting an $\alpha_2\beta\beta'$ core and the additional proteins ρ and σ.

RNA polymerase; *(Streptomyces aureofaciens)*

1. INTRODUCTION

In our study of the metabolism of the chlorotetracycline producing strain of *S. aureofaciens* [1–3], we have detected a RNA polymerase (EC 2.7.7.6) during the early phase of growth of this organism [4]. We report the isolation of this enzyme and some of its properties in order to elucidate the possible function of chlorotetracycline during transcription.

2. MATERIALS AND METHODS

ATP, GTP, CTP, UTP, DEAE-cellulose and electrophoresis reagents were purchased from Serva (Heidelberg, FRG), [^{3}H]UTP (0.462 TBq/mol) from Radiochemical Centre (Amersham, England), Coomassie brilliant blue R-250, phenylmethylsulfonyl fluoride and ammonium sulfate from Merck (Darmstadt, FRG), ppGpp from Sanraku-Ocean (Tokyo, Japan), chlorotetracycline from Biotika (Slovenská Ľupča, Czechoslovakia) and membrane filter 'Synpor 6' from Synthesia, Pardubice, Czechoslovakia. Polymin P was kindly donated by BASF (Ludwigshafen, FRG). A 100 ml stock solution of 5% Polymin P (pH 7.4) was prepared as in [5], heparin-Sepharose as in [6], DNA from *S. aureofaciens* as in [7], and DNA-aminooxybutyl-cellulose as in [8,9].

RNA polymerase from exponentially growing cells of *S. aureofaciens* BM-K [10], was purified as in [2,5]. 500 g of cells was suspended in 600 ml extraction buffer A (0.01 M Tris-HCl, pH 7.5, 0.01 M $(NH_4)_2SO_4$, 6 mM $MgCl_2$, 0.01 M EDTA, 5 mM 2-mercaptoethanol, 0.1 mM phenylmethyl-

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sulfonyl fluoride and 5% glycerol) and homogenized using a blender with internal cooling at 4°C. Then the mixture was centrifuged at 3000 × *g* for 20 min. The cell debris was then sonicated on a MSE ultrasonic disintegrator Mkz at 12 U for 3 min in the presence of buffer A; after centrifugation both supernatants were pooled and fractionated with 5% (v/v) Polymin P [2]. The RNA polymerase was extracted from the precipitate by suspending it in 1500 ml of buffer A containing 0.2 M $(NH_4)_2SO_4$. The suspension was centrifuged at 30000 × *g* for 30 min. The supernatant solution (1400 ml) was diluted by 1400 ml of buffer A and then applied to a DEAE-cellulose column (3 × 18 cm) equilibrated with buffer A containing 0.1 M $(NH_4)_2SO_4$. RNA polymerase was eluted with 600 ml of a linear gradient of $(NH_4)_2SO_4$ from 0.1–0.5 M in buffer A at a flow rate of 70 ml/h. 8 ml fractions were collected and aliquots of 10 μl were assayed. The active fractions were pooled and dialyzed (10 h) against buffer B (0.01 M Tris-HCl, pH 7.9, 0.1 mM EDTA, 10 mM 2-mercaptoethanol, 1 mM $(NH_4)_2SO_4$, 0.05 mM phenylmethylsulfonyl fluoride, 5% glycerol) containing 0.1 M NaCl and applied onto a heparin-Sepharose column (1.5 × 12 cm) [6]. The elution was performed with 400 ml of a linear gradient of 0.1–1.2 M NaCl in buffer B at a flow rate of 20 ml/h. 5.5 ml fractions were collected and 5 μl samples assayed. The active fractions were diluted with buffer B to the concentration of 0.1 M NaCl and applied to a column (1.5 × 8 cm) of *S. aureofaciens* DNA immobilized on aminooxybutyl-cellulose [8,9]. The column was first washed with buffer B containing 0.1 M NaCl until the A_{280} of the effluent was less than 0.05. A linear gradient of 200 ml of 0.1–1.7 M NaCl in buffer B was sufficient to elute the enzyme. Active fractions were pooled, dialyzed overnight against buffer B containing 0.2 M NaCl and 50% glycerol and stored in 0.1 ml portions in liquid nitrogen.

The RNA polymerase assay contained in 50 μl: 10 mM Tris-HCl, pH 8.2, 10 mM $MgCl_2$, 0.1 mM EDTA, 0.2 mM K_2HPO_4, 0.1 mM 2-mercaptoethanol, 0.1 mM GTP, ATP and CTP and 2.5–7 × 10^5 nmol [^{3}H]UTP and 1–5 μg *S. aureofaciens* DNA. One unit of RNA polymerase activity is defined as the amount of enzyme catalyzing the incorporation of 1 nmol UMP into acid precipitable form in 30 min at 37°C. SDS-polyacrylamide slab gels (7.5%) and samples (5 μg of each enzyme was used) were prepared and the electrophoreses carried out as in [11].

3. RESULTS AND DISCUSSION

3.1. *Purification of* S. aureofaciens *RNA polymerase*

Chromatography on DEAE-cellulose gave one major peak of activity (fig.1, upper panel), and sometimes additional small peaks at 0.22 and 0.38 M $(NH_4)_2SO_4$ (not shown). Using heparin-Sepharose chromatography (fig.1, lower panel) an additional > 10-fold purification was achieved with a specific activity of ~150 units/mg. Finally, affinity chromatography on DNA-cellulose gave only one protein peak at 0.4 M NaCl overlapping with the enzyme activity (not shown). The specific activity is 10% greater than after heparin-Sepharose chromatography caused by the removal of two minor proteins (fig.2) which apparently do not contribute directly to the activity of the

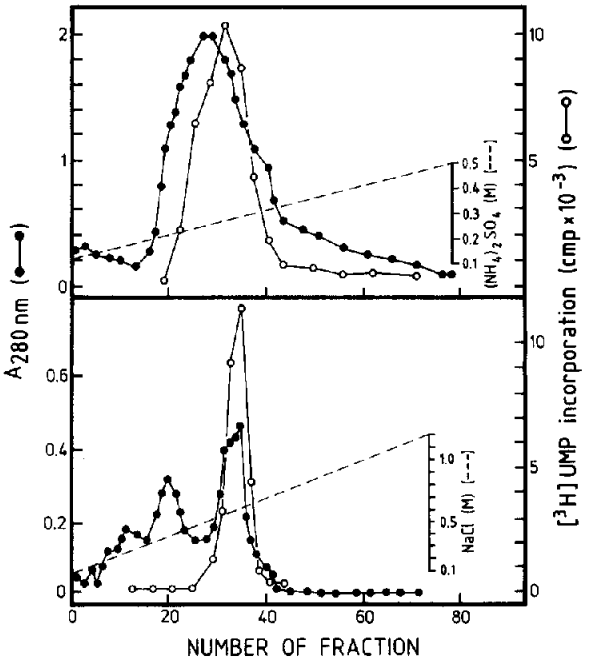

Fig.1. (Upper) DEAE-cellulose chromatography of *S. aureofaciens* RNA polymerase. (Lower) Heparin-Sepharose chromatography of *S. aureofaciens* RNA polymerase. For experimental details see section 2.

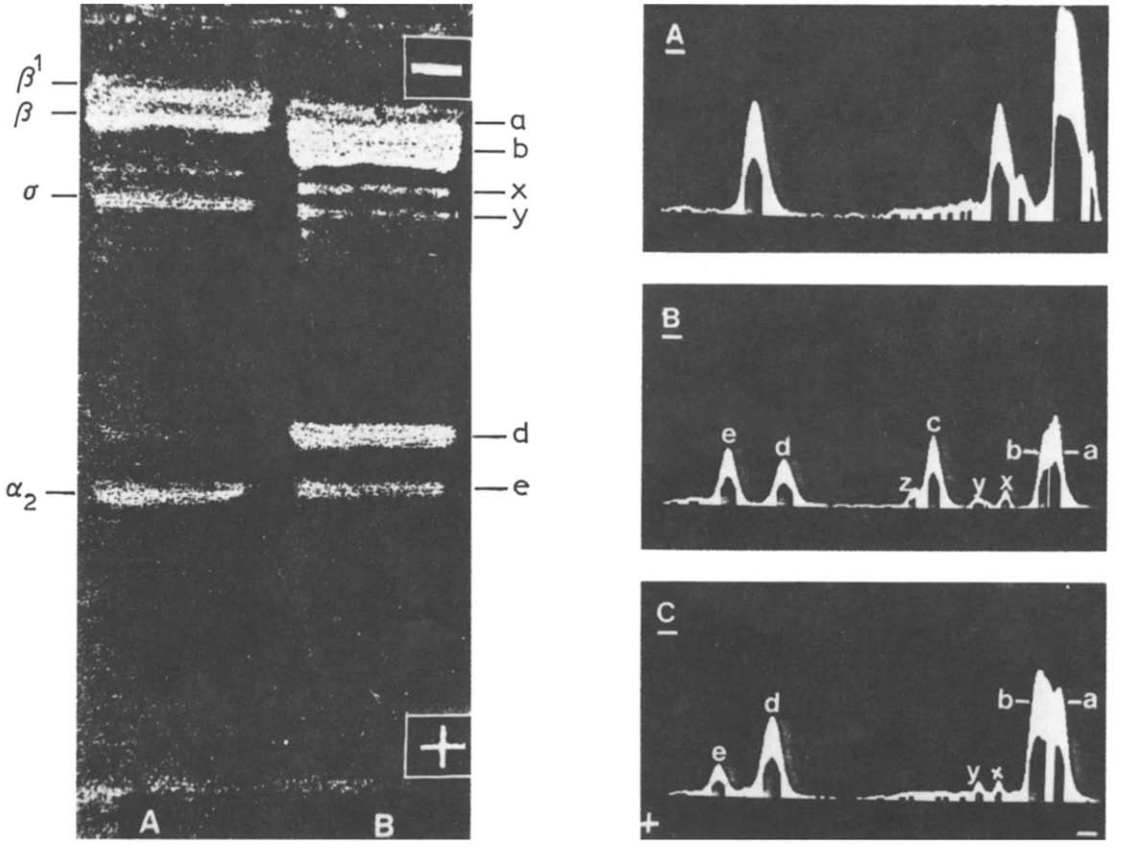

Fig.2. (Left) Electrophoresis of RNA polymerases from *E. coli* (A) and *S. aureofaciens* after chromatography on DNA-aminooxybutyl-cellulose (B). (Right) Videoscanner (E 599 nm scanner with high differentiating ability, equipped with video display, laser recorder and computer [13]) displays of RNA polymerase from *E. coli* (A), from *S. aureofaciens* after heparin-Sepharose chromatography (B) and after chromatography on DNA-aminooxybutyl-cellulose (C).

polymerase. The overall yield is ~4 mg of RNA polymerase from 0.5 kg wet wt of mycelium.

3.2. *General properties of* S. aureofaciens *RNA polymerase*

All four NTPs, DNA and a divalent cation are necessary for activity (table 1). From cation titration curves a sharp optimum at 3 mM $MnCl_2$, pH 7.8, and a broad optimum at 5–10 mM $MgCl_2$, pH 8.2, are observed in the presence of 10 mM NaCl (not shown). The enzyme activity is inhibited in the presence of similar concentrations of rifampicin, chlorotetracycline and ppGpp as other bacterial RNA polymerases (table 1). The in vitro inhibition of the purified enzyme by chlorotetracycline is in agreement with the in vivo inhibition of RNA synthesis by chlorotetracycline [10]. Whether the RNA polymerase in vivo is protected against the endogenous chlorotetracycline is still a matter of investigations. The rapid reduction of RNA synthesis and the beginning of the synthesis of highly phosphorylated nucleotides could correspond to the inhibition of RNA polymerase by ppGpp in vitro (table 1). The finding that in *E. coli* [12] high concentrations of tetracycline mimic ppGpp in specifically inhibiting rRNA synthesis strengthens this consideration.

3.3. *Subunit composition*

S. aureofaciens RNA polymerase displays after heparin-Sepharose chromatography five major (a,b,c,d,e) and three minor (x,y,z) proteins (fig.2). This is similar to the subunit composition of *Bacillus subtilis* [14] and *S. granicolor* [15] RNA

Table 1
Requirements for transcription by *S. aureofaciens* RNA polymerase

Reaction conditions	Nucleotide incorporated (%)
Complete	100
Omit DNA	0.9
Omit CTP (omit GTP/ATP)	0.2 (0.7/0.8)
Add rifampicin[a] (0.07 mM)	0
Add chlorotetracycline[a] [0.5 mM (1 mM)]	33 (0)
Add ppGpp[a] [0.5 mM (1 mM)]	65 (39)

[a] These components were added to the enzyme before DNA and NTP

The assay uses native *S. aureofaciens* DNA and 0.2 unit of enzyme; total nucleotide incorporation in the complete reaction was 0.25 nmol

Table 2
Molecular masses (kDa) of RNA polymerase components from *S. aureofaciens*

Component	*S. aureofaciens*	*E. coli*
a	145	160 β'
b	136	155 β
x	110	110 ϱ
y	84	85 σ
c	68	–
z	59	–
d	44	40 α
e	39	–

The molecular masses were calculated from the mobilities of the subunits of RNA polymerase from *E. coli*

polymerases. After the affinity chromatography the c and z proteins are absent and the quantity of e protein decreased. Occasionally both the d and e proteins were absent. The variable amount or the absence of these proteins do not affect the activity of the enzyme in vitro. The variability of these proteins could be due to uncontrolled changes during isolation or could be caused by the considerable physiological variability of *S. aureofaciens*.

Perhaps these proteins are subject to regulation of distinct promotor regions in connection with the production of chlorotetracycline by the organism.

SDS-PAGE electropherograms were analyzed densitometrically at 599 nm using a scanner with high differentiating ability [13]. The data showed that the proteins a, b and d are present in the ratio a:b:d = 1:1:2. Table 2 shows the approximate molecular mass of *S. aureofaciens* RNA polymerase subunits compared with *E. coli* RNA polymerase. This corresponds to prokaryotic RNA polymerases of the type $\alpha_2\beta,\beta'$ [16]. Preliminary experiments regarding the function of the single subunits of *S. aureofaciens* RNA polymerase by DNA recombinant technology are in agreement with recent studies of *S. coelicolor* RNA polymerase [17,18].

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