LOCALIZATION OF KANAMYCIN SENSITIVITY IN THE 23S CORE OF 30S RIBOSOMES OF *E. COLI*

Sir:

It has been demonstrated in an *E. coli* system that streptomycin inhibits polypeptide synthesis if the 30S ribosomes are derived from sensitive organisms, but inhibits it much less if the 30S comes from resistant organisms, regardless of the source of the 50S ribosomes. It indicates that the determinant of streptomycin sensitivity is associated with the 30S ribosomes[1,2]. The investigations have been extended to the subunits, and streptomycin sensitivity has been related to the 23S ribonucleoprotein core of 30S ribosomes[3,4].

In previous studies, it was observed that the sensitivity to kanamycin, another aminoglycosidic antibiotic is associated with the ribosomes, but not with the supernatant factors[5]. The determinant of kanamycin sensitivity was further studied with respect to the ribosomes and their subunits of sensitive *E. coli* cells and those of resistant cells. The results are presented in this communication.

The sensitive strain employed was *E. coli* B and kanamycin-resistant mutant was derived from *E. coli* NIHJ. The minimal growth-inhibitory concentrations of kanamycin were 5 μg/ml and 2,000 μg/ml respectively. The preparation of 50S and 30S ribosomes, and 23S core and split protein (SP30), and their reconstitution principally followed the method of HOSOKAWA *et al.*[6] and STAEHELIN and MESELSON[7]. The ribosomes were separated into 30S and 50S fractions by dialysis against low Mg^{++} solution, and were purified by two successive sucrose density gradient (5~20 %) centrifugations. The 30S ribosomes were dissociated to SP30 and 23S core by centrifugation to equilibrium in 5.2 M CsCl solution with 0.04 M Mg^{++} and 2×10^{-4} M EDTA.

The 30S and 50S ribosomes from sensitive cells and from resistant cells were mixed, and poly U-directed incorporation of phenylalanine and isoleucine was examined in the reaction mixture, as described in the legend of Table 1. Kanamycin inhibited polyphenylalanine synthesis by sensitive 30S but not by resistant 30S, in combination with sensitive or resistant 50S. The stimula-

Table 1. Poly U-dependent incorporation of phenylalanine and isoleucine by reconstituted ribosomes of *E. coli*

Ribosomal subunits				Phenylalanine incorporated			Isoleucine incorporated		
50S	30S	23S	SP30	No KM	+KM	% Inhibit.	No KM	+KM	% Stimulat.
S	S			63.8*	32.7*	49	0.76	8.84	1163
S	R			57.2	54.9	4	0.76	4.80	632
R	S			47.6	29.4	38	0.63	5.42	860
R	R			45.4	51.1	—	0.88	2.53	288
S		S	S	48.0	25.4	47	0.76	4.17	549
S		S	R	55.5	31.3	47	0.76	3.53	464
S		R	S	47.1	45.2	4	0.63	1.52	241
S		R	R	47.6	46.0	4	0.63	1.39	221
R		S	S	37.7	22.9	39			
R		S	R	38.2	24.2	37			
R		R	S	34.0	32.4	5			
R		R	R	32.1	29.0	10			

* The number represents $\mu\mu$moles incorporated per mg of protein.

S: sensitive, R: resistant, SP30: split protein of 30S ribosomes, KM: kanamycin 2×10^{-5}M.

The reaction mixture contains, in 0.5 ml, 50S 200 μg, 30S 120 μg, 23S core 70 μg, SP30 60 μg, *E. coli* B 105,000$\times g$ supernatant 150 μg, *E. coli* B sRNA 150 μg, poly U 20 μg, ATP 1 μmole, GTP 0.05 μmoles, creatine phosphate 2 μmoles, creatine phosphokinase 50 μg, ^{14}C-phenylalanine (297 mC/mM) or ^{14}C-isoleucine (79.2 mC/mM) 0.1 μC, and kanamycin 0.01 μmoles. The buffer used consists of $MgCl_2$ 10 mM, NH_4Cl 50 mM, 2-mercaptoethanol 6 mM, and Tris-HCl 10 mM, pH 7.6. It is incubated at 37°C for 30 minutes.

tion by kanamycin of isoleucine incorporation was observed more markedly with sensitive 30S than with resistant 30S. The results are summarized in Table 1. They indicate that the determinant of kanamycin sensitivity resides on 30S ribosomes but not on 50S.

For the purpose of learning whether kanamycin sensitivity is determined by SP30 or 23S core of 30S ribosomes, they were mixed in various combinations and dialyzed. The sensitivity of the resultant reconstituted 30S ribosomes to kanamycin was investigated with sensitive or resistant 50S. Polyphenylalanine synthesis was approximately at the same level in the 4 different reconstitution mixtures. Kanamycin inhibited polyphenylalanine synthesis by sensitive 23S core but not by resistant 23S core in combination with sensitive or resistant SP30 and 50S. The origin of SP30 did not affect the kanamycin sensitivity of the system. The stimulation by kanamycin of isoleucine incorporation was greater with sensitive 23S core than with resistant 23S core. The results are presented in Table 1. They indicate that kanamycin sensitivity lies in 23S core of 30S ribosomes, but not in SP30 (split protein). In summary, localization of kanamycin sensitivity in the ribosomal subunits is similar to that of streptomycin sensitivity, although they seem to be different because no cross resistance is observed[5,8].

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