Physico-chemical study of complex formation of DNA with wild-type and mutant *E. coli* RNA polymerases

Recognition properties of β-subunit

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Received 14 January 1986

Complex formation of T_7 DNA with RNA polymerase from *E. coli* B/r WU-36-10-11-12 (*E. coli* W12) and its rifampicin-resistant mutant rpoB409 was studied. The rpoB409 mutant possesses a highly pleiotropic effect due to alteration in the RNA polymerase β-subunit structure. The two RNA polymerases have been previously shown to differ in gene selection during RNA synthesis on T_7 DNA. In this study it was found that the change in selective properties of the mutant RNA polymerase occurs during its interaction with DNA, the general ability of the enzyme to melt DNA being unaffected.

RNA polymerase *Subunit structure* *Promoter recognition*

1. INTRODUCTION

Gene activity in bacteria is regulated by numerous mechanisms. The initiation of transcription alone is controlled by: (i) special regulatory proteins (activators and repressors), (ii) RNA polymerase itself and (iii) DNA structure (e.g. at the level of supercoiling) [1–7]. This multicomponent control of genome expression appears to be vital to the cell, providing a labile, coordinated and economical adaptation to alterations in the environment. It should be noted that, unlike the well-studied mechanisms of transcription control by regulatory proteins, the molecular mechanisms of two other types have remained practically unstudied.

To investigate the regulatory properties of RNA polymerase, a special search of RNA polymerase mutants with altered transcriptional specificity has been suggested [3,4]. This approach consists of obtaining those mutations in RNA polymerase subunits that have a highly pleiotropic effect. We obtained 4 rifampicin-resistant (rif-r) mutants of *E. coli* B/r WU-36-10-11-12 (rpoB401, rpoB402, rpoB403, rpoB409) possessing a number of new phenotypic properties, along with resistance to rifampicin [3–5]. All new properties of the mutants should be attributed solely to the change in the RNA polymerase β-subunit induced by the corresponding rif-r mutations [4]. In [4,5,8,9] it has been shown that the new properties of the mutants can be due to several factors, the most significant of which is the change in RNA synthesis specificity induced by alterations in RNA polymerase. Such a manifestation of rif-r mutations was proved for RNA polymerase from the rpoB409 mutant [5].

The present study was undertaken to elucidate the molecular mechanism of the influence of the rpoB409 mutation on the RNA polymerase-DNA interaction. The stage of open promoter complex formation was studied by a direct method for detecting conformational changes, viz. spin-label modification. Alteration of selective properties of rpoB409 RNA polymerase was shown to occur at the stage of interaction with DNA. The mutational alteration of RNA polymerase has no effect on its ability to melt DNA in the transition to open pro-

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00145793/86/$3.50

moter complexes. The data obtained show that the RNA polymerase β-subunit is involved in the selection of heterogeneous promoters.

2. MATERIALS AND METHODS

2.1. *Bacterial strains*

Wild-type *E. coli* WU-36-10-11-12 (leu_{am}, tyr_{oc}, $SupE_{oc}$) – (*E. coli* W12) and its rif-r mutant rpoB409 with a highly pleiotropic effect were used [3,4].

2.2. *RNA polymerases from* E. coli *W12 and rpoB409*

These were isolated according to [10]. The specific activity of the two enzymes determined with T_2 DNA as a template as in [10] was 7000–7500 and 6000 units, respectively.

2.3. *RNA polymerase-T_7 [^{14}C]DNA complexes*

These were prepared according to [11] with minor modifications. The standard reaction mixture (0.1 ml) contained 12 μg T_7 [^{14}C]DNA (150000 cpm), 5 μg RNA polymerase, 0.04 M Tris-HCl (pH 8.0), 0.01 M $MgCl_2$, 5 μg albumin, 4 mM β-mercaptoethanol, 5×10^{-5} M EDTA and NaCl at varying concentrations, as noted in the figure legends. After incubation for 10 min at a given temperature, 2 ml of a solution of the same salt concentration were added at the same temperature. The complexes were then collected on filters and washed twice with 2 ml of the same solution. The filters (Synpor, N9, Czechoslovakia) were preliminarily washed on both sides with 0.04 M Tris-HCl (pH 8.0), 0.01 M $MgCl_2$, 10^{-4} M EDTA and stored in the same solution.

2.4. *Preincubation of RNA polymerase-T_7 DNA complexes at various temperatures followed by RNA synthesis*

The reaction mixture (0.09 ml) was identical with that above except that it contained no albumin and the concentration of NaCl was 0.1 M. After incubation for 10 min at various temperatures, as noted in the figure legends, 0.09 ml of the same salt solution containing [^{14}C]ATP, GTP, CTP, UTP (3 mM each) was added and the mixture incubated for 60 s at the same temperature. NaCl (0.02 ml) was then added up to 0.35 M to prevent reinitiation of RNA synthesis. The mixtures were further incubated for 10 min at 35°C, cooled, and 0.2 ml (5 mg/ml) albumin and 2 ml of 5% trichloroacetic acid added. The pellet was washed on nitrocellulose filters (AUFs, Czechoslovakia) with 30 ml of 5% trichloroacetic acid and 5 ml of 96% ethanol, dried and the radioactivity determined. The amount of complexes formed during preincubation was ascertained from the incorporation of [^{14}C]AMP into the acid-insoluble fraction. In the control assay, preincubation and RNA synthesis were carried out at 0.35 M NaCl.

2.5. *Modification of RNA polymerase with the spin label 2,2',6,6'-tetramethyl-4-*p*-chloromercuricarbobenzoxypiperidino-1-oxyl (I)*

This was carried out as reported in [12]. In [12], it was shown that the modification occurs with one type of RNA polymerase SH group and that it does not affect the first two stages of RNA synthesis: interaction of the enzyme with DNA and initiation of transcription. ESR spectra of spin-labeled RNA polymerase and its complex with DNA were measured on a Varian E104 ESR spectrometer. τ_c values were calculated from ESR spectra according to the equation:

$$\tau_c = 6.06\Delta H_0 \left[\sqrt{I_0/I_{+1}} + \sqrt{I_0/I_{-1}} - 2\right] \times 10^{-10} \text{ s} \quad [13]$$

where ΔH_0 is the linewidth (in G) of the central component; I_{+1}, I_0 and I_{-1} are the peak heights (in arbitrary units) of the low-field, central and high-field components, respectively.

3. RESULTS AND DISCUSSION

By investigating the selective transcription of particular gene groups on phage T_4 and T_7 DNA it was found that RNA polymerases from *E. coli* wild-type strain W12 and rpoB409 mutant differ in the relative efficiency of synthesis for the same RNA types [5]. In addition, on the same DNAs as well as on T_7 and λ DNA restriction fragments containing a limited number of known promoters, the normal and mutant enzymes were shown to differ in the relative number of RNA starts from the two initiating purines, ATP and GTP [14]. This is evidence that mutational alteration of the structure

of RNA polymerase produces a change in transcription specificity, which takes place at some stage of the transcription cycle preceding RNA chain elongation.

3.1. *Effect of rpoB409 mutation on RNA polymerase-DNA complex formation*

To determine whether the rpoB409 mutation affects the interaction of RNA polymerase with DNA, complex formation of native and mutant enzymes with T_7 [^{14}C]DNA was studied using the filter binding assay [11].

Fig.1 presents the temperature dependence of binding of the normal and mutant enzymes with T_7 [^{14}C]DNA. It can be seen that the maximum amount of radioactive DNA is retained on filters at the same temperature (about 30°C) for both enzymes. At low temperatures, however, the mutant enzyme retains radioactive DNA more efficiently, i.e. at low temperatures rpoB409 RNA polymerase forms a greater number of tight complexes with T_7 DNA than normal enzyme. It should be noted that under the conditions of a low enzyme/DNA ratio and a long (about 5 min) washing procedure the retention of DNA on filters is chiefly due to the formation of tight, long-lived complexes of RNA polymerase with some of the early T_7 DNA promoters, their set being obviously altered depending upon the temperature of complex formation [15,16].

Analogous results were obtained in experiments with preliminary incubation of both kinds of RNA polymerase with T_7 DNA at various temperatures followed by RNA synthesis at 35°C at high ionic strength (0.35 M NaCl) (fig.2). In such experiments only rapidly initiating, productive RNA polymerase-DNA complexes formed during preincubation contribute to RNA synthesis.

Thus, the mutation-induced changes in the selective properties of RNA polymerase are manifested as early as at the stage of complex formation with the template as judged from the increased ability

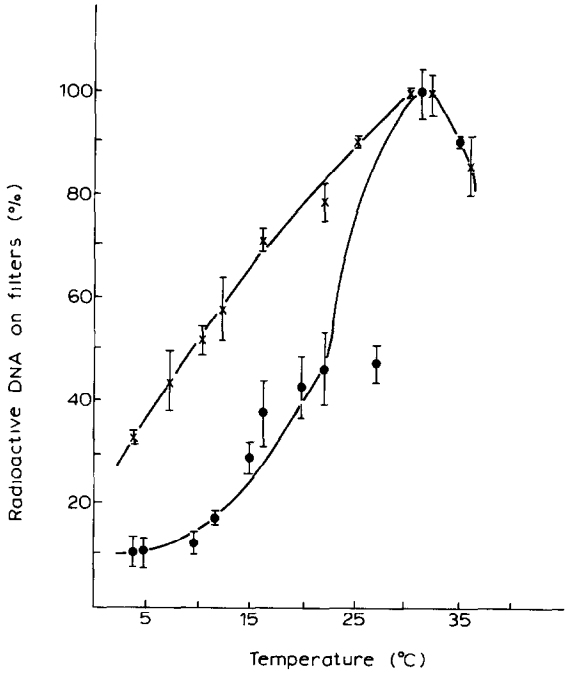

Fig.1. Temperature dependence of complex formation between T_7 [^{14}C]DNA and RNA polymerases from *E. coli* W12 (●) and rpoB409 mutant (×). Complexes were formed at 0.1 M NaCl. Results are given in per cent of the maximal retention of [^{14}C]DNA in complex on filters (88486 cpm for *E. coli* W12 RNA polymerase and 109080 for rpoB409 RNA polymerase).

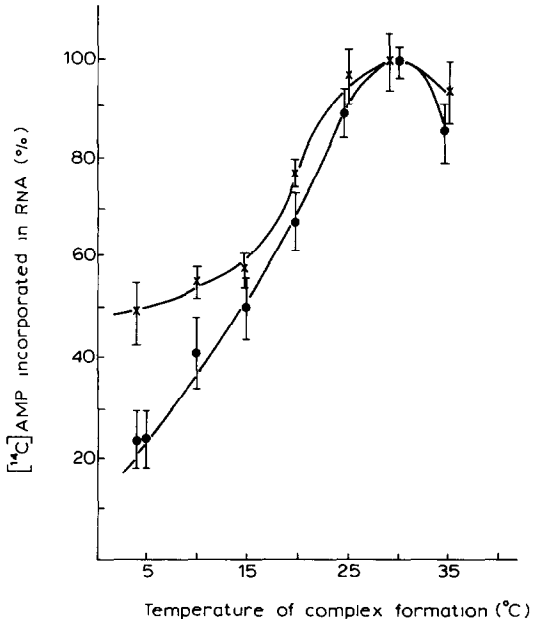

Fig.2. Formation of productive complexes of *E. coli* W12 (●) and rpoB409 (×) RNA polymerases with T_7 DNA on preincubation of the complexes at different temperatures and subsequent RNA synthesis. Results are given in per cent of maximal incorporation at 30°C and are the average of data from 6 different experiments.

of the mutant enzyme to form tight, productive complexes with T_7 DNA promoters at low temperatures.

3.2. *Conformational changes of RNA polymerase upon formation of open promoter complexes on T_7 DNA*

Recent studies on the formation of productive promoter-polymerase complexes have demonstrated that this process consists of more than the two usually accepted steps, their exact number and nature being unknown and, most likely, different for various promoters [17–19]. Evidently, any subsequent step of the process after location of RNA polymerase on a promoter should involve conformational changes in RNA polymerase and promoter.

The rpoB409 mutation can in principle exert its effect at the level of RNA polymerase binding to promoters and/or of conformational changes in the components of initial complexes that occur in the transition to final productive complexes. For further characterization of the observed differences in complex formation between normal and mutant polymerases, conformational changes of the enzyme in T_7 DNA promoter complexes were studied using ESR. For this purpose *E. coli* W12 and rpoB409 RNA polymerases were modified with spin label I (see section 2).

Fig.3 shows the ESR signals of the free spin label and of the label bound to both kinds of RNA polymerase. The spin-labeled RNA polymerases can be seen to have much more retarded ESR signals than the free label. The ESR spectra were characterized by means of the rotational diffusion parameter, τ_c, i.e. the time required for a label to rotate through an angle of about 40°. The mobility of the spin label bound to rpoB409 RNA polymerase proved to be greater than that of label bound to normal enzyme. At 20°C, τ_c was $3.65–3.84 \times 10^{-9}$ and $2.74–3.1 \times 10^{-9}$ s for the normal and mutant enzymes, respectively, indicating a different microenvironment of the label in the mutant enzyme. This indicates that the label is sensitive to mutation-induced structural changes in RNA polymerase.

The interaction of spin-labeled *E. coli* W12 and rpoB409 RNA polymerases with T_7 DNA over the range 5–45°C was studied. Local melting of a promoter site in the conversion of intermediate to

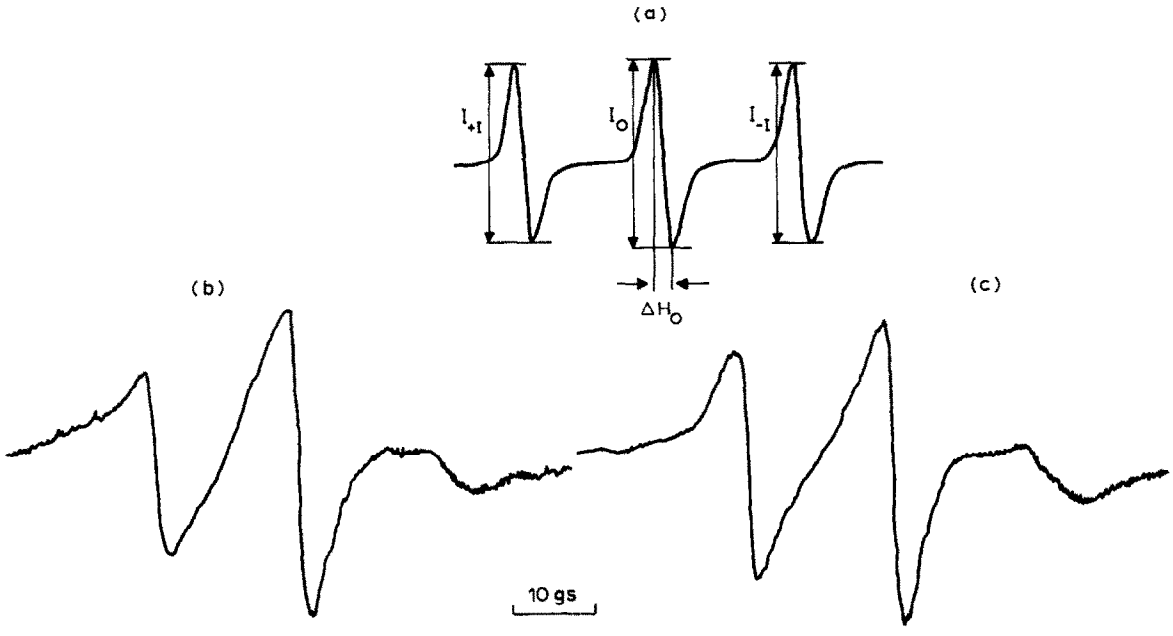

Fig.3. ESR spectra of free spin label (a), spin-labeled *E. coli* W12 (b) and rpoB409 (c) RNA polymerases. Spectra were registered at 20°C.

open complexes has previously been found to respond differently to temperature for various promoters. Specifically, isomerization to open complexes at some particular T_7 DNA promoters in the DNA body can be followed by the temperature change [15,16].

To avoid the effect of specific changes in promoter selection by the mutant enzyme and of the change in location of RNA polymerase molecules on promoters at various temperatures, complexes were allowed to form at those RNA polymerase concentrations saturating all early promoters of T_7 DNA.

The results are presented in fig.4a. The τ_c values are seen to decrease monotonically with increasing temperature from 5 to 15°C and from 25 to 45°C. In the temperature range 15–25°C in which most of the main T_7 DNA promoters form open complexes due to local DNA melting by RNA polymerase [19,20], the spin label behaves abnormally – τ_c increasing with temperature – in both spin-labeled enzymes. This is evidence that between 15 and 25°C RNA polymerase undergoes conformational changes affecting the microenvironment of the spin label. It was shown that the conformational changes in RNA polymerase occur at a higher temperature with increasing ionic strength which stabilizes the DNA double helix (cf. curves 2 and 3 in fig.4a), none of these being observed in the absence of DNA (fig.4b). Therefore, the observed conformational changes in RNA polymerase bound with DNA can be considered as corresponding to the transition into open complexes which is characterized by local DNA melting. As the process involving promoter melting is observed for both RNA polymerases at the same temperature, the general ability of the mutant enzyme to melt the DNA double helix appears to be unchanged.

Circumstantial evidence for the above is provid-

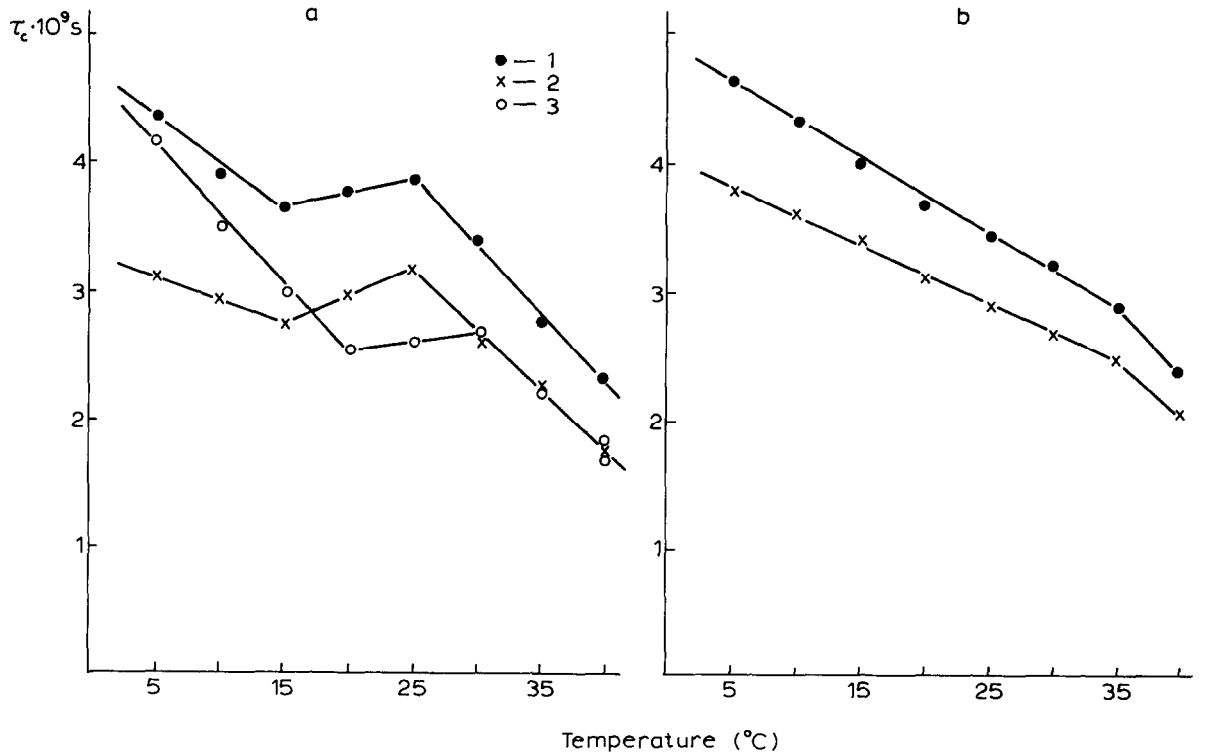

Fig.4. Temperature dependence of correlation time, τ_c, of spin-labeled RNA polymerase [free (b) and in complex with T_7 DNA (a)]. For complex formation, 100 μg T_7 DNA was added to the reaction mixture (0.1 ml) containing 0.01 M Tris-HCl (pH 8.0), 0.01 M $MgCl_2$, 0.1 M NaCl (curves 1,2) or 0.2 M NaCl (curve 3) and 100 μg spin-labeled *E. coli* W12 (1) or rpoB409 (2,3) RNA polymerase at 0°C. The ESR spectra were taken while increasing the temperature by 5°C and incubating the samples for 3 min before each recording.

ed by the fact that maximal retention of T_7 [^{14}C]DNA on nitrocellulose filters by both enzymes is observed at the same temperature (fig.1) as well as by the findings presented in fig.5. If the rpoB409 mutation influenced the ability of RNA polymerase to melt the DNA double helix, stabilization of the double helix by high ionic strength would be expected to influence to different degrees complex formation of DNA with *E. coli* W12 and rpoB409 RNA polymerases. However, the dependence of the amount of complexes retained on nitrocellulose filters on ionic strength proved to be similar for both enzymes (fig.5).

Therefore, the observed difference in the ability of the normal and mutant enzyme to form complexes with T_7 DNA promoters at low temperatures most likely results from a change in the selective properties of the mutant RNA polymerase toward some promoters which are preferentially used at these temperatures.

It should be mentioned that the two methods (filter binding, ESR) detect different features of RNA polymerase-T_7 DNA complexes. In addition, in accordance with the aims of this study their use was dependent on the enzyme/DNA ratio in the complexes (see above). This could be the reason why the difference in behaviour of the complexes for the two enzymes at low temperature on filters was not registered by ESR (cf. figs 1, 2 with fig.4a).

Thus, the study of RNA polymerase mutants with a pleiotropic effect enabled us to demonstrate the strain (rpoB409) with a changed transcription specificity [5]. The change in selective properties of the rpoB409 RNA polymerase takes place at the stage of the complex formation with DNA promoters. This indicates that the RNA polymerase β-subunit plays a role in specific recognition of heterogenous promoters. This means that bacterial RNA polymerase in the absence of regulatory proteins can control the relative expression of operons (primarily, constitutive ones) depending on the structure of their promoters and environmental factors affecting the enzyme structure.

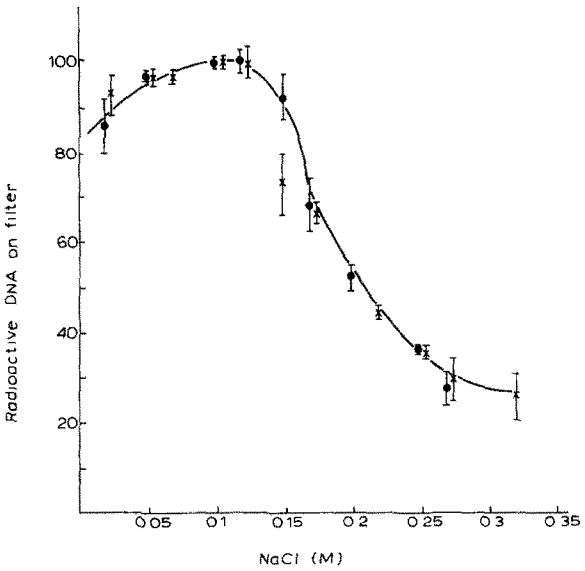

Fig.5. Efficiency of retention of T_7 [^{14}C]DNA-*E. coli* W12 (●) and rpoB409 (×) RNA polymerase complexes on nitrocellulose filters vs ionic strength. Complexes were formed at 35°C. Results are given in per cent of maximal DNA retained at 0.1 M NaCl (83415 cpm for *E. coli* W12 RNA polymerase and 121575 cpm for rpoB409 RNA polymerase).

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