A derivative of rifamycin SV inhibiting rifampicin-resistant RNA polymerase of *Escherichia coli*

V.G. Nikiforov, A.A. Trapkova, A.I. Gragerov and D.N. Maslin*

Institute of Molecular Genetics, USSR Academy of Sciences, Moscow and **All-Union Institute of Antibiotics, USSR Ministry of Medical Industry, Moscow, USSR*

Received 28 October 1982

RNA polymerase *Rifamycin derivative SV* *Antibiotic resistance* *RM41* *DNA-binding*

1. INTRODUCTION

The antibiotic rifampicin, a semi-synthetic derivative of rifamycin SV, inhibits bacterial RNA polymerase by forming a very stable complex with the enzyme in a 1 : 1 ratio [1]. In the search for rifamycins which would affect rifampicin-resistant bacteria, a large number of derivatives with bulky lipophilic side-chains were synthesized. Many of them proved capable of inhibiting the RNA polymerase of rifampicin-resistant *E.coli* mutants [2] and a number of other enzymes responsible for the polymerization of nucleotides [3]. However, the mechanism of action of these compounds against rifampicin-resistant RNA polymerases is different from the mechanism whereby rifampicin acts on the normal RNA polymerase. For example, the best-studied of these rifamycin derivatives, AF/013, unlike rifampicin prevents the binding of RNA polymerase to DNA [2,4].

The first rifamycin derivatives inhibiting a rifampicin-resistant *Escherichia coli* RNA polymerase by a similar mechanism to the action of rifampicin on a sensitive RNA polymerase were found among the dimeric rifamycins [5]. We have synthesized a number of derivatives of rifamycin SV with various substitutions in the 3rd position of the naphthoquinone nucleus; some of them proved capable of inhibiting rifampicin-resistant RNA polymerase of *E.coli* mutants. This paper describes a derivative of rifamycin SV, 3-(6-bromo-4-phenyl-2-hydrazonomethyl-quinazoline)-rifamycin SV (fig. 1) which inhibits a rifampicin-resistant RNA polymerase by a similar mechanism to that of the action of rifampicin on a sensitive RNA polymerase.

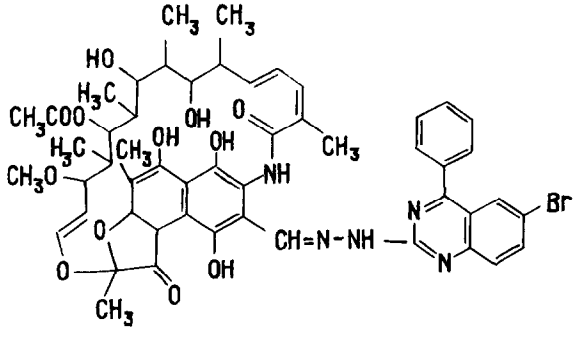

Fig. 1. 3-(6-bromo-4-phenyl-2-hydrazonomethyl-quinazoline)-Rifamycin SV (RM41).

2. MATERIALS AND METHODS

Purified RNA polymerase was obtained from rifampicin-resistant mutant *E.coli* K12 RpoB255 and RpoB4 cells by the method in [6] as described [7]. RNA polymerase activity was assayed in 200 μl reaction samples containing the buffer solution: 0.01 M Tris (pH 80), 0.1 M NaCl, 0.01 M $MgCl_2$. Phage T2 DNA (100 μg/ml) was used as the template. The order in which the samples were made up and incubated is specified in the figure legends. The binding of RNA polymerase to T2

Published by Elsevier Biomedical Press
00145793/82/0000—0000/$2.75

DNA was assayed with the help of nitrocellulose filters as in [7]. 3-(6-bromo-4-phenyl-2-hydrazonomethyl-quinazoline)-Rifamycin SV (the code name RM41) was synthesized by mixing equimolar amounts of 3-formyl rifamycin SV and 6-bromo-4-phenyl-2-hydrazonomethyl-quinazoline in tetrahydrofuran with subsequent solvent removal and recrystallization of the product from ethyl acetate. The product was analytically pure: silica gel chromatography in a chloroform–methanol (9:1) system showed one spot. Rifamycins were dissolved in dimethylsulfoxide (10 mg/ml).

3. RESULTS AND DISCUSSION

Fig. 2 shows the effects of rifampicin and RM41 on rifampicin-resistant and -sensitive RNA polymerases. RM41 completely inhibits the rifampicin-resistant RNA polymerase, though this requires appreciably higher concentrations than the inhibition of the sensitive RNA polymerase. It is interesting that, although RM41 is much more effective than rifampicin in inhibiting the rifampicin-resistant mutant RNA polymerase, the action of RM41 on the rifampicin-sensitive RNA polymerase is less effective than that of rifampicin (fig. 2). RM41 does not seem to penetrate into intact *E.coli* cells, for it does not affect their reproduction.

To elucidate the mechanism of action of RM41 on the resistant RNA polymerase, we studied its effect on various stages of transcription. RNA polymerase is known to display a varying sensitivity to rifampicin at different stages of transcription. The greatest inhibiting effect is observed if rifampicin is added to a free RNA polymerase, while an RNA polymerase that has started RNA synthesis is rifampicin-resistant. After RNA polymerase gets bound to DNA and forms open promoter complexes, rifampicin is much more slow to attack it than in the case of a free enzyme. Therefore when rifampicin and substrates are simultaneously added to open complexes, the majority of the RNA polymerase molecules manage to start RNA synthesis and avoid the inhibiting effect [8]. The same effect is observed in the case of RM41 and rifampicin-resistant RNA polymerase (fig. 3): when RM41 and substrates are added to an RNA polymerase–T2 DNA complex formed at 37°C, the inhibition is far less pronounced than with RM41 added to a free RNA polymerase. The number of rifampicin-resistant open complexes is known to diminish considerably with a fall in temperature and an increase in ionic strength. Fig. 4 shows that the same dependences are observed with regard to the action of RM41 on the resistant RNA polymerase.

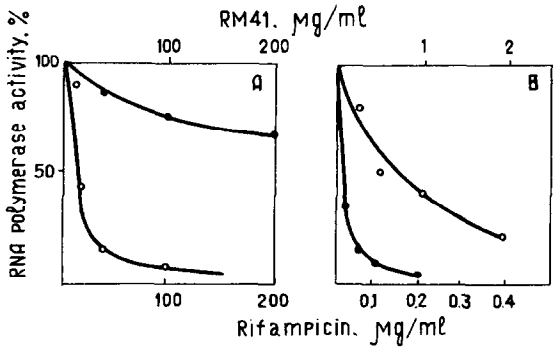

Fig. 2. Effects of rifampicin and RM41 on rifampicin-resistant (A) and sensitive (B) RNA polymerases. The reaction mixture was made up in the following order: buffer solution, RNA polymerase, antibiotic, DNA, substrates. Activity is plotted in % of the activity without antibiotics: (●) rifampicin; (○) RM41.

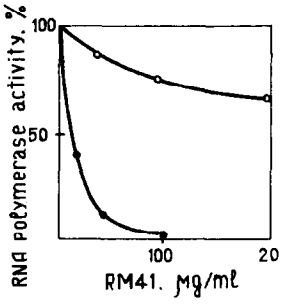

Fig. 3. Protection of RNA polymerase from RM41 after the formation of an open complex with DNA. (○) RNA polymerase and DNA were incubated in the buffer solution for 7 min at 37°C to form open complexes, then the antibiotic (to 100 μg/ml) and substrates were added simultaneously, and the samples were incubated for another 10 min. (●) The antibiotic was added to RNA polymerase in the buffer solution, then the other components were added and the samples were incubated for 10 min at 37°C. Activity is plotted in % of the enzyme activity without the antibiotic.

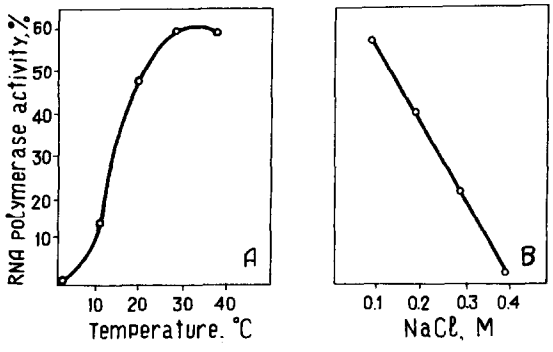

Fig. 4. Influence of temperature and ionic strength on the resistance of DNA–RNA polymerase complexes to RM41. (A) RNA polymerase and DNA were incubated in the buffer solution for 7 min at the temperature shown, then RM41 (to 100 μg/ml) and substrates were added simultaneously and the samples were transferred to 37°C for 10 min. RNA polymerase activity is plotted in % of the activity without the antibiotic. (B) RNA polymerase and DNA were incubated in the buffer solution with NaCl in the concentration shown for 7 min at 37°C. Then RM41 and substrates were added simultaneously, and the samples were incubated for 10 min at 37°C. Activity is plotted in % of the activity without the antibiotic at 0.1 M NaCl.

A study of the RNA polymerase–DNA binding using nitrocellulose filters has shown that RM41 in inhibiting concentrations (100 μg/ml) does not affect the enzyme's binding to DNA (not shown). Thus all our results indicate that RM4, unlike derivatives of the AF/013 type, acts on a resistant RNA polymerase by a mechanism similar to the action of rifampicin upon a sensitive RNA polymerase, possibly through interaction with the same enzyme centre. Therefore RM41 may prove useful for studies of the structure and functioning of that centre.

ACKNOWLEDGEMENTS

The authors are grateful to Professor R.B. Khesin for the interest shown in their work and to Dr O.Yu. Chertov for the preparation of RpoB255 RNA polymerase.

REFERENCES

[1] Riva, S. and Silvestri, L.G. (1972) Annu. Rev. Microbiol. 26, 199–224.
[2] Riva, S., Fietta, A. and Silvestri, L.G. (1972) Biochem. Biophys. Res. Commun. 49, 1263–1271.
[3] Sethi, V.S. and Okano, P. (1976) Biochim. Biophys. Acta 454, 230–247.
[4] Meilhac, M., Tysper, Z. and Chambon, P. (1972) Eur. J. Biochem. 28, 291–300.
[5] Fietta, A.M. and Silvestri, L.G. (1975) Eur. J. Biochem. 52, 391–400.
[6] Burgess, R.R. and Jendrisak, J.J. (1975) Biochemistry 14, 4634–4639.
[7] Larionov, O.A., Gragerov, A.I., Kalyaeva, E.S. and Nikiforov, V.G. (1979) Mol. Gen. Genet. 176, 105–111.
[8] Chamberlin, M. (1976) in: RNA polymerase (Losick, R. and Chamberlin, M. eds) pp. 159–191, Cold Spring Harbor Laboratory, New York.