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Truncated elongation factor G lacking the G domain promotes translocation of the 3′ end but not of the anticodon domain of peptidyl-tRNA

(ribosome/GTPase)

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ABSTRACT **The mechanism by which elongation factor G (EF-G) catalyzes the translocation of tRNAs and mRNA on the ribosome is not known. The reaction requires GTP, which is hydrolyzed to GDP. Here we show that EF-G from *Escherichia coli* lacking the G domain still catalyzed partial translocation in that it promoted the transfer of the 3′ end of peptidyl-tRNA to the P site on the 50S ribosomal subunit into a puromycin-reactive state in a slow-turnover reaction. In contrast, it did not bring about translocation on the 30S subunit, since (*i*) deacylated tRNA was not released from the P site and (*ii*) the A site remained blocked for aminoacyl-tRNA binding during and after partial translocation. The reaction probably represents the first EF-G-dependent step of translocation that follows the spontaneous formation of the A/P state that is not puromycin-reactive [Moazed, D. & Noller, H. F. (1989) *Nature (London)* 342, 142–148]. In the complete system—i.e., with intact EF-G and GTP—the 50S phase of translocation is rapidly followed by the 30S phase during which the tRNAs together with the mRNA are shifted on the small ribosomal subunit, and GTP is hydrolyzed. As to the mechanism of EF-G function, the results show that the G domain has an important role, presumably exerted through interactions with other domains of EF-G, in the promotion of translocation on the small ribosomal subunit. The G domain's intramolecular interactions are likely to be modulated by GTP binding and hydrolysis.**

The mechanism of elongation factor G (EF-G)-catalyzed translocation of tRNAs and mRNA on the ribosome is not known. It is assumed that binding of EF-G·GTP to the pretranslocation ribosome induces a structural transition in the ribosome that allows translocation, and that the reaction is completed by GTP hydrolysis, which results in EF-G·GDP dissociation from the ribosome (1). It is likely that EF-G functions like other GTPases which, upon GTP hydrolysis, switch from the active GTP-bound to the inactive GDP-bound conformation (2).

The structures of both nucleotide-free and GDP-bound EF-G from *Thermus thermophilus* have been determined recently and are nearly identical (3, 4). The molecule, which is among the largest GTP-binding proteins (73 kDa), consists of five structural domains. Domain I (the G domain) together with domain II (domain III is not well defined in the crystals) form a structural unit resembling EF-Tu in the GTP form (5, 6), while domains IV and V form another unit. The structure of the GTP form of EF-G has not been determined yet. The comparison of the EF-G structure with the recently determined structure of the ternary complex EF-Tu·GTP[β,γ-NH]·Phe-tRNA^{Phe}, where GTP[β,γ-NH] is guanosine 5′-[β,γ-imido]triphosphate, shows that the anticodon domain occupies the same position as domain IV of EF-G (7), lending support to the idea that domain IV may have a role in translocation catalysis (3). As to the function of the other domains of EF-G, there is evidence suggesting that the C-terminal domain V is required for ribosome binding (8, 9). Domain I is interacting with both domains II and V; these interactions are probably functionally important because numerous fusidic acid resistance mutations have been found at the respective interfaces (10).

To study the role of EF-G domains for translocation catalysis, we have prepared several deletion mutants that were truncated from the N terminus, lacking domain I or domains I–III; the isolated G domain was also studied. The function of the EF-G fragments in promoting translocation was studied with pretranslocation complexes carrying deacylated tRNA^{fMet} in the P site and peptidyl-tRNA (fMetPhe–tRNA^{Phe}) in the A site of ribosomes programmed with heteropolymeric mRNA. Some experiments were also performed with poly(U)-programmed ribosomes. The reaction was assayed by measuring (*i*) the puromycin reaction, (*ii*) the release of deacylated tRNA from the P site, and (*iii*) the binding of another aminoacyl-tRNA to the A site and formation of tripeptide. Thereby, the translocation of the peptidyl end of peptidyl-tRNA on the 50S subunit and of the anticodon domain on the 30S subunit could be monitored separately. We found that a fragment of EF-G that lacked the G domain retained the ability to translocate the 3′ end of peptidyl-tRNA on the 50S subunit, whereas it was inactive in displacing the anticodon domain of peptidyl-tRNA on the 30S subunit. Fragments with larger deletions were entirely inactive.

MATERIALS AND METHODS

Buffers and Reagents. Buffer A was 25 mM Tris·HCl (pH 7.5)/50 mM NH_4Cl/10 mM $MgCl_2$/0.5 mM EDTA/0.5 mM dithiothreitol. Buffer B was 50 mM Tris·HCl (pH 7.5)/70 mM NH_4Cl/30 mM KCl/7 mM $MgCl_2$/1 mM dithioerythritol/0.5 mM EDTA. Biochemicals were from Boehringer Mannheim, ^3H-labeled amino acids were from Amersham, and ^{14}C-labeled amino acids from ICN.

tRNAs, Ribosomes, and Factors. Materials not mentioned in the following were prepared and characterized as described (11). Ac[^{14}C]Phe-tRNA^{Phe} (800 dpm/pmol, 1.75 nmol/A_{260} unit) and [^{14}C]Phe-tRNA^{Phe} (1012 dpm/pmol, 1.65 nmol/A_{260} unit) were prepared from *Escherichia coli* tRNA^{Phe}; the latter as well as *E. coli* EF-Tu·Ts were gifts of Y. Semenkov (St. Petersburg Nuclear Physics Institute, St. Petersburg, Russia). *E. coli* initiation factors were donated by C. Gualerzi (University of Camerino, Camerino, Italy).

mRNAs. Two different mRNAs were used, each about 120 nucleotides long with a ribosome binding site and the coding

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Abbreviations: EF, elongation factor; GTP[β,γ-NH], guanosine 5′-[β,γ-imido]triphosphate.

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sequence AUGUUUAGG... (Met-Phe-Thr..., MFT-mRNA) and AUGUUUUUU... (Met-Phe-Phe..., MFF-mRNA), respectively. The sequence coding for MFT... was cloned into the polylinker region of plasmid pTZ18R (Pharmacia) via *Eco*RI and *Hin*dIII sites as described (12); the original plasmid (pXR022) was provided by C. Gualerzi. The mutation leading to the sequence coding for MFF... was introduced by site-directed mutagenesis (R. Spurio, University of Camerino, personal communication). The plasmids were linearized with *Hin*dIII, and the respective mRNA was obtained by T7 RNA polymerase runoff transcription (12). The transcripts were purified by fast protein liquid chromatography on MonoQ (Pharmacia).

EF-G Fragments. Deletions were introduced into the EF-G gene (*fus*) carried in the *Kpn* I site of plasmid pTZ18R (Pharmacia). The *Kpn* I fragment (3 kbp) was obtained from plasmid pLL145 (13). EF-G fragments truncated from the amino terminus were prepared by PCR using primers of 28 (5′) and 40 (3′) nucleotides and *Pfu* DNA polymerase (Stratagene). The PCR products were cloned into the *Bam*HI and *Xho* I sites of plasmid pRSET B (Invitrogen). Constructs were verified by sequencing using a sequenase kit (United States Biochemical). The EF-G fragments, which at the N terminus comprised about 30 amino acids from the vector, including six histines, were expressed in *E. coli* JM109. EF-GΔ1–474 was purified from the cell extract by affinity chromatography on Ni^{2+}–nitrilotriacetic acid agarose (Diagen, Düsseldorf, Germany) following the manufacturers protocol. The larger fragment, EF-GΔ1–307, was adsorbed to the affinity-column in the presence of 8 M urea; for renaturation, the urea was gradually diluted out, and the protein was finally eluted as above. Following concentration by ultrafiltration (Amicon), the protein was purified by gel filtration on Superdex 75 HiLoad (Pharmacia) in buffer A containing 0.35 M KCl.

EF-GΔ308–703 was prepared by oligonucleotide-directed mutagenesis of plasmid pTZ18R containing the EF-G gene. According to sequencing, the construct coded for amino acids 1–303 of EF-G plus four amino acids from the vector (14). The protein was expressed in *E. coli* Bl21DE3 and purified from the cell extract by ammonium sulfate fractionation (35–45%), chromatography on DEAE Sepharose CL 6B (Pharmacia), gel filtration on Superdex 75 HiLoad, fast protein liquid chromatography on MonoQ (Pharmacia), and dialysis into buffer A containing 10 μM GDP, adopting a procedure described for the purification of the G domain of EF-Tu (15).

Ribosome Complex Formation and Translocation Assays. Ribosomes (0.4 μM) in buffer B were programmed with MFF–mRNA (1 μM) (or, when indicated, MFT–mRNA) by incubation with f[^{3}H]Met-tRNA^{fMet} (0.6 μM) and *E. coli* initiation factors 1, 2, and 3 (0.6 μM each) in the presence of GTP (1 mM) (11); subsequently, [^{14}C]Phe-tRNA^{Phe} (1 μM) was bound to the A site in the presence of EF-Tu·EF-Ts (0.4 μM). According to nitrocellulose filtration, peptide analysis on HPLC (11), and puromycin assay, 95% of the ribosomes (10 pmol) had bound f[^{3}H]Met[^{14}C]Phe–tRNA^{Phe} to the A site. Incubation with EF-G or EF-GΔ1–307 (2 pmol, 0.08 μM) was at 37°C in 25 μl. At the indicated times, puromycin (1 mM) was added and the incubation continued for 10 s. After the addition of 0.5 ml 1.5 M sodium acetate saturated with magnesium sulfate (pH 5), f[^{3}H]Met[^{14}C]Phe–puromycin was extracted into ethyl acetate and counted. The analogous procedure was applied in assays with poly(U)-programmed ribosomes carrying tRNA^{Phe} in the P site and Ac[^{14}C]Phe–tRNA^{Phe} (or $AcPhe_2$–tRNA^{Phe}) in the A site.

To measure the amount of f[^{3}H]Met-, or [^{14}C]aminoacyl-, or peptidyl-tRNA bound to the ribosome, aliquots were rapidly filtrated through nitrocellulose filters (Sartorius) without prior dilution, followed by extensive washing of the filters with 15 ml of buffer A. For counting, the filters were dissolved in QS361 (Zinsser, Frankfurt, Germany). Radioactivity was measured in a Packard 2500 TR scintillation counter using a double label program.

RESULTS

EF-G Mutants. Deletion mutants of *E. coli* EF-G (Fig. 1*A*) were prepared by excising parts of the gene (*fus*) carried on a plasmid by either oligonucleotide-directed mutagenesis or PCR (see *Materials and Methods*). EF-G fragments comprising either domains II–V or domains IV and V, as well as the isolated G domain (domain I), were prepared and studied. In the two mutants truncated from the amino terminus, the latter was extended by additional amino acids including six histidines to facilitate the rigorous purification of the proteins expressed in *E. coli* from intact EF-G by affinity chromatography on Ni^{2+}–nitrilotriacetic acid agarose (Fig. 1*B*). All constructs were verified by DNA sequencing.

Translocation on Poly(U)-Programmed Ribosomes Assayed by Puromycin. Initial experiments were performed with poly(U)-programmed ribosomes which carried deacylated tRNA^{Phe} in the P site and Ac[^{14}C]Phe-tRNA^{Phe} in the A site (Fig. 2). Only the mutant lacking domain I (G domain), EF-GΔ1–307, induced puromycin reactivity, albeit slowly. The smaller fragment, EF-GΔ1–474, lacking domains I–III, and

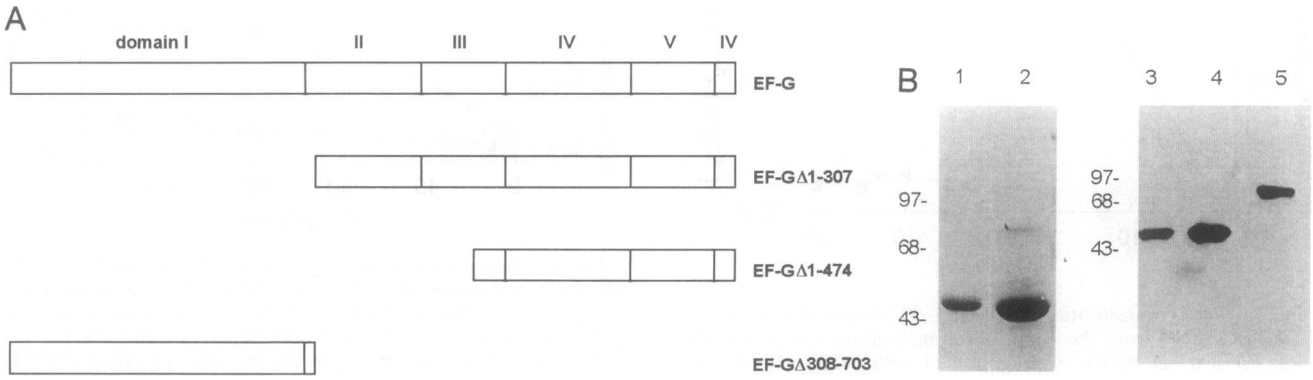

FIG. 1. Deletion mutants of *E. coli* EF-G. (*A*) EF-G fragments. The domains of EF-G (Roman numerals) as derived from the crystal structure of the *T. thermophilus* protein (3, 4) are indicated by vertical bars; domain I is the G domain. EF-G from *T. thermophilus* consists of 691 amino acids, the *E. coli* protein of 703 amino acids; therefore, the assignment of structural domains of the latter on the basis of the known structure of the former may not be precise. (*B*) Characterization of EF-GΔ1–307. The purified fragment was run on SDS/12% PAGE and the gels stained with either Coomassie (lanes 1 and 2) or by immunoblot analysis (lanes 3 and 4); lane 5, intact EF-G prepared from *E. coli MRE600*. Proteins were blotted to nitrocellulose and stained by rabbit anti-EF-G (8) and an anti-rabbit antibody conjugated with peroxidase (Dianova, Hamburg, Germany); detection was by enhanced chemiluminescence. The positions of molecular weight markers are indicated in kDa.

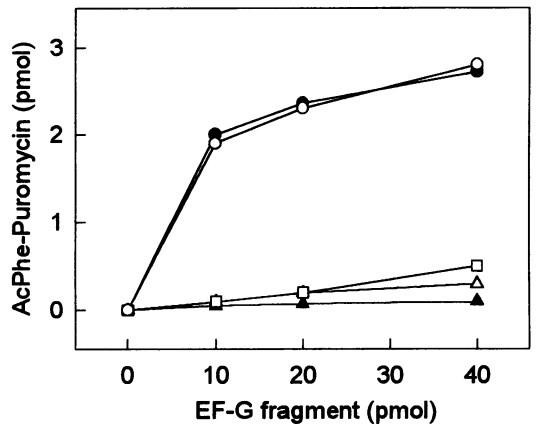

FIG. 2. Puromycin reactivity of A site-bound AcPhe-tRNA^{Phe} upon incubation with EF-G fragments. *E. coli* ribosomes (10 pmol) were successively incubated with poly(U) (0.5 A_{260} units/ml), *E. coli* tRNA^{Phe} (10 pmol), and *E. coli* Ac[^{14}C]Phe-tRNA^{Phe} (12 pmol) for 5 min each at 37°C in 100 μl buffer A; 7.6 pmol was bound according to nitrocellulose filtration. Incubation with the indicated amounts of EF-G fragments was performed at 37°C for 40 min in the presence of puromycin (1 mM). With intact EF-G (1 pmol), 4.7 pmol Ac[^{14}C]Phe-puromycin was formed. Blank values obtained with mock-treated samples (2.7 pmol) were subtracted throughout. ●, EF-GΔ1–307; ▲, EF-GΔ1–474; △, EF-GΔ308–703; ○, EF-GΔ1–307 plus EF-GΔ308–703 (1:1); and □, EF-GΔ1–474 plus EF-GΔ308–703 (1:1).

the isolated G domain, EF-GΔ308–703, were inactive; the G domain neither stimulated the activity of EF-GΔ1–307 nor induced any activity of EF-GΔ1–474 (Fig. 2).

tRNA Release Assay for Translocation. To monitor the release of tRNA^{fMet} from the P site, the pretranslocation complex on MFT-mRNA was set up with [^{14}C]tRNA^{fMet} in the P site and fMet[^{14}C]Phe-tRNA^{Phe} in the A site. Upon addition of EF-GΔ1–307, the peptidyl-tRNA became fully puromycin-reactive, as above, while no tRNA^{fMet} was released from the P site (Fig. 3*A*). In the control experiment with EF-G, all tRNA^{fMet} was released rapidly in parallel with the appearance of puromycin reactivity (Fig. 3*B*), indicating that full translocation had taken place. The lack of tRNA release in the experiment with EF-GΔ1–307 suggests that the appearance of puromycin reactivity is due to partial translocation, which is restricted to the 50S subunit, and that the fragment is not able to bring about the rearrangement on the 30S subunit and tRNA^{fMet} release from the ribosome.

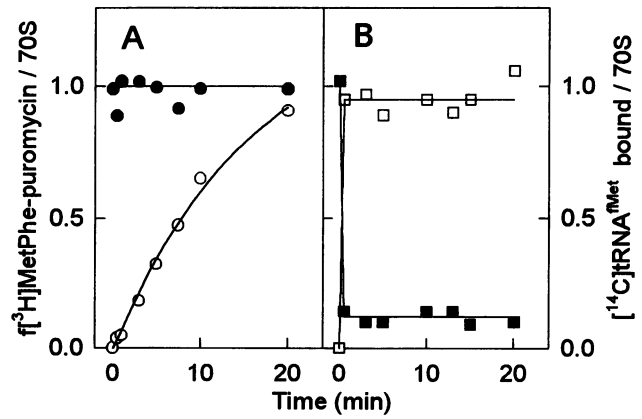

FIG. 3. Partial translocation by EF-GΔ1–307 assayed by the release of [^{14}C]tRNA^{fMet} from the P site. Pretranslocation complexes (10 pmol; 0.4 μM) in buffer A programmed with MFT-mRNA and containing [^{14}C]tRNA^{fMet} in the P site and f[^{3}H]Met[^{14}C]Phe-tRNA^{Phe} in the A site were incubated with (*A*) EF-GΔ1–307 or (*B*) EF-G in the presence of excess [^{14}C]Phe-tRNA^{Phe} and EF-Tu·Ts. Incubation with EF-GΔ1–307 (2 pmol; 0.08 μM) (●, ○) or EF-G (■, □) was at 37°C in 25 μl. At the indicated times, puromycin (1 mM) was added and the incubation continued for 10 s. Puromycin reactivity in mock-treated samples amounted to <5% and was subtracted. ● and ■, Ribosome-bound [^{14}C]tRNA^{fMet}; ○, and □, f[^{3}H]MetPhe-puromycin.

Assaying Translocation by A-Site Binding and Peptide Analysis. The study was extended to the translocation of A site-bound fMetPhe-tRNA^{Phe} on ribosomes programmed with heteropolymeric mRNA and carrying tRNA^{fMet} in the P site. Compared to the poly(U) system, mRNA-programmed ribosomes have the advantage that the extent of spontaneous translocation (16) is very low, even at 37°C; an additional advantage is the high rate of the puromycin reaction, which is completed within 10 s. By measuring the reactivity toward puromycin, the position of the 3′ end of the peptidyl-tRNA in either the A site (unreactive) or the P site (reactive) on the 50S ribosomal subunit was determined. In addition, translocation on the 30S subunit was followed by the binding of EF-Tu·GTP·Phe-tRNA^{Phe} to the A site vacated by the displacement of the peptidyl-tRNA and subsequent tripeptide formation. The latter is the most reliable measure of the status of the 30S A site with respect to tRNA binding. The lack of tripeptide formation is indicative of peptidyl-tRNA still being bound to the 30S A site, provided one can exclude that the inhibition is due to the EF-G fragment remaining bound after the displacement of the peptidyl-tRNA. This condition is met when there is turnover.

When an excess of the pretranslocation complex was incubated with EF-GΔ1–307, a slow increase of the puromycin reactivity up to the level obtained with intact EF-G was observed, indicating the translocation of the peptidyl end of fMetPhe-tRNA^{Phe} on the 50S subunit (Fig. 4). At the same time, the peptide analysis showed that, in the reaction with EF-GΔ1–307, only the dipeptide, fMet[^{14}C]Phe, was present, indicating that a second molecule of Phe-tRNA^{Phe} could not bind and form tripeptide. In contrast, in the control with intact EF-G, the tripeptide fMet[^{14}C]Phe[^{14}C]Phe was formed in nearly quantitative yield. Qualitatively the same result was obtained with poly(U)-programmed ribosomes carrying tRNA^{Phe} in the P site and AcPhe$_2$-tRNA^{Phe} in the A site—that is, the appearance of full puromycin reactivity of AcPhe$_2$-

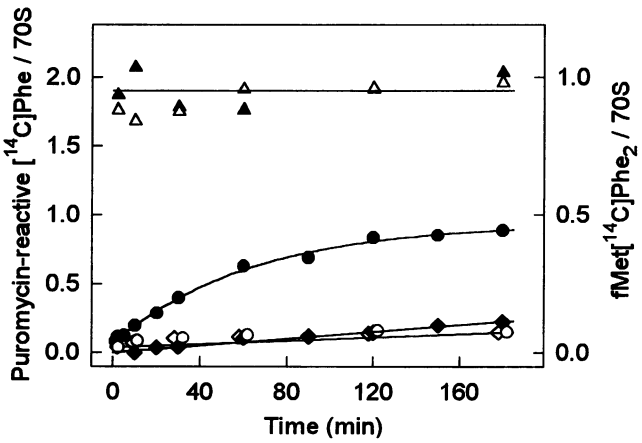

FIG. 4. Partial translocation by EF-GΔ1–307 of peptidyl-tRNA on ribosomes programmed with MFF-mRNA. Pretranslocation ribosomes carrying f[^{3}H]Met[^{14}C]Phe-tRNA^{Phe} in the A site (10 pmol; 0.4 μM) were incubated with EF-GΔ1–307 (●, ○) or with EF-G (▲, △), or without added protein (◆, ◇) in the presence of excess [^{14}C]Phe-tRNA^{Phe} and EF-Tu·EF-Ts, and puromycin-reactive [^{14}C]Phe (solid symbols) and the total amount of fMet[^{14}C]Phe$_2$ (open symbols) were determined. Pretranslocation ribosomes were prepared as in Fig. 3, except that f[^{3}H]Met-tRNA^{fMet} was used. Ninety-five percent of the ribosomes had bound f[^{3}H]Met[^{14}C]Phe-tRNA^{Phe} to the A site. Incubation with EF-G or EF-GΔ1–307 (2 pmol; 0.08 μM) was at 37°C in 25 μl. At the indicated times, puromycin (1 mM) was added and the incubation continued for 10 s.

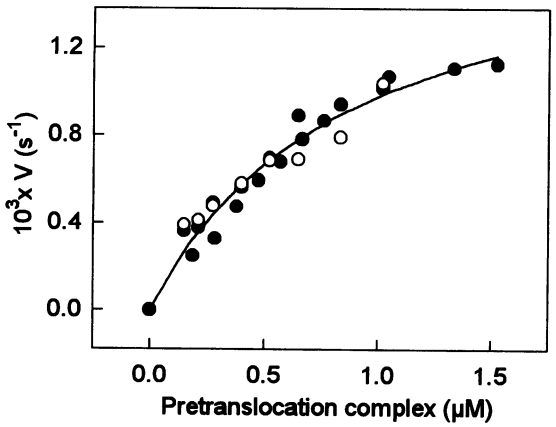

FIG. 5. Michaelis–Menten titration of EF-GΔ1–307 with pretranslocation complex. EF-GΔ1–307 (2 pmol) alone (●) or together with the same amount of EF-GΔ308–703 (○) was incubated for 20 min (initial rate conditions) with increasing amounts of pretranslocation complex and the puromycin reactivity measured. k_{cat} and K_m were estimated by fitting to the data the Michaelis–Menten equation. The pretranslocation complex was prepared and the assay performed as in Fig. 4, except that MFT–mRNA (coding sequence AUGUUUACG...) was used and [^{14}C]Phe-tRNA^{Phe} was added equimolar to ribosomes (0.5 μM) to avoid any tripeptide formation.

tRNA^{Phe} and no tripeptide formation upon incubation with EF-GΔ1–307 (data not shown).

It is to be noted that the amount of puromycin-reactive ribosomes formed exceeded the amount of EF-G fragment present several-fold (Fig. 4). This means that the truncated EF-G performed a turnover reaction, implying (*i*) that the inaccessibility of the A site was not due to the protein remaining bound to the ribosome, and (*ii*) that the puromycin-reactive intermediate translocation state of the ribosomes, brought about by the action of EF-GΔ1–307, was stable without the protein bound to it. Thus, we conclude that EF-GΔ1–307 brought about translocation on the 50S ribosomal subunit, in that it promoted the transfer of the 3′ end of fMetPhe–tRNA^{Phe} to the P site, thereby rendering it reactive toward puromycin. The remainder of the fMetPhe–tRNA^{Phe} molecule, that is the anticodon arm bound to the mRNA on the 30S ribosomal subunit, however, apparently was not moved out of the 30S A site—just like tRNA^{fMet} was not moved out of the 30S P site—thus blocking further binding of Phe-tRNA^{Phe} and subsequent elongation cycles.

K_m and k_{cat} of Partial Translocation. When the fragment EF-GΔ1–307 was titrated with increasing amounts of the pretranslocation complex, the initial rate of partial translocation showed saturating behavior (Fig. 5), yielding $K_m = 0.8 \pm 0.2$ μM and $k_{cat} = (2 \pm 0.5) \cdot 10^{-3} \cdot s^{-1}$. The corresponding parameters for complete translocation catalyzed by intact EF-G·GTP are about 0.15 μM (data not shown) and 50 s^{-1} (17), respectively. Hence, the slowness of the reaction brought about by the fragment, compared to intact EF-G, is mainly due to a 10^4-fold decrease in k_{cat}, rather than to the small decrease in the affinity of binding to the ribosome. As in the poly(U) system, the activity of EF-GΔ1–307 was not stimulated by the presence of the isolated G domain, EF-GΔ308–703 (Fig. 5).

DISCUSSION

The intermediate state of translocation identified by the present experiments resembles the hybrid A/P state of peptidyl-tRNA which, according to chemical footprinting data, forms when the peptidyl residue is transferred from the P site to the A site-bound tRNA (18). In this state, a P-site location is imposed to the 3′ end of the peptidyl-tRNA remaining in the A site and, at the same time, an E-site location to the flexible 3′ end of the P site-bound deacylated tRNA. However, in the A/P state, the peptidyl residue of the A site-bound AcPhe-tRNA is not reactive toward puromycin; also, the footprinting pattern on 23S rRNA is somewhat different in the A/P state as compared to the P/P state, notably at residue A2602 (18). Therefore, the A/P state is different both functionally and structurally from, and probably precedes, the intermediate translocation state described here. In the latter state, the peptidyl end of the A site-bound tRNA has been transferred into a puromycin-reactive location, presumably by fixation in the 50S P site that is induced by EF-G, or by EF-GΔ1–307.

The 3′ ends of both P site and E site-bound tRNAs appear to interact with 23S rRNA (19), and a functionally essential base pair between the penultimate base in the terminal CCA sequence of P site-bound peptidyl-tRNA with G2252 of 23S rRNA has been demonstrated recently (20). It is likely that EF-G or EF-GΔ1–307, by binding to the pretranslocation ribosome, promotes the translocation of the 3′ ends of both tRNAs by making available for the interaction the respective region of 23S rRNA (21). The present results show that the binding to the ribosome of the EF-G fragment lacking the G domain is sufficient to bring about the rearrangement of the 50S subunit, although the reaction is very slow, k_{cat} being about 10^4-fold lower than the k_{cat} of the EF-G-catalyzed (full) translocation. According to preliminary single-turnover results (data not shown), the turnover rate of the partial reaction is limited by the rearrangement step rather than by the dissociation of the protein. Nevertheless, the observation that, in the intermediate translocation state, the peptidyl–tRNA reacts very rapidly (within 10 s) with puromycin, as in the posttranslocation state induced by EF-G and GTP, strongly suggests that it represents a true intermediate of translocation, rather than the product of an aberrant translocation.

The finding that EF-GΔ1–307 is active in promoting partial translocation, while EF-GΔ1–474, which lacks domains I–III and comprises only domains IV and V, is not (Fig. 2), probably means that domains II and/or III are important either for ribosome binding or for the activity on the ribosome. Binding experiments performed by centrifugation showed the binding of EF-GΔ1–307 to vacant ribosomes, whereas binding of the smaller fragment could not be detected (data not shown). Thus, domains II and/or III in fact seem to be necessary for the binding, in keeping with results reported for EF-Tu, suggesting that domain II is involved in ribosome binding (22, 23). In addition, there is evidence suggesting that also domain V is important for ribosome binding of EF-G (8, 9). On the other hand, the similar affinity of EF-G and EF-GΔ1–307 for the ribosome indicates that the G domain does not form strong interactions with the ribosome.

It has been discussed that domain IV of EF-G, which is the most prominent structural difference between EF-G and EF-Tu (5, 6), may have an important role in translocation catalysis on the small ribosomal subunit (3, 4). The proposal is based upon the findings that ADP-ribosylation of a residue in homologous position inactivates eukaryotic EF-2 (14, 15) and that kanamycin resistance mutations are located in domain IV (8). The idea has been reinforced by the finding that in the tertiary structure of EF-Tu·GTP[β,γ-NH]·Phe-tRNA^{Phe} the anticodon domain occupies the same position as domain IV in EF-G and nearly exactly mimics both its shape and charge distribution (7). The present results, however, show that the presence of domain IV in EF-GΔ1–307 is not sufficient to induce translocation on the 30S subunit. A possible explanation is that, for EF-G to be fully active, the presence of the G domain is necessary to provide intramolecular interactions with other domains of EF-G, notably with the functionally essential domain V (8, 9) and with domain II. The importance for EF-G function of intramolecular interactions of domain I with those two domains is underlined by mapping at the respective interface sides the mutations conferring fusidic acid

resistance to EF-G (10). The modulation of those interactions by GTP binding and hydrolysis in the G domain may have a major influence on the function of EF-G, for instance by determining the position of domain IV relative to the other domains.

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