Differential inhibition of transcription from σ^{70}- and σ^{32}-dependent promoters by rifampicin

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Abstract **Rifampicin is an antibiotic which binds to the β subunit of prokaryotic RNA polymerases and prevents initiation of transcription. It was found previously that production of heat shock proteins in *Escherichia coli* cells after a shift from 30°C to 43°C is not completely inhibited by this antibiotic. Here we demonstrate that while activity of a p_L-*lacZ* fusion (p_L is a σ^{70}-dependent promoter) in *E. coli* cells is strongly inhibited by rifampicin, a p_{groE}-*lacZ* fusion, whose activity is dependent on the σ^{32} factor, retains significant residual activity even at relatively high rifampicin concentrations. Differential sensitivity to this antibiotic of RNA polymerase holoenzymes containing either the σ^{70} or the σ^{32} subunit was confirmed in vitro. Since the effects of an antibiotic that binds to the β subunit can be modulated by the presence of either the σ^{70} or the σ^{32} subunit in the holoenzyme, it is tempting to speculate that binding of various σ factors to the core of RNA polymerase results in different conformations of particular holoenzymes, including changes in the core enzyme.**

Key words: RNA polymerase (*Escherichia coli*); Initiation of transcription; Sigma factor; Rifampicin

1. Introduction

Escherichia coli RNA polymerase holoenzyme is composed of the core enzyme (E) and a σ factor. The core enzyme consists of a dimer of α subunits and monomers of β and β′ subunits (for a review see [1]). There are at least seven alternative σ factors in *E. coli* cells, responsible for recognizing different types of promoters [2]. Studies on the structure of the RNA polymerase reported to date concerned predominantly the holoenzyme containing the most abundant σ factor, σ^{70}, responsible for recognition of most *E. coli* genes (such a holoenzyme is abbreviated Eσ^{70}) [1]. However, it is not known whether binding of different σ subunits to the core enzyme is a simple exchange of a factor without any effect on the core structure, or conformation of the core may vary considerably upon binding of different σ subunits. In fact, in simplified models, RNA polymerase holoenzyme molecules are usually presented as an unchanged core bound to various σ factors of different sizes [3].

Rifampicin is an antibiotic which binds to the β subunit of RNA polymerase [4,5], either as the free protein or in its complex with DNA (for a review see [6]). In the presence of this antibiotic, RNA polymerase is able to initiate RNA chains but is unable to make products that are longer than three residues, and transcription elongation is not affected by rifampicin [6,7]. It is also worth mentioning that studies on the mechanism of rifampicin-mediated inhibition of transcription were performed predominantly using Eσ^{70}. Since rifampicin binds to the β subunit, it might be assumed that holoenzymes bearing other σ factors should be similarly affected by this antibiotic.

Recent studies led to the unexpected observation that considerable synthesis of at least some heat shock proteins occurs in the presence of rifampicin after shifting an *E. coli* culture from 30°C to 43°C [8]. Transcription of most heat shock genes is dependent on the σ^{32} factor [2]. Therefore, in this work we investigated the sensitivity to rifampicin of RNA polymerase holoenzymes containing either the σ^{70} or the σ^{32} subunit (i.e. Eσ^{70} or Eσ^{32} respectively).

2. Materials and methods

2.1. Bacterial strains and fusions

Escherichia coli strain WAM106 (F$^-$, *araD139, Δ(argF-lac)U169, Δ(his-gnd), thi, rpsL150, gltS$_o$, flbB5301, relA1, deoC1, rbsR*) has already been described [9]. Single copy fusions bearing the *lacZ* gene under the control of the σ^{70}-dependent p_L promoter (kindly provided by D. Court, see also [8]) or σ^{32}-dependent p_{groE} promoter [10] were used.

2.2. Measurement of β-galactosidase activity

Activity of β-galactosidase was measured according to Miller [11].

2.3. Measurement of total RNA synthesis

Total RNA synthesis was measured as described previously [12]. Briefly, [^{3}H]uridine was added to the bacterial cultures to a final concentration of 2 μCi/5 μg/ml. Samples were withdrawn at indicated times and transferred onto paper filters. The filters were placed immediately in 10% ice-cold trichloroacetic acid (TCA) for 10 min and transferred to 5% TCA for 10 min. Then the samples were washed twice with 96% ethanol, and dried at room temperature. The radioactivity of the samples was estimated in a scintillation counter.

2.4. In vitro transcription

Run off in vitro transcription assays were performed as described previously [13,14]. The bands on autoradiograms were quantitated by densitometry. *E. coli* RNA polymerase core enzyme and holoenzyme saturated with the σ^{70} subunit were purchased from Epicentre Technologies. The σ^{32} factor was purified as described previously [15]. Plasmid pOF39 [16] cleaved with *Sac*II was used as a template for in vitro transcription assays.

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3. Results

3.1. Sensitivity of p_L-lacZ and p_{groE}-lacZ fusions to rifampicin in E. coli cells

We studied activities of p_L-*lacZ* and p_{groE}-*lacZ* fusions in *E. coli* cells. The p_L promoter of bacteriophage λ is a typical σ^{70}-dependent promoter. The *E. coli groE* operon is under the control of both σ^{70}- and σ^{32}-dependent promoters, however the fusion used in this study contains exclusively the p_{groE} promoter recognized by $E\sigma^{32}$ (see [10] for details). Transcription initiation from σ^{32}-dependent promoters is effective upon shifting the bacterial cultures to a high temperature (for example 43°C). In order to keep the same conditions for measuring activities of both fusions, bacteria harboring either the σ^{70}-dependent p_L-*lacZ* or the σ^{32}-dependent p_{groE}-*lacZ* fusion were cultivated at 30°C and then shifted to 43°C. The p_L promoter activity was repressed at 30°C by a thermosensitive product of the *cI857* gene present on a cryptic prophage. Therefore, upon temperature shift, activities of both promoters were stimulated. We found that addition of rifampicin to a final concentration of 25 μg/ml resulted in almost total inhibition of transcription from the σ^{70}-dependent p_L promoter, whereas the σ^{32}-dependent p_{groE} promoter retained considerable activity under these conditions (Fig. 1).

In order to compare sensitivities of both fusions to rifampicin in more detail, we measured their activities at different concentrations of this antibiotic. We found that transcription from the σ^{70}-dependent p_L promoter is inhibited at significantly lower rifampicin concentrations than transcription from the σ^{32}-dependent p_{groE} promoter (Fig. 2).

3.2. Total in vivo RNA synthesis in the presence of rifampicin

In experiments presented in Figs. 1 and 2, rifampicin was added to bacterial cultures at the time of the temperature shift. As rifampicin must enter bacteria through the cell envelope to mediate inhibition of transcription, this process may take some time before effects caused by this antibiotic can be observed. Therefore, in control experiments we measured total RNA synthesis at different times after rifampicin addition under the same conditions as employed for experiments depicted in Figs. 1 and 2. As shown in Fig. 3, significant rifampicin-mediated inhibition of RNA synthesis was observed as soon as a few minutes after addition of the antibiotic to the bacterial culture. Since in this type of experiments we monitored mainly synthesis of stable RNAs, these results also indicate efficient inhibition by rifampicin of σ^{70}-dependent promoters for synthesis of rRNA and tRNA.

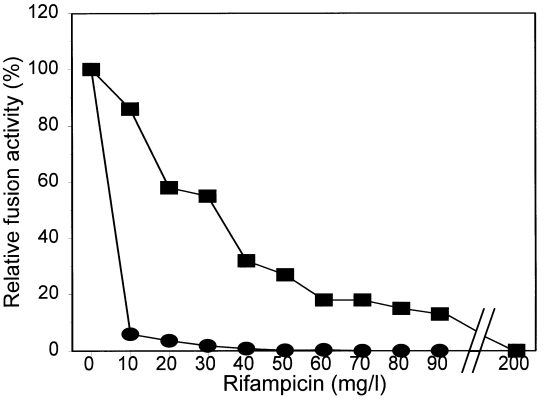

Fig. 2. Activities of σ^{70}-dependent p_L-*lacZ* (circles) and σ^{32}-dependent p_{groE}-*lacZ* (squares) fusions in *E. coli* cells treated with indicated concentrations of rifampicin. Both addition of rifampicin and temperature shift (from 30°C to 43°C) were performed at time = 0, and activities of β-galactosidase were measured 60 min later. Activities obtained with cultures non-treated with rifampicin were assumed to be 100% and other values reflect these values. The results are the average of at least two independent measurements.

3.3. Inhibition of transcription from σ^{70}- and σ^{32}-dependent promoters by rifampicin in vitro

The in vivo effects of rifampicin on activities of the fusions bearing the *lacZ* reporter gene under control of σ^{70}- or σ^{32}-dependent promoter (Figs. 1 and 2) may be direct, but one could also imagine an indirect inhibition of transcription from both promoters. Therefore, we aimed to investigate the sensitivity to rifampicin of transcription by $E\sigma^{70}$ and by $E\sigma^{32}$ in vitro. The wild-type *groE* operon of *E. coli*, present also on plasmid pOF39 [16], is under the control of both σ^{70}- and σ^{32}-dependent promoters. Thus, transcription from both promoters may be investigated using the same template – plasmid pOF39 digested with *Sac*II [13]. The results presented in Fig. 4 demonstrate that in vitro $E\sigma^{70}$-mediated transcription is con-

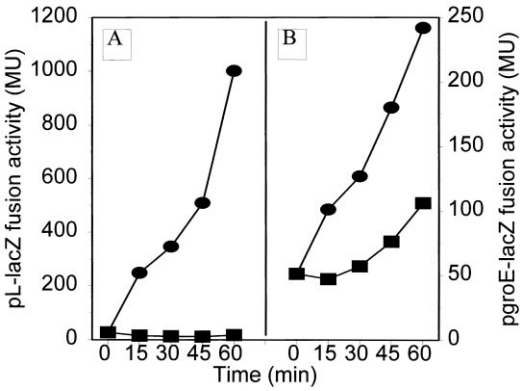

Fig. 1. Activities of σ^{70}-dependent p_L-*lacZ* (A) and σ^{32}-dependent p_{groE}-*lacZ* (B) fusions in *E. coli* cells non-treated (circles) and treated with rifampicin (squares) at final concentration of 25 μg/ml. Both addition of rifampicin and temperature shift (from 30°C to 43°C) were performed at time = 0. β-Galactosidase activities are shown in Miller units (MU).

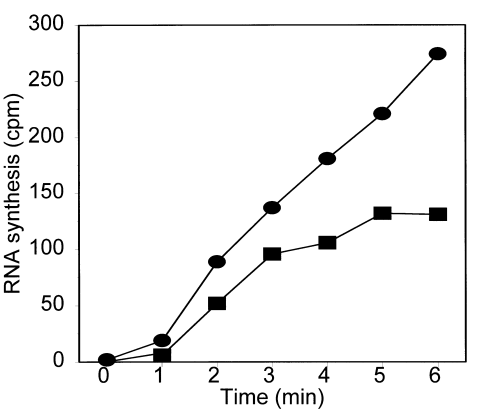

Fig. 3. Synthesis of total RNA (measured by incorporation of [^{3}H]uridine to TCA-precipitable material) in *E. coli* cells non-treated (circles) and treated with rifampicin (squares) at a final concentration of 25 μg/ml. Both addition of rifampicin and temperature shift (from 30°C to 43°C) were performed at time = 0.

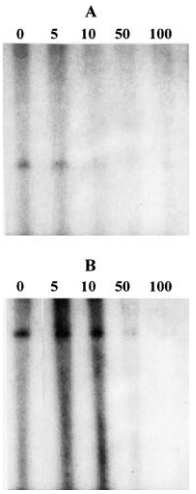

Fig. 4. In vitro transcription from the σ^{70}-dependent p_{groE} promoter (A) and the σ^{32}-dependent p_{groE} promoter (B) in the presence of rifampicin. Rifampicin concentrations (in ng/ml) are provided above the lanes. 1 U of Eσ^{70} (Epicentre Technologies) per reaction (A), and 1 U of the core enzyme (Epicentre Technologies) mixed with 0.16 μg of purified σ^{32} factor per reaction (B) were used. 0.5 μg of plasmid pOF39 digested with *Sac*II was used as a template in the in vitro run off reactions.

siderably more sensitive to rifampicin than Eσ^{32}-mediated transcription. At 5 ng/ml, rifampicin had no significant effect on transcription from both σ^{70}- and σ^{32}-dependent promoters. However, densitometric analysis of the bands on autoradiograms presented in Fig. 4 revealed that at 10 ng/ml of rifampicin, the efficiency of transcription from the σ^{70}-dependent promoter was only about 10% of that observed without this antibiotic, whereas the impairment of efficiency of transcription from the σ^{32}-dependent promoter was negligible. Significant transcription from the σ^{32}-dependent promoter (about 20% of that found in the sample without antibiotic) was observed even at 50 ng/ml of rifampicin. Nevertheless, at very high rifampicin concentrations transcription from both promoters was inhibited, similarly to the results obtained in vivo (compare Figs. 2 and 4).

4. Discussion

It was found previously that significant synthesis of at least some heat shock proteins proceeds in *E. coli* cells upon a shift from 30°C to 43°C in the presence of rifampicin [8]. Here we demonstrate that, contrary to σ^{70}-dependent promoters (the p_L promoter, promoters for stable RNA synthesis, σ^{70}-dependent p_{groE} promoter), transcription from the σ^{32}-dependent p_{groE} promoter proceeds relatively efficiently even at high concentrations of rifampicin, both in vivo and in vitro.

Rifampicin binds to the β subunit of RNA polymerase [4,5] and inhibits transcription at the initiation stage (production of RNA chains longer than three nucleotides is prevented) without significant effects on elongation [6,7]. Therefore, one might assume that the effect of rifampicin on transcription should be independent of the kind of σ factor bound to the core of RNA polymerase. However, the results presented in this report indicate that Eσ^{70} may be significantly more sensitive to rifampicin than Eσ^{32}. One possible hypothesis to explain this phenomenon is that RNA polymerase holoenzymes bearing different σ factors differ considerably in their structure (including the structure of the core enzyme or at least the β subunit). Thus, either the efficiency of binding of rifampicin to the β subunit or its effects on transcription may be different for various RNA polymerase holoenzymes. In generally accepted models of the structure of RNA polymerase, it is assumed that the structure of the core enzyme is not significantly changed when bound to different σ subunits [3]. This might not necessarily be true if the above hypothesis is correct. It is also possible that access to the rifampicin binding site is more occluded when σ^{32} is bound to the core enzyme than when σ^{70} is bound. However, since σ^{32} is a considerably smaller polypeptide than σ^{70}, we assume that the former hypothesis is more probable.

An alternative, though in our opinion less likely, explanation for the results presented in this report is that differences in the structure of promoters recognized by various RNA polymerase holoenzymes may somehow influence the efficiency of rifampicin-mediated inhibition of transcription initiation.

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