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# Bovine seminal plasmin is a DNA unwinding protein

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The duplex DNA unwinding ability of seminal plasmin from bovine semen was examined by treatment of plasmid-protein complexes with calf thymus topoisomerase I and resolution of the topoisomer distributions by agarose gel electrophoresis. Binding of seminal plasmin results in a moderate degree of unwinding of supercoiled plasmid. The elongation of the RNA chain by *E.coli* RNA polymerase over promoter containing template is not inhibited by seminal plasmin. However, the reinitiation of transcription is blocked in such cases indicating that seminal plasmin inhibits transcription by binding to the initiation site of RNA polymerase.

Topoisomer; Unwinding; Seminal plasmin; RNA polymerase; Elongation

## 1. INTRODUCTION

Seminal plasmin (SPLN), an antimicrobial protein from bovine seminal plasma, has been shown to be an inhibitor of transcription in *Escherichia coli* and it does so in vitro by binding strongly to RNA polymerase [1,2]. Surprisingly, it has also been noted that SPLN binds strongly to DNA, a maximum of 1 molecule of SPLN being bound per 8 base pairs in the case of poly(dA-dT) [2]. Incubation of SPLN with the RNA polymerase before the start of transcription was necessary for the optimal inhibition of transcription. If the template DNA was pretreated with SPLN or if it was added at the same time at which the transcription was started, no inhibition of RNA synthesis was observed [2]. The above experiments indicated that once bound to the template, SPLN is no longer available for binding with the enzyme and hence does not inhibit transcription.

*E. coli* RNA polymerase has two catalytic sites: the substrate binding site or the initiation site and the template binding site or the elongation site [3]. It appears therefore that SPLN predominantly acts on the initiation site of the enzyme and blocks the substrate binding. It has been further shown by one of us that SPLN competes with rifampicin, a specific inhibitor of the initiation of RNA synthesis by bacterial RNA polymerase, for binding to the enzyme [4]. We report here that SPLN does not inhibit the elongation of transcription and in fact may facilitate it by unwinding the template.

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## 2. EXPERIMENTAL

*E. coli* RNA polymerase was purified as described [5] from 250 g of MRE600, RNase I$^-$ cells, grown to the log phase in a 40-l fermentor. The 500 kDa enzyme with the subunit composition $\alpha_2\beta\beta'\sigma$ had a specific activity of 600 units/mg. SPLN was purified following the standard procedure developed in this laboratory [1], and was found to be completely free from RNases. All other chemicals used were of the purest grade available. [$^3$H]UTP used for RNA polymerase assay was obtained from New England Nuclear. Plasmid pBR322 from *E. coli* strain HB101 was purified after lysing the cells in the presence of lysozyme, 1% Brij 35 and 0.4% deoxycholate, and subsequently banding over a CsCl-ethidium bromide density gradient as described [6]. Calf thymus topoisomerase I was obtained from Bethesda Research Laboratory, USA.

The DNA unwinding assay was done according to Hanas et al. [7]. Supercoiled plasmid was either initially relaxed by topoisomerase I, or pretreated with the unwinding protein before the relaxation reaction. The topoisomers thus generated were electrophoresed on 1.2% agarose gel in Tris-acetate buffer [8]. Electrophoresis was conducted at 40 V at room temperature

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in a BRL minigel setup and the gel was stained with 1 $\mu$g/ml of ethidium bromide and photographed. A film of topoisomer distribution was scanned with a Biomed densitometer using a soft laser source. The mean weighted averages of the respective distributions were calculated assuming that the individual peak heights are proportional to DNA topoisomer concentration [9]. SPLN-resistant elongation of RNA chain by *E. coli* RNA polymerase was performed over $\Delta D_{111}T_7$ DNA, as described [10].

## 3. RESULTS

The method employed here to detect the specific unwinding of the DNA helix by SPLN is as in [11], which compares the distributions of DNA topoisomers resulting from nicking and closing the DNA in the presence and the absence of a DNA-binding protein. Fig.1 depicts the unwinding effect of SPLN on the topoisomer distribution of pBR322. Unwinding is seen as an upward shift in the topoisomer distribution. In the middle lane the concentration of topoisomerase I was selected such that the complete relaxation of the plasmid was obtained. It is seen clearly from the figure that SPLN induces an upward shift in the band distribution of the topoisomers (right lane). Fig.2 depicts the laser scan data on the unwinding of the plasmid by SPLN. The unwinding ability of SPLN essentially can be calculated from these scans following the method in [11]. It is seen from the arrows in fig.2a and b that the centre of the Gaussian distribution lies in between the two most intense bands in the case of topoisomerase treated pBR322 (fig.2a). This is about 1½ turns away (540°) from the centre of the band pattern when plasmid was relaxed in the presence of 20 times molar excess of SPLN (fig.2b).

As mentioned, the template DNA pretreated with SPLN can be transcribed efficiently by RNA polymerase. It was of interest to find out whether such observations merely account for the elongation of RNA chains over a natural or synthetic but non-specific DNA chain. Fig.3 shows the in vitro RNA synthesis over $\Delta D_{111}T_7$ DNA, which has a single strong promoter ($T_7A_1$) that gives rise to a discrete RNA of 6100 nucleotides [12]. The template was preincubated with RNA polymerase before the addition of the inhibitor or triphosphates. It can be seen from fig.3 that within 12 min of the start of the reaction from $A_1$ pro-

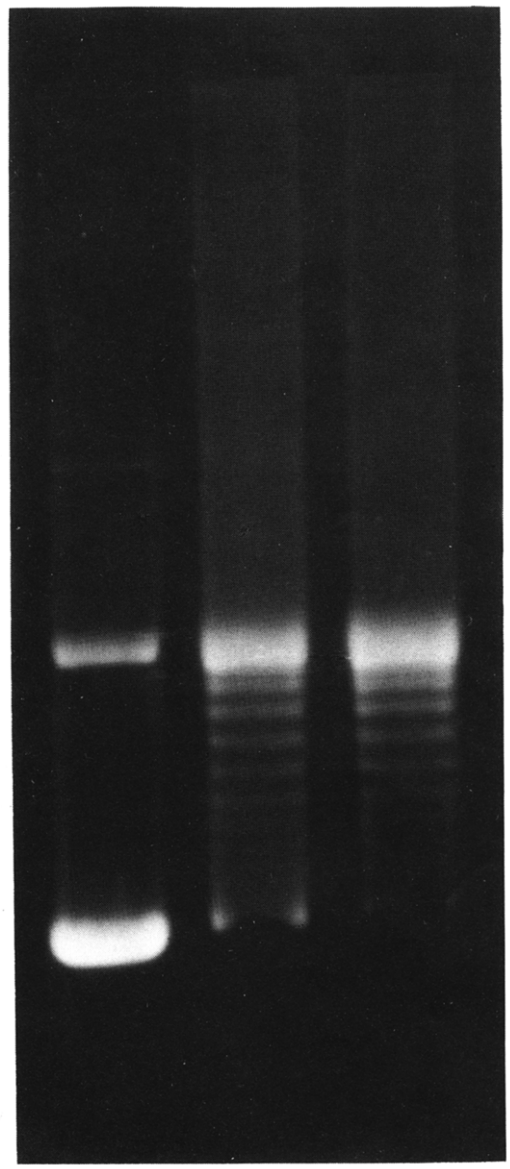

Fig.1. Unwinding by bovine seminal plasmin of plasmid pBR322. Left lane, pBR322 (80 nM); middle lane, pBR322 (80 nM) incubated with calf thymus topoisomerase I (10 units) at 30°C for 30 min in buffer A containing 70 mM Tris-HCl, pH 7.5, 0.5 mM DTT, 0.15 M NaCl, 10% glycerol and 10 mM $MgCl_2$; right lane, pBR322 (80 nM) pretreated with seminal plasmin (1.6 $\mu$M) in buffer A for 30 min at 37°C before the addition of topoisomerase I (10 units), and incubated further in the same buffer for 30 min at 30°C. The reaction was stopped by the addition of 0.5% sodium dodecyl sulfate and 0.05% bromophenol blue.

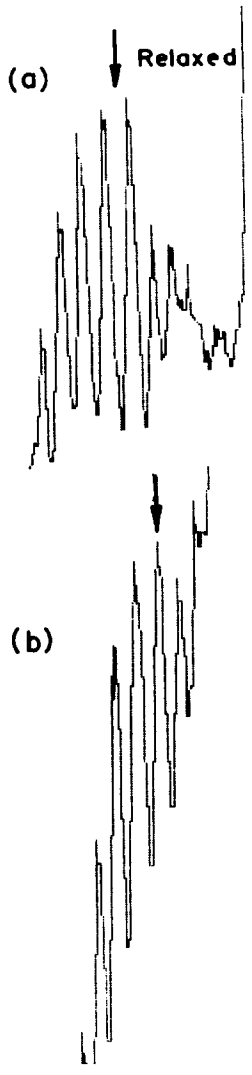


Fig.2. Densitometric trace of the topoisomeric distribution of plasmid pBR322 (80 nM) in the absence (a) and presence of seminal plasmin (1.6 $\mu$M).

moters one round of RNA synthesis is coming to an end and the residual increase in incorporation of UTP to RNA product is due to the escape of some polymerase molecules from the termination signal at 6100 nucleotides [10]. In any event, no reinitiation of RNA synthesis is taking place. However, in the absence of SPLN, transcription continuously goes on which was characterized by monotonous increase in incorporation of UTP to the RNA product (not shown).

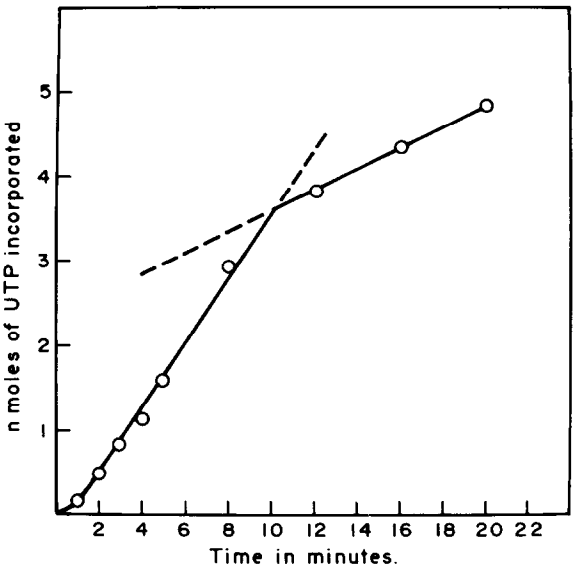


Fig.3. Seminal plasmin-resistant elongation of RNA synthesis over $\Delta D_{111}T_7$ DNA. DNA (5 nM) was preincubated with *E. coli* RNA polymerase (8 nM) for 3 min to allow the promoter search. Later, seminal plasmin (100 nM) was added along with the substrates and the incorporation [$^3$H]UTP to the RNA product at defined time points were measured.

## 4. DISCUSSION

Bovine seminal plasmin binds both to the DNA and bacterial RNA polymerase and therefore the exact mechanism by which it inhibits transcription could not be established. Normal rate of transcription over SPLN treated template further raised a question whether SPLN bound to the template DNA is available for the binding with the enzyme. We show here that SPLN is a DNA unwinding protein and therefore it should facilitate the movement of RNA polymerase over the DNA chain. Using the protocol already established by Chamberlin and his group we have carried out transcription over specific promoter containing DNA both in the presence and absence of SPLN. It is clear from the result that the elongation of RNA synthesis is not affected in the presence of SPLN. However, the initiation of RNA synthesis is inhibited. Therefore, it appears that the transcription observed by other workers in the past over SPLN-treated templates are mainly the elongation of non-specific RNA molecules.

It is tempting to conclude from the results

presented here that SPLN binds to the substrate binding site of the enzyme and thereby inhibits the transcription. It also explains the competition of SPLN with rifampicin for binding to *E. coli* RNA polymerase [4].

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