CONSERVED ANTIGENIC DETERMINANTS IN THE VICINITY OF THE DNA-BINDING CENTER OF BACTERIAL RNA POLYMERASE

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1. Introduction

RNA polymerases purified from a variety of bacteria have the same subunit composition of $\alpha_2\beta\beta'\sigma$ [1]. Despite differences in primary structure [2–4] there is a considerable functional and structural homology between RNA polymerases from different bacteria. The enzyme is able to recognize promoters in heterologous DNA [5] and active hybrid RNA polymerase can be reconstituted from the subunits of taxonomically distant bacteria [6–8].

To get an insight into the nature of this homology we compared the antigenic properties of RNA polymerases from different bacteria [3]. Here we report the effects of anti-RNA polymerase monovalent antibodies on the RNA polymerization and DNA-binding activities of RNA polymerases from *Escherichia coli* and *Pseudomonas putida*. Conserved antigenic determinants were detected in the vicinity of the DNA-binding center of the enzyme. At least part of these determinants were located in the β-subunit.

2. Experimental

RNA polymerase from *E. coli* and *P. putida* was purified by the method in [9] as described [10]. RNA polymerization and DNA-binding activities of RNA polymerase were assayed as described in the figure legends. Antibodies were obtained by immunizing rabbits as in [11]. Monovalent antibodies were obtained by pepsin digestion of the IgG fraction of sera purified by DEAE-cellulose chromatography [12]. To prevent reassociation monovalent fragments were treated with iodoacetamide. The absence of intact antibodies in the monovalent antibody preparations was checked by SDS electrophoresis.

3. Results and discussion

Fig.1 shows the influence of monovalent antibodies against *E. coli* core RNA polymerase on RNA polymerization and DNA-binding activities of the homologous holoenzyme. The inhibition depends on the antibody–enzyme ratio rather than on the absolute concentration of antibodies. The inhibition of the binding to DNA requires a considerably higher antibody–enzyme ratio than the inhibition of RNA synthesis. Hence the inhibition of the over-all reaction of RNA synthesis mainly results from the interaction of antibodies with the enzyme sites which are not involved in the binding to DNA. The binding to DNA seems to be inhibited predominantly through direct blocking of the DNA-binding center of the enzyme, since antibodies added after the RNA polymerase–RNA complex is formed have a much weaker effect than those added before complex formation (fig.1). Moreover, antibodies added to the RNA polymerase–DNA complex stabilize it, i.e., decrease the rate of its dissociation (fig.2). This suggests that antibodies might change the conformation of the RNA polymerase molecule, leading to a stabilization of its interaction with DNA. One can also suspect that the inhibition of the over-all RNA polymerase reaction may result not only from a direct blocking of the active centers but from conformational effects induced by antibodies.

Monovalent antibodies against *E. coli* core enzyme are much less effective in inhibiting the over-all reaction of *P. putida* RNA polymerase than that of *E. coli* RNA polymerase. However, the inhibition of the binding to DNA is the same for *P. putida* RNA polymerase and *E. coli* RNA polymerase. Moreover, monovalent antibodies against *E. coli* core inhibit the RNA polymerization and DNA-binding activities of

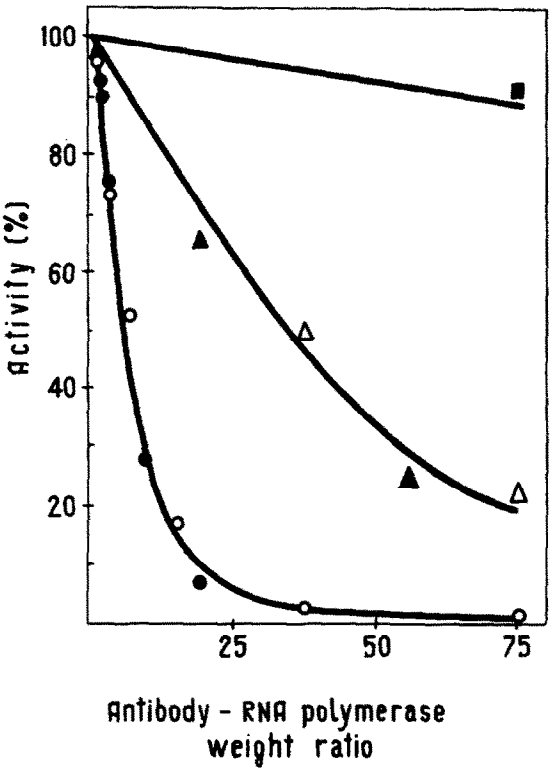

Fig.1. Inhibition of *E. coli* RNA polymerase holoenzyme by anti-*E. coli* core polymerase monovalent antibodies. RNA polymerization activity was assayed in the binding buffer of [13] with a saturating amount of T2 DNA as template by measuring [^{14}C]UMP incorporation into RNA as in [10]. Assay mixtures were incubated at 37°C for 10 min. DNA-binding activity was assayed in the same buffer at 37°C as in [10] by measuring the radioactivity of T2 [^{14}C]-DNA retained on nitrocellulose filters. DNA polymerization activity was determined either with 5 μg/ml (○) or with 20 μg/ml (●) of RNA polymerase. DNA-binding activity was determined either with 1 μg/ml (△,■) or with 4 μg/ml (▲) of RNA polymerase. Antibodies at indicated weight ratios were added to assay mixtures containing RNA polymerase either before (○,●,△,▲)or after (■) adding DNA. Data are plotted in % of antibody-free control. Controls with non-immune preparations have 100% activities (not shown).

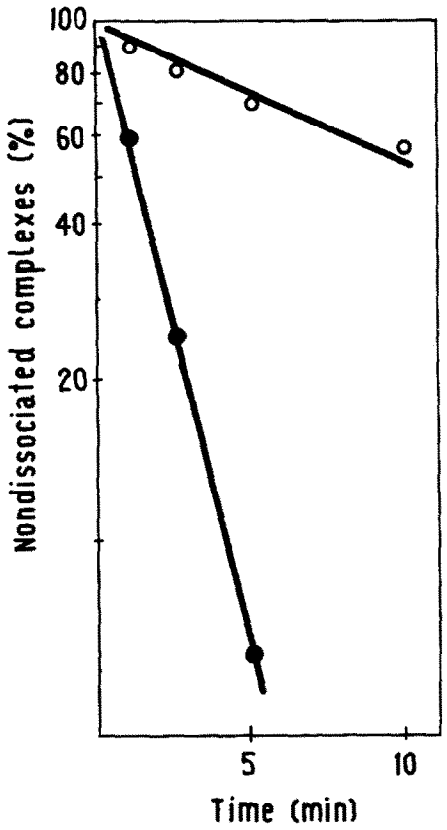

Fig.2. Effect of anti-*E. coli* core polymerase monovalent antibodies on the kinetics of *E. coli* RNA polymerase holoenzyme–T2 DNA closed complex dissociation. Dissociation was followed by the nitrocellulose filter binding method [13] at 4°C in the binding buffer containing 0.15 M NaCl after adding an excess of unlabelled DNA as in [10]. (○) No antibodies. (●) Antibodies added after complex formation at weight ratio to polymerase of 50. Data are plotted in % of T2 [^{14}C]DNA retained on filters at zero time.

P. putida RNA polymerase with equal efficiency (fig.3A). This shows that in the case of *P. putida* RNA polymerase the inhibition of the over-all reaction is solely due to the blocking of the DNA-binding center. Thus RNA polymerases from *E. coli* and *P. putida* have the same antigenic determinants in the vicinity of the DNA-binding center, while all the other determinants involved in RNA polymerization are different. This conclusion was confirmed in reciprocal experiments: anti-*P. putida* RNA polymerase antibodies inhibited the DNA-binding activity of *E. coli* RNA polymerase with the same efficiency as its RNA polymerization activity, while the RNA polymerization activity of *P. putida* enzyme was inhibited at a much lower concentration of antibodies than its DNA-binding activity (fig.3B).

It should be noted that, because of the varying specificity of antibodies produced by different rabbits, some preparations of antibodies do not recognize the conserved antigenic determinants detected in the above experiments. We obtained a preparation of anti-*E. coli* core antibodies which inhibited the binding to DNA of *E. coli* but not of *P. putida* RNA poly-

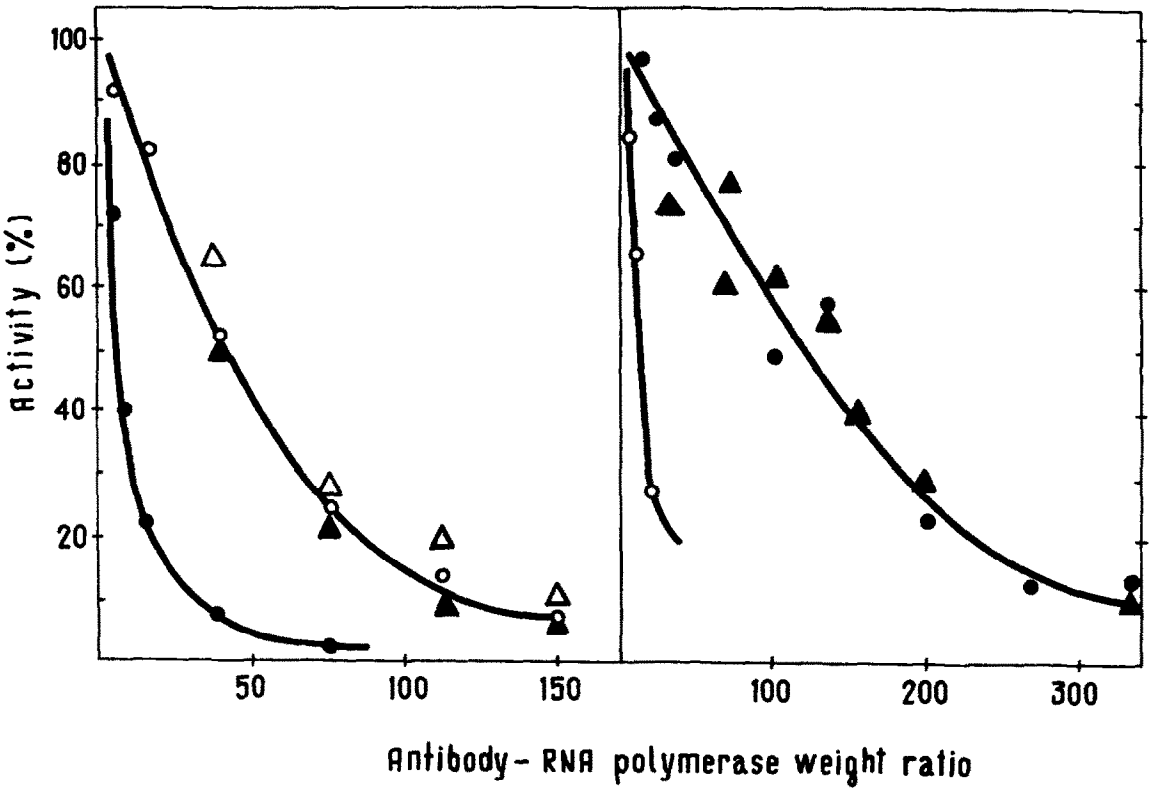

Fig.3. Inhibition of RNA polymerase by heterologous monovalent antibodies. Experiments were run as in fig.1. (A) Anti-*E. coli* RNA polymerase antibodies. (B) Anti-*P. putida* RNA polymerase antibodies. RNA polymerization activity of *E. coli* (●) and *P. putida* (○) RNA polymerase. DNA-binding activity of *E. coli* (▲) and *P. putida* (△) RNA polymerase.

merase. This shows that some antigenic determinants in the vicinity of the DNA-binding center are not conserved. Neither did this particular antibody preparation inhibit RNA synthesis by *P. putida* RNA polymerase. This shows once again that all the hitherto detected antigenic determinants which are involved in the over-all RNA polymerase reaction but not in DNA-binding are not conserved.

To find out which of the RNA polymerase subunits bear antigenic determinants essential to DNA-binding, we used antibodies against individual core polymerase subunits from *E. coli*. Anti-β monovalent antibodies from two rabbits tested were found to inhibit the binding of *P. putida* holoenzyme to DNA. One preparation inhibited the binding of *P. putida* RNA polymerase as efficiently as that of *E. coli* polymerase while another preparation inhibited *P. putida* RNA polymerase less efficiently than the *E. coli* enzyme. Thus the β-subunit bears both conserved and unconserved antigenic determinants in the vicinity of the DNA-binding center. In the β'-subunit we were able to detect unconserved determinants only, since anti-β' antibodies (1 prep.) inhibited the binding of *E. coli* but not of *P. putida* RNA polymerase. These data suggest the involvement of the β- and β'-subunits in the binding to DNA. This accords with our genetic data [10,14].

Anti-α antibodies (2 preps.) did not inhibit either the DNA-binding or the over-all reaction of RNA polymerase from *E. coli* or *P. putida*. The functional 'unimportance' of the α-subunit's antigenic determinants revealed in these experiments does not prove the functional unimportance of the α-subunit itself. One can speculate, for instance, that the functionally important sites in α are well separated from the antigenic determinants which react with the antibodies tested. In view of the variability of antibodies from different rabbits, it is not unlikely that by testing a sufficient number of immunized rabbits one would obtain an antibody preparation which would react

with the 'functional' sites of the α subunit. Inhibition of the RNA polymerization activity by anti-α bivalent antibodies has been reported [2], but it is not clear whether the bivalent antibodies inhibit RNA polymerase by blocking functional sites or merely by precipitating the enzyme.

Despite their apparent unimportance, antigenic determinants in the α-subunit are highly conserved. Radioimmunoassay experiments carried out by Lebedev and Nikiforov (cited in [3]) have shown that ~50% of anti-*E. coli* α-subunit antibodies reacting with the *E. coli* RNA polymerase react with the *P. putida* RNA polymerase (the β- and β′-subunits of *E. coli* and *P. putida* show only ~10% homology in such assays). Thus, at least in the α-subunit, there are antigenic determinants which are conserved but may be blocked by antibodies without impairing the enzyme's function.

The results obtained suggest that the ability of RNA polymerase to recognize promoters in heterologous DNAs is at least partially based on the strong structural conservation of the enzyme's DNA-binding center. The other stages of the RNA polymerase reaction do not seem to depend on the conservation of the antigenic structure. The significance of the conserved antigenic determinants which appear to be unessential to the enzyme's functioning remains to be elucidated.

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