MACROLIDE RESISTANCE IN *STAPHYLOCOCCUS AUREUS*

DECREASE OF SPIRAMYCIN-BINDING TO 50S RIBOSOMAL SUBUNIT IN MACROLIDE RESISTANT STRAINS OF STAPHYLOCOCCI

Sir:

It was reported that the ribosomes from *in vitro* developed erythromycin (EM)-resistant strains decrease their affinity for EM in *Bacillus subtilis*[1] and *Escherichia coli*[2]. It was further indicated that the decrease in ribosomal affinity for EM was accountable for by the alteration of 50S ribosomal protein component in EM-resistant strain of *E. coli*[2].

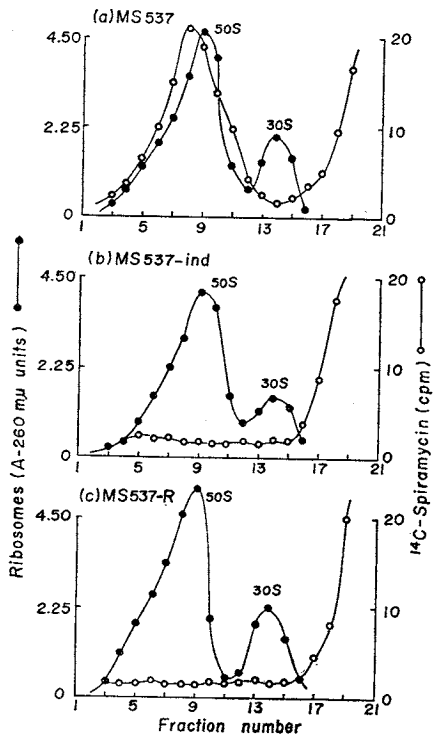
It has been known, however, that there are differences in biochemical mechanisms of drug-resistance between *in vitro* developed resistant strains and those isolated from clinical sources in either chloramphencol or streptomycin resistance[3,4,5].

Therefore we have started studies of the biochemical mechanisms of macrolide resistance in staphylococci of clinical sources, indicating that the ribosomes form macrolide (Mac)-resistant strains of staphylococci decrease their affinity for either EM[6,7] or spiramycin (SP)[8,9]. We have also indicated that the correlation between SP-binding to ribosomes and inhibition of polypeptide synthesis by SP in a cell-free system exists in strains of various levels of Mac resistance[10,11]. By contrast, it was reported that SP resistance did not affect the SP-binding to the ribosomes in *Bacillus subtilis*[2].

S. aureus MS537 is a strain carrying inducible resistance to Mac which becomes resistant to Mac when treated with sub-inhibitory concentrations of EM. However, the induced population becomes sensitive to Mac when grown without inducers[8]. MS 537-R is a constitutive-resistant mutant derived from MS537[8]. SP resistance of MS537 is 0.8 mcg/ml, but MS537(*ind*) after induction and MS537-R are resistant to more than 400 mcg/ml SP.

Preparation of 50S and 30S ribosomal subunits was performed as described by MAO[13]. The 20-30 A_{260} units of dissociated ribosomes and 20 mcg of ^{14}C-SP (0.24 μCi/mg)/ml in medium B (0.01 M Tris-acetate (pH 7.5), 0.1 mM Mg acetate, 50 mM NH_4Cl and 0.1 mM dithioerythreitol) were incubated at 37°C for 10 minutes. A 0.15 ml sample of the reaction mixture was then layered on the top of 4.5 ml linear sucrose gradient (5~17 % sucrose in medium B). Samples were centrifuged at 35,000 r.p.m. for 180 minutes in RPS-40 rotor (Hitachi). One drop from the bottom of the tube was used for the assay in 260 mμ and three drops for the radioactive sasay as described previously[10].

Fig. 1. Binding of ^{14}C-spiramycin to ribosomes.



As shown in Fig. 1, 50S ribosomal subunit from a sensitive strain of MS 537 had the binding affinity for SP, but those from either resistant strains of MS537 (*ind*) or MS537-R had decreased their affinity for SP. These results indicate that 50S ribosomal subunit was altered not only in MS537-R but also in MS537 (*ind*) after induction. However, it is not answered yet that component(s) of 50S subunit is altered after induction. Furthermore, the biochemical mechanisms of reversible alteration in 50S subunit still remain to be studied.

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