# THE FORMATION OF A COMPLEX CONTAINING RIBOSOMES, TRANSFER FACTOR G AND A GUANOSINE NUCLEOTIDE

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Summary

Several guanosine nucleotides stimulate the binding of transfer factor G to E. coli ribosomes. A binding of these nucleotides to the ribosome-factor G complex has also been demonstrated.

Recent studies have focused on the role of GTP in relation to the mechanism of action of the transfer factors Ts, Tu and G** from E. coli (1) which are required for amino acid polymerization (2-13). Evidence has been presented that factor Ts catalyzes the formation of a Tu-GTP complex which is involved in the transfer of aminoacyl-tRNA to the ribosome (5). Transfer factor G (which catalyzes a ribosomal dependent hydrolysis of GTP) requires GTP for the movement or translocation of the growing polypeptide chain from the aminoacyl site on the ribosome to the peptidyl site (10-13). It is in the latter reaction that GTP is believed to be hydrolyzed (14). Recent studies by Skogerson and Moldave (15) have shown that the binding of rat liver aminoacyl transferase II (which appears to catalyze a similar reaction to E. coli transfer factor G) to ribosomes was dependent upon the presence of GTP, although GDP, and the GTP analogue GDPCP could partially be substituted for GTP.

The present report describes results of experiments with E. coli factor G which show that the binding of this protein to ribosomes is stimulated by GTP

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**Abbreviations - Ts, Tu, G, soluble E. coli transfer factors referred to by Lucas-Lenard and Lipmann (1); GDPCP, 5'-guanylyl methylenediphosphonate; DTT, dithiothreitol.

and certain other guanosine nucleotides. In addition, a factor G dependent binding of guanosine nucleotides to ribosomes has been observed.

## Experimental

E. coli cells were obtained from the Grain Processing Corp. Ammonium chloride washed ribosomes (free of transfer factors) and purified factor G were prepared as previously described (4) except that the factor G preparation was rechromatographed on a Sephadex G-200 column. The purified factor G preparations, when initially prepared, catalyzed the ribosomal dependent hydrolysis of between 8.5-11.5 μmoles of GTP per mg of protein in 10 min as assayed by the procedure described by Conway and Lipmann (16).

Sephadex Chromatography Experiments - The incubation mixture contained in a final volume of 0.35 ml; 92 μg of factor G, 25-30 $A_{260}$ ribosomes, 200 μμmoles of the radioactive nucleotide, 0.01 M Tris-Cl buffer pH 7.0, .01 M magnesium acetate; 0.01 M ammonium acetate; and 0.001 M DTT (buffer A). The incubation was carried out for 5 min at 0° and the mixture was chromatographed on a Sephadex G-25 column (1.5 x 22.5 cm) which had been equilibrated with buffer A. One ml samples were collected and assayed for radioactivity in a Beckman LS-100 Liquid Scintillation Spectrometer using the counting fluid described by Bray (17).

Centrifugation Studies - Each incubation was performed in a 13 ml nalgene screw cap centrifuge tube and contained in 0.35 mlof buffer A; 58 μg of factor G, 17 $A_{260}$ ribosomes and 3 mμmoles of radioactive nucleotide. After incubating for 5 min at 0°, the tubes were centrifuged at 160,000 x g in a Beckman LS-65 ultracentrifuge. The supernatant fluid was siphoned, and the ribosomal pellet was rinsed with two 0.5 ml aliquots of buffer A. The ribosomal pellet was then suspended in 0.3 ml of a buffer containing 0.06 M Tris-HCl pH 7.4; 0.012 M $NH_4Cl$; and 0.012 M DTT. Aliquots were removed from each sample for the determination of $A_{260}$ (recovery of ribosomes), radioactivity (nucleotide bound) and factor G activity. The amount of factor G present in the ribosomal pellet was assayed by its ability to hydrolyze GTP (16). Since the recovery of $A_{260}$

in the ribosomal pellet was not always uniform, the aliquots that were removed for the assay of factor G were adjusted so that the amount of ribosomes in the assay were saturating (1.9-2.1 $A_{260}$), and the activity was expressed as mμmoles GTP hydrolyzed per $A_{260}$ of ribosomes in 10 min at 37°.

GTP-γ-$P^{32}$ and GPT-β-γ-$P^{32}$ were purchased from International Chemical and Nuclear Corp., while the other radioactive nucleotides were obtained from Schwarz BioResearch Inc. Unlabeled GDPCP was purchased from Miles Corp.

## Results and Discussion

Figure 1 shows the results of experiments in which $^3$H-GTP was incubated at 0° for 5 min with ribosomes and factor G and then chromatographed on Sephadex G-25. The formation of a complex of GTP with a high molecular weight substance (tubes 15 to 18) was dependent upon the presence of both ribosomes and factor G. Factor G or ribosomes alone did not react with $^3$H-GTP to a significant extent. In other experiments GTP-γ-$P^{32}$ and GTP-β-γ-$P^{32}$ were used to determine whether hydrolysis of GTP occurred during the binding reaction.

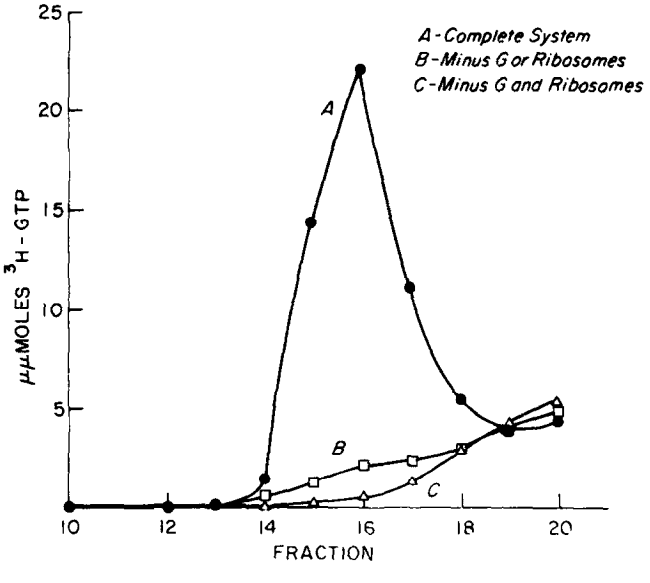


Fig. 1. The formation of a $^3$H-GTP high molecular weight complex by Sephadex G-25. Conditions and experimental details are described in the text.

Under conditions where 35.2 μμmole of $^{3}$H-GTP were found to be associated with a high molecular weight fraction, no appreciable GTP-$\gamma$-$P^{32}$ was bound; however, 30.5 μμmoles of GTP-$\beta$-$\gamma$-$P^{32}$ were detected. These results indicated that the nucleotide contained in the complex isolated by Sephadex chromatography under the conditions used was probably GDP and not GTP.

The ability of other nucleotides to form a high molecular weight complex is compared in Table I. GDP was as effective as GTP, while GMP, ATP, CTP, and UTP all showed very low activity.

Table I. The Binding of Nucleotides to a High Molecular Weight Fraction in the Presence of Factor G and Ribosomes.

| Nucleotide | Sp. Act. C/mmole | μμmoles bound |
|---|---|---|
| $^{3}$H-GTP | 1.1 | 36.1 |
| $^{14}$C-GTP | 0.1 | 35.5 |
| $^{14}$C-GDP | 0.1 | 40.0 |
| $^{3}$H-GMP | 1.0 | 6.3 |
| $^{3}$H-ATP | 3.8 | 4.2 |
| $^{3}$H-UTP | 2.0 | 4.1 |
| $^{3}$H-CTP | 1.0 | 4.0 |

The conditions for Sephadex chromatography are described in the text. The total μμmoles in fractions 15 to 18 (see Fig. 1) were determined with each nucleotide.

In an attempt to determine whether the nucleotide bound was associated with the ribosomes and also if this binding were accompanied by a binding of factor G to the ribosomes, the ribosomes were isolated from the reaction mixture by centrifugation as described above. These results, shown in Table II, extend and corroborate the data obtained by Sephadex chromatography. Thus radioactive GTP, GDP and dGTP bind to the ribosomes to about the same extent. It is also seen in Table II that only those nucleotides which bind to the ribosomes stimulate the binding of transfer factor G to the ribosomes. An interesting observation was that GDPCP was 2.5 times as effective as GTP in stimulating the binding of transfer factor G to ribosomes.

Table II. The Binding of Transfer Factor G and Various Nucleotides to Ribosomes.

| Nucleotide | Nucleotide-bound μμmoles per $A_{260}$ ribosomes | Enzymatic activity* |
|---|---|---|
| None | - | 0.20 |
| GTP | 3.7 | 0.90 |
| GDP | 3.2 | 0.88 |
| GMP | 0.3 | 0.22 |
| dGTP | 2.9 | 0.68 |
| GDPCP** | - | 2.13 |
| ATP | 0.2 | 0.17 |
| UTP | 0.3 | 0.22 |
| CTP | 0.3 | 0.29 |

*Values are expressed as mμmoles GTP hydrolyzed per $A_{260}$ ribosomes per 10 min at 37°. Conditions and experimental details are described in the text.

**Labeled GDPCP was not available so the binding of this compound to ribosomes could not be determined.

The specific activity of the enzyme at the time of these experiments had deteriorated to 1.1 μmoles GTP hydrolyzed per mg protein per 10 min at 37°.

The present results suggest another possible role for GTP in protein synthesis. Thus a sequence of events might be envisaged in which there is an initial GTP dependent binding of transfer factor G to the ribosomes. This binding apparently does not require hydrolysis of GTP since GDPCP is even more effective than GTP, although under the conditions employed hydrolysis of GTP did occur in these experiments. In addition to factor G being bound to the ribosomes the nucleotide is also bound, perhaps in a ternary complex. After binding to the ribosomes factor G could catalyze the translocation of peptidyl-tRNA from the aminoacyl site to the peptidyl site on the ribosome.

The results and conclusions presented above on the binding of factor G to ribosomes are very similar to those reported by Skogerson and Moldave using a rat liver system (15).

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