INTERACTIONS BETWEEN THE ELONGATION FACTORS: THE DISPLACEMENT OF GDP FROM THE TU-GDP COMPLEX BY FACTOR Ts

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SUMMARY: Increasing the amount of Ts in a reaction mixture containing Tu, Ts, and GDP causes a proportionate decrease in the amount of GDP bound as Tu-GDP when measured by gel filtration. It appears that Ts displaces an equivalent amount of GDP from Tu-GDP, forming a Ts-Tu complex. This observation provides a means to remove GDP from Tu, and suggests a role for Ts in protein biosynthesis.

In bacterial systems the binding of aminoacyl-tRNA (AA-tRNA) to ribosomes requires GTP and two protein factors, denoted Ts[1] and Tu (2-5). Apparently a Tu-GTP complex is initially formed (5-9) which can interact with AA-tRNA to form a ternary complex, AA-tRNA-Tu-GTP (4-8). When this complex interacts with ribosomes in the presence of messenger, AA-tRNA is transferred to the ribosome, GTP is hydrolyzed, and Tu-GDP is released[2] (5,10,11).

Since Tu-GDP does not react with AA-tRNA (4,7,14), the regeneration of Tu-GTP is required for Tu to function catalytically in AA-tRNA transfer. However, GTP does not readily exchange with Tu-GDP, nor does Tu-GDP rapidly dissociate (15,16). It now appears that factor Ts is involved in the regeneration of Tu-GTP. A recent study has shown that Ts stimulates the exchange of GDP bound to Tu with unbound GTP (16). The present report demonstrates that Ts can displace GDP from Tu.

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[1] Ts and Tu, soluble transfer factors originally separated and named by Lucas-Lenard and Lipmann (1). It should be noted that other symbols have been used by various workers to denote these factors (see references 4 and 5).

[2] For recent reviews of this subject see references 12 and 13.

MATERIALS AND METHODS: The techniques for assaying Tu and Ts activities by GDP binding have been described previously (9). The unit of Ts activity is based on the rate of exchange of ^{3}H-GDP with Tu-GDP measured by the nitrocellulose filter assay (9). One unit is that quantity of Ts which catalyzes the exchange of 1 pmole of ^{3}H-GDP with Tu-GDP in 5 min at 0°. The reaction mixtures contained 35 units of Tu-GDP, 500 pmoles of ^{3}H-GDP, 0.01 M $MgCl_2$, 0.05 M Tris-HCl, pH 7.4, 0.10 M NH_4Cl, and 0.005 M dithiothreitol in a total volume of 0.2 ml. A unit of Tu is that quantity of protein which binds 1 pmole of ^{3}H-GDP. The Tu and Ts factors were prepared by procedures similar to those reported elsewhere (9,17,18). The Tu preparation was estimated to be 95% pure by gel electrophoresis, and it bound 20 mμmoles of ^{3}H-GDP per mg of protein. The Ts preparation, which was generously supplied by Dr. John Hachmann, had been purified through Sephadex G-100 chromatography (9) and was estimated to be 50% pure (specific activity of 2.5×10^5 units per mg).

The Tu-Ts complex was prepared from Tu and Ts as described previously and purified by Sephadex G-100 chromatography (19). This procedure separates the Tu-Ts complex from most of the impurities originally present in the Ts preparation because the Ts-Tu complex emerges ahead of the fractions in which the Ts previously had been collected. Disc gel electrophoresis of the Tu-Ts complex showed one major band containing 90% of the protein (determined by photoelectric scanning of the stained gel). This preparation, thought to be composed of equimolar amounts of Ts and Tu, was found to contain 30-35 units of Ts per unit of Tu.

Gel filtration chromatography was performed on a 26 x 1.5 cm column of Sephadex G-25 equilibrated with a buffer consisting of 0.05 M Tris-HCl, pH 8.0; 10^{-3} M $MgCl_2$, and 10^{-3} M dithriothreitol. Reaction mixtures (0.1 ml) containing the preceding buffer constituents, 1000 units of Tu (50 μg protein), 5000 pmoles ^{3}H-GDP, (700 cpm/pmole), and varying amounts of Ts were incubated for 10 min at 23° prior to chromatography. The reaction mixture was applied to the column and was eluted at a rate of 0.7 ml/min

using the equilibration buffer. Fractions of 0.57 ml were collected, and aliquots of the effluent were assayed for total ^{3}H-GDP, ^{3}H-GDP bound to Tu, and total Tu. Tu-^{3}H-GDP (measured by the filter assay) was generally found to be 5-10% lower than the total ^{3}H-GDP in the fractions emerging with Tu. Dissociation of Tu-^{3}H-GDP and the incomplete retention of the Tu-^{3}H-GDP complex on the nitrocellulose filter may account for this small discrepancy.

RESULTS AND DISCUSSION: Fig. 1 shows the effect of increasing amounts of Ts on the quantity of ^{3}H-GDP eluted as Tu-^{3}H-GDP. Part A shows that in the presence of a small amount of Ts (500 units), a Tu-^{3}H-GDP complex containing over 90% of the Tu complexed to GDP can be eluted from the column. The ^{3}H-GDP emerging in the effluent with Tu is bound to Tu since 90% of the radioactivity is retained on a nitrocellulose filter. However, the addition of larger amounts of Ts to the reaction mixture (parts B and C) decreases the amount of Tu-^{3}H-GDP found in the effluent.

Adding a large amount of Ts does not lower the amount of Tu-GDP formed in the reaction mixture as assayed by the nitrocellulose filter procedure. From the data in Table I, one can compare the effect of Ts on the amounts of Tu-^{3}H-GDP found in the reaction mixture by the filter assay before gel chromatography with those observed in the effluent from the column. Even with an amount of Ts which removes 90% of the ^{3}H-GDP by the Sephadex procedure, there is little effect on the amount of Tu-^{3}H-GDP found in the reaction mixture as assayed by the filter method. This difference is presumed to be a manifestation of the higher affinity of Tu for GDP than for Ts at the concentrations employed in the reaction mixture. However, when the mixture is chromatographed on Sephadex G-25, the excess GDP separates from the proteins, and Ts, traveling with Tu, displaces some of the GDP bound to Tu. The GDP which is released from Tu also separates from the protein on the column.

The Tu-^{3}H-GDP in the column effluent was stable. The amount of Tu-^{3}H-GDP in the effluent from the column represented in Part B, Fig. 1 did not

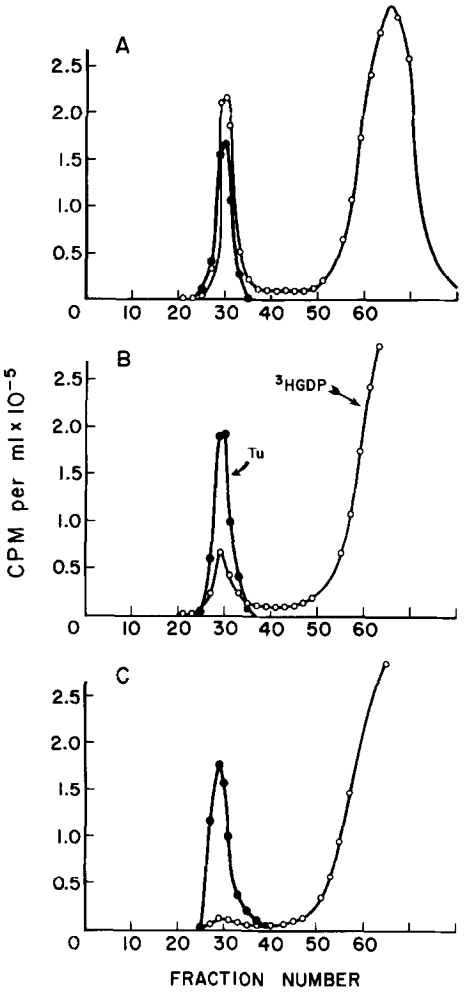

FIG. 1: Displacement of GDP from Tu-^{3}H-GDP by Ts as shown by gel filtration. Tu (1000 units) ^{3}H-GDP (5000 p moles) and three different amounts of Ts were mixed and applied to a Sephadex G-25 column and eluted as described in the text.

A: 500 units Ts; B. 14,000 units Ts; C: 25,000 units Ts.

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decrease appreciably during a period of three days after chromatography. Furthermore, the ratio Tu-^{3}H-GDP/Tu_{total} in the effluent was little affected by rechromatography on the Sephadex G-25 column. When a portion of the effluent containing a ratio Tu-^{3}H-GDP/Tu_{total} of 0.58 was rechromatographed on Sephadex G-25, the ratio of the species recovered in the effluent was 0.50

The present study could explain why other workers employing a "T" fraction (containing Ts and Tu), have been unable to demonstrate the formation of a Tu-GDP complex by Sephadex Chromatography (7,15,20). The "T" fraction presumably contains one equivalent of Ts bound to Tu, and the Ts effectively competes with GDP for Tu during the chromatographic separation. To verify this, a Ts-Tu complex was prepared by Sephadex G-100 chromatography as described previously (19). When this complex was incubated in the presence of ^{3}H-GDP, a Tu-^{3}H-GDP complex could be demonstrated by the nitrocellulose filter assay, but only a small amount of Tu-^{3}H-GDP could be isolated by Sephadex G-25 chromatography (Table I, Exp. D).

From the result that one unit of Tu forms a complex with 30-35 units of Ts (see Methods), it can be calculated that the fractions of Tu that could be complexed by Ts in Fig. 1, parts A, B, and C, are 0.02, 0.5, and 0.9, respectively. These are roughly the ratios of $(Tu_{total}\text{-}Tu\text{-}^{3}H\text{-}GDP)/Tu_{total}$ found

TABLE I

COMPARISON OF TU-GDP FORMATION BY THE FILTER METHOD AND SEPHADEX G-25 CHROMATOGRAPHY

Exp.	Amounts of Ts and Tu added		Tu-^{3}H-GDP formed (pmoles)	
	Tu (pmoles)	Ts (units)	Filter Assay	Column Effluent
A	1000	500	1050	880
B	1000	14,500	900	450
C	1000	25,000	800	100
D	1500	40,000	1200	120

Mixtures of Tu, ^{3}H-GDP, and Ts were assayed for Tu-^{3}H-GDP by the nitrocellulose filter method (9) and by chromatography on Sephadex G-25. In Exp. D a Ts-Tu complex was prepared as described previously (19) and incubated with ^{3}H-GDP as described in the text.

in the effluents. Thus, the amount of GDP displaced equals the amount of Ts added.

The preceding experiments indicate that Ts can displace GDP from Tu-GDP to form a Tu-Ts complex. Previous studies demonstrated the formation of a Tu-Ts complex and suggested that GDP can dissociate a Tu-Ts complex when the complex is chromatographed on a Sephadex G-100 column with GDP present in the eluting fluid (19). Thus, reaction 1 (below) can be made to proceed in either direction. The Ts catalysis of the exchange between free GDP and Tu-GDP can be viewed as the rapid equilibration of reaction 1.

$$\text{Ts} + \text{Tu-GDP} \rightleftharpoons \text{Ts-Tu} + \text{GDP} \quad (1)$$

$$\text{Tu-Ts} + \text{GTP} \rightleftharpoons \text{Tu-GTP} + \text{Ts} \quad (2)$$

$$\text{Tu-GTP} + \text{AA-tRNA} \rightleftharpoons \text{AA-tRNA-Tu-GTP} \quad (3)$$

$$\text{AA-tRNA-Tu-GTP} \xrightarrow[\text{messenger}]{\text{ribosome}} (\text{AA-tRNA-messenger-ribosome}) + \text{Tu-GDP} + \text{Pi} \quad (4)$$

We have recently demonstrated (16) that Ts stimulates the exchange of GTP with GDP bound to Tu (reactions 1 and 2) and the formation of the AA-tRNA-Tu-GTP complex when AA-tRNA and GTP are incubated with Tu-GDP (reactions 1-3). However, Ts did not stimulate the formation of the AA-tRNA-Tu-GTP complex starting with Tu-GTP (reaction 3), suggesting that Ts is only required when Tu-GDP is present. Since the transfer of AA-tRNA from the AA-tRNA-Tu-GTP complex to ribosome (reaction 4) yields Tu-GDP and Pi (5,10,11), the Tu-GDP formed in reaction 4 must be converted to Tu-GTP in order to function catalytically in AA-tRNA binding. The results presented in this communication suggest that the role of Ts in protein synthesis may be to provide a means to remove GDP from Tu-GDP by forming a Tu-Ts complex.

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