Polypeptide Synthesis with Ribosomes from Streptomycin-Resistant and Dependent E. Coli*

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In the preceding paper (1) we have shown that streptomycin (SM) interferes with the polyuridylate (poly U) stimulated incorporation of L-phenylalanine-C^{14} into acid-insoluble polypeptide when measured with the amino acid incorporating system recently described by Nirenberg and Matthaei (2). Spotts and Stanier (3) have recently proposed a theory of streptomycin action which attempts tc explain the various epiphenomena associated with streptomycin. Their pro± posal is satisfying on genetic grounds and at the basis of the argument is the postulate that the ribosomes of the various strains have different affinities for, or dependencies upon, streptomycin. We offer evidence in this report that the basic postulate regarding differences in ribosomal affinity for streptomycin indeed appears to be true. However, not all of the predictions of the hypothesis are borne out and a number of the biological effects of streptomycin remain to be explained. An important finding prior to this work is the observation of Erdös and Ullmann (4) that cell-free extracts from a streptomycin-resistant Mycobacterium incorporated labeled amino acids into protein in the presence of the antibiotic whereas an extract from sensitive cells was inhibited.

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Streptomycin-dependent (B/Sd-9) and streptomycin-resistant (B/Sr-11) spontaneous mutants derived from our parent strain B were isolated in this laboratory. They are both resistant to high levels of streptomycin (1000 µg./ml.) in the growth medium and their physiology is similar to that of other such strains which have been reported (3, 5). The organisms were grown in a yeast extract-tryptone supplemented medium containing 200 µg./ml. of streptomycin. Washed ribosomes and the supernatant fraction were prepared from these strains by the procedure of Nirenberg and Matthaei (2), as well as from the parent sensitive strain grown in the absence of streptomycin.

The Table shows the results of an experiment in which the ribosomes of the three strains were tested with their own supernatant fraction, and in the case of the ribosomes from resistant and dependent cells, with the supernatant fraction from sensitive cells. The concentration of streptomycin present in the assay is indicated in column 3. The results are recorded in column 4 as the stimulation by poly U of the incorporation of L-phenylalanine-C^{14} into acid-insoluble polypeptide.

Three facts are readily apparent. First, it is clear that with ribosomes from resistant or dependent cells it makes little difference whether they are incubated with their own supernatant fraction or with one derived from sensitive cells. This is true whether streptomycin is present or not. These results imply that the differences in behavior to streptomycin reside in the ribosomes and do not involve the enzymes concerned with amino acid activation or amino acyl transfer to the ribosome. This finding is in agreement with the conclusion of the first paper and strongly supports the basic postulate of the Spotts and Stanier hypothesis.

Secondly, it is apparent that ribosomes from resistant and from dependent cells are resistant to levels of streptomycin (1×10^{-6} M) which inhibit the

Table

L-Phenylalanine-C^{14} Incorporation with Ribosomes from Sensitive, Dependent and Resistant Strains of E. Coli.

Additions to System			L-Phenylalanine Incorporation*	%
Ribosome Source	Supernatant Source	Streptomycin (M)	CPM/mg. Protein	Inhibition
Sensitive	Sensitive	0	786	-
"	"	1×10^{-6}	126	84
"	"	1×10^{-3}	101	87
Dependent	Dependent	0	581	-
"	"	1×10^{-6}	584	0
"	"	1×10^{-3}	263	55
Dependent	Sensitive	0	610	-
"	"	1×10^{-6}	516	15
"	"	1×10^{-3}	200	67
Resistant	Resistant	0	568	-
"	"	1×10^{-6}	586	0
"	"	1×10^{-3}	157	72
Resistant	Sensitive	0	593	-
"	"	1×10^{-6}	560	6
"	"	1×10^{-3}	284	52

The complete incubation system is that described by Nirenberg and Matthaei (2), with ribosomal (2.0 mg.) and supernatant (1.0 mg.) protein, 0.3 mg. of transfer-RNA and 13.4 µg. of poly U present in each vessel. The poly U was added at 20 minutes and Streptomycin at 0 minutes. The vessels were incubated at 37° for 45 minutes.

* Corrected for CPM/mg. protein incorporated in the absence of added poly U.

ribosomes from sensitive cells about 85%. However, at higher levels of streptomycin (1×10^{-3} M) both resistant and dependent ribosomes are inhibited about 50 to 70%. Barring an effect on poly U directly, or some other competitive phenomenon, it would seem that the ribosomes from resistant and dependent cells have about a 1000-fold lower sensitivity to inhibition by the antibiotic than ribosomes from sensitive cells.

Finally, we are unable to demonstrate that washed ribosomes isolated from streptomycin-dependent cells are dependent for their activity upon the presence of streptomycin in the incubation system. The cells, however, were grown in the presence of 200 μg./ml. of streptomycin and may have taken up enough of the antibiotic during growth to obscure any stimulation on further addition. In this regard, Spotts and Stanier (3) found that radioactive streptomycin taken up by dependent cells during growth was not easily removed.

Discussion. The results of this and the preceding paper support some of the main points of the Spotts and Stanier hypothesis of streptomycin action. As mentioned previously however, the theory does not adequately explain the permeability alterations associated with streptomycin action on sensitive cells, and more recently reported with dependent cells (6, 7). In the results presented here we have been impressed with the similarity in behavior of the ribosomes isolated from resistant and from dependent cells. They both show a similar inhibition at high levels of streptomycin and are equally active in the absence of the antibiotic. It is clear that further work is required to clarify the phenomenon of dependency.

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