INHIBITION OF RIFAMPICIN-RESISTANT RNA SYNTHESIS BY RIFAMPICIN–RNA POLYMERASE COMPLEXES

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1. Introduction

Biochemical and electron microscopic evidence has shown that *Escherichia coli* RNA polymerase holoenzyme forms in vitro, in the absence of nucleoside triphosphates, a stable and specific complex with bacteriophage T7 DNA in the early promoter region of the chromosome [1,2]. Neither the stability nor the specificity of this complex is altered by inactivation of the RNA polymerase by the antibiotic rifampicin [2,3]. In work described in this letter T7 DNA was first complexed with rifampicin-inactivated *E. coli* wild type RNA polymerase and was then used as template for an in vitro transcription assay. RNA synthesis in the presence of rifampicin was catalysed by *E. coli* rifampicin resistant RNA polymerase or by bacteriophage T7 RNA polymerase. The results show that rifampicin-inactivated *E. coli* RNA polymerase inhibits the activity of *E. coli* rifampicin resistant enzyme but does not affect the activity of bacteriophage T7 RNA polymerase. The mechanism of this inhibition is discussed along with its relation to the mechanism of dominance of rifampicin sensitivity over rifampicin resistance observed in bacteria heterozygous for the rifampicin alleles [4–7].

2. Materials and methods

E. coli B wild type (rifampicin sensitive) RNA polymerase holoenzyme was prepared according to Burgess [8] or Chamberlin and Berg [9]. *E. coli* rifampicin resistant RNA polymerase (strain K-12 RFS 524 from R. F. Schleif) and bacteriophage T7 RNA polymerase (rifampicin resistant) were generous gifts from R. Burgess and M. Chamberlin respectively. T7 bacteriophage was grown as described in ref. [10] and purified on CsCl gradients. T7 DNA was extracted with phenol. Rifampicin was purchased from Ciba-Geigy A.G., Basel.

The reaction mixture for RNA synthesis by *E. coli* rifampicin resistant RNA polymerase contained 10 mM Tris–HCl pH 7.9, 50 mM NaCl, 10 mM $MgCl_2$, 0.2 mM dithiothreitol, 0.1 mM EDTA, 100 μg/ml rifampicin, 60–100 μg/ml T7 DNA and various amounts of wild type RNA polymerase. The same reaction mixture containing 20 mM $MgCl_2$ and 1 mg/ml bovine serum albumin was used for RNA synthesis by bacteriophage T7 RNA polymerase. Rifampicin resistant RNA synthesis was started by simultaneous addition of either *E. coli* or bacteriophage T7 rifampicin resistant RNA polymerase and the four nucleoside triphosphates to a reaction mixture containing T7 DNA complexed with various amounts of rifampicin-inactivated *E. coli* wild type RNA polymerase.

3. Results

In a first experiment, *E. coli* wild type RNA polymerase was inactivated with rifampicin. Complexes were formed between varying amounts of the inactivated enzyme and a constant amount of T7 DNA. RNA synthesis was started by simultaneously adding to these solutions the four nucleoside triphosphates and a constant amount of either *E. coli* rifampicin resistant RNA polymerase or bacteriophage

T7 RNA polymerase. RNA synthesis was stopped after 2 min at 37°C. As shown in fig. 1, rifampicin-inactivated *E. coli* RNA polymerase inhibits efficiently RNA synthesis by *E. coli* rifampicin resistant enzyme, whereas it affects only slightly total RNA synthesis by bacteriophage T7 RNA polymerase. It is important to note that in this experiment and in the following one RNA synthesis was not limited by the amount of DNA present in the reaction mixture. Control experiments showed that with the highest amount of wild type RNA polymerase used in these experiments the RNA synthesis was, in absence of rifampicin, proportional to the amount of enzyme present in the reaction mixture.

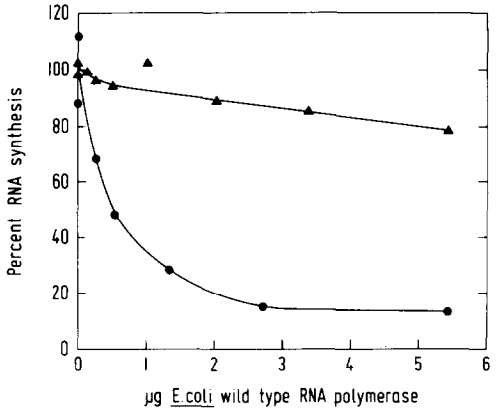


Fig. 1. Inhibition of RNA synthesis by rifampicin-inactivated RNA polymerase. 0 to 5.4 µg wild type *E. coli* RNA polymerase (about 80% active) were incubated 12 min at 0°C in the buffer described in Materials and methods in the presence of rifampicin. 13 µg T7 DNA were added and the incubation was continued for 10 min at 37°C. The four nucleoside triphosphates, containing [^{14}C]ATP at 4200 cpm/nmol, were added to a final concentration of 200 µM each. In one series of assays 1.0 µg *E. coli* rifampicin-resistant RNA polymerase was added with the nucleoside triphosphates and the incubation was continued for 2 min 15 sec at 37°C in a final volume of 230 µl. In a series of blanks *E. coli* rifampicin resistant RNA polymerase was omitted. In the other series of assays 0.55 µg T7 RNA polymerase was added and the reaction was continued for 1 min 45 sec at 37°C in a final volume of 135 µl. Here again in a series of blanks T7 RNA polymerase was omitted. The reactions were stopped by addition of 500 nmol ATP and 250 µg bovine serum albumin followed by 5% trichloroacetic acid. The acid insoluble [^{14}C]AMP was determined. After subtraction of the blank values (constant over the whole range of concentration of wild type RNA polymerase) the RNA synthesis obtained in the absence of inactivated wild type RNA polymerase was taken as 100% and the other amounts were expressed as a percent of this value. The *E. coli* rifampicin resistant RNA polymerase and the bacteriophage T7 RNA polymerase incorporated respectively 0.13 nmol and 0.28 nmol AMP into RNA in the absence of *E. coli* wild type RNA polymerase. The blank value was equivalent to 0.02 nmol ATP. RNA synthesis by *E. coli* rifampicin resistant RNA polymerase: ——●——; RNA synthesis by bacteriophage T7 RNA polymerase: ——▲——.

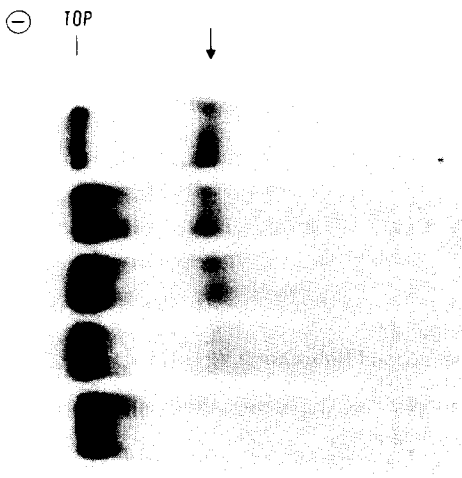
In a second experiment, *E. coli* wild type RNA polymerase was allowed to form complexes with T7 DNA in the presence of rifampicin. As above, RNA synthesis was started by the simultaneous addition of the four nucleoside triphosphates and *E. coli* rifampicin resistant RNA polymerase. After 16 min at 37°C, the reaction was stopped and the RNA synthesized was analyzed by polyacrylamide gel electrophoresis. The electrophoresis resolved the major specific RNA transcript made in vitro on T7 DNA by *E. coli* RNA polymerase holoenzyme (see fig. 2). This RNA has a molecular weight of about 2.2×10^6 daltons [11] and is transcribed from the DNA sequences expressed in vivo early after the bacteriophage infection [12,13].

The relative amount of the 2.2×10^6 dRNA was determined in each sample by scanning the autoradiogram with a microdensitometer, and is also shown in fig. 2. These data show that the synthesis of the 2.2×10^6 dT7 early RNA catalysed by *E. coli* rifampicin resistant RNA polymerase is completely inhibited in vitro by rifampicin-inactivated wild type RNA polymerase. In a separate experiment I have shown with T7 RNA pre-synthesized in vitro that, in the conditions of the experiment shown in fig. 2, the inhibition of synthesis of the 2.2×10^6 dRNA was not due to ribonuclease contamination of the wild type RNA polymerase preparation.

4. Discussion

The experiments I have presented show that specific RNA synthesis catalysed in vitro by *E. coli* rifampicin resistant RNA polymerase on a non-limiting amount of T7 DNA is inhibited by rifampicin-inactivated wild type RNA polymerase.

The mechanism of this inhibition may be discussed in the light of two observations. The first is that rifampicin-inactivated *E. coli* RNA polymerase keeps its specificity of interaction with T7 DNA. Inactivated



SAMPLE	E.COLI WILD TYPE RNA POLYMERASE (μg)	RIFAMPICIN AND RIF-RESISTANT RNA POLYMERASE	RELATIVE AMOUNT OF EARLY RNA
1	4	–	199
2	0	+	100
3	1	+	72
4	4	+	17
5	8	+	<6

Fig. 2. Inhibition of the in vitro T7 early RNA synthesis. In the five sample 0–8 μg wild type *E. coli* RNA polymerase (about 20% active) were mixed with rifampicin and 13 μg T7 DNA in the reaction mixture described in Materials and methods. Rifampicin was omitted in sample 1. After 6 min at 37°C, 2.5 μg *E. coli* rifampicin resistant RNA polymerase was added together with ATP, GTP and UTP at a final concentration of 400 μM each and α-labelled ^{32}P CTP (97 cpm/pmol) at a final concentration of 200 μM in a final volume of 125 μl. Rifampicin resistant RNA polymerase was omitted in sample 1. The incubation was continued for 16 min at 37°C and terminated by addition of EDTA and SDS. 100 μl of each sample was electrophoresed on a 2% polyacrylamide gel for 18 hr as described in ref. [20]. The autoradiogram was exposed 18 hr. The arrow indicates the 2.2 × 10^6 dT7 early RNA synthesized in vitro. The relative amount of this RNA was determined by scanning the autoradiogram with a microdensitometer.

enzyme recognizes and binds to the same early promotor sites as it does in the absence of rifampicin [2]. Secondly, the rate of dissociation of an RNA polymerase–DNA complex is not afffected by inactivation of the enzyme with rifampicin [3]. The half-life of an RNA polymerase-promotor complex is about 60 hr for T7 DNA in the conditions used in these experiments [1], a time very long compared with the times of incubation. Thus incubation of DNA with rifampicin-inactivated RNA polymerase or inactivation of DNA-bound enzyme should result in the formation of a tight binding complex of rifampicin, enzyme and DNA located in the early promotor region of the chromosome. As rifampicin-inactivated enzyme is not able to initiate RNA synthesis in the presence of nucleoside triphosphates, it should block access to the early promoter binding sites. For lack of an accessible binding site in the early promoter region rifampicin resistant RNA polymerase would be unable to initiate specific RNA synthesis [1,14].

RNA synthesis by bacteriophage T7 RNA polymerase is only slightly inhibited by rifampicin-inactivated *E. coli* RNA polymerase. On the basis of the mechanism described above this result is to be expected since *E. coli* RNA polymerase and bacteriophage T7 RNA polymerase use different promoters to initiate RNA synthesis in vitro on T7 RNA [15].

The mechanism presented does not exclude this possibility that rifampicin-inactivated RNA polymerase inhibits elongation of RNA chains. A ternary transcription complex of RNA polymerase and RNA on DNA could conceivably be unable to displace a rifampicin-inactivated RNA polymerase molecule from a tight binding site on the same DNA molecule. It has indeed been shown that there are three or four strong binding sites [2] and three sites of RNA synthesis initiation [16,17] in the early promotor region of the T7 chromosome.

Rifampicin-inactivated RNA polymerase has also been shown to inhibit the synthesis in vitro of rifampicin resistant RNA on bacteriophage T2 DNA [4,5,18]. This inhibition has, however, only been observed at a high enzyme to DNA ratio such that DNA was present at limiting concentration in the

reaction. The inhibition in this case may have been due rather to an inability of the rifampicin resistant RNA polymerase to elongate efficiently the nascent RNA chains than to an inability to initiate RNA synthesis [18].

Bacteria heterozygous for the rifampicin alleles (rif-sensitive/rif-resistant) are generally sensitive to rifampicin [4–7] although about half the RNA polymerase molecules in such cells are known to be resistant in vitro to the antibiotic [4–6]. Austin and Khesin and their collaborators have proposed that phenotypic dominance of sensitivity to rifampicin is due to blocking of the DNA template [6] or, more specifically, to blocking of promoters [4,5], by rifampicin-inactivated rifampicin sensitive RNA polymerase. The data presented above support the hypothesis that initiation of RNA synthesis at certain promoters could be inhibited in heterodiploid cells by rifampicin-inactivated RNA polymerase molecules. It is, however, likely that the synthesis of RNA initiated at promoters with weak RNA polymerase binding sites would not be efficiently blocked by the inactivated enzyme. The slowness of inhibition of cell growth [6] and of RNA synthesis [19], after addition of rifampicin to rif-s/rif-r bacteria, the differential inhibition of protein synthesis [19] and the relative resistance of bacteriophage T2 development [5] show that gross RNA synthesis is not efficiently blocked in vivo by this mechanism. The known rifampicin sensitivity of rif-s/rif-r cells could be due to blockade of only a few genes which have strong promoter binding sites and whose expression is essential for growth.

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