EFFECTS OF STREPTOMYCIN ON THE TRANSLATION OF TURNIP YELLOW MOSAIC VIRUS RNA IN VITRO

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Recent experiments have led to a new concept concerning the action of SM*. Gorini et al. (1964) and Lederberg et al. (1964) found certain classes of E.coli mutants resistant to SM in which a genetic defect could be phenotypically suppressed by the antibiotic. It was assumed that such a suppression was related to a SM induced alteration of the ribosome structure which might influence the accuracy of messenger RNA reading. Phenotypic repair by SM of RNA bacteriophage mutants was interpreted in a similar fashion (Valentine and Zinder, 1964).

Subsequently Davies et al. (1964) demonstrated that SM may influence the reading of poly U in cell-free extracts of E.coli leading to a strong inhibition of phenylalanine incorporation and an extensive incorporation of isoleucine into polypeptide. Other instances of a wrong translation of triplets were also described by these authors.

In our laboratory anomalous effects of SM on polypeptide formation in cell-free extracts of E.coli were noted, namely strong stimulation as well as inhibition of this process (van Knippenberg et al., 1964; van Knippenberg et al., 1965). These effects depended on the nature of the messenger and the Mg^{2+} concentration of the medium and the question arose whether they could also be explained in terms of misreading. When TYMV-RNA was used as a messenger in a system containing ribosomes from

* Abbreviations: SM, streptomycin; TYMV, turnip yellow mosaic virus.

SM-sensitive E.coli cells and a mixture of ^{14}C labelled amino acids, inhibition of polypeptide formation by SM occurred in the presence of 0.008 M - 0.012 M Mg^{2+}. Stimulation was observed from 0.014 M Mg^{2+} onwards. If it is supposed that in the latter range of Mg^{2+} concentrations 3 U triplets code for phenylalanine and isoleucine when SM is present in the mixture, one could expect the enhancement of isoleucine incorporation to be accompanied by a lowering of phenylalanine incorporation. This was not found to be the case as is illustrated in Figure 1. The incorporation profiles of the two amino acids are very similar and reflect the findings obtained with a mixture of ^{14}C amino acids in the presence of TYMV-RNA (van Knippenberg et al., 1964; van Knippenberg et al., 1965). The stimulation of phenylalanine incorporation by SM could also be due to coding for phenylalanine by triplets different from 3 U, in such a way that the lowering of phenylalanine incorporation, resulting from misreading of 3 U triplets, is more than compensated. We now have studied, however, 12 different labelled amino acids by following the incorporation of each in the presence of 19 other cold ones. All yielded essentially the same profile as can be seen from Figure 1. These data indicate that stimulation of polypeptide synthesis by SM cannot merely be explained by the substitution of certain amino acids by others.

TYMV-RNA is supposed to be a polycistronic messenger (Voorma et al., 1964; Voorma et al., 1965) and the effect of SM on polypeptide formation could be a consequence of an altered accesibility of cistrons. The association between TYMV-RNA and isolated ribosomes from E.coli has been studied by Voorma et al. (1965) at $0^{\circ}C$ by means of sucrose gradient centrifugation. By varying the ribosome/viral RNA ratio it was found that the association reaches a maximum at 10 - 15 ribosomes added per messenger. We have performed similar experiments at 0.020 M Mg^{2+} in the presence and absence of SM. As may be seen from Figure 2 the two association curves coincide. It has been demonstrated (Voorma et al., 1964; Voorma et al.,

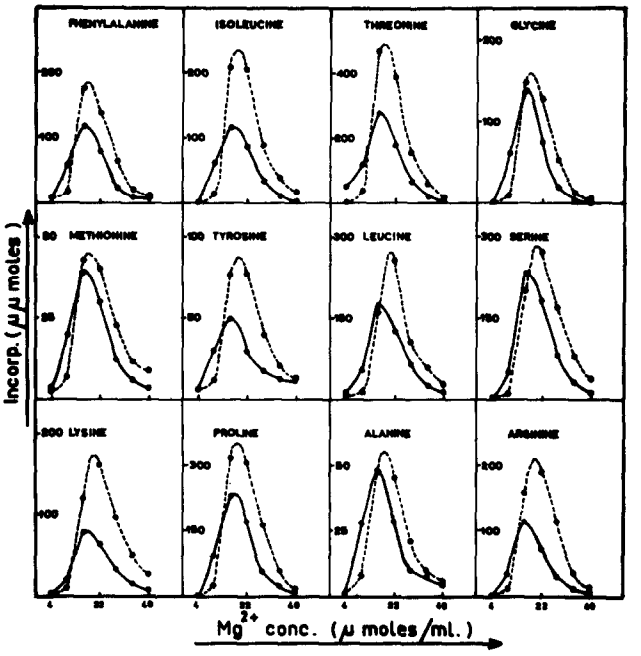

Figure 1. The influence of the Mg^{2+} concentration on the effect exerted by SM on the incorporation of different amino acids by a TYMV-RNA directed system from SM-sensitive E.coli B. Solid lines: no SM, dotted lines: with 0.001 μmoles SM per ml (SM/ribosome ratio = ± 3). Ca. 10 mμmoles of the specified ^{14}C amino acid were added together with 10 mμmoles of each of the other cold amino acids. Other components were as described previously (van Knippenberg et al., 1965). The values for phenylalanine, isoleucine, threonine, glycine, methionine and tyrosine incorporation were obtained in one experiment. The same holds true for the incorporation of leucine, serine and lysine and of proline, alanine and arginine.

1965) that the ribosomal aggregates formed under these conditions initiate polypeptide synthesis when supplied with amino acids, soluble enzymes and cofactors. The association process thus recorded is supposed therefore to represent the primary step of messenger translation at least under in vitro conditions. If this is so, SM does not seem to alter the accessibility of the messenger or parts of it for the ribosomes, but may act by an enhancement of the rate of messenger translation. Evidence for the latter possibility has been obtained and presently we wish to report about one of the experiments carried out so far, which

has also general relevance for the reading mechanism of TYMV-RNA in the system here employed.

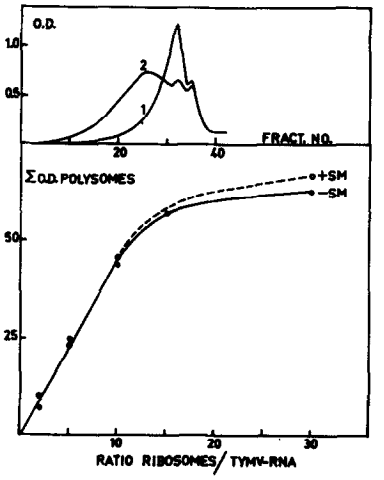

Figure 2. The formation of polysomes at different ratios of ribosomes and TYMV-RNA, without and with 0.001 µmoles SM per ml. Ribosomes and TYMV-RNA were mixed at 0°C in different ratios (in 0.010 M tris, 0.050 M KCl, 0.020 M magnesium acetate pH 7,8) and the mixtures were analyzed on a sucrose density gradient. In the upper part of the figure the patterns recorded with free 70 S particles (curve 1) and with 5 ribosomes per TYMV-RNA molecule (curve 2) are depicted. The absorbancies at 260 mµ of the polysome fractions were enumerated after the subtraction of the absorbancies due to free 70 S particles and plotted against the ribosome/TYMV-RNA ratio (lower part of the figure).

Figure 3 illustrates the incorporation of a mixture of ^{14}C labelled amino acids under the direction of TYMV-RNA when followed for various periods of time.

Polypeptide formation slows down after a while and the incorporation reaches its maximum after about 60 min. Protein synthesis is resumed when the system is replenished with messenger (Voorma and Bosch, 1965), indicating that polypeptide formation stops due to messenger breakdown. That degradation of the messenger is not complete at 60 min., however, is clearly shown by the fact that amino acid incorporation starts again, when SM is added at that time. Moreover it could be shown, that such

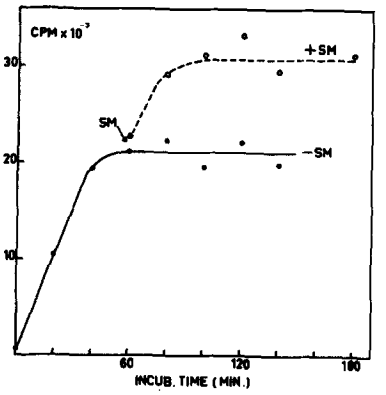

Figure 3. Time course of amino acid (^{14}C-algal protein hydrolysate) incorporation directed by TYMV-RNA and the effect of SM (0.001 µmoles/ml) after 60 min. of incubation. The experiment was performed with a purified system described by Voorma and Bosch (1965) at 0.020 M Mg^{2+}. Other components were as described previously (van Knippenberg et al., 1965).

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an addition of the antibiotic induces a further release of polypeptides from the ribosomes, as well as a more extensive loading of these particles with radioactive nascent chains.

Apparently there must be an additional reason for the cessation of amino acid incorporation, which has escaped attention so far. The possibility may be envisaged that this cessation is due to an extreme type of what has been called modulation by Ames and Hartman (1963).

Other interpretations are also possible, however.

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