

## EXCHANGE OF THE SIGMA SUBUNIT OF RNA POLYMERASE

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### 1. Introduction

The DNA dependent RNA polymerase from *Escherichia coli* can be separated into two components, the core polymerase and the sigma subunit [1]. The core polymerase contains the enzymic machinery for the synthesis of inter-nucleotide bonds [2] and has the subunit composition,  $\alpha_2\beta\beta'\omega$  [3] giving a minimum mol. wt of 400 000. The sigma subunit,  $\sigma$ , is required for the accurate initiation of RNA synthesis in vitro at many promoters [4–6] and is a single polypeptide of mol. wt 86 000 [7]. The core polymerase and sigma subunit combine to form a macromolecular complex of subunit composition  $\alpha_2\beta\beta'\omega\sigma$  [2], termed RNA polymerase holoenzyme, which can again be dissociated by chromatography on phosphocellulose [1] or by the addition of single stranded polynucleotides [8]. Dissociation also occurs during the initiation of transcription [2]. I show here that the dissociation of holoenzyme into core and sigma factor can occur in the absence of such agents and can be detected by the exchange of sigma subunit activity between distinguishable core polymerase molecules. The *E. coli* RNA polymerase holoenzyme thus exists in a state of dynamic equilibrium.

### 2. Materials and methods

#### 2.1. RNA polymerase

RNA polymerase holoenzyme and core enzyme were prepared from a rifampicin sensitive strain of *E. coli*, RFS 57 ( $F^-$  thr $^-$  leu $^-$  lac $^-$  mal $^-$  man $^-$  gal $^-$  xyl $^-$  ara $^-$  str $^r$ , az $^r$ , thi $^-$  rif $^s$ ) and its rifampicin

resistant isogenic derivative RFS 338 as described by Burgess and Travers [9]. The polymerases so prepared contained  $< 2$  mol phosphate per mol enzyme.

$^{35}\text{S}$ -labelled core polymerase was prepared by the following procedure. RFS 338 was grown to  $2 \times 10^8$  cells/ml in 100 ml medium containing 0.04 disodium hydrogen phosphate, 0.017 M potassium dihydrogen phosphate, 0.0125 M sodium chloride, 0.02 M ammonium chloride, 0.001 M magnesium chloride, 0.00005 M magnesium sulfate and carrier free inorganic sulfate at 1 mCi/5 ml. After harvesting the cell pellet was mixed with 2 g unlabelled RFS 338. The cells were resuspended in 2 ml 0.05 M Tris-HCl pH 7.5, 0.01 M magnesium chloride, 0.2 M potassium chloride, 0.1 mM dithiothreitol, 5% glycerol and briefly sonicated. 20  $\mu\text{g}$  DNase I were added and the suspension was allowed to stand for 15 min at 4°C. Core polymerase was then prepared by the method of Burgess [10]. The  $^{35}\text{S}$ -labelled polymerase was further diluted with unlabelled core polymerase to give a final specific activity of 46 cpm/ $\mu\text{g}$ .

#### 2.2. RNA polymerase assay

20  $\mu\text{l}$  aliquots of the gradient fractions were assayed for RNA polymerase activity as previously described [11] except that the labelled ribonucleoside triphosphate was ATP (s.a. 2 Ci/mole).

#### 2.3. Zone sedimentation

A 200  $\mu\text{l}$  sample was layered on a 12 ml linear 10–30% glycerol density gradient containing 0.01 M Tris-HCl pH 7.9, 0.01 M magnesium chloride, 0.36 M potassium chloride, 0.0001 M EDTA and 0.001 M 2 mercaptoethanol. The gradient was centrifuged for 24 hr at 39 000 rev/min in an International SB 283

rotor at 4°C. 0.24 ml fractions ( $\sim 50$  fractions/tube) were collected. Molecular weight markers (*E. coli*  $\beta$ -galactosidase, catalase and human hemoglobin) with sedimentation coefficients of 16 S, 11 S and 4.6 S respectively) were centrifuged on a parallel gradient. The determined S values for different RNA polymerase preparations varied by  $\pm 0.3$  S.

### 3. Results and discussion

At high ionic strength ( $\mu > 0.2$ ) and low concentration RNA polymerase core polymerase and holoenzyme sediment at 15.0 S and 12.6 S respectively [12]. However when a mixture of equivalent weight of core polymerase and holoenzyme were sedimented on a glycerol gradient only a single peak of RNA polymerase activity was detected using calf thymus DNA as template (fig.1). This single peak sedimented at

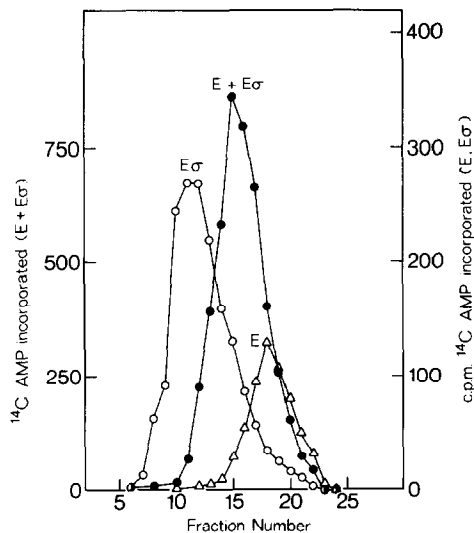


Fig.1. Composite figure showing activity profiles of RNA polymerase preparations assayed with calf thymus DNA as template after zone sedimentation on parallel gradients as described in Materials and methods. The gradients were loaded with 200  $\mu\text{g}$  RFS 57 holoenzyme ( $\circ-\circ-\circ$ ), 160  $\mu\text{g}$  RFS 57 core polymerase ( $\triangle-\triangle-\triangle$ ) and 200  $\mu\text{g}$  RFS 57 holoenzyme + 160  $\mu\text{g}$  RFS 57 core polymerase ( $\bullet-\bullet-\bullet$ ). In a parallel marker gradient of 52 fractions  $\beta$  galactosidase, catalase and hemoglobin packed in fractions 9, 22 and 40 respectively. All gradients separated into 52 fractions numbered from the bottom.

13.2 S in contrast to values of 14.1 S and 12.4 S recorded for holoenzyme and core polymerase sedimented separately at the same concentration in parallel gradients. This result is consistent with the observation that holoenzyme containing less than stoichiometric amounts of the sigma subunit sediments with an S value intermediate between that of core polymerase and holoenzyme [12]. The failure to resolve the mixture of core polymerase and holoenzyme into separate activity peaks suggests that these two forms of the enzyme may equilibrate with each other by the exchange of a subunit or subunits.

Evidence that such exchange occurs was obtained by sedimenting a mixture of rifampicin resistant core polymerase prepared from *E. coli* RFS 338 and rifampicin sensitive holoenzyme prepared from *E. coli* RFS 57. These enzymes probably differ only in the  $\beta$  subunit of the core component [13]. In contrast to the polymerase activity profile observed on mixing core enzyme and holoenzyme from the same source, the polymerase activity in the experiment was resolved in two peaks (fig.2). When assayed with calf thymus DNA, which is a template for both core polymerase and holoenzyme [1] a major peak of polymerase activity was observed sedimenting at 13.9 S and a minor peak at 12.2 S. When assayed with T4 DNA, which is a template for only the holoenzyme [1] a single peak of activity was observed at 13.9 S. In parallel control gradients holoenzyme sedimented at 14.1 S and core polymerase at 12.4 S. Thus by the criteria of template specificity and S value the 13.9 S and 12.2 S peaks must correspond to holoenzyme and core polymerase respectively.

To determine whether the subunit responsible for the rifampicin resistant phenotype sedimented with the core enzyme or holoenzyme activity or with both the gradient was assayed with calf thymus DNA as template at a rifampicin concentration which inhibited sensitive polymerase by  $\sim 95\%$  and the resistant polymerase by  $\sim 35\%$ . This assay revealed the 92% of the rifampicin resistant activity as determined by total RNA synthesis cosedimented with the 13.9 S peak (fig.2). Since the specific activity of holoenzyme with calf thymus DNA was 2.1 times that of core polymerase then approximately 84% of rifampicin resistant activity was recovered from the gradient as holoenzyme even though this activity was originally added as core polymerase. Conversely the 12.2 S core

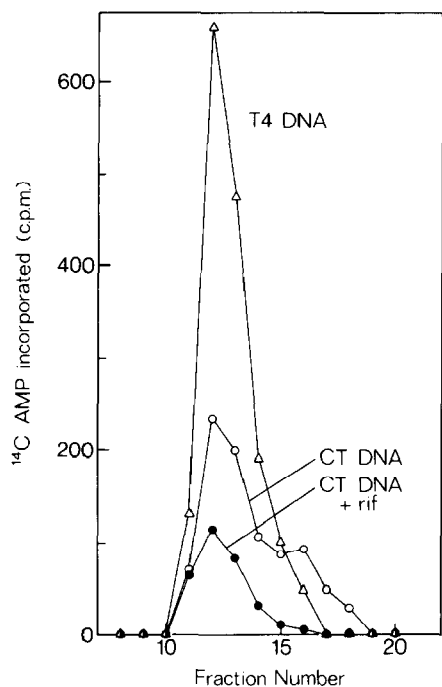


Fig.2. Zone sedimentation of a mixture of 100  $\mu$ g RFS 57 holoenzyme and 80  $\mu$ g RFS 338 core polymerase. In a parallel marker gradient of 54 fractions  $\beta$  galactosidase, catalase and hemoglobin packed in fractions 5, 19 and 41 respectively. Polymerase activity was assayed with T4 DNA ( $\Delta$ - $\Delta$ - $\Delta$ ), and calf thymus DNA with ( $\bullet$ - $\bullet$ - $\bullet$ ) and without ( $\circ$ - $\circ$ - $\circ$ ) rifampicin at 1.0  $\mu$ g/ml.

polymerase peak contained a high proportion of rifampicin sensitive polymerase activity even though this activity was originally added as holoenzyme. In a reciprocal experiment in which rifampicin resistant holoenzyme prepared from RFS 338 and rifampicin sensitive core polymerase prepared from RFS 57 were sedimented together a virtually identical profile of rifampicin resistant and sensitive polymerase activities to those shown in fig.2 were obtained (data not shown).

The result of this exchange is a preferred association of the sigma subunit with the mutated core subunit conferring the rifampicin resistant phenotype rather than with the corresponding wild type subunit. Such an affinity difference would explain the observed separation of core polymerase and holoenzyme activities on a single gradient. These experiments confirm that subunit exchange between RNA polymerase molecules occurs. To determine whether the observed

exchange involved the core polymerase as a whole or merely a component subunit or subunits,  $^{35}$ S-labelled rifampicin resistant core polymerase was mixed with a slight excess of unlabelled rifampicin sensitive holoenzyme and sedimented as before. Analysis of the resulting gradient revealed that the radioactivity was split between the core polymerase and holoenzyme peaks in the ratio 5:95 and that the radioactivity profile was that characteristic of a single peak (fig.3). This result strongly suggests that the observed subunit exchange involves the core polymerase as a whole and is thus a consequence of the rapid exchange of sigma subunit molecules between core polymerase molecules. The data do not however exclude the possibility of the slow exchange of core subunits between individual core polymerases, although such exchange has not been detected *in vivo* or *in vitro* [14].

The rifampicin resistant core polymerase of RFS

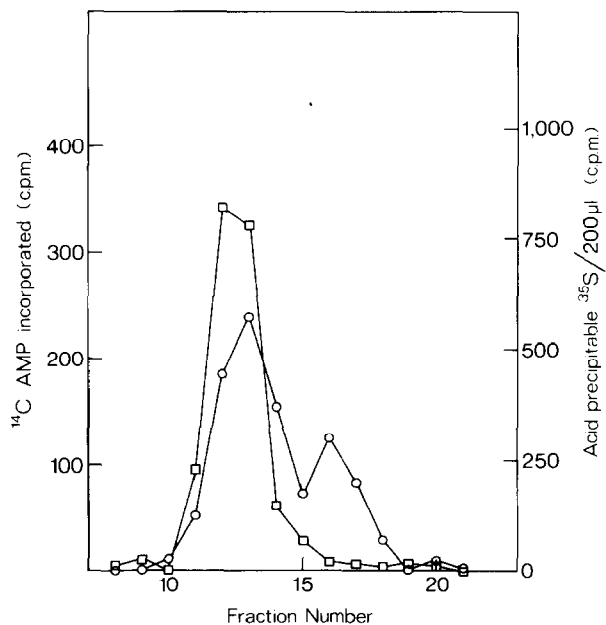


Fig.3. Zone sedimentation of a mixture of 120  $\mu$ g RFS 57 holoenzyme and 80  $\mu$ g  $^{35}$ S-labelled RFS 338 core polymerase (total added radioactivity 3680 cpm). In a parallel marker gradient of 54 fractions  $\beta$  galactosidase catalase and hemoglobin packed in fractions 5, 18 and 38 respectively. 20  $\mu$ l aliquots of the gradient fractions were assayed for RNA polymerase activity with calf thymus DNA as template ( $\circ$ - $\circ$ - $\circ$ ) and 200  $\mu$ l aliquots for acid precipitable  $^{35}$ S label ( $\square$ - $\square$ - $\square$ ). The additional  $^{35}$ S background was subtracted from the RNA synthesis assay results.

338 must have a greater affinity for a sigma subunit than the rifampicin sensitive core polymerase of RFS 57. This conclusion is consistent with the observation (Burgess, personal communication) that holoenzyme prepared from RFS 338 is much less readily dissociated by phosphocellulose than that prepared from RFS 57. The enhanced affinity for the sigma subunit demonstrated by the RFS 338 core polymerase does not seem to be a general property of rifampicin core polymerases as two other such enzymes prepared from other rifampicin resistant strains of *E. coli* did not, when cosedimented with the holoenzyme of the corresponding wild type strain, permit separation of core polymerase and holoenzyme activities into distinguishable peaks.

The experiments described here show that the sigma subunit, an initiation factor, can exchange between core RNA polymerase molecules in the absence of transcription or a DNA template. The polymerase must thus, in vitro at least, exist in a dynamic state. Such a state is a requirement of models postulating that the polymerase holoenzyme can possess variable initiation specificities as a consequence of different conformations of the enzyme [14].

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