GTP INTERACTION WITH A PROTEIN SYNTHESIS INITIATION FACTOR PREPARATION FROM ESCHERICHIA COLI

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Received June 5, 1967

Although ten years have elapsed since the discovery of GTP as a requirement for protein synthesis (5) the exact role of this nucleotide remains unclear. Schweet and his collaborators (1), working with a rabbit reticulocyte system first reported a requirement for GTP and an enzymatic factor in the binding of aminoacyl-sRNA to ribosomes in the presence of messenger RNA. Recently a similar requirement for GTP and initiation factors has been observed in an Escherichia coli system although a clear consensus on the involvement of GTP has not been achieved (4,7,9,10). The binding reaction, because of its relative simplicity as compared with protein or polypeptide synthesis, may be the preferred tool to study the function of GTP in protein synthesis.

In the present communication, we wish to report a definite requirement for both GTP and initiation factors in N-formylmethionyl-sRNA (F-Met-sRNA) binding to ribosomes directed by ApUpG (AUG), and an interaction between a protein present in the initiation factor preparation and GTP.

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METHODS

Ribosomes from *E. coli* B or *E. coli* MRE-600 (a low nuclease strain originally obtained from Dr. Wade) were isolated and washed by a procedure similar to that described by Hershey and Thach (4). The ribosomal particles were stored at 4°. The unfractionated preparation of the crude initiation factors was obtained by ammonium sulfate precipitation (35-65% saturation) of the first ammonium chloride wash of the ribosomes as described by Stanley *et al* (12). This fraction was dialyzed against a buffer containing 0.01 M Tris-HCl pH 7.7, 0.01 M Mg acetate and 0.06 M KCl and stored in liquid nitrogen. Separation of factors F_1 and F_2 by DEAE-cellulose chromatography was accomplished by the procedure of Stanley *et al* (12).

F-Met-sRNA, labelled in the methionine moiety (^{14}C-198 μc/μmole) or in the formyl group (^{14}C-50 μc/μmole) was prepared using a purified *E. coli* transformylase as described previously (3).

F-Met-sRNA binding to ribosomes in the presence of the triplet AUG (kindly supplied by Dr. M. Nirenberg) was measured by the method of Nirenberg and Leder (8), except that the mixture contained 5 mM Mg acetate and the incubation was carried out at room temperature for 4 minutes.

The formation of a complex between the initiation factor preparation and GTP was measured by incubating the factor preparation (10 μg-150 μg of protein) with ^{3}H-GTP 15 μM (1 mc/μmole), 50 mM Tris pH 7.4, 10 mM Mg acetate, and 50 mM NH_4 acetate in an incubation volume of 0.2 ml at 0° for 5 minutes. The incubations were terminated by the addition of 3 ml of cold 0.01 M Tris pH 7.4 and 0.01 M Mg acetate buffer and passed through a millipore filter (HA 0.45 μ pore size). The filter was

thoroughly washed with the same buffer, dried and counted in a Packard scintillation spectrometer.

RESULTS

Requirements for binding of F-Met-sRNA to ribosomes. Table 1 demonstrates that under the conditions employed, the binding of F-Met-sRNA to ribosomes has an almost absolute requirement for GTP and initiation factors. The two initiation factors are complementary in nature, but F_2 has partial activity alone.

Table 1

Requirements for Binding of F-Met-sRNA to Ribosomes

Experiment		μμmoles bound
1	Complete system	4.65
	- AUG	0.22
	- Initiation factors	0.80
	- GTP	0.20
	- Ribosomes	0.06
2	Complete system	8.02
	- F_1	3.03
	- F_2	0.90
	- F_1 - F_2	0.71

The binding reaction (Expt. 1) contained in a 50 μl incubation: 5 mM Mg acetate, 50 mM Tris-acetate pH 7.4, 50 mM K acetate, 1 mM GTP; washed ribosomes (2 A_{260}), AUG (0.18 A_{260}), unfractionated F-^{14}C-Met-sRNA (9.9 μμmoles, 0.73 A_{260}), and initiation factors (160 μg of protein). In Expt. 2, F_1 (4.6 μg of protein) and F_2 (26 μg of protein) separated by DEAE-cellulose chromatography were used in place of the unfractionated initiator factor preparation. The incubation was at 24° for 4 minutes.

Formation of a complex between guanosine triphosphate and the initiation factor preparation. The requirement for GTP in the binding reaction suggested that a clue to its role might be found by testing

the capacity of radioactive GTP to label the ribosome-triplet-amino-acyl-sRNA complex which was retained on a millipore membrane. The results obtained were surprising. As shown in Table 2, a considerable amount of the radioactivity supplied as ^{3}H-GTP in the F-Met-sRNA

Table 2

Interaction of ^{3}H-GTP with the Components of the Binding Mixture

System	μμmoles ^{3}H-GTP bound
Complete system	300
- ribosomes	244
- AUG	255
- F-Met-sRNA	370
- Initiation factors	13

The conditions for the binding reaction were similar to those described in Table 1, except that the incubations were in 200 μl, the initiation factors contained 180 μg of protein, the F-Met-sRNA (50 μμmoles) was labelled in the formyl group (50 μc/μmole), and ^{3}H-GTP (1 mc/μmole) was used at a concentration of 50 μM.

binding incubation was retained on the filter membrane, but the phenomenon only required the presence of the initiation factors. In other experiments it was shown that the binding of ^{3}H-GTP was proportional to the concentration of initiation factor. The component in the initiation factor preparation which combined with GTP was inactivated by heating at 100° for 2 minutes at neutral pH suggesting that the active fraction was a protein. The reaction with GTP occurred very rapidly even at 0°. Specificity studies with other nucleoside triphosphates and other guanosine derivatives have shown that seven times more binding was obtained with GTP as compared with ATP, CTP, UTP, and GMP. ATP and GMP at concentrations 10 and 50 times larger, respectively, did not inhibit the reaction with GTP. GDP, however, was able to bind to the protein as well as GTP, and was a potent inhibitor

of the GTP reaction. Separation of the GTP complex could be obtained by passing the reaction mixture through a column of Sephadex G-50. A radioactive fraction travelling with the protein, clearly separated from free GTP. This protein-bound radioactivity could be removed by heating at 100° for 2 minutes or treatment with 5% TCA or NH_4OH pH 11, but was unaffected by pancreatic RNase, T_1 RNase, and DNase. Addition of deacylated sRNA also caused breakdown of the GTP complex. In an attempt to see whether the factor that complexed with GTP might also be involved in the binding of F-Met-sRNA to ribosomes the initiation factor preparation was further purified by DEAE-cellulose chromatography as shown in Fig. 1. Assay of GTP binding capacity shows that it appears as a discreet peak which coincides with initiation factor 2 (F_2) activity as assayed by the latter's capacity to stimulate F-Met-sRNA binding to ribosomes in the presence of F_1.

Under the conditions employed in the present study it has been shown that 1) GTP is required for the binding of F-Met-sRNA to ribosomes and 2) GTP is specifically bound to a heat labile component in the initiator factor preparation. Several facts suggest that the two observations are related. The GTP binding fraction is eluted together with F_2 (a factor required for F-Met-sRNA binding to ribosomes and for protein synthesis with natural messengers) on DEAE-cellulose chromatography. The only other nucleotide, of those tested, which interacts with the protein to a degree similar to GTP is GDP. Conway and Lipmann (2) reported strong inhibition of polypeptide synthesis by GDP. In addition, the GTP complex was found to be labilized in the presence of deacylated sRNA. Deacylated sRNA also has been reported to inhibit the binding of aminoacyl-sRNA to ribosomes, and this inhibition is

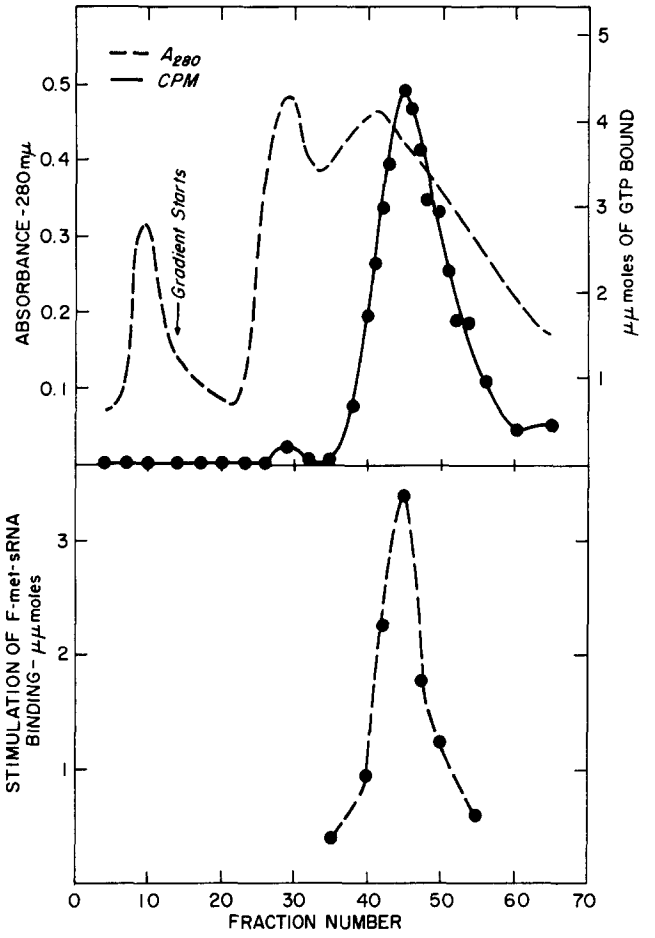

Fig. 1. DEAE-Cellulose chromatography of the initiation factors. Approximately 45 mg of crude initiation factor protein was applied to a DEAE-cellulose column (2.2 x 10 cm) that had been equilibrated with 0.01 M potassium phosphate buffer pH 7.5 and 0.01 M β-mercaptoethanol. The column was washed with 40 ml of the same buffer containing 0.025 M NH_4Cl and eluted with a linear gradient from 0.1 M to 0.3 M NH_4Cl in the above buffer (100 ml in each chamber). Fractions of 3 ml were collected and GTP binding activity was assayed with 20 μl aliquots as described in Methods. The effect of 10 μl aliquots on the binding of F-Met-sRNA to ribosomes was assayed as described in Table 1, except that F_1 (10 μg) was included in the mix. The values obtained with F_1 alone (2.3 μμmoles) were subtracted from those shown in the figure.

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reversed by GTP (6,11). In this regard it may be that the degree of GTP dependency for the binding of F-Met-sRNA to ribosomes is related

to the amount of deacylated sRNA present. More direct evidence, as well as further purification and characterization of the components involved in the GTP-protein interaction are required before its role in protein synthesis is established.

ACKNOWLEDGMENTS

The authors express their thanks to Mrs. B. Redfield and Mrs. A. Wortsman for their technical assistance and Dr. M. Nirenberg for his advice and support.

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