Interaction of Elongation Factor Tu with the Aminoacyl Transfer Ribonucleic Acid Dimer Phe-tRNA–Glu-tRNA†

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ABSTRACT: The effect of EF-Tu·GTP on the codon–anticodon interaction of AA-tRNA was studied by using as a model system the interaction of AA-tRNAs with complementary anticodons, namely, dimerization between yeast or *Escherichia coli* Phe-tRNA^{Phe} (anticodon GmAA) and *E. coli* Glu-tRNA^{Glu} (anticodon s^{2}UUC) or nonacylated tRNA^{Glu} in the presence or absence of EF-Tu·GTP. The present data indicate that the ternary complexes Phe-tRNA–EF-Tu·GTP and Glu-tRNA–EF-Tu·GTP can form dimers with a binding constant of $(0.9 \pm 0.2) \times 10^6$ M^{-1}, which is identical in magnitude with that of the dimer of the nonacylated tRNAs tRNA^{Phe}–tRNA^{Glu} and also with that of the complex Phe-tRNA–EF-Tu·GTP with nonacylated tRNA^{Glu}. These results show that the anticodon region is not affected by complexation with EF-Tu·GTP; however, this conclusion does not preclude the possibility of structural changes in the anticodon loop that have no effect in energetic terms. In addition, this model codon–anticodon interaction does not stimulate hydrolysis of the GTP bound in the ternary complex.

The function of elongation factor Tu (EF-Tu)[1] in protein biosynthesis is to select elongator AA-tRNAs by forming AA-tRNA–EF-Tu·GTP and to bring this ternary complex to the acceptor site on the mRNA–ribosome complex, (Ravel, 1967; Lucas-Lenard & Haenni, 1968; Ertel et al., 1968; Ono et al., 1969). The mechanism of this binding reaction is unknown. A possible result of the AA-tRNA–EF-Tu·GTP interaction might be that the tertiary structure of AA-tRNA is altered to a conformation that interacts strongly with the codon. On the other hand, the codon–anticodon interaction might weaken the binding of EF-Tu·GTP or stimulate the EF-Tu-dependent GTPase and promote the protein's release from the ribosome.

At present there is little evidence that EF-Tu·GTP binding affects the codon–anticodon interaction. EF-Tu·GTP might be expected not to bind directly with the anticodon, because this region of the nucleic acid must be free to interact with mRNA. In fact, studies on the nuclease-digested complex of AA-tRNA and EF-Tu·GTP have shown that the anticodon loop is not a critical point for EF-Tu·GTP binding (Jekowsky et al., 1977). However, EF-Tu·GTP reduces the optimal Mg^{2+} concentration for the binding of AA-tRNA to the mRNA–ribosome complex (Ravel et al., 1969), and this might be due to a conformational change of the anticodon region of tRNA induced by EF-Tu·GTP binding.

We have undertaken to study the effect of EF-Tu·GTP upon a covenient model of the codon–anticodon interaction, the interaction of AA-tRNAs with complementary anticodons, in order to examine the energetic significance of any anticodon structural changes which take place. It is known that two tRNAs whose codons have base complementarity can form a complex, as first suggested by M. Guêron (Eisinger, 1971; Eisinger & Gross, 1975), and a thorough study of this interaction has been carried out for 21 pairs of tRNAs (Grosjean et al., 1976, 1978).

According to kinetic studies, the dimerization of complementary tRNAs occurs with a single relaxation and therefore does not involve any slow reorganization of the tRNA molecule (Grosjean et al., 1976, 1978). The reaction is relatively specific and restricted to the anticodon loop of the tRNA molecule. The stabilization effect in a tRNA–tRNA complex is due mainly to its slow rate of dissociation. The association rate constant (3×10^6 M^{-1} s^{-1} at 23 °C) (Grosjean et al., 1976, 1978) is typical of values observed for bimolecular oligonucleotide double helix formation, but for the complex formed between tRNA^{Phe} (anticodon GmAA) and tRNA^{Glu} (anticodon s^{2}UUC), the dissociation rate constant lies around 5 s^{-1} at room temperature. This value is lower by a factor of $\sim 10^4$ than would be predicted by the theoretical stability of a double helix of trinucleotides with the complementary anticodon composition GAA-UUC (Uhlenbeck et al., 1973; Pörschke & Eigen, 1971; Craig et al., 1971).

This report contains our study of the interaction between yeast and *Escherichia coli* Phe-tRNA^{Phe} (anticodon GmAA) and *E. coli* Glu-tRNA^{Glu} (anticodon s^{2}UUC) or uncharged tRNA^{Glu} in the presence or absence of EF-Tu·GTP. This system was selected for the present study because it has the largest known affinity (lifetime = 1640 ms) of any complex so far examined (Grosjean et al., 1976, 1978) and at the same time the fluorescence yield of Y base of yeast tRNA^{Phe} is affected by the anticodon binding (Eisinger, 1971) and provides a convenient binding assay. Our objective was to determine whether these complementary tRNAs can still interact in the presence of EF-Tu·GTP and if so, what its quantative effect of ternary complex formation upon this model codon–anticodon interaction is.

Methods and Materials

Yeast tRNA^{Phe} (anticodon GmAA, acceptance act. of 1029 pmol/A_{260}) was purchased from Boehringer Mannheim. *E.*

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[1] Abbreviations used: EF-Tu, elongation factor Tu; AA-tRNA, aminoacyl transfer ribonucleic acid; mRNA, messenger RNA; GTPase, guanosine 5′-triphosphatase; Tris, tris(hydroxymethyl)aminomethane; DTT, dithiothreitol.

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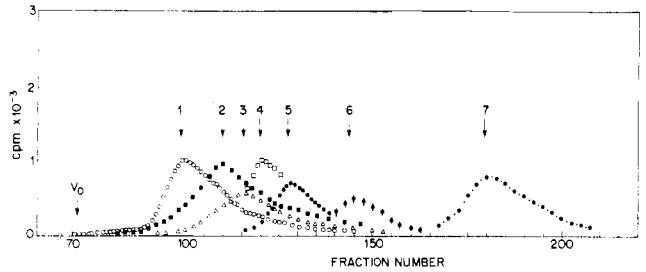

FIGURE 1: Gel chromatography (1.5 × 30 cm) of Phe-tRNA^{Phe}, Glu-tRNA^{Glu}, and Tu·GTP complexes on LKB Ultrogel AcA 44. Complexes were prepared as previously described (Miller & Weissbach, 1974); namely, to convert EF-Tu·GDP to EF-Tu·GTP, EF-Tu·GDP (1000 pmol), GTP (5000 pmol), phosphoenolpyruvate (0.5 μmol), and pyruvate kinase (2 μg) were incubated for 10 min at 25 °C in 150 μL of 50 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$, and 100 mM NH_4Cl. Aminoacyl-tRNA (1000 pmol) was added, and the mixture was kept in an ice bath for 5 min. For removal of the excess GTP, the solution was passed through a Sephadex G-25 column (1 × 25 cm) equilibrated with 50 mM Tris-HCl (pH 7.4) plus 10 mM $MgCl_2$. The column (Ultrogel AcA 44) was equilibrated with a buffer containing 300 mM NaCl, 50 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$, 75 mM KCl, and 5 mM DTT. For resolution of the ternary complexes, 5 μM GTP and 0.1 μM EF-Tu·GTP were also added to the equilibrium buffer except when [^{3}H]Glu-tRNA was chromatographed. Fractions of 0.16 mL were collected, and the radioactivity was counted on a scintillation counter. The void volume was determined with Blue Dextran 2000 (Pharmacia). (1) EF-Tu·GTP–[^{14}C]Phe-tRNA (118 pmol) + Tu·GTP–[^{3}H]Glu-tRNA (120 pmol) (○); (2) EF-Tu·GTP–[^{14}C]Phe-tRNA (118 pmol) + tRNA^{Glu} (150 pmol) (■); (3) EF-Tu·GTP–[^{14}C]Phe-tRNA (118 pmol) (△); (4) [^{14}C]Phe-tRNA (120 pmol) + tRNA^{Glu} (150 pmol) (□); (5) EF-Tu·[^{3}H]GTP (●); (6) [^{3}H]Glu-tRNA (◆); (7) [^{14}C]Phe (●·●).

coli tRNA^{Glu} (anticodon s^{2}UUC, acceptance act. of 1200 pmol/A_{260}) was provided by Dr. A. D. Kelmers of the Oak Ridge National Laboratory, Oak Ridge, TN. This tRNA was annealed at 65 °C for 3 min to ensure renaturation (Grosjean et al., 1976).

EF-Tu·GTP was prepared as described before (Miller & Weissbach, 1974), and for removal of the excess [^{3}H]GTP, the solution was passed through a Sephadex G-25 column (1 × 25 cm) equilibrated with 50 mM Tris-HCl (pH 7.4) plus 10 mM $MgCl_2$. Radioactive [^{14}C]Phe and [^{3}H]Glu were obtained from New England Nuclear Corp.

Column chromatography (1.5 × 30 cm, LKB Ultrogel AcA 44) was used for the detection of complex formation between complementary tRNAs in the presence and absence of EF-Tu. Conditions used are described in the legends to the figures. Fluorescence measurements were carried out with Perkin-Elmer Fluorescence Spectrophotometer, Model MPF-4, at room temperature (21–23 °C) with excitation and emission monochromators slits of 6 nm. The same cell (0.3-cm optical path and 200-μL volume) was used throughout the experiment and the fluorescence intensity was corrected for the light absorbed by components other than tRNA^{Phe} (Eisinger, 1971). All samples had an absorbance of <0.2 at 313 nm, the excitation wavelength. Emission spectra were recorded from 400 to 450 nm.

Results

Complex formation between complementary tRNAs can be detected by gel filtration chromatography as is demonstrated in Figure 1, where the tRNA^{Glu} dimer (peak 4) emerges well ahead of monomeric tRNA (peak 6). Peaks containing the ternary complex could be resolved because the column was equilibrated with EF-Tu·GTP and free GTP. If EF-Tu·GTP were not present in order to prevent dissociation, the ternary complex would emerge with EF-Tu·GTP. NaCl (300 mM),

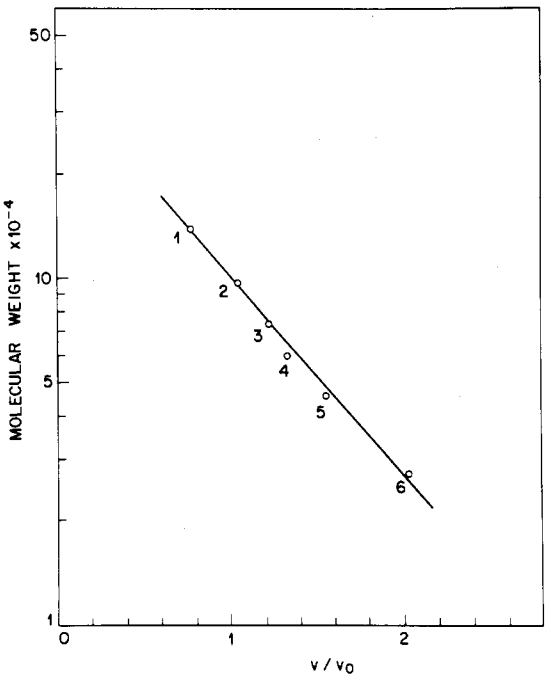

FIGURE 2: The ratio of elution volume to void volume, V/V_0, at 0 °C and molecular weight of complexes. Sample numbers are from Figure 1.

added to the buffer to stabilize the tRNA dimers, did not weaken the ternary complex. The early emergence of the peaks containing EF-Tu·GTP, Phe-tRNA, and tRNA^{Glu} or Glu-tRNA suggests that the dimer Phe-tRNA–tRNA^{Glu} will interact with EF-Tu·GTP and, in addition, the ternary complexes containing the complementary tRNAs will dimerize.

A linear correlation was obtained between the log of molecular weight of a compound and the ratio of its elution volume, V, to the void volume, V_0, of the column, as shown in Figure 2 for tRNA and its complexes. This linear correlation supports our identification of the peaks (Whitaker, 1963), and we conclude that the complementary anticodon interaction is unimpaired even when both tRNAs are bound to EF-Tu·GTP.

The interaction between nonacylated *E. coli* tRNA^{Glu} and yeast tRNA^{Phe} has been previously studied by using a fluorescence titration method which made use of the fact that the fluorescence of the Y base (adjacent to the anticodon of tRNA^{Phe}) is quenched when the anticodon–anticodon complex is formed (Eisinger, 1971). It is difficult to be certain that the quenching is indeed complete, but a linear relationship between fluorescence change and extent of binding was assumed for the data analysis.

Figure 3 shows the plot of fluorescence intensity as a function of the molar ratios tRNA^{Glu}/tRNA^{Phe}, in the presence and absence of EF-Tu where f is the fluorescence intensity and f_0 is the tRNA^{Phe} fluorescence in the absence of tRNA^{Glu}. The fluorescence intensity f_0 of tRNA^{Phe} was unaltered by aminoacylation or binding to EF-Tu·GTP (Beres & Lucas-Lenard, 1973; H. Grosjean, personal communication). The ratio f/f_0 gives the fraction of Phe-tRNA^{Phe} complexed with Glu-tRNA^{Glu}, and from this the concentrations of the free Glu-tRNA^{Glu} and Phe-tRNA^{Phe} can be calculated. Figure 4 shows the plot of concentration of the complex vs. the concentration of the product of unbound tRNAs for the three cases studied: (a) tRNA^{Phe} and tRNA^{Glu}; (b) Phe-tRNA–EF-Tu·GTP plus tRNA^{Glu}; (c) Phe-tRNA–EF-Tu·GTP plus Glu-tRNA–EF-Tu·GTP. The slope ([complex]/[tRNA^{Phe}]$_{free}$) [tRNA^{Glu}]$_{free}$ should give the association constant of the complex in the

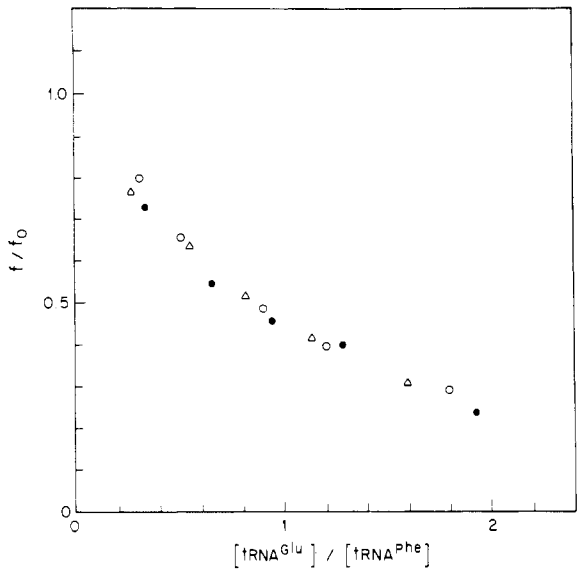

FIGURE 3: The relative fluorescence intensity of $tRNA^{Phe}$; Phe-$tRNA^{Phe}$ and Phe-tRNA–Tu·GTP as a function of $tRNA^{Glu}$ or Glu-tRNA–Tu·GTP concentration. The $tRNA^{Phe}$ concentration was kept constant for each set of experiments, ~2.5 μM. The buffer in Figure 1, minus NaCl, was used. No difference in the fluorescence yield was observed between $tRNA^{Phe}$, acylated and nonacylated and when bound to Tu·GTP. (○) $tRNA^{Phe}$–$tRNA^{Glu}$; (●) Tu·GTP–Phe-tRNA + $tRNA^{Glu}$; (△) Tu·GTP–Phe-tRNA + Glu-tRNA–Tu·GTP.

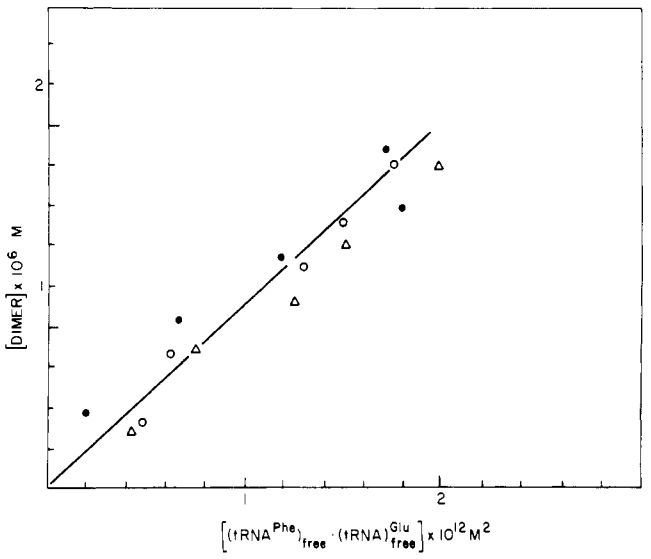

FIGURE 4: Plot of [dimer complex] vs. $[tRNA^{Phe}]_{free}[tRNA^{Glu}]_{free}$. Data calculated from Figure 3. (○) $tRNA^{Phe}$–$tRNA^{Glu}$; (●) Tu·GTP–Phe-tRNA + $tRNA^{Glu}$; (△) Tu·GTP–Phe-tRNA + Glu-tRNA–Tu·GTP.

absence and presence of EF-Tu·GTP. All data points fall within the slope of $(0.9 \pm 0.2) \times 10^6$ M^{-1}, in good agreement with published values for the interaction between the nonacylated tRNAs determined by gel electrophoresis (Eisinger & Gross, 1975) and temperature-jump methods (Grosjean et al., 1976).

Interaction of the ternary complex with codons associated with ribosomes results in the hydrolysis of GTP. This hydrolysis reaction is dependent upon the codon–anticodon interaction and precedes the stable binding of aminoacyl-tRNA to ribosomes (Thompson & Stone, 1977). Adding $tRNA^{Glu}$ to Phe-tRNA–EF-Tu·GTP at a concentration that allows extensive interaction between the tRNAs does not induce a significant increase in the rate of GTP hydrolysis (Table I). Thus, the GTPase activity accompanying binding of the ternary complex to mRNA-programmed ribosomes is not initiated solely by the codon–anticodon interaction.

Table I: Effect of Phe-tRNA and $tRNA^{Glu}$ upon Hydrolysis of EF-Tu·GTP[a]

	fraction of EF-Tu·GTP hydrolyzed/h
EF-Tu·GTP	0.16
EF-Tu·GTP + Phe-tRNA	0.14
EF-Tu·GTP + $tRNA^{Glu}$	0.12
EF-Tu·GTP + Phe-tRNA + $tRNA^{Glu}$	0.17

[a] Each reaction mixture contained 1 μM EF-Tu and 5 μM [γ-^{32}P]GTP, and 1.25 μM Phe-tRNA or 1.8 μM $tRNA^{Glu}$ where indicated, in a buffer composed of 50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 160 mM NH_4Cl, and 1 mM DTT. At 30-min intervals aliquots were removed and assayed for radioactive orthophosphate by the method of Modollell & Vazquez (1973).

Discussion

Models have been proposed for the mechanism of protein synthesis involving conformational changes of the tRNA (Woese, 1970; Kurland et al., 1975; Sprinzl et al., 1976; Lake, 1977; Thach & Thach, 1971; Crothers & Cole, 1978). These models generally assume a rearrangement of the tertiary structure of the tRNA involving the codon–anticodon interaction. Conformational changes of tRNA with possible biological significance have been observed by equilibrium dialysis and spectrophotometric experiments which indicate that the codon–anticodon interaction triggers exposure of the TψCG sequence (residues 54–57) for the binding to oligonucleotide CGAA (Schwarz et al., 1976; Möller et al., 1979). However, no conformational change involving residue T-54 could be detected by NMR of $tRNA^{Phe}$ upon complexation with $tRNA^{Glu}$ (Davanloo et al., 1979). A similar conclusion has been reported concerning an NMR study of the interaction of the anticodon of yeast $tRNA^{Phe}$ with the oligonucleotide UUCA (Geerdes et al., 1978); in both cases no disruption of hydrogen bonds was observed, implying that the interaction between the DHU and TψC loops remained intact. These contradictory results can probably be reconciled by assuming that in the thermal average over equilibrium configuration only a small percentage of the tRNA molecules undergoes the disruption of the DHU–TψC loops' interaction and is thus undetected by NMR.

Does the EF-Tu·GTP binding of AA-tRNA affect the anticodon region? ESR studies of a spin-label on s^2C-32 of Arg-$tRNA^{Arg}$ have shown that the mobility of the free radical becomes somewhat immobilized upon ternary complex formation with EF-Tu·GTP (Kruse et al., 1978). On the other hand, analysis of a nuclease-digested complex of Phe-$tRNA^{Phe}$ and the elongation factor suggested that EF-Tu binds primarily along the amino acid acceptor TψC helix and avoids contact with the various tRNA loops (Jekowsky et al., 1977). One definitive conclusion of these and other studies (Krauskopf et al., 1972) was that the anticodon loop is not a critical point for the elongation factor binding. In addition, an NMR study of the effect of EF-Tu·GTP upon the double-helical structure of AA-tRNA has been carried out, and no alterations in base pairing were observed (Shulman et al., 1974).

The present data firmly indicate that the ternary complexes Phe-tRNA–EF-Tu·GTP and Glu-tRNA–EF-Tu·GTP can form dimers with a binding constant of $(0.9 \pm 0.2) \times 10^6$ M^{-1}, which is, within experimental error, identical in magnitude with that of the dimer of the nonacylated tRNAs, $tRNA^{Phe}$–$tRNA^{Glu}$, and also with that of the complex Phe-tRNA–EF-

Tu·GTP with nonacylated $tRNA^{Glu}$. Since the association constant obtained is in quite good agreement with values determined by other methods, the assumption taken on linearity between fluorescence change and binding is valid, a posteriori. The low value observed previously by the fluorescence method might be due to the low ionic strength used (Eisinger, 1971). The Y base fluorescence is known to be very sensitive to the salt concentration (Beardsly et al., 1970; Leroy et al., 1977). From the present data, one is justified in assuming that the anticodon region is not affected by complexation with EF-Tu·GTP; if its conformation does change, the difference should be rather small, undetectable by the Y base fluorescence change. A similar observation has been previously made (H. Grosjean, personal communication). This conclusion does not preclude the possibility of structure changes in the anticodon loop but shows that such changes have no significance in energetic terms. The results suggest that the increased binding to the message–ribosome complex of AA-tRNA–EF-Tu·GTP (compared to tRNA) is probably not due to differences in the anticodon loop structures of these species.

Other sites besides its anticodon contribute to binding AA-tRNA to the ribosomes (Gorini, 1971; Ninio, 1974; Hopfield, 1974). This is clearly demonstrated by the example of the UGA suppressor derived from the mutation of G-25 to C in *E. coli* $tRNA^{Trp}$ (Hirsch, 1971), where the anticodon structure of the suppressor is identical with that of the wild-type $tRNA^{Trp}$. This is shown by a temperature-jump kinetic study of the interaction of these tRNAs with *E. coli* $tRNA^{Pro}$ bearing the complementary anticodon (H. Grosjean, personal communication). The relaxation times of the dimers formed between $tRNA^{Pro}$ (anticodon U*GG) and suppressing or non-suppressing forms of $tRNA^{Trp}$ (anticodon CCA) were identical. Thus, the explanation of the suppressor activity must be that the tRNA region near the site of the mutation (base 25) is involved in binding to the ribosome. Similarly, EF-Tu·GTP may promote the ribosomal binding of AA-tRNA in several ways. The protein may itself have binding contacts with the ribosome and may alter the conformation of the tRNA to change the tRNA–ribosome interaction. Our results show that if it does the latter, it does so not by restructuring of the anticodon but rather by reordering other regions in the tRNA.

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