ACTIVITY OF RIBOSOMES FROM KANAMYCIN-RESISTANT E. COLI

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Kanamycin(KM) is a basic glycoside antibiotic and the biological activity is similar to streptomycin(SM). Concerning the mechanism of action of the antibiotics, an effect on aerobic respiration, a damage of cytoplasmic membrane, a reaction with nucleic acid, or an inhibition of protein synthesis has been reported; no difference has been observed between both antibiotics.

Morikubo(1958, 1959) described a one-way cross resistance of microorganisms with SM and KM: i.e. the SM-resistant mutant is sensitive to KM, but the KM-resistant mutant is resistant to SM.

In the present study, the activity of KM and SM on ^{14}C-leucine incorporation into protein and polyuridylate(poly U)-stimulated polyphenylalanine synthesis with ribosomes from sensitive and antibiotic-resistant strains of E. coli were investigated, with particular reference to the mechanism of the one-way cross resistance, and a certain difference was observed between the actions of these antibiotics.

Sensitivity of the organisms to antibiotics: Antibiotic-resistant mutants were derived from E. coli NIHJ by successive transfer through media containing SM or KM. They were grown on nutrient agar supplemented with 4×10^{-4} M of the antibiotic. The minimal growth-inhibitory concentration of each antibiotic against the parent strain was found to be 0.5×10^{-5} M, that of KM against the SM-resistant mutant 10^{-5} M and that of SM against the KM-resistant mutant 4×10^{-4} M. Therefore, the SM-resistant mutant was sensitive to KM, but the KM-resistant one was resistant to SM.

Effects of KM and SM on cell-free protein synthesis systems from sensitive and resistant cells: The soluble and ribosomal fractions were obtained from exponentially growing cultures. The cells were disrupted by grinding with siliceous sand and extracted with a buffer. The extract was, after removal of cell debris, separated into the soluble and the ribosomal fractions by ultracentrifugation at 105,000 x g for 2 hours. The incorporation of ^{14}C-leucine into the hot acid-insoluble material was carried out in the following reaction mixture(in 1 ml): soluble fraction 1-1.5 mg protein, ribosomal fraction 2-4 mg protein, ATP 1 μmoles, phosphocreatine 5 μmoles, creatine kinase 0.05 mg, 19 different amino acids 0.3 μmoles(each), ^{14}C-L-leucine 0.4 μc, KCl 100 μmoles, $MgCl_2$ 10 μmoles, tris(hydroxymethyl)-aminomethane (pH 7.6) 30 μmoles and β-mercaptoethanol 3 μmoles.

Table 1 presents the results of experiments, in which the ribosomes of the three strains were examined with their own soluble fraction. Both antibiotics were observed to interfere with protein synthesis by the parent cell system. Inhibition by KM was more marked than that by SM. The leucine incorporation by the SM-resistant cell extract was significantly inhibited by KM but not by SM. The synthesis by KM-resistant cell system was not significantly affected

Table 1. Effect of KM and SM on ^{14}C-leucine incorporation into protein in cell-free systems obtained from sensitive, SM-resistant and KM-resistant strains of E. coli.

Antibiotics		Cell-free system obtained from		
		Parent strain	SM-resistant mutant	KM-resistant mutant
No antibiotic		16.0	18.7	36.6
Streptomycin	10^{-4}M	11.2 (29.6)	18.1 (3.3)	32.1 (12.3)
"	10^{-5}M	12.4 (22.5)	18.9 (0.)	35.4 (3.3)
Kanamycin	10^{-4}M	5.10(68.1)	8.58(54.1)	27.3 (25.4)
"	10^{-5}M	7.32(54.1)	8.70(53.5)	35.7 (2.4)

The number shows ^{14}C-leucine incorporation(cpm/μg protein). The number in the bracket represents % inhibition.

by 10^{-5} M of each antibiotic, but slightly by 10^{-4} M. The results established that the protein synthesis system from SM-resistant organism is sensitive to KM and resistant to SM; but that from KM-resistant cells is resistant to both antibiotics.

As summarized in Table 2, protein synthesis with the system in combination of the sensitive cell ribosome and the KM-resistant cell supernatant was markedly inhibited by the two antibiotics. However, leucine incorporation with the KM-resistant ribosome and the sensitive cell supernatant was not affected by either antibiotic. With the KM-resistant cell ribosome, it made little difference whether it was incubated with its own supernatant or with the one derived from the sensitive cells. The results indicated that KM- and SM-resistant changes of KM-resistant cells are involved in the ribosome, but not in the soluble part.

Table 2. ^{14}C-leucine incorporation with ribosomes from sensitive and resistant strains of E. coli.

Cell-free systems		Antibiotics	cpm/μg protein	% inhibition
Ribosome	Soluble fr.			
Parent strain	KM-resistant mutant	0	13.5	
		SM 10^{-5}M	11.8	12.5
		KM 10^{-5}M	8.04	40.4
KM-resistant mutant	Parent strain	0	26.9	
		SM 10^{-5}M	27.1	0.
		KM 10^{-5}M	26.6	1.2

Activity of kanamycin and streptomycin on poly U-stimulated polyphenylalanine synthesis: The incorporation of ^{14}C-phenylalanine was performed by the method of Nirenberg and Matthaei(1961) in the same reaction mixture as above, in which ^{14}C-L-phenylalanine 0.2 μc, poly U 20 μg, soluble RNA of E. coli 0.5 mg and GTP 0.03 μmoles were replaced for ^{14}C-L-leucine. Both antibiotics were found to inhibit polyphenylalanine synthesis with the system from the sensitive

parent strain. The peptide synthesis with the SM-resistant cell extract was resistant to SM but sensitive to KM. On the contrary, the one with the KM-resistant cell system was resistant to KM but sensitive to SM. The results are presented in Table 3. From the results of the experiments in which the soluble and ribosomal fractions from the sensitive and resistant organisms were combined, it was concluded that the changes responsible for antibiotic-resistance occurred in the ribosomes but not in the soluble part (Table 4). This is in accordance with the observation of the SM-resistant ribosomes by Flaks et al(1962).

Table 3. Activity of KM and SM on polyphenylalanine synthesis with poly U as messenger.

Antibiotics	Cell-free system obtained from		
	Parent strain	SM-resistant mutant	KM-resistant mutant
No antibiotic	27.5	5.55	21.2
SM 10^{-5}M	4.89 (82.2)	5.40 (2.7)	5.16 (75.8)
" 10^{-6}M	10.4 (62.2)	6.73 (0.)	8.71 (63.6)
KM 10^{-5}M	2.01 (92.5)	0.81 (85.2)	8.43 (60.2)
" 10^{-6}M	8.04 (70.8)	3.30 (40.5)	18.7 (11.6)

The number shows ^{14}C-L-phenylalanine incorporation (cpm/μg protein). The number in the bracket represents % inhibition.

From the results of the present study, the following conclusions may be obtained. The localization of the resistant changes in the ribosome indicated that the primary sites of action of both antibiotics may be the ribosome. SM markedly inhibited poly U-stimulated peptide synthesis but only slightly leucine incorporation into protein, which is presumably due to messengers already attached to the ribosome. However, KM markedly inhibited both syntheses. This appeared to be the difference in the action of the two antibiotics.

No cross resistance was observed with polyphenylalanine synthesis to both antibiotics suggested that the binding site

Table 4. Polyphenylalanine synthesis with ribosomes from sensitive and resistant strains of E. coli.

Cell-free systems		Antibiotic	cpm/μg protein	% inhibition
Ribosome	Soluble fr.			
Parent strain	SM-resistant mutant	0	8.37	
		KM 10^{-6}M	3.60	57.0
		SM 10^{-6}M	5.04	39.8
SM-resistant mutant	Parent strain	0	6.30	
		KM 10^{-6}M	1.68	73.2
		SM 10^{-6}M	6.77	0.
Parent strain	KM-resistant mutant	0	20.1	
		KM 10^{-6}M	8.10	59.8
		SM 10^{-6}M	6.60	67.1
KM-resistant mutant	Parent strain	0	18.1	
		KM 10^{-6}M	13.1	27.5
		SM 10^{-6}M	7.26	59.9

of KM is different from that of SM, or that both antibiotics are bound to different ribosomes.

On the contrary, the same one-way cross resistance was observed in the inhibition of the protein synthesis, in which native messengers participate, as in the case of antibacterial activity.

Reference

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