LOCALIZATION OF CO-RESISTANCE TO STREPTOMYCIN, KANAMYCIN, CAPREOMYCIN, AND TUBERACTINOMYCIN IN CORE PARTICLES DERIVED FROM RIBOSOMES OF VIOMYCIN-RESISTANT *MYCOBACTERIUM SMEGMATIS*

Sir:

Since viomycin-resistant mutants with altered ribosomes could be isolated from *Mycobacterium smegmatis* but not from *Escherichia coli*,[1] further studies were carried out on viomycin-resistant ribosomes of *M. smegmatis*. Viomycin-resistant strains of *M. smegmatis* were isolated by serial transfers of parental cells to media containing increasing concentration of antibiotic. The isolation of mutants by a one-step selection was without success, except for the appearance of low level resistant strains (5 μg/ml). To isolate mutants with high level resistance (more than 50 μg/ml), multiple-step selection on viomycin was necessary. These viomycin-resistant mutants were always associated with co-resistance to streptomycin, capreomycin, tuberactinomycin, or kanamycin except one mutant which was sensitive to kanamycin, although they were selected with viomycin only.[2] Ribosomes and ribosomal subunits were prepared from the mutants for testing localization of drug resistance in a subcellular system. It was found that there are two kinds of mutants of *M. smegmatis* in which viomycin resistance was due to altered 50S, and altered 30S subunits, respectively.[1] Further study on localization of viomycin resistance in 30S ribosomes revealed that viomycin resistance is localized on core particles of 30S ribosomes.[3]

As a continuation of preceding works, the study on localization of co-resistance to streptomycin, kanamycin, capreomycin, and tuberactinomycin has been carried out. The results reported in this paper showed that co-resistance to streptomycin, kanamycin, capreomycin, and tuberactinomycin is localized on core particles of 30S ribosomes as well as viomycin resistance.

M. smegmatis ATCC 14468 and a viomycin-resistant mutant designated as AC-16 were used in this study. The mutant was resistant not only to viomycin but also to kanamycin, streptomycin, capreomycin, and tuberactinomycin at 20,000 μg/ml. These organisms were cultured at 37°C in media containing the following ingredients, per liter of distilled water: meat extract, 10 g; Polypeptone, 10 g; NaCl, 2 g; glycerol, 40 ml. The final pH was adjusted to 7.0 with 10% NaOH. Cultures were harvested at the exponential phase and washed three times with a standard buffer[0.01 M tris(hydroxymethyl)aminomethane (Tris), pH 7.8; 0.06 M NH_4Cl; 0.006 M 2-mercaptoethanol] containing 0.01 M $MgCl_2$. The cells suspended in two volumes of the same buffer were disrupted by sonic oscillation (Tomy model UR-1501/Japan). Ribosomes and ribosomal subunits were prepared as described previously.[1] Reconstitution of 30S ribosomal subunits from core particles and split proteins were carried out as described by TRAUB *et al.* with a few modifications.[4] The 30S ribosomal subunits were dissociated into core particles and split proteins by centrifugation in cesium chloride solution consisting of 82 % cesium chloride, 20 mM Tris-HCl buffer (pH 7.8), 45 mM $MgCl_2$, 3 mM 2-mercaptoethanol, and 15 mM NH_4Cl at 39,000 rpm for 40 hours (7°C). The gradients were fractionated from the bottom of the centrifuge tubes. Small aliquots were taken from each fraction, mixed with distilled water and analyzed for their optical density at 260 nm. Fractions containing core particles were pooled for use. Split proteins forming a meniscus attached to the tube wall, were dissolved in 10 mM Tris-HCl buffer (pH, 7.5), containing 30 mM $MgCl_2$, 6 mM 2-mercaptoethanol, and 1 M LiCl. The core particles and split proteins of viomycin-sensitive and -resistant strains of *M. smegmatis* were prepared and interchanged. These mixtures were dialyzed against standard buffer containing 0.2 M LiCl and 0.01 M $MgCl_2$ for 20 hours to reconstitute 30S particles. The resulting reconstituted 30S particles were tested in a subcellular system for their drug susceptibility. Experimental conditions for poly(U)-directed polyphenylalanine synthesis in cell-free system were as described previously.[1]

As shown in Table 1, phenylalanine incorporation was distinctly inhibited in the presence of streptomycin, kanamycin, capreomycin, and tuberactinomycin, when the 30S ribosomes were reconstituted from core particles of the parental strain and split proteins of the resistant mutant. A similar inhibition was observed with the 30S ribosomes reconstituted from core particles and split proteins both from the viomycin-sensitive strain. However, the phenylalanine incorporation by ribosomes formed from core particles of the

resistant mutant and split proteins of the parental strain was resistant to the inhibition by these drugs, as with core particles and split proteins both from the resistant mutant. The inhibition of poly(U)-directed polyphenylalanine synthesis by streptomycin is not good index of sensitivity since somewhat variable results are obtained. The inhibition of polypeptide synthesis stimulated by bacteriophage RNA by streptomycin would be more reliable, but this system is not available in mycobacteria.

From the observations described above, it is concluded that co-resistance to streptomycin, kanamycin, capreomycin, and tuberactinomycin of viomycin-resistant mutants with altered 30S ribosomes is localized on the core particles. We suggest that phenotypic expression for viomycin-resistance with high level might require various ribosomal change in core particles of 30S ribosomes, since genetic studies have indicated that the viomycin resistance is mapped at a locus different from streptomycin and kanamycin resistance loci and both determine a component of 30S ribosomes.[5,6,7]

Table 1. Sensitivity to SM, KM, CPRM and TUM of 30S ribosomal particles reconstituted from core particles (CP) and split proteins(SP).[a]

Source of		Antibiotics[b] (μg/ml)	Phenylalanine incorporated[c] (%)
CP	SP		
Sensitive	Sensitive	—	100
Sensitive	Sensitive	SM(5)	25
Sensitive	Sensitive	KM(5)	27
Sensitive	Sensitive	CPRM(1)	11
Sensitive	Sensitive	TUM(1)	6
Resistant	Resistant	—	100
Resistant	Resistant	SM(5)	83
Resistant	Resistant	KM(5)	110
Resistant	Resistant	CPRM(1)	96
Resistant	Resistant	TUM(1)	100
Sensitive	Resistant	—	100
Sensitive	Resistant	SM(5)	40
Sensitive	Resistant	KM(5)	29
Sensitive	Resistant	CPRM(1)	11
Sensitive	Resistant	TUM(1)	11
Resistant	Sensitive	—	100
Resistant	Sensitive	SM(5)	64
Resistant	Sensitive	KM(5)	95
Resistant	Sensitive	CPRM(1)	84
Resistant	Sensitive	TUM(1)	67

a) Poly(U)-dependent incorporation of ^{14}C-phenylalanine was carried out by mixing one OD_{260} unit of reconstituted 30S ribosomes and 2 OD_{260} units of 50S ribosomes of parental strain. The cell-free system was as described previously.[1]
SM, streptomycin; KM, kanamycin; CPRM, capreomycin; TUM, tuberactinomycin.

b) Antibiotics were added at the start of incubation.

c) Results are expressed as percent of phenylalanine incorporation. Incorporation of 100% corresponds to that observed on respective ribosomes in the absence of antibiotic. The radioactivity in the absence of poly(U) was subtracted from radioactivity of each reaction. The values are expressed as the average of duplicates.

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