INTERACTION OF BRAIN TRANSFERASE I WITH GUANOSINE NUCLEOTIDES AND AMINOACYL-tRNA

H.M. Moon and Herbert Weissbach
Roche Institute of Molecular Biology, Nutley, N.J. 07110

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SUMMARY: Brain transferase I has been purified, and its interactions with GTP and aminoacyl-tRNA have been studied. The data suggest that a transferase I-GTP complex can be formed which interacts with aminoacyl-tRNA to yield an aminoacyl-tRNA-transferase I-GTP complex.

Studies with the bacterial transfer factor Tu have shown that this protein is required for aminoacyl-tRNA (AA-tRNA) binding to ribosomes (1-6). The sequence of reactions that appears to be involved in this process is shown below:

$$\text{Tu-GTP} + \text{AA-tRNA} \longrightarrow \text{AA-tRNA-Tu-GTP} \quad (1)$$

$$\text{AA-tRNA-Tu-GTP} \xrightarrow[\text{ribosomes}]{\text{mRNA}} \text{AA-tRNA-mRNA-Rib} + \text{Tu-GDP} + \text{Pi} \quad (2)$$

Reaction 1 shows the formation of the AA-tRNA-Tu-GTP complex which reacts with the ribosome-messenger complex (Reaction 2). In the latter reaction the AA-tRNA is bound to the ribosome, and Tu-GDP and inorganic phosphate are formed. Transfer factor Ts appears to function in the regeneration of Tu-GTP from Tu-GDP as shown in Reactions 3 and 4 (7-10), although Tu can also react directly with GTP (7) to form Tu-GTP (Reaction 5).

$$\text{Tu-GDP} + \text{Ts} \longrightarrow \text{Tu-Ts} + \text{GDP} \quad (3)$$

$$\text{Tu-Ts} + \text{GTP} \longrightarrow \text{Tu-GTP} + \text{Ts} \quad (4)$$

$$\text{Tu} + \text{GTP} \longrightarrow \text{Tu-GTP} \quad (5)$$

There is less information available on the role of the eukaryote factor, transferase I (T_1), in AA-tRNA binding to ribosomes. Partially purified T_1 preparations from wheat germ and yeast have been shown to react with GTP and AA-tRNA to form a ternary complex (11-13). However, with T_1 from animal tissues, which is required for AA-tRNA binding to ribosomes (14-17), very little information on

the partial reactions in this process has been obtained. Ibuki et al. (18) have shown that T_1 from liver is more heat labile in the presence of GTP, suggesting that a protein nucleotide interaction takes place. In addition, Rao and Moldave (19) have also presented data showing that liver T_1 interacts with AA-tRNA and tRNA, and Dawkins et al. (20) reported in an abstract that reticulocyte T_1 interacts with GTP and AA-tRNA. However, it is still not clear whether animal T_1 reacts with guanosine nucleotides and AA-tRNA similar to what has been seen with factor Tu. We have recently purified T_1 from brain and would like to present evidence that this protein can react with GTP to form a T_1-GTP complex which can react with AA-tRNA to form an AA-tRNA-T_1-GTP complex.

MATERIALS AND METHODS: Unless stated otherwise, all materials were obtained from commercial sources, and the details of the incubations and assay conditions are described in the appropriate legends.

RESULTS: Nucleotide Binding to T_1 - The presence of soluble factors in animal tissues that interact with GTP and other nucleotides to form complexes that are retained on nitrocellulose filters has been described previously (21). The large extent of unrelated binding of adenosine and guanosine nucleotides in partially purified preparations of T_1 masked any interaction of GTP and/or AA-tRNA with the soluble transfer factor. Only after the electrofocusing step (see Table I) was it possible to obtain a T_1 preparation that bound GTP or GDP to a greater extent than ATP or ADP. As seen in Table I, the T_1 preparation used for the studies in this report had a ratio of GTP/ATP bound of 2. Other results suggest that the GTP and ATP binding components are different. With one preparation of T_1 there was essentially no ATP binding, and the ratio of GTP/ATP bound was greater than 20. In addition, it was noted that the presence of 10 equivalents of unlabeled ATP to an incubation containing T_1 and ^{3}H-GTP did not decrease the amount of ^{3}H-GTP bound, although the same amount of unlabeled GDP or GTP reduced the amount of ^{3}H-GTP bound by greater than 80%. It should be noted that similar binding results were obtained when ^{3}H-GTP was replaced with γ-^{32}P-GTP, showing that GTP hydrolysis was not occurring during this reaction.

TABLE I

Nucleotide Binding to the Purified T_1 Preparation

Nucleotide added	pmoles Bound
^{3}H-GTP	7.3
^{3}H-GDP	6.3
^{3}H-ATP	3.5
^{3}H-ADP	3.2

T_1 was purified from calf brain by a procedure which will be described in detail elsewhere but which involved the following steps: Brains (from slaughterhouse) were homogenized in a buffer containing 0.05 M Tris, pH 7.4; 0.01 M $MgCl_2$, 0.025 M KCl, and 0.25 M sucrose, and the homogenate was centrifuged at 8000 x g. The supernatant was treated with $(NH_4)_2SO_4$, and the proteins that precipitated between 35-65% saturation were dialyzed against buffer A (0.05 M Tris, pH 7.4; 0.0001 M EDTA and 0.001 M DTT). T_1 was then adsorbed to calcium phosphate gel and eluted with buffer A containing 30% saturated $(NH_4)_2SO_4$. The eluate was dialyzed against buffer A, and T_1 was separated from T_2 by Sepharose 6 B gel filtration. The T_1-rich fractions were combined, and after dialysis against potassium phosphate buffer, pH 7.0 (0.01 M), the protein was chromatographed on hydroxylapatite using a potassium phosphate gradient from 0.01 M, pH 7.0 to 0.5 M, pH 7.0. The fractions containing T_1 were combined and concentrated by Diaflow membrane filtration. T_1 was then passed through a Sepharose 4 B column, and the protein in the fraction containing the T_1 activity was precipitated by the addition of ammonium sulfate to 0.7 saturation. T_1 was extracted from the precipitate with a solution of 50% saturated ammonium sulfate solution in buffer A. The extract was dialyzed against buffer A, and T_1 was further purified by isoelectric focusing using ampholites having a pH range between 5 and 7.

The results of two purifications showed that the T_1 preparations were purified 80 and 40 fold, respectively. The final preparations were able to polymerize between 7 and 14 pmoles of phenylalanine per μg of protein in 10 min at 37° in a standard polymerization system (22).

A nitrocellulose filter was used to assay for the interaction of T_1 with GTP and other nucleotides. The incubations contained in a total volume of 200 μl: 0.05 M Tris-Cl buffer, pH 7.4; 0.05 M NH_4Cl, 0.01 M $MgCl_2$, 3.75 x 10^{-3} M phosphoenolpyruvate (PEP), 10 μg pyruvate kinase (PK), 10 μg of T_1 protein (7 pmoles of T_1 based on GTP binding) and 2.5 x 10^{-6} M ^{3}H-GTP or ^{3}H-ATP. When the diphosphates were used in place of the triphosphates, the PEP and PK were omitted. The incubations were for 2 min at 37° and were terminated by the addition of 2 ml of a cold wash buffer containing 0.01 M Tris-Cl, pH 7.4; $MgCl_2$ and NH_4Cl. The diluted reaction mixture was filtered through a nitrocellulose filter (Millipore Corp. 25 mm, 0.45 μ), and the filter was washed with 15 ml of the wash buffer. The filter was dissolved in a scintillation fluid (23) and assayed for radioactivity.

Phosphoenolpyruvate (PEP) and pyruvate kinase (PK) were routinely added to the incubations containing the triphosphates, since this kinase system increased the extent of binding by about 20%. Fig. 1 shows the rate of ^{3}H-GTP binding and the

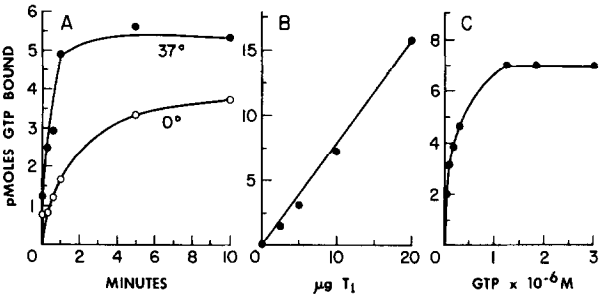

Fig. 1: GTP binding to T_1 as a function of time, T_1 concentration and GTP concentration. A. 10 μg of T_1 protein were used. B. A two minute incubation was used. C. 10 μg of T_1 protein and a 2 min incubation were used.

effect of T_1 and GTP concentrations on the extent of the reaction. The binding of GTP is essentially complete in 1 min at 37° and occurs at 0° at less than 1/2 the rate seen at 37°. The reaction is dependent on T_1 concentration (Fig. 1B), and levels of GTP above 1.2×10^{-6} M resulted in a maximal extent of binding (Fig. 1C).

Interaction of T_1 with AA-tRNA - Bacterial transfer factor Tu reacts with GTP to form a Tu-GTP complex which can interact with AA-tRNA to form an AA-tRNA-Tu-GTP complex (Reaction 1). Based on the analogous reaction seen with factor Tu, a T_1-GTP complex, if formed, should also interact with AA-tRNA. With the bacterial factor, the formation of the AA-tRNA-Tu-GTP complex is readily assayed by the loss of ^{3}H-GTP retained on a nitrocellulose filter, since the AA-tRNA-Tu-GTP complex, unlike Tu-GTP, is not retained on the filter. A similar filter assay was attempted and found to be suitable for the interaction of T_1-GTP with AA-tRNA. Table II shows the effect of 16.5 pmoles of tRNA on the T_1-dependent retention of ^{3}H-GTP on a nitrocellulose filter. In the presence of purified *E. coli* Phe-tRNA, there is a 65% decrease (7 pmoles to 2.6 pmoles) in the amount of ^{3}H-GTP retained on the filter. A similar amount of deacylated $tRNA^{Phe}$ did not

result in a significant loss of radioactivity retained on the filter. When ^{3}H-GTP was replaced by ^{3}H-GDP (Exp. 2), there was also no reaction with Phe-tRNA as measured by loss of filter-bound radioactivity. However, when the ^{3}H-GDP was incubated with T_1 in the presence of PEP and PK (to convert GDP ——> GTP), the subsequent addition of Phe-tRNA resulted in a large loss in the amount of radioactivity retained on the filter. The binding of ATP by this T_1 preparation was not affected by the presence of Phe-tRNA (Exp. 3). Fig. 2 shows the effect of Phe-tRNA concentration on the loss of ^{3}H-GTP bound to the filter in the presence of T_1. For these experiments 22 pmoles of T_1 (assumed value based on GTP binding) were used. In the presence of excess Phe-tRNA (55 pmoles) a 75% drop in ^{3}H-GTP retained by the filter was obtained. Limiting levels of Phe-tRNA (5.5 pmoles) caused a loss in the filter bound radioactivity of about an equivalent

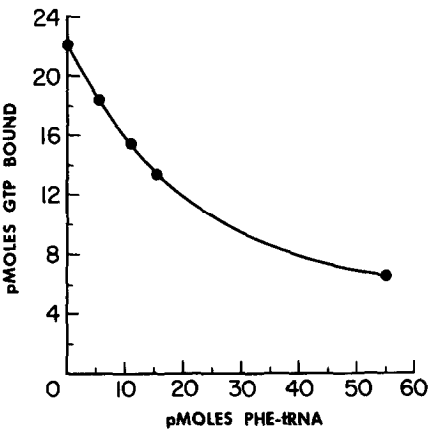

Fig. 2: Effect of Phe-tRNA on the retention of ^{3}H-GTP on a nitrocellulose filter in the presence of T_1. The incubations contained 30 μg of T_1 protein, and the assay is described in the legends to Tables I and II.

amount of ^{3}H-GTP (5 pmoles). The decrease in GTP binding in the presence of AA-tRNA seen in both Table II and Fig. 2 suggests that an AA-tRNA-T_1-GTP complex is formed which, like the AA-tRNA-Tu-GTP complex, is not retained on a nitrocellulose filter. This point could be examined by determining whether T_1 activity was present in the filtrate under conditions where a ternary complex should be formed. The results of these experiments are shown in Table III.

TABLE II

Interaction of T_1 with GTP and Phe-tRNA

Exp.	Additions	pmoles ^{3}H-Nucleotide Bound
1	T_1 + ^{3}H-GTP	7.0
	T_1 + ^{3}H-GTP + Phe-tRNA	2.6
	T_1 + ^{3}H-GTP + Deacylated $tRNA^{Phe}$	6.9
2	T_1 + ^{3}H-GDP	4.9
	T_1 + ^{3}H-GDP + Phe-tRNA	4.9
	T_1 + ^{3}H-GDP (+PEP,PK)	6.4
	T_1 + ^{3}H-GDP + Phe-tRNA (+PEP,PK)	2.1
3	T_1 + ^{3}H-ATP	6.9
	T_1 + ^{3}H-ATP + Phe-tRNA	7.0

The experiments were performed as described in the legend to Table I for nucleotide binding. 10 μg of T_1 protein were used except for experiment 3 where 20 μg were added. After the incubation at 37°, the tubes were placed on ice, and where indicated, 16.5 pmoles of Phe-tRNA or deacylated tRNA were added. After a 2 min incubation at 0°, the reaction mixtures were filtered as described in Table I.

The purified preparation of E. coli $tRNA^{Phe}$ (80% pure) was prepared by BD cellulose chromatography by a modification of the procedure of Gillam et al. (24). It was acylated with ^{14}C-phenylalanine by the procedure of Conway (25). Deacylation was accomplished by incubating the acylated tRNA at pH 9 (37°) until there was complete loss of TCA insoluble radioactivity.

In Table III, Exp. 1, it is seen that T_1 activity is found in the filtrate when T_1 is incubated with GTP and Phe-tRNA and the incubation passed through a nitrocellulose filter. The presence of T_1 in the filtrate was dependent on Phe-tRNA (2nd line) but did not seem to require GTP (line 3). These results suggested that GTP was not required for the interaction of T_1 with Phe-tRNA, but it should be noted that PEP and PK were present in Exp. 1. If there were trace levels of GDP in the T_1 preparation, the GDP could be converted to GTP by the kinase sys-

TABLE III

Requirements for the Presence of T_1 in the Nitrocellulose Membrane Filtrate

		PEP + PK	T_1 in Filtrate
			pmoles phenylalanine polymerized
1	T_1 + GTP + Phe-tRNA	+	8.1
	T_1 + GTP + ---	+	0.2
	T_1 + --- + Phe-tRNA	+	5.0
2	T_1 + GTP + Phe-tRNA	-	4.4
	T_1 + GTP + ---	-	0.2
	T_1 + --- + Phe-tRNA	-	0.3

Incubations were performed (see Tables I and II) in a total volume of 0.2 ml and terminated by the addition of 0.8 ml of the wash buffer. The diluted mixture was filtered through a nitrocellulose filter and the filtrate collected. The filter was then washed 2 times with 0.5 ml of wash buffer, and the washes were combined with the initial filtrate (2 ml total volume). To make the filtrates comparable, GTP and Phe-tRNA were added to the filtrates which lacked these components because they were omitted from the incubations. For these experiments, 10 μg of protein T_1, 22 pmoles of Phe-tRNA, 2.5×10^{-6} M GTP, 3.75×10^{-3} M PEP and 10 μg PK were added where indicated.

The T_1 in the filtrate was assayed in polyphenylalanine synthesis (22). These incubations contained in a total volume of 1 ml: 500 μl of the filtrate obtained as described above, 0.05 M Tris buffer, pH 7.4; 0.01 M $MgCl_2$, 0.05 M NH_4Cl, 40 μg poly U, 32 μg T_2 (22), 1×10^{-4} M GTP, 11 A_{260} calf brain ribosomes prepared by modification of existing procedures (26,27), 0.75×10^{-4} M PEP, 10 μg PK, and 80 pmoles purified yeast Phe-tRNA. Incubations were for 10 min at 37°.

tem and combine with T_1 to form T_1-GTP. The T_1-GTP could then react with Phe-tRNA. To test this possibility, Exp. 2 in Table III was performed in the absence of PEP and PK, and the results, in fact, suggest that there was sufficient GDP in the T_1 preparation to account for the lack of GTP requirement seen in Exp. 1. Thus, when T_1 was incubated with Phe-tRNA in the absence of PEP and PK, both

GTP and Phe-tRNA were required in order for T_1 to pass through the filter.*

All of the results presented here indicate that brain transferase I behaves similarly to bacterial factor Tu and wheat and yeast factor T_1. Thus, the animal factor reacts with both GDP and GTP to form a complex that is retained on a nitrocellulos filter, but only T_1-GTP appears to react with AA-tRNA. The use of a nitrocellulose filter assay has greatly facilitated the present studies. It was unexpected that T_1 would behave so similarly to Tu with regard to its retention on nitrocellulose filters, especially in the presence of AA-tRNA. However, this characteristic of T_1 should greatly facilitate studies on its interaction with guanosine nucleotides, AA-tRNA and ribosomes. Since the T_1 preparation used here is not homogeneous (estimated to be about 10-15% pure), and a ternary complex has not been isolated, the present results should be considered as preliminary evidence that an AA-tRNA-T_1-GTP complex can be formed. A more detailed study on the AA-tRNA specificity of the reaction is warranted, and attempts to isolate the ternary complex and study its interaction with ribosomes are in progress.

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*It was noted in the experiments in Table III that T_1 in the filtrate lost its activity in 4-6 hrs. It was necessary, therefore, to assay the T_1 in the filtrate as soon as it was prepared.

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