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PRELIMINARY REPORT

BINDING OF DIHYDROSTREPTOMYCIN TO RIBOSOMES FROM VIOMYCIN-SENSITIVE AND -RESISTANT STRAINS OF *MYCOBACTERIUM SMEGMATIS*

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In our laboratory, multiple drug-resistant strains of *Mycobacterium smegmatis* have been isolated by selection with viomycin. These strains were resistant not only to viomycin but also to tuberactinomycin-N, capreomycin, streptomycin and kanamycin. Recent work has suggested that the multiple drug-resistance in these strains is due to an alteration of ribosomes (Yamada et al., 1973). However, it is not yet known whether the alteration is associated with changes in the capacity of the ribosomes to bind antibiotics.

To clarify this, the binding of dihydrostreptomycin to the ribosomes from viomycin-sensitive and -resistant strains was studied.

The results showed that ribosomes isolated from the multiple drug-resistant strains bound very little dihydrostreptomycin.

Four multiple drug-resistant strains of *M. smegmatis* ATCC 14468 (parent strain) were designated as E, M, A and O-1. The three former strains (Yamada et al., 1972) and O-1 were isolated by multi-step selection with viomycin. A streptomycin-resistant strain, designated as K-1, was also isolated from the parent strain by selection with a high concn of streptomycin. A streptomycin-resistant strain of *Escherichia coli*, designated as ES02, was a gift from Dr. S. Osawa through Dr. H. Teraoka. Another streptomycin-resistant strain of *E. coli* was also isolated from *E. coli* A19 by one-step selection.

Culture conditions and procedures for isolation of 70S ribosomes were as described in previous paper (Yamada et al., 1972).

Tritium labelled dihydrostreptomycin was purchased from the Radiochemical Centre, England. The purity of this dihydrostreptomycin was checked by paper chromatography (solvent: NaOH, 15 g; H_2O, 150 ml; pentachlorophenol, 30 g; butanol, 850 ml).

The procedures used for binding of dihydrostreptomycin to ribosomes were as described by Teraoka and Tanaka (1972) with a few modifications. The reaction mixture contained the following components in a final volume of 100 μl; 50 mM tris (hydroxymethyl)aminomethane(Tris), pH 7.8, 5 mM $MgCl_2$, 70 mM KCl, 4.1×10^{-5} M ^{3}H-dihydrostreptomycin(7.2×10^5 count/min) and 200 μg of the respective ribosomes.

The reaction was carried out at 37 C for 10 min and stopped by quickly chilling the mixture in ice and adding 2 ml of cold buffer (50 mM Tris-HCl, pH 7.8, 5 mM $MgCl_2$, 70 mM KCl). The diluted mixture was then

filtered through a Millipore Filter (pore size, 0.45 μm) and ribosomes retained on the membrane were washed with 20 ml of the same cold buffer. The amounts of ^{3}H-dihydrostreptomycin bound to the ribosomes were then counted in a liquid scintillation counter.

The binding of ^{3}H-dihydrostreptomycin to ribosomes from viomycin-sensitive and -resistant strains was tested under the optimal experimental conditions described above. As shown in the accompanying Table, ^{3}H-dihydrostreptomycin was bound very little to ribosomes isolated from strains M, A and O–1 of *M. smegmatis*. The molar ratio of ^{3}H-dihydrostreptomycin bound to parent ribosomes was 0.7–0.8. The ribosomes from strain E, was very low resistant to streptomycin in culture, bound an appreciable amount of ^{3}H-dihydrostreptomycin, but less than those from the parent strain of *M. smegmatis*. To ribosomes from the steptomycin-resistant strain K-1, ^{3}H-dihydrostreptomycin was also bound very little.

For comparison, ribosomes were isolated from both the parent and streptomycin-resistant strains of *E. coli* A19. Ribosomes were also isolated from a streptomycin-resistant strain of *E. coli* ES02. Very little dihydrostreptomycin was bound to the ribosomes from the resistant strains compared to the amount bound to parental *E. coli* ribosomes.

It appears therefore that streptomycin resistance in the multiple drug-resistant strains of *M. smegmatis* employed might be due to a mutational alteration of the ribosomes resulting in a reduced capacity to bind streptomycin.

TABLE 1. *Binding of ^{3}H-dihydrostreptomycin to 70S ribosomes*[a]

Strain	^{3}H-dihydrostreptomycin bound	
	count/min[b]	%
Mycobacterium smegmatis (parent)	7143	100
Viomycin-resistant E (Streptomycin low resistant)	4073	57
Viomycin-resistant M (Streptomycin high resistant)	522	7
Viomycin-resistant A (Streptomycin high resistant)	501	7
Viomycin-resistant O-1 (Streptomycin high resistant)	862	12
Streptomycin-resistant K-1 (Viomycin sensitive)	983	14
Escherichia coli A19 (parent)	10227	100
Streptomycin high resistant	476	5
E. coli ES02 (Streptomycin high resistant)	1075	11

[a] Experimental procedures were described in the text.
[b] Radioactivity (800–1000 count/min) in the absence of the ribosomes was subtracted from radioactivity of each reaction.

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