AN EFFECT OF STREPTOMYCIN ON THE DISSOCIATION OF ESCHERICHIA COLI 70 S RIBOSOMES.

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It has been suggested by Spotts and Stanier (1961) that the primary site of action of streptomycin (Sm) is the ribosomes. According to their hypothesis, the different alleles at the streptomycin locus would direct the synthesis of structurally different ribosomes, and this would be responsible for sensitivity (Sm^s), resistance (Sm^r) or dependence (Sm^d) towards the antibiotic.

This hypothesis was reinforced by reports from several laboratories (Flaks et al., 1962 ; Mager et al., 1962 ; Speyer et al., 1962) that in cell-free amino acids incorporating systems, streptomycin exerts a strong inhibitory effect, when the ribosomal fraction has been purified from a Sm^s strain, but that it has only a small effect when the ribosomes originate from Sm^r or Sm^d bacteria.

The aim of this work was to look for a possible effect of streptomycin on the centrifugation patterns of ribosomes, using a Sm^s and a Sm^r strain of E. coli.

MATERIAL AND METHODS.

Two strains of E. coli K 12 were used :
C 600 (Appleyard, 1954), which is Sm^s, and
C 600 R 4 (Thomas and Lambert, 1962), one of its single step Sm^r mutants (of the "temperator" type).

The bacteria were grown at 37°C, with aeration in tryptone medium (Kaiser, 1955) complemented with thiamine (1 µg/ml) and $MgSO_4$ (10^{-2}M) to a concentration of about

3×10^8 cells/ml. The culture was then rapidly chilled and centrifuged. The sedimented cells were washed twice with Tris buffer (Tris 10^{-2}M, $MgSO_4$ 10^{-3}M, adjusted to pH 7.4), broken either with a French pressure cell or by grinding with alumina, and extracted in the same buffer to obtain approximately 5 mg ribosomal material per ml of extract.

Streptomycin (dihydrostreptomycin sulfate, Pfizer) was added either to the growing culture, at various times before chilling, or (control) just after chilling, at a concentration of 50 µg/ml. In other experiments, the antibiotic was added to the extract, at concentrations ranging from 100 µg to 4 mg per ml.

In some experiments, Chloramphenicol (Chloromycetin, generous gift of Parke & Davis) was added alone or together with streptomycin, to the growing culture, at a concentration of 100 µg/ml.

The ultracentrifugal behaviour of the ribosomes in the extracts was tested either with a preparative ultracentrifuge (Spinco L 50, rotor SW 39) in sucrose density gradients, or with an analytical ultracentrifuge (Spinco E, equipped with Schlieren optics).

RESULTS.

1) Treating a growing culture of the Sm^s strain with Sm (5 to 500 µg/ml) consistently results in a characteristic alteration of the ultracentrifugal pattern. Whereas, in the extracts from untreated cultures, most of the ribosomes are dissociated into their 50 S and 30 S subunits when the Mg^{++} concentration is 10^{-3}M, in the extracts from Sm-treated cultures, an important fraction of the ribosomes remain associated in the 70 S form (see figure 1).

This effect (which will be referred to as the "sticking effect" of Sm) develops rapidly and reaches about half of its final magnitude when the cultures have been exposed to Sm (50 µg/ml) for 4 minutes at 37°C (see figure 2).

2) No "sticking effect" is found when the Sm^r strain is treated the same way.

Figure 1. Sedimentation patterns of ribosomes from Sm^S E. coli Centrifugation from left to right, at 31,410 rpm. At left, extract from an untreated culture. At right, extract from a culture treated for 16 min. with Sm (50 μg/ml).

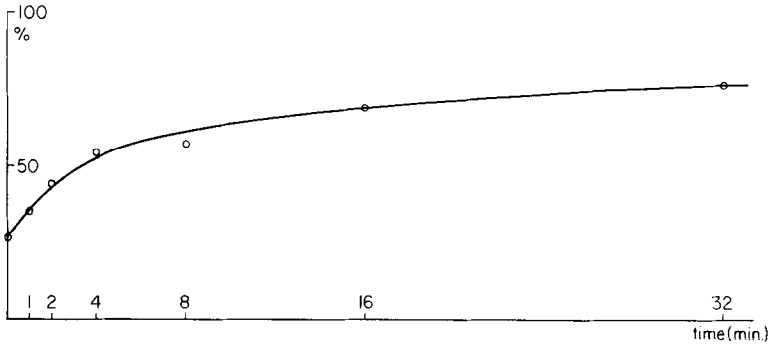

Figure 2. Kinetic of appearance of the "sticking effect" of Sm Abscissa : time ; ordinate : ratio of the area under the 70 S peak and the total area under the three ribosomal peaks, on Schlieren patterns observed after 20 min. centrifugation at 31,410 rpm, for extracts obtained from cultures chilled at variable time after addition of Sm (50 μg/ml).

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3) In vitro treatment of the extract with streptomycin does not reveal any effect on the ultracentrifugation patterns (except a non-specific one, at very high Sm concentrations, just before the beginning of precipitation). This is true even when the extracts are prepared in high Mg^{++} concentrations (10^{-2}M) to prevent dissociation of the ribosomes, then treated with Sm, and thereafter diluted to lower the Mg^{++} concentration to 10^{-3}M.

4) Addition of chloramphenicol (100 μg/ml) at the same time as Sm completely prevents the development of the "sticking effect" (see figure 3).

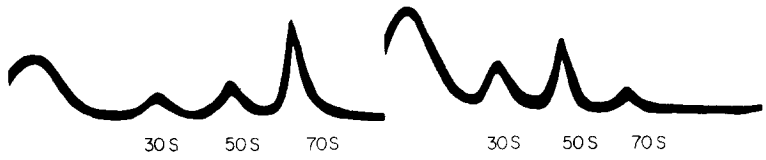

Figure 3. At left, extract from a Sm treated culture (50 μg/ml for 16 min.). At right, extract from a culture treated with both Sm (50 μg/ml) and chloramphenicol (100 μg/ml). The peak at left represents "chloramphenicol particles" (Nomura and Watson, 1959).

DISCUSSION AND CONCLUSIONS.

A short treatment of a culture of a Sm^S E. coli strain with streptomycin results in a sharp alteration of the ultracentrifugal behaviour of the ribosomes : whereas most ribosomes from an untreated culture dissociate into their 50 S and 30 S components in 10^{-3}M Mg^{++}, a large part of the ribosomes from the Sm treated cultures remain stuck in the 70 S form in the same environment.

That dissociation of the 70 S ribosomes in low Mg^{++} is not prevented by Sm if the antibiotic is added after disruption of the cells and before lowering the Mg^{++} concentration, suggests that the interaction between ribosomes and Sm may not be simple. The possible nature of this complexity is indicated by the fact that the expression of the "sticking effect" is prevented by chloramphenicol : the interaction of ribosomes with Sm apparently is thus associated with a step of protein synthesis following that inhibited by chloramphenicol.

These results are consistent with the idea that the ribosomes are essential sites of Sm action (Spotts and Stanier, 1961). The"sticking effect" might very well be a critical event in the lethal action of the antibiotic, since :

1°. This effect develops very early after the addition of the antibiotic (under our conditions, 50% of the maximum effect develops in the first 4 minutes).

2°. Prevention of the early-occuring "sticking effect" by chloramphenicol is paralleled by the already described (Plotz and Davis, 1962) prevention by chloramphenicol of the lethal effect of Sm.

The fact that Sm does inhibit protein synthesis in amino acid incorporating systems, whereas the "sticking effect" cannot be observed with ribosomes treated in vitro with Sm seems to contradict the hypothesis that the sticking effect of Sm is the primary one. But it must be emphasised that, in the amino acid incorporating system, the conditions are such as to allow protein synthesis to start, while this is not the case in the conditions used here.

In conclusion, we assume that streptomycin exerts its lethal effect by interacting with the ribosomes, and that this interaction can only take place when the ribosomes are already involved in some way in actual protein synthesis.

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