STIMULATION OF CELL FREE POLYPHENYLALANINE SYNTHESIS BY 30S RIBOSOMAL SUBUNITS IN ESCHERICHIA COLI

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SUMMARY

In cell free polyphenylalanine synthesis in Escherichia coli, an addition of 30S ribosomal subunits to pure 70S ribosomes stimulates the synthesis several times. Such a stimulating effect is not observed with an addition of 50S ribosomal subunits.

Role of 30S ribosomal subunits in protein synthesis has been well studied. Nomura and Lowry (1) proposed a hypothesis that the first step in the initiation of protein synthesis is formation of an initiation complex consisting of the 30S ribosomal subunit, mRNA and formyl-methionyl-$tRNA_F$, and the 50S ribosomal subunit next joins this complex, followed by the binding of a second aminoacyl-tRNA. This proposal is now well accepted and several authors have presented data supporting this hypothesis (2-6).

This communication reports that an addition of 30S ribosomal subunits to a conventional ribosome preparation, which contains only 70S ribosomes, stimulates polyphenylalanine synthesis several times compared to the amount of polyphenylalanine synthesized with 70S ribosomes alone.

MATERIALS AND METHODS

Preparation of pure 70S ribosomes

Ribosomes of E. coli B or Q13 were prepared according to the method described previously (7). They were washed

three times with buffer containing 0.5 M NH_4Cl, 0.1 M Tris-HCl pH 7.8, 0.01 M magnesium acetate and 0.006 M 2-mercaptoethanol. About 20 mg of this preparation (washed ribosomes) were placed on a linear sucrose density gradient (10-25% in a buffer containing 0.01 M Tris-HCl pH 7.1, 0.06 M NH_4Cl and 0.01 M magnesium acetate, the total volume being 28 ml) and the preparations were centrifuged at 18,000 rev/min for 15 hours in a SW 25.1 rotor. The fractions containing 70S ribosomes were pooled and concentrated by centrifugation. This purification procedure was repeated two or three times. The final preparation (pure 70S ribosomes) was suspended in a buffer containing 0.01 M Tris-HCl pH 7.8, 0.06 M NH_4Cl, 0.01 M magnesium acetate and 0.006 M 2-mercaptoethanol at a concentration of 2.4 mg/ml (assuming 1 A_{260} unit equals 0.06 mg/ml). The preparations were stored at -80°C until used. These preparations were not contaminated with ribosomal subunits as shown in Fig. 1.

Preparation of ribosomal subunits

Ribosomal subunits were prepared as described previously (8). About 50 mg of washed ribosomes were suspended in

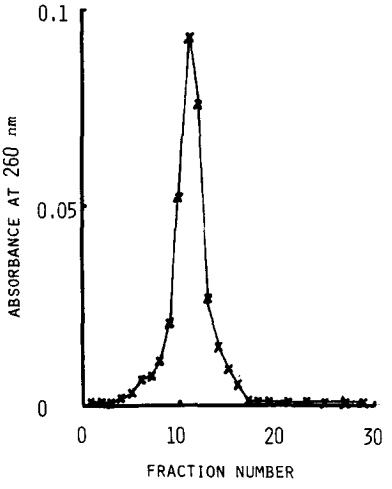

Fig. 1. Purity of the pure 70S ribosomes. A 30 µl aliquot of pure 70S ribosome preparation (2.4 mg/ml) was placed on a linear sucrose density gradient (5-20% in a buffer containing 0.01 M Tris-HCl pH 7.8, 0.06 M NH_4Cl, 0.01 M magnesium acetate, the total volume being 4.6 ml) and the preparation was centrifuged at 38,000 rev/min for 80 minutes in a SW 50.1 rotor. A 2 drops fractionation was carried out and absorbance at 260 mu was measured with samples of each fraction diluted twelve fold.

0.01 M potassium phosphate buffer (pH 7.0) containing 10^{-4} M magnesium acetate. They were placed on a linear sucrose density gradient (5-20% in a buffer containing 0.01 M Tris-HCl pH 7.1 and 10^{-4} M magnesium acetate, the total volume being 28 ml) and the preparation was centrifuged at 23,000 rev/min for 12 hours in a SW 25.1 rotor. The fractions containing 30S and 50S ribosomal subunits were pooled and concentrated separately by use of collodion bag. The concentrations of 30S and 50S ribosomal subunits were 2 mg/ml and 4 mg/ml, respectively. They were stored in small aliquots at -80°C until used. The purity of these preparations were shown in Fig. 2.

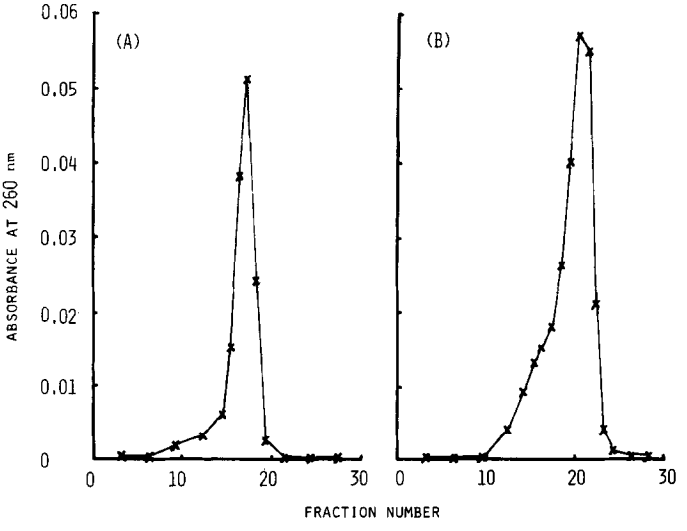

Fig. 2. Purity of the ribosomal subunits. (A) A 10 µl aliquot of 50S ribosomal subunits (4mg/ml) was placed on a linear sucrose gradient (5-20% in a buffer containing 0.01 M Tris-HCl pH 7.8, 0.06 M NH_4Cl, 10^{-4} M magnesium acetate, the total volume being 4.6 ml). Centrifugation and fractionation were carried out as described in the legend of Fig. 1. Absorbance at 260 mu was measured with samples of each fraction diluted twenty fold. (B) A 20 µl aliquot of 30S ribosomal subunits (2 mg/ml) was used for fractionation on sucrose density gradient. Other experimental conditions are as described in (A).

Preparation of enzyme fraction for polyphenylalanine synthesis

The supernatant fraction (S-100) obtained according to the method described previously (7) was further centrifuged

at 220,000 x g for 3 hours in the presence of 0.02 M magnesium acetate. The upper four-fifths of the supernatant was used as enzyme fraction. This preparation contained 9.2 A_{280} units and 14.0 A_{260} units per ml. With this preparation, no significant polyphenylalanine synthesis was observed when either 30S or 50S ribosomal subunits alone was added, indicating this preparation contained few, if any, 30S or 50S ribosomal subunits.

Assay for polyphenylalanine synthesis

The standard reaction mixture (0.1 ml) for polyphenylalanine synthesis contained the following: 0.01 M Tris-HCl pH 7.8, 0.06 M NH_4Cl, 0.012 M magnesium acetate, 1 mM ATP, 0.02 mM GTP, 6 mM 2-mercaptoethanol, 4 mM phosphoenolpyruvate, 3 µg pyruvate kinase, 21.2 µM ^{14}C-phenylalanine (23.5 µC/µmole), 20 µg poly U (Miles Chemical Co.), 100 µg E. coli B tRNA (General Biochemicals), 10 µl of enzyme fraction and ribo-

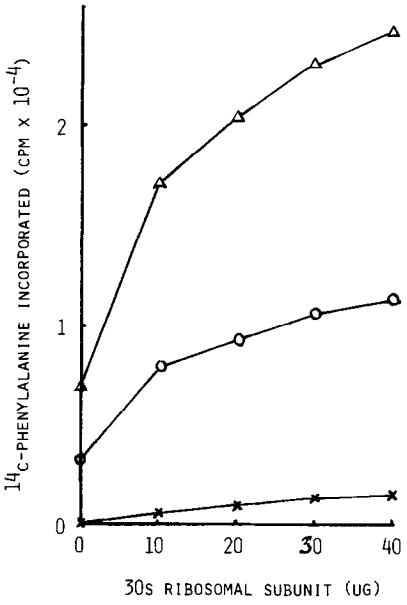

Fig. 3. Stimulation of polyphenylalanine synthesis by 30S ribosomal subunits. The components of the reaction mixture and the assay procedure for polyphenylalanine synthesis are described in the text. Pure 70S ribosomes added are 48 µg (Δ — Δ), 24 ug (o — o) and none (x — x). In addition, several amounts of 30S ribosomal subunits as indicated are added. Hot trichloroacetic acid insoluble radioactivity without adding poly U (350 cpm at maximum) was subtracted from each value, which is an average of duplicate determinations.

some fractions as indicated in the figures. The reaction mixtures were incubated at 35°C for 45 minutes. The assay procedure is described previously (7).

RESULTS

Stimulation of polyphenylalanine synthesis by 30S ribosomal subunits

Experimental data presented in Fig. 3 show that 30S ribosomal subunits added in the reaction mixture in addition to 70S ribosomes stimulate polyphenylalanine synthesis. With 24 µg of 70S ribosomes, 3,030 cpm of polyphenylalanine was synthesized, while 7,935 cpm and 11,393 cpm of polyphenylalanine synthesis were observed by the addition of either 10 µg or 40 µg of 30S ribosomal subunits, respectively. A similar stimulation by 30S ribosomal subunits was observed

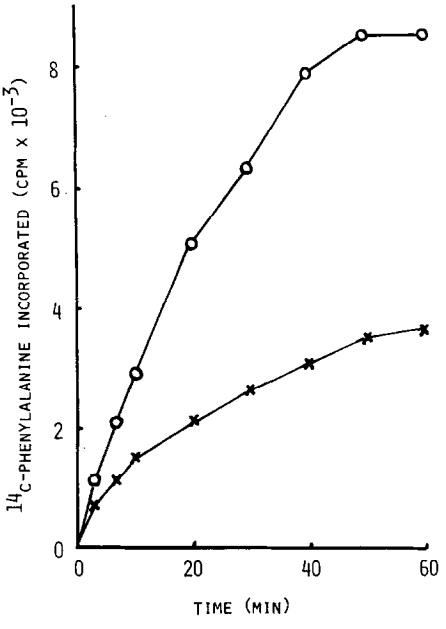

Fig. 4. Kinetics of polyphenylalanine synthesis in the absence and in the presence of the additional 30S ribosomal subunits. The components of the reaction mixture are as described in the text. No 30S ribosomal subunit was added in the one experiment (x — x) and 20 µg of 30S ribosomal subunits per 0.1 ml of the reaction mixture were added in the other (o — o). The amount of 70S ribosomes added is 24 µg. At various time intervals as indicated, a 0.1 ml aliquot is taken out of 1 ml of the reaction mixture and poured into 1 ml of 10% cold trichloroacetic acid solution. The assay was carried out as described in the text.

binding, or if it is due to double infection, cannot be determined. A partial detachment of membrane-bound DNA during the lysis procedure seems unlikely, since the sedimentation profile of the cytoplasmic DNA in a neutral CsCl gradient clearly differs from the sedimentation behaviour of membrane-bound DNA, which is discussed below.

Fig. 2 shows the distribution of parental DNA between the membrane and the soluble fraction during the first hour after infection. The total intracellular parental label decreases after 15' due to transfer of parental DNA into progeny phages (2). There is a slow decrease of membrane-bound parental DNA, but the percentage of intracellular parental DNA which is associated with the membrane stays fairly constant. Attachment of the parental DNA strand to the cell membrane might therefore be the main limitation for the complete transfer of parental DNA to progeny phages.

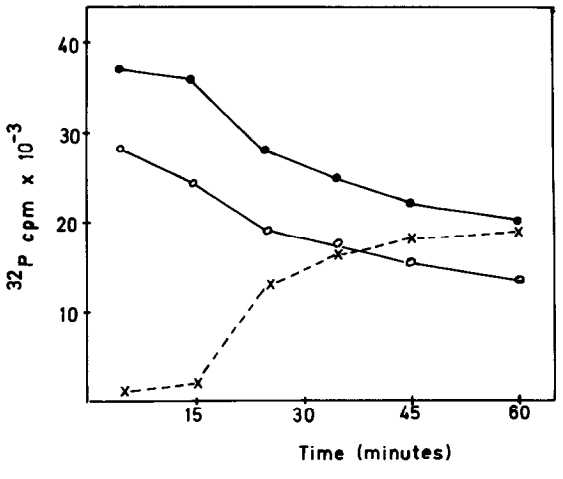

Fig. 2. Turnover of membrane-bound ^{32}P-labeled parental DNA. Total intracellular parental DNA was calculated by summing up the radioactive material in the fractions from the sucrose gradients. Transfer of parental DNA to progeny phages was assayed as described previously (2). o——o, membrane-bound parental DNA; ●——●, total intracellular parental DNA; x----x, parental DNA transferred into progeny phages.

tiation of polypeptide synthesis occurred through a complex of the 30S ribosomal subunit, mRNA and formyl-methionyl-$tRNA_F$ (1-6). If this is the case, conventional ribosome preparation is not favorable to polypeptide synthesis, since the dissociation into subunits is necessary step for 70S ribosomes to participate in polypeptide synthesis.

Recently, we found that streptomycin-sensitive 30S ribosomal subunits added to streptomycin-resistant 70S ribosomes form a hybrid 70S ribosomes with streptomycin-resistant 50S ribosomal subunits prior to the initiation of polyphenylalaninie synthesis (9). This indicates that dissociation of 70S ribosomes into subunits occurrs in cell free polyphenylalanine synthesis also. During the course of this study, we have assumed that the addition of extra 30S ribosomal subunits to conventional ribosome preparation may be favorable to polypeptide synthesis. Present experiments were carried out to demonstrate this assumption.

There may be several explanations for the finding that the addition of 30S ribosomal subunits to 70S ribosomes stimulate polyphenylalanine synthesis several times. It is possible that factor(s) which dissociate 70S ribosomes exist in 30S ribosomal subunits added. These factor(s) dissociate 70S ribosomes, resulting in the stimulation of polyphenylalanine synthesis. A dissociation factor (DF) proposed by Subramanian et al. (10) exists only on "native" 30S ribosomal subunits but not on derived 30S ribosomal subunits (10,11). Since the 30S ribosomal subunits used in this experiments contain few "native" 30S ribosomal subunits, there is little possibility that the preparation is contaminated with DF. However, further study is necessary to elucidate a possible participation of dissociation factor(s) in the stimulation of polyphenylalanine by 30S ribosomal subunits.

There is a possibility that 70S ribosomes are contaminated with 50S ribosomal subunits, thus the addition of 30S ribosomal subunits stimulates polyphenylalanine synthesis. This may not be the case since the pure 70S ribosomes used in the experiments contained few, if any, 50S ribosomal subunits (Fig. 1).

Whether 70S ribosomes is an artifact or not is still confusing. Mangiarotti and Schlessinger (12) proposed that

there is no 70S ribosomes in vivo. However, in vivo demonstration of 70S ribosomes have been reported from several laboratories (13-17). Recently, Kaempfer (18) reported that 70S ribosomes do not exist during rapid protein synthesis but exist when protein synthesis slow down. If 70S ribosomes exist in vivo, a question arises how 70S ribosomes participate in protein synthesis. Present findings suggest that the participation of 70S ribosomes in polypeptide synthesis can be controlled by the existence of 30S ribosomal subunits.

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