SHORT COMMUNICATION

Susceptibility of Protein Synthesis to Neomycin in Neomycin-producing *Streptomyces fradiae*

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A cell-free protein-synthesizing system from *Streptomyces fradiae* was developed by preparing ribosomes and an S-150 fraction with precautions to prevent protease action. Using this system, the ribosomes of this organism were shown to be susceptible to its own product, neomycin.

INTRODUCTION

Neomycin inhibits protein synthesis in sensitive bacteria. The neomycin-producing *Streptomyces fradiae* possesses the same type of ribosomes (70S) as sensitive bacteria, but it has not previously been determined whether the ribosomes of *S. fradiae* are sensitive or resistant to neomycin. In this paper, the sensitivity of the ribosomes of *S. fradiae* to neomycin was investigated using an in-vitro system which we have developed.

METHODS

Micro-organism. *Streptomyces fradiae* HUT6096 (IMRU 3554) was used as a neomycin-producing strain and was grown in the medium described by Majumdar & Majumdar (1969) at 28 °C until the mid-exponential phase of growth.

Preparation of the ribosomes and the S-150 fraction. The harvested cells were washed twice with buffer I [10 mM-Tris/HCl (pH 7.65), 30 mM-NH₄Cl, 10 mM-magnesium acetate and 6 mM-2-mercaptoethanol], then ground with quartz sand and extracted with similar buffer containing 2 µg deoxyribonuclease I ml⁻¹. The extract was centrifuged at 30000 g for 30 min. From the supernatant, the ribosomes were sedimented by centrifugation at 150000 g for 3 h, then washed with buffer II [20 mM-Tris/HCl (pH 7.65), 1 M-NH₄Cl, 10 mM-magnesium acetate, 6 mM-2-mercaptoethanol and 1.1 M-sucrose] by the methods of Fahnestock *et al.* (1974). The 150000 g supernatant fluid was dialysed against buffer I and used as the S-150 fraction.

Assay of protein synthesis in vitro. The reaction mixture and conditions for assay of polyphenylalanine synthesis were the same as those described by Kobayashi *et al.* (1977) except that spermidine and the 19 amino acids other than phenylalanine were present at 0.4 mM and 0.004 mM, respectively. The tRNA was derived from *Escherichia coli* MRE600 (Boehringer).

RESULTS AND DISCUSSION

When polyphenylalanine-synthesizing activity was measured *in vitro* using the ribosomes and the S-150 fraction from *S. fradiae*, no activity was detected (results not shown).

In *Streptomyces griseus*, we previously found (Sugiyama *et al.*, 1980) that polyphenylalanine synthesis by the ribosomes and the S-150 fraction was inhibited by a protease

Fig. 1. Effect of neomycin on polyphenylalanine synthesis by a cell-free system prepared from exponential phase cells of *S. fradiae*: ○, without neomycin; ●, with 100 µg neomycin ml⁻¹ added to the reaction mixture. Amounts of ribosomes and S-150 fraction employed were 60 µg and 258 µg (as protein), respectively.

produced by the organism itself. By eliminating the protease activity from these two fractions, polyphenylalanine synthesis was successfully observed.

Protease activity was eliminated from the ribosomes and the S-150 fraction of *S. fradiae* by adding 5 mM-EDTA K₂Mg salt, 3.45 mM-phenylmethylsulphonyl fluoride and 0.2 mM-di-isopropyl fluorophosphate to buffer I and buffer II as described in the earlier experiments (Sugiyama *et al.*, 1980). However, in the present experiments the ribosomes and the S-150 fraction were dialysed against buffer I just before use for polyphenylalanine synthesis, whereas in the earlier experiments the buffer for dialysis contained the above protease inhibitors. As shown in Fig. 1, polyphenylalanine synthesis by the ribosomes and the S-150 fraction of *S. fradiae* was clearly observed, showing that protease was probably an inhibitory factor in this in-vitro protein-synthesizing system also. Furthermore, polyphenylalanine synthesis was obviously inhibited by neomycin, showing an inhibition of approximately 70% by 100 µg neomycin ml⁻¹ after 30 min incubation at 37 °C. From these results, we conclude that the ribosomes of *S. fradiae* are susceptible to neomycin. In *S. griseus*, polyphenylalanine synthesis by cell-free systems prepared from exponential phase cells and also stationary phase cells was shown to be susceptible to streptomycin. Aminoglycoside antibiotic-producing *Streptomyces* seem to have some mechanism of resistance against their products other than the ribosomal resistance.

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