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Correlation between the DNA supercoiling and the initiation of transcription by *Escherichia coli* RNA polymerase in vitro: role of the sequences upstream of the promoter region

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Binding of *Escherichia coli* RNA polymerase and the abortive initiation of transcription at the A2 promoter of bacteriophage T7, separately cloned in pBR322, was found to be strongly dependent on the degree of supercoiling of the plasmid. Supercoiling does not seem to play any role in the initiation of transcription at the T7A1 promoter under identical conditions. Plasmid containing T7A2 promoter was found to be less amenable to S1 nuclease in comparison to that having T7A1. Sequence comparison reveals a high G/C content upstream to the −35 region of T7A2 which by extra duplex stability probably renders the initiation of transcription more dependent on the state of supercoiling of the template.

T7 promoter; RNA polymerase; Supercoiling; Abortive initiation; Upstream sequence; (*E. coli*)

1. INTRODUCTION

Chromosomal DNA carefully isolated from bacteria is always negatively supercoiled and it has been implicated as an important factor for the regulation of the expression of various genes [1,2]. Energy required for the DNA strand separation can indeed be accounted for the free energy associated with the negative supercoiling in the DNA [3]. Thus, any process like initiation of transcription, which requires local unwinding of DNA, is known to be facilitated by the template supercoiling [4]. As many of the *E. coli* promoters show increased activity due to the transition from the relaxed to the supercoiled state [5–7], the rate of formation of the open complex between RNA polymerase and DNA [8,9] is thought to be the causative factor for the supercoiling induced regulation of gene expression. However, this concept is not without doubt mainly because of the wide range of supercoiling response of different promoters [10]. Thus, we have no clear picture till today on the interrelationship between the template supercoiling and the initiation of transcription.

In order to follow the mechanism of initiation of transcription by *E. coli* RNA polymerase, we have felt the necessity of understanding the role of DNA supercoiling in initiation and elongation of transcription separately. This has been possible because of the availability of many strong *E. coli* promoters individually cloned in a suitable plasmid and also different well-standardized assays for the initiation of RNA chains. We report here that dependence of initiation of transcription on supercoiling is not a universal phenomenon, and it is probably dependent on the sequence of bases upstream of the promoter regions.

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2. MATERIALS AND METHODS

All the chemicals and buffers used in this study were of the purest grade available. Nucleotides were purchased from Boehringer (U.S.A.). Heparin was a product of Sigma. Radionucleotides used in this study to assay the RNA polymerase activity were obtained from Amersham and Bhabha Atomic Research Centre (India) and were checked before use [11]. *E. coli* RNA polymerase was purified from mid-log phase cells of a RNaseI$^-$ strain (MRE600) as described before [12]. S1 nuclease was purchased from BRL. Wheat-germ topoisomerase I was a kind gift from Dr S.K. Brahmachari's laboratory, Bangalore. Derivatives of *E. coli* strain HMS 174, containing plasmid pAR1435 (T7A1) or pAR1539 (T7A2) were kindly provided by Dr A.H. Rosenberg and Dr F.W. Studier of Brookhaven Natl. Laboratory, U.S.A. These plasmids have single early promoter of T7 DNA as mentioned in the parenthesis [13] cloned in the *Bam*H1 site of pBR322. Synthesis of the dinucleotide tetraphosphates from different promoters was carried out following the protocol of Johnston and McClure [14,15] for the abortive initiation reaction.

For the preparation of the DNA-protein complex, *E. coli* RNA polymerase was incubated with T7A1, T7A2 promoter-containing plasmids for 10 min at 37°C in buffer containing 40 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 100 mM KCl, 0.1 mM dithiothreitol and 0.1 mM EDTA. The molar ratio between the DNA and the enzyme was between 1:2 and 1:8. The complex was analyzed in a 1% agarose gel in Tris-borate-EDTA buffer (pH 8.3) by the gel retardation technique [16]. Different topological distribution of plasmid pAR1539 was generated using DNA topoisomerase I in presence of varying concentrations of ethidium bromide [17]. S1-nuclease cleavage of different plasmid DNA was carried out in a total incubation volume of 10 μl containing plasmid DNA (12 nM), 25 mM potassium acetate (pH 5.5), 25 mM KCl, 4 mM $MgCl_2$, 1 mM $ZnCl_2$, 5 mM β-mercaptoethanol and varying concentrations of S1 nuclease. The in-

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cubation was carried out for 10 min at 37°C and loaded directly on a 1% agarose gel in Tris-borate-EDTA buffer (pH 8.3).

3. RESULTS

Fig.1 shows the analysis of the complex formed between the plasmid pAR1435, pAR1539 with *E. coli* RNA polymerase. The relaxed DNA band was moving slower with increasing concentration of the enzyme in the case of plasmid containing T7A1 promoter unlike that of T7A2. However, the supercoiled DNA band was retarded in both cases. The degree of retardation was more with T7A1, which was expected as T7A1 is known to be the strongest promoter for *E. coli* RNA polymerase in vitro [18]. We observed that in both the above cases, there was a heterogeneous distribution of the retarded supercoiled DNA band in the presence of the enzyme (vide fig.1). This we attributed to the known DNA-unwinding property of RNA polymerase or the heterogeneous molar ratios of complexation between the DNA and the enzyme.

We intended to find out whether the supercoiling dependent complexation between the T7A2 promoter and *E. coli* RNA polymerase has any role to play in the initiation of transcription. Under the condition of abortive initiation of transcription [14,15] dinucleotides pppApU and pppGpC were formed under the control of T7A1 and T7A2 promoters, respectively. Characterization of these products was carried out extensively by removing the terminal phosphates with alkaline phosphatase and comparing the product with commercial ApU or GpC. The total synthesis of pppApU from T7A1 was insensitive to the nature of the template DNA, relaxed, linear or supercoiled (not shown). However, such was not the case with T7A2. Relaxed plasmid bearing the T7A2 promoter showed minimal level of pppGpC synthesis catalyzed by RNA polymerase. This was further proved by checking the degree of abortive initiation from T7A2 at different degrees of supercoiling. Fig.2a shows the different groups of DNA topoisomers used as templates in the abortive initiation assay. Average number of supercoils represent the mean of the Gaussian distribution in each group. Interestingly, it was observed that the supercoil response in initiation of transcription was sigmoidal (fig.2b). Here initiation with pAR1539 having natural superhelical density is taken as 100%. This template will have approximately 25 negative supercoils considering one such supercoil per 200 bp of DNA [19].

Plasmid pAR1539 bearing the T7A2 promoter was found to be significantly less sensitive to S1 nuclease in comparison to the plasmid pAR1435 having the T7A1 promoter (fig.3). The susceptibility of pBR322 to S1 nuclease was even less than that of pAR1539. It can be seen from fig.3 that a large excess of S1 nuclease was necessary to generate open circular and linear DNA in the case of pAR1539. Fig.4a shows that among the

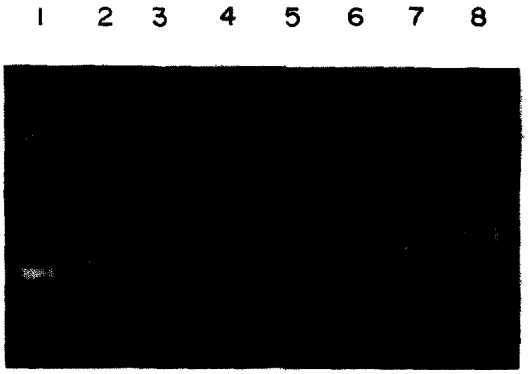
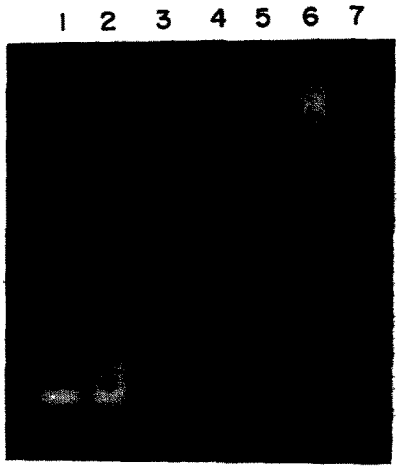
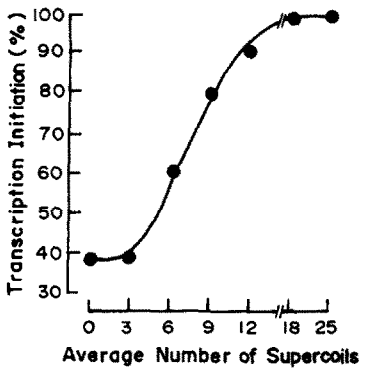


Fig.1. Analysis of the DNA-RNA polymerase complex in 1% agarose gel. The amount of DNA in all cases was 8 nM. Lanes 1–4 were pAR 1435 (T7A1) and 5–8 were pAR1539 (T7A2). In lanes 1 and 5 only DNA was present. In lanes 2 and 6, 3 and 7 and 4 and 8, DNA was complexed with *E. coli* RNA polymerase with DNA/protein molar ratios 1:2, 1:4 and 1:8, respectively.



a



b

Fig.2. Degree of supercoiling dependence of the abortive initiation of transcription from pAR1539. (a) 1% Agarose gel electrophoresis of the different topoisomers from supercoiled to relaxed pAR1539 DNA, lanes 1–7. (b) Each point represents percent of abortive initiation of transcription catalysed by *E. coli* RNA polymerase from each group of topoisomers as shown in (a).

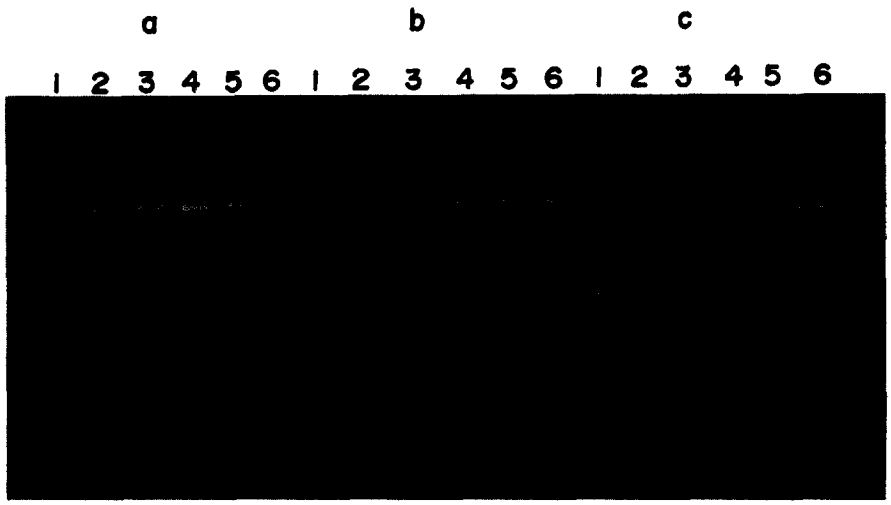


Fig.3. Analysis of S1-nuclease cleavage of the plasmids pAR1435 (a), pAR1539 (b) and pBR322 (c). Lane 1, DNA alone, lanes 2-6, plasmid after incubation with 0.58, 1.16, 2.90, 5.80, 8.70 units of S1-nuclease respectively.

topoisomers distribution of the plasmid pAR1539, only those with more number of negative supercoils were preferentially digested by S1. Interestingly *E. coli* RNA polymerase also favoured binding to these topoisomers (fig.4b).

4. DISCUSSION

We have shown in this study that the two strongest promoters of *E. coli* RNA polymerase i.e. T7A1 and T7A2 [18] behave differently towards the binding with the enzyme and initiation of transcription at them in vitro. However, supercoiled form of the plasmids bearing these two promoters also show difference in their susceptibility towards S1-nuclease. It has been demonstrated by Margalit et al. [20] that the instability or the melting property of the promoter region plays an important role in its strength and function. It is now well established that negative supercoiling induces spontaneous unwinding preferably in promoter regions, rendering them S1 nuclease sensitive [21]. Thus, we postulated that the promoter region of T7A1 is probably more meltable resulting in better binding with RNA polymerase. However, in the case of T7A2, DNA supercoiling is necessary to contribute the extra energy

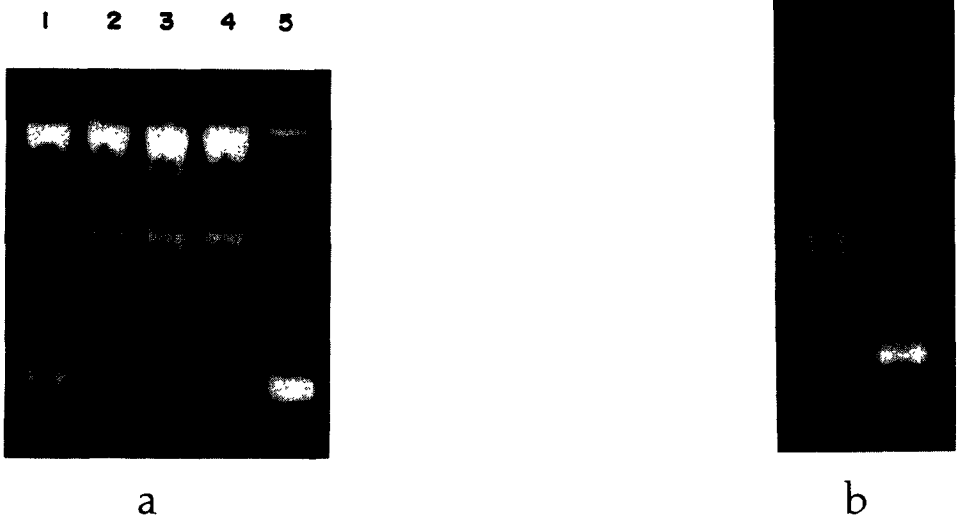


Fig.4. (a) Analysis of the preferential S1 susceptibility of the topoisomer distribution of T7A2. Lane 1, template topoisomer profile, lanes 2-4, treated with 2.9, 5.8 and 8.7 units of S1 respectively, lane 5, control plasmid. (b) Preferential binding of *E. coli* RNA polymerase to higher negatively supercoiled template (lane 2), where lane 1 is the control topoprofile of T7A2 containing plasmid.

required to unwind the template and help the polymerase molecule to bind and initiate transcription. Therefore, neither the host vector pBR322 or its derivative pAR1435 (T7A1) showed any preferential binding with the enzyme in supercoiled form unlike pAR1539 (T7A2). It was further proved from our experiments where we found that RNA polymerase favours binding to the DNA topoisomers having higher number of supercoils which are also more sensitive to S1-nuclease (fig.4). On comparing the sequence between -35 and -55 region of T7A1 and T7A2 [13], we found that, indeed a remarkably high G/C rich region exists in T7A2 unlike T7A1, which probably explains our results. These observations probably establish a relationship between the initiation of transcription and supercoiling specified by the sequence of promoter upstream to -35 regions.

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