BIOCHEMICAL MECHANISM OF ACTION OF SUBSTANCE B 44 P

Sir:

A pigmented antibiotic substance B 44 P was isolated in our laboratory from a culture broth of *Streptomyces* species No. B 44-P 1 and later was found to be identical with streptovaricin[1]. Seven components were detected in substance B 44 P by thin-layer chromatography: two main components A, B and five minor ones C, D, E, F and G. Substance B 44 P is inhibitory againt Gram positive bacteria, mycobacteria and a few kinds of Gram negative bacteria *in vitro*. It exhibits chemotherapeutic activity against staphylococcal infection in mice caused by drug-sensitive and multi-drug-resistant strains. The toxicity of this antibiotic is relatively low.

We investigated the biochemical mechanism of action of substance B 44 P using intact cells of *E. coli* B and *S. aureus* 209 P. *E. coli* B and *S. aureus* 209 P were shake-cultured at 37°C in STEPHENSON-WHETHAM's medium and tryptose-phosphate broth, respectively. Three hours after the initiation of the culture, the substance B 44 P was added to the culture at a final concentration of 10 mcg/ml in case of *E. coli* and of 0.5 mcg/ml or 0.25 mcg/ml in case of *S. aureus*. At definite times, aliquots of the cultures were withdrawn for the determination of protein, RNA and DNA content. The biosynthesis of protein and nucleic acids in the samples was stopped with TCA at a final concentration of 5 %. Protein, RNA and DNA were determined by LOWRY's method, orcinol reaction and diphenylamine reaction, respectively. As seen in Table 1, substance B 44 P inhibited the biosyntheses of protein and RNA both in *E. coli* B and in *S. aureus* 209 P. The RNA synthesis was more strongly inhibited than the protein synthesis. DNA synthesis was rather accelerated in *E. coli* and in the first 30 minutes in *S. aureus*.

Table 1. Inhibitory effect of the substance B44P against the biosynthesis of protein, RNA and DNA in *E. coli* B and *S. aureus* 209P

Target organism	Concentration of B44P mcg/ml	Pertinent subject	% Inhibition reacting period in hour 0.5	1	2	3
E. coli B	10	Protein		33.3	49.2	26.0
		RNA		50.0	35.7	38.5
		DNA		Acc.	Acc.	Acc.
S. aureus 209P	0.5	Protein	72.2	91.7	68.1	
		RNA	113.5	98.4	82.8	
		DNA	Acc.	42.0	64.3	
	0.25	Protein	54.9	70.8	47.3	
		RNA	92.5	82.0	64.4	
		DNA	Acc.	0	10.4	

Acc. : accelerated

E. coli B and *S. aureus* 209P were shake-cultured at 37°C in STEPHENSON-WHETHAM's medium and tryptose-phosphate broth, respectively. After 3 hours, B44P was added. Samples were taken for the determination of protein, RNA and DNA at the indicated times. The protein, RNA and DNA content was measured by LOWRY's method, orcinol reaction and diphenylamine reaction, respectively.

Similar results were obtained in another experiment in which we tested substance B 44 P for its effect on the incorporation of ^{14}C-amino acids, ^{3}H-uridine and ^{3}H-thymidine into protein, RNA and DNA fractions in *E. coli* B and *S. aureus* 209 P. These bacteria were cultured in the same way as mentioned above. After 3 hours, the following was added together with B 44 P or actinomycin D: for ^{14}C-amino acid incorporation, ^{14}C-chlorella hydrolysate (6.47 mc/mMC) 0.25 μc/ml (in *E. coli* experiment) or 0.1 μc/ml (in *S. aureus* experiment) and casamino acids 0.5 mcg/ml; for ^{3}H-uridine incorporation, ^{3}H-uridine (2.7 c/mM) 0.1 μc/ml and uracil 0.5 mcg/ml; for ^{3}H-thymidine incorporation, ^{3}H-thymidine (5.0 c/mM) 0.1 μc/ml and cold thymidine 0.025 mcg/ml. The substance B 44 P was added to the *E. coli* culture and to the *S. aureus* culture at final concentrations of 10 mcg/ml and 0.5 mcg/ml, respectively. The concentration of actinomycin D added to the culture of *S. aureus* was 1.0 mcg/ml. After 5, 10, 15, 20 and 30 minutes, aliquots of the cultures were taken as test samples, after stopping the reaction with TCA. The precipitate produced by the addition of TCA

Table 2. Inhibitory effect of substance B44P and actinomycin D against thes incorporation of ^{14}C-amino acids, ^{3}H-uridine and ^{3}H-thymidine into protein, RNA and DNA fractions in *E. coli* B and *S. aureus* 209P

Target organism	Antibiotic added	Labeled precursor incorporated	% Inhibition reacting period in minute				
			5	10	15	20	30
E. coli B	B44P 10 mcg/ml	^{14}C-amino acids	26.8	33.3	31.6	35.9	34.2
		^{3}H-uridine	72.8	69.2	54.6	42.3	13.3
		^{3}H-thymidine	Acc.	Acc.	Acc.	Acc.	Acc.
S. aureus 209P	B44P 0.5 mcg/ml	^{14}C-amino acids	44.4	48.6	60.6	63.4	70.4
		^{3}H-uridine	86.7	83.3	84.1	84.5	82.3
		^{3}H-thymidine	15.7	32.9	31.6	33.3	49.4
	Actinomycin D 1.0 mcg/ml	^{14}C-amino acids	4.8	15.5	28.1	31.7	39.4
		^{3}H-uridine	65.0	63.3	69.4	69.1	62.3
		^{3}H-thymidine	54.9	56.1	59.7	60.6	53.7

Acc. : accelerated

E. coli B and *S. aureus* 209P were shake-cultured at 37°C in STEPHENSON-WHETHAM's medium and tryptose-phosphate broth, respectively. After 3 hours, the followings were added together with B44P or actinomycin D :

for ^{14}C-amino acid incorporation : ^{14}C-chlorella hydrolysate 0.25 μc/ml (*E. coli* B) or 0.1 μc/ml (*S. aureus* 209P)+casamino acids 0.5 mcg/ml

for ^{3}H-uridine incorporation : ^{3}H-uridine 0.1 μc/ml+uracil 0.5 mcg/ml

for ^{3}H-thymidine incorporation : ^{3}H-thymidine 0.1 μc/ml+thymidine 0.025 mcg/ml.

Samples were taken at the indicated times for counting the radioactivity incorporated in protein, RNA and DNA fractions.

solution was washed with 5 % TCA. The radioactivity incorporated into hot-TCA insoluble and cold-TCA insoluble portions of the samples was measured with a windowless gas flow counter. The results are shown in Table 2. In both the *E. coli* and *S. aureus*, the incorporation of ^{3}H-uridine was strongly inhibited by substance B 44 P in the shorter reacting periods, whereas the inhibition of ^{14}C-amino acid incorporation was moderate. The low inhibition percentage of ^{3}H-uridine incorporation in 20 and 30 minutes in *E. coli* B is due to the decrease in the incorporated radioactivity counts in the control which might be caused by exhaustion of labeled uridine. Although 1.0 mcg/ml of actinomycin D showed virtually no inhibition against ^{14}C-amino acid incorporation in 5 minutes, 0.5 mcg/ml of substance B 44 P displayed 44.4 % inhibition in the same period of reacting time.

In order to determine whether the inhibition of protein synthesis by substance B 44 P was a primarily or secondly effect, we tested substance B 44 P for its effect on ^{14}C-amino acid incorporation under conditions where RNA synthesis did not occur using a cell free system. The reaction mixture consisted of Tris-HCl (pH 7.8) 50 μmoles, magnesium acetate 5 μmoles, KCl 30 μmoles, β-mercaptoethanol 3 μmoles, ATP 0.5 μmoles, creatine phosphate 2.5 μmoles, creatine kinase 25 mcg, GTP 0.05 μmoles, ^{14}C-amino acids (mixture of ^{14}C-lysine, ^{14}C-leucine, ^{14}C-glycine, ^{14}C-proline and ^{14}C-phenylalanine) 0.18 μc, 15 other unlabeled amino acids 25 mμmoles each and *E. coli* S-30 fraction (DNAase-treated) 1.7 mg protein in a total volume of 0.5 ml. Substance B 44 P was added prior to the S-30 fraction. After 30 minutes-incubation at 37°C, ^{14}C-amino acid incorporation was measured. In this system, substance B 44 P was found not to affect protein synthesis even at a concentration of 50 mcg/ml in cell free system of *E. coli* B where RNA synthesis did not occur.

Through the three series of experiments mentioned above, we would like to conclude that the mechanism of the action of substance B 44 P might be on the inhibition of RNA systhesis in bacteria.

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