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Differences in Contacts of RNA Polymerases from *Escherichia coli* and *Thermus aquaticus* with *lac*UV5 Promoter Are Determined by Core-Enzyme of RNA Polymerase

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Abstract—The interaction of RNA polymerases from *Escherichia coli* and *Thermus aquaticus* with *lac*UV5 promoter was studied at various temperatures. Using DNA–protein cross-linking induced by formaldehyde, it was demonstrated that each RNA polymerase formed a unique pattern of contacts with DNA in the open promoter complex. In the case of *E. coli* RNA polymerase, β′ and σ subunits were involved into formation of cross-links with the promoter, whereas in the case of *T. aquaticus* RNA polymerase its β subunit formed the cross-links with the promoter. A cross-linking pattern in promoter complexes of a hybrid holoenzyme comprised of the core-enzyme of *E. coli* and σ subunit of *T. aquaticus* was similar to that of the *E. coli* holoenzyme. This suggests that DNA–protein contacts in the promoter complex are primarily determined by the core-enzyme of RNA polymerase. However, temperature-dependent behavior of contact formation is determined by the σ subunit. Results of the present study indicate that the method of formaldehyde cross-linking can be employed for elucidation of differences in the structure of promoter complexes of RNA polymerases from various bacteria.

Key words: RNA polymerase, promoter complex, formaldehyde cross-linking

Initiation of transcription in a bacterial cell involves RNA polymerase holoenzyme, which consists of a core-enzyme and σ subunit. The core-enzyme consists of five subunits: two α, β, β′, and ω subunits. This enzyme exhibits catalytic activity but it cannot specifically interact with promoters. Promoter recognition requires the σ subunit. During the initiation process, the holoenzyme initially interacts with double stranded promoter DNA and then induces DNA melting around a starting point of transcription. The promoter RNA polymerase complex in which DNA strands are melted is known as an open complex, whereas an intermediate complex containing double strand DNA is known as a closed complex [1-3].

Initiation of transcription has been the most extensively studied using *Escherichia coli* (*Eco*) RNA polymerase [1, 2, 4]. Studies employing RNA polymerases from other bacteria revealed that basic mechanisms of initiation are similar in all bacterial cells. Promoters of all studied bacteria have similar consensus structure; in many cases, RNA polymerases of one bacteria can use heterologous promoter taken from other bacteria [3-7, 8]. However, promoter complexes of RNA polymerases from different bacteria exhibit certain structural and functional differences. In contract to *Eco* RNA polymerase, the RNA polymerases from *Bacillus subtilis* (*Bsu*) and *Rickettsia prowazekii* form unstable complexes with most promoters [6, 9-11]. It was also found that in promoter complexes *Bsu* RNA polymerase does not interact with DNA downstream to the start site of transcription. This may possibly explain lowered stability of promoter complexes formed by this polymerase [6, 10, 12].

It has recently been demonstrated that RNA polymerase from the thermophilic bacterium *Thermus aquaticus* (*Taq*) also forms unstable promoter complexes, which readily dissociate in the presence of heparin, a competitive inhibitor of DNA binding [13]. Studies of DNA melting in promoter complexes formed by *Eco* and *Taq* RNA polymerases by footprinting with $KMnO_4$, which modifies thymine residues of single stranded DNA, revealed that these polymerases differ neither by the size of the melted promoter region nor by distribution of modified thymine residues [13]. Thus, it remains unclear whether promoter complexes of *Taq* RNA polymerase exhibit any structural differences compared with *Eco* RNA polymerase.

Abbreviations: *Eco*) *Escherichia coli*; *Taq*) *Thermus aquaticus*; *Bsu*) *Bacillus subtilis.*

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In this study, we have employed the method of formaldehyde-induced DNA–protein cross-linking for evaluation of structural differences between promoter complexes formed by *Eco* and *Taq* RNA polymerases. This method allows the detection of tight contacts of RNA polymerase with single stranded DNA in the melted promoter region [14, 15]. This study revealed that both RNA polymerases formed characteristic patterns of contacts with DNA fixed by the formaldehyde cross-linking. In the open promoter complex of *Eco* RNA polymerase, cross-linking with DNA involves β′ and σ^{70} subunits [14]. In the promoter complex with *Taq* RNA polymerase, the cross-link is formed with β subunit, but not with σ subunit. Thus, the method of formaldehyde cross-linking reveals differences in the structure of promoter complex of *Eco* and *Taq* RNA polymerases that are not detectable by other methods. It is possible that these differences underlie the difference in stabilities of promoter complexes of these polymerases.

MATERIALS AND METHODS

Plasmids and enzymes. The plasmid pET28ABCZ encoding all subunits of the core-enzyme of *Taq* RNA polymerase and the plasmid pET28TaD carrying the gene encoding the *Taq* σ subunit were generous gifts by K. Severinov and L. Minakhin [16]. The plasmid pMRG8 was used for expression of *Eco* σ subunit [17]. *Eco* and *Taq* σ subunits were isolated from *Eco* cells (containing corresponding plasmids) as described earlier [16, 18]. The core-enzyme of *Eco* RNA polymerase was isolated using the methods published in [18, 19]. The core-enzyme of *Taq* RNA polymerase was isolated from *Eco* cells, strain BL21(DE3), carrying the plasmid pET28ABCZ [16].

Formaldehyde cross-linking of RNA polymerase with *lac*UV5 promoter. A 167 bp long DNA fragment containing *lac*UV5 promoter was radioactively labeled at 3′-end of the non-template strand as described earlier [14]. In cross-linking experiments, core-enzyme (50 nM) and σ subunit (300 nM) were incubated in 10 μl of 40 mM Tris-HCl buffer, pH 7.9, containing 40 mM KCl, 10 mM $MgCl_2$, and 5% glycerol at 37°C for 5 min; samples were then cooled in ice and the labeled promoter fragment was added to the final concentration 10 nM. The samples were incubated at required temperature for 7 min and formaldehyde (Sigma, USA) was added to the final concentration 20 mM. Samples were incubated for 20 sec and the reaction was terminated by adding an equal amount of the stop-buffer containing 2% SDS, 2 mM β-mercaptoethanol, 0.25 M Tris-HCl, pH 6.8, 10% glycerol. Cross-linked products were separated by SDS electrophoresis in 5% polyacrylamide gel. DNA–protein complexes were analyzed using Phosphoimager (Amersham Biosciences, USA). Efficacy of cross-linking was defined as percentage of radioactively labeled DNA in DNA–protein complex to total amount of radioactively labeled DNA in a sample.

RESULTS

Contacts of *Eco* and *Taq* RNA polymerases with the melted region of *lac*UV5-promoter were investigated by the method of formaldehyde-induced DNA–protein cross-linking. Figure 1 shows results of an experiment on temperature-dependence of covalent cross-linking between radioactively labeled DNA fragment containing *lac*UV5-promoter and RNA polymerase subunits. Figure 2 summarizes quantitative data of this experiment. In the case of *Eco* holoenzyme, cross-linking with the DNA promoter region involves β′ and σ^{70} subunits [14], whereas core-enzyme does not form cross-links (Fig. 1a). Efficacy of holoenzyme cross-linking increases with temperature and reaches maximal values at 60°C (4.2 and 1.8% for β′ and σ subunits, respectively) (Fig. 2). At higher temperature the cross-link disappears; this probably corresponds to denaturation of RNA polymerase and dissociation of the open complex.

In the case of the hybrid holoenzyme consisting of *Eco* core-enzyme and *Taq* σ^{A} subunit, the cross-linking pattern was similar to that of *Eco* holoenzyme (Fig. 1). The cross-link was also formed with β′ and σ subunits of RNA polymerase and efficacy of its formation roughly corresponded to that observed with *Eco* RNA polymerase (Figs. 1a and 2). Consequently, the general architecture of contacts with the melted promoter region was very similar for RNA polymerases containing *Eco* core-enzyme and various σ subunits. However, in contrast to *Eco* holoenzyme the hybrid RNA polymerase does not cross-link with the promoter at low temperature (≤30°C).

A completely different pattern of DNA–protein contacts was observed in the case of promoter complexes with *Taq* RNA polymerase. The position of the observed DNA–protein complex was between complexes containing β′ subunit (protein molecular mass, M_r, of 155 kD) and σ^{70} subunit ($M_r \approx 70$ kD) of *Eco* RNA polymerase (Fig. 1a). This suggests that cross-linking involves *Taq* RNA polymerase β subunit ($M_r \approx 130$ kD), because *Taq* RNA polymerase β′ subunit has larger sizes than *Eco* RNA polymerase β′ subunit ($M_r \approx 160$ kD). The cross-link with β subunit was observed in promoter complex of *Taq* RNA polymerase at temperatures above 37°C. Further temperature increase was accompanied by sharp increase in cross-linking efficiency; the maximal value of 50% was observed at 70°C (Figs. 1b and 2).

The hybrid holoenzyme containing *Taq* core-enzyme and *Eco* σ^{70} subunit does not form cross-link at any temperature (Fig. 1b). Thus, such RNA polymerase cannot form the open complex on *lac*UV5-promoter. This is consistent with a published observation that σ^{70}

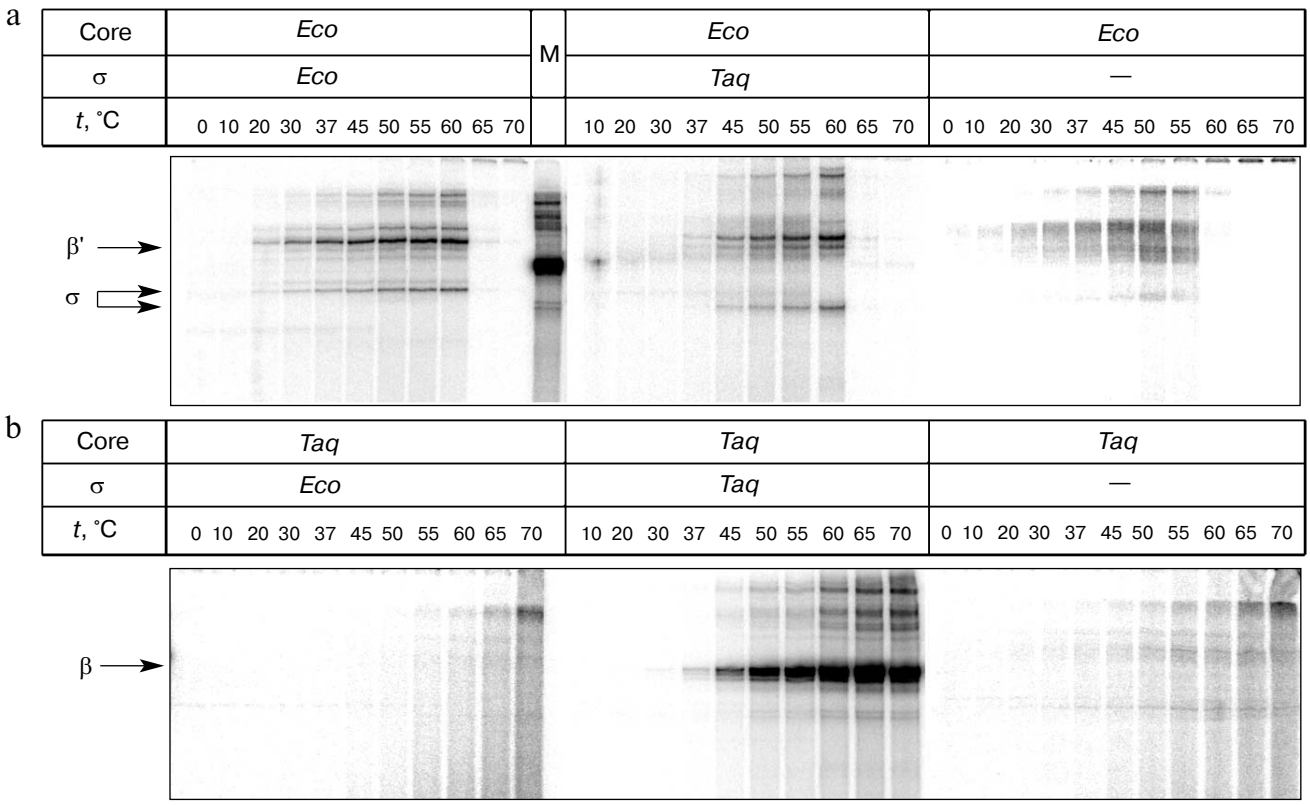

Fig. 1. Formation of formaldehyde cross-links of RNA polymerase holoenzyme with *lac*UV5 promoter at various temperatures. a) Cross-linking in promoter complexes of RNA polymerases containing *Eco* core-enzyme; b) cross-linking in RNA polymerase holoenzyme complexes containing *Taq* core-enzyme. RNA polymerase subunits forming cross-links with promoter are indicated on the left. Lane M contains a sample corresponding to the cross-link of *Taq* RNA polymerase at 65°C.

subunit cannot activate *Taq* core-enzyme in the transcription test *in vitro* [16, 20].

DISCUSSION

In the present study, we have investigated structural differences in promoter complexes of RNA polymerases of mesophilic *Eco* bacterium and thermophilic *Taq* bacterium using the method of formaldehyde cross-linking. Our results clearly demonstrate the existence of significant differences in DNA–protein contacts formed by these RNA polymerases with *lac*UV5-promoter in the open complex. In promoter complexes with *Eco* RNA polymerase, cross-linking involves β′ and σ subunits, whereas in promoter complexes with *Taq* RNA polymerase cross-linking involves β subunit. Thus, the method of formaldehyde cross-linking revealed differences in the promoter complexes formed by *Eco* and *Taq* RNA polymerase that had not been detected by other methods (e.g., $KMnO_4$ footprinting [13]).

It should be noted that differences in the cross-linking patterns of *Eco* and *Taq* RNA polymerases are mainly

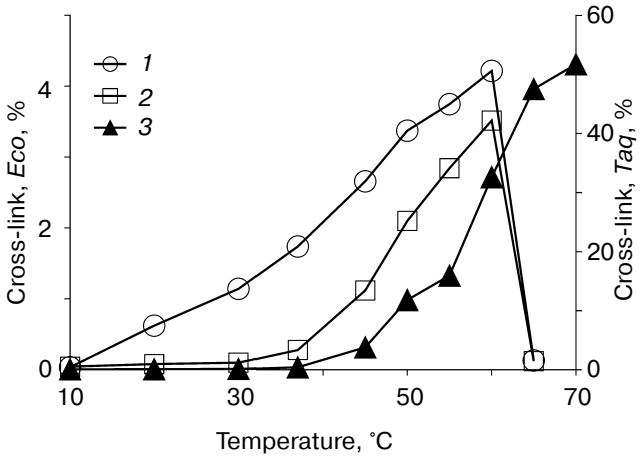

Fig. 2. Temperature dependence of formaldehyde-induced cross-linking of various RNA polymerases with *lac*UV5 promoter. Efficacy of cross-linking with the β′ subunit of *Eco* RNA polymerase (*1*) and the hybrid holoenzyme (*2*) is shown on the left ordinate axis; efficacy of cross-linking with *Taq* RNA polymerase β subunit (*3*) is shown on the right ordinate axis. The efficacy of cross-linking is shown in percent of total DNA amount added to the sample.

attributed to differences in the open complex structure rather than to differences in amino acid sequences of the protein regions contacting with DNA. Indeed, one could suggest that lack of cross-linking with σ subunit in *Taq* RNA polymerase promoter complexes was due to the absence of certain amino acid residues required for reaction with formaldehyde (and therefore cross-link formation). However, in the hybrid RNA polymerase containing *Eco* core-enzyme *Taq* σ subunit does form cross-link with DNA. The latter implies that core-enzyme determines specific conformation of σ subunit and structure of RNA polymerase contacts with the promoter.

Effectiveness of cross-linking is also determined by a core-enzyme irrespectively to the nature of the σ subunit present in the RNA polymerase holoenzyme. Maximal cross-linking efficiency for *Eco* RNA polymerase and the hybrid holoenzyme observed at 50-60°C is just a few percent. Under the same experimental conditions effectiveness of *Taq* RNA polymerase cross-linking is much higher (~50%). This probably suggests the presence of tight DNA–protein contacts. Such contacts may be required for maintenance of promoter complex structure at high temperatures.

Study of temperature dependence of formaldehyde cross-linking demonstrates that appearance of specific DNA–protein contacts in the promoter complexes formed by various polymerases correlates with DNA melting around the starting site of transcription and open complex formation. Analysis of DNA melting in promoter complexes revealed that *Taq* RNA polymerase and the hybrid holoenzyme containing *Eco* core-enzyme and *Taq* σ subunit form the open complex on *lac*UV5-promoter at temperatures ≥37°C. However, in contrast to *Eco* RNA-polymerase they do not open the promoter at 20°C [13]. In accordance with this observation, DNA cross-links in the promoter complexes formed by *Eco* RNA polymerase are detected at temperatures ≥20°C, whereas *Taq* RNA-polymerase and the hybrid holoenzyme form the cross-link only at temperatures above 37°C. Since the hybrid holoenzyme containing *Taq* σ subunit does not form cross-links with promoter at low temperature, we can conclude that temperature dependence of *Taq* RNA polymerase promoter opening is apparently determined by the σ subunit [13].

It was demonstrated earlier that *Taq* RNA polymerase promoter complexes are characterized by lower stability than *Eco* RNA polymerase complexes [13] (see the introductory section). Different stabilities of *Eco* and *Taq* RNA polymerase complexes may be attributed to the difference in the structures of contacts of these RNA polymerases with DNA found in this study. It was recently shown that promoter complexes of other RNA polymerases (e.g., *Bsu* RNA polymerase) also exhibit lower stability than *Eco* RNA polymerase complexes (see the introductory section). It was suggested that many functional differences of *Bsu* and *Eco* RNA polymerases may be attributed to properties of core-enzyme. Particularly, they may be explained by differences in interactions with DNA downstream of the enzyme active site [10]. It is reasonable to suppose that the differences in characteristics of the promoter complexes formed by *Eco* and *Taq* RNA polymerases are also due to different structures of RNA polymerase contacts with downstream DNA duplex.

In conclusion, it should be noted that the method of formaldehyde cross-linking is highly sensitive for detection of DNA melting in the process of open promoter complex formation and studying of structural differences of promoter complexes of various RNA-polymerases, which are not detected by other methods. Thus, this method can be used in future studies of mechanisms of promoter opening and in analysis of structural differences of promoter complexes formed by RNA polymerases from various bacteria.

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