CONFORMATIONAL CHANGES OF *ESCHERICHIA COLI* RNA POLYMERASE ON BINDING OF TEMPLATES

Walter STENDER

Physiologisch-Chemisches Institut der Universität Würzburg, Koellikerstrasse 2, D-8700 Würzburg, FRG

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1. Introduction

One way to monitor conformational changes of proteins on ligand binding is to quantitate the response of specific antibodies before and after binding by the micro complement fixation technique (C′ fixation) [1,2]. Using antibodies against RNA polymerase we have found that DNA from various sources and poly-[d(A–T)] have a very pronounced effect on C′ fixation of RNA polymerase–antibody complexes whereas rifampicin, an inhibitor of RNA synthesis, has not.

2. Experimental

Poly[d(A–T)], calf thymus DNA, adenylyluridine (ApU) and uridylyladenosine (UpA) were from Boehringer, Mannheim; *E. coli* DNA was from Serva, Heidelberg, and anti sheep erythrocytes serum (Ambozeptor) and guinea pig complement from Behringwerke AG, Marburg. Sheep erythrocytes were kindly supplied by the laboratory of Professor Dr Emmerling, Institut für Hygiene und Mikrobiologie, Universität Würzburg.

2.1. *Micro complement fixation*

The assay was carried out according to [1], scaled down by a factor of 10. Gelatine instead of bovine serum albumin was used in the diluent buffer.

2.2. *Enzymes*

RNA polymerase from *E. coli* was prepared according to [3] as modified [4]. The holo enzyme had spec. act. 10 200 units/mg when assayed with calf thymus DNA. One unit here is defined as incorporation of 1 nmol nucleoside monophosphate into acid-insoluble material per mg protein per 60 min. RNA polymerase core enzyme was prepared by Bio-Rex chromatography [3] and had spec. act. 4500 units/mg when assayed with poly[d(A–T)]. The enzymes were >95% pure as judged from dodecylsulfate–polyacrylamide gel electrophoresis.

2.3. *Preparation of antiserum*

Rabbits (4) were immunized by administration of ~1 mg RNA polymerase holo enzyme in phosphate buffered saline mixed with an equal volume of Freund's complete adjuvant subcutaneously into the back of each rabbit. After 4 weeks the sera contained antibodies against the enzyme, assayed by Ouchterlony agar gel double diffusion and micro complement fixation. Sera taken before administration of antigen did not react. Two weeks after a booster injection of again 1 mg antigen, blood was collected from the ear vein of the immunized rabbit. The booster and bleeding was repeated 4 months later. γ-Globulin from the pooled sera was purified by ammonium sulfate precipitation at 40% saturation and subsequent gel filtration on Sephadex G-150.

2.4. *Purification of antibodies*

RNA polymerase core enzyme was coupled to Sepharose 4-B with cyanogen bromide according to [5]. After binding of the antibodies to the affinity adsorbent elution was brought about by 0.1 M acetic acid. The eluate was dialyzed against phosphate-buffered saline (pH 7.4) and concentrated to ~1 mg/ml by ultrafiltration.

3. Results and discussion

Micro complement fixation showed no difference in antibody binding to RNA polymerase or to the RNA polymerase–rifampicin complex (fig.1). The results were the same with crude or purified immunoglobulins and apply to rifampicin binding to holo or core polymerase at 0°C and 37°C. On the other hand, the binding of DNA or model templates, e.g., poly-[d(A–T)] to RNA polymerase interfered with the complement fixation response. This agrees with experiments with *E. coli* DNA polymerase, where in the presence of DNA maximal complement fixation was decreased and shifted to lower antigen concentrations [6].

A more pronounced effect can be seen if the specific antibodies from the crude immunoglobulin fraction were isolated by prior adsorption to Sepharose-bound core enzyme. In fig.2 the C′ fixation of holoenzyme in the presence or absence of poly[d(A–T)] shows a much larger effect than in the case of total immunoglobulins, and a marked temperature dependence if the enzyme–template complex (prior to addition of antibodies and complement) was incubated at 0°C (A) or at 37°C (B).

The interactions of holo enzyme with calf thymus

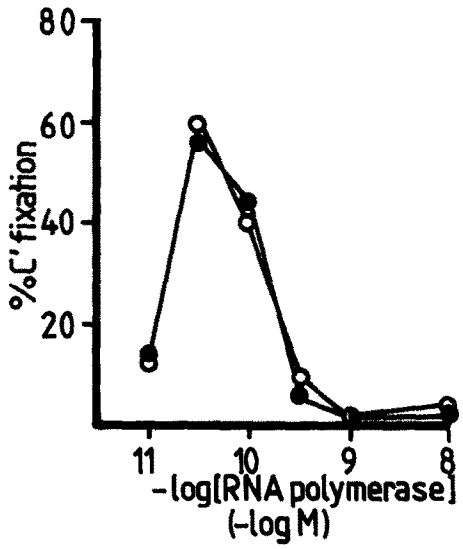

Fig.1. C′ fixation of anti-RNA polymerase antibodies with holo or core RNA polymerase with 7×10^{-7} M rifampicin (○) and without (●).

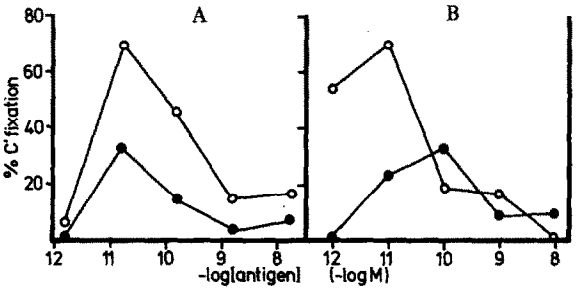

Fig.2. C′ fixation of purified anti-RNA polymerase antibodies with holo enzyme with 1.3×10^{-5} M poly[d(A–T)] (○) and without (●). Pretreatment prior to C′ fixation with template was at 0°C in A and 37°C in B.

DNA and *E. coli* DNA are shown in fig.3A and 3B, respectively. The changes in the C′ fixation are less dramatic, *E. coli* DNA exhibiting almost no effect. Analogous experiments with core enzyme are seen in fig.4A and 4B and in fig.5A and 5B. Contrary to holo enzyme (fig.2B) the core enzyme is less reactive and the C′ fixation in fig.4A shifts to higher antigen concentrations on incubation with poly[d(A–T)] at 37°C.

Molecular details of binding and recognition of DNA binding enzymes are still not fully explained, although conformational changes in the protein on binding to DNA have been suggested [7]. There is no direct approach at hand (such as X-ray crystallography) to investigate the molecular basis of conformational

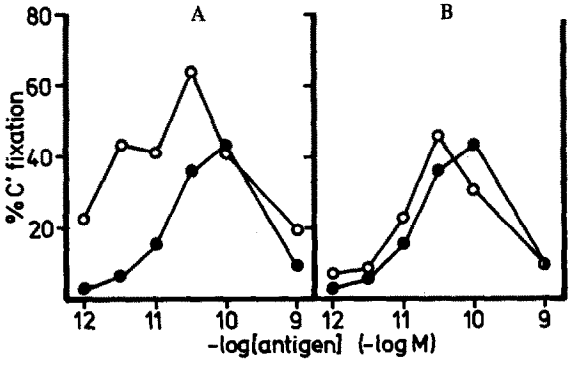

Fig.3. C′ fixation of antibodies (see legend to fig.2) with holo enzyme. In A the enzyme was incubated with 5×10^{-6} M calf thymus DNA (○) and without (●); and in B at 0°C prior to C′ fixation with 1.7×10^{-6} M *E. coli* DNA (○) and without (●).

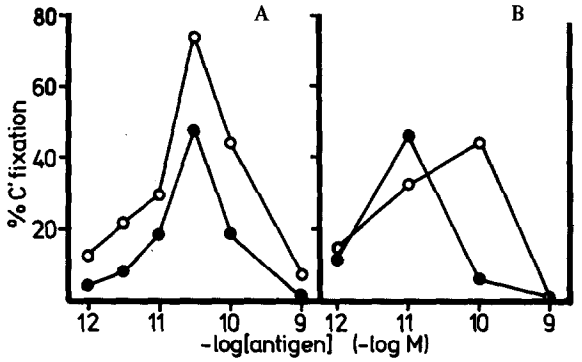

Fig.4. C′ fixation of antibodies (see legend to fig.2) with core enzyme. Conditions were the same as for holo enzyme in fig.2.

changes in RNA polymerase on binding to templates. Recently it has been shown that in the course of RNA–protein assembly in tobacco mosaic virus interaction of RNA with two flexible protein loops from the two axial protein subunits in the interior of the virus particle abolishes flexibility [8]. If the conformational change in RNA polymerase on binding the template is envisioned likewise to consist in reduction of the flexibility of subunits one would interpret our results as follows:

Flexible loops from the protein subunits surround the DNA binding site. At low concentrations of antigen and antibody (10^{-11}–10^{-10} M), where the C′ fixation experiments are carried out, parts of the antibodies may be prevented from binding because the antigenic region in the unliganded enzyme is not readily accessible due to its high flexibility. Binding of DNA fixes the protein loops and makes them accessible to antibodies.

A comparison of C′ fixation of holo enzyme–poly [d(A–T)]–antibody complexes at different temperatures (fig.2A and 2B) indicates a major conformational change of the enzyme at the higher temperature.

It is known that binding of RNA polymerase to promoters is temperature dependent [9,10] and that the dissociation rate of rifampicin derivatives from the enzyme shows a discontinuity between 20°C and 25°C [11]. The same transition was found by following the fluorescence of a GTP analogue bound to RNA polymerase [12]. We therefore propose that the difference in the C′ fixation between fig.2A and 2B reflect two conformational states on binding of DNA at different temperatures. This phenomenon was ascribed to the formation of closed and open complexes at temperatures below and above the transition, which depends on the template. Interactions between RNA polymerase and calf thymus DNA and *E. coli* DNA, respectively, are reduced and reveal a slight lateral shift similar to the situation in fig.2B, although the experiment was performed at 0°C (cf. fig.3A and 3B). This suggests that templates like calf thymus DNA or *E. coli* DNA have additional specific binding sites for RNA polymerase holo enzyme compared to poly [d(A–T)] and shift the equilibrium in favor of the low temperature conformation.

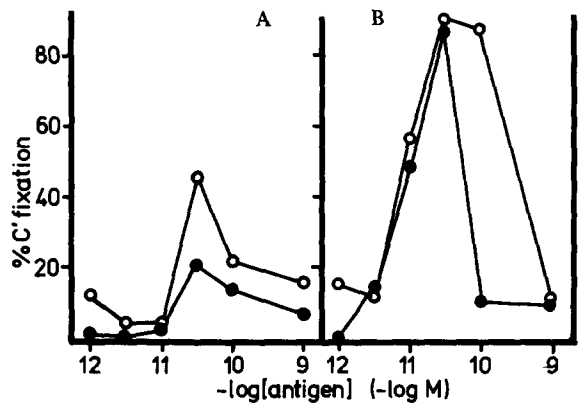

Fig.5. C′ fixation of antibodies (see legend to fig.2) with core enzyme. Conditions were the same as for holo enzyme in fig.3.

The corresponding experiments with core enzyme are shown in fig.4 and 5: a lateral shift in opposite direction to higher antigen concentration at 37°C points to a weakened antigen–antibody interaction (cf. fig.4), indicating that the σ-factor plays an important part in the conformational change on forming the enzyme–template complex at 37°C. Accordingly the conformational change resulting on binding of core enzyme to calf thymus DNA and *E. coli* DNA (cf. fig.5) is weaker than for the holo enzyme. Primer analogues ApU or UpA at 1.3×10^{-6} M had no effect.

RNA polymerase core and holo enzyme tend to aggregate at low ionic strength forming dimeric and higher aggregates [13]. The aggregation state of the respective enzymes was determined in the analytical

ultracentrifuge. In the buffer used in the C′ fixation which contains 0.14 M NaCl the *S*-value for holo enzyme was 14 S and therefore not indicative of dimerisation but the 16 S value for the core enzyme suggests partial aggregation. However it should be kept in mind that the *S* values were determined at 10^{-6} M core enzyme. Therefore increased C′ fixation on adding DNA cannot be attributed to additional antigenic determinants exposed on monomerisation of higher aggregates of core enzyme because the C′ fixation experiments were carried out at very low concentrations of antigen (see above) where aggregation is minimal. Moreover holo enzyme behaved like core enzyme although there was no indication of dimer formation even at higher concentrations.

There was no change in C′ fixation on binding of rifampicin to polymerase. This favours models in which either rifampicin is bound in an essentially irreversible second-order reaction or which assume that the polymerase exists in two forms in tautomeric equilibrium with different reactivities for rifampicin, and that this equilibrium is not or little changed by rifampicin [14].

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