STREPTOVARICIN- AND RIFAMPICIN-RESISTANCE OF RNA POLYMERASE IN A RESISTANT CLONE OF *ESCHERICHIA COLI* B

Sir:

Previously we demonstrated that the primary action of streptovaricin and rifamycin on bacteria was the inhibition of DNA-dependent RNA synthesis, and showed, in *Escherichia coli* B, that the inhibiting site was the initiation of nucleotide-polymerization[1,2,3,4]. Other experimental results of ours indicated that these antibiotics had no inhibitory effects on DNA-dependent RNA polymerase isolated from EHRLICH ascites tumor cells and we suggested that the initiation of RNA synthesis by the mammalian enzyme must proceed in a different manner from the bacterial enzyme[2,3,4].

In the present studies, we have observed that the reaction of the DNA-dependent RNA polymerase obtained from a resistant clone of *E. coli* B was not inhibited by streptovaricin and rifampicin, a derivative of rifamycin. The resistant clone was obtained by plating susceptible *E. coli* B on heart infusion broth agar plates containing 1,000 mcg/ml of streptovaricin. Only one clone was obtained. It showed resistance not only against streptovaricin but also against rifampicin at 1,000 mcg/ml. The original *E. coli* B was completely inhibited by streptovaricin and rifampicin at 25 mcg/ml and 12.5 mcg/ml, respectively. The clone was checked and ascertained to be *E. coli* by Dr. K. MISE, Department of Bacteriology I in the National Institute of Health, Tokyo. The RNA polymerase was extracted and purified by CHAMBERLIN and BERG's method[5] from both the susceptible *E. coli* B and the resistant clone. The experimental procedures and the chemical reagents were the same as used in our previous studies[4] except for rifamycin. The rifampicin used in the present study was rifampicin kindly supplied by Ciba Seihin Co., Takarazuka, Japan.

As shown in Table 1, the reaction of the RNA polymerase prepared from the resistant

Table 1. Effect of streptovaricin and rifampicin on the reaction of RNA polymerase prepared from the resistant *E. coli*.

	^{3}H-CMP incorporated cpm/10 min.
Exp. 1	
Enz(R) control	2,814
+Act D 0.5 mcg/ml	1,296
+Act D 1.0	686
+Act D 2.0	366
+SV 5 mcg/ml	2,746
+SV 20	2,734
+SV 50	2,756
+SV 100	1,642
+RM 0.2 mcg/ml	2,862
+RM 0.5	2,462
+RM 1.0	3,024
+RM 2.0	2,514
Enz(S) control	16,310
+SV 5 mcg/ml	5,184
+RM 0.2	6,040
Enz(R)+Enz(S) control	19,950
+SV 5 mcg/ml	7,520
+RM 0.2	7,070
Exp. 2	
Enz(R) control	2,094
+SV 5 mcg/ml	2,004
+RM 0.2	1,926
Enz(S) control	1,434
+SV 5 mcg/ml	297
+RM 0.2	160
Enz(R)+Enz(S) control	3,970
+SV 5 mcg/ml	2,522
+RM 0.2	2,170

Enz(R): RNA polymerase from resistant *E. coli*, 11 mcg protein.
Enz(S): RNA polymerase from sensitive *E. coli*, 69 mcg protein in Exp. 1 and 11 mcg protein in Exp. 2.
RM: rifampicin
SV: streptovaricin
Act D: actinomycin D

RNA polymerase from susceptible and resistant *E. coli* was prepared by the method of CHAMBERLIN and BERG (Fraction 4). The reaction mixture 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 μmole (3,000 cpm/mμmole); calf thymus DNA, 30 mcg and indicated amount of RNA polymerase. Incubation was at 37°C for 10 min. The reaction was terminated by adding 2 ml of 5 % trichloroacetic acid and 0.1 ml of 1 % bovine serum albumin as a carrier. After standing for 30 min. at 0°C, the precipitate was washed 3 times with 2 ml of 5 % trichloroacetic acid. The acid-insoluble material obtained was dissolved in 1.0 ml of 2 N NH_4OH and 0.1 ml aliquot was added to 10 ml of dioxane scintillation fluid. The radioactivity was assayed in a liquid scintillation spectrometer (Beckmann, LS-200B).

clone (Enz(R)) was not affected by 50 mcg/ml of streptovaricin nor by 2 mcg/ml of rifampicin, whereas the RNA polymerase from susceptible *E. coli* B (Enz(S)) was greatly inhibited by 5 mcg/ml of streptovaricin or 0.2 mcg/ml of rifampicin. As the mixture of Enz(S) and Enz(R) showed additive effects in the absence and the presence of the antibiotics, it would appear that the preparation of the Enz(R) did not contain a streptovaricin- and rifampicin-inhibiting factor as a contaminant. The results cannot be explained by the inactivation of the antibiotics by the enzyme solutions of the resistant cells, since the whole cell extract of the resistant cells failed to reduce the antimicrobial activity of streptovaricin and rifampicin.

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