EFFECT OF RIFAMPICIN ON IN VITRO RNA SYNTHESIS OF STREPTOMYCES MEDITERRANEI

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SUMMARY

DNA-dependent- and actinomycin D sensitive-RNA synthesis by the enzyme preparation from a rifamycin-producing organism, Streptomyces mediterranei, was highly resistant to rifampicin as compared with those from Streptomyces griseus B_3 and Escherichia coli Q13. Rifampicin was not inactivated by the incubation with Streptomyces mediterranei enzyme preparation. Thus the extensively high resistance of RNA synthesis by the enzyme preparation to rifampicin may be due to the resistance of RNA polymerase of Streptomyces mediterranei.

It is well known that rifamycin-antibiotics inhibit DNA-dependent RNA synthesis interacting with RNA polymerase (1,2). Rifampicin is the most widely used semi-synthetic derivative of rifamycin for clinical purposes and biochemical studies (1,2). We have recently observed that Streptomyces griseus B_3, which produces streptomycin but not rifamycin (3), cannot grow in the presence of a low concentration of rifampicin, but Streptomyces mediterranei, rifamycin producer, can grow without any impairment even at a high concentration of rifampicin. In this paper we report that RNA polymerase of Streptomyces mediterranei is highly resistant to rifampicin compared with those of Streptomyces griseus B_3 and Escherichia coli Q13.

MATERIALS AND METHODS

Streptomyces mediterranei (a rifamycin producer) and Streptomyces griseus B_3 (a streptomycin producer) were provided by Dr. Seino, Kaken Chemical Co., Ltd.,

Tokyo, Japan. These microorganisms were grown in a medium containing the following components per liter (pH 7.3): 2 g of Difco yeast extract, 1 g of beef extract, 2 g of Casamino Acids and 10 g of glucose. After cultivation at 28°C for 20 hours with aeration by shaking, the mycelia were harvested by filtration, washed once with saline and stored at -20°C until use. Escherichia coli Q13 cells were grown at 28°C for 18 hours in a medium containing the following components per liter (pH 7.0): 10 g of Difco Bacto-Tryptone, 5 g of NaCl and 0.5 mg of Vitamin B_1. The cells were harvested by centrifugation, washed with 0.05 M Tris buffer containing 0.01 M $MgCl_2$, 0.2 M KCl, 0.1 mM dithiothreitol, 0.1 mM EDTA and 5% (v/v) glycerol, and kept frozen until use. Cells of Streptomyces mediterranei, Streptomyces griseus B_3 and Escherichia coli Q13 were disrupted by grinding with alumina or quartz sand, and then the crude RNA polymerase preparations were obtained by DEAE-cellulose column chromatography according to the procedure of Burgess (4). To the pooled fractions from DEAE-cellulose column (Fraction 4 in Burgess' method) one and a half volumes of saturated ammonium sulfate solution was added and the resulted precipitate was collected by centrifugation. The pellet was dissolved in a storage buffer containing 50% glycerol (4). The protein concentrations for the preparations from Streptomyces mediterranei, Streptomyces griseus B_3 and Escherichia coli Q13 measured by the method of Lowry et al. (5) were 8.2, 12.1 and 16.2 mg/ml respectively.

The complete reaction mixture for the assay of RNA polymerase activity contained in 0.2 ml the following components unless otherwise indicated: 50 mM Tris-HCl (pH 7.9), 10 mM $MgCl_2$, 200 mM KCl, 1 mM dithiothreitol, 0.2 mM each of ATP, UTP, CTP and [^{3}H]GTP (1 μc), 0.8 mM potassium phosphate (pH 7.5), 30 μg of calf thymus DNA and enzyme fractions as stated in the legends to the Tables and Figure. The mixtures were incubated at 30°C for 15 minutes, chilled in ice, added 0.005 ml of bovine serum albumin (10 mg/ml) and 2 ml of cold 5% trichlor-

acetic acid. After at least 15 minutes, the precipitates were collected on Whatman GF/C glass fiber filters, washed 4 times with 5 ml of cold 5% trichloracetic acid and once with 1% trichloracetic acid. The filters were dried and counted by liquid-scintillation spectrometer using conventional toluene scintillation mixture.

[^{3}H]GTP was purchased from Schwarz/mann: calf-thymus DNA, ATP and GTP from Sigma Chemical Company: UTP, CTP and rifampicin from Boehringer mannheim GmbH: Deoxyribonuclease from Worthington Biochemical Corp.: and DEAE-cellulose DE52 from Whatman Biochemicals Ltd.

RESULTS

As reported for E. coli (4), RNA polymerase activities from Streptomyces mediterranei and Streptomyces griseus B_3 were eluted from DEAE-cellulose column with 0.23 M KCl. The Streptomyces enzymes, however, seemed to be less stable even in the presence of glycerol and dithiothreitol. The incorporation of [^{3}H]GTP into acid-insoluble fraction by any one of these crude enzyme preparations from Streptomyces mediterranei, Streptomyces griseus B_3 and Escherichia coli Q13 is considered to be DNA-dependent RNA synthesis since the incorporation depends on the presence of DNA and nucleoside triphosphates and is completely inhibited by actinomycin D as shown in Table 1. It was also confirmed that acid-insoluble products were completely hydrolyzed by alkaline treatment (0.3 M NaOH for 90 min at 44°C (6)).

To test the effect of rifampicin on RNA synthesis by the enzyme preparations from different microorganisms, we chose each enzyme concentration within the ranges in which the [^{3}H]GTP incorporation increases linearly with the amounts of enzyme and with incubation time. As shown in Table 1, enzyme preparations from Streptomyces cells showed rather weak activity as compared with the preparation from Escherichia

Table 1

Requirements and antibiotic-susceptibility in RNA synthesis by enzyme preparations from S. mediterranei, S. griseus B_3 and E. coli Q13

Enzyme preparations	S. mediterranei (41 μg)		S. griseus (61 μg)		E. coli (81 μg)	
Exp. no.	I	II	I	II	I	II
Conditions						
Complete	100% (1989 c.p.m.)	100% (1362 c.p.m.)	100% (1173 c.p.m.)	100% (801 c.p.m.)	100% (8878 c.p.m.)	100% (12782 c.p.m.)
-Enzyme	31.0	14.7	26.3	12.1	1.9	1.9
+Actinomycin D (AmD)	24.1	21.4	-	23.5	8.0	-
+Rifampicin (Rif)	120*	99.7	32.0	17.1	3.8	3.2
+Rif and AmD	31.8*	32.7	-	34.0	-	-
-DNA	27.0	-	-	28.6	4.6	-
-UTP, ATP and CTP	41.4	-	-	43.6	6.9	-

The concentrations of antibiotics used were 2.5 μg or 1.0 μg(*) per ml for rifampicin and 20 μg per ml for actinomycin D. More detailed experimental conditions are as described in MATERIALS AND METHODS.

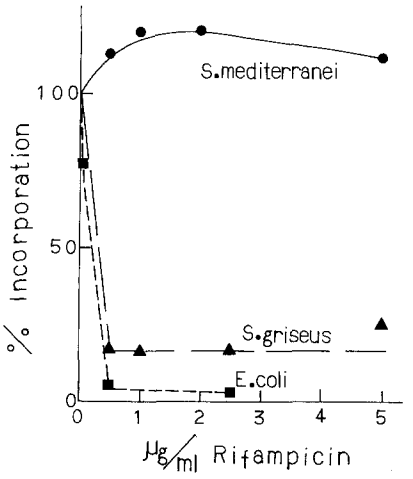

Fig. 1. Effects of rifampicin on RNA synthesis by enzyme preparations from S. mediterranei, S. griseus and E. coli.

The reaction mixture (0.2 ml) contained rifampicin as indicated. Enzyme preparations used were 41 μg for S. mediterranei, 61 μg for S. griseus and 81 μg for E. coli. The other conditions were the same with those described in the legend to Table 1. 100% represents 1989 c.p.m., 801 c.p.m. and 12782 c.p.m. for S. mediterranei, S. griseus and E. coli enzymes respectively.

coli and the lower activities observed in the second experiment indicated the unstability of these Streptomyces enzyme preparations. The resistance to rifampicin, however, could be clearly observed for the preparation from Streptomyces mediterranei. This was more precisely examined in the experiments shown in Fig. 1. The RNA synthesis by the enzyme preparations from Streptomyces griseus B_3 and Escherichia coli Q13 were completely inhibited by 0 5 μg/ml of rifampicin, but that by the preparation from Streptomyces mediterranei was not repressed even at 5 μg/ml of rifampicin. Since the acid-insoluble product formed by Streptomyces mediterranei enzyme preparation in the presence of rifampicin was completely hydrolyzed by alkaline treatment (data are not shown) and its synthesis was greatly inhibited by actinomycin D (Table 1), the incorporation of [^{3}H]GTP in the presence of rifampicin represents DNA dependent RNA synthesis as that observed in the absence of rifampicin. Thus, it is concluded that in

Table 2

Effect of rifampicin preincubated with S. mediterranei enzyme preparation on RNA synthesis by E. coli RNA polymerase preparation

Incubation	RNA synthesized c.p.m.	%
Complete + S. mediterranei (S. med) enzyme preincubated without rifampicin	7853	100
Complete + rifampicin preincubated without S. med enzyme	397	5.1
Complete + rifampicin preincubated with S. med enzyme	631	8.0
Complete - E. coli enzyme + S. med enzyme preincubated without rifampicin	399	5.1

The enzyme preparation from S. mediterranei was preincubated in the presence or absence of rifampicin at 30°C for 15 min, followed heat-treatment at 70°C for 1.5 min. To 0.165 ml of the preincubated mixture, DNA, nucleoside triphosphates and E. coli enzyme (81 μg) were added to give complete reaction mixtures (0.2 ml). Then the incubation for RNA synthesis and the assays were carried out as described in the legend to Table I. When rifampicin was added, the amount was controlled to give the final concentration of 2.5 μg/ml and protein content of S. mediterranei enzyme added was 41 μg per tube.

vitro RNA synthesis of Streptomyces mediterranei is extensively resistant to rifampicin comparing with those of Streptomyces griseus B_3 and Escherichia coli Q13.

Since rather crude enzyme preparations were used in the experiments described above, we examined whether rifampicin is inactivated by the enzyme preparation from Streptomyces mediterranei. As indicated in Table 2, the RNA synthesis by Escherichia coli enzyme was completely inhibited by rifampicin which was preincubated with the enzyme preparation from Streptomyces mediterranei. We also confirmed that the antibacterial activity of rifamycin SV was not decreased by the incubation with supernatant fraction from Streptomyces mediterranei in the presence of ATP (data are not shown). These facts suggested that resistance to rifampicin of RNA synthesis by the enzyme preparation from Streptomyces mediterranei is not due to the inactivation of the antibiotic by the contaminated enzyme, but to the resistance of RNA polymerase to rifampicin.

DISCUSSION

Antibiotic-producing microorganisms which can actively grow in the presence of their own antibiotic must acquire some mechanism of resistance to avoid suicide (7,8). Several antibiotic-producing microorganisms have been shown to possess the antibiotic-modifying enzymes (7) or to be impermeable to exogenous antibiotics (8,10). Furthermore, there is another type of resistant mechanism. Teraoka and Tanaka (9) have shown that the ribosomes of erythromycin-producing Streptomyces erythreus have very little affinity to erythromycin and analogous finding was subsequently reported by Dixon et al. (10) for thiostrepton-producing microorganism, Streptomyces azureus. And here we suggested that the resistance of Streptomyces mediterranei to rifamycin may be due to the resistance of RNA polymerase of this microorganism. Since these producers possessing antibiotic-resistant target are extensively resistant to the antibiotic, they may probably be advantageous in antibiotic-production.

In connection with the rifampicin-resistance of RNA polymerase, Chater (11) reported that rifampicin-resistant mutant of Streptomyces coelicolor isolated at a high concentration (200 μg/ml) of the drug seems to possess RNA polymerase resistant to this antibiotic.

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