Conversion of Escherichia coli RNA polymerase to a template independent enzyme

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ABSTRACT
Preparations of RNA polymerase (E.C.2.7.7.6) from uninfected Escherichia coli, T^ infected Escherichia coli, and Acinetobacter calcoaceticus when centrifuged in sucrose gradients in the absence of magnesium ions gave rise to five peaks, all of which were able to form polymers from ribonucleoside 5'-triphosphates in the absence of template or primer. All of the peaks obtained from the Escherichia coli enzyme appeared to contain the subunit a and p' and, in addition, polypeptides which appeared to be derived from the subunit p.

INTRODUCTION
RNA polymerase from Escherichia coli (E.C.2.7.7.6) consists of the subunits a, p, p' and p in the stoichiometric relationship a_p_p_p (1). Some preparations contain a polypeptide unit of molecular weight approximately 60 000 daltons. This unit is designed a' and has p-like properties (2). The a' containing enzyme has been reported to be able to form poly(A)·poly(U) in the absence of template (3). RNA polymerase have been dissociated reversibly (4-9). The a particle may have the ability to catalyze the formation of poly(A) (10,11). To our knowledge none of the other subunits or combinations of subunits has been claimed to form polymers from ribonucleoside 5'-triphosphates (12,13,14).

In this work we have shown that E.coli polymerase, under conditions which evidently involve dissociation and aggregation of subunits of the enzyme, can be converted into entities which polymerize ATP, GTP and UTP in the absence of DNA template or RNA primer (OTP has not been tested). The original enzyme preparations were completely inactive without DNA added. Similar results were obtained with RNA polymerase preparations from T^ infected E.coli and Acinetobacter calcoaceticus.
In the course of this work Fukasawa and Mizuochi (15) reported that RNA polymerase from Cauliflower inflorescence was transformed to a template independent enzyme when dialyzed against a buffer not containing magnesium ions.

MATERIALS AND METHODS

All chemicals used were of the highest commercial grade. ATP, CTP, GTP, UTP, pancreatic RNAsase I, pancreatic DNAase I, calf thymus DNA, and rifampicin were obtained from Sigma Chemical Company. Phenylmethylsulfonyl fluoride and dithiothreitol were from CalBioChem and N,N'-methylene bis-acrylamide was from British Drug House Ltd. N,N,N', N'-tetramethylene diamine and acrylamide were products of Eastman Organic Chemicals. [3H]ATP, [3H]UTP and [3H]GDP were purchased from The Radiochemical Centre, England. [3H]GTP and [3H]ADP were from New England Nuclear, West Germany.

RNA polymerase from E. coli K 12 (MRE 600) and from Acinetobacter calcoaceticus were gifts from Dr. A.K. Abraham (16) and Dr. R.K. Kleppe (17), respectively.

RNA polymerase was prepared from uninfected E. coli B as described by Berg et al. (18). Filtration on Biogel P-300 was omitted. The activity of this enzyme was 3520 nmoles per hr per mg protein. RNA polymerase from T4 infected E. coli B (6 min infection) was isolated essentially as described by Abraham (15).

All but one of the RNA polymerase preparations contained a 60 000 daltons unit. This unit was separated from the enzyme by centrifugation in a glycerol gradient in the presence of a high salt concentration (19).

RNA polymerase activity was measured in a 500 µl reaction mixture containing 550 yM ATP, 330 yM CTP, 330 µM GTP, 130 µM [3H]UTP corresponding to 2 µCi, 50 mM Tris-maleate-KOH (pH 7.2), 50 mM KCl, 8 mM MgCl2, 5 mM 2-mercaptoethanol, and 25 µg DNA of calf thymus DNA. Polymerization in the absence of template was determined under otherwise the same conditions. When ATP, GTP, or UTP were used separately as substrates, their concentration was 130 µM.

The radioactive filters were dried and counted as described.
Sucrose gradients (5-45 %) were prepared in a buffer containing 20 mM Tris-HCl (pH 7.3), 5 mM 2-mercaptoethanol, 25 mM KCl, and in addition 0.1 mM Mg++ when specified. Centrifugation was performed for 12 hours at 27 000 rpm (4°C) in a 3x20 ml swing out rotor (MSE ultracentrifuge). Trichloroacetic acid (final concentration 5%) was added to the fractions from the gradient, the precipitates spun down, washed three times with ether, and finally dissolved in the buffer just described (without Mg++). Proteins were then denatured by incubation at 80°C for 10 min in the presence of 1.0% SDS and 10 mM dithiothreitol. Glycerol and bromphenolblue were added to final concentrations of 12% and 0.002% respectively, and 70 - 100 µl of the mixture applied to a gel containing 7.5% acrylamide, 0.1% SDS and 0.38 M Tris base (pH 8.5). The gels were prepared in 12 cm tubes with a diameter of 0.5 cm and run at room temperature for 2.5-3 hours at 4 mA per tube. Fixation and staining were performed as described by Shapiro et al. (21) and by Burgess (4), respectively. Destaining was carried out in a mixture of methanol-acetic acid-water (5:1:5). The gels were scanned with a Shimadzu recording spectrophotometer (MPS-50L).

Protein concentration was determined according to the method of Lowry et al. (22) using bovine plasma albumin as a standard.

**RESULTS**

A preparation of RNA polymerase isolated from *T₄* infected *E.coli* was analyzed by sucrose gradient centrifugation. *E. coli* RNA polymerase has a sedimentation coefficient of 14-15 s (1,22). At low ionic strength, however, the enzyme is known to form aggregates (23,24). Under the conditions used in our experiments, minute amounts of four peaks were obtained in addition to the major peak of 14-15 s. One of these peaks had a molecular weight less than that of the major peak, the others were possibly polymers of the enzyme (Fig. 1). There was no activity in the absence of template; 75% of the activity could be recovered.

When Mg++ was omitted from the sucrose gradient solution, again five peaks were obtained which were located roughly as
Fig. 1. Sedimentation of RNA polymerase from T₄ infected E. coli in the presence of Mg²⁺.

The enzyme preparation (4 mg) was sedimented through a linear (5-15 % w/v) sucrose gradient (Δ-Δ). Fractions were collected and 200 μl assayed for polymerizing activity in the presence (Δ-Δ) and absence (Δ-Δ) of added DNA (see Methods).

Fig. 2. Sedimentation of RNA polymerase from T₄ infected E. coli in the absence of Mg²⁺.

The enzyme preparation (40 μg) was sedimented through a linear (5-45 % w/v) sucrose gradient x-x. Fractions were collected and 150 μl assayed for polymerizing activity in the presence (Δ-Δ) and absence (Δ-Δ) of added DNA (see Methods).
those obtained with Mg$^{++}$ added. The sedimentation coefficient of peaks I, II, III, IV and V were approximately 7, 15, 26, 44 and 72 S, respectively (Fig. 2). In this case, however, all the peaks were of about the same size. Furthermore, template addition had no effect on the activity of peaks I, III, IV and V. Only peak II, with a sedimentation coefficient like that of the main fraction of the original enzyme, was stimulated slightly by template in some of the experiments. The polymerizing activity of all the peaks added together was roughly 30% of the DNA directed RNA polymerase activity added to the sucrose gradient (see Methods). Centrifugation for 6, instead of the normal 12 hours, was not sufficient for the completion of the conversion to template independent RNA polymerase activity with a consequently lower yield of activity. Increasing centrifugation from 12 to 23 hours did not result in further increase of activity.

Similar results were obtained when various preparations of RNA polymerase from uninfected E. coli were tested (see Methods). One example is shown in Fig. 3.

Fig. 3. Sedimentation of RNA polymerase from uninfected E. coli in the absence of Mg$^{++}$. The highly purified enzyme preparation (40 μg) was sedimented through a linear (5-45% w/v) sucrose gradient. Fractions were collected and 100 μl assayed for polymerizing activity in the presence (○-○) and absence (△-△) of added DNA (see Methods).
Likewise, a preparation from *Acinetobacter calcoaceticus* was converted into five polymerizing peaks independent of added template. This preparation had no σ factor.

The activity of the template independent polymerase (peak I, II, III, IV and V) obtained from the highly purified preparations of RNA polymerase from *E. coli* with differing substrates can be summarized as follows:

1. The rate of polymer formation was approximately the same with ATP, GTP and UTP as substrates.
2. The rate of polymer formation with UTP as labelled substrate was not influenced significantly by the addition of ATP, CTP and GTP to the reaction mixture.
3. ADP and GDP did not function as substrates.
4. Mixtures of different peaks did not restore the template dependent polymerizing activity.

The products of the polymerizing reaction, obtained with the simultaneous presence of all four triphosphate substrates, were sensitive to treatment with either RNase I or 0.3 N KOH at room temperature. The reaction was not inhibited by rifampicin (20 μg/ml) (1).

Upon polyacrylamide electrophoresis it was seen that all fractions with polymerizing activity contained the alpha band and beta band, while these polypeptides were not seen between the peaks of activity. Peaks I, III, IV and V appeared to contain only one beta band, while two bands were obtained in peak II. The slower moving of these, β, was present in small and varying amounts. Scanning diagrams of the original enzyme and of peaks II and III are shown in Figs. 4 a, b and c, respectively.

Approximately 50% of the beta band (β + β') was broken down during centrifugation, and low molecular weight polypeptides appeared (approximately 20 000 daltons). The low molecular weight polypeptides were principally found in the peaks with polymerizing activity. There was no significant decrease in the α band. The 60 000 daltons unit was found in the peaks with polymerizing activity and, in addition, between peaks III and IV, and between peaks IV and V (25).
Sodium dodecyl sulphate gel electrophoresis of E.coli RNA polymerase (Fig. 4 a) and of peak II (Fig. 4 b) and peak III (Fig. 4 c) obtained from the same enzyme preparation (see Fig. 3.)

RNA polymerase and peaks II and III, isolated from RNA polymerase as shown in Fig. 3, were analyzed by electrophoresis on 0.1 % sodium dodecyl sulphate/7.5 % acrylamide gels, stained with 0.2 % Coomassie Blue in 9 % acetic acid/46 % methanol and scanned with a Shimadzu recording spectrophotometer (MPS-50L) (see Methods).
DISCUSSION

The conversion of RNA polymerase to a template independent, rifampicin resistant enzyme evidently involves the dissociation of the original enzyme into smaller units (peak I), aggregation to larger entities (peaks III, IV and V), and selective proteolytic degradation of the \( \beta \) subunit. The subunits \( \alpha, \beta' \) and \( \beta \) are reported to have unrelated sequences which differ in resistance to proteolysis by trypsin, chymotrypsin, subtilicin, and clostripain (26).

In the absence of Mg\( ^ {\text{++}} \) and at low ionic strengths, RNA polymerase could dissociate in the following manner:

\[
a_{\beta'} \beta \leftrightarrow a_{\beta'} + a_{\beta} \leftrightarrow 2a + \beta' + \beta
\]

The \( \beta \) subunit, either as free \( \beta \) or as \( a_{\beta} \), or as both, is preferentially attacked by the proteolytic enzyme which was present even in our purest preparations. This would result in the conversion of either \( \beta \) to \( \beta_D \), or \( a_{\beta} \) to \( a_{\beta_D} \), or both, where \( \beta_D \) is the resistant nucleus of \( \beta \) which still can associate with the \( \alpha \) subunit. \( a_{\beta_D} \) may then unite with \( a_{\beta} \) to form \( a_{\beta'} \beta_D \) which would be the active complex. Peak I could be a mixture of \( a_{\beta'} \) and \( a_{\beta_D} \) which forms \( a_{\beta'} \beta_D \) under the assay conditions. The separation of the aggregates from the monomer in the sucrose gradient perhaps is a driving force in the conversion of RNA polymerase to template independency.

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