CHROMATOGRAPHY OF RNA POLYMERASE FROM ESCHERICHIA COLI ON SINGLE STRANDED DNA-AGAROSE COLUMNS

Christiane Nüsslein and Bertold Heyden
Max-Planck-Institut für Virusforschung,
Biochemische Abteilung,
Tübingen, Germany.

Received March 13, 1972

Summary:

The purification of RNA polymerase from E. coli on single-stranded DNA-agarose columns is described. On these columns the RNA polymerase can be separated into two fractions (1). One fraction contains core enzyme and, though inactive on T_4 DNA, a protein with the molecular weight as that of σ. The other fraction is shown to be RNA polymerase holo enzyme, based on template specificity and subunit composition. Since σ is not separated from the holo enzyme, this chromatographic method is useful for the preparation of large quantities of holo enzyme.

Introduction:

RNA polymerase from E. coli can be obtained in two functional states, the holo enzyme with the subunit composition $\alpha_2\beta\beta'\sigma$ and the core enzyme ($\alpha_2\beta\beta'$), which lacks the σ subunit and with it the ability to initiate RNA synthesis effectively on specific sites of an intact DNA template like T_4 DNA (2, for review see Ref. 3). Most of the isolation procedures for RNA polymerase yield an enzyme which contains σ, but usually in less than equimolar amounts (4,5). Whether this reflects different amounts of the core subunits and σ in the cell, or a preferential loss of σ during the purification of the enzyme, is not known. Upon chromatography on phosphocellulose σ is released from the polymerase and core enzyme is obtained.

A σ-saturated enzyme (holo enzyme) can be prepared by complementation with isolated σ (6,7).

In this communication we describe a direct method for the preparation of large quantities of σ-saturated enzyme, which does not involve the isolation of σ.

Materials and Methods:

Calf thymus DNA, type V, and agarose ("for electrophoresis") were purchased from Sigma, St. Louis, U.S.A. Biogel A 1.5 m, 200-400 mesh, was obtained from BioRad, Richmond, California, U.S.A., polyethylene glycol 20 000 from Roth, Karlsruhe, Germany. E. coli cells, strain W, were a gift from Farbenfabriken Bayer, Leverkusen, Germany. Single-stranded DNA-agarose was prepared as described (1). The final 2% agarose gel contained 3.5 mg denatured calf thymus DNA per ml bed volume.

RNA polymerase was assayed according to Burgess (8), using ^{14}C-UTP (10^6 cpm/μmole) as radioactive nucleotide and calf thymus DNA, prepared according to Kay, Simmons and Dounce (9) as template. When indicated, calf thymus DNA was replaced by T_4 DNA at a concentration of 9 μg/ml. DNA polymerase activity was assayed as described (1).

The preparation of RNA polymerase up to Fr. 4 (DEAE fraction) and the final Biogel A 1.5 m chromatography was done essentially as described by Burgess (8).

DNA-agarose chromatography: Fr. 4 (45 000 units RNA polymerase in 95 ml buffer C (8), 0.23 M KCl, corresponding to a preparation out of 250 g E. coli cells) was pumped onto the bottom of a 3.1 cm^2 × 15 cm column of DNA-agarose, equilibrated with 0.01 M Tris, pH 8.0; 10^{-3} M EDTA, 10^{-4} M dithioerythritol, 5% glycerol (standard buffer) and 0.25 M KCl. The column was washed with the same buffer and the

RNA polymerase eluted by upflow chromatography with a linear gradient of 0.25 M - 1.25 M KCl in standard buffer (total volume 600 ml). A constant flow rate during loading and elution was maintained by use of a peristaltic pump. 10 ml fractions were collected and assayed for RNA polymerase activity, using calf thymus DNA and T_4 DNA as template. Peak fractions with relatively low (Fr. A, 10 000 units) and high (Fr. B, 23 000 units) activity on T_4 DNA were pooled, concentrated by dialysis against a 20% polyethylene glycol 20 000 solution, followed by 50% glycerol in buffer A (8), 0.1 M KCl and stored at -20°C. The total recovery of the RNA polymerase activity from the column was 80%.

Protein was determined using the procedure of Lowry et al. (10) with bovine serum albumin as a standard. SDS polyacrylamide gel electrophoresis was performed according to Weber and Osborn (11). Samples were dialysed against 0.01 M sodium phosphate, pH 7.2, 10% glycerol, 0.1% SDS, 0.14 M mercaptoethanol, heated to 65°C for 10 min., and applied in a 0.1 ml portion to the gels. Electrophoresis was conducted at room temperature, using 7.5% acrylamide gels (0.5 × 10 cm), at 4 mAmpère per tube. The gels were stained with coomassie brillant blue, destained electrophoretically and scanned with a Gilford spectrophotometer. The relative molar amount of each subunit was calculated, assuming that each protein band stains equally on the basis of mass, using the molecular weights of Berg and Chamberlin (4).

Results:

As reported earlier (1), the RNA polymerase activity appears in two peaks, when eluted with a linear salt gradient from a single-stranded DNA-agarose column (Fig. 1).

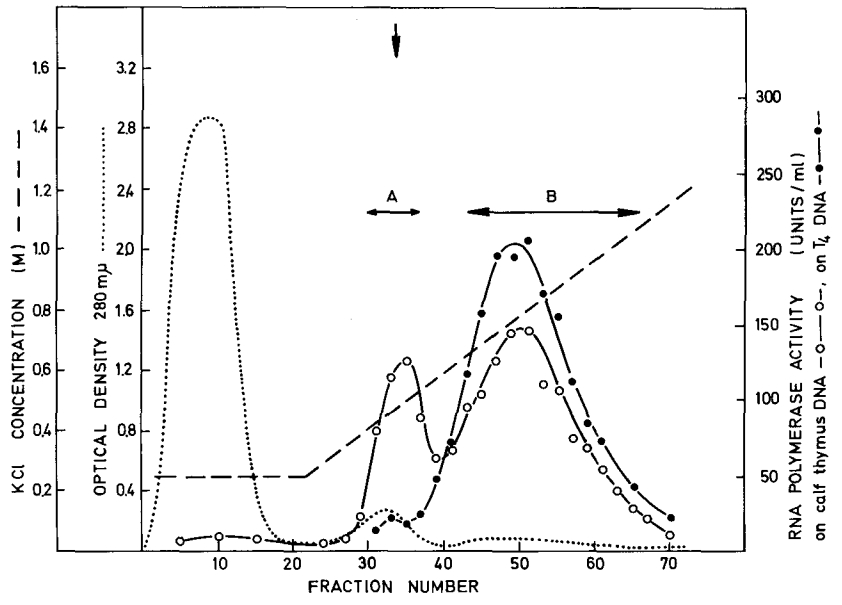

Fig. 1: Chromatography of RNA polymerase on DNA-agarose: 45 000 units RNA polymerase, Fr. 4, were applied to a 3.1 cm^2 × 15 cm DNA-agarose column and eluted with a 600 ml linear gradient of 0.25 - 1.25 M KCl in standard buffer, as described in Materials and Methods. 10 ml fractions were collected. The elution position of the DNA polymerase I marker is indicated by the arrow. Fractions were pooled as indicated to give Fr. A and B, respectively.

The first peak (Fr. A), eluting around 0.5 M KCl, contains polymerase with the template specificity of the core enzyme, i.e. it is essentially inactive with T_4 DNA as template. The enzyme eluting between 0.6 M and 1 M KCl in the second rather broad peak (Fr. B) is more active on T_4 DNA than on calf thymus DNA, suggesting that this is RNA polymerase holo enzyme.

To test whether the two peaks are an artefact due to a partial release of σ from holo enzyme during binding to the single-stranded DNA, the fractions were rechromatographed. Fig. 2 shows, that both Fr. A and B elute homogeneously at their characteristic salt concentrations. The eluted Fr. B polymerase has the same σ content as the enzyme applied (data not shown).

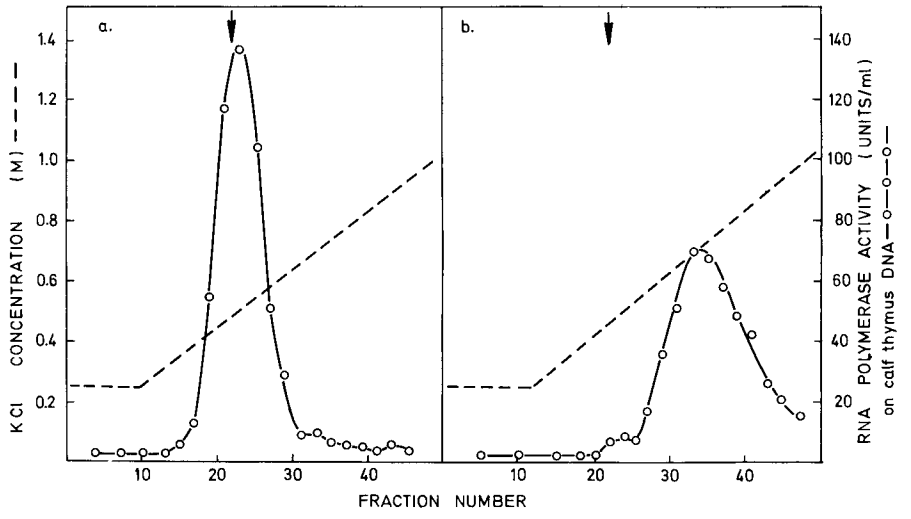

Fig. 2: Rechromatography of the DNA-agarose fractions: 5 000 units each of Fr. A (a) and B (b) were applied to a 0.8 cm^2 × 15 cm DNA-agarose column and eluted with a 120 ml linear gradient of 0.25 - 1.25 M KCl in standard buffer. 2.5 ml fractions were collected. The elution position of DNA polymerase I is indicated by the arrow.

Thus the holo enzyme appears to remain stable during binding to single-stranded DNA-agarose columns, and the heterogeneity of the RNA polymerase activity reflects low σ content of the starting material rather than σ release.

Purity and subunit structure of the different fractions were analysed by polyacrylamide gel electrophoresis. As shown in Fig. 3 a,b almost all proteins in Fr. 4 besides RNA polymerase are not retained by the column.

The gel pattern of Fr. A (the fraction inactive on T_4 DNA) (Fig. 3 c) shows besides the core subunits β,β' and α two further bands, a very strong ω protein (2) band and a band in the σ position. After A 1.5 m gel filtration [x] the ω protein completely disappears,

x) When an A 1.5 m gel filtration step precedes the DEAE chromatography, essentially the same pattern as in Fig. 3 e,f are obtained from the two DNA-agarose fractions A and B, respectively.

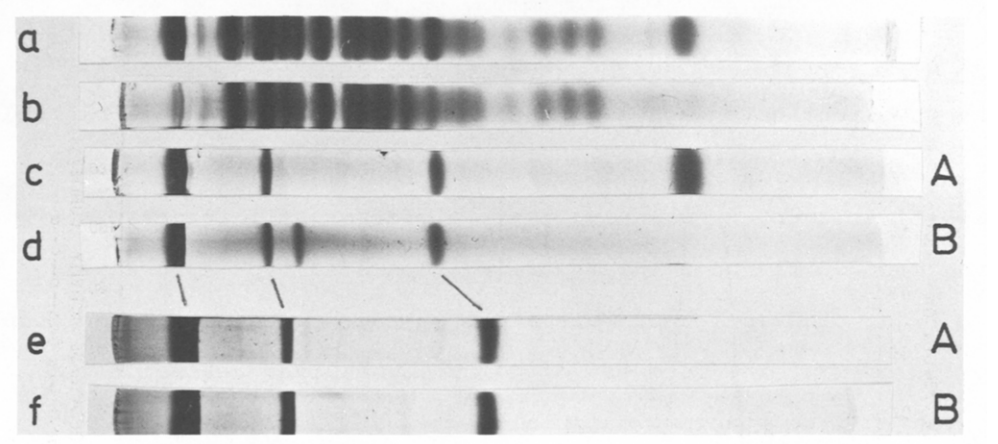

Fig. 3: SDS polyacrylamide gel analysis of the different fractions: Gel electrophoresis was performed as described in Materials and Methods. Migration was from left to right. a) Fr. 4, DEAE fraction (35 µg); b) flow through of the DNA-agarose column, tube 9, Fig. 1 (30 µg); the DNA-agarose fractions c) A (7 µg) and d) B (6 µg), and the same fractions after A 1.5 m gel filtration e) A (10 µg) and f) B (10 µg).

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while the "σ-like protein" is only slightly diminished (from 0.8 moles to 0.7 moles per two moles of α)(Fig. 3 e). Its electrophoretic mobility in urea gels appears to be different from that of σ (preliminary results).

The relative molar amount of this protein in Fr. A varied in different preparations between 0.3 and 0.8, while the elution position of peak A was not significantly changed, thus the presence of this protein appears not to alter the binding of core polymerase to single-stranded DNA.

Fr. B represents essentially pure RNA polymerase holo enzyme. The only remaining impurity can be removed by A 1.5 m gel filtration [x)] (Fig. 3 d,f). The molar proportions of the different subunits, normalised to α = 2, are $(\beta+\beta')_{2.4} : \alpha_2 : \sigma_{1.2}$ both before and after gel filtration.

Discussion:

A method for the quick and efficient preparation of RNA polymer-

ase holo enzyme from rather crude enzyme fractions is described. The purification is achieved on a single-stranded DNA-agarose column, to which most of the contaminating proteins do not bind under the conditions used. By means of their different affinities to single-stranded DNA the holo enzyme can be separated from a core polymerase fraction. Since σ is not released from the holo enzyme during binding to the column, the recovery of holo enzyme might reflect the σ content of the starting material.

Our finding, that the holo enzyme remains stable during chromatography on single-stranded DNA-agarose columns, is in contrast to the results of Krakow and von der Helm (12), who observed a release of σ from RNA polymerase of Acetobacter vinelandii in the presence of single-stranded polynucleotides. The possibility that we observe the enzyme binding to renatured double-stranded regions in the calf thymus DNA-agarose can be excluded, since similar results were obtained using single-stranded fd DNA-agarose columns.

The core polymerase fraction contains a protein which is indistinguishible from σ in SDS polyacrylamide gels. This protein is not σ, since it does not allow the transcription of T_4 DNA, it seems not to alter the binding of core polymerase to single-stranded DNA, and it is different from σ in charge. However, since it copurifies to some extent with the core polymerase, it may be some modified or inactivated form of the σ subunit. A protein with the molecular weight of σ has also been observed by Burgess (quoted in Ref. 2) as a contaminant in RNA polymerase preparations.

Acknowledgements:

We thank Ch. Kurz for preparing the DNA-agarose, S. Mrozek for helpful advice in gel electrophoresis and Drs. H. Schaller, C.N.

David and F. Bonhoeffer for discussion and comments on the manuscript.

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