RELATIONSHIP OF RIBOSOMAL BINDING AND ANTIBACTERIAL PROPERTIES OF TYLOSIN-TYPE ANTIBIOTICS

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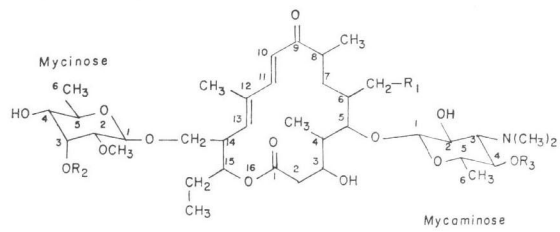
Macrocin, relomycin and tylosin are structurally related macrolide antibiotics produced by *Streptomyces fradiae*[1,2,8]. In addition to these three substances, desmycosin[4], a compound similar to tylosin but lacking mycarose, has also been described. The structures of these antibiotics are presented as Fig. 1. All four of the compounds have recently been shown to be interrelated in a biosynthetic pathway[2]. Since macrolide antibiotics are known to inhibit bacterial protein synthesis by binding to ribosomes[5], a study was undertaken to determine if there is a correlation between the ability of these four natural products to bind to ribosomes and to inhibit bacterial growth. Similar studies using other antibiotics and their semi-synthetic derivatives have been reported[6,7,8].

To test the effect of the antibiotics on the growth of *Bacillus subtilis* 168, 100 ml of trypticase-soy broth was inoculated from a slant culture and incubated 18 hours at 37°C on a shaker (2.54 cm throw, 250 rpm). One ml of this culture was then added to 10 ml trypticase-soy broth in a 26×150 mm tube. To this mixture was also added one ml of an antibiotic solution of appropriate concentration and the cultures incubated as above for 20 hours. Growth was estimated by observing the optical density at 660 nm and compared to a culture to which no antibiotic had been added. All antibiotics were dissolved in 0.05 M potassium phosphate buffer pH 7.0 and sterilized by filtration. The binding of the antibiotics to ribosomes was estimated by their ability to interfere with the binding of radioactive erythromycin A to the same particle. Procedures for the preparation of ribosomes and the determination of binding have been previously described[8,9]. Tylosin, desmycosin, macrocin and relomycin were obtained from I. TAYLOR (Eli Lilly and Company). Erythromycin A-^{14}C was kindly supplied by J. MAO (Abbott Laboratories).

When the ability of the antibiotics to inhibit the growth of *Bacillus subtilis* 168 was compared, macrocin and tylosin were found to have similar activity (Fig. 2). The least active compound tested was observed to be desmycosin, while relomycin was found to have intermediate activity. In contrast, when the same antibiotics were examined for their ability to bind to ribosomes, desmycosin appeared to be the most active (Fig. 3). In this test, little difference was observed in the binding properties of macrocin, relomycin and tylosin. One may assume that the overall potency of a macrolide antibiotic is related to two major properties; one its ability to effectively interact with the ribosomal target and the other its ability to reach that target in sufficiently high concentration. It follows that if a series of compounds are of similar potency in both the cell-free ribosomal binding assay and the whole cell growth inhibition assay the structure-activity relationships among the antibiotics do not differ significantly with respect to either barrier. On the other hand, any major difference between the cell-free and whole cell assays reflect a change either in the accumulation of the antibiotic at the target site or in its ability to react at the target. Previous studies with some derivatives of erythromycin A have indicated that there is little difference among them with respect to

Fig. 1. Structure of desmycosin, macrocin, relomycin and tylosin



	R_1	R_2	R_3
1. Tylosin	-CHO	$-CH_3$	Mycarosyl
2. Desmycosin	-CHO	$-CH_3$	-H
3. Macrocin	-CHO	-H	Mycarosyl
4. Relomycin	$-CH_2OH$	$-CH_3$	Mycarosyl

these indicators of activity[6]. The present study indicates two differences of probable significance among the tylosin family of antibiotics. The most striking exception is that of desmycosin which although the least potent antibiotic in terms of its inhibition of whole *B. subtilis* multiplication is the most active compound in the ribosomal binding assay. The other difference noted is between relomycin as compared with tylosin and macrocin. These three antibiotics are nearly identical in their ability to interact with ribosomes; however, relomycin is less potent than the other two antibiotics against whole *B. subtilis* cells. Desmycosin differs from the other three structures in lacking the neutral sugar, mycarose. Relomycin differs from tylosin and macrocin by having a branch primary alcohol function in place of an aldehyde group. One interpretation of the results is that the neutral sugar is important for the accumulation of desmycosin within the *B. subtilis* cells but is of little importance for potency at the ribosomal target. A similar explanation may be valid for the lesser difference between relomycin and the other two antibiotics. If so, the branch aldehyde group may be more important for the accumulation of these antibiotics than for inhibition at the ribosome. An explanation similar to this has been offered for the erythromycin family of antibiotic[8]. In that series, the basic monoglycoside desosaminyl-erythronolide B which lacks a neutral sugar is active in cell-free assays using *B. subtilis* but is not effective against the whole bacteria.

Fig. 2. Inhibition of *B. subtilis* by tylosin, desmycosin, macrocin, and relomycin

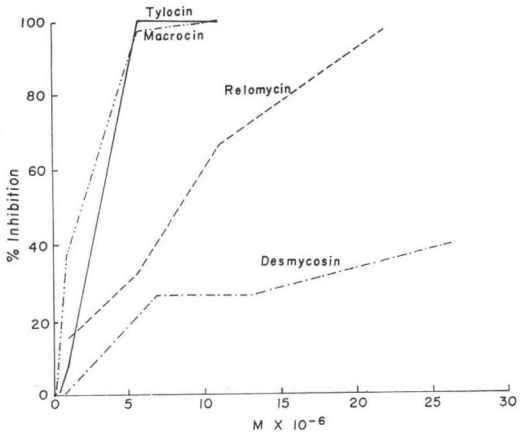
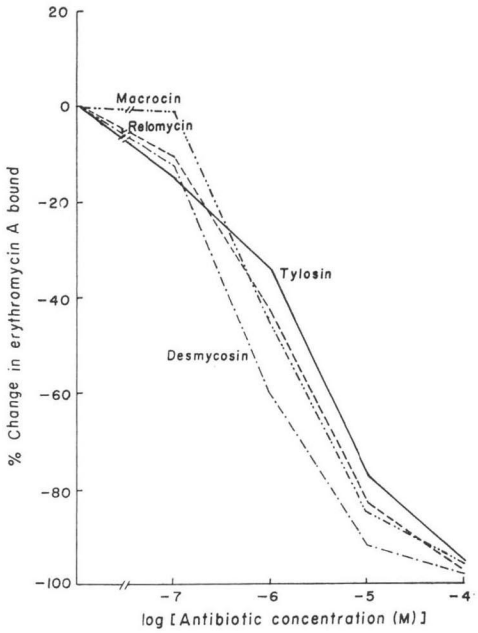


Fig. 3. Effect of various antibiotics on the binding of ^{14}C erythromycin A to sensitive ribosomes of *Bacillus subtilis*
At EryTh.A—^{14}C 10^{-6} M (6.4 μCi/μmole)



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