Coupling of GTP Hydrolysis by Elongation Factor G to Translocation and Factor Recycling on the Ribosome†

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ABSTRACT: The translocation step of elongation entails the coordinated movement of tRNA and mRNA on the ribosome. Translocation is promoted by elongation factor G (EF-G) and accompanied by GTP hydrolysis, which affects both translocation and turnover of EF-G. Both reactions are much slower (50−100-fold) when GTP is replaced with non-hydrolyzable GTP analogues or GDP, indicating that the reaction rates are determined by conformational transitions induced by GTP hydrolysis. Compared to the rate of uncatalyzed, spontaneous translocation, ribosome binding of EF-G with any guanine nucleotide reduces the free energy of activation by about 18 kJ/mol, whereas GTP hydrolysis contributes another 10 kJ/mol. The acceleration by GTP hydrolysis is due to large decrease in activation enthalpy by about 30 kJ/mol, compared to the reaction with GTP analogues or GDP, whereas the activation entropy becomes unfavorable and is lowered by about 20 kJ/mol (37 °C). The data suggest that GTP hydrolysis induces, by a conformational change of EF-G, a rapid conformational rearrangement of the ribosome ("unlocking") which determines the rates of both tRNA-mRNA translocation and recycling of the factor.

During the translocation step of the elongation cycle, two tRNAs together with the mRNA move synchronously and rapidly on the ribosome. The movement is catalyzed by the binding of elongation factor G (EF-G)[1] and GTP hydrolysis. Before translocation, deacylated tRNA is bound to the P site and peptidyl-tRNA to the A site of the ribosome. Immediately following the binding of EF-G·GTP to the pretranslocation complex, GTP is hydrolyzed (*1*). GTP hydrolysis and subsequent release of P_i cause conformational changes of EF-G (*2*) which, in turn, induce the formation of the transition state of the ribosome (*2*, *3*). In this state, the movement of the tRNA·mRNA complex takes place. Then, the ribosome returns to the ground state and EF-G assumes the GDP-bound conformation. Finally, EF-G·GDP and deacylated tRNA dissociate, leaving the ribosome in the post-translocation state with peptidyl-tRNA in the P site and a free A site.

The role of GTP binding and hydrolysis for EF-G function on the ribosome is insufficiently studied. Early experiments showed that translocation can take place in the presence of non-hydrolyzable GTP analogues but not GDP; the turnover of EF-G on the ribosome was inhibited in the absence of GTP hydrolysis (*4* and references therein). On the basis of these data, as well as functional analogy with the elongation factor Tu (EF-Tu) and structural homology to EF-Tu and other GTP-binding proteins (*5*), it was concluded that EF-G functions according to the canonical GTPase switch mechanism. It was suggested that EF-G·GTP has high affinity to the ribosome, whereas EF-G·GDP has low affinity, in analogy to other GTP-binding proteins (*4*). According to this model, translocation is brought about by the binding of EF-G·GTP to the ribosome, and GTP hydrolysis is necessary only for the release of EF-G·GDP after completion of translocation. However, early steady-state studies suggested fairly similar ribosome-binding affinities of EF-G·GTP and EF-G·GDP, 0.22 and 0.15 μM, respectively (*6*, *7*). Rapid kinetic experiments showed that, in contrast to the switch model, EF-G-dependent GTP hydrolysis precedes, and greatly accelerates, the rearrangement of the ribosome that leads to translocation (*1*). Furthermore, slower, but efficient translocation was found with EF-G·GDP, which is inconsistent with the GTP/GDP affinity switch model.

In the present work, we determined rate constants of partial reactions of translocation catalyzed by EF-G in the presence of non-hydrolyzable GTP analogues or GDP. Different steps of translocation were studied under single-round conditions, using fluorescence stopped-flow and a newly developed rapid puromycin assay, and under steady-state multiple-turnover conditions using the conventional puromycin reaction and nitrocellulose filtration methods. The comparison of the rate constants with those of the spontaneous EF-G-independent reaction and with translocation in the presence of GTP provides an estimate for the contributions of EF-G binding and GTP hydrolysis to translocation catalysis. Transition state

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[1] Abbreviations: EF-G, elongation factor G; EF-Tu, elongation factor Tu; caged GTP, P3-[1-(2-Nitrophenyl)ethyl]guanosine-5′-triphosphate; GTPγS, guanosine 5′-[γ-thio]triphosphate; GDPNP, guanosine 5′-[($\beta\gamma$)-imido]triphosphate; methyl-O-GTP, guanosine 5′-[γ-methyl]triphosphate; mant-GDP/GTP, 3′(2′)-O-(*N*-methylanthraniloyl)-guanosine diphosphate/triphosphate; GAP, GTPase activating protein.

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parameters for translocation catalyzed by EF-G without and with GTP hydrolysis are also reported.

EXPERIMENTAL PROCEDURES

Materials. Experiments were carried out in buffer A (50 mM Tris·HCl, pH 7.5, 70 mM NH_4Cl, 30 mM KCl, 7 mM $MgCl_2$, 1 mM DTT) at 37 °C. Biochemicals were from Roche Diagnostics, ^{3}H-methionine from Amersham, and ^{14}C-phenylalanine from ICN. Non-hydrolyzable GTP analogues GTPγS and GDPNP were from Roche Diagnostics, and caged-GTP and methyl-O-GTP were donated by R. Goody (MPI Dortmund).

[^{14}C]tRNA^{fMet} (600 dpm/pmol) was labeled at the 3′-terminal adenosine (*8*). f[^{3}H]Met-tRNA^{fMet}, f[^{3}H]Met-[^{14}C]-tRNA^{fMet}, [^{14}C]Phe-tRNA^{Phe}, and unlabeled Phe-tRNA^{Phe} were prepared as described (*9*, *10*), as were ribosomes, initiation factors, mRNA, EF-Tu, and EF-G (*11*). The GDP/GTP contents of EF-G and non-hydrolyzable GTP analogues and the GTP content of GDP were determined by RP-HPLC on C_{18}·MNP (Macherey-Nagel) in 65 mM potassium phosphate, pH 6.2, 2 mM tetrabutylammonium hydrogen sulfate, and 15% acetonitril (*12*). EF-G preparations contained up to 10% GDP. The GTP content of EF-G, non-hydrolyzable GTP analogues, or GDP was below the detection limit (5%).

Biochemical Assays. To prepare 70S initiation complexes, ribosomes (0.5 μM) were programmed with a 3-fold excess of MFTI- or MFFI-mRNA (*11*) in the presence of 0.75 μM IF1, IF2, IF3, and f[^{3}H]Met-tRNA^{fMet} and 1 mM GTP in buffer A for 30 min at 37 °C. Initiation complex was purified from GTP, initiation factors, and unbound f[^{3}H]Met-tRNA^{fMet} by ultracentrifugation through 400 μL of 1.1 M sucrose in buffer A for 1 h at 295 000*g* in a Sorvall M120GX centrifuge. The ternary complex, EF-Tu·GTP·[^{14}C]Phe-tRNA^{Phe}, was prepared by incubating 15 μM EF-Tu with 1 mM GTP, 3 mM phosphoenol pyruvate, 0.5 mg/l pyruvate kinase, and 7.5 μM [^{14}C]Phe-tRNA^{Phe} for 30 min at 37 °C, and it was purified by gel filtration on Superdex 75 (Pharmacia) as described (*13*). The ternary complex was added to the initiation complex, and incubated for 1 min at 37 °C to form pretranslocation complex; practically all ribosomes formed pretranslocation complex. The amount of [^{14}C]Phe- tRNA^{Phe}, f[^{3}H]Met-tRNA^{fMet}, or [^{14}C]tRNA^{fMet} bound to ribosomes was determined by filtration, applying aliquots of the reaction mixture to nitrocellulose filters (Sartorius) and washing with buffer A. Filters were dissolved and radioactivity measured in QS361 (Zinsser) scintillation cocktail. To induce translocation, EF-G preincubated with 1 mM of guanine nucleotide for 15 min at 37 °C was added to the pretranslocation complex.

Rapid Kinetic Methods. Fluorescence stopped-flow measurements were performed on an SX-18MV Spectrometer (Applied Photophysics) and the data evaluated as described previously (*1*). Fluorescence changes of proflavin attached to positions 16/17 of fMetPhe-tRNA^{Phe} upon translocation from the A site to the P site was monitored. Fluorescence was excited at 460 nm and measured after passing KV500 filters (Schott). Experiments were performed in buffer A at 37 °C by rapidly mixing equal volumes (60 μL each) of the pretranslocation complex and EF-G·GTP. Time constants of up to 500 s^{-1} could be measured. The data were evaluated by fitting to a sum of two exponential functions with the characteristic time constants, k_{app1} and k_{app2}, the amplitudes, A_1 and A_2, and another variable for the final signal.

Quench-flow experiments were performed at 37 °C as described (*1*) using a KinTek quench-flow apparatus. Experiments were performed in buffer A at 37 °C by rapidly mixing equal volumes (15 μL each) of pretranslocation complex and EF-G·GTP in the presence of excess puromycin. Reactions were stopped by addition of 0.5 M KOH, hydrolyzed 30 min at 37 °C, and neutralized by addition of acetic acid. The amount of f[^{3}H]MetPhe-puromycin was determined by HPLC on Lichrosphere C_8 using an adapted 0−65% acetonitril gradient in 0.1% TFA. The data were analyzed by single-exponential fitting using TableCurve software (Jandel Scientific).

RESULTS

Kinetics of EF-G Binding to Pretranslocation Ribosomes. Binding of EF-G to pretranslocation ribosomes and translocation was monitored by the fluorescence of A site-bound fMetPhe-tRNA^{Phe}(Prf16/17) (Figure 1A). Pretranslocation complexes were rapidly mixed with EF-G and a non-hydrolyzable GTP analogue or GDP in a stopped-flow apparatus, and time courses were followed by the changes in proflavin fluorescence. The affinities of non-hydrolyzable GTP analogues, GTP, and GDP to EF-G were in the range of 5−10 μM (data not shown), as measured by exchange of mant-GTP from EF-G after addition increasing concentrations of nonlabeled non-hydrolyzable nucleotide (*14*, *15*); thus, at the nucleotide concentration used (1 mM), EF-G was saturated with nucleotide.

In the presence of caged-GTP and GDP (Figure 1B) or of other non-hydrolyzable GTP analogues (not shown), a biphasic change in fluorescence was observed: a rapid decrease of the fluorescence of about 10% was followed by an increase of about 20%. The first phase is attributed to the binding of EF-G to the ribosome (*1*). As expected for a bimolecular binding reaction, the concentration dependence of the rate of the first step was linear (Figure 1C). From the slope and the *Y*-axis intercept of the linear plot of Figure 1C, rate constants of the association-dissociation equilibrium were determined as $k_1 = 1.5 \times 10^8$ $M^{-1}s^{-1}$ and $k_{-1} = 140$ s^{-1} for caged-GTP and $k_1 = 9 \times 10^7$ $M^{-1}s^{-1}$ and $k_{-1} = 90$ s^{-1} for GDP, similar to previously determined values for GTP, $k_1 = 1.4 \times 10^8$ $M^{-1}s^{-1}$ and $k_{-1} = 70$ s^{-1} (*1*). Thus, the rapid association-dissociation equilibrium of EF-G with the ribosome is independent of the nucleotide bound to the factor.

Kinetics of Translocation. The second phase of the fluorescence change in the time course of Figure 1B is due to translocation of fMetPhe-tRNA^{Phe}(Prf16/17) (*1*). Rate constants estimated from the fluorescence increase were in the range of 0.2−0.8 s^{-1} with GDP or any non-hydrolyzable GTP analogue (Figure 1B). Essentially no signal change due to translocation was observed when no nucleotide was present (data not shown), consistent with the biochemical analysis (*1*).

The results obtained by fluorescence stopped-flow were verified by measuring the kinetics of translocation using a rapid quench-flow puromycin assay (Figure 2A). Purified pretranslocation complex carrying puromycin-unreactive fMetPhe-tRNA^{Phe} in the A site was rapidly mixed with EF-G

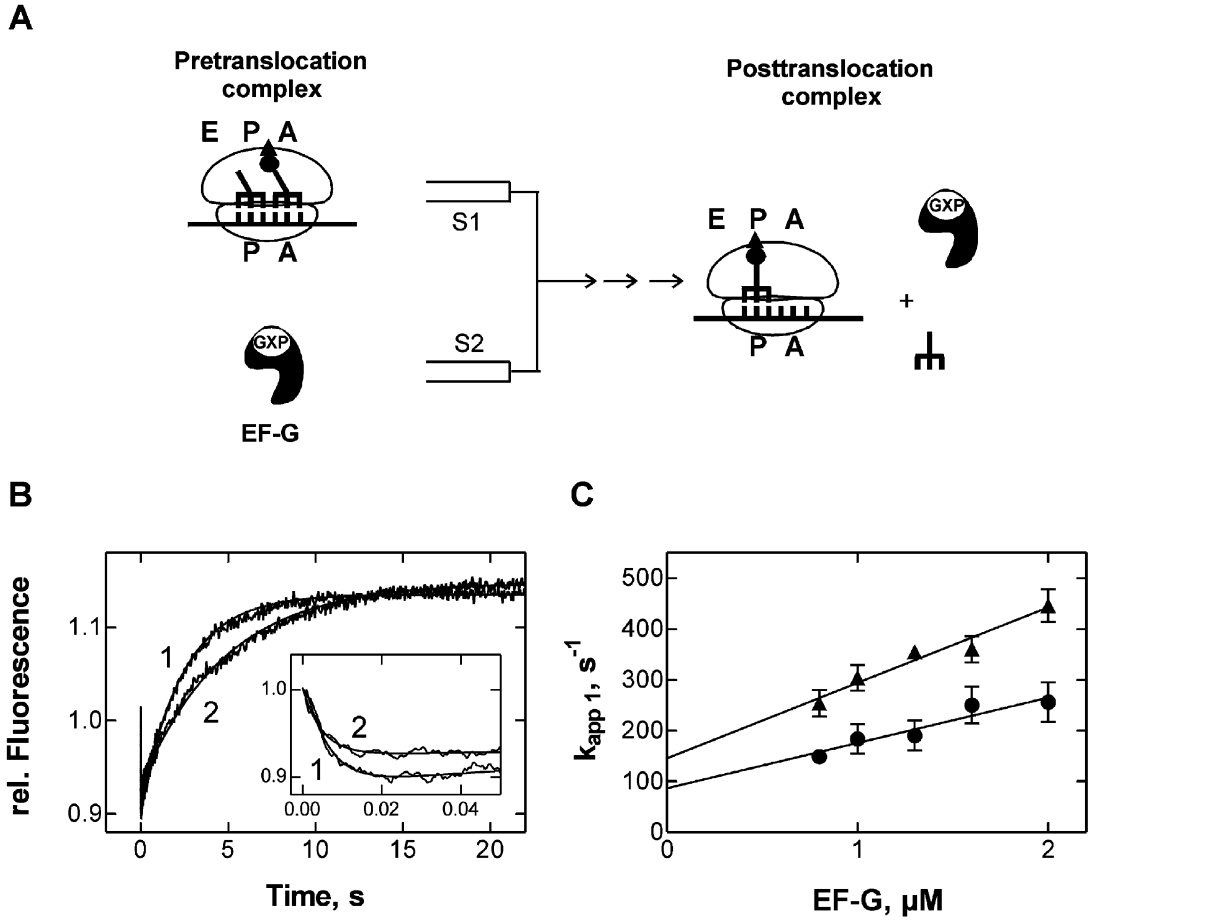

FIGURE 1: EF-G-dependent translocation measured by fluorescence stopped-flow. (A) Experimental approach. Pretranslocation complex (0.2 μM) with deacylated $tRNA^{fMet}$ in the P site and f[^{3}H]Met[^{14}C]Phe-$tRNA^{Phe}$(Prf16/17) in the A site was mixed with EF-G (0.8−2.0 μM) preincubated with caged-GTP or GDP (1 mM) in the stopped-flow apparatus. (B) Time courses of translocation with caged-GTP (trace 1) or GDP (trace 2) and EF-G (1 μM). Inset: Short time window showing the time course of EF-G binding. Parameters of two-exponential fits: caged-GTP, $k_{app1} = 230 \pm 10\ s^{-1}$ (EF-G binding), $k_{app2} = 0.4\ s^{-1}$ (translocation); GDP, $k_{app1} = 180 \pm 20\ s^{-1}$ (EF-G binding), $k_{app2} = 0.2\ s^{-1}$ (translocation). (C) Concentration dependence of k_{app1} with caged-GTP (triangles) or GDP (circles). Rate constants were calculated from the slope and *Y*-axis intercept of the linear fit, respectively: $k_1 = 150 \pm 20\ \mu M^{-1}s^{-1}$ and $k_{-1} = 140 \pm 30\ s^{-1}$ with caged-GTP, and $k_1 = 90 \pm 30\ \mu M^{-1}s^{-1}$ and $k_{-1} = 90 \pm 40\ s^{-1}$ with GDP. The concentration dependence of k_{app2} (translocation) is included in Figure 2.

in the presence of puromycin at sufficiently high concentration (10 mM) to saturate the kinetics. After translocation to the P site, fMetPhe-$tRNA^{Phe}$ rapidly reacted with puromycin, yielding fMetPhe-puromycin which was analyzed by HPLC. Under the present experimental conditions, the rate constant of the reaction with puromycin was about 20 s^{-1} (*16*), fast enough to measure translocation rates of up to about 2 s^{-1}.

Time courses of translocation with GDP, caged-GTP, GTPγS, GDPNP, and methyl-O-GTP measured by the quench-flow puromycin assay are shown in Figure 2B,C. Rate constants of translocation measured in the presence of EF-G at saturating concentration (5 μM, see below) were in the range of 0.2−0.8 s^{-1}, depending on the nucleotide, in agreement with the values obtained by fluorescence stopped-flow. The extent of translocation, i.e., the maximum amount of fMetPhe-$tRNA^{Phe}$ per ribosome that became puromycin-reactive, was >85% relative to the amount of initially bound fMetPhe-$tRNA^{Phe}$ in all cases (not shown), except in the absence of nucleotide, where there was essentially no reaction, as shown previously (*1*).

To determine the ribosome-binding affinity of EF-G, translocation rates were measured at increasing concentrations of EF-G. Rates obtained by the puromycin quench-flow assay or by fluorescence stopped-flow were practically identical (closed and open symbols in Figure 2D,E, respectively). The K_d of the EF-G·ribosome complex, given by the concentration of EF-G at half-maximum rate, was about 1 μM with caged-GTP (Figure 2D) or GDP (Figure 2E), consistent with K_d values calculated from rate constants determined by stopped-flow, $140/1.5 \times 10^8 = 0.9\ \mu M$ (caged-GTP) and $90/9 \times 10^7 = 1\ \mu M$ (GDP) (Figure 1C). Affinities in the same range (0.1−0.8 μM) were previously reported for GTP (*1*, *6*, *7*, *17*, *18*). At saturating EF-G concentration, rate constants of translocation were 0.7 s^{-1} (caged-GTP) and 0.5 s^{-1} (GDP); similar rate constants were observed with GTPγS (0.9 s^{-1}), GDPNP (0.8 s^{-1}), and methyl-O-GTP (0.2 s^{-1}) (Figure 2C). Thus, rate constants of translocation without GTP hydrolysis are 25−100 times lower than those with GTP, 25 s^{-1} (*1*), whereas the ribosome-binding affinity of EF-G is about the same with any guanine nucleotide.

EF-G Turnover. The rate of EF-G turnover on the ribosome was studied using catalytic amounts of EF-G and increasing amounts of pretranslocation complex. The concentration of the factor was chosen such that during the reaction time (3 min) there were several rounds of translo-

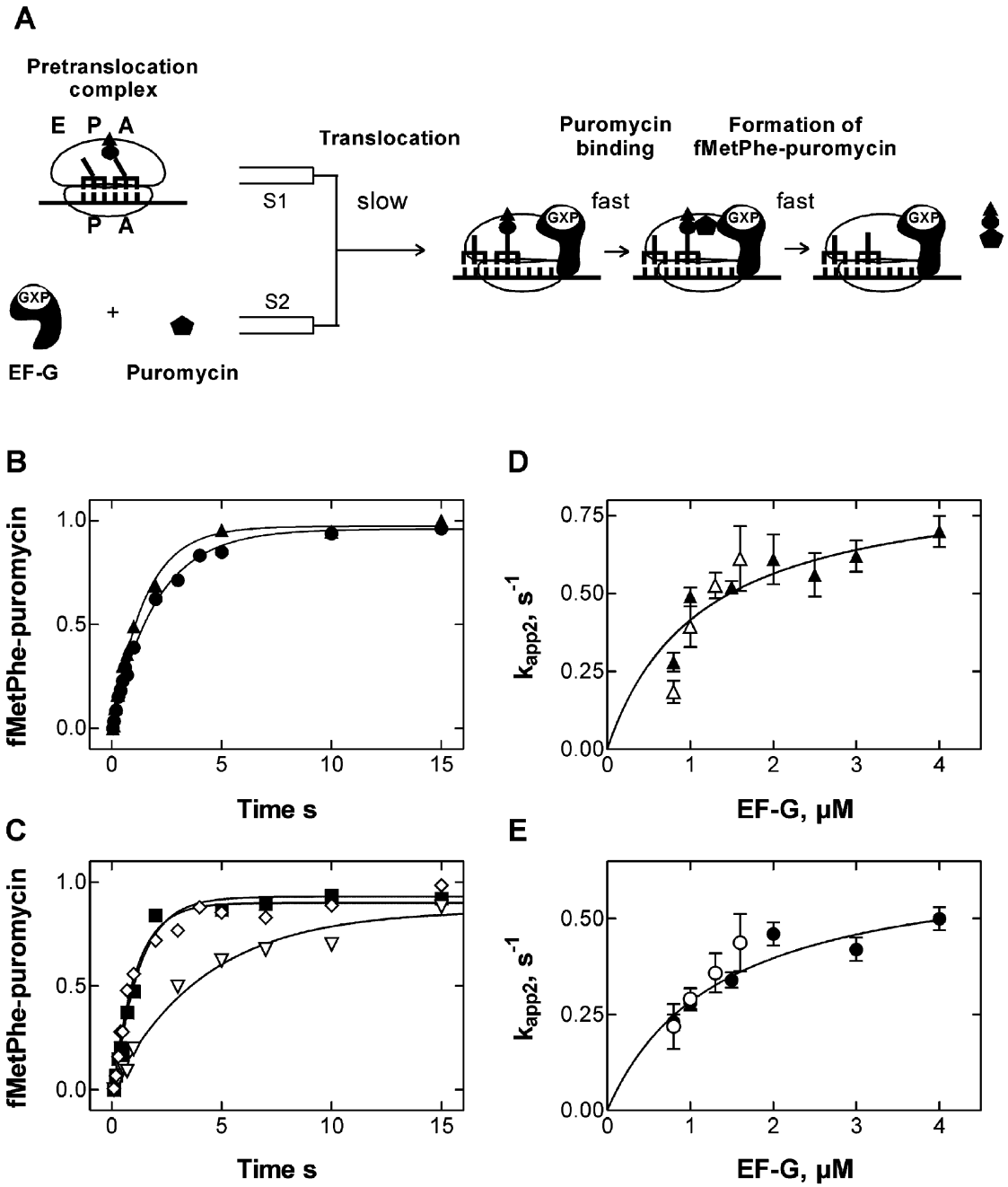

FIGURE 2: Kinetics of translocation. (A) Quench-flow puromycin assay of translocation. Pretranslocation complex (0.4μM) with deacylated $tRNA^{fMet}$ in the P site and f[^{3}H]Met[^{14}C]Phe-$tRNA^{Phe}$ in the A site was mixed with EF-G (0.8−5.0 μM) preincubated with a non-hydrolyzable GTP analogue or GDP (1 mM) in the presence of puromycin (10 mM) in the quench-flow apparatus. (B) Time courses of translocation with caged-GTP (closed circles) or GDP (closed triangles) and EF-G (4 μM). The extent of translocation (>85%) is normalized to 1.0. Time constants of single-exponential fits: caged-GTP, 0.7 s^{-1}; GDP, 0.5 s^{-1}. (C) Time courses of translocation with GTPγS (open diamonds), GDPNP (closed squares), or methyl-O-GTP (open triangles) and EF-G (5 μM). Time constants of single-exponential fits: GTPγS, 0.9 s^{-1}; GDPNP, 0.8 s^{-1}; methyl-O-GTP, 0.2 s^{-1}. (D) Concentration dependence of the translocation rate with caged-GTP measured by fluorescence stopped-flow (k_{app2}, Figure 1; open triangles) or quench-flow puromycin assay (closed triangles). (E) Concentration dependence of the translocation rate with GDP measured by fluorescence (k_{app2} as in panel D; open circles) or puromycin (closed circles).

cation, but only a small portion of the pretranslocation complex was consumed (initial velocity conditions). Under these conditions, the Michaelis−Menten formalism can be used to determine both K_M value of EF-G for the ribosome and turnover rate constant. Translocation was monitored either by the puromycin reaction (10 s incubation at 37 °C, 1 mM puromycin) or by the dissociation of [^{14}C]$tRNA^{fMet}$ from the ribosome (*19*).

Turnover rates of EF-G were very low, 0.02−0.03 s^{-1}, when caged-GTP or GDP were present (Figure 3A,B), about 50 times lower than those observed with GTP (k_{cat} = 1.3 ± 0.2 s^{-1}; Figure 3C). Furthermore, turnover rates in the presence of non-hydrolyzable GTP analogues or GDP were about 30 times lower than the respective rates of translocation, suggesting that a step following tRNA movement, presumably a rearrangement of the ribosome−EF-G complex (see Discussion), limits the rate of EF-G turnover. K_M values of 0.5−0.7 and 1.5 μM were determined by measuring puromycin reactivity and $tRNA^{fMet}$ release, respectively, in good agreement with K_d values around 1 μM determined by

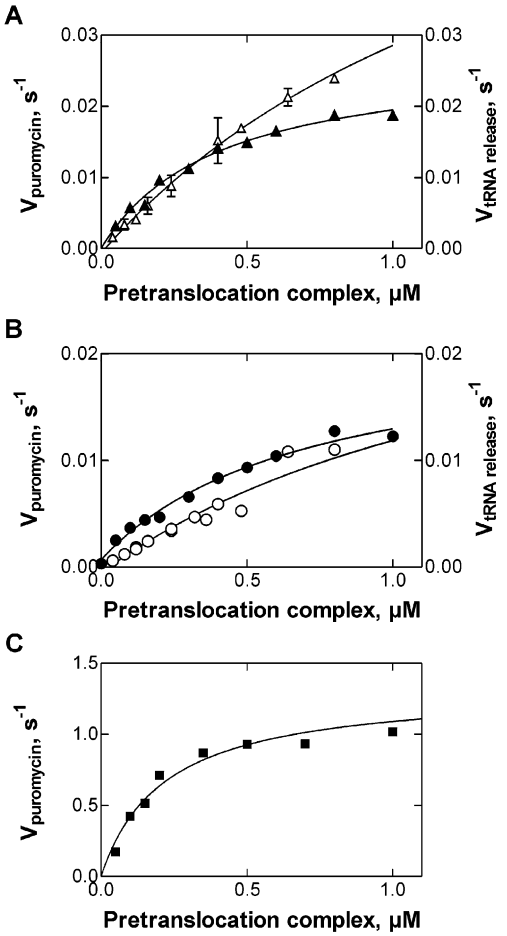

FIGURE 3: EF-G turnover in translocation. (A) Concentration dependence of the initial velocity with caged-GTP. Multiple turnover translocation was measured with a catalytic amount of EF-G (0.02 μM) and caged-GTP. Puromycin reaction (closed triangles) or release of deacylated [^{14}C]tRNA^{fMet} from the ribosome (open triangles) were measured. (B) Concentration dependence of the initial velocity with GDP. Multiple turnover translocation was measured with a catalytic amount of EF-G (0.02 μM) with GDP. Puromycin reaction (closed circles) or release of deacylated [^{14}C]-tRNA^{fMet} from the ribosome (open circles) were measured. (C) Concentration dependence of the initial velocity with GTP. Multiple turnover translocation was measured with a catalytic amount of EF-G (0.005 μM) by the puromycin reaction.

single-turnover kinetics. The K_M value for the reaction with GTP, 0.2 μM (Figure 3C), was somewhat lower than the K_d value calculated from kinetic constants ($K_d = k_{-1}/k_1 = 70$ $s^{-1}/(1.4 \times 10^8\ M^{-1}\ s^{-1} = 0.5\ \mu M$) because the steps following the binding of EF-G to the ribosome, i.e., GTP hydrolysis, Pi release, and translocation, contribute to the value of K_M.

Transition State Parameters of Translocation. The temperature dependence of the translocation reaction was measured with GTP, caged GTP, and GDP. Linear Arrhenius plots of ln(k_{pep}) versus 1/T were obtained in the temperature range between 15 and 37 °C (Figure 4). From the plots, the thermodynamic parameters of the transition state at 37 °C were calculated (Table 1). GTP hydrolysis reduces the free energy of activation, $\Delta G^{\ddagger}$, by about 10 kJ/mol. The difference results from a large decrease of the activation enthalpy and an unfavorable decrease of the activation entropy. At all conditions, the free energy of activation is dominated by the enthalpy term. The entropy term is small but negative

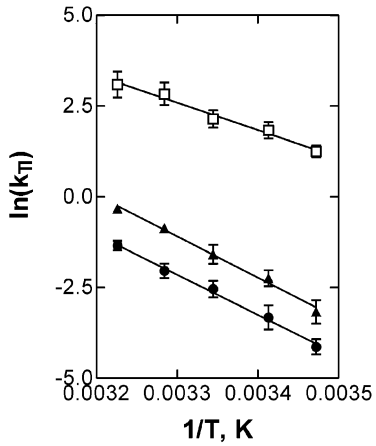

FIGURE 4: Temperature dependence of translocation. Translocation rates were measured at 15, 20, 26, 32, and 37 °C with GTP (squares), caged-GTP (triangles), and GDP (circles) at saturating concentration of EF-G (5 μM).

Table 1: Transition State Parameters of Translocation (37 °C)

nucleotide	$\Delta G^{\ddagger}$ (kJ/mol)	$\Delta H^{\ddagger}$ (kJ/mol)	$T\Delta S^{\ddagger}$ (kJ/mol)
GTP	68 ± 7	60 ± 5	−8 ± 1
caged-GTP	77 ± 6	92 ± 5	15 ± 1
GDP	79 ± 5	89 ± 4	10 ± 1

(unfavorable) for translocation with GTP, whereas it is positive (favorable) with caged-GTP and GDP, indicating that the reaction proceeds by different pathways with and without GTP hydrolysis.

DISCUSSION

Energetics of Translocation Catalysis. Spontaneous translocation of peptidyl-tRNA in the absence of EF-G on mRNA-programmed ribosomes is very slow; the rate may be estimated to 5×10^{-4} s^{-1} (37 °C) (*20*, *21*). Relative to this estimated number, binding of EF-G with non-hydrolyzable GTP analogues or GDP to the ribosome accelerates translocation about 1000-fold, to 0.5 s^{-1} (summarized in Figure 5A), i.e., lowers the free energy of activation, $\Delta G^{\ddagger}$, by about 18 kJ/mol. The same rate was observed with a GTPase-inactive mutant of EF-G in the presence of GTP (*15*). GTP hydrolysis increases the rate of translocation by a factor of about 50, to 25 s^{-1} (*1*). Assuming the energetic contributions of EF-G binding and GTP hydrolysis to be additive, this corresponds to a reduction of the free energy of activation by another 10 kJ/mol (Figure 5B).

The transition state of translocation, as induced by EF-G binding to the ribosome, without GTP hydrolysis is characterized by a large $\Delta H^{\ddagger}$ of about 90 kJ/mol and a positive activation entropy, $T\Delta S^{\ddagger}$, of 10−15 kJ/mol. The acceleration of translocation by GTP hydrolysis is purely enthalpic, i.e., the activation enthalpy is decreased by about 30 kJ/mol, whereas the entropic term becomes unfavorable and is lowered by about 20 kJ/mol. As to the mechanism of catalysis, this can be interpreted in two different ways. The decrease in the activation enthalpy may indicate that in the transition state of translocation after GTP hydrolysis additional interactions are formed, compared to the transition state formed in the absence of GTP hydrolysis, thus lowering the energy of the transition state. This model implies that a substantial fraction of the free energy of GTP hydrolysis is

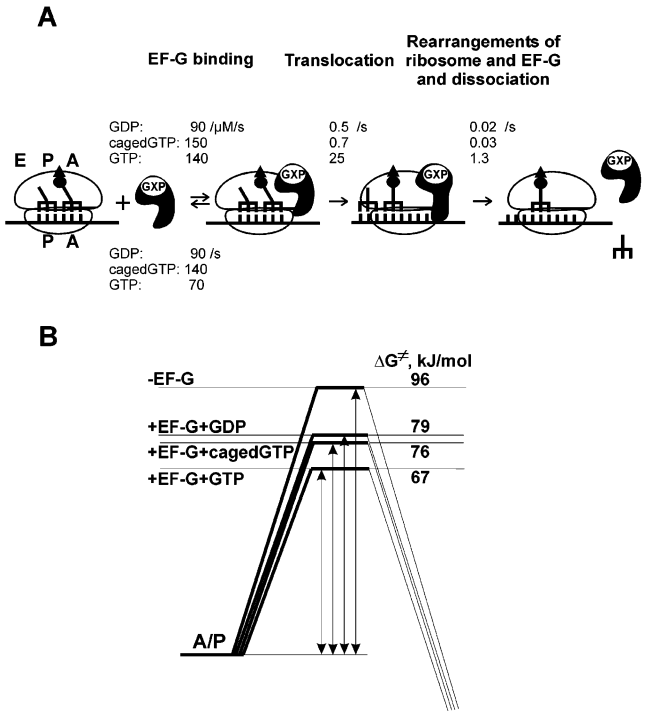

FIGURE 5: Kinetic parameters of translocation. (A) Summary of rate constants. (B) Contributions of EF-G binding and GTP hydrolysis to translocation catalysis. The rate of spontaneous translocation (−EF-G) at comparable conditions was estimated from (*20*, *21*). $\Delta G^{\ddagger}$ (37 °C) was derived from the translocation rate constant according to the equation: $\Delta G^{\ddagger} = -RT \ln(kh/k_B/T)$ (*27*).

retained in the complex and promotes the formation of the transition state by providing part of the activation enthalpy. The accompanying loss of entropy would be consistent with the formation of new binding interactions between EF-G and the ribosome. Alternatively, the decrease of the activation enthalpy may be due to destabilization of the reaction intermediate before translocation. In such a case, the free energy of GTP hydrolysis would serve to resolve binding interactions in the ribosome·tRNA·EF-G complex prior to movement, thereby facilitating movement. Such a model could also explain why the turnover of EF-G is accelerated by GTP hydrolysis. However, the unfavorable change in the activation entropy due to GTP hydrolysis is difficult to rationalize in terms of the latter model. It appears likely, therefore, that both mechanisms are effective, i.e., that the intermediate immediately preceding translocation is destabilized and the transition state of translocation is stabilized by GTP hydrolysis, resulting in a net decrease of the free energy of activation that is composed of favorable enthalpy and unfavorable entropy changes.

Coupling of GTP Hydrolysis to Translocation and EF-G Turnover. It was shown previously that coupling between GTP hydrolysis and translocation requires (i) the presence of domain 4 of EF-G, and particularly the conserved amino acids at the tip of domain 4 (*1*, *18*), (ii) conformational flexibility of EF-G (*22*), and (iii) conformational flexibility of the thiostrepton-binding region of 23S rRNA and/or correct interactions of EF-G with this region (*11*, *22*). By analogy to other GTP binding proteins, the structure of the switch I and II regions of EF-G is expected to change upon GTP hydrolysis. Because the switch regions are located at the interface between domain 1 and 5, such a change may affect the interactions between the domains and lead to a different arrangement of the two domains relative to each other which will also result in repositioning of domain 4. The formation of additional contacts between EF-G and the 50S subunit in the transition state of translocation after GTP hydrolysis, together with the movement of domain 4, could provide the driving force for changing the conformation of the 30S subunit (*2*, *23*) and/or of the relative positions of the 30S and 50S subunits (*3*). In turn, these changes are likely to be crucial for the unlocking the tRNA-mRNA complexes in the A and P sites on the 30S subunit and facilitating the tRNA movement. During translocation, domain 4 of EF-G enters the 30S A site vacated by the tRNA, and the conformation of EF-G changes (*2*, *24*). This may result in destabilization of EF-G contacts with the 50S subunit, thereby accelerating the dissociation of the factor from the ribosome. All these steps, i.e., the reorganization of the subunit interface, tRNA translocation, and EF-G dissociation, take place also when there is no GTP hydrolysis (*1*, *3*; present paper), but slowly and by a different pathway, as indicated by substantial differences in the activation parameters.

It is known that the turnover of EF-G on the ribosome, which is rapid when GTP is present, is rather slow with non-hydrolyzable GTP analogues (*4*, *25*, *26*). The present quantitative analysis shows that turnover is inhibited about 50-fold, from 1.3 s^{-1} with GTP to 0.02−0.03 s^{-1} with non-hydrolyzable GTP analogues and GDP, i.e., to about the same extent as translocation. Interestingly, there is essentially no difference between non-hydrolyzable GTP analogues and GDP. The same is true for the affinity of EF-G binding to ribosomes in the pre- translocation state, as determined from the rate constants of EF-G binding to the ribosome and the concentration dependence of the turnover rate. Affinities are about the same, 0.5−1.0 μM, with non-hydrolyzable GTP analogues, GDP, and GTP, in agreement with previous values determined for GTP and GDP (*6*, *7*). Thus, it is not an affinity difference that causes the different turnover rates observed when GTP and GDP are compared, in particular, as in both cases it is EF-G·GDP that eventually dissociates from the ribosome. This indicates that the observed turnover rate is determined by a rearrangement of the ribosome-EF-G complex that precedes dissociation, rather than by the dissociation step itself, and it is this rearrangement which is accelerated, or induced, by GTP hydrolysis. Thus, the energy of GTP hydrolysis in EF-G on the ribosome is used to form a complex in which the factor and the ribosome are conformationally coupled. Conformational changes induced by GTP hydrolysis result in an unlocked state in which both the movement of the tRNA−mRNA complex and the dissociation of EF-G from the ribosome are facilitated.

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