PROPERTIES OF THE CRYSTALLINE AMINO ACID POLYMERIZATION FACTORS FROM ESCHERICHIA COLI : BINDING OF G TO RIBOSOMES

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In the past few years a great number of independent reports have indicated the requirement of the protein factors G and T for ribosomal polypeptide synthesis in the in vitro system from E. coli (1-9). With the availability of electrophoretically homogeneous, crystalline G factor (7), the importance of studying the direct interaction of this enzyme with ribosomes became evident. Moldave and co-workers already observed in the rat liver system that aminoacyltransferase II - comparable to G - binds to ribosomes in the presence of a guanine nucleotide (10,11). In this communication, we extend our observations (12,13) on the formation of a G-ribosome complex.

METHODS

G and T were purified and crystallized from E. coli A19 (7). Ribosomes were washed 5 times with 0.010 M Tris-HCl, pH 7.8, containing in the first wash 1 M NH_4Cl and 0.002 M $MgCl_2$ and in the remaining 4 washes 0.5 M NH_4Cl and 0.010 M $MgCl_2$.

Binding of G to ribosomes - One ml of the reaction mixture contained: 25-50 A_{260} units of ribosomes (~0.6-1.2 mμmoles); 100-200 μg (~1.2-2.4 mμmoles) of crystalline G factor (20-30 units/mg protein); 50 μmoles Tris-HCl, pH 7.8; 12 μmoles $MgCl_2$;

80 μmoles NH_4Cl; 100 μmoles KCl; 3 μmoles 2-mercaptoethanol (ME); 2 μmoles dithiothreitol (DTT); nucleotide and poly U as indicated in the legends. After incubation at 30° for the selected interval of time, the reaction mixture was layered on a sucrose gradient and centrifuged for 3 hours at 45,000 rpm with the No. 50 rotor in a Spinco ultracentrifuge. Discontinuous (4ml of a 10% + 4ml of a 20% sucrose solution) or continuous (5-20% sucrose solution) gradients were used. The sucrose solution contained : 0.050 M Tris-HCl, pH 7.8; 0.010 M $MgCl_2$; 0.003 M ME; 0.001 M DTT. The ribosome pellets, yielding about 60-80% of the original ribosomes, were dissolved in a small amount (50-75 μl) of 0.050 M Tris-HCl, pH 7.8, containing 0.010 M $MgCl_2$, 0.003 M ME and 0.001 M DTT. Prior to the use, the ribosome concentration was adjusted to 480 A_{260} units. The activity of the sedimented ribosomes was assayed either by determining their GTP hydrolysis or by measuring their ability to complement the added T factor in the poly U-directed polyphenylalanine synthesis as already described (7), except that the addition of G factor was omitted. One unit of G as ribosome-dependent GTPase was the activity liberating 1 μmole of P_i in 10 min at 30°.

30S and 50S ribosomal particles were purified by sucrose gradient centrifugation (14). When they were assayed for their functional ability in poly U-directed phenylalanine polymerization, the 30S particles were found to be free from 50S particles, while the 50S particles were slightly contaminated (2-5%) with 30S particles.

γ-^{32}P-GTP was synthesized from GDP and $^{32}P_i$ by spinach chloroplasts (15) and had a spec. act. of 0.1-2.0 mc/μmole. Poly U, from Boehringer, was dialyzed against 0.001 M EDTA and, thereafter, thoroughly against H_2O. GTP, GDP, GMP and ATP were from

Boehringer, ^{14}C-GTP from Schwarz BioResearch. 5'-guanylylmethylenediphosphonate (GOPOPCP) was purchased from Miles Labs and sucrose, ribonuclease-free, from Serva.

RESULTS AND DISCUSSION

When 5 X NH_4Cl-washed ribosomes, G factor and a guanine nucleotide (GTP, GOPOPCP or GDP) were incubated and the ribosomes sedimented through a sucrose gradient, they showed a significant GTPase activity and catalyzed poly U-directed polyphenylalanine synthesis in the presence of only the T factor without further addition of G. These results indicate a binding of G to ribosomes. As shown in Fig. 1, the GTP analogue GOPOPCP was the most effective of the nucleotides tested in promoting the binding of G to ribosomes. GTP and GDP were less effective, while GMP and ATP were essentially inactive. The time course of the reaction showed that at zero time some enzyme was already

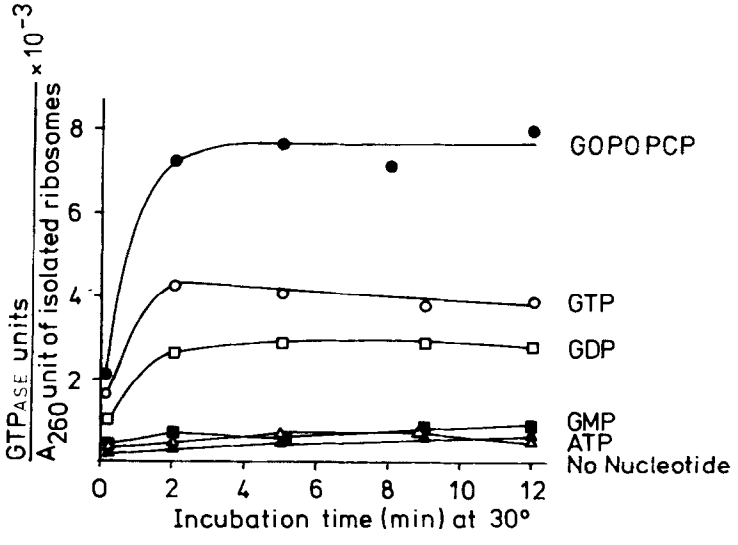

Figure 1. Effect of various nucleotides on the binding of G to ribosomes. The ribosomes were incubated with nucleotide (2 x 10^{-4} M) and G as described in methods. The GTPase activity of the ribosomes sedimented through a sucrose gradient was then tested.

bound, and the entire reaction was completed within 2 min at 30°.

As shown in Fig. 2, the presence of poly U enhanced the formation of the complex. The amount of G bound to ribosomes was increased of about 10-50%. This result is similar to the observation that poly U stimulates the ribosome-linked GTPase activity of G (16,17). In the absence of GOPOPCP or GTP, the synthetic messenger was essentially uneffective.

When 30S and 50S ribosomal particles - which had been incubated with G, poly U and GOPOPCP- were sedimented through a su-

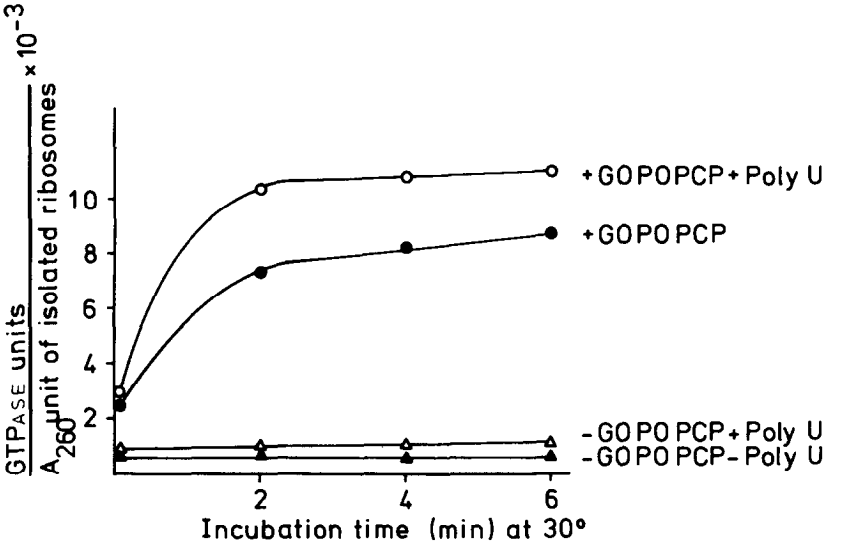

Figure 2. Effect of GOPOPCP and poly U on the binding of G to ribosomes. The ribosomes, incubated with GOPOPCP (2×10^{-4} M), poly U (30 μg/ml) and G as indicated, were isolated through a sucrose gradient and their GTPase activity was tested. Similar results were obtained when GTP was used.

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crose gradient, they did not show any significant GTPase activity when tested in the presence of the complementing ribosomal subunits which had not been previously incubated (Table I). These results suggest that the 70S particle is needed for G to bind to ribosomes.

The concomitant hydrolysis of GTP might explain the minor effectiveness of GTP - compared to the non hydrolyzable analogue GOPOPCP - in promoting the binding of G to ribosomes. In fact, as shown in Table II, the presence of GDP inhibits the effect of GOPOPCP on the binding of G to ribosomes.

If ^{14}C-GOPOPCP (synthesized by Dr. F. Eckstein) or ^{14}C-GTP were used, some radioactivity was found to be associated to the ribosomal pellet (18). These results suggest that the nucleotide may be part of the G-ribosome complex.

For the same nucleotide, variations in the amount of the bound G seem to depend on the quality of the NH_4Cl-washed ribosome preparation.

Using a molecular weight for G of 84,800, as determined by low-speed equilibrium centrifugation (19), it was calculated that 100 μμmoles of ribosomes can bind, in the presence of GOPOPCP, from 10 to 25 μμmoles of the enzyme, while in the presence of GTP from 2 to 10 μμmoles enzyme were bound. The K_m of GOPOPCP for this reaction was found to be about 4.3×10^{-5}M.

The effectiveness of GOPOPCP in promoting the formation of the G-ribosome complex indicates that concomitant hydrolysis is not required for this reaction. This behaviour is a further indication of the different roles played by GTP in the polypeptide synthesis.

Gordon(20), Lucas-Lenard and Haenni (6) and Ravel et al. (21) observed the formation of a GTP-T-phe-tRNA complex which can readily interact with ribosomes. It will be of interest to study a possible interaction between this complex and the observed G-ribosome complex.

In conclusion, similarly to the observations of Moldave and co-workers (10,11) for the rat liver system, a stable complex is

Table I

EFFECT OF GOPOPCP ON THE BINDING OF G TO RIBOSOMAL SUBUNITS

	$\frac{\text{GTPase units} \times 10^{-3}}{A_{260} \text{ unit ribosomes}}$
70S (incubated)	10.3
30S (incubated) + 50S (not incubated)	0.3
30S (not incubated) + 50S (incubated)	0.8
30S (incubated) + 50S (incubated)	0.9
*30S (incubated) + 50S (incubated) + G	12.6
*30S (not incubated) + 50S (not incubated) + G	11.8

30S and 50S subunits - obtained from 5 x NH_4Cl-washed ribosomes - and 70S particles were incubated with G, GOPOPCP (2×10^{-4} M) and poly U (30 μg/ml) for 6 min at 30° as described in methods. The GTPase activity of the 30S and 50S ribosomal subunits was assayed by adding, respectively, 50S and 30S subunits which had not been incubated with nucleotide and poly U. The A_{260} relationship of the added 30S and 50S particles was 2 to 3.

*As control, 0.5 μg from the same G batch per A_{260} unit of 30S + 50S ribosomal particles were added in the assay.

Table II

GDP INHIBITION OF THE GOPOPCP EFFECT ON THE BINDING OF G TO RIBOSOMES

	$\frac{\text{GTPase units} \times 10^{-3}}{A_{260} \text{ unit ribosomes}}$
COMPLETE + GOPOPCP (0.2 mM)	8.8
COMPLETE + GDP (0.2 mM)	2.8
COMPLETE	0.6
COMPLETE + GOPOPCP (0.2 mM) + GDP (0.2 mM)	6.4
COMPLETE + GOPOPCP (0.2 mM) + GDP (0.5 mM)	4.7
COMPLETE + GOPOPCP (0.2 mM) + GDP (1.0 mM)	3.1

The ribosomes, incubated with G, poly U (30 μg/ml) and nucleotides, were sedimented through a sucrose gradient and tested for their GTPase activity as described in methods.

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formed by G and ribosomes in the presence of either GTP, GOPOPCP or GDP. The formation of this complex takes place only in the

presence of both ribosomal subunits and does not require the hydrolysis of GTP. The role of this complex in the translocation process of the polypeptide synthesis is under investigation.

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