Kinetics and Thermodynamics of the Interaction of Elongation Factor Tu with Elongation Factor Ts, Guanine Nucleotides, and AminoacyltRNA

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The exchange of elongation factor Tu (EF-Tu)-bound GTP in the presence and absence of elongation factor Ts (EF-Ts) was monitored by equilibrium exchange kinetic procedures. The kinetics of the exchange reaction were found to be consistent with the formation of a ternary complex EF-Tu-GTP-EF-Ts. The equilibrium association constants of EF-Ts to the EF-Tu-GTP complex and of GTP to EF-Tu-EF-Ts were calculated to be $7 \times 10^8$ and $2 \times 10^8$ M$^{-1}$s$^{-1}$, respectively. The dissociation rate constant of GTP from the ternary complex was found to be $13$ s$^{-1}$. This is 500 times larger than the GTP dissociation rate constant from the EF-Tu-GTP complex ($2.5 \times 10^6$ s$^{-1}$).

A procedure based on the observation that EF-Tu-GTP protects the aminoacyl-tRNA molecule from phosphodiesterase I-catalyzed hydrolysis was used to study the interactions of EF-Tu-GTP with Val-tRNA$^{Val}$ and Phe-tRNA$^{Phe}$. Binding constants of Phe-tRNA$^{Phe}$ and Val-tRNA$^{Val}$ to EF-Tu-GTP of $4.8 \times 10^8$ and $1.2 \times 10^8$ M$^{-1}$s$^{-1}$, respectively, were obtained.

The exchange of bound GTP with GTP in solution in the presence of EF-Ts was also examined. The kinetics of the reaction were found to be consistent with a rapid equilibrium mechanism. It was observed that the exchange of bound GTP with free GTP in the presence of a large excess of the latter was accelerated by the addition of aminoacyl-tRNA. On the basis of these observations, a complete mechanism to explain the interactions among EF-Tu, EF-Ts, guanine nucleotides, and aminoacyl-tRNA has been developed.

The supply of substrate for ribosomal peptide synthesis in prokaryotic organisms is controlled by an extraribosomal mechanism. In this work, we describe the interactions of EF-Tu with GTP in the presence and absence of EF-Ts and aa-tRNA.

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The abbreviations used are: EF-Tu, polypeptide elongation factor Tu; EF-Ts, polypeptide elongation factor Ts; aa-tRNA, aminoacyltransfer ribonucleic acid.
reported previously (10). In this work, we have determined the equilibrium constants of Reactions 3, 4, and 5. The rate constant \( k_{\text{ex}} \) was measured experimentally. A detailed mechanism for the overall reaction is discussed.

**Experimental Procedures**

**Materials**

*Escherichia coli* K12 cells were obtained as frozen cell paste from Grain Processing Co., Muscatine, IA, and stored at -20 °C until use. [\(^{3}P\)]GTP was prepared elsewhere (10). [\(^{32}P\)]GTP was prepared by a method described by Johnson and Wallach (12). Radiolabeled EF-Tu.GDP was prepared by incubation of EF-Tu.GDP with [\(^{3}H\)]GDP and [\(^{32}P\)]GTP in the presence of pyruvate kinase, phosphoenolpyruvate, valine- and phenylalanine-specific tRNAs, and unfractionated tRNA were obtained from Boehringer Mannheim. All other chemicals used were of analytical grade.

**Methods**

Preparation of Radiolabeled EF-Tu. Nucleotide Complexes—EF-Tu was purified as described previously (10). EF-Tu.[\(^{3}H\)]GDP was prepared by incubation of EF-Tu.GDP with tritiated GDP as described elsewhere (10). [\(^{3}P\)]GTP was prepared by a method described by Johnson and Wallach (12). Radiolabeled EF-Tu.GTP was prepared by incubation of EF-Tu.GDP with [\(^{3}H\)]GDP and [\(^{32}P\)]GTP in the presence of pyruvate kinase (10 μg/ml) in 0.02 M phosphate buffer (pH 7.2), 0.1 M NaCl, 0.01 M MgCl₂, and 1 mM dithiothreitol (reaction buffer). The phosphorylation of the nucleotide diphosphate was monitored by measuring the amount of radioactive material retained on nitrocellulose filters. The filter-bound \(^{32}P\)-labeled material reached a constant value. This material was further examined by thin layer chromatography in polyethyleneimine-cellulose.

The filter-bound material was extracted either by dissolution of the filter in 0.5 ml of 2-methoxyethanol or by incubation of EF-Tu.GDP with [\(^{3}H\)]GDP and [\(^{32}P\)]GTP in the presence of pyruvate kinase (2 mM) and pyruvate kinase (100 μg/ml) in 0.02 M phosphate buffer (pH 7.2). The mixture was then incubated at 37 °C for 10 min and the filter-bound material was extracted with 0.5 ml of 2-methoxyethanol. The extract was evaporated to dryness, redissolved in 0.5 ml of 2-methoxyethanol, and spotted on plastic TLC plates and dried in air. After two to three applications, the TLC plates were developed in acetate buffer, and lyophilized. The aminoacylated tRNA cannot be stored over long periods of time and thus were used within 48 h after their preparation.

Exchange Kinetics—Radiolabeled EF-Tu. nucleotide complexes were used to study the kinetics of the exchange of bound nucleotide with nucleotide free in the solution. Three different types of exchange experiments were performed: (a) the exchange of bound radioactive GTP with free unlabeled GTP under equilibrium conditions (10); (b) the exchange of bound GTP with free GDP; and (c) the exchange of bound GDP with free GTP. All experiments were performed at 21 °C in 20 mM phosphate (pH 7.2) containing 0.1 M NaCl, 10 mM MgCl₂, and 1 mM dithiothreitol using a rapid filtration apparatus as described elsewhere (10).

Hydrolysis of aa-tRNA by Snake Venom Phosphodiesterase—A rapid, simple method was used to study the interactions of aa-tRNA and EF-Tu. was developed. The method was based on the observation that the observed rate of hydrolysis of aa-tRNA by C. adamantanus phosphodiesterase I decreases by at least 2 orders of magnitude when the nucleic acid is bound to EF-Tu.GTP (15).

**Results**

**Kinetics of the GTP Exchange Reaction**

When a solution containing EF-Tu.[\(^{3}H\)]GTP or EF-Tu.[\(^{3}P\)]GTP is mixed with a large excess of unlabeled GTP, the bound ligand exchanges very rapidly with the free nucleotide (Fig. 1). The addition of 1.25 nm EF-Ts accelerates the reaction significantly. The time course of the reaction follows first order kinetics whether or not EF-Ts is present in the reaction mixture (Fig. 2). The observation of first order rate constants of the GTP exchange reactions were found to be 0.025 and 0.102 s⁻¹ in the absence and presence of EF-Ts (1.25 nm), respectively.

The observed kinetic behavior of the GTP exchange reaction is analogous to the GDP exchange reaction previously studied in this laboratory (10). It is, therefore, susceptible to a detailed analysis under equilibrium exchange assumptions. Since the EF-Tu concentration in the experiments shown in Fig. 2 is 100-fold in excess over the EF-Ts concentration, the

![Fig. 1. The time course of the GTP exchange reaction.](image-url)
Interaction of EF-Tu with EF-Ts, GDP, GTP, and aa-tRNA

FIG. 2. The effect of EF-Ts in the GTP exchange reaction. The reaction mixture contained initially a mixture of EF-Tu-[3H]GTP and EF-Tu-[γ-32P]GTP (0.125 μM total), 1.25 nM EF-Tu-EF-Ts, and 1 mM GTP. The solid curves were obtained by fitting the data to a first order rate equation using a fitting routine which included a base-line correction factor as described under "Methods." The first order rate constant, $k_{obs}$, was found to be 0.102 s$^{-1}$. The top panel shows a graphic representation of the data. The bottom panel shows the data after base-line correction plotted in semilogarithmic form.

The observed rate of exchange of bound GTP in the presence of EF-Ts is the result of two independent reactions: (a) the spontaneous dissociation of the radiolabeled ligand from the EF-Tu-GTP complex, and (b) the dissociation of GTP from the EF-Tu-GTP-EF-Ts ternary complex. Therefore, the observed rate of exchange can be written as:

$$
\frac{d[\text{EF-Tu-GTP}^*]}{dt} = k_{GTP}[\text{EF-Tu-GTP}^*] + k_{cat}[\text{EF-Tu-GTP}^*]_{T}
$$

where $k_{GTP}$ is the association equilibrium constant of EF-Ts with the EF-Tu-GTP complex.

According to Equation 7, $k_{obs}$ should be a linear function of the concentration of EF-Ts. This is indeed the case, as shown in Fig. 3. In these experiments, the concentration of EF-Ts was varied between 0.2 and 1.5 nM while the concentrations of EF-Tu-GTP and GTP were kept constant at 200 nM and 1 mM, respectively. The $y$ intercept of Fig. 3 is the dissociation rate constant of GTP from the EF-Tu-GTP complex, $k_{GTP}$, calculated from these data, was found to be 0.023 s$^{-1}$, which compares very well with the value obtained by measuring directly the EF-Tu-GTP dissociation rate in the absence of EF-Ts (Fig. 1).

Equation 7 can be transformed into

$$
\frac{1}{k_{obs} - k_{GTP}} = \frac{1}{[\text{EF-Ts}]} \left[ \frac{1}{k_{cat}K_4} + \frac{[\text{EF-Tu-GTP}^*]_{T}}{k_{cat}} \right]
$$

This expression predicts a linear relationship between the variable $1/(k_{obs} - k_{GTP})$ and the total concentration of EF-Tu-GTP. Thus, $K_4$ and $k_{cat}$ can be estimated by determining the effect of $[\text{EF-Tu-GTP}^*]_{T}$ on the apparent rate constant of the exchange reaction. This effect is shown in Fig. 4. The values of $k_{cat}$ and $K_4$ obtained from a least squares fit of the

![Graph showing the dependence of the observed rate of exchange on the concentration of EF-Ts.](image)

FIG. 3. The dependence of the observed rate of exchange of bound GTP on the concentration of EF-Ts. The concentration of EF-Tu-GTP was maintained constant at 200 nM whereas the concentration of EF-Tu-EF-Ts was varied from 0.2 to 1.5 nM. The GTP concentration was 1 mM in all the experiments.

![Graph showing the dependence of the rate of exchange of GTP on the EF-Tu-GTP concentration.](image)

FIG. 4. The dependence of the rate of exchange of GTP on the EF-Tu-GTP concentration. The data are plotted according to Equation 8 (see text).
Heterogeneous Exchange Reactions

The experiments described above show that EF-Tu, EF-Ts, and GTP interact in a rapid equilibrium system similar to the equilibrium established with the nucleoside diphosphate GDP described previously (10). However, there are two important questions that the equilibrium exchange experiments cannot answer per se. First, the value of the rate constant \( k_{on} \) cannot be unequivocally established by the equilibrium exchange technique; and second, it is necessary to show that bound GDP can be exchanged with GTP free in solution in order to determine whether or not the set of reactions studied are relevant to the mechanism of EF-Tu turnover during peptide elongation. Therefore, studies of the exchange of bound GDP with free GTP in the solution and the exchange of bound GTP with free GDP were initiated.

The Exchange of Bound GDP with GTP—When EF-Tu-\([^{3}H]\)GDP (60 nM) is incubated with 1 mM in the absence of EF-Ts, the bound GDP exchanges with a first-order rate constant of \(1.8 \times 10^7\) s\(^{-1}\) (Fig. 5, upper curve). The exchange reaction in the presence of EF-Ts did not follow first order kinetics and appeared to be a biphasic process. A fraction of the bound GDP exchanges initially very rapidly, as shown in Fig. 5 (lower curve). The remaining GDP exchanges at a much slower rate. This observation can be explained in terms of the relative affinity of EF-Ts to EF-Tu.GTP and EF-Tu.GDP. As shown above, EF-Ts binds to EF-Tu.GTP with a binding constant of \(7.2 \times 10^5\) M\(^{-1}\), whereas the binding constant of EF-Ts to EF-Tu.GDP is about 400 times smaller \((K_3 = 1.8 \times 10^6\ M^{-1})\). Thus, a comparison of these constants indicates that the EF-Tu.GTP complex formed as a result of the exchange reaction will behave as a strong inhibitor of the EF-Ts-catalyzed GDP exchange reaction.

Additional evidence of this “product inhibition” was obtained by incubating a mixture of EF-Tu-\([^{3}H]\)GDP and EF-Tu-\([^{3}H,^{14}P]\)GTP with 1.25 nM EF-Ts and 62 nM EF-Tu.\([^{3}H]GTP\), 62 nM EF-Tu.\([^{3}P]GTP\), and 1.25 nM EF-Tu.EF-Ts. Panel a shows the composition of the filter-bound radioactive material analyzed by TLC on polyethyleneimine-cellulose after 1 s of incubation with 1 mM GTP. Panel b shows the same material after 30 s of incubation with GTP.
Interaction of EF-Tu with EF-Ts, GDP, GTP, and aa-tRNA

When [3H]aa-tRNA is incubated in the presence of C. adamanteus phosphodiesterase (0.3 unit/ml), the amount of trichloroacetic acid-insoluble material decreases rapidly, the reaction being apparently complete within 1 min. Preincubation of the aa-tRNA with EF-Tu-GTP decreases the observed rate of hydrolysis of aa-tRNA by about 2 orders of magnitude (data not shown and Ref. 15). A simple assay based on this observation was developed in order to obtain a direct measurement of the equilibrium association constant of aa-tRNA to EF-Tu.

Fig. 8 shows the results obtained by incubating Phe-tRNA\textsuperscript{Ph} and Val-tRNA\textsuperscript{Va} with increasing amounts of EF-Tu-GTP for 5 min at 21 °C in the presence of 1 mM GTP and phosphodiesterase (0.3 unit/ml). The fraction of bound aa-tRNA, f\textsubscript{b}, was calculated from the ratio of cold trichloroacetic acid-insoluble radioactive material remaining after the incubation period to the total amount of insoluble radioactive material added initially to the reaction mixture. The solid lines represent the best fit of the data to the equation

$$f_b = \frac{K_s [\text{EF-Tu-GTP}]}{1 + K_s [\text{EF-Tu-GTP}]}$$

where K\textsubscript{s} is the equilibrium association constant of aa-tRNA to EF-Tu-GTP (K\textsubscript{s} = k\textsubscript{a}/k\textsubscript{d}). The free EF-Tu-GTP concentration was calculated from mass conservation equations of the system. The values obtained for K\textsubscript{s} were 4.8 \times 10\textsuperscript{7} and 1.2 \times 10\textsuperscript{8} M\textsuperscript{-1} for Phe-tRNA\textsuperscript{Ph} and Val-tRNA\textsuperscript{Va}, respectively.

The Interactions of EF-Tu-GTP with Aminoacyl-tRNA

With [3H]aa-tRNA incubated in the presence of 1 mM GDP, the amount of trichloroacetic acid-insoluble material decreases rapidly, the reaction being apparently complete within 1 min. Preincubation of the aa-tRNA with EF-Tu-GTP decreases the observed rate of hydrolysis of aa-tRNA by about 2 orders of magnitude (data not shown and Ref. 15). A simple assay based on this observation was developed in order to obtain a direct measurement of the equilibrium association constant of aa-tRNA to EF-Tu.

Fig. 8 shows the results obtained by incubating Phe-tRNA\textsuperscript{Ph} and Val-tRNA\textsuperscript{Va} with increasing amounts of EF-Tu-GTP for 5 min at 21 °C in the presence of 1 mM GTP and phosphodiesterase (0.3 unit/ml). The fraction of bound aa-tRNA, f\textsubscript{b}, was calculated from the ratio of cold trichloroacetic acid-insoluble radioactive material remaining after the incubation period to the total amount of insoluble radioactive material added initially to the reaction mixture. The solid lines represent the best fit of the data to the equation

$$f_b = \frac{K_s [\text{EF-Tu-GTP}]}{1 + K_s [\text{EF-Tu-GTP}]}$$

where K\textsubscript{s} is the equilibrium association constant of aa-tRNA to EF-Tu-GTP (K\textsubscript{s} = k\textsubscript{a}/k\textsubscript{d}). The free EF-Tu-GTP concentration was calculated from mass conservation equations of the system. The values obtained for K\textsubscript{s} were 4.8 \times 10\textsuperscript{7} and 1.2 \times 10\textsuperscript{8} M\textsuperscript{-1} for Phe-tRNA\textsuperscript{Ph} and Val-tRNA\textsuperscript{Va}, respectively.

The Interactions of EF-Tu-GTP with Aminoacyl-tRNA

With [3H]aa-tRNA incubated in the presence of 1 mM GDP, the amount of trichloroacetic acid-insoluble material decreases rapidly, the reaction being apparently complete within 1 min. Preincubation of the aa-tRNA with EF-Tu-GTP decreases the observed rate of hydrolysis of aa-tRNA by about 2 orders of magnitude (data not shown and Ref. 15). A simple assay based on this observation was developed in order to obtain a direct measurement of the equilibrium association constant of aa-tRNA to EF-Tu.

Fig. 8 shows the results obtained by incubating Phe-tRNA\textsuperscript{Ph} and Val-tRNA\textsuperscript{Va} with increasing amounts of EF-Tu-GTP for 5 min at 21 °C in the presence of 1 mM GTP and phosphodiesterase (0.3 unit/ml). The fraction of bound aa-tRNA, f\textsubscript{b}, was calculated from the ratio of cold trichloroacetic acid-insoluble radioactive material remaining after the incubation period to the total amount of insoluble radioactive material added initially to the reaction mixture. The solid lines represent the best fit of the data to the equation

$$f_b = \frac{K_s [\text{EF-Tu-GTP}]}{1 + K_s [\text{EF-Tu-GTP}]}$$

where K\textsubscript{s} is the equilibrium association constant of aa-tRNA to EF-Tu-GTP (K\textsubscript{s} = k\textsubscript{a}/k\textsubscript{d}). The free EF-Tu-GTP concentration was calculated from mass conservation equations of the system. The values obtained for K\textsubscript{s} were 4.8 \times 10\textsuperscript{7} and 1.2 \times 10\textsuperscript{8} M\textsuperscript{-1} for Phe-tRNA\textsuperscript{Ph} and Val-tRNA\textsuperscript{Va}, respectively.

The Interactions of EF-Tu-GTP with Aminoacyl-tRNA

With [3H]aa-tRNA incubated in the presence of 1 mM GDP, the amount of trichloroacetic acid-insoluble material decreases rapidly, the reaction being apparently complete within 1 min. Preincubation of the aa-tRNA with EF-Tu-GTP decreases the observed rate of hydrolysis of aa-tRNA by about 2 orders of magnitude (data not shown and Ref. 15). A simple assay based on this observation was developed in order to obtain a direct measurement of the equilibrium association constant of aa-tRNA to EF-Tu.

Fig. 8 shows the results obtained by incubating Phe-tRNA\textsuperscript{Ph} and Val-tRNA\textsuperscript{Va} with increasing amounts of EF-Tu-GTP for 5 min at 21 °C in the presence of 1 mM GTP and phosphodiesterase (0.3 unit/ml). The fraction of bound aa-tRNA, f\textsubscript{b}, was calculated from the ratio of cold trichloroacetic acid-insoluble radioactive material remaining after the incubation period to the total amount of insoluble radioactive material added initially to the reaction mixture. The solid lines represent the best fit of the data to the equation

$$f_b = \frac{K_s [\text{EF-Tu-GTP}]}{1 + K_s [\text{EF-Tu-GTP}]}$$

where K\textsubscript{s} is the equilibrium association constant of aa-tRNA to EF-Tu-GTP (K\textsubscript{s} = k\textsubscript{a}/k\textsubscript{d}). The free EF-Tu-GTP concentration was calculated from mass conservation equations of the system. The values obtained for K\textsubscript{s} were 4.8 \times 10\textsuperscript{7} and 1.2 \times 10\textsuperscript{8} M\textsuperscript{-1} for Phe-tRNA\textsuperscript{Ph} and Val-tRNA\textsuperscript{Va}, respectively.
These values are in close agreement with previously published results (16).

The Effect of aa-tRNA on the Rate of Exchange of EF-Tu-bound GDP with GTP

We have shown above that when EF-Tu-GDP is incubated with excess GTP in the presence of EF-Ts the observed rate of dissociation of the bound nucleotide decreases with time as the reaction proceeds. This decrease of the nucleotide dissociation rate occurs because of the higher binding constant of EF-Ts to the EF-Tu-GTP complex which is formed during the exchange reaction. However, when Val-tRNA\(^{\text{Val}}\) is included in these exchange experiments, essentially all the bound GDP exchanges at a very fast rate.

Fig. 9 shows the rate of exchange of EF-Tu-bound GDP (60 \(\text{nM}\)) in the presence of EF-Ts (0.625 \(\text{nM}\)) and GTP (1 \(\text{mM}\)), with (open circles) and without (closed circles) 90 \(\mu\text{M}\) Val-tRNA\(^{\text{Val}}\). Both solid lines were obtained by numerical integration of Equation 13. These data were analyzed according to a model which assumed that the binding of aa-tRNA to EF-Tu-GTP was not rate-limiting. The results of this analysis lead to the conclusion that the EF-Tu-GDP complex can be recycled rapidly during the polypeptide elongation reaction only when the concentration of aa-tRNA is sufficiently high to drive the reaction in the direction of the formation of the EF-Tu-GTP-aa-tRNA ternary complex.

DISCUSSION

When the ternary complex EF-Tu-GTP-aa-tRNA interacts with the ribosome to induce binding of aa-tRNA to the ribosomal A site, EF-Tu-GDP is released as a product of the reaction (4, 5). Since the intracellular concentration of EF-Tu is about 10-fold greater than that of the ribosomes (20, 21), the overall rate of the reaction that enables EF-Tu-GDP to dissociate and reform the EF-Tu-GTP-aa-tRNA complex must be at least \(\frac{1}{10}\) the rate of protein synthesis. The in vivo rate of protein synthesis has been estimated to be about 10 amino acids/s (11). Therefore, the in vivo half-life of the formation of the ternary complex EF-Tu-GTP-aa-tRNA from EF-Tu-GDP must be less than 1 s. However, the rate constant for dissociation of GDP from the EF-Tu-GDP complex is only about \(1.7 \times 10^{-5}\text{ s}^{-1}\), which is equivalent to a half-life of about 400 s (10). Thus, the elongation factor Ts is required to accelerate GDP dissociation to within limits consistent with the rate requirements of the elongation process.

We have previously demonstrated that the EF-Ts-catalyzed dissociation of EF-Tu-GDP proceeds via the formation of a ternary complex EF-Tu-GTP-EF-Ts, which dissociates rapidly into GDP and EF-Tu-EF-Ts (10). In this report, we have examined the remaining reactions of the EF-Tu turnover reaction using equilibrium and kinetic experimental procedures and analyses.

A quantitative description of the interactions of EF-Tu-GTP and EF-Ts has been obtained. We have shown that EF-Tu-GTP and EF-Ts participate in the formation of a ternary complex EF-Tu-GTP-EF-Ts, which exists in rapid equilibrium with its components as shown below:

\[
\text{EF-Tu-GTP} + \text{EF-Ts} \rightleftharpoons \text{EF-Tu-EF-Ts} + \text{GTP}
\]

The equilibrium constant \(K_e\) and the rate constant \(k_3\) were determined by analysis of kinetic data, their values being \(7 \times 10^7\text{ s}^{-1}\) and 13 \(\text{s}^{-1}\), respectively. \(K_e\) could not be determined directly. However, from our data and the EF-Tu-EF-Ts association equilibrium constant reported by Miller and Weissbach (18), \(K_e\) was calculated to be \(2 \times 10^6\text{ M}^{-1}\).

The interactions of EF-Tu-GTP and aa-tRNA were studied using a procedure based on the C. adamanteus phosphodiesterase I-catalyzed hydrolysis of aa-tRNA. The procedure was based on the observation that EF-Tu-GTP decreased the apparent rate of hydrolysis of aa-tRNA by 2 orders of magnitude. The equilibrium association constants of Phe-tRNA\(^{\text{Ph}}\) and Val-tRNA\(^{\text{Val}}\) were found to be \(4.8 \times 10^7\) and \(1.2 \times 10^7\) \(\text{M}^{-1}\), respectively. We also attempted to use this procedure to study the interaction of EF-Tu-GTP with aa-tRNA, but at the EF-Tu-GDP concentrations employed, we were unable to detect any significant changes in the rate of phosphodiesterase-catalyzed aa-tRNA hydrolysis.

The rate of dissociation of aa-tRNA from the ternary complex EF-Tu-GTP-aa-tRNA cannot be unequivocally determined from hydrolysis protection experiments such as those described in this paper as well as in others (16, 17). This is due to the fact that these procedures fail to distinguish between the hydrolysis of EF-Tu-GTP-bound aa-tRNA and the dissociation of the nucleic acid followed by hydrolysis of the free aa-tRNA. However, the rate of dissociation of the aa-tRNA from the ternary complex cannot be much faster than the rate of hydrolysis measured during protection experiments. Since the rate of hydrolysis decreases by at least 50-fold in the presence of EF-Tu-GTP, the rate-limiting step of the hydrolysis reaction is either the dissociation of aa-tRNA or the hydrolysis of the bound aa-tRNA, whichever is faster. Thus, an upper limit of \(k_3\), the dissociation rate constant of aa-tRNA from the ternary complex, can be calculated from hydrolysis experiments. These values vary from \(8 \times 10^{-6}\) to \(6 \times 10^{-5}\text{ s}^{-1}\) for different species of aa-tRNA (16, 17).

Table I summarizes the thermodynamic and kinetic parameters for the interactions among EF-Tu, guanine nucleotides, EF-Ts, and aminoacyl-tRNA. Although further information is required, a general description of the mechanism of the
Interaction of EF-Tu with EF-Ts, GDP, GTP, and aa-tRNA

Table I

Equilibrium and kinetic parameters of the interactions of EF-Tu with EF-Ts, GDP, GTP, and aa-tRNA in solution.

| Reaction | Parameter | Reference |
|----------|-----------|-----------|
| EF-Tu + GDP ⇔ EF-Tu.GDP | \( k_{\text{GDP}} \) \( \equiv \frac{9 \times 10^4 \text{ M}^{-1} \text{s}^{-1}}{} \) | 15, 19 |
| EF-Tu + GDP ⇔ EF-Tu.GDP | \( k_{-\text{GDP}} \) \( \equiv \frac{1.7 \times 10^3 \text{ M}^{-1} \text{s}^{-1}}{} \) | 10, 19 |
| EF-Tu + GDP ⇔ EF-Tu.GDP | \( K_{\text{GDP}} \) \( \equiv \frac{1 \times 10^4 \text{ M}^{-1}}{} \) | 15, 18, 19 |
| EF-Tu + GTP ⇔ EF-Tu.GTP | \( k_{\text{GTP}} \) \( \equiv \frac{2 \times 10^5 \text{ M}^{-1}}{} \) | 15 |
| EF-Tu + GTP ⇔ EF-Tu.GTP | \( k_{-\text{GTP}} \) \( \equiv \frac{2.5 \times 10^2 \text{ s}^{-1}}{} \) | 15, this work |
| EF-Tu + GTP ⇔ EF-Tu.GTP | \( K_{\text{GTP}} \) \( \equiv \frac{9 \times 10^4 \text{ M}^{-1}}{} \) | 15, 18 |
| EF-Tu + EF-Ts ⇔ EF-Tu.EF-Ts | \( K_{\text{TS}} \) \( \equiv \frac{3 \times 10^4 \text{ M}^{-1}}{} \) | 18 |
| EF-Tu.GDP + EF-Ts ⇔ EF-Tu.GDP.EF-Ts | \( k_{1} \) \( \equiv \frac{2.3 \times 10^9 \text{ M}^{-1} \text{s}^{-1}}{} \) | 10 |
| EF-Tu.GDP + EF-Ts ⇔ EF-Tu.GDP.EF-Ts | \( k_{-1} \) \( \equiv \frac{1270 \text{ s}^{-1}}{} \) | 10 |
| EF-Tu.GDP + EF-Ts ⇔ EF-Tu.GDP.EF-Ts | \( K_{1} \) \( \equiv \frac{1.8 \times 10^9 \text{ M}^{-1}}{} \) | 10 |
| EF-Tu.EF-Ts + GDP ⇔ EF-Tu.EF-Ts.GDP | \( k_{2} \) \( \equiv \frac{8.7 \times 10^7 \text{ M}^{-1} \text{s}^{-1}}{} \) | 10 |
| EF-Tu.EF-Ts + GDP ⇔ EF-Tu.EF-Ts.GDP | \( k_{-2} \) \( \equiv \frac{1270 \text{ s}^{-1}}{} \) | 10 |
| EF-Tu.EF-Ts + GDP ⇔ EF-Tu.EF-Ts.GDP | \( K_{2} \) \( \equiv \frac{6.9 \times 10^4 \text{ M}^{-1}}{} \) | 10 |
| EF-Tu.EF-Ts + GTP ⇔ EF-Tu.EF-Ts.GTP-EF-Ts | \( k_{3} \) \( \equiv \frac{2.5 \times 10^5 \text{ M}^{-1} \text{s}^{-1}}{} \) | This work |
| EF-Tu.EF-Ts + GTP ⇔ EF-Tu.EF-Ts.GTP-EF-Ts | \( k_{-3} \) \( \equiv \frac{13 \text{ s}^{-1}}{} \) | This work |
| EF-Tu.EF-Ts + GTP ⇔ EF-Tu.EF-Ts.GTP-EF-Ts | \( K_{3} \) \( \equiv \frac{2 \times 10^4 \text{ M}^{-1}}{} \) | This work |
| EF-Tu.GTP + EF-Ts ⇔ EF-Tu.GTP.EF-Ts | \( k_{4} \) \( \equiv \frac{9.1 \times 10^9 \text{ M}^{-1} \text{s}^{-1}}{} \) | This work |
| EF-Tu.GTP + EF-Ts ⇔ EF-Tu.GTP.EF-Ts | \( k_{-4} \) \( \equiv \frac{13 \text{ s}^{-1}}{} \) | This work |
| EF-Tu.GTP + EF-Ts ⇔ EF-Tu.GTP.EF-Ts | \( K_{4} \) \( \equiv \frac{7 \times 10^4 \text{ M}^{-1}}{} \) | This work |
| EF-Tu.GTP + aa-tRNA ⇔ EF-Tu.GTP-aa-tRNA | \( k_{5} \) \( \equiv \frac{1 \times 10^2-10^3 \text{ M}^{-1} \text{s}^{-1}}{} \) | 16, 17, this work |
| EF-Tu.GTP + aa-tRNA ⇔ EF-Tu.GTP-aa-tRNA | \( k_{-5} \) \( \equiv \frac{8 \times 10^{-10}-6 \times 10^{-9} \text{ s}^{-1}}{} \) | 16, 17, this work |
| EF-Tu.GTP + aa-tRNA ⇔ EF-Tu.GTP-aa-tRNA | \( K_{5} \) \( \equiv \frac{10^6-5 \times 10^5 \text{ M}^{-1}}{} \) | 16, 17, this work |
| EF-Tu.GDP + aa-tRNA ⇔ EF-Tu.GDP-aa-tRNA | \( K_{6} \) \( \equiv \frac{7 \times 10^4 \text{ M}^{-1}}{} \) | 22 |

Elongation substrate supply reaction can be developed from the available data. Thus, the sequence of events which follow the release of EF-Tu-GDP complex from the ribosome can be represented by the scheme shown in Fig. 10. EF-Tu·GDP reacts rapidly with EF-Ts to form a complex EF-Tu·GDP·EF-Ts which dissociates into EF-Tu·EF-Ts within 1 ms. GTP then reacts with EF-Tu·EF-Ts forming a second ternary complex, EF-Tu·GTP·EF-Ts. The formation of the ternary complexes EF-Tu·GDP·EF-Ts and EF-Tu·GTP·EF-Ts involves about the same Gibbs energy change. In the next step of the reaction, the EF-Tu·GTP·EF-Ts complex dissociates into EF-Tu·GTP and EF-Ts and, in the presence of aa-tRNA, the ternary complex EF-Tu·GTP·aa-tRNA is formed. The latter complex exhibits thermodynamic stability similar to the two previously described complexes. The latter complex is, however, extremely long lived (\( t_{\text{H}} > 2 \) h) in the absence of active, initiated ribosomes.

The complete reaction appears to be kinetically controlled by the presence of aa-tRNA. Although GTP can displace EF-Tu-bound GDP under equilibrium conditions when GTP is in large excess, the exchange reaction does not proceed at a rate consistent with the rate of protein elongation unless a sufficient amount of aa-tRNA is present in the system. This is because of the high affinity of EF-Tu·GTP·EF-Ts, which results in the depletion of free EF-Ts and a consequent deceleration of the GDP exchange reaction.

A report of the binding of aa-tRNA to EF-Tu·GDP has been published (22). This finding suggests an additional role of aa-tRNA in the substrate supply reaction, namely the acceleration of GDP dissociation in a manner analogous to that proposed for EF-Ts. We investigated this possibility by incubating EF-Tu·[\( ^{3}H \)]GDP with up to 1 mM GDP, but were unable to detect any effect on the kinetics of the GDP exchange reaction. This result rules out this possibility, at least within the concentrations of aa-tRNA used in this study. The mechanism discussed herein thus remains the most likely pathway of the polypeptide elongation substrate supply reaction.

References

1. Haenni, A. L., Lucas-Lerard, J., and Gordon, J. (1968) Fed. Proc. 27, 397
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2. Ono, Y., Skoultchi, A., Waterson, J., and Lengyel, P. (1969) Nature (Lond.) 223, 697–701
3. Weissbach, H., Redfield, B., and Brot, N. (1971) Arch. Biochem. Biophys. 145, 676–684
4. Shorey, R. L., Ravel, J. M., Garner, C. W., and Shive, W. (1969) J. Biol. Chem. 244, 4555–4564
5. Lucas-Lenard, J., Tao, P., and Haenni, A. L. (1969) Cold Spring Harbor Symp. Quant. Biol. 34, 455–462
6. Gordon, J. (1969) J. Biol. Chem. 244, 5680–5686
7. Skoultchi, A., Ono, Y., Waterson, J., and Lengyel, P. (1969) Cold Spring Harbor Symp. Quant. Biol. 34, 437–454
8. Weissbach, H., Brot, N., Miller, D., Rosman, M., and Ertel, R. (1969) Cold Spring Harbor Symp. Quant. Biol. 34, 419–431
9. Arai, K.-I., Kawakita, M., and Kaziro, Y. (1970) J. Biol. Chem. 247, 7029–7037
10. Chau, V., Romero, G., and Biltonen, R. L. (1981) J. Biol. Chem. 256, 5591–5596
11. Kennell, D., and Riezman, H. (1977) J. Mol. Biol. 114, 1–21
12. Johnson, R., and Walseth, T. F. (1979) Adv. Cyclic Nucleotide Res. 10, 135–167
13. Littauer, U. Z. (1971) Methods Enzymol. 20, 70–74
14. Hildebrand, F. B. (1956) Numerical Analysis, pp. 233–239, McGraw-Hill, New York
15. Romero, G. (1980) Ph.D. dissertation, University of Virginia
16. Pingoud, A., and Urbanke, C. (1980) Biochemistry 19, 2108–2122
17. Antonsson, B., and Leberman, R. (1982) Biochimie 64, 1035–1040
18. Miller, D. L., and Weissbach, H. (1970) Arch. Biochem. Biophys. 141, 26–37
19. Arai, K.-I., Kawakita, M., and Kaziro, Y. (1974) J. Biochem. (Tokyo) 92, 521–531
20. Furano, A. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 4780–4784
21. Jacobson, G. R., and Rosenbusch, J. P. (1976) Nature 261, 23–29
22. Pingoud, A., Block, W., Wittinghofer, A., Wolf, H., and Fischer, E. (1982) J. Biol. Chem. 257, 11261–11267