The Elongation Factor Tu Binds Aminoacyl-tRNA in the Presence of GDP*

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Escherichia coli elongation factor (EF-Tu) binds aminoacyl-tRNAs (aa-tRNA) not only in the presence of GTP but also in the presence of GDP. Complex formation leads to a protection of the aa-tRNA against non-enzymatic deacylation and digestion by pancreatic ribonuclease, as well as to a protection of EF-Tu against proteolysis by trypsin. The equilibrium constant for the binding of Phe-tRNAPhe to EF-Tu-GDP has been determined to be $0.7 \times 10^{2} \text{ M}^{-1}$, which is 2 orders of magnitude lower than the equilibrium constant for Phe-tRNAPhe binding to EF-Tu-GTP.

In the presence of kirromycin, aminoacyl-tRNA binding to EF-Tu-GDP is not affected as much: Phe-tRNAPhe is bound with an equilibrium constant of $3 \times 10^{5} \text{ M}^{-1}$. While there is also a measurable interaction between EF-Tu-GTP and tRNA, such an interaction cannot be detected with EF-Tu-GDP and tRNA, not even at millimolar concentrations.

A so far undetected complex formation between aminoacyl-tRNA and EF-Tu-GTP in the presence of puromycin, however, could be detected.

The results are discussed in terms of the structural requirements of ternary complex formation and in the light of proofreading schemes involving A-site binding on the E. coli ribosome.

Reviews on protein biosynthesis state that the Escherichia coli elongation factor Tu interacts with aminoacyl-tRNA only when complexed with GTP (e.g. Refs. 1–3). This statement is based on results obtained in the late 1960s by Gordon (4), Skoulitchi et al. (5), and Ravel et al. (6), who have carried out nitrocellulose filtration and gel permeation chromatography experiments on mixtures of EF-Tu, aminoacyl-tRNA, and GTP or GDP, respectively, from which it was concluded that only EF-Tu-GTP, not, however, EF-Tu-GDP, forms a ternary complex with aminoacyl-tRNAs. This result has been confirmed numerous times by various laboratories, including our respective ones, since then. It is nevertheless a somewhat surprising result from three view points. Firstly, there does not seem to be a biological necessity for a rigorous exclusion of the EF-Tu-GDP complex from interacting with aminoacyl-tRNAs, since normally the intracellular concentration of GDP exceeds that of GDP so much that EF-Tu is predominantly complexed with GTP. Secondly, although the conformations of EF-Tu-GTP and EF-Tu-GDP are different, as can be demonstrated by various techniques (7–14), the differences probably are not too pronounced because they do not show up when investigated by optical rotatory dispersion or circular dichroism (8, 11). On the other hand, one would like to assume large conformational differences between EF-Tu-GDP and EF-Tu-GTP in order to rationalize the inactivity of EF-Tu-GDP regarding formation of a ternary complex with aminoacyl-tRNAs, since it is an interaction of two macromolecules that one is examining, which comprises many points of contact. Unless the conformational change from EF-Tu-GTP to EF-Tu-GDP is such that most of these contacts cannot be formed anymore, one would expect a reduced but measurable affinity between EF-Tu-GDP and aminoacyl-tRNAs. Thirdly, a complex between EF-Tu-GDP and aminoacyl-tRNAs has been implicated in the elongation cycle as being necessary for proofreading of the codon-anticodon interaction on ribosomes (37, 38). Such a complex, however, may be produced only on the ribosome, which may stabilize the otherwise unstable complex. These considerations, as well as recent results obtained by Ringer and Chladek (15) and Jonak et al. (16, 17), according to which EF-Tu-GDP interacts with $2'3'\text{-}O$-aminoacyl oligonucleotides as analogues of the 3' terminus of aminoacyl-tRNAs, and preliminary results of neutron diffraction studies have led us to reinvestigate the problem of whether EF-Tu-GDP can form a ternary complex with aminoacyl-tRNAs. As a corollary to this problem, the finding that aminoacyl-tRNAs do not bind to EF-Tu-GTP in the presence of puromycin (18) also needs to be questioned, since a variety of experiments indicate that EF-Tu-GTP in the presence of puromycin behaves like EF-Tu-GDP (18, 19).

**Experimental Procedures**

**Materials**

5'-GDP, 5'-GTP, phosphoenolpyruvate, pyruvate kinase (rabbit muscle), ribonuclease A (bovine pancreas), and tRNA$^{\text{E}}$ were purchased from Boehringer Mannheim. tRNA$^{\text{E}}$ and tRNA$^{\text{P}}$ were isolated from E. coli B (Plenum Scientific) by chromatography on BD-cellulose, DEAE-Sepharose CI-6B, and Sepharose 4B to a specific

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1 The abbreviations used are: EF-Tu, Escherichia coli elongation factor Tu; aa, aminoacyl.
acceptor activity of 1.36 and 1.25 mmol/Amg, respectively. [3H]EF-Tu-GDP, t-[U-14C]phenylalanine, t-[phenyl[2-3H]alanine, and [U-14C]valine were obtained from Amerham-Buchler.

Methods

**Gel Permeation Chromatography**—Gel permeation chromatography was carried out at 4 °C on AcA 44 (LKB) columns (0.4 × 31 cm), equilibrated with 75 mM Tris-HCl, pH 7.4, 75 mM NH₄Cl, 15 mM MgCl₂, 7.5 mM 1,4-dithioerythritol, GDP or GTP, phosphoenolpyruvate, pyruvate kinase, and [3H]Phe-tRNA in the presence of EF-Tu. GDP or EF-Tu. GTP as specified in the text. Fifty-µl samples containing EF-Tu, Phe-tRNA, GDP, or GTP were applied to the column. Fractions comprising one drop were collected and assayed for Phe-tRNA and EF-Tu content. Phe-tRNA content was determined by measuring the acid-precipitable radioactivity in a way similar to that described for the hydrolysis protection experiments (see below). EF-Tu content was determined using the ninhydrin filter assay (27) and spectrophotometrically with ε₉₅₀₅₅ = 23,900 (23).

**Hydropolysis Protection**—Hydropolysis protection experiments (22) were carried out at 25 °C in 110 mM Tris-HCl, pH 8.4, 75 mm NH₄Cl, 15 mm MgCl₂, 7.5 mm 1,4-dithioerythritol, and EF-Tu [3H]Phe-tRNA, GDP or GTP, phosphoenolpyruvate, pyruvate kinase in the absence or presence of EF-Tu. GDP or EF-Tu. GTP as indicated. Twenty-µl aliquots were withdrawn and applied to Whatman No. 3MM filter discs, which were immediately immersed into cold 10% trichloroacetic acid and subsequently washed with 5% trichloroacetic acid and then with an ethanol/ether mixture (1:1, v/v). The radioactivity of the dried filter discs was measured in a liquid scintillation counter. For a quantitative analysis, hydropolysis progress curves were analyzed as described in Ref. 22 using an analogue computer (RA 741, ABG-Telefunken).

**Tryptic Digestion of EF-Tu**—Various amounts of EF-Tu as either EF-Tu-GDP or EF-Tu-GTP were digested in the absence or presence of uncharged or charged tRNA with 3 µg of trypsin in 97 mM 4-[2-hydroxyethyl]-1-piperazinepropanesulfonic acid, 20 mM NaOH, 10 mM MgCl₂, pH 7.5 at 25 °C. At various time intervals, a 10-µl aliquot was taken from the 100-µl reaction mixture and pipetted into 10 µl of a 70 µg/ml soybean trypsin inhibitor solution in 1 mM HCl. 7.5 µg of EF-Tu were subjected to electrophoresis on polyacrylamide gels in the presence of sodium dodecyl sulfate (21). The gels were stained with Coomassie brilliant blue, destained, and scanned with a Beckman Model CDS-200 computing densitometer. The areas under the peaks for uncleaved EF-Tu and for fragments A and B (M₀ = 37,800 and 36,000, respectively) were integrated automatically. The trypptic digestion of EF-Tu-GTP in the presence of pyruvate kinase was carried out and analyzed as described in the legend to Fig. 10.

Ribonuclease Digestion—One hundred thirty µg of [3H]Val-tRNA in the presence or absence of either EF-Tu-GDP or EF-Tu-GTP in 97 mM 4-[2-hydroxyethyl]-1-piperazinepropanesulfonic acid, 20 mM NaOH, 10 mM MgCl₂, pH 7.3 at 25 °C, was incubated with 6 µg/ml of ribonuclease A at 0 °C in a total volume of 30 µl. At various time intervals, 5-µl aliquots were applied to glass fiber filters (GF/C, Whatman) which were immediately immersed in 5% trichloroacetic acid and then washed consecutively with 5% trichloroacetic acid, ethanol, ethanol/ether (1:1, v/v), and ether, dried, and counted for precipitated [3H]Val-tRNA.

For all gel filtration or protection experiments in which the affinity of EF-Tu-GDP or EF-Tu-GTP toward aminoacyl-tRNA was compared, it was demonstrated in control experiments that the presence of phosphoenolpyruvate or pyruvate kinase did not produce artifacts.

**RESULTS AND DISCUSSION**

The original experiments, in particular the gel permeation chromatography experiments (4, 5), which led to the conclusion that EF-Tu-GDP does not interact with aminoacyl-tRNA, were carried out under conditions which would not have allowed detection of a weak interaction. It was manda-
complex and the EF-Tu-GTP-pulvomycin complex in binding aminoacyl-tRNAs, we carried out three different kinds of protection experiments. 1) EF-Tu-GTP protects the ester bond between the aminoacyl moiety and the tRNA against hydrolysis, an effect that can be used for the determination of the equilibrium constant of ternary complex formation (22, 23). 2) Aminoacyl-tRNAs protect EF-Tu-GTP against digestion with trypsin (29). EF-Tu-GDP and EF-Tu-GTP are rapidly cleaved by trypsin at amino acid residues Arg-44 and Arg-58 (21, 35). Concomitant to the proteolytic degradation, the aminoacyl-tRNA binding activity is diminished. If EF-Tu is complexed with aminoacyl-tRNA, the rate of the proteolytic degradation is considerably reduced (29, 34). 3) It has been reported by Jekowsky et al. (30), Knowlton and Yarus (31), and Tanada et al. (32) that EF-Tu-GTP is able to protect aminoacyl-tRNA from digestion by pancreatic ribonuclease and that this can be used to study in detail the interaction between elongation factor Tu and aminoacylated tRNA.

A prerequisite for the use of these techniques for the purpose of investigating the binding of aminoacyl-tRNA to EF-Tu-GDP and to EF-Tu-GTP in the presence of pulvomycin is of course that the mutual protection is also observed with EF-Tu-GDP or with EF-Tu-GTP in the presence of pulvomycin, respectively.

Fig. 3 shows a set of hydrolysis protection experiments with EF-Tu-GDP and with EF-Tu-GTP as reference. At low concentrations of both EF-Tu and Phe-tRNA<sup>Phe</sup>, no protection is seen with EF-Tu-GDP, while EF-Tu-GTP fully protects the aminoacyl-tRNA (Fig. 3A). At higher concentrations of EF-Tu, a protective effect is also seen with EF-Tu-GDP (Fig. 3C). The quantitative evaluation of the hydrolysis protection experiments yields an equilibrium constant of $K_{asso} = 0.7 \times 10^5$ M for the binding of Phe-tRNA<sup>Phe</sup> to EF-Tu-GDP. The statement, hence, that EF-Tu-GDP does not protect aminoacyl-tRNA from deacylation (22) is not true for concentrations of EF-Tu and aminoacyl-tRNA higher than approximately 10 $\mu$M. As shown in Fig. 4, a similar correction must be applied to the result that EF-Tu-GDP, when complexed with the antibiotic kirromycin, does not protect aminoacyl-tRNAs from deacylation (33). This is true only for concentrations of EF-Tu-GDP and aminoacyl-tRNA below approximately 1 $\mu$M. Kirromycin, which lowers the protective effect that EF-Tu-GTP affords to the aminoacyl-tRNA (33), affects EF-Tu-GDP in a similar manner (Fig. 4C). The quantitative evaluation of the hydrolysis protection experiments yields an equilibrium constant $K_{asso} = 3 \times 10^5$ M for the binding of Phe-tRNA<sup>Phe</sup> to the EF-Tu-GDP complex in the presence of kirromycin. Fig. 5 shows that a similar analysis cannot be carried out for the hydrolysis protection experiments in the presence of pulvomycin. Due to the action of the antibiotic, aminoacyl-tRNA is prone to hydrolysis in the presence of EF-Tu-GTP exactly as aminoacyl-tRNA alone (18). The plots of residual amounts of aminoacyl-tRNA versus time are congruent under both conditions. Without pulvomycin, on the other hand, no substantial hydrolysis of aminoacyl-tRNA can be measured in the presence of EF-Tu-GTP (20, 22). Apparently, EF-Tu is hindered by the antibiotic from interacting properly with aminoacyl-tRNA and thereby protecting the amino acid-charged 3'-end against deacylation. Although the time course of hydrolysis in the presence of pulvomycin is very similar to the one with aminoacyl-tRNA alone, the gel permeation experiment shown in Fig. 2 demonstrates that

**Figs. 2-4.** Hydrolysis protection experiments with EF-Tu-GDP and EF-Tu-GTP in the presence of kirromycin. Incubations were carried out as described in the legend to Fig. 3, with the modification that 215 $\mu$M kirromycin was present in the assay.
EF-Tu-GDP BINDS AMINOACYL-tRNAs

By trypsin in the presence of aminoacyl-tRNA, which is shown, the rate, there is another difference in the way EF-Tu is cleaved. The proteolysis of EF-Tu-GDP for aminoacyl-tRNA is much lower than for EF-Tu. GTP, is cleaved, the proteolytic product, fragment A (Mr = 27,500), remains uncleaved in the absence of tRNA’s’ in Fig. 6A.

An indication that the interaction of EF-Tu-GDP with aminoacyl-tRNA has otherwise similar structural requirements as that of EF-Tu-GTP can be deduced from the observation that Phe-tRNA_Phe under similar concentrations has a higher protective effect than Val-tRNA.Val (Fig. 8A). For EF-Tu-GTP, the binding constant for Phe-tRNA_Phe has been investigated. When EF-Tu.GDP in the absence or presence of 1 eq of aminoacylated tRNA, however, 75% of the EF-Tu remains uncleaved. In the presence of 1 eq of aminoacylated tRNA, however, 75% of the EF-Tu.GTP remains uncleaved. Besides this difference in reaction rate, there is another difference in the way EF-Tu is cleaved by trypsin in the presence of aminoacyl-tRNA, which is shown in Fig. 7. In Fig. 7A, the densitometric tracing of a gel loaded with tryptically digested EF-Tu.GTP shows the characteristic peaks for EF-Tu (Mr = 43,200), fragment A* (Mr = 37,800), and fragment A (Mr = 36,000) (21). The product from a tryptic digestion of EF-Tu.GTP in the presence of Val-tRNA^Val in Fig. 7B does not show a peak for the intermediate cleavage product. This means that we have to discuss a different pathway for the tryptic digestion of the ternary complex EF-Tu.GTP-an-tRNA, where the protein is either not cleaved at Arg-44 or is cleaved too fast to be detected as an intermediate product of the reaction. This is currently under investigation. When EF-Tu.GDP in the absence or presence of 1 eq of aminoacyl-tRNA, under otherwise identical reaction conditions as for EF-Tu.GTP, is cleaved, the protective effect of aminoacylated tRNA is much less pronounced (Fig. 6B). This is independent evidence for the finding that EF-Tu.GDP can bind aminoacyl-tRNAs, but that the affinity of EF-Tu.GDP for aminoacyl-tRNA is much lower than for EF-Tu.GTP. At higher concentrations of EF-Tu.GDP and aminoacyl-tRNA, however, the protective effect of Val-tRNA^Val on the tryptic cleavage of EF-Tu.GDP is much more pronounced (Fig. 8A). By comparison of the data shown in Fig. 8A and those performed with EF-Tu.GTP under more dilute conditions (data not shown) those shown in Fig. 6A, we can estimate that the binding affinity for Val-tRNA^Val is about 50 times lower for EF-Tu.GDP than for EF-Tu.GTP. The tryptic cleavage of the EF-Tu.GDP-an-tRNA complex is different from the EF-Tu.GTP complex in another aspect, as shown in Fig. 8B: in that the cleavage reaction of the protein proceeds at Arg-44 and Arg-56, as can be seen from the gel tracing, where we have a peak pattern as for uncleaved EF-Tu, i.e., the sequence of aminoacylated EF-Tu, i.e., where the intermediate cleavage product, fragment A*, is seen (cf. Fig. 7A and Ref. 35).

FIG. 5. Hydrolysis protection experiments with EF-Tu.GDP and EF-Tu.GTP in the presence of pulvomycin. Incubations were carried out as described in the legend to Fig. 3, with the modification that 337 nM pulvomycin was present in the assay.

pulvomycin rather impairs, but does not abolish aminoacyl-tRNA binding to EF-Tu.GTP. That the lack of protection against decapacitation of aminoacyl-tRNA is not a consequence of the lack of binding to EF-Tu.GTP will be demonstrated again later (see below).

The finding that aminoacyl-tRNAs also cleave with EF-Tu.GDP can be shown by another kind of protection experiment in which the tryptically-catalyzed cleavage of EF-Tu is investigated. The results are given in Figs. 6-9. Fig. 6A shows the time course of the cleavage reaction of 69 nM EF-Tu.GDP with 12 nM trypsin which demonstrates that after 4 min less than 10% of the EF-Tu remains uncleaved. In the presence of 1 eq of aminoacylated tRNA, however, 75% of the EF-Tu.GTP remains uncleaved. Besides this difference in reaction rate, there is another difference in the way EF-Tu is cleaved by trypsin in the presence of aminoacyl-tRNA, which is shown in Fig. 7. In Fig. 7A, the densitometric tracing of a gel loaded with tryptically digested EF-Tu.GTP shows the characteristic peaks for EF-Tu (Mr = 43,200), fragment A* (Mr = 37,800), and fragment A (Mr = 36,000) (21). The product from a tryptic digestion of EF-Tu.GTP in the presence of Val-tRNA^Val in Fig. 7B does not show a peak for the intermediate cleavage product. This means that we have to discuss a different pathway for the tryptic digestion of the ternary complex EF-Tu.GTP-an-tRNA, where the protein is either not cleaved at Arg-44 or is cleaved too fast to be detected as an intermediate product of the reaction. This is currently under investigation. When EF-Tu.GDP in the absence or presence of 1 eq of aminoacyl-tRNA, under otherwise identical reaction conditions as for EF-Tu.GTP, is cleaved, the protective effect of aminoacylated tRNA is much less pronounced (Fig. 6B). This is independent evidence for the finding that EF-Tu.GDP can bind aminoacyl-tRNAs, but that the affinity of EF-Tu.GDP for aminoacyl-tRNA is much lower than for EF-Tu.GTP. At higher concentrations of EF-Tu.GDP and aminoacyl-tRNA, however, the protective effect of Val-tRNA^Val on the tryptic cleavage of EF-Tu.GDP is much more pronounced (Fig. 8A). By comparison of the data shown in Fig. 8A and those performed with EF-Tu.GTP under more dilute conditions (data not shown) those shown in Fig. 6A, we can estimate that the binding affinity for Val-tRNA^Val is about 50 times lower for EF-Tu.GDP than for EF-Tu.GTP. The tryptic cleavage of the EF-Tu.GDP-an-tRNA complex is different from the EF-Tu.GTP complex in another aspect, as shown in Fig. 8B: in that the cleavage reaction of the protein proceeds at Arg-44 and Arg-56, as can be seen from the gel tracing, where we have a peak pattern as for uncleaved EF-Tu, i.e., where the intermediate cleavage product, fragment A*, is seen (cf. Fig. 7A and Ref. 35).

FIG. 6. Time course of trypsin cleavage of EF-Tu in the presence and absence of aminoacyl-tRNA. The 100-pl reaction mixture contained 69 nM EF-Tu.GTP (A) or EF-Tu.GDP (B) and, where indicated, 65 nM [^3]HVal-tRNA^Val. Digestion with 3 nM of trypsin was performed at 25 °C. At the indicated times, 10-pl samples were removed, pipetted into 10 pl of a solution containing 70 nM/ml of soybean trypsin inhibitor, and treated as described under "Methods." The polypeptide band corresponding to uncleaved EF-Tu and the high molecular weight fragments A* and A were quantitated as described under "Methods." Data are presented as per cent EF-Tu remaining in the absence (◆) and presence (○) of aminoacyl-tRNA.

FIG. 7. Cleavage pattern of EF-Tu.GTP in the presence and absence of aminoacyl-tRNA. Densitometric tracing of the gel which was loaded with the 1-min tryptic digestion product of EF-Tu.GTP in the absence (A) and presence (B) of valyl-tRNA^Val. These scans were used for the data points indicated in Fig. 6 by an arrow. The three peaks correspond to EF-Tu, fragment A*, and fragment A (from left to right) (21). OD, optical density.

FIG. 8. Cleavage of EF-Tu.GDP in the absence and presence of aminoacyl-tRNA. EF-Tu.GDP was digested with 30 nM/ml of trypsin at 25 °C under the conditions described in the legend to Fig. 6. A, time course of the cleavage reaction carried out with 60 nM EF-Tu.GDP in the absence ef aa-tRNA (×) or 140 nM EF-Tu.GDP in the presence of either 800 nM (C) or 230 nM (○) [^3]HVal-tRNA^Val. B, densitometric tracing of the gel loaded with the 1-min tryptic digestion product from the reaction indicated in A by an arrow. OD, optical density.
EF-Tu-GDP Binds Aminoacyl-tRNAs

The affinity of aminoacyl-tRNA for EF-Tu. The rate of EF-Tu-GDP conversion to fragment A was determined to be much higher than for Val-tRNA\textsuperscript{Val} (20, 21) and our experiments also show that for EF-Tu-GDP we have the same order of affinities.

That uncharged tRNA can form a complex with EF-Tu-GTP, as was first shown by Shulman et al. (36), on the basis of NMR experiments with EF-Tu-GTP and tRNA\textsuperscript{Val}, both in millimolar concentrations, is also demonstrated by trypsin digestion experiments. Fig. 9A shows that uncharged tRNA\textsuperscript{Val} slows down the trypsin-catalyzed cleavage of EF-Tu-GTP. It can be seen in Fig. 9B that uncharged tRNA\textsuperscript{Val} does not influence the cleavage of EF-Tu-GDP, since complex formation between EF-Tu-GDP and tRNA presumably is much weaker than that between EF-Tu-GTP and tRNA.

These experiments show that for a strong interaction to occur, EF-Tu must be complexed with GTP and the tRNA must be aminoacylated. When either one requirement is relaxed, there is still an appreciable interaction between protein and nucleic acid and only when both requirements for strong interaction are absent can an interaction not be detected anymore.

We have also used the diminished susceptibility of the aminoacyl-tRNA-EF-Tu-GTP complex, as compared to EF-Tu-GTP toward proteolysis by trypsin to investigate the action of pulvomycin on ternary complex formation. If pulvomycin were to prevent EF-Tu from interacting with aminoacyl-tRNA, the amino acid residues Arg-44 and Arg-58 should be accessible to trypsin, irrespective of the presence or absence of aminoacyl-tRNA. Our results clearly show, however, that in the presence of aminoacyl-tRNA no fragment A is formed, despite the presence of pulvomycin (Fig. 10). Virtually no difference was found in the resistance of aminoacyl-tRNA-EF-Tu-GTP toward proteolysis in the absence (B) and presence (C) of pulvomycin under conditions in which, in the absence of aminoacyl-tRNA (A), a considerable amount of the elongation factor was converted to fragment A.

The results from the trypsin degradation experiment (Fig. 10), as well as the gel filtration experiment (Fig. 2), demonstrate that binding of aminoacyl-tRNA to EF-Tu does occur in the presence of pulvomycin also. The apparent discrepancy between these results and the absence of the protection against deacylation of aminoacyl-tRNA (18) and Fig. 5) is reconciled by the assumption that pulvomycin eliminates the interaction of the amino acid bearing 3'-end of aminoacyl-tRNA and the corresponding domain of the aminoacyl-tRNA binding site on EF-Tu. The affinity of aminoacyl-tRNA for the other domain of the aminoacyl-tRNA binding site—with residues Arg-44 and Arg-58 being part of it—is not affected by the antibiotic. Such a specific inactivation of a differentiable part of the aminoacyl-tRNA binding site could explain both experimental findings. In light of this interpretation, the failure to detect ternary complex formation in experiments involving filtration on nitrocellulose filters or by conventional gel chromatography becomes comprehensible. By abolishing essential sites of the interaction, pulvomycin weakens the affinity of aminoacyl-tRNA for EF-Tu-GTP. That complexes of the aminoacyl-tRNA and EF-Tu-GTP occur, even in the presence of pulvomycin, however, has been shown unequivocally in the experiments described above.

Last but not least, the complex formation between EF-Tu-GDP and aminoacyl-tRNA can be demonstrated by nuclease protection experiments. Since with these experiments we probe a similar site on the aminoacyl-tRNA, as with the hydrolysis protection experiments, namely the aminoacylated terminus of the tRNA, one should expect similar results. Fig. 11 shows that free Val-tRNA\textsuperscript{Val} under our conditions is completely digested within 15 s, while the addition of 1 or 2 eq of EF-Tu-GTP protects between 70 and 90% of the tRNA.
Tu. GDP and aminoacyl-tRNA does indeed form, but that it
pany the stringent response
regulatory function: it will prevent aminoacyl residues from
being incorporated into a growing polypeptide chain under
Tu. GDP is
factor-mediated A-site binding of aminoacyl-tRNAs to the
presumably is an obligatory intermediate in the process of the
existence of such an intermediate has not been demonstrated as yet, although an elevated GTP hydrolysis has been measured during the interaction of noncognate EF-Tu-GTP-
aminacyl-tRNA complexes and poly(U) programmed ribo-
somes (38), as well as during the interaction of EF-Tu-GTP-
Phe-tRNA complexes and AUGU2 or AUGU programmed ribosomes having a fMet-tRNA bound in the P-site (41). The elevated GTP hydrolysis is the necessary requirement of a kinetic proofreading scheme (37), but it can be proven only by the identification of the postulated intermediates and the assessment of the rate constants that characterize their conversion. We have shown here that an EF-Tu-GDP-aminocy-
acyl-tRNA complex exists in the absence of a programmed ribosome. It remains to be established whether the ribosome can bind such a complex, furthermore, whether and to what extent the ribosome affects the stability of this complex. It is also conceivable that the ribosome does not interact with such a complex, unless it is produced in situ (A-site).

FIG. 11. Time course of a ribonuclease digestion of
Val-tRNA\(^{\text{Val}}\) in the presence and absence of EF-Tu. Thirty \(\mu\)l of a 130 \(\mu\)M [\(^{14}C\)]Val-tRNA\(^{\text{Val}}\) solution in the presence (—) and presence of either 130 \(\mu\)M (■) or 260 \(\mu\)M (■) EF-Tu-GTP (A) or in the presence of either 270 \(\mu\)M (□) or 480 \(\mu\)M (□) EF-Tu-GDP (B) were digested with 0.2 \(\mu\)g of pancreatic ribonuclease at 0 °C. At the indicated times, 5-\(\mu\)l samples were removed and analyzed for trichlo-
roacetic acid (TCA)-precipitable radioactive material as described under “Methods.” A trichloroacetic acid-precipitable radioactivity of 100% corresponds to 14,900 cpm.

against digestion. EF-Tu-GDP under identical conditions
indeed has an albeit smaller protective effect on the ribonuclease
digestion reaction, proving again that a complex between EF-
Tu-GDP and aminoacyl-tRNA does indeed form, but that it
has a much lower stability as compared to the EF-Tu-GTP
aminacyl-tRNA complex.

CONCLUSIONS

We have established, by using several different techniques, that EF-Tu-GDP binds aminoacyl-tRNAs. The equilibrium constant for the binding of Phe-tRNA\(^{\text{Val}}\), for example, to EF-
Tu-GDP is 0.7 \(\times\) 10\(^{-7}\) M\(^{-1}\) and, therefore, sufficiently high with the given in vitro concentrations of EF-Tu and aminoacy-
RNAs (39, 42), around 100 \(\mu\)M, to allow complex formation between EF-Tu-GDP and aminoacyl-tRNAs. This will, nevertheless, not happen under normal growth conditions, since the high excess of GTP over GDP will prevent the accumulation of EF-Tu-GDP. Under energy shift down conditions, however, when the GTP concentration is diminished, a considerable amount of the “incorrect” ternary complex, EF-Tu-GDP-
ata-tRNA, might be formed. Presumably, this complex is in-
active in protein biosynthesis and might, therefore, have a regulatory function: it will prevent aminoacyl residues from being incorporated into a growing polypeptide chain under conditions in which this is not desirable. This effect may accompany the stringent response (40) elicited in many prokaryotic organisms upon amino acid starvation, energy shift down, and several other conditions of environmental stress.

The fact that EF-Tu-GDP can form a fairly stable complex
with aminoacyl-tRNAs has to be discussed in another impor-
tant context. The EF-Tu-GDP-aminacyl-tRNA complex
presumably is an obligatory intermediate in the process of the
factor-mediated A-site binding of aminoacyl-tRNAs to the
ribosome, which leads either to stable binding of cognate or
rejection of noncognate aminoacyl-tRNA (38).

Rib-mRNA + EF-Tu-GTP aatRNA

Rib-mRNA + EF-Tu-GDP aatRNA + P

Rib-mRNA + EF-Tu-GDP aatRNA

Rib-mRNA + EF-Tu-GDP aatRNA

Rib-mRNA + EF-Tu-GTP aatRNA

Rib-mRNA + EF-Tu-GDP aatRNA

Rib-mRNA + EF-Tu-GTP aatRNA

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