DISSOCIATION OF DNA-DEPENDENT RNA-POLYMERASE FROM *E. COLI* IN LITHIUM CHLORIDE

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1. Introduction

DNA-dependent RNA-polymerase from *E. coli* occurs in the dimeric form (MW ∿ 900,000) at low ionic strength, whereas at high salt concentration it dissociates into the monomeric form (MW ∿ 450,000) [1, 2]. The enzyme can be further dissociated into smaller subunits at high pH or with urea [3, 4]. These subunits have been designated as β' (MW ∿ 160,000), β (∿ 145,000), σ (∿ 85,000), α (∿ 40,000) and ω* (∿ 12,000) in the order of increasing mobility in polyacrylamide gel electrophoresis in 6 M urea and 0.1% SDS [5, 6]. The σ subunit, which acts at the initiation level, can be separated from the core enzyme by cellulose phosphate column chromatography [5]. The subunits of the core enzyme have been separated by chromatography on Sephadex G-200 in 1% SDS and on DEAE-cellulose in 8 M urea [7]. All subunits of the enzyme have been isolated in pure state by electrophoresis on preparative cellulose acetate sheets in 6 M urea [6]. This paper reports the dissociation of enzyme into its subunits at high lithium chloride concentrations.

2. Materials and methods

The purification and assay of DNA-dependent RNA-polymerase from *E. coli* K12 Hfr (λ) followed procedures described in [3, 8]; core enzyme was prepared by cellulose phosphate chromatography [5]. ^{14}C-labelled ATP was from Amersham, Radiochemical Centre, England, other substrates were from C.F. Boehringer u. Soehne, Mannheim, LiCl p.A. from E. Merck, Darmstadt. TMA buffer pH 7.3 contains (mM) 100 tris acetate, 100 Mg acetate, 22 NH_4Cl, 0.25 EDTA and 0.1 mercaptoethanol. Polyacrylamide gel electrophoresis was performed in gels containing 7.5% acrylamide, 0.1% sodium dodecylsulfate (SDS), 6 M urea and 0.4 M tris pH 9.5. Analytical electrophoresis on cellulose acetate sheets ("Cellogel" from Chemetron, Milano, Italy) was performed at pH 9.2 in 6 M urea containing 0.5 M ammonium borate, 10^{-2} M EDTA and 10^{-2} M mercaptoethanol as in [9]. Aquacid was from Calbiochem, USA.

3. Results and discussion

It was observed that on incubation of RNA-polymerase in the presence of high concentrations of lithium cloride at 4°C, even after thorough dialysis, enzymic activity is lost. This appeared to be correlated to a dissociation of enzyme into its subunits. In 7–8 M LiCl, for example, the β' and β components of the enzyme are precipitated, whereas α and ω remain in solution; the subunit σ is found in both fractions. Fig. 1 shows the sedimentation pattern of the enzyme in 4 M LiCl in a sucrose gradient as well as the distribution of the subunits ($\beta' + \beta$, σ, α and ω) as determined by polyacrylamide gel electrophoresis of all 25 fractions. The gel electrophoresis of fractions 2, 6, 11, 13, 15 and 23 is documented in fig. 2A. In order to differentiate between β' and β on the gradient, electrophoresis of frac-

* It is still a matter of discussion whether ω is a subunit of the enzyme [6].

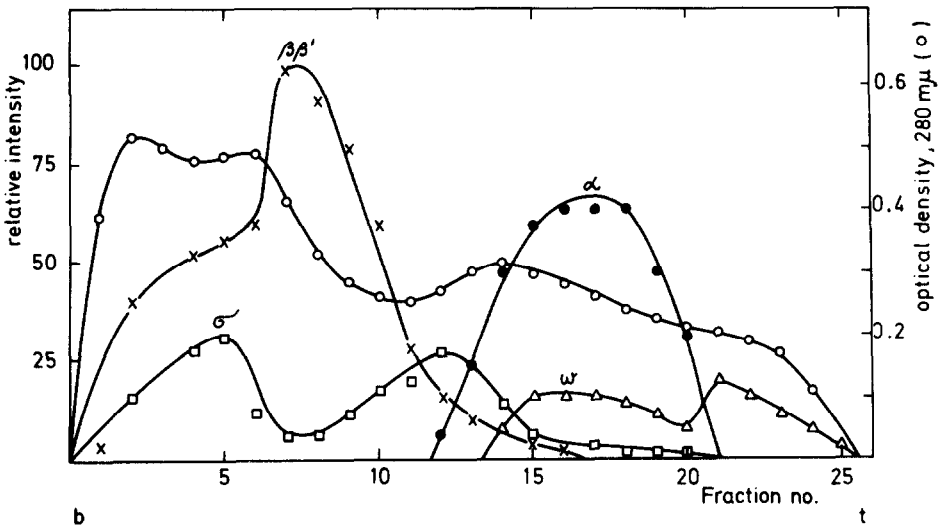

Fig. 1. Sedimentation pattern of RNA-polymerase in a sucrose gradient containing 4 M LiCl. 3.0 mg of the enzyme in 0.2 ml of 2 M LiCl TMA buffer were incubated for 15 min at 37°C; layered on a 5–20% linear sucrose gradient containing 4 M LiCl in TMA buffer and spun for 22 hr at 50,000 rpm in rotor SW 50 (Beckmann-Spinco) at 4°C. 0.2 ml fractions were collected from the bottom and the intensity (–o–o–) measured in a flow through cuvette at 280 nm in a Zeiss PMQII Spectrophotometer equiped with an automatic recorder. 50 μl of each fraction were analysed by polyacrylamide gel electrophoresis in 6 M urea and 0.1% SDS. After staining with Amido Schwarz and destaining in 7% acetic acid, the intensities of the bands were recorded with a Joyce, Loebl Chromoscan MKII Densitometer. The relative intensities of the subunits ($\beta' + \beta$ in the fraction 7 = 100) were plotted against fraction numbers. $\beta\beta'$(x–x), σ(□–□), α(●–●), ω(△–△).

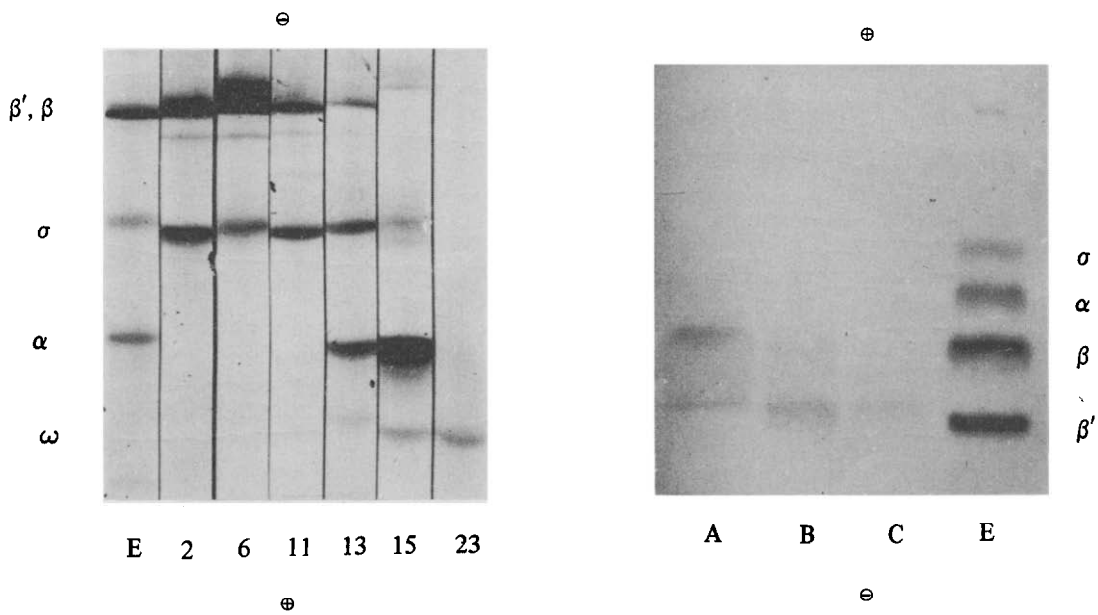

Fig. 2. A. Polyacrylamide gel electrophoresis of enzyme (E) and fractions (2, 6, 11, 13, 15 and 23) in 6 M urea and 0.1% SDS. 20 μg of the enzyme and 50 μl of the fractions were subjected to electrophoresis for 2 hr at 4 mA/tube.

B. Electrophoresis of the enzyme (E) and fractions 1–3 (A), 4–6 (B) and 7–9 (C) on cellulose acetate sheets in 6 M urea. The fractions A, B and C were dialysed for 20 hr against TMA buffer containing 5% glycerol at 4°C and then concentrated in Aquacid to about 0.1–0.2 ml. 2–4 μl of the samples were applied on "Cellogel" and the electrophoresis carried out for 45 min at 800 V.

tions 1–3 (A), 4–6 (B) and 7–9 (C) were carried out on cellogel. Fig. 2B shows that fraction A contains more β than β' and fraction B and C contain more β' than β.

It is evident from fig. 1 that $\beta' + \beta$, which could not be determined separately in disc electrophoresis, appear in two separate positions, a heavy shoulder close to the bottom of the tube and a sharp peak just above it. The shoulder coincides with a peak of σ, which is also present in another peak above $\beta' + \beta$ in the middle of the gradient. Since separation in sucrose gradient centrifugation is due to sedimentation velocity differences, it is reasonable to conclude that the heavy shoulder indicates a complex of either β' or β or both with σ, whereas the $\beta' + \beta$ peak above corresponds to a mixture of free β' and β subunits. From cellogel electrophoresis (fig. 2B) it is clear that the material under the shoulder contains a much higher amount of β than of β', whereas the peak above the shoulder contains an excess of β'. It appears probable therefore that the shoulder corresponds to a complex of β with σ, contaminated by a tail of β' from the peak above, and the lesser amount of β in this peak is due to the existence of the complex. Because of their small molecular weight difference a separation of the free β' and β on the sucrose gradient appears unlikely. The existence of the $\beta\sigma$ complex indicates a specific affinity between these two subunits. However, it cannot be decided at present whether the complex preexists in the native enzyme or is formed during dissociation. The σ band above $\beta' + \beta$ is followed, in the direction to the top, by a band of α which probably corresponds to the free subunit or a complex of α with ω. Similar to σ, the ω subunit appears in two positions, namely under the α-band and at the top of the gradient.

The fractions containing σ were tested for the activation of core enzyme. The $\beta\sigma$ fraction showed a weak stimulating activity whereas the free σ band exhibited high activation. The weak activity could be due to the inactivation of σ in the bound state or due to the nonavailability of free sigma from the complex under reaction conditions.

A systematic study of the loss of the enzyme activity with increasing concentrations of LiCl shows that after a rise in the enzyme activity, with an optimum around 1 M LiCl, there is a sharp decline of activity, which reaches an almost constant low level at 4 M LiCl and above (fig. 3). The rise around 1 M LiCl can be explained by assuming a reactivation of inactive aggregates of enzyme at high ionic strength. There is strong evidence of dissociation of the enzyme to different complexes of the subunits below 4 M LiCl [10]. The dissociation of the enzyme by LiCl in contrast to urea occurs in steps and thus this method appears to be promising in studying the arrangement of subunits in the enzyme.

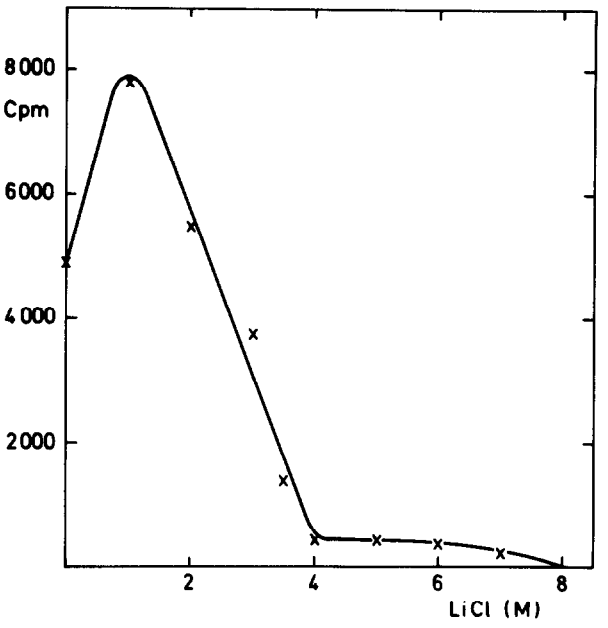

Fig. 3. Loss of enzyme activity of RNA-polymerase with increasing concentrations of lithium chloride. 1 mg/ml of enzyme solutions in TMA buffer with 5% sucrose and 2.5% glycerol containing different amounts of LiCl were kept for 30 min at 4°C and then dialysed thoroughly for 20 hr against TMA buffer containing 5% glycerol. The samples were incubated for 30 min at 37°C and specific activities were determined.

Acknowledgements

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