STRUCTURAL AND ENZYMATIC PROPERTIES OF THE *E. COLI* RNA POLYMERASE SUBUNITS

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1. Introduction

The different methods [1–4] used to purify RNA polymerase lead to enzyme preparations which differ by their subunits composition and their specificity of transcription.

Depending on the method of preparation used, the number of polypeptides chains, as identified by electrophoresis in polyacrylamide gel, in the presence of sodium dodecylsulfate (SDS), varies from two to six [5, 6], the sedimentation coefficient at low ionic strength varies from 13*S* to 25*S* [2], and the relative rates of transcription of T4 or T7 DNA and of thymus DNA are different [3, 5]. Factors might be lost during the preparation and it is known that some of them play a critical role in the regulation of the enzymatic activity [5, 6]. Thus RNA polymerase activity cannot be ascribed to a unique, well defined, molecular species.

In this letter we describe and characterise some physicochemical, and enzymatic properties of the DNA dependent RNA polymerase of *E. coli* A19, purified, as previously described, by a method of phase partition [1]. This preparation (P) can be resolved into two fractions, A and B, by chromatography on phosphocellulose column [5]. B is a single species which has the RNA polymerase activity. A, devoid of this activity, consists of several polypeptide chains as seen by electrophoresis in polyacrylamide gel, in the presence of SDS. The addition of A to B enhances the polymerase activity of B by a factor of 3.5, when the template is T4 DNA. In addition, B aggregates at low ionic strength, but this aggregation is prevented by the presence of A. Thus, fraction A modifies both the enzymatic activity and the sedimentation properties of RNA polymerase.

2. Materials and methods

Enzyme P, prepared by Babinet's procedure [1] was further purified on a phosphocellulose column as described by Burgess [5]. Only two peaks of proteins were obtained with this type of preparation. One of these peaks was eluted with 0.05 M KCl (peak A), the second (peak B) was eluted at 0.3 M KCl. The RNA polymerase activity was determined as previously described [7] using ATP-^{3}H with a specific activity of 1660 cpm/mμM, and a concentration of T4 or Calf thymus DNA of 20 μg/ml. Poly A synthetase activity was measured as described by Chamberlin et al. [8]. Polynucleotide phosphorylase activity was measured as described by Grunberg Manago et al. [9]. T4 DNA was prepared by M. Yaniv following the procedure of Kaiser et al. [10] and of Marmur [11].

Calf thymus DNA was prepared according to Kay procedure [12] followed by digestion by pronase, treatment with lauryl sulfate and deproteinisation [13].

Polyacrylamide gel electrophoresis in sodium dedecyl sulfate was performed as described by Maizel [14]. Each sample (200 to 250 μg in 100 μl) was denatured in SDS 10% for 30 min at 40°C in presence of 10 mM of mercaptoethanol followed by addition of 60 mM of iodo-acetate. The sample then sat overnight and electrophoresis was performed the following morning,

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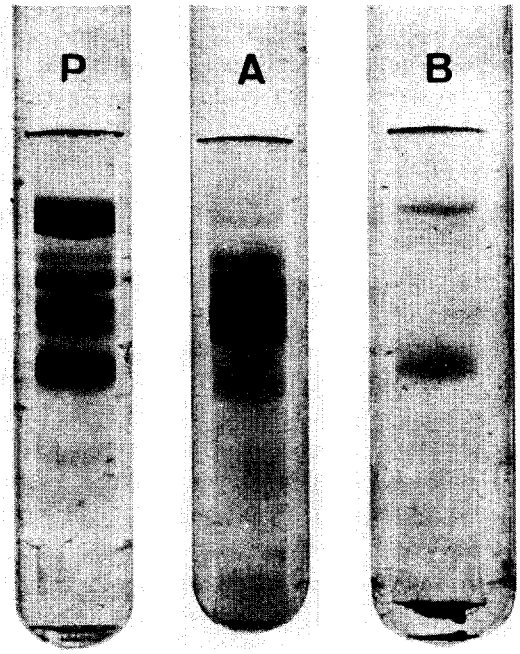

Fig. 1. Polyacrylamide gel analysis of RNA polymerase and of fractions A and B. SDS 0.1% gels pH 7.4, containing 5% acrylamide were used. Enzyme P 100 μg, A 50 μg and B 30 μg.

as described in ref. [14]. After electrophoresis the gels were stained at least for 2 hr in a solution of 0.1% of comassie brillant blue and then distained in 10% acetic acid.

3. Results

I. As shown in fig. 1, the preparation (P) of RNA polymerase gives at least six bands by electrophoresis in polyacrylamide gel in the presence of 0.1% SDS. Upon further fractionation of P through a phospho-cellulose column (see methods) two fractions A and B are separated. Disc gel electrophoresis of B in 0.1% SDS gives two bands which can be assigned to the α and β bands described by Burgess [5]. Fraction A is rather more complex: in addition to traces of β and α, the other 4 bands of P are present. Since P and A shows some polynucleotide phosphorylase activity (see below) it is likely that at least one component of A is unrelated to RNA polymerase.

II. The sedimentation properties of P have been determined in sucrose gradient at low (0.05 M) and high (0.5 M) KCl concentration. The activity was measured using T4 bacteriophage DNA and calf thymus DNA as templates. At high KCl concentration the peak of activity is sharp and corresponds to a sedimentation coefficient of 13*S*, using catalase (11*S*) as a marker. The shape and position of the peak are the same when either template (T4 DNA or claf thymus DNA) is used to detect the enzyme. At low ionic strength, the peak of activity has its maximum around 14*S*, but is broader with thymus DNA than with T4 DNA.

When the same analysis is carried out with B using thymus DNA as the template, B behaves as P at high ionic strength, but at low ionic strength all of the activity appears in a single peak of 25*S* (fig. 2).

Thus the sedimentation properties of B are very different at high and at low salt concentrations, which is not the case with P. Since P is made up of A and B, one might expect that addition of A to B would give back a complex behaving as P at low ionic strength. Indeed, A added to B prevents the formation of the 25*S* component and the complex thus formed has the same sedimentation properties as P, i.e. 13–14*S*.

III. We then looked for the catalytic properties of

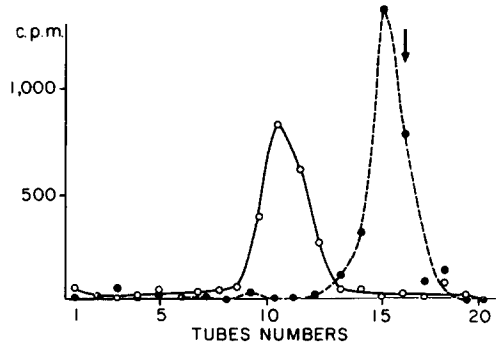

Fig. 2. Sucrose gradient profiles of fraction B. 150 μl containing 200 μg of protein was layered on top of a 5 ml 5–20% sucrose gradient containing 0.01 M tris pH 7.4, β mercaptoethanol 5×10^{-3} M and KCl 0.5 M (- - - -) or KCl 0.05 M (——). The samples had been dialysed overnight at high or low salt concentration against the above buffers. The gradients were centrifuged at 63000 rpm in a Spinco SW 65 rotor for two hours at 4°C. Template used in the tests of activity was calf thymus DNA. The arrow indicates the position of catalase. The tubes are numbered from bottom to top.

Table 1

Fractions	DNA T4	DNA Calf thymus
Enzyme P, 2 μg	1.4	1.1
Fraction B, 2 μg	0.4	0.5
Fraction A, 1 μg	0.005	0.06
A 1 μg + B 2 μg	1.3	0.5
A heated, 1 μg + B, 2μg	0.4	0.4
A, 1 μg + B heated 2 μg	0.03	0

Enzymatic activities for enzyme P, fractions A and B and a mixture of A and B. Inactivation of fractions A and B was made by heating for 10 min at 60°C. Activities are expressed in mμM of AMP ^{3}H incorporated in twenty minutes at 37°C.

fractions A and B and found that the RNA polymerase and the poly A synthetase activities are restricted to B while fraction A has the contaminating polynucleotide phosphorylase activity. In addition RNA polymerase activity tested with T4 DNA as template is 3.5 times higher for P than for B. The addition of fraction A to fraction B restores the optimal level of activity with T4 DNA, but does not increase the activity of fraction B when thymus DNA or λ DNA are used as templates. Heating of fraction A for 10 min at 60°C abolishes this stimulatory effect (table 1).

4. Discussion

Purification of RNA polymerase by the method of phase partition [1] yields a preparation P composed of several different polypeptide chains. In the native enzyme these polypeptide chains are associated into subunits which can be separated into two fractions (A and B) by chromatography. B is enzymatically active. The other, A, which does not show any RNA polymerase activity alters both the enzymatic activity and the quaternary structure of B.

We have shown that enzymatic activity of B, can be related to a single molecular species; we do not know whether the two above properties of A belong to only one [5, 6] or to both main components present in this fraction. The isolation of the various components of A should let us determine whether the modifications elicited by A are due to a single transition of structure or to a sequential series of events.

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