INHIBITION OF RNA POLYMERASE OF *ESCHERICHIA COLI* BY AN ANTIMICROBIAL SUBSTANCE B44P (STREPTOVARICIN)

Sir:

Previously we found that a pigmented antibiotic substance B44P (streptovaricin) inhibited the syntheses of RNA and protein in growing cells of *E. coli* B and *S. aureus* 209P but in cell-free system of *E. coli* B, protein synthesis was not inhibited by B44P. From these facts, we concluded that the primary action of B44P might be the inhibition of RNA synthesis in bacterial cells.

In order to clarify the synthesis inhibition of RNA by B44P, we studied the effect of this antibiotic on RNA polymerase (EC 2.7.7.6) of *E. coli*.

RNA polymerase was extracted by the method of CHAMBERLIN and BERG[1] from *E. coli* cells in the late logarithmic growth phase. The activity of the enzyme was measured by determining the radioactivity of labeled precursor incorporated into the acid insoluble fraction in the test system. The reaction mixture contained in 0.3 ml, Tris-HCl (pH 8.1) 15 μmoles, β-mercaptoethanol 3.6 μmoles, $MgCl_2$ 1.2 μmoles, $MnCl_2$ 0.3 μmoles, ATP 0.1 μmole, GTP 0.1 μmole, UTP 0.1 μmole, ^{3}H-CTP 0.05 μmoles (3×10^6 cpm/μmole), calf thymus DNA 20 mcg and the enzyme 1.5 units (1 unit of the enzyme catalyzes the incorporation of 1 mμmole of CMP per 10 minutes in the presence of 20 mcg of DNA). The reaction mixture was incubated at 37°C for 10 minutes and then the reaction was stopped by heating in boiling water. After 3 minutes, the mixture was cooled in ice-water for 5 minutes and 0.1 ml of bovine serum albumin (10 mg/ml) was added as a carrier followed by 2.0 ml of 5 % TCA. The mixture was allowed to stand for 30 minutes at 0°C and the precipitate produced was submitted to radioactivity counting after washing four times with 2.0 ml of 5% TCA. Radioactivity was counted with a windowless gas-flow counter. Since the omission of ATP, GTP, UTP, primer DNA or the enzyme preparation from the reaction system or the addition of either RNAase or DNAase almost completely depressed the incorporation of the labeled precursor into the acid-insoluble fraction, it was considered that this enzyme preparation could be used as RNA polymerase and that this system was satisfactory enough for the assay of the RNA polymerase activity. Various concentrations of B44P and actinomycin D were added to the reaction mixture prior to the enzyme and the primer DNA. As seen in Fig. 1, similar inhibition patterns were observed with these two antibiotics. At a concentration of 3 mcg/ml, these antibiotics showed 77 % inhibition. The inhibition by B44P varied depending upon the time of the addition of the drug. An example in which 2 mcg/ml of B44P and 2.1 units of RNA polymerase were used is shown in Table 1. This result suggested that B44P does not affect RNA polymerase reaction, once the polymerization of nucleotides into RNA has started.

Fig. 1. Inhibition of RNA polymerase reaction by B44P and actinomycin D

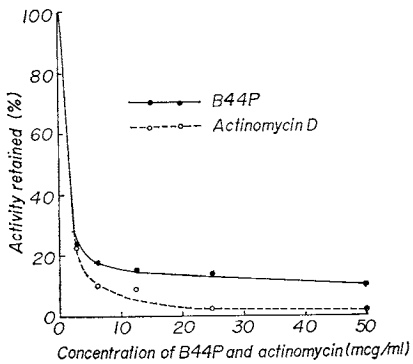

Table 1. Effect of time of addition of B44P on RNA polymerase reaction

Time of addition of B44P after the start of incubation at 37°C	Inhibition
0 min.	60.5 %
1	11.5 〃
2	0.4 〃

Kinetic studies on the inhibition of the RNA polymerase reaction by B44P showed that it was non-competitive with respect to DNA or substrate.

Actinomycin D is known to inhibit RNA synthesis by binding to template DNA. We examined binding ability of B44P to DNA

in order to determine whether the same mechanism worked on the inhibition of the RNA polymerase reaction by B44P. Four methods were employed : (1) measuring the difference in the spectrum between the B44P solution and the mixture of DNA and B44P, (2) comparing the thermal transition curves of *E. coli* DNA in the presence and absence of B44P, (3) comparing the antibacterial activities of B44P in the presence and absence of DNA, and (4) sucrose gradient ultracentrifugation analysis of the mixture of tritiated B44P and DNA. Through these four experiments, we could not obtain any evidence for binding of B44P to DNA.

In conclusion, B44P inhibited the function of RNA polymerase in *E. coli* without binding to DNA and the inhibition occurs before the initiation of the polymerization of nucleotides into RNA. The type of the inhibition was non-competitive with regard to B44P and substrate or DNA.

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Reference

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