Interaction of bovine seminalplasmin with *Escherichia coli* RNA polymerase in the presence of rifampicin

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The interaction of bovine seminalplasmin and rifampicin with *E.coli* RNA polymerase was studied using fluorescence spectroscopy. Both seminalplasmin and rifampicin are known to be the inhibitors for the initiation of RNA synthesis in *E.coli*. Rifampicin quenced the intrinsic fluorescence of RNA polymerase and seminalplasmin when excited at 280 nm. However, excess of seminalplasmin reversed the quenching of RNA polymerase fluorescence by rifampicin. Upon addition of rifampicin to the seminalplasmin-RNA polymerase complex, no change in fluorescence spectrum was observed. It appeared that although rifampicin could form complexes with RNA polymerase and seminalplasmin alone, no binding domain was available for rifampicin in the RNA polymerase-seminalplasmin complex. These observations are discussed in the light of the 'initiation site' of *E.coli* RNA polymerase.

RNA polymerase *Inhibitor* *Seminalplasmin* *Rifampicin* *Fluorescence*

1. INTRODUCTION

Seminalplasmin, a 6-kDa 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]. It has been shown further that seminalplasmin inhibits the formation of the first phosphodiester bond or the initiation of RNA synthesis, at least in the case of certain promoters of T_7 DNA [2].

It is now well established that, unlike many other compounds that interfere with transcription [3], the antibiotic rifampicin, a specific inhibitor of bacterial RNA polymerase acts by direct interaction with the enzyme [4], forming a remarkably stable, stoichiometric complex. Probably, rifampicin inhibits RNA synthesis at the level of initiation [5,6]. In the light of these observations, we thought it of interest to study the competitive binding of seminalplasmin and rifampicin with *E. coli* RNA polymerase under non-transcribing conditions, i.e., in the absence of both template and substrate.

We attempted to study the interaction of seminalplasmin and rifampicin with *E. coli* RNA polymerase by using fluorescence spectroscopy. Rifampicin quenches the intrinsic fluorescence of RNA polymerase through energy transfer [7]; the known amino acid sequence of seminalplasmin shows that it has a single tryptophan making the protein intrinsically fluorescent [8].

2. EXPERIMENTAL PROCEDURE

E. coli RNA polymerase was purified as described before [9] from 250 g cells (MRE 600, RNase 1^-) grown up to the log phase in a 40-l fermentor. The 500-kDa enzyme with subunit composition $\alpha_2\beta\beta'\sigma$ was found to be more than 90% pure on SDS-polyacrylamide gel electrophoresis (not shown) and had a specific activity of 1200 units/ mg. Seminalplasmin was purified following the standard procedure developed in this laboratory [1]. Rifampicin bought from Sigma was used without further purification. All the

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nucleoside triphosphates and other reagents used were of the purest grade available. [^{3}H]UTP used for assaying RNA polymerase was obtained from New England Nuclear. Uncorrected fluorescence emission spectra were recorded on a 650-10S Hitachi spectrofluorimeter. The concentrations of the enzyme, seminalplasmin and rifampicin were fixed spectrophotometrically, using the values $A_{280}^{1\,mg/ml} = 0.62$ for RNA polymerase, $A_{280}^{1\,mg/ml} = 1.07$ for seminalplasmin and the molar extinction coefficient of 28 000 for rifampicin at 334 nm.

3. RESULTS

All the fluorescence experiments were performed such that the total absorbance at the excitation wavelength was always less than 0.1 so as to avoid the inner filter effect. The measurements were carried out at 25°C in a buffer containing 0.1 M KCl and 10 mM Tris-HCl (pH 8). Fig.1 shows the typical fluorescence emission spectrum of RNA polymerase when excited at 280 nm. Upon addition of an equimolar quantity of rifampicin, the 340 nm emission band of RNA polymerase was quenched due to complexation, as expected. Fig.1 also shows the emission spectrum of seminalplasmin and that of RNA polymerase with seminalplasmin when the latter is in 10-fold molar excess, the excitation being at 280 nm. It is seen that the emission spectrum is simply additive of the contributions of the constituents. Interestingly, it was observed that the addition of rifampicin to this

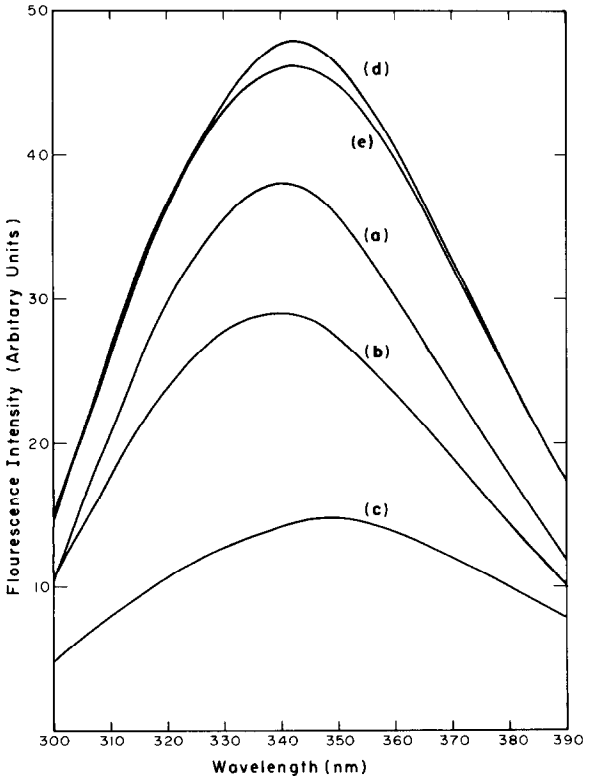

Fig.1. Fluorescence emission spectra of *E. coli* RNA polymerase in the presence of rifampicin and bovine seminalplasmin at 24°C (excitation wavelength 280 nm). a, RNA polymerase (2.6×10^{-8} M); b, (a) + rifampicin (3×10^{-8} M); c, seminalplasmin (2.8×10^{-7} M); d, RNA polymerase (2.6×10^{-8} M) + seminalplasmin (2.8×10^{-7} M); e, (d) + rifampicin (3×10^{-8} M).

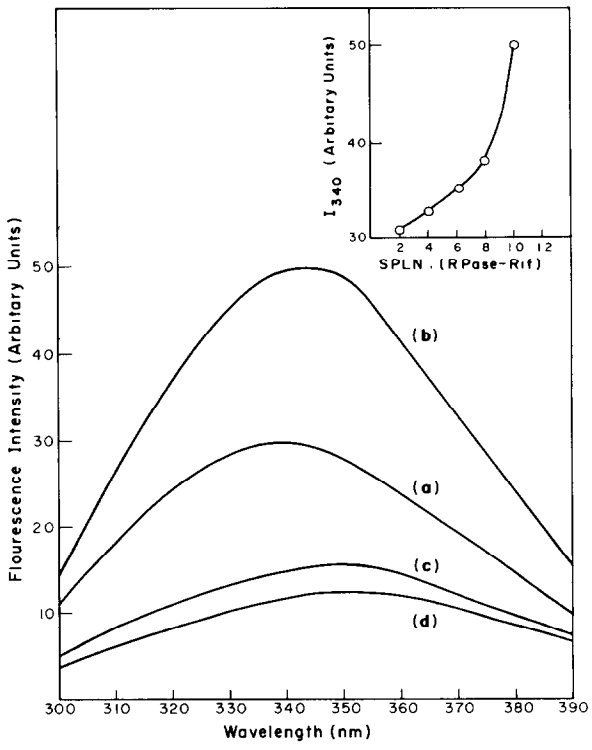

Fig.2. Fluorescence emission spectra of *E. coli* RNA polymerase, and bovine seminalplasmin in the presence of rifampicin, at 24°C (excitation wavelength, 280 nm). a, RNA polymerase (2.6×10^{-8} M) + rifampicin (3×10^{-8} M); b, (a) + seminalplasmin (2.8×10^{-7} M); c, seminalplasmin (2.8×10^{-7} M); d, (c) + rifampicin (3×10^{-8} M); the inset shows the variation of the fluorescence intensity *(I)* at 340 nm with different molar ratios of seminalplasmin (SPLN) to the RNA polymerase (RPase) – rifampicin (Rif) complex.

mixture of seminalplasmin and RNA polymerase caused little quenching of the 340 nm band (fig. 1e). This leads us to infer that rifampicin, which otherwise forms a strong complex with RNA polymerase, may not be able to bind to the RNA polymerase-seminalplasmin complex. To substantiate this inference, the effect of the addition of seminalplasmin to the preformed RNA polymerase rifampicin complex was studied (vide, fig.2). The concentration of seminalplasmin was varied up to a 10-fold molar excess to that of RNA polymerase alone (inset, fig.2). At lower molar ratios of seminalplasmin:(RNA polymerase-rifampicin), the emission spectra were the summation of that of enzyme-rifampicin complex and that of seminalplasmin. However, at 10-fold molar excess of seminalplasmin, the quenching effect of rifampicin on RNA polymerase was abolished, generating the emission spectrum (fig.2b) as that shown previously for the seminalplasmin-RNA polymerase complex (see fig.1d). However, it was observed that rifampicin is able to quench the emission band of seminalplasmin alone when excited at 280 nm (vide, fig.2d). This observation suggests that a direct interaction exists between free seminalplasmin and the antibiotic rifampicin.

4. DISCUSSION

The reversible nature of rifampicin binding to RNA polymerase has been established earlier through fluorescence titration [4]. Both the association and dissociation reaction rates are biphasic, and $MgCl_2$ and DNA influence the fast or slow phases in different proportions. It has also been postulated by Bahr et al. [4] that there exist two putative structural states of the enzyme for binding to the antibiotic. Our measurements were done at equilibrium and thus represent a gross change of the enzyme upon binding to rifampicin. The emission spectrum of seminalplasmin, typical of that of tryptophan fluorescence, is quite weak in the concentration range we have studied. However, on addition of the seminalplasmin to RNA polymerase, the emission band was found to be additive, indicating thereby that any interaction between these two species could not be followed by fluorescence spectroscopy.

It is important to note at this stage that both rifampicin and seminalplasmin are inhibitors for the initiation of RNA synthesis. Our spectral data indicate that in the presence of an excess of seminalplasmin, rifampicin cannot bind to the enzyme and that seminalplasmin can remove rifampicin from the vicinity of RNA polymerase. Are they competing for the same site on the enzyme?

It has been established previously that *E. coli* RNA polymerase has two sites, the initiation and the elongation sites, for RNA synthesis [10]. Both inhibitors are thought to interact with the initiation site of the enzyme, although no detailed physical mapping is available in either case. From the comparison of the M_r values (seminalplasmin, M_r 6000; rifampicin, M_r 823), and from the fact that at least 10-fold molar excess of seminalplasmin is required to remove rifampicin, it is difficult to envisage any competitive site binding between seminalplasmin and rifampicin. However, since the 'initiation domain' of *E. coli* RNA polymerase is not available for the drug in the presence of seminalplasmin, an extended region of interaction between the enzyme and seminalplasmin cannot be ruled out.

Out of the two zinc atoms that are tightly bound to *E. coli* RNA polymerase, the one in the β-subunit is involved in RNA chain initiation and directly participates in the reaction [11]. Moreover, it is the β-subunit of RNA polymerase which carries the binding site for rifampicin [12]. It is thus tempting to speculate that this zinc-containing region of the β-subunit is mainly responsible for the binding of both seminalplasmin and rifampicin. It should be mentioned here that seminalplasmin is also an inhibitor for reverse transcriptase which again is a Zn-containing enzyme [13]. Whether seminalplasmin has any special affinity towards Zn-containing sites in proteins is currently being studied in our laboratory.

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