THE AFFINITY OF *E. COLI* RNA POLYMERASE TO MATRIX BOUND RIFAMYCIN

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1. Introduction

The antibiotic rifamycin and its various derivatives strongly inhibit procaryotic DNA dependent RNA polymerases [1]. Presently it is believed that the antibiotic complexes with free RNA polymerase molecules which then become unable to bind the initial nucleoside triphosphate thus preventing transcription [2–5]. Studies with *E. coli* RNA polymerase revealed that both core ($\beta'\beta\alpha_2$) and holo enzyme ($\beta'\beta\alpha_2\sigma$) are inhibited by rifamycin derivatives [1]. Although the mechanism of action of rifamycin has been the subject of many studies, there remained open questions. We were interested in the problem, whether certain protein subunits, like σ or ω are displaced from holo enzyme upon binding of rifamycin. The experimental approach which we employed, was to investigate the binding of RNA polymerase from *E. coli* to matrix bound rifamycin [6].

2. Materials and methods

E. coli RNA polymerase holo enzyme was isolated according to literature [7,8]. 3-(2-aminoethyl)-thiorifamycin SV was synthesized following a published procedure [9]. SDS gel electrophoresis was performed according to [10]. *E. coli* cells, strain MRE 600, were purchased from Fa. E. Merck. Rifamycin-Sepharose: 10 ml Sepharose 4B (Pharmacia) were activated with CNBr as described [11]. 3-(2-aminoethyl)-thio-rifamycin (10 mg) was dissolved in a mixture of 7 ml DMF and 2.5 ml 0.065 M phosphate buffer pH 7. This solution was added to the activated Sepharose and the suspension stirred over night at 4°C. The rifamycin-Sepharose was extensively washed with a mixture of H_2O–DMSO (3:1, v/v). From the absorbance (450 nm) of the combined filtrates a yield of 95% was estimated for the coupling reaction.

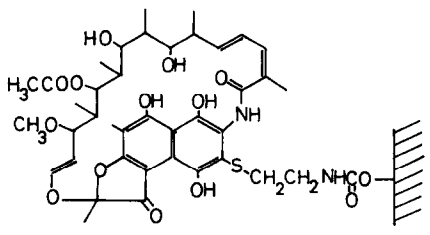
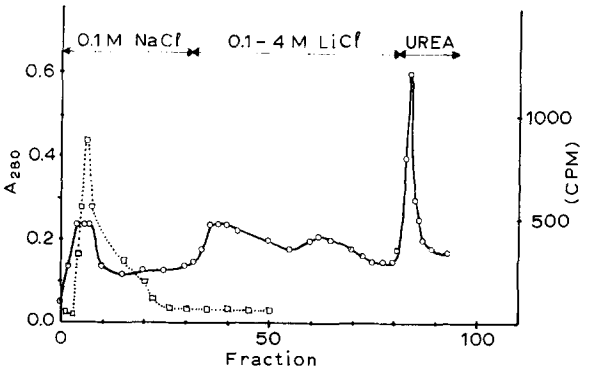


Fig.1. 3-(2-aminoethyl)-thiorifamycin–Sepharose.

3. Results and discussion

A procedure is described in the literature which allows the derivatization of rifamycin SV to 3-(2-aminoethyl)-thiorifamycin SV [9]. This derivative, bearing a reactive primary amino group, covalently reacted with Sepharose, activated by CNBr. Prolonged washing of rifamycin-Sepharose at 4°C did not remove appreciable amounts of rifamycin, as judged from attempts to inhibit RNA polymerase by these filtrates. A rather impure preparation of RNA polymerase holo enzyme, containing both σ-subunit and ω-protein component, was applied to a rifamycin-Sepharose column. A gradient of 0.1–4 M LiCl removed only traces of enzymes. Under denaturating conditions, using 6 M urea–mercaptoethanol, a protein fraction was eluted, which according to SDS gel electrophoresis contained the protein components of pure RNA polymerase holo enzyme: $\beta'\beta\alpha_2\sigma$ (fig. 2a,b). The application of large amounts of proteins of the urea fraction to SDS gels did not reveal traces of the protein component ω.



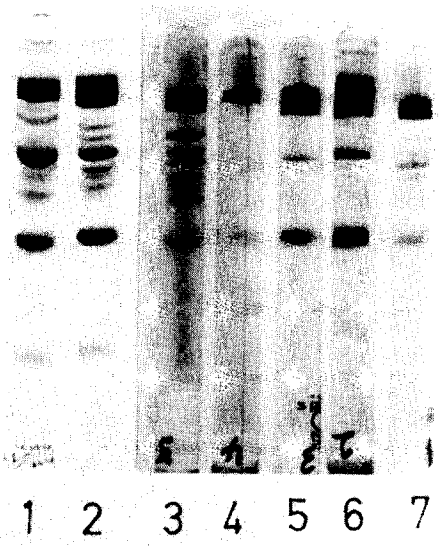


Fig.2. a) RNA polymerase holo enzyme (2 mg) in 2 ml 0.1 M NaCl, 10 mM Tris pH 7.9 was applied to a 1 ml rifamycin–Sepharose column at 4°C. The column was washed with 5 ml 0.1 M NaCl, followed by a linear gradient of 0.1–4 M LiCl in 10 mM Tris pH 7.9. Finally the Sepharose was eluted with 6 M urea, 2 mM mercaptoethanol. The enzymatic activity was measured in a standard assay [7]. Appropriate fractions were dialysed against 10 mM Tris pH 7.9, concentrated by vacuum dialysis and subjected to SDS gel electrophoresis. (□–□–□) enzymatic activity, (o–o–o) absorbance at 280 nm. b) SDS gels: 1, fraction 35–45; 2, RNA polymerase; 3, 0.1 M NaCl fraction; 4–6, 6 M urea fraction; 7, RNA polymerase.

Employing an excess of rifamycin–Sepharose, it was observed that a certain amount of RNA polymerase, varying from preparation to preparation, did not bind. This protein fraction, although displaying the correct subunit pattern in SDS gel electrophoresis of RNA polymerase holo enzyme, was enzymatically inactive.

Sometimes preparations of RNA polymerase holo enzyme contain an additional protein component with mol. wt of approx. 110 000. Stetter et al. who termed this component 'x' assume that it might be identical with protein factor 'τ' of Burgess et al. [13] and the 'inactive σ' of Nüsslein et al. [14]. An enzyme preparation containing 'x' was chromatographed on rifamycin–Sepharose.

The column was washed with either 0.1 or 4 M NaCl, then proteins were eluted with 6 M urea. SDS gels of the respective urea fractions showed that the protein component 'x' was still present. Hence high ionic strength did not displace this protein component from holo enzyme bound to rifamycin–Sepharose (fig.3).

The results can be summarized as follows: purified,

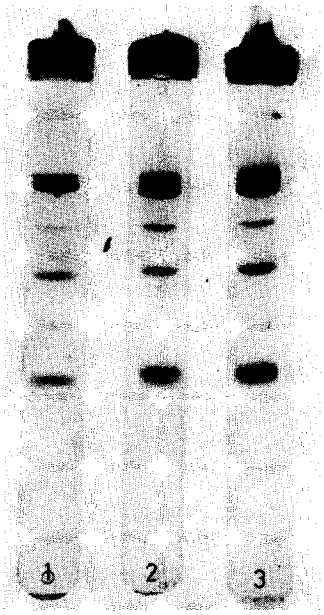


Fig.3. RNA polymerase, (0.5 mg), containing protein component 'x' was applied to a 1 ml rifamycin–Sepharose column. The column was washed with either 0.1 M NaCl or 4 M NaCl. Protein was eluted by 6 M urea, 2 mM mercaptoethanol. SDS gels of urea fractions: 3, treatment with 4 M NaCl; 2, treatment with 0.1 M NaCl; 1, RNA polymerase, unchromatographed.

native *E. coli* RNA polymerase strongly bound to rifamycin–Sepharose, enzymatically inactive enzyme did not. RNA polymerase protein was eluted by 6 M urea. Subunit σ, in contrast to protein component ω, was not displaced by high ionic strength from holo enzyme bound to rifamycin–Sepharose. A protein component 'x' of mol. wt 110 000, sometimes found in RNA polymerase preparations, was likewise not displaced by high ionic strength from holo enzyme. From this we conclude that the protein component 'x' is part of the native *E. coli* RNA polymerase. Its function remains to be elucidated.

As shown by Zillig et al. [15] *E. coli* RNA polymerase can be dissociated by LiCl into different complexes of subunits. The dissociation of the enzyme seemed to start below 4 M LiCl. RNA polymerase holo enzyme bound to rifamycin–Sepharose, however, could not be removed by elution with a linear gradient of 0.1–4 M LiCl. From this observation we conclude that binding of rifamycin to RNA polymerase stabilizes the interactions between subunits. Attempts to use rifamycin–Sepharose for the isolation of RNA polymerase from crude extracts of bacteria are presently undertaken.

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