# SEPARATION OF RNA POLYMERASE HOLOENZYME FROM CORE ENZYME ON DNA-CELLULOSE COLUMN

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Summary

A new and simple method was presented to isolate purified holoenzyme of *E. coli* RNA polymerase. When a purified enzyme preparation was chromatographed on a DNA-cellulose column equilibrated with a buffer containing 10mM $MgCl_2$, holoenzyme was separated from core enzyme. Thus holoenzyme was eluted at 0.15M KCl and core enzyme at 0.25M KCl.

RNA polymerase has been purified from *E. coli* almost free from any other contaminating proteins. However, a problem is still remained because the most of these purified enzyme preparations are the mixtures of holoenzyme and core enzyme, that is they contain less than one equimolar amount of σ subunit ( 1, 2, 3, 4 ). We have also observed 0.6 - 0.7 equivalent of σ in our preparations from *E. coli* B. Because core enzyme is different from holoenzyme in binding to DNA and initiation of transcription ( 3, 5, 6, 7 ), those preparations containing less than one equimolar amount of σ often make experimental results ambiguous. Preparation of σ-saturated enzyme by complementation with isolated σ is time consuming and nuisance.

Nüsslein and Heyden reported that holoenzyme was separated from core enzyme on a single-stranded DNA-agarose column (8). In this communication we present another simple method to separate them.

The σ content of the enzyme was estimated as follows. Molecular weights of subunits of RNA polymerase, β', β, σ, and α, were estimated from their relative lengths of migration during polyacrylamide gel electrophoresis following denaturation with sodium dodecyl sulfate (SDS) as described by Shapiro et al. (9). They were 160,000, 150,000, 87,000 and 41,000 respectively. Proteins used as

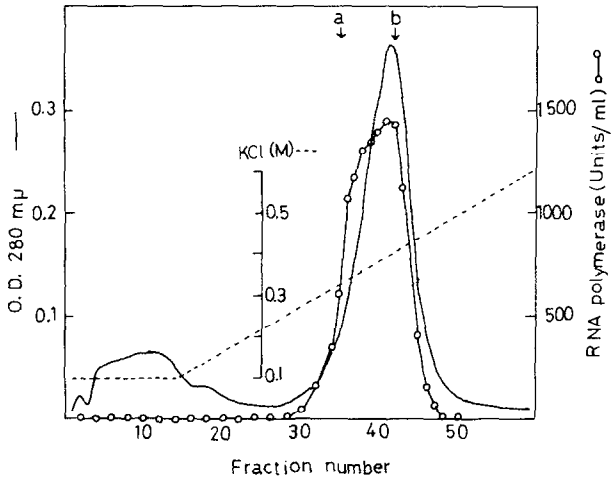


Fig. 1. Chromatography of RNA polymerase on DNA-cellulose.
5.6 O. D. Unit (280mμ) of the sample were applied to T4-DNA-cellulose column (1.5 x 2.5 cm, 400μg DNA/packed ml) and eluted with a 120 ml of linear gradient from 0.1M to 1.0M KCl in the buffer M. RNA polymerase activity was measured using T7 DNA as a template. One unit was 1mμmole AMP incorporated /hr in the standard reaction mixture (10).

references for molecular weight measurements are γ-globulin, 160,000; human serum albumin, 69,000; ovalbumin 45,000; hemoglobin, 16,000. Relative amounts of each component were then estimated by scanning stained proteins in the gels with a recording densitometer (Joyce Loebel Chromoscann). These values, normalized by the estimated molecular weights, gave approximate molar ratios of the components assuming that the each enzyme molecule contains 2 molar equivalents of α subunit.

The RNA polymerase was purified from *E. coli* B(G) as descrived by Kameyama et al. (10). In outline, cells were disrupted by RIBI Cell Fractionator (Sorvall, Inc.) and the extract was fractionated sequentially with protamin sulfate, ammonium sulfate and through a chromatography on DEAE-Sephadex A50.

This enzyme preparation, which contained 0.6 equivalent of σ, was further purified by sheared* T4-DNA-cellulose column chromatography with a linear gradient of KCl according to the method described by Alberts et al. (11). As shown in Fig. 1, RNA polymerase was eluted around 0.4M KCl when buffer M (20mM

* One-tenth of native DNA in length produced by stirring in warring blender.

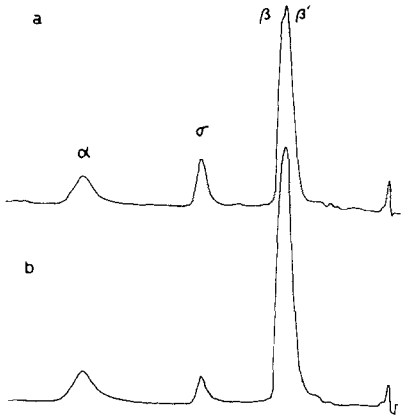


Fig. 2. SDS polyacrylamide gel analysis of fractions indicated in Fig. 1. Gel electrophoresis was performed as described by Shapiro et al.(9). The gels were stained with Coomassie brilliant blue and scanned by a Joyce Loebel Chromoscann at 620mμ.

Tris-HCl pH 7.8, 0.1mM EDTA, 0.1mM Dithiothreitol, 5% glycerol) was used. The enzyme activity was found overlapped with the optical density peak, but slightly skewed towards its front edge. Each fraction was analyzed by SDS polyacrylamide gel electrophoresis as shown in Fig. 2. The front edge of the peak contained 0.8 equimolar amount of σ, and the σ content decreased gradually to 0.5 equivalent at the trailing edge. In contract to this data, McConnel and Bonner (4) found a lower activities and lesser σ contents towards the front edge of the optical density peak. The reason of this discrepancy is not clear at this moment.

In the course of this study we found a striking effect of magnesium ion on the chromatography through DNA-cellulose column. Fractions from No.35 to No.46 in Fig. 1 were collected, dialyzed against the buffer M containing 0.1M KCl and 10mM $MgCl_2$ and charged on T4-DNA-cellulose column equilibrated with the same buffer. In the presence of magnesium ion, RNA polymerase was eluted in two peaks around 0.15M and 0.25M KCl as shown in Fig. 3. The first peak showed higher specific activity than the other, when native T7 DNA was used as a template. Approximate molar ratios of the components of the first peak were (β' + β) : σ : α = 2.0 : 1.0 : 2, while the second peak did not contain σ as determined by SDS polyacrylamide gel electrophoresis (Fig. 4). It was concluded that the first peak was holoenzyme and the second one was core enzyme.

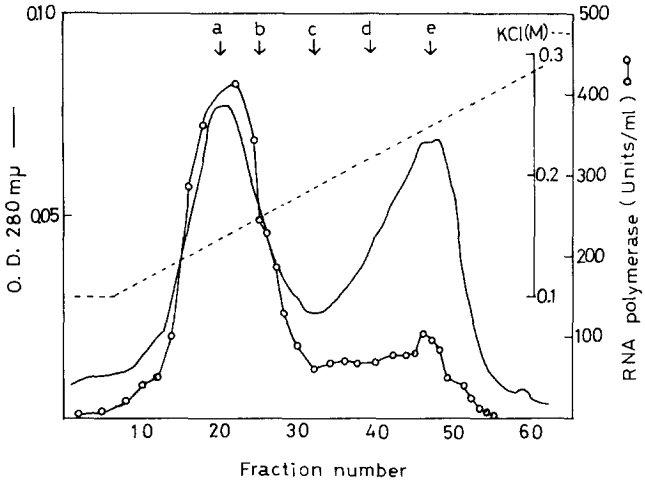


Fig. 3. Chromatography of RNA polymerase on DNA-cellulose in the buffer containing magnesium ion. Fractions from No.35 to No.46 in Fig. 1 were applied to T4-DNA-cellulose column (1.5 x 2.5 cm) eluted with a 80ml of linear gradient from 0.1M to 0.3M KCl in the buffer M containing 10mM $MgCl_2$.

For preparative purposes, the chromatography on T4-DNA-cellulose in 10mM $MgCl_2$ was carried out immediately after the chromatography on DEAE-Sephadex. The holoenzyme fraction was further purified by a chromatography on DEAE-Sephadex and a glycerol gradient centrifugation in 0.2M KCl. Throughout these procedures one equivalent of σ was retained in the enzyme.

DNA-celluloses prepared by native T4 DNA, sheared T4 DNA or native T7 DNA gave essentially the same results. Single-stranded DNA-cellulose has not been tried, though on single-stranded DNA-agarose column, core enzyme was eluted at lower ionic strength than holoenzyme in the absence of magnesium ion (8).

The conventional buffer for DNA-cellulose chromatography (11) does not contain magnesium ion, instead does contain EDTA in order to inhibit DNases in the preparation. Even with this buffer, holoenzyme tended to be eluted earlier than core enzyme (Fig. 1, 2). However, in the presence of magnesium ion, holoenzyme was completely separated from core enzyme. Magnesium ion increases melting temperature of DNA by binding to phosphate, thus stabilizing the double helix (12). This comformational change of DNA seems to affect binding of holoenzyme and core enzyme to DNA. Our data preserted here clearly indicate

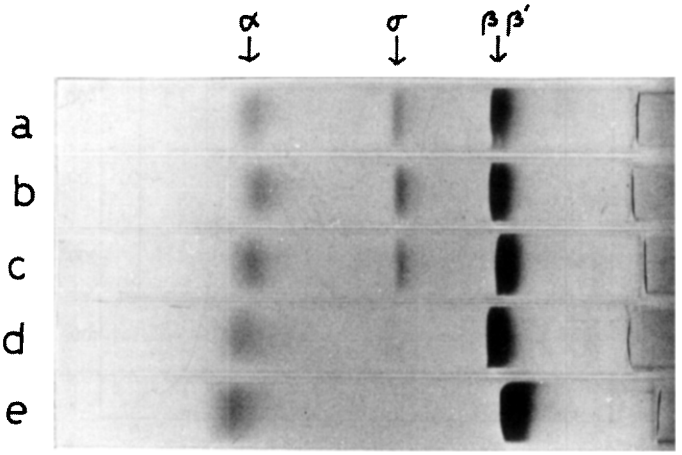


Fig. 4. SDS polyacrylamide gel analysis of fractions indicated in Fig. 3.

that in the presence of magnesium ion, holoenzyme-DNA complex was more labile to ionic strength than core enzyme-DNA complex at low temperature (5°C). At 37°C, however, holoenzyme-DNA complex was more stable to ionic strength than core enzyme-DNA complex as described by Hinkle and Chamberlin (3).

Using holoenzyme thus obtained, starting nucleotide sequences of RNA synthesized on T7 DNA were determined (13). The major terminal sequence was pppApUpCpGp--, while the enzyme containing 0.7 equivalent of σ produced RNAs with various terminal sequences.

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