INHIBITION of POLYPEPTIDE SYNTHESIS by STREPTOMYCIN*

Joel G. Flaks+, Edward C. Cox and James R. White++

Dept. of Biochemistry, University of Pennsylvania School of Medicine, Philadelphia 4, Pa.

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There is evidence from several laboratories that at least one of the early effects of streptomycin on sensitive bacteria is an inhibition of protein synthesis (1-3). We have recently obtained evidence that the synthesis of both ribosomal and soluble protein is completely inhibited 5 minutes after the addition of bactericidal levels of streptomycin (SM) to exponentially growing cultures of E. Coli B in a salts-glucose medium (4, 5). Further incorporation of RNA and protein into the ribosomal fraction is stopped, and shortly thereafter an accumulation of RNA occurs in the supernatant fraction. A portion of the RNA which accumulates has the properties of messenger-RNA (6).

These observations suggested a possible interference in the attachment and/or function of messenger-RNA. A direct test of this has now been sought by use of the cell-free amino acid incorporating system from E. coli which has recently been described by Nirenberg and Matthaei (7). Figure 1 shows an experiment in which the time-course of L-phenylalanine-C^{14} incorporation into acid-insoluble polypeptide was measured under the conditions described by Nirenberg and Matthaei. In the absence of added polyuridylate (poly U)

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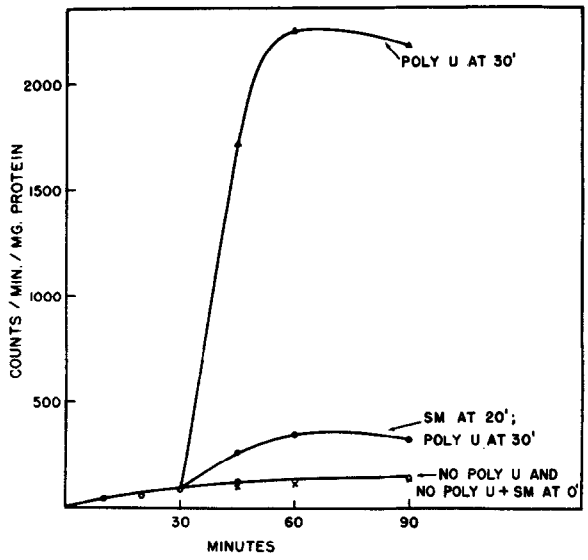

Figure 1. Time-course of L-phenylalanine-C^{14} incorporation into acid-insoluble protein. The complete system is that described by Nirenberg and Matthaei (7), with 3.0 mg. of ribosomal protein (washed ribosomes), 1.5 mg. of soluble protein and 0.5 mg. of transfer-RNA. Where added: poly U, 13.4 µg. of uridine nucleotide; streptomycin (SM), 1×10^{-5} M.

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as a messenger, there is a significant incorporation of L-phenylalanine into protein which is unaffected by the presence of streptomycin at levels as high as 5×10^{-4} M. This incorporation is presumed to be directed by the presence of templates (messengers) already attached to the ribosomes isolated from bacterial cells. When poly U is added to the complete system at 30 minutes in the absence of streptomycin, there is a rapid incorporation of L-phenylalanine over the next 15 to 20 minutes. If streptomycin (1×10^{-5} M) is added 10 minutes prior to the poly U addition, the stimulation of phenylalanine incorporation obtained with poly U is inhibited by 95%. There are two requirements for the inhibition. The antibiotic must be added to the ribosomes prior to the addition of the poly U, and the intact antibiotic is required for activity. Streptidine (5×10^{-4}M), streptobiosamine (1×10^{-3}M), or a combination of both compounds are not inhibitory. Streptomycin, dihydrostreptomycin and

hydroxystreptomycin are equally effective as inhibitors. Up to the present time reversal of the inhibition has not been obtained. The polyamines spermidine and putrescine, alone, or in combination, at levels of 1×10^{-6} to 1×10^{-3} M do not reverse the inhibition, nor does a 10-fold excess of poly U or an excess of Mg^{++}.

We believe the inhibition is due to binding of streptomycin to the ribosome. When ribosomes are incubated in a complete system with unlabeled L-phenylalanine and streptomycin (1×10^{-5} M), then isolated by centrifugation, resuspended and tested as in Figure 1, but in the absence of streptomycin, the stimulation normally obtained with poly U does not occur. Further evidence for this view is given in the accompanying paper dealing with ribosomes isolated from streptomycin-dependent and resistant bacteria.

The inhibition is obtained with very low levels of streptomycin. Figure 2 shows the activity of the complete system with added poly U as a function of the streptomycin concentration. Half-maximal inhibition obtains at about 5×10^{-7} M streptomycin and is the same with a 10-fold excess of poly U. It is of interest to note that at the 50% inhibition point the number of molecules of streptomycin per ribosome is about 0.3, while at 1×10^{-6} M streptomycin where the poly U stimulation is inhibited about 90% the ratio SM/ribosome is about 0.6. Thus if all of the streptomycin in the system is bound to the ribosomes, of the order of 1 to 2 molecules per ribosome suffices to inhibit expression of the added messenger-RNA. It is also of interest to compare this figure with the number of molecules of streptomycin taken up by sensitive cells at the time when protein synthesis in the cell is interrupted (about 5 minutes). Binding studies with labeled streptomycin by Davis and co-workers (8), and in this laboratory (4, 5), indicate an initial rapid uptake of 1 to 2.5 µg. of streptomycin per 10^{10} bacteria. Assuming 10^{4} ribosomes per cell, this gives a value of

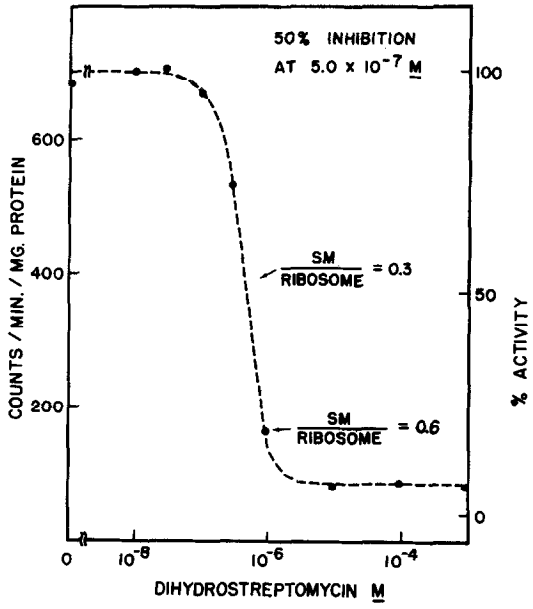

Figure 2. Activity with poly U as messenger as a function of streptomycin concentration. The conditions are the same as those of Figure 1 with streptomycin added at 0 minutes and poly U at 20 minutes. The vessels were incubated at 37° for 45 minutes.

1,000 to 2,500 molecules of streptomycin/ribosome, certainly in excess of that required for full inhibition.

Chloramphenicol appears to behave in a manner similar to streptomycin in blocking the stimulation with poly U but not the incorporation in the absence of poly U. However, the level required is much higher (55% inhibition at 9×10^{-4} M), and possibly another site is involved. Puromycin at 1×10^{-3} M inhibited the incorporation whether or not poly U was present.

It should be pointed out that the present observations with streptomycin do not adequately explain some of its important biological effects. Permeability alterations are a well documented effect of streptomycin (9), and it is difficult at present to see how inhibition of protein synthesis, per se, would lead to such changes. One should then expect to see such permeability altera-

tions with chloramphenicol and puromycin, but such have not been recorded. In fact, chloramphenicol blocks the subsequent permeability changes associated with streptomycin administration to bacteria (10). There is also the lethal event which must be considered.

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