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ROLES OF STREPTOMYCIN 6 -KINASE AND RIBOSOMAL AFFINITY TO STREPTOMYCIN IN SELF-PROTECTION OF STREPTOMYCIN PRODUCER

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Summary. In streptomycin (SM)-producing organisms, the lower affinity of ribosomes for SM gives rise to a lower susceptibility of protein synthesis to SM. But, even in a strain with considerably low affinity of ribosomes for SM, phosphorylation of SM in the cells by SM 6-kinase is necessary for the protein synthesis to be fully tolerant to SM.

Introduction. The ribosomes of streptomycin(SM)-producting microorganism are susceptible to SM (Valu and Szabo, 1979; Sugiyama el al., 1980), though this organism is tolerant to its own extracellular product. Since external SM is permeable into the cells of SM producers (Cella and Vining, 1975; Piwowarski and Shaw, 1979), there must be some mechanism to protect the protein-synthesizing system from SM. In this view, we already showed that ribosome-bound SM was effectively released from the ribomsome on phosphorylation with SM 6-kinase in the SM producer cells (submitted to J. Antibiotics). It was also shown that the inhibition of protein synthesis by SM was apparently reduced with the reduction of ribosomal affinity for SM in later stages of growth.

In the present study, the affinity of ribosomes for SM and SM 6-kinase activity were compared among a highly SM-tolerant mutant, a SM-susceptible mutant and the parent strain. Furthermore, hybrid protein-synthesizing systems prepared with ribosomes and S-150 fractions derived from different strains were used to compare the roles of the ribosomal affinity for SM and the SM 6-kinase activity in protection of the protein synthesis. As a result, SM 6-kinase was confirmed as indispensable for protection of protein synthesis. In addition, even the ribosomes having very low affinity to SM required cooperation of SM 6-kinase for the protein-synthesizing system to be fully SM-tolerant.

Materials and Methods. SM-producing Streptomyces griseus HUT 6037 was used as a parent strain. A highly SM-tolerant mutant 1500-1 was derived from the parent strain by successive transfers on Bennet-agar slants containing increasing concentrations of SM. A SM-susceptible mutant KSN, a threonine auxotroph, was isolated from the parent strain by treatment with ultraviolet radiation. Characteristics of these strains are shown in Table 1. Preparation of ribosomes and the S-150 fraction, assay of protein synthesis in vitro and of SM 6-kinase activity, and measurement of binding affinity of dihydro-SM to ribosomes were the same as previously described (submitted to J. Antibiotics). The protein synthesis in vitro was determined using polyuridylic acid-directed polyphenylalanine synthesis.

Results and Discussion. Inhibition of in-vitro polyphenylalanine synthesis by SM was first compared among the three strains using the exponential phase cells grown in 1% glucose-meat extract-peptone (GMP) medium (Nimi et al., 1976). The results are shown in Fig. 1 (a, e and i). The degree of inhibition in the parent strain and mutant 1500-1 was about 50% and 10%, respectively, in the presence of 100 μg SM ml^{-1}, while the inhibition in the mutant KSN was about 65% in the presence of only 5 μg SM ml^{-1}.

To know the reason for such differences in SM tolerance of the in-vitro protein synthesis, the affinity of ribosomes for SM and the activity of SM 6-kinase in the S-150 fraction employed in the above experiment were determined. The results are shown in Fig. 2 and Table 2. The affinity of ribosomes to SM in the mutants 1500-1 and KSN were, respectively, one-third of a and a little higher than that of the parent strain. The specific activity of SM 6-kinase in the S-150 fraction was highest in the mutant 1500-1, followed by the parent strain, while the mutant KSN showed no SM 6-kinase activity. This SM-non-producing mutant did not produce the enzyme even in the stationary phase of growth (data not shown). From these results, it can be pointed out that the more SM-tolerant cells showed both lower affinity of ribosomes for SM and in addition higher specific activity of SM 6-kinase. Thus, the SM susceptibility of protein synthesis must be controlled by the ribosomal affinity for SM as well as by the activity of SM 6-kinase in the cell.

To elucidate which of the two factors is more essential for protection of ribosomes from SM, mixed protein-synthesizing systems prepared by combination of the ribosomes and the S-150 fraction derived from the different

strains were compared with the native protein-synthesizing systems. The results are shown in Fig. 1, in which a, e and i indicate polyphenylalanine synthesis by the native systems and b, c, d, f, g and h by the hybrid systems. The following observations were noteworthy.

1. Though the specific activity of SM 6-kinase in the S-150 fraction was different between the parent strain and mutant 1500-1 (Table 2), the influence of this difference on in-vitro polyphenylalanine synthesis was not significant (cf. a and b; d and c; g and h).
2. However, when SM 6-kinase was absent in the S-150 fraction, the in--vitro polyphenylalanine synthesis was highly susceptible to SM irrespective of the extent of ribosomal affinity to SM (cf. a and c; e and f), though a slightly less susceptibility was observed with decrease in the affinity (cf. c, f and i).
3. Decrease in the affinity of ribosomes to SM gave rise to reduction in SM-susceptibility of the in-vitro polyphenylalanine synthesis as long as the SM 6-kinase was contained in the system (cf. a and d; b and e).
4. Even if ribosomal affinity to SM was very high (KSN in Fig. 2), the presence of SM 6-kinase in the S-150 fraction brought significant decrease in SM-susceptibility of the in-vitro polyphenylalanine synthesis (cf. g, h and i). Namely, SM-susceptible mutant, KSN, could be SM-tolerant to the same extent as the parent strain, if it produced SM 6-kinase.

These results can be summerized as follows. SM 6-kinase is inevitably required for protein synthesis to be protected from lethal effect of SM. Though decrease in ribosomal affinity to SM is helpful for reduction of SM-susceptibility of protein-synthesizing machinery, cooperation of SM 6-kinase is indispensable for the machinery to be highly tolerant to SM.

Table 1. Characteristics of three strains used.

	Parent	1500-1	KSN
SM production* ($\mu g\ ml^{-1}$)	200	30	0
SM tolerance** ($\mu g\ ml^{-1}$)	60	>2500	<20

* SM production was determined using GMP medium. ** SM tolerance was defined as the maximal SM concentration allowing significant growth after 24 hr cultivation in SM-supplemented GMP medium at 28°C with shaking.

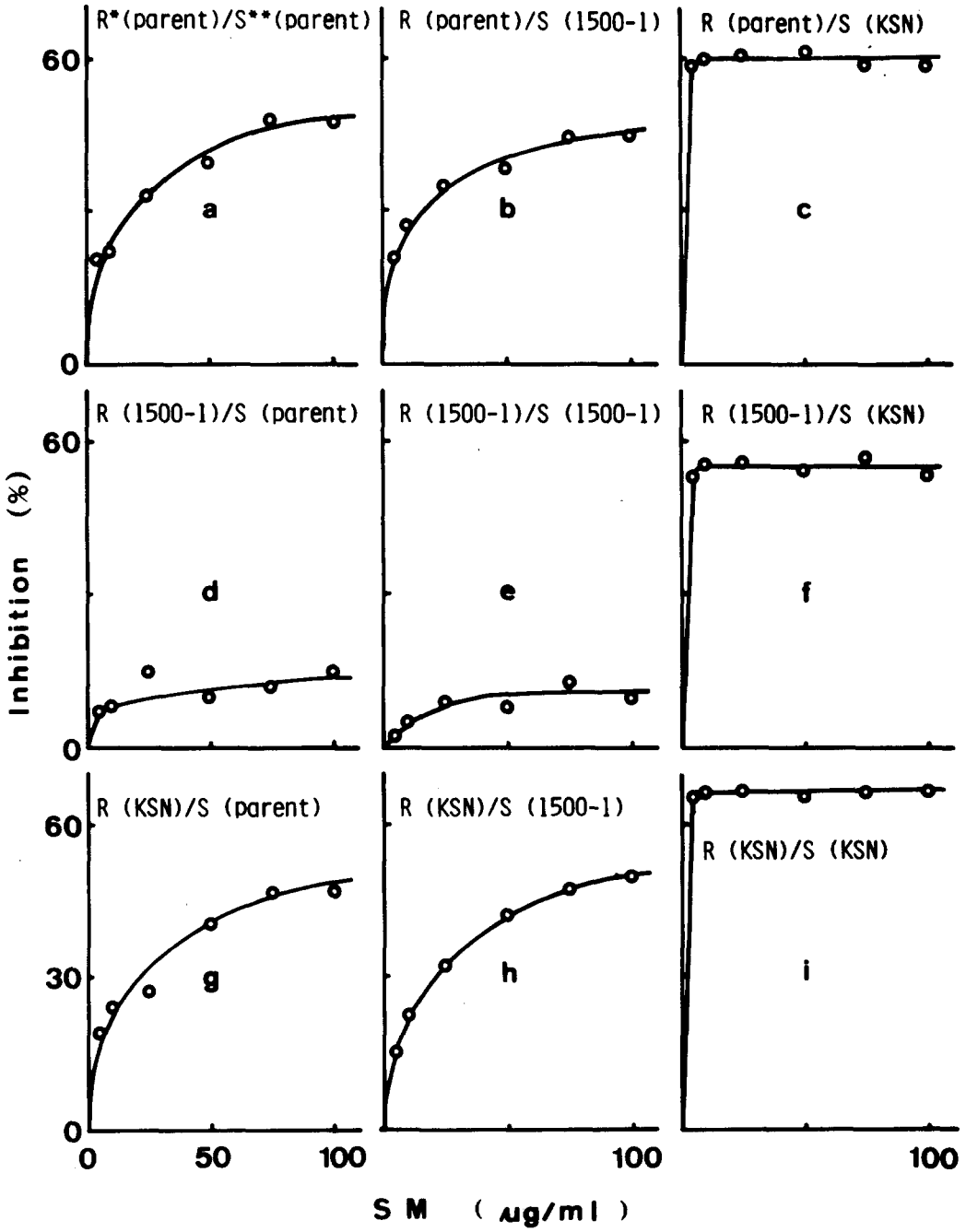

Fig. 1. Inhibition of polyphenylalanine synthesis by SM in in-vitro systems prepared by combinating ribosomes and S-150 fraction derived from three strains.

Inhibition (%) by a given concentration of SM was expressed as the ratio of the reduced amount of polyphenylalanine in the presence of SM to the amount synthesized in the SM-free reaction mixture. Amount of the ribosomes and S-150 fraction used were 60 μg and 240 μg, respectively, in all systems. * R: ribosomes, ** S: S-150 fraction

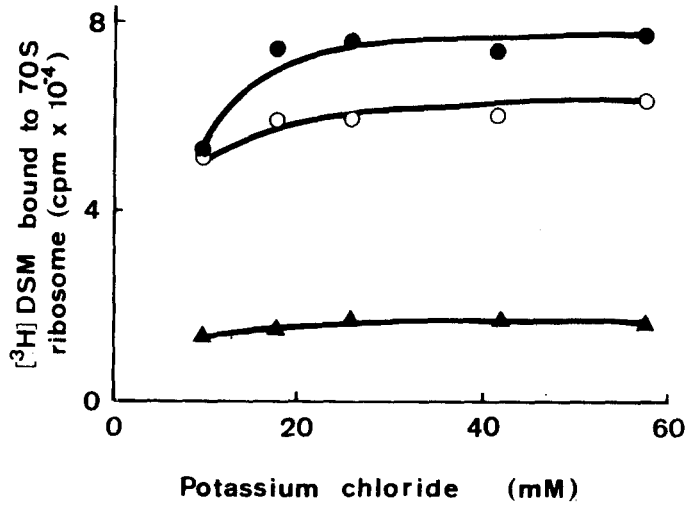

Fig. 2. Binding of [^{3}H]dihydrostreptomycin to the ribosomes from three strains.

The reaction mixture was made up of 50 mM Tris/HCl(pH 7.65), 16 mM magnesium acetate, 140 μg ribosomes, 605,000 cpm (329 pmol) [^{3}H]dihydrostreptomycin ([^{3}H]DSM) and indicated concentration of potassium chloride. The reaction mixture was incubated at 28°C for 20 min. The marks, ●, ○ and ▲ indicate the ribosomes from KSN, parent and 1500-1 strains, respectively.

Table 2. Activity of SM 6-kinase in S-150 fractions from three strains used.

Strains	Specific activity
parent	0.67
1500-1	1.43
KSN	0

Specific activity of enzyme was expressed as μmol of SM 6-phosphate produced from SM in one hr by one mg protein in S-150 fraction.

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