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A TEMPLATE INDEPENDENT, RIFAMPICIN SENSITIVE POLY (A)·POLY (U) SYNTHESIZING ACTIVITY PRESENT IN BACILLUS SUBTILIS

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SUMMARY: A template independent poly (A)·poly (U) synthesizing activity has been isolated from Bacillus subtilis. This activity is eluted from a DNA-cellulose column along with DNA-dependent RNA polymerase. The column fractions which exhibit this activity contain RNA polymerase holoenzyme plus a polypeptide which is slightly larger than sigma factor; pure RNA polymerase holoenzyme did not synthesize poly (A)·poly (U). The activity was dependent on the presence of ATP, UTP, and Mn^{++} (Mg^{++} could not substitute), and was inhibited by rifampicin, streptolydigin, and Cibacron Blue. The incorporation of nucleotides was not linear with time, but appeared after a lag period. The results suggest that a modified form of DNA-dependent RNA polymerase analogous to Escherichia coli holoenzyme II is catalyzing the synthesis of poly (A)·poly (U).

INTRODUCTION

Recent studies have shown that Bacillus subtilis DNA-dependent RNA polymerase can be purified in at least two forms from a DNA-cellulose column (1,2). One form which eluted at about 0.4-0.5 M KCl consisted of a 21,000 dalton polypeptide (δ) associated with core enzyme ($\alpha_2\beta\beta'$) ; the other major form was the holoenzyme ($\alpha_2\beta\beta'\sigma$) which eluted at about 0.55-0.65 M KCl. We report here a minor fraction eluting with the holoenzyme peak which contained in addition to holoenzyme a polypeptide (58K) with a molecular weight of about 58,000 daltons. This 58K-containing fraction, but not the pure holoenzyme, exhibits a template independent poly (A)·poly (U) synthesizing activity which was inhibited by RNA polymerase specific antibiotics and Cibacron Blue. The properties of this activity are similar to that reported for Escherichia coli holoenzyme II, which had σ' factor associated with the core instead of σ factor (3,4).

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MATERIALS AND METHODS

Bacillus subtilis W168 was grown in super rich medium as described previously (5) except that the culture was harvested in late exponential phase at 1400 or 1100 Klett Units. When the Klett Units reached 750, 1.5 ml of 6 N NH_4OH was added per liter to raise the pH. RNA polymerase was purified from 175 grams of cells by phase extraction followed by chromatography on DEAE-cellulose and then DNA-cellulose columns as described previously (1,5). Phosphocellulose column chromatography was used as previously described (5).

The assay system for poly (A)·poly (U) synthesis was essentially that of Iwakura and associates (4). The reaction mixture (0.25 ml) contained 120 mM Tris·HCl buffer (pH 7.9 at 22 C); 4 mM $MnSO_4$; 5 mM dithiothreitol; 0.4 mM each of ATP and [^{3}H]UTP (2.8 x 10^4 cpm/nmole). For [^{3}H]AMP incorporation into poly (A)·poly (U) the reaction mixture contained 0.4 mM of UTP and [^{3}H]ATP (2.8 x 10^4 cpm/nmole). Modifications of the assay are noted in the legends. The reaction was carried out at 37 C for the indicated times and the incorporation of [^{3}H]UMP or [^{3}H]AMP into acid precipitable counts was determined as described previously (6).

The anionic SDS-polyacrylamide urea gel system of Wu and Bruening (7) was used as modified previously (1).

RESULTS AND DISCUSSION

Isolation of poly (A)·poly (U) synthesizing activity

The final step of purification of the DNA-dependent RNA polymerase was its elution from a DNA-cellulose column by a KCl gradient (Figure 1). The holoenzyme eluted after the δ-core enzyme fractions as reported previously (1,2). When the holoenzyme began to elute from the DNA-cellulose column, there appeared in addition to holoenzyme subunits, a polypeptide (58K) which migrated more slowly than the sigma factor in an urea-SDS-polyacrylamide gel electrophoresis system (Fig. 1, fractions 82,84). Previously a polypeptide with a molecular weight of 60,000 daltons which also migrated more slowly than sigma factor was found to be associated with RNA polymerase from B. subtilis (8). As with this polypeptide our 58K polypeptide was isolated with the RNA polymerase from late logarithmic cultures of B. subtilis. This polypeptide was also similar in size to the sigma prime factor of Escherichia coli holoenzyme II which exhibited template independent poly (A)·poly (U) synthesizing activity (4). Enzymes prepared from a number of cultures grown to late log phase con-

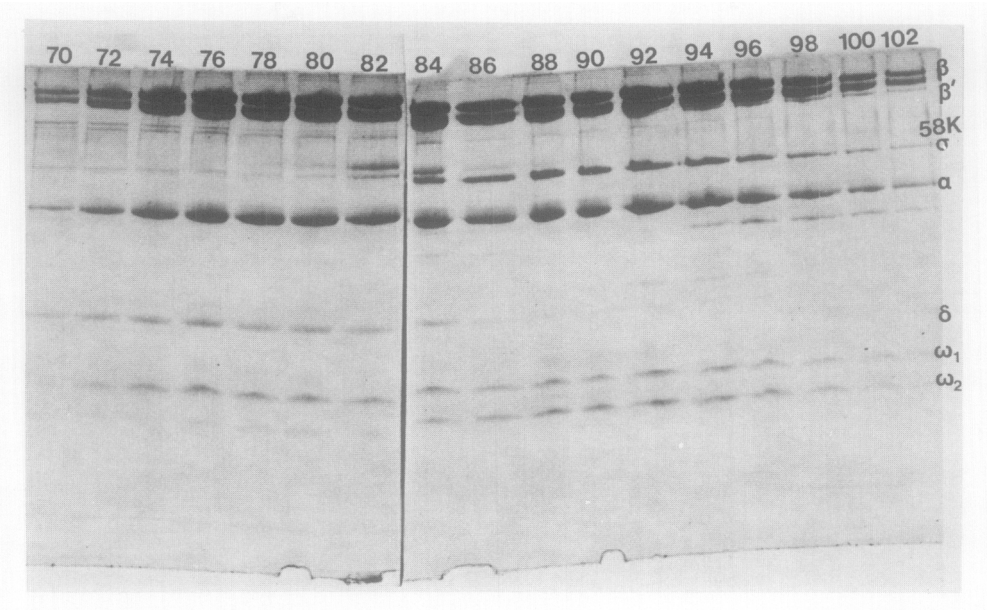
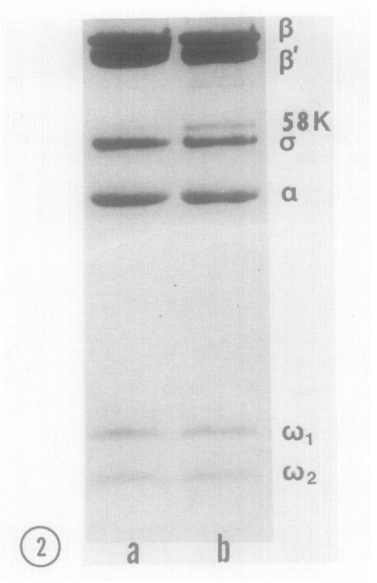


Figure 1. A typical urea-SDS-polyacrylamide (10%) slab gel electrophoresis pattern of RNA polymerase eluted by use of a salt gradient from a DNA-cellulose column (5). These cells were grown to 1400 Klett Units.

tained the 58K-polypeptide associated with RNA polymerase holoenzyme fractions (Fig. 2). These fractions showed poly (A)·poly (U) synthesizing activity. Those fractions containing pure holoenzyme or core enzyme did not exhibit this activity (Table 1).

Characterization of the poly (A)·poly (U) synthesizing activity

The poly (A)·poly (U) synthesizing activity did not require a DNA template, but required ATP, UTP, and Mn^{++}, and was inhibited by rifampicin, streptolydigin, and Cibacron Blue (Table 1). The incorporation of nucleotide into poly (A)·poly (U) occurred only after a lag period (Fig. 3). All of these properties and requirements are identical to the requirements for the E. coli holoenzyme II (4).
In addition when the fractions shown in Fig. 1 were pooled and chromatographed on phosphocellulose columns, most of the σ and δ factors were found in the flow-through fractions (Fig. 4, fractions 4-18), while the 58K polypeptide remained on the column and was step eluted at higher ionic strengths with core subunits (Fig. 4, fractions 40-50). The σ' subunit of holoenzyme II of E. coli is also



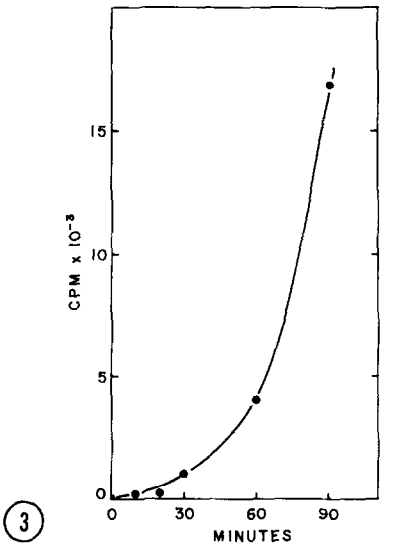


Figure 2. Urea-SDS-polyacrylamide (10%) slab gel electrophoresis pattern of RNA polymerase (a) holoenzyme and (b) the enzyme fraction containing poly (A)·poly (U) synthesizing activity. These cells were grown to 1100 Klett Units.

Figure 3. Kinetics of incorporation of ^{3}H-UMP into poly (A)·poly (U).

not removed by phosphocellulose column chromatography (4).

The inhibition by rifampicin and streptolydigin indicate that the poly (A)·poly (U) synthesizing activity is due at least in part to RNA polymerase core activity, since recent studies with B. subtilis RNA polymerase have shown that rifampicin resistance is due to a mutation in the β subunit (1) and streptolydigin resistance is due to a mutation in the β' subunit (Halling, Burtis, & Doi, submitted for publication). Cibacron Blue has also been shown to be an inhibitor of RNA polymerase (9) and is an effective inhibitor of poly (A)·poly (U) synthesis. Since pure holoenzyme and core enzyme do not exhibit the activity (Table 1), the activity may be due to the presence of the 58K protein in association with either the holoenzyme ($\alpha_2\beta\beta'\sigma$58K) or the core ($\alpha_2\beta\beta'$58K) The small amount of 58K-containing fractions in vegetative cells has so far precluded a reconstitution study between 58K protein and RNA polymerase core or holoenzyme.

Table 1

Requirements for the Synthesis of Poly (A)·poly (U)

Conditions	[^{3}H]UMP Incorporation Exp. 1	Exp. 2	[^{3}H]AMP Incorporation Exp. 3
	(cpm)	(cpm)	(cpm)
Complete	1,870	7,487	1,724
- ATP	189	--	--
- UTP	--	--	21
- Mn^{++}, + Mg^{++} (4 mM)	332	--	--
+ Rifampicin (1 µg)	14	--	3
+ Streptolydigin (12 µg)	--	278	--
+ Cibacron Blue (8 µM)	--	--	12
- enzyme, + holoenzyme (10.5 µg)	83	--	--
- enzyme, + δ-core enzyme (8 µg)	40	--	--

The incubation period was 60min in Exp. 1 and 120 min in Exp. 2 and 3. The reaction mixtures contained 1.88 µg of the enzyme fraction shown in Fig. 1, lane b. Both antibiotics were added last to the reaction mixtures. The background of 289 cpm was subtracted from all the values reported. The cpm values represent the average values of three reaction mixtures.

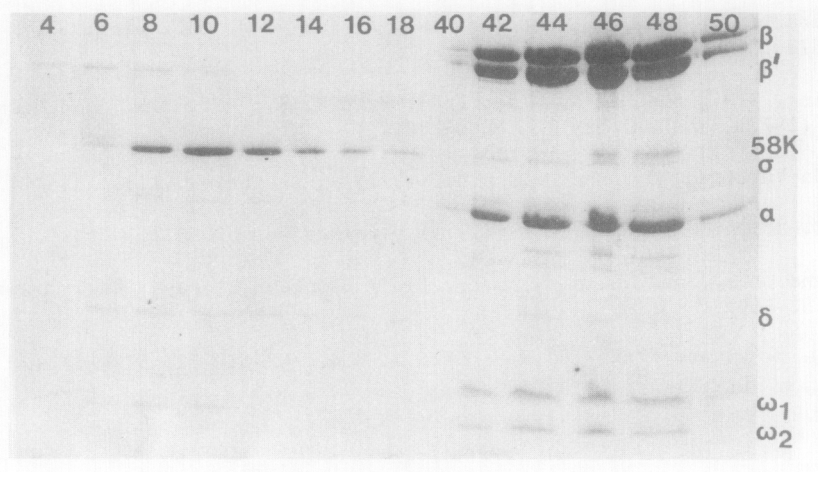


Figure 4. Urea-SDS-polyacrylamide (10%) slab gel electrophoresis pattern of fractions eluted from a phosphocellulose column.

The identification of poly (A)·poly (U) synthesizing activity in B. subtilis and in E. coli indicates that this may be a general activity for a portion of the RNA polymerase in the cell. Since this activity appears in late logarithmic cells, an investigation of stationary phase B. subtilis cells has been undertaken. Preliminary results indicate that the 58K-core enzyme predominates over the holoenzyme and is a major form of the RNA polymerase found in stationary phase and sporulating cells (Dooley, Halling, & Doi, unpublished results).

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