REVERSAL BY GTP OF SOLUBLE RNA INHIBITION OF POLYPHENYLALANINE SYNTHESIS*

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Deacylated soluble ribonucleic acid (sRNA) has been reported to inhibit the binding of phenylalanyl-sRNA (phe-sRNA) to polyuridylate (poly U) containing ribosomes (Nirenberg and Leder, 1964) in one of the early reactions involved in peptide synthesis. Kaji and Kaji (1965) have also demonstrated that deacylated phenylalanine-acceptor RNA is capable of binding to the same group of ribosomes that attaches phe-sRNA, presumably by binding at the same site. The sRNA binding reaction, as studied in Escherichia coli, does not usually require the addition of soluble transfer enzymes or guanosine triphosphate (Nakamoto et al., 1963; Nirenberg and Leder, 1964) and can be regarded as an early reaction preceding the peptide bond forming steps. Thus it seemed surprising that under our usual assay conditions synthesis of polyphenylalanine from phe-sRNA was hardly affected by rather large amounts of deacylated sRNA. It will be shown here, however, that when the rate of phenylalanine incorporation is limited by the concentration of guanosine triphosphate (GTP), deacylated sRNA becomes very inhibitory. We will further show that the inhibition appears to be competitively reversed by GTP and that a soluble enzyme fraction and GTP will increase the amount of phe-sRNA bound to the ribosomes in the presence of deacylated sRNA.

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Materials and Methods. E. coli B early log cells (Grain Processing Corp., Muscatine, Iowa) were used to prepare ribosomes, soluble transfer enzymes and C^{14}-phenylalanyl-sRNA by methods that have been described (Conway and Lipmann, 1964). Soluble RNA was prepared from either fully grown E. coli B cells (von Ehrenstein and Lipmann, 1961) or obtained commercially. Soluble RNA from both sources was deacylated by incubation at 37° for 30 min in 0.2 M Tris-base, pH 9.5. The C^{14}-phenylalanyl-sRNA was prepared from a portion of the same batch of deacylated sRNA used as inhibitor.

Assay of the ribosomal bound C^{14}-phe-sRNA was according to a modified procedure of Nirenberg and Leder (1964). After incubation, the reaction mixtures were terminated with 3 ml of cold 0.1 M Tris-chloride, pH 7.4; 0.05 M NH_4Cl; 0.02 M $MgCl_2$ buffer. The diluted mixture was poured onto two cellulose nitrate membranes, filtered under gentle suction, and washed with three 3 ml portions of cold buffer. The filters were dried and counted in a liquid scintillation counter at 65% efficiency. Polyphenylalanine synthesis was determined as previously described (Conway and Lipmann, 1964) except liquid scintillation counting was used to assay radioactivity.

Immediately before an experiment ribosomes were preincubated at 37° for 30 min in 0.5 M NH_4Cl, 0.005 M Tris-chloride, pH 7.4, and 0.005 M $MgCl_2$ (T. Nakamoto, personal communication). Ribosomes so treated were more active for both binding and incorporation. Polyuridylate stimulated incorporation 100-fold and the binding reaction 25-fold.

Results. Deacylated sRNA is an effective inhibitor of the rate of poly U directed binding of phe-sRNA to E. coli ribosomes as illustrated in Figure 1. A similar sort of inhibition has been reported by Hardesty et al. (1963) for reticulocyte ribosomes and by Nirenberg and Leder (1964) for an E. coli system. The latter workers also reported that the attachment of phe-sRNA was almost irreversible because once bound it was not easily displaced by deacylated sRNA. We have confirmed this observation and in addition have

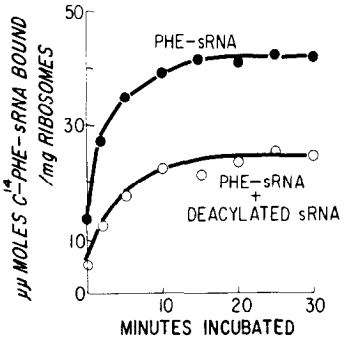

Figure 1. Inhibition of the binding of C^{14}-phenylalanyl-sRNA to ribosomes by deacylated sRNA. Aliquots of 0.10 ml were taken at the indicated times from each of the two reaction mixtures and terminated with hot 5% trichloroacetic acid. The composition of the mixtures was the same except that one contained 2.17 mg of deacylated sRNA. Both contained 65 μmoles of Tris-Cl, pH 7.4; 200 μmoles of NH_4Cl, 16 μmoles of $MgCl_2$, 50 μg of poly U, 1.29 mg of preincubated 3-times washed ribosomes, and 0.5 mg of C^{14}-phenylalanyl-sRNA (62 μC/μmole; 13,400 cpm) in a final volume of 1.25 ml.

learned that previously bound deacylated sRNA can be displaced by C^{14}-phe-sRNA to where binding is about 50% of an uninhibited control.

Under our usual assay conditions, it was not possible to show an inhibition of polyphenylalanine synthesis by deacylated sRNA. Since deacylated sRNA can be thought of as one of the products of peptide formation from amino acyl-sRNA, we looked for an effect of the soluble enzymes or GTP on reversal of this inhibition. Figure 2 shows the results of an experiment where the synthesis of polyphenylalanine was measured in a system completely dependent on added GTP. As can be seen, deacylated sRNA is a very effective inhibitor of polyphenylalanine synthesis when the GTP concentration is low. The effectiveness of small amounts of the nucleoside triphosphate in reversing the inhibition accounts for the inability of deacylated sRNA to inhibit peptide synthesis under our usual assay conditions where GTP is present in saturating amounts.

Because GTP appeared responsible for allowing peptide synthesis in the presence of deacylated sRNA, we examined its effect on the similarly inhibited binding reaction. To minimize polyphenylalanine synthesis we used a partially heat-denatured enzyme (Conway and Lipmann, 1964) and a higher magnesium ion

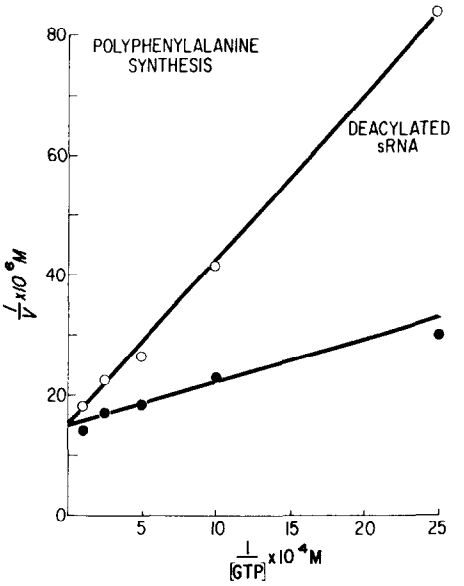

Figure 2. Reversal of sRNA inhibited polyphenylalanine synthesis by guanosine triphosphate. The assay mixtures contained the following in 0.25 ml: 12.5 μmoles of Tris-Cl, pH 7.4; 40 μmoles of NH_4Cl; 8 μmoles of $MgCl_2$; 3 μmoles of 2-mercaptoethanol; 10 μg of poly U; 0.20 mg of washed ribosomes; 0.11 mg of polymerizing enzymes (65% AS fraction of Conway and Lipmann, 1964); 0.11 mg of C^{14}-phenylalanyl-sRNA (62 μC/μmole, 2960 cpm); GTP as indicated and where indicated 0.429 mg of deacylated sRNA. Reaction mixtures were incubated for 7 min at 30° and terminated with 2.5 ml of 5% TCA. Velocity is expressed as moles phenylalanine polymerized per liter per 7 min.

concentration that allows binding to occur but severely inhibits polymerization. Table I describes the results of one such experiment. The data shows that both GTP and soluble enzymes are required for partial reversal of the inhibition. As well, it can be seen that added GTP and soluble enzymes do not stimulate the binding of the phe-sRNA in the uninhibited controls.

Discussion. Phenylalanyl-sRNA binding to E. coli ribosomes (Nirenberg and Leder, 1964) and to reticulocyte ribosomes (Hardesty et al., 1963) is more efficient at magnesium ion concentrations higher than those optimal for polymerization. At the lower magnesium concentration binding to reticulocyte ribosomes requires a soluble enzyme together with GTP (Hardesty et al., 1963), and as might be predicted from the data presented here, is insensitive to

TABLE I

Reversal of the Deacylated-sRNA Inhibited Binding Reaction

	Composition	C.P.M. C^{14}-phe-sRNA Bound*
1.	Complete	1526
2.	Complete + enzyme	1437
3.	Complete + enzyme + deacylated sRNA	627
4.	Complete + deacylated s RNA	704
5.	Complete + GTP	1554
6.	Complete + GTP + deacylated sRNA	716
7.	Complete + enzyme + GTP	1364
8.	Complete + enzyme + GTP + deacylated sRNA	1042

*Data has been corrected for hot TCA insoluble material as follows: no. 5, 83 cpm; no. 7, 279 cpm; no. 8, 12 cpm. No corrections were necessary for the others.

The complete reaction mixture contained in 0.25 ml: 50 μmoles of Tris-Cl, pH 7.4; 80 μmoles of NH_4Cl; 16 μmoles of $MgCl_2$; 0.01 mg of poly U; 0.4 mg of preincubated ribosomes, and 0.11 mg of C^{14}-phenylalanyl-sRNA (62 μC/μmole). Additions were made in the following amounts: 0.11 mg protein of a soluble fraction (65% AS fraction of Conway and Lipmann, 1964) that had been heated for 4 min at 65°, 50 mμmoles of GTP, and 0.429 mg of deacylated sRNA. Reaction mixtures were incubated for 15 min at 30° and terminated as described in the text.

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inhibition by deacylated sRNA. Thus, in respect to phe-sRNA binding, the E. coli system can be made to resemble the reticulocyte system by inhibiting it with deacylated sRNA.

We have shown a close relationship of deacylated sRNA to GTP that suggests a role for the nucleoside triphosphate in removal of deacylated sRNA from the ribosome. In an efficiently operating cell one would expect that most of the deacylated sRNA would be quickly charged so that only one GTP would be required to remove it when the peptide bond is formed. This would be consistent with the stoichiometry of one phosphoryl cleaved per phenylalanine incorporated that is indicated by the work of Nishizuka and Lipmann (1966). Neither would the proposed role for GTP conflict with the notion that the nucleoside tri-

phosphate is involved in advancing the messenger RNA on the ribosome since release of deacylated sRNA could be either the cause or the consequence of messenger RNA movement.

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