Communications to the Editors

INHIBITION OF DNA-DEPENDENT RNA SYNTHESIS BY RIFAMYCINS

Sir:

In our previous studies on the mode of action of an antibiotic B44P which was isolated in our laboratory and identified with streptovaricins[1], we found that the antibiotic B44P inhibited initiation process on RNA synthesis by DNA-dependent RNA polymerase (EC 2. 7. 7. 6) of *E. coli*[2], but did not affect RNA synthesis by the corresponding enzyme of EHRLICH ascites carcinoma cells[3]. This unique mode of action of B44P appeared to give rise to a new basis for chemotherapy against bacterial diseases.

The chemical structure of streptovaricin A recently reported tempted us to examine whether rifamycins have the same mode of action on DNA-dependent RNA synthesis of *E. coli* because of their structural resemblance[4].

In this communication, we report the mode of action of rifamycin B and rifamycin SV on DNA-dependent RNA polymerase reactions of both *E. coli* and EHRLICH ascites carcinoma cells.

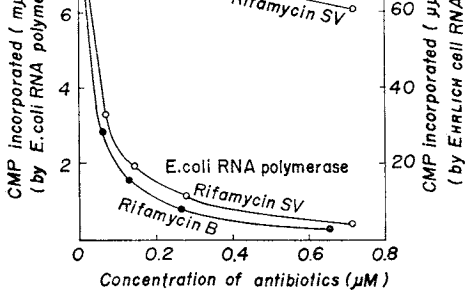
DNA-dependent RNA polymerase from *E. coli* B was prepared by the method of CHAMBERLIN and BERG[5] and that from EHRLICH ascites cells was extracted as reported previously[3]. The procedure for the assay of RNA polymerase was the same as described in a previous paper[2].

Rifamycin B and rifamycin SV were kindly given by Dr. P. SENSI, Research Laboratories, Lepetit, S.p.A., Milan, Italy.

Fig. 1. Effect of rifamycins on RNA polymerase reactions of *E. coli* and EHRLICH carcinoma cells.

The reaction mixture for the assay of *E. coli* RNA polymerase contained (0.3 ml) Tris-HCl, pH 8.0, 15 μmoles; β-mercaptoethanol, 3.6 μmoles; $MgCl_2$, 1.2 μmoles; $MnCl_2$, 0.3 μmoles; ATP, GTP and UTP, 0.1 μmole each; ^{3}H-CTP, 0.05 μmoles (3,000 cpm/mμmole); calf thymus DNA, 30 μg, enzyme, 20 μg. Incubation was at 37°C for 10 minutes.

The mixture for the assay of EHRLICH cell RNA polymerase contained (0.3 ml) Tris-HCl, pH 7.8, 15 μmoles; β-mercaptoethanol, 3 μmoles; $MgCl_2$, 2.4 μmoles; $MnCl_2$, 0.6 μmole; ATP, GTP and UTP, 0.1 μmole each; ^{3}H-CTP, 0.012 μmole (30,000 cpm/mμmole); calf thymus DNA, 25 μg; enzyme, 0.34 mg. Incubation was at 37°C for 20 minutes.



In our laboratory, the effect of rifamycins was studied on the next three processes of RNA synthesis: (1) binding of enzyme to DNA template to form DNA-enzyme complex, (2) initiation of RNA synthesis, and (3) polymerization of four kinds of nucleotides into RNA chains.

The effect of rifamycins on the binding of enzyme to DNA was examined by the membrane filter technique[6]. The result indicated that rifamycins did not exert any

influence on the binding reaction as seen in Fig. 2.

To examine the action of rifamycins on polymerization of nucleotides, the effect of time of addition of rifamycins was studied on ^{3}H-CMP incorporation by RNA polymerase of *E. coli*. As indicated in Fig. 3, when rifamycins were added to the complete reaction mixture for RNA synthesis at 0°C and thereafter the reaction was carried ont at 37°C, strong inhibition of overall RNA synthesis was observed, but only slight inhibition was shown if the antibiotics were added after the incubation of the complete reaction mixture at 37°C for 2 minutes. These experimental results indicated that the inhibitory action by rifamycins was exerted on initiation of RNA synthesis.

Concerning the initiation of RNA synthesis, the effect of rifamycins on the incorporation of $\beta\gamma$-^{32}P-GTP into acid-insoluble fraction by RNA polymerase of *E. coli* was examined and compared with the effect on the incorporation of ^{3}H-CMP which represented the total RNA synthesis. As seen in Table 1, rifamycins markedly inhibited the incorporations of $\beta\gamma$-^{32}P-GTP and ^{3}H-CMP in almost the same degree. The rate of the inhibition of $\beta\gamma$-^{32}P-GTP incorporation by rifamycins could fully explain the inhibition of overall RNA synthesis represented by ^{3}H-CMP incorporation, that is, the observed inhibition of overall RNA synthesis was considered to result from the inhibition of initiation of RNA synthesis starting from 5′-terminus. Accordingly, this experimental result indicates that initiation process of RNA synthesis is the site of action of rifamycins.

In summary, rifamycins inhibited initiation of DNA-dependent RNA synthesis in *E. coli*, but did not have any influence on RNA synthesis in EHRLICH ascites cells.

A more detailed paper will be published elsewhere.

Fig. 2. Effect of rifamycins on RNA polymerase-dependent retention of ^{32}P-labeled *E. coli* DNA.

The reaction mixture (0.2 ml) contained 1.2 μg of ^{32}P-labeled DNA (4,600 cpm), Tris-HCl, pH 8.0, 10 μmoles; $MgCl_2$, 1 μmole; β-mercaptoethanol, 2 μmoles and indicated amount of enzyme protein. Incubation was at 37°C for 2 minutes.

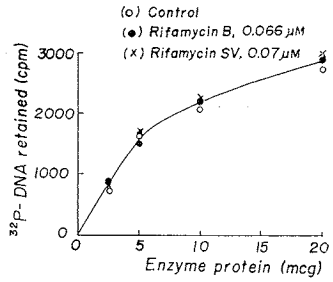


Fig. 3. Effect of time of addition of rifamycins on RNA polymerase reaction of *E. coli* (Rifamycin B, 0.13 μM; rifamycin SV, 0.14 μM)

The reaction mixture was the same as that of Fig. 1.

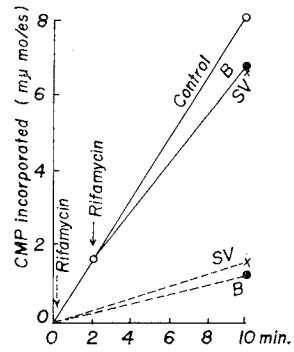


Table 1. Inhibitory effect of rifamycins on the incorporation of $\beta\gamma$-^{32}P-GTP and ^{3}H-CMP

	$\beta\gamma$-^{32}P-GTP ($\mu\mu$moles)	^{3}H-CMP (mμmoles)
Control	1.01	7.2
Rifamycin B, 0.13 μM	0.16(84.2)	1.3(82.0)
Rifamycin SV, 0.14 μM	0.14(86.1)	1.7(76.4)

The numbers in parentheses represent percent inhibition. The reaction mixture was the same as that of Fig. 1 except ATP, GTP, UTP and CTP, 50 mμmoles each; $\beta\gamma$-^{32}P-GTP (5×10^{5} cpm/mμmole) substituted for GTP for measurement of triphosphate terminus or ^{3}H-CTP (3,000 cpm/mμmole) substituted for CTP for measurement of the total RNA synthesis. Incubation was at 37°C for 10 minutes.

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Addendum

After we had sent this communication to the editors, "Biochimica et Biophysica Acta, Vol. 145, No. 3, October 1967" reached us, in which HARTMANN *et al.* reported "The specific inhibition of the DNA-directed RNA synthesis by rifamycin" (pp. 843～844). Their results agree with ours, but the inhibited step of RNA synthesis is not described in their paper.

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