Changes in Aminoacyl Transfer Ribonucleic Acid Conformation upon Association with Elongation Factor Tu–Guanosine 5′-Triphosphate. Fluorescence Studies of Ternary Complex Conformation and Topology†

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ABSTRACT: The association of aminoacyl-tRNA (aa-tRNA) with elongation factor Tu·GTP to form an aa-tRNA·EF-Tu·GTP ternary complex was investigated by using two different fluorescent probes, both of which monitored structural changes near the juncture of the two arms of the L-shaped tRNA. Aminoacylation of $tRNA^{Phe}$–F^8, a functionally active analogue of $tRNA^{Phe}$ with a fluorescein moiety covalently attached to the s^4U-8 base, did not cause a change in the fluorescence emission intensity. However, when EF-Tu·GTP bound to Phe-$tRNA^{Phe}$–F^8, the emission intensity increased by approximately 30%, depending upon the solvent conditions. About half of this increase in fluorescence was due to an increase in the molar absorptivity of the fluorescein dye. Ternary complex formation did not alter the rate of iodide ion quenching of the Phe-$tRNA^{Phe}$–F^8 fluorescence. Since solvent access to fluorescein was not reduced when EF-Tu·GTP was bound to Phe-$tRNA^{Phe}$–F^8, the fluorescence intensity change noted above was not caused by a direct interaction between fluorescein and EF-Tu. Instead, the binding of EF-Tu·GTP to the aa-tRNA resulted in a conformational change in the aa-tRNA near s^4U-8. Ternary complex formation also altered the nature of the single strong binding site for ethidium in unfractionated and unmodified aa-tRNA. However, ethidium binding to its strong site was not blocked. These results indicate that only the acceptor-TΨC arm of aa-tRNA interacts directly with EF-Tu·GTP and that the anticodon-D arm is available for direct interaction with the ribosome during recognition. Our data also suggest that EF-Tu facilitates protein biosynthesis by ensuring that every aa-tRNA is in a particular (possibly the same) conformation prior to initiation of the recognition process at the ribosomal complex.

Aminoacyl-tRNA (aa-tRNA)[1] normally binds to the ribosomal complex as part of a ternary complex containing GTP and a nonribosomal protein (Miller & Weissbach, 1977; Grunberg-Manago et al., 1978). This protein, designated elongation factor Tu or EF-Tu in *Escherichia coli*, catalyzes protein biosynthesis in vitro, but the mechanism(s) by which EF-Tu facilitates polypeptide chain elongation is (are) not known. In this paper, we focus on two aspects of ternary complex structure which will dictate the nature of that mechanism(s): ternary complex topology and EF-Tu·GTP-induced conformational changes in aa-tRNA.

It is clear that the topology of the ternary complex will determine which molecular interactions can be involved in ternary complex function at the ribosome. At one extreme, it is conceivable that only the aminoacyl end of the aa-tRNA is bound to the EF-Tu. In this arrangement, the ribosome would have the maximum opportunity to "read" the tRNA structure and any conformational changes during the recognition process. Alternatively, at the other extreme, the EF-Tu and aa-tRNA would be arranged so that only the anticodon of the aa-tRNA is exposed. This would minimize aa-tRNA interaction with the ribosome and would thereby reduce the chances of the aa-tRNA becoming bound to a high-affinity site on the ribosome until the codon–anticodon recognition process was complete.

The aminoacyl end of the aa-tRNA is known to be involved in ternary complex formation because EF-Tu·GTP associates much more strongly with aa-tRNA than with peptidyl-tRNA, unacylated tRNA, or some tRNAs modified at their aminoacyl ends [reviewed in Miller & Weissbach (1977) and Grunberg-Manago et al. (1978)] and because complex formation is sensitive to the nature of the amino acid side chain of the aa-tRNA (Pingoud et al., 1977b; Knowlton & Yarus, 1980; Pingoud & Urbanke, 1980; Wagner & Sprinzl, 1980). A direct interaction between EF-Tu·GTP and the aminoacyl end of the aa-tRNA is also shown by the extremely efficient affinity labeling of the protein with N^{ϵ}-(bromoacetyl)-Lys-tRNA (Johnson et al., 1978), the protection of the -CCA end against nuclease digestion (Jekowsky et al., 1977), and the experiments with analogues of the 3′ terminus of aa-tRNA [see Joňak et al. (1980) and Bhuta et al. (1982) and references cited therein]. Partial digestion studies with double-strand-specific cobra venom ribonuclease indicate that EF-Tu also interacts with the amino acid acceptor and TΨC helices (Boutorin et al., 1981) and kethoxal modification studies suggest that EF-Tu covers the D loop and stem (Bertram & Wagner, 1982). On the other hand, limited T_1 ribonuclease digestion studies have shown that the anticodon, TΨC, and D loops are exposed in the ternary complex (Jekowsky et al., 1977). Covalent attachment of probes to s^4U-8 (Schwartz et al., 1975; Johnson et al., 1982), to X-47 in the extra loop (Sprinzl & Faulhammer, 1978; T. H. Kao, D. L. Miller, M. Abo, and J. Ofengand, unpublished results), or to i^6A-37 (Weygand-Durasevic et al., 1981), s^2C-32 (Kruse et al., 1978), or Q-34 (Pingoud et al., 1977a) in the anticodon loop does not prevent

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[1] Abbreviations: EF-Tu, elongation factor Tu; aa-tRNA, aminoacyl-tRNA; IAAF, 5-(iodoacetamido)fluorescein; s^4U, 4-thiouridine; $tRNA^{Phe}$–F^8, adduct between IAAF and the s^4U-8 base of $tRNA^{Phe}$; PEP, phosphoenolpyruvate; EDTA, ethylenediaminetetraacetic acid; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane.

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complex formation and, in some cases, the subsequent interaction of the ternary complex with the ribosome (Schwartz et al., 1975; Sprinzl & Faulhammer, 1978; Johnson et al., 1982).

These studies demonstrate that the aminoacyl arm of the L-shaped aa-tRNA interacts directly with EF-Tu·GTP, but they do not define the limits of the interaction of EF-Tu with the midsection of the tRNA. Since EF-Tu·GTP associates only weakly with an aa-tRNA involved in cell wall biosynthesis that has a normal acceptor stem (Tanada et al., 1981), structural features of the aa-tRNA other than the acceptor stem are presumably involved in some way in ternary complex formation.

The involvement of conformational changes in tRNA function seems likely [reviewed by Crothers & Cole (1978)], and it is conceivable that ternary complex formation and/or function causes a conformational change in the aa-tRNA. Tritium exchange [cited in Miller & Weissbach (1977)], NMR (Schulman et al., 1974), and oligonucleotide binding (Schwarz et al., 1976; Liesch, 1977; Kruse et al., 1980) experiments have not revealed any significant changes in aa-tRNA structure upon association with EF-Tu·GTP, aside from differences at the -CAA terminus (Liesch, 1977; Kruse et al., 1980). Nor did a fluorescent probe in the anticodon exhibit a change in fluorescence emission upon ternary complex formation (Beres & Lucas-Lenard, 1973). However, the rotational correlation times of nitroxide spin-labels attached to two different bases in the anticodon loop were increased substantially when the modified aa-tRNAs were complexed with EF-Tu·GTP (Kruse et al., 1978; Weygand-Durasevic et al., 1981). The immobilization of the spin-labels was interpreted to be the result of a conformational change in the anticodon region when the aa-tRNA associated with EF-Tu·GTP.

We have utilized a functionally active aa-tRNA with a fluorescent probe covalently attached to the s^4U-8 base to examine ternary complex topology and possible EF-Tu·GTP-induced conformational changes near the midsection or "elbow" of the aa-tRNA. The effects of ternary complex formation on ethidium binding to aa-tRNA were also investigated.

Experimental Procedures

EF-Tu, tRNA, and Enzymes. *Escherichia coli* $tRNA^{Phe}$ (Plenum Scientific, Hackensack, NJ) was reacted with IAAF (Molecular Probes, Plano, TX) as described previously (Johnson et al., 1982), except that reaction times were increased to 17 h. The tRNA species with the fluorescein dye covalently attached to s^4U-8, $tRNA^{Phe}$–F^8, was purified by RPC-5 chromatography (Johnson et al., 1982). Aminoacylation of modified and unmodified $tRNA^{Phe}$ was carried out as described earlier (Johnson et al., 1982) by using S-100 enzymes prepared according to Johnson et al. (1976) and [^{14}C]Phe purchased from ICN (Irvine, CA). Gel filtration and dialysis of the aa-tRNAs in 1 mM potassium acetate (pH 5.0)–5 mM $MgCl_2$ were followed by storage at −70 °C or in liquid nitrogen.

Unfractionated *E. coli* MRE 600 tRNA (Boehringer, Indianapolis, IN) was totally aminoacylated in 2.0-mL incubations containing 100 mM Hepes (pH 8.0), 10 mM $MgSO_4$, 10 mM KCl, 1 mM dithiothreitol, 4 mM ATP, 0.1 mM CTP, 100 A_{260} units of tRNA, 200 μg of S-100 enzymes, 19 nonradioactive amino acids (minus Phe) at 8.3 μM, and a 1:20 dilution of a mixture of ^{3}H-labeled amino acids (NET-250, New England Nuclear). After a 37 °C, 30-min incubation, this unfractionated aa-tRNA was processed in the same manner as the pure Phe-tRNA (Johnson et al., 1982).

Crystalline EF-Tu·GDP was purified from *E. coli* B as described elsewhere (Miller & Weissbach, 1974). Pyruvate kinase was purchased from Sigma Chemical Co. (St. Louis, MO). Partially purified phenylalanyl-tRNA synthetase was prepared according to Wagner & Sprinzl (1979).

Protection Assays. Final incubations contained in 160 μL in 1.5-mL capped polypropylene tubes 10 mM Hepes (pH 7.4), 10 mM magnesium acetate, 50 mM NH_4Cl, 0.1 mM dithiothreitol, 0.1 mM $Na_2S_2O_3$, 10 μM GDP, 1 mM PEP, 3.1 μg of pyruvate kinase, KI or KCl as indicated, EF-Tu as indicated, and aa-tRNA as indicated. Prior to the addition of aa-tRNA, KI, and KCl, the samples were preincubated at 37 °C for 10 min to convert GDP into GTP with pyruvate kinase and PEP, and hence convert EF-Tu·GDP into EF-Tu·GTP. For EF-Tu·GDP (no ternary complex) controls, the PEP was omitted. Following the addition of aa-tRNA, and prior to any KCl or KI addition, samples were incubated in ice for 5 min to allow ternary complexes to form. Immediately after KI or KCl addition, two 25-μL aliquots were removed from each sample for assay by cold trichloroacetic acid precipitation using 0.74 A_{260} unit of ribosomal RNA as carrier. The sample tube was then capped, placed at 30 °C, and assayed again as indicated.

The effect of ethidium bromide on ternary complex formation was investigated by using the same procedure as above, except that all incubations contained 125 mM NaCl and did not contain $Na_2S_2O_3$, KI, or KCl. Ethidium bromide (Sigma) was added to the appropriate samples after their 5-min incubation in ice.

Fluorescence Measurements. All fluorescence measurements were made by using a Spex Fluorolog spectrofluorometer, a photon counting instrument equipped with a 450-W xenon lamp, and double monochromators in both the excitation and emission light paths. Emission correction factors were determined by using a standard lamp (Optronic Laboratories, Orlando, FL). The band-pass was 5 nm on both excitation and emission for all experiments. Slits were kept closed except during scans to avoid photodegradation of the sample. Temperature control was maintained with a Lauda K2R circulating bath attached to the cell holder. All spectral measurements were done at 30 °C. A Tektronix 31 programmable calculator was interfaced to the Spex spectrofluorometer to provide operational control, data collection, and data manipulation.

Fluorescein-containing samples were excited at 480 nm. Emission intensity was quantified by integration of the corrected fluorescence signal at 1-nm intervals from 495 to 680 nm. In the iodide ion quenching experiments, uncorrected intensities were integrated from 495 to 600 nm. The signal from samples which lacked a $tRNA^{Phe}$–F^8 species was insignificant, so it was not necessary to subtract a background signal from the fluorescein spectra. Ethidium-containing samples were excited at 520 nm, and their uncorrected emission was quantified by integration at 2-nm intervals from 540 to 750 nm. Prior to integration, the emission spectrum of a sample in the absence of ethidium was always subtracted from its spectrum in the presence of ethidium. All data were corrected for dilution due to the addition of protein or titrant.

Spectral Changes upon Aminoacylation and Association with EF-Tu·GTP. Each sample (2.25 mL) contained, initially, 100 mM Hepes (pH 8.0), 20 mM magnesium acetate, 100 μM dithiothreitol, 10 μM [^{14}C]Phe (405–450 Ci/mol), 4 mM ATP, 0.1 mM CTP, 10 μM GDP, 11.4 μg of pyruvate kinase, 1 mM PEP, and 10^{-7} M $tRNA^{Phe}$–F^8 (using $\epsilon_{260} = 6.25 \times 10^5$ M^{-1} cm^{-1}). After the fluorescence emission of a sample had been measured, partially purified phenylalanyl-tRNA

synthetase (6.3 μg in 15 μL) was added. Aminoacylation was allowed to proceed for at least 20 min, and then the spectral measurements were repeated. EF-Tu·GDP was then added to the cuvette to a final concentration of 3.0 μM. Ternary complex formation was allowed to proceed for at least 15 min before the fluorescence emission of the sample was again measured. Control samples either contained 4 mM AMP and 50 μM PP_i (instead of ATP) to prevent aminoacylation or lacked PEP to prevent ternary complex formation. Samples which lacked $tRNA^{Phe}$–F^8 had no significant fluorescence. Three 50-μL aliquots were assayed for cold trichloroacetic acid insoluble radioactivity after the spectral measurements in the presence of synthetase and of EF-Tu.

When both fluorescence and absorbance measurements were to be made on a sample, the $tRNA^{Phe}$–F^8 concentration was increased to 1 μM and the EF-Tu concentration to 4.7 μM. Absorbance was measured by using a Hitachi 100-80 spectrophotometer after each set of emission scans. Each sample remained in the same cuvette for both fluorescence and absorbance measurements. Samples were maintained at 30 °C throughout.

In all cases, emission scans were repeated until a sample exhibited the same fluorescence intensity on two or three consecutive scans (typically separated by 10 min). The sample was then presumed to have reached equilibrium.

Quenching Experiments. Initially, samples (2.04 mL) contained buffer A [10 mM Hepes (pH 7.4), 10 mM magnesium acetate, 50 mM NH_4Cl, 0.1 mM dithiothreitol, and 0.1 mM $Na_2S_2O_3$ (to prevent I_3^- formation)] and 10^{-7} M fluorescein, added as IAAF, disodium fluorescein, $tRNA^{Phe}$–F^8, Phe-$tRNA^{Phe}$–F^8, or the covalent adduct of s^4U and IAAF (Johnson et al., 1982) as indicated. EF-Tu-containing samples also contained 1 mM PEP (where indicated), 10 μM GDP, 19.2 μg of pyruvate kinase, and EF-Tu at 2.0 μM. All samples were incubated in the cell compartment at 30 °C for 15 min to allow for temperature equilibration and for the formation of GTP and the ternary complex in those samples containing PEP. It was necessary to shake the cuvettes gently several times during this time period in order to obtain a stable F_0 measurement. Samples were titrated with iodide (or chloride) ions by the sequential addition of (usually) 10-μL aliquots of buffer A containing 4 M KI (or 4 M KCl). Fluorescence emission was measured 5 min after each addition of KI (or KCl). Before and after each quenching experiment involving Phe-$tRNA^{Phe}$–F^8, two 20-μL samples were removed from each cuvette and assayed for cold trichloroacetic acid precipitable radioactivity. Individual experiments were repeated between 2 and 10 times, and K_{sv} values were reproducible to within 0.1 M^{-1}.

Gel Filtration Chromatography. Following the completion of the spectral measurements and the acid precipitation assays, the remainder of each sample (approximately 2 mL) was applied directly to an Ultrogel AcA 44 (LKB Instruments) column (1.8 cm i.d. × 30 cm) column equilibrated in 50 mM Tris-HCl (pH 7.4), 10 mM magnesium acetate, 50 mM NH_4Cl, and 1 mM 2-mercaptoethanol. Fractions (1.35 mL) were collected at room temperature, and 0.4 mL of each was counted by using a Triton-containing scintillator.

Titrations of Ethidium. Samples (2.0 mL) initially contained 1 μM ethidium bromide and 19.2 μg of pyruvate kinase in buffer A plus 125 mM NaCl. GTP (10 μM) and PEP (1 mM) were included in samples destined to contain ternary complex, while control samples contained 10 μM GDP. Prior to titration, EF-Tu was incubated for 10 min at 37 °C in a 50-μL volume of buffer A plus 125 mM NaCl containing 168 μg of EF-Tu, 4.8 μg of pyruvate kinase, and either 10 μM GDP (control samples) or 10 μM GTP and 1 mM PEP (ternary complex samples). Unfractionated aa-tRNA (0.69 A_{260} unit; totally aminoacylated with a mixture that included 15 ^3H-labeled amino acids) was included in the initial 2.0-mL volume when aa-tRNA–ethidium was titrated with EF-Tu, and in the initial 50-μL volume when ethidium was titrated with ternary complex or aa-tRNA plus EF-Tu·GDP. Analysis of samples by acid-insoluble radioactivity and by gel filtration chromatography was done as described above.

The amount of bound ethidium was calculated from the fluorescence intensity of the samples as follows. The emission intensity of free ethidium at a given concentration was defined to be F_F. The fluorescence intensity of the same ethidium sample when the dyes are totally bound to tRNA was defined to be F_B. When the fraction of dye that is bound to tRNA is set equal to b, then

$$F = bF_B + (1 - b)F_F$$

where F is the measured intensity of the sample. Rearrangement gives

$$b = (F/F_F - 1)/(F_B/F_F - 1)$$

F_B/F_F was determined by titrating 1 μM ethidium with aa-tRNA until the fluorescence intensity was no longer increased by the addition of aa-tRNA. F_B/F_F was 18.0 at 10 °C and 16.2 at 30 °C for ethidium binding to aa-tRNA under our conditions. F_B/F_F for ethidium binding to aa-tRNA·EF-Tu·GTP was assumed to be the same. Since F_F was measured prior to the addition of tRNA, measurements of F (corrected for dilution) provided the data necessary to calculate b and the amount of bound ethidium in the sample during the titration.

Titrations with Ethidium. Samples (2.0 mL; 30 °C) contained 370 μg of EF-Tu and 3.4 A_{260} units of unfractionated [^3H]aa-tRNA. The aa-tRNA and the components of the GTP- and GDP-containing samples were as described above for the titrations of ethidium. Ethidium bromide was added to samples as indicated. F_F values for varying concentrations of ethidium were obtained from a control sample lacking aa-tRNA and EF-Tu that was titrated with ethidium. Background fluorescence was subtracted as noted earlier. The amount of bound ethidium was calculated as described above.

Results

Phe-$tRNA^{Phe}$–F^8·EF-Tu·GTP Complex Formation. Fluorescent-labeled $tRNA^{Phe}$–F^8 has been shown to be nearly as active as the unmodified $tRNA^{Phe}$ in both aminoacylation and EF-Tu-dependent binding to ribosomes (Johnson et al., 1982). In order to demonstrate directly that the modified tRNA also interacts normally with EF-Tu·GTP, we compared the rates of [^{14}C]Phe hydrolysis from Phe-$tRNA^{Phe}$ and Phe-$tRNA^{Phe}$–F^8 under conditions which either prevented (+GDP) or promoted (+GTP, converted from GDP by pyruvate kinase and phosphenolpyruvate) association with EF-Tu. This approach has been widely used to demonstrate ternary complex formation between an aa-tRNA and EF-Tu·GTP [e.g., see Beres & Lucas-Lenard (1973), Pingoud et al. (1977b), and Johnson et al. (1978)].

As shown in Figure 1, the EF-Tu·GTP protection of the Phe-$tRNA^{Phe}$–F^8 aminoacyl bond is nearly equivalent to that of the Phe-$tRNA^{Phe}$ aminoacyl bond. Thus, EF-Tu·GTP has the same affinity for Phe-$tRNA^{Phe}$ and Phe-$tRNA^{Phe}$–F^8. This is true even at a considerably higher ionic strength and in the presence of chaotropic iodide ions (Figure 1). Hence, the

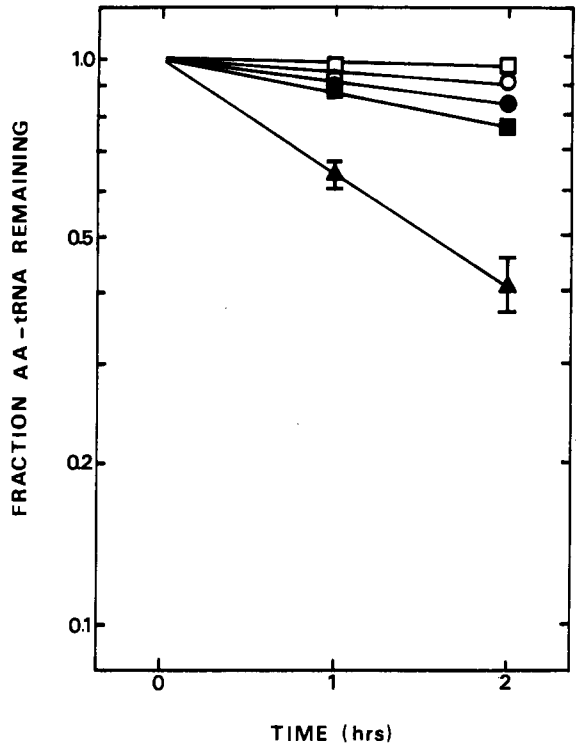

FIGURE 1: EF-Tu·GTP protection of the Phe-tRNA^{Phe}–F^8 aminoacyl bond. Incubations contained 1.76 μg of EF-Tu, either [^{3}H]Phe-tRNA^{Phe} (7260 dpm/pmol of Phe; 868 pmol of Phe/A_{260} unit of tRNA; 0.011 A_{260} unit) (●, ○) or [^{3}H]Phe-tRNA^{Phe}–F^8 (11 750 dpm/pmol of Phe; 1085 pmol of Phe/A_{260} unit of tRNA; 0.010 A_{260} unit) (■, □), and either no KI (○, □) or 100 mM KI (●, ■). Assay procedures are described under Experimental Procedures. The range of values obtained from parallel samples which lacked PEP (and therefore ternary complex) is also shown, with the solid triangle indicating the average value.

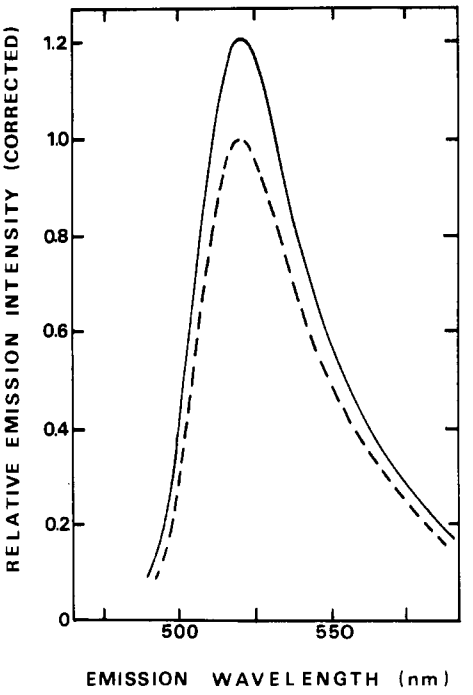

FIGURE 2: Fluorescence change upon ternary complex formation. Corrected emission spectra for 1 μM tRNA^{Phe}–F^8 (aminoacylated in the cuvette as described under Experimental Procedures) before (---) and after (—) the addition of 4.7 μM EF-Tu. Excitation was at 480 nm. The emission intensity of the sample prior to EF-Tu addition was arbitrarily defined to be 1.0.

fluorescein moiety covalently attached to the s^{4}U base does not block ternary complex formation and has little effect on the strength of the association between the aa-tRNA and EF-Tu·GTP.

We did find, however, that the extent of Phe-tRNA^{Phe}–F^8 aminoacyl bond protection varied somewhat from sample to sample. Some preparations deacylated faster than Phe-tRNA when protection assays were done with a low molar excess of EF-Tu·GTP over aa-tRNA (not shown). We also found that Phe-tRNA^{Phe} prepared from sham-reacted tRNA^{Phe} (tRNA^{Phe} carried through the fluorescent-labeling procedures except that IAAF was omitted from the reaction) was protected from hydrolysis in 100 mM KI slightly less well than was unreacted Phe-tRNA (not shown). The latter result shows that tRNA molecules may be altered during the labeling reaction procedure and suggests a cause for the variability in Phe-tRNA^{Phe}–F^8 protection. Some preparations of Phe-tRNA^{Phe}–F^8 apparently contain aa-tRNA molecules with a reduced capacity to interact with EF-Tu·GTP. The copurification of such molecules with active tRNA^{Phe}–F^8 suggests that one must define the tRNA^{Phe}–F^8 peak stringently during purification by RPC-5 chromatography.

Fluorescence Change upon Ternary Complex Formation. The fluorescence emission intensity of Phe-tRNA^{Phe}–F^8 is considerably enhanced upon association with EF-Tu·GTP. This is shown most directly by monitoring the emission of a single sample of tRNA^{Phe}–F^8 before its aminoacylation, after its aminoacylation, and after its binding to EF-Tu·GTP.

The addition of partially purified phenylalanyl-tRNA synthetase to a cuvette containing 10^{-7} M tRNA^{Phe}–F^8 and its subsequent aminoacylation to between 900 and 1200 pmol of Phe/A_{260} unit of tRNA did not alter the fluorescence emission of the fluorescein (not shown). When synthetase was added to samples containing a higher concentration of tRNA^{Phe}–F^8 (10^{-6} M), a slight fluorescence increase (2%) was observed. However, since an equivalent fluorescence increase was obtained in a parallel control sample (containing AMP + PP$_i$ instead of ATP), this emission intensity increase was not due to aminoacylation. One possible explanation for this small spectral change is that the tRNA and the synthetase enzyme are associated to some extent under these conditions.

In contrast to the insensitivity of fluorescein emission to aminoacylation, the fluorescence intensity of Phe-tRNA^{Phe}–F^8 increased substantially when EF-Tu was added to the cuvette. A typical result is shown in Figure 2. In this experiment, the total change in emission intensity amounted to 18.1%. Since this sample was 72% aminoacylated (1150 pmol of Phe/A_{260} unit of tRNA), this fluorescence increase corresponds to a 25.2% increase per Phe-tRNA^{Phe}–F^8 molecule. Each Phe-tRNA^{Phe}–F^8 molecule was associated with EF-Tu·GTP because the EF-Tu was present, intentionally, at a high concentration to assure ternary complex formation. Five separate experiments gave an average normalized fluorescence change of 28%. Thus, association of a Phe-tRNA^{Phe}–F^8 molecule with EF-Tu·GTP to form a ternary complex causes, either directly or indirectly, a 28% increase in fluorescein emission intensity.

No changes in spectral shape were observed, either upon aminoacylation or upon ternary complex formation. The wavelength of maximum fluorescence emission (corrected) remained at 519–520 nm throughout our experiments.

Origin of Fluorescence Intensity Increase. An increase in fluorescence emission intensity may result from an increase in either the quantum yield or the molar absorptivity of the dye. Chen (1969) has observed that changes in the fluorescence of fluorescein conjugates are usually due to changes in fluorophore absorptivity. Because the origin of the change affects the interpretation of experiments discussed below, we

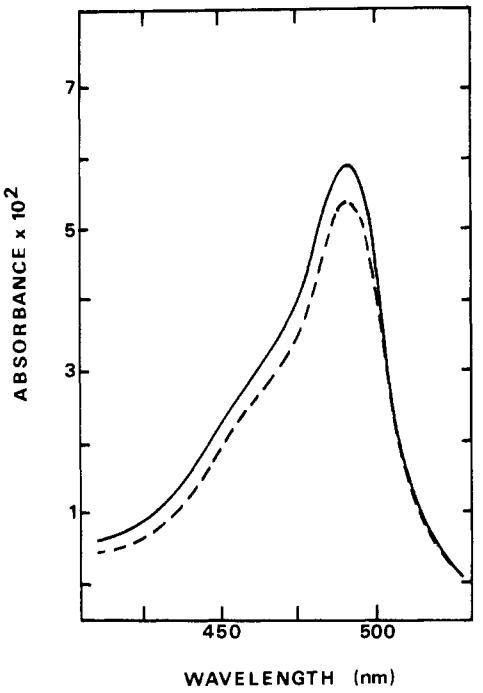

FIGURE 3: Absorbance change upon ternary complex formation. Fluorescein absorbance in the sample of Figure 2 before (---) and after (—) EF-Tu addition.

measured the absorbance of the fluorescein in $tRNA^{Phe}$–F^8 before aminoacylation, after aminoacylation, and after binding to EF-Tu·GTP.

The absorbance at 480 nm did not change when 0.1 μM $tRNA^{Phe}$–F^8 was aminoacylated (not shown). The absorbance increase in 1 μM $tRNA^{Phe}$–F^8 samples was the same as the fluorescence increase, but, as noted earlier, this increase was not due to aminoacylation.

However, when EF-Tu·GTP was added to aminoacylated $tRNA^{Phe}$–F^8, the absorbance of fluorescein increased markedly. The absorbance change was 10.7% (Figure 3) for the sample used to obtain the fluorescent spectra in Figure 2, and this corresponds to a 14.8% increase in fluorescein absorbance whenever a Phe-$tRNA^{Phe}$–F^8 associated with EF-Tu·GTP. The average normalized absorbance change for four separate experiments was 13%.

It is clear from these results that about half of the increase in fluorescence intensity which accompanies the association of Phe-$tRNA^{Phe}$–F^8 and EF-Tu·GTP results from an increase in the molar absorptivity of the fluorescein moiety.

Fluorescence Change Requirements and Reversibility. Several control experiments were performed in order to demonstrate that the fluorescence change depicted in Figure 2 resulted from the association of Phe-$tRNA^{Phe}$–F^8 and EF-Tu·GTP. With the exception of the sample of Figure 2 and its controls, the $tRNA^{Phe}$–F^8 concentration was 10^{-7} M in the following experiments. Other concentrations are given below or under Experimental Procedures.

(*i*) *Aminoacyl-tRNA Is Required.* Aminoacylation was prevented in a sample investigated in parallel with that of Figure 2 by using AMP and pyrophosphate instead of ATP. No fluorescence change was observed when either synthetase or EF-Tu·GTP was added to this sample (not shown). Hence, at these concentrations, unacylated $tRNA^{Phe}$–F^8 did not interact with EF-Tu·GTP, and Phe-$tRNA^{Phe}$–F^8 was required to obtain a spectral change.

(*ii*) *GTP Is Required.* When Phe-$tRNA^{Phe}$–F^8 was added to each of two cuvettes, one containing 3.5 μM EF-Tu·GTP (+PEP) and the other 3.5 μM EF-Tu·GDP (–PEP), the fluorescence intensity per aa-tRNA was 33% higher in the sample containing EF-Tu·GTP (not shown). The 33% figure is an average value for five separate experiments and is probably higher than the above 28% value because of differences in sample pH and content. Thus, it is clear that the fluorescence change was dependent upon ternary complex formation.

(*iii*) *The Spectral Change Is Reversible.* For demonstration of the reversibility of the fluorescence change, unfractionated nonfluorescent aa-tRNA was added to a sample to compete with Phe-$tRNA^{Phe}$–F^8 for binding to EF-Tu·GTP. When a large excess of aa-tRNA was added to a sample of Phe-$tRNA^{Phe}$–F^8·EF-Tu·GTP, the emission intensity decreased to the same level found in a control sample which lacked EF-Tu (not shown). When an intermediate amount of aa-tRNA was added to the sample, the amount of the fluorescence intensity decrease was consistent with the amount of Phe-$tRNA^{Phe}$–F^8 expected to be displaced from the ternary complex.

We also found that the fluorescence increase could be reversed when GDP was added to compete with aa-tRNA and GTP for binding to EF-Tu in the absence of EF-Ts. After the addition of GDP (to 2 mM) to samples similar to that of Figure 2, the fluorescence decrease was substantial, amounting within 10 min to an average of 30% of the fluorescence increase observed upon the addition of EF-Tu (not shown). Thereafter, the fluorescence intensity began increasing since added GDP was being converted to GTP in the sample.

Iodide Ion Quenching of Fluorescence. Collisions between fluorescent chromophores and certain solutes, such as iodide ions, result in a quenching of fluorescence (Lehrer & Leavis, 1978). Thus, the exposure of a particular fluorophore to the solvent and dissolved solute can be examined by measuring the fluorescence intensity or lifetime as a function of quencher concentration. For steady-state collisional quenching of fluorescence, a linear plot is obtained when data are analyzed according to the Stern–Volmer law:

$$F_0/F = K_{sv}[Q] + 1$$

where F_0 is the fluorescence intensity in the absence of quencher, F is the fluorescence intensity in the presence of quencher at concentration [Q], and K_{sv} is the Stern–Volmer quenching constant. K_{sv} is equal to $k_q\tau_0$, where k_q is the bimolecular quenching constant and τ_0 is the fluorescence lifetime in the absence of quencher. The k_q value found for a dye covalently attached to a macromolecule depends upon the steric accessibility of the quencher to the dye and may be affected by the electrostatic environment of the dye, among other things. A major change in macromolecular topology or conformation near the dye is therefore likely to cause a major alteration in the k_q value of such a fluorophore.

Iodide ion quenching of fluorescence was the same for both free IAAF and fluorescein covalently attached to an s^4U nucleoside (Figure 4). The magnitude of the Stern–Volmer constant, 9.8 M^{-1}, is consistent with a diffusion-controlled interaction between dye and quencher. As expected, the covalent attachment of fluorescein to tRNA substantially reduced the rate of collisional quenching of fluorescence (Figure 4). Phe-$tRNA^{Phe}$–F^8 and $tRNA^{Phe}$–F^8 exhibited a small, but reproducible, difference in iodide ion quenching (Figure 4). This suggests that under these conditions aminoacylated $tRNA^{Phe}$ has a slightly different conformation than unacylated $tRNA^{Phe}$ near the s^4U base. The linearity of the plots demonstrates that the decrease in fluorescence with increasing $[I^-]$ was due primarily to collisional quenching over a wide range of iodide ion concentrations. As expected, digestion of the tRNA by ribonuclease or denaturation of the tRNA in EDTA

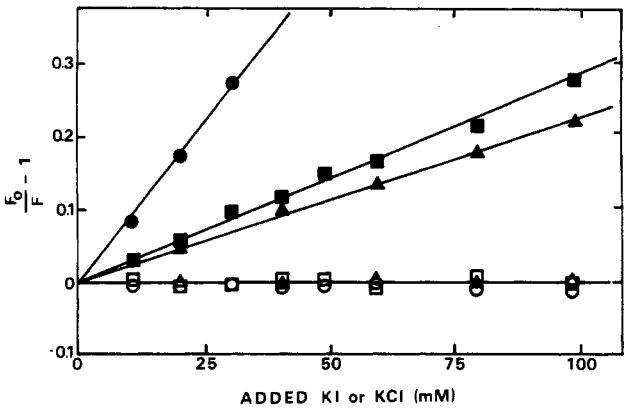

FIGURE 4: Iodide ion quenching of fluorescence. Experimental procedures are detailed under Experimental Procedures. KI (closed symbols) or KCl (open symbols) was added to samples as indicated. Samples contained 10^{-7} M fluorescein, added as IAAF (○, ●), $tRNA^{Phe}$–F^8 (□, ■), or Phe-$tRNA^{Phe}$–F^8 (△, ▲).

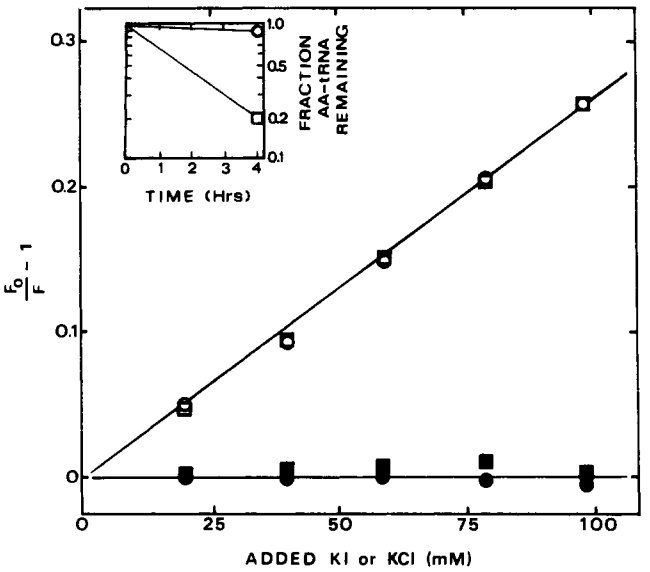

FIGURE 5: Effect of ternary complex formation upon iodide ion quenching of Phe-$tRNA^{Phe}$–F^8 fluorescence. Details are given under Experimental Procedures. KI (open symbols) or KCl (closed symbols) was added as indicated. Each sample contained 0.133 A_{260} unit of [^{14}C]Phe-$tRNA^{Phe}$–F^8 (932 dpm/pmol of Phe; 1170 pmol of Phe/A_{260} unit of tRNA) and differed only in the presence (○, ●) or absence (□, ■) of 1 mM PEP (and therefore ternary complex).

greatly increased the K_{sv} of iodide ion quenching of fluorescein.

Solvent Accessibility of Fluorescein in Phe-$tRNA^{Phe}$–F^8·EF-Tu·GTP. In order to determine whether the binding of EF-Tu·GTP to Phe-$tRNA^{Phe}$–F^8 reduced the exposure of the fluorescein dye to the solvent, we examined the sensitivity of the fluorescence emission to quenching with iodide ions under conditions which either prevented or promoted ternary complex formation. The latter conditions were identical with the former except for the presence of phosphoenolpyruvate, which permitted the conversion of GDP into GTP by pyruvate kinase and hence the association of Phe-$tRNA^{Phe}$–F^8 with EF-Tu·GTP. As shown in Figure 5, the Stern–Volmer constant of fluorescence quenching is the same for both Phe-$tRNA^{Phe}$–F^8 bound to EF-Tu·GTP and Phe-$tRNA^{Phe}$–F^8 free in a solution containing EF-Tu·GDP. Equally important, the K_{sv} value for Phe-$tRNA^{Phe}$–F^8 is the same in the presence or absence of EF-Tu.

The F_0 values in samples containing EF-Tu·GTP were 33% higher than those in parallel samples containing EF-Tu·GDP (see above). As noted earlier (cf. Figures 2 and 3), about half

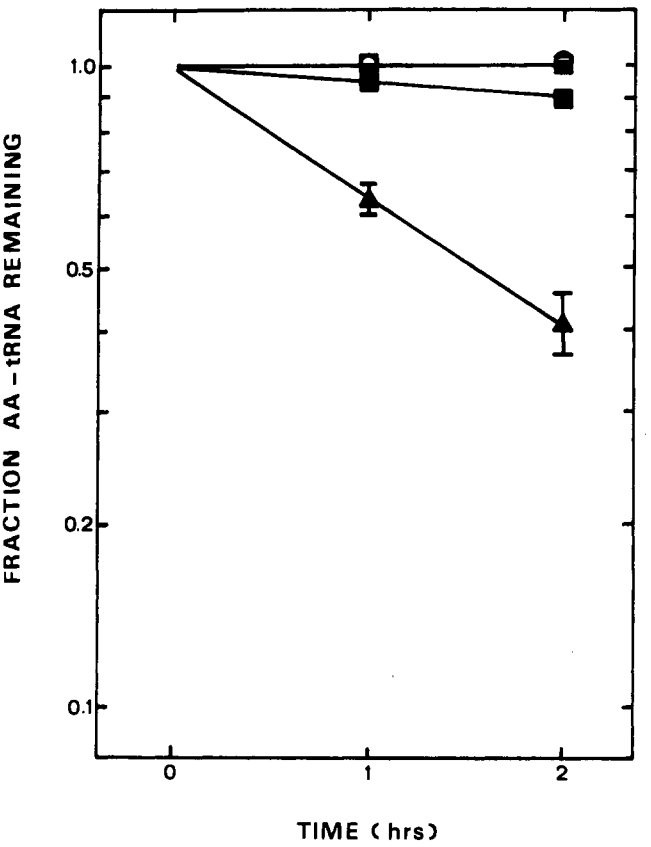

FIGURE 6: EF-Tu dependence of ternary complex stability in 100 mM KI. Assay procedures are described under Experimental Procedures. Samples contained [^{14}C]Phe-$tRNA^{Phe}$–F^8 (899 dpm/pmol of Phe; 905 pmol of Phe/A_{260} unit of tRNA; 0.012 A_{260} unit), either 4.1 μg (□, ■) or 24.4 μg (○, ●) of EF-Tu, and either 0 (○, □) or 100 (●, ■) mM KI. The range of values obtained from parallel samples which lacked PEP (and therefore ternary complex) is also shown, with the solid triangle indicating the average value.

of this difference was caused by an increase in the molar absorptivity of fluorescein when Phe-$tRNA^{Phe}$–F^8 bound to EF-Tu·GTP rather than by an increase in the quantum yield and fluorescence lifetime of the fluorescein. Therefore, the τ_0 values for Phe-$tRNA^{Phe}$–F^8 in the presence of EF-Tu·GTP or EF-Tu·GDP differ by, at most, about 16%.

The observed decrease in fluorescence is not an ionic strength effect because the emission intensity did not change when KCl was added to the solution instead of KI (Figure 5). This was confirmed by adding Phe-$tRNA^{Phe}$–F^8·EF-Tu·GTP to a set of samples in which [KI] varied from 0 to 118 mM, while [KI] + [KCl] was kept constant at 118 mM. The Stern–Volmer constant obtained by using this procedure was exactly the same as that obtained by using the addition procedure described under Experimental Procedures.

Stability of the Ternary Complex in Quenching Experiments. High ionic strengths, and iodide ions in particular, destabilize the ternary complex (not shown). As a result, iodide ion concentrations were limited to 100 mM in the above quenching experiments. In addition, EF-Tu·GTP was present in the above samples at a 25-fold molar excess over Phe-$tRNA^{Phe}$–F^8, at a concentration high enough (2.0 μM) to assure ternary complex formation with every Phe-$tRNA^{Phe}$–F^8. At such a high concentration of EF-Tu, there was essentially no deacylation of the aa-tRNA after 2 h (Figure 6).

The extent of ternary complex formation was routinely ascertained following each quenching experiment. Two independent methods were used to determine the extent to which [^{14}C]Phe-$tRNA^{Phe}$–F^8 was protected from hydrolysis in each

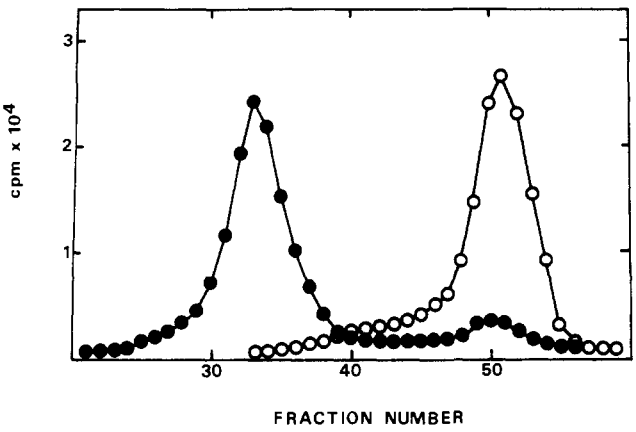

FIGURE 7: Gel filtration of samples following quenching experiments. The samples of Figure 5 were analyzed by gel filtration chromatography as detailed under Experimental Procedures. Standardization of the Ultrogel AcA 44 column gave the following elution positions: blue dextran at fraction 25; bovine serum albumin at 36; EF-Tu·GDP at 39; lysine at 51. (●) +PEP; (○) −PEP.

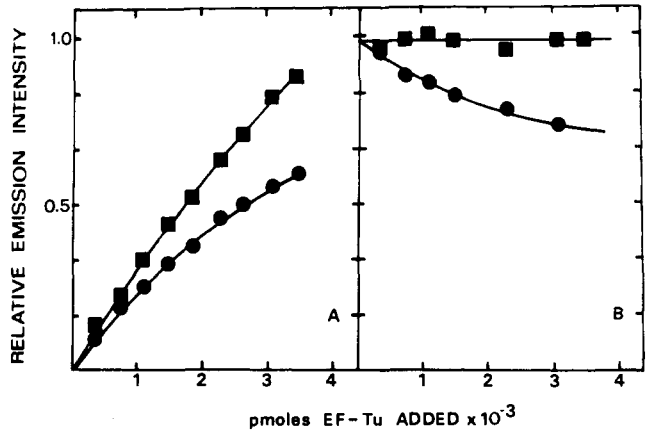

FIGURE 8: Effect of ternary complex formation on ethidium binding to aa-tRNA. Sample contents are detailed under Experimental Procedures, as are the specifics of the spectral measurements. In (A), 1 μM ethidium bromide was titrated with either aa-tRNA·EF-Tu·GTP (●) or aa-tRNA + EF-Tu·GDP (■). In (B), preformed aa-tRNA–ethidium complexes were titrated with either EF-Tu·GTP (●) or EF-Tu·GDP (■). These titrations were done at 10 °C.

sample. Measurements of acid-precipitable radioactivity before and after the spectral measurements demonstrated that little hydrolysis (usually <10%) occurred in samples containing EF-Tu·GTP, whereas substantial hydrolysis occurred, as expected, in samples containing EF-Tu·GDP (Figure 5, inset). Gel filtration following the quenching experiments directly confirmed the presence of ternary complex in the GTP-containing sample. As shown in Figure 7, nearly all of the [^{14}C]Phe (>90%) elutes from an Ultrogel AcA 44 column at a position consistent with the elution of [^{14}C]Phe-tRNA^{Phe}–F^{8}·EF-Tu·GTP. In contrast, most of the [^{14}C]Phe (approximately 80%, depending upon the length of the experiment) elutes with the included volume, presumably as free Phe, when GTP is absent from the sample (Figure 7). The remainder (20% or less) eluted at the position expected for aa-tRNA.

Thus, under our conditions, essentially every Phe-tRNA^{Phe}–F^{8} molecule was bound to an EF-Tu·GTP throughout the experiments measuring the iodide ion quenching of the ternary complex. These experiments were done at 30 °C because of the ease of demonstrating the presence or absence or ternary complex formation in parallel samples (Figure 7).

Effect of Ethidium Bromide on Ternary Complex Formation. Aminoacyl bond protection assays were done in the presence of EF-Tu·GTP by using unfractionated tRNA which had been totally aminoacylated (15 of the amino acids were tritiated; see Experimental Procedures). If ethidium inhibited ternary complex formation, then the overall rate of deacylation would be higher in ethidium-containing samples because of the reduced concentration of aa-tRNA·EF-Tu·GTP. However, the overall rate of hydrolysis of amino acids from tRNA was found to be the same in the presence and absence of ethidium bromide at concentrations up to 19 μM (not shown). Ethidium bromide did not alter the hydrolysis rate even when EF-Tu·GTP was present at a low (1.7-fold) molar excess over aa-tRNA. Thus, ternary complex formation was not significantly perturbed by the presence of ethidium bromide at concentrations high enough to permit ethidium binding to its strong binding site on each aa-tRNA.

Fluorescence Detection of Ethidium Binding to tRNA. When excess tRNA was added to a solution of ethidium bromide, a dramatic change in fluorescence was observed. The total fluorescence emission intensity increased by 16-fold at 30 °C (18-fold at 10 °C), and the wavelength of maximum emission shifted 30 nm to the blue (not shown). Scatchard plots of the data obtained from titrations of ethidium bromide with tRNA at 10 °C yielded an association constant of 1.5 × 10^{6} M^{-1} for ethidium association with unfractionated tRNA (not shown). This agrees well with the association constants obtained by others using somewhat different temperature and solvent conditions (Bittman, 1969; Tao et al., 1970; Wells & Cantor, 1977).

Effect of Ternary Complex Formation on Ethidium Binding to tRNA. When either aa-tRNA·EF-Tu·GTP or aa-tRNA + EF-Tu·GDP was added to a solution of ethidium bromide, the intensity of fluorescence emission increased (Figure 8A). This change in fluorescence was not due to an interaction between ethidium and protein because ethidium fluorescence was not changed when the same amount of either EF-Tu·GTP or EF-Tu·GDP was added to a cuvette containing 1.0 μM ethidium bromide (not shown). Thus, the fluorescence increase resulted from binding of ethidium to aa-tRNA.

The difference in fluorescence intensity observed in the two samples of Figure 8A is caused by a reduction in ethidium emission intensity upon ternary complex formation. This is shown by the data in Figure 8B, where the addition of EF-Tu·GDP to a sample containing the preformed ethidium–aa-tRNA complex did not affect the fluorescence, while the ethidium fluorescence intensity was reduced when EF-Tu·GTP was added to a parallel sample. It is clear from these results that the nature of the ethidium binding site is altered when EF-Tu·GTP associates with aa-tRNA.

This difference in ethidium binding is observed over a wide range of ethidium concentrations, as can be seen by parallel titrations with ethidium of free aa-tRNA and of aa-tRNA·EF-Tu·GTP (Figure 9). At low ethidium concentrations, when aa-tRNA was much in excess over ethidium and secondary binding by ethidium was negligible, the difference in ethidium fluorescence in the aa-tRNA and the ternary complex samples was clearly evident (Figure 9). At high ethidium concentrations, the fluorescence intensity in the free aa-tRNA sample was slightly more than that expected for full occupation of the tight ethidium binding sites (given by the dashed line in Figure 9) and also slightly more than that observed in the sample containing ternary complex (Figure 9). On the other hand, since the difference in ethidium fluorescence is relatively small for the aa-tRNA and aa-tRNA·EF-Tu·GTP samples (Figure 9), it appears that ethidium binds to its high-affinity

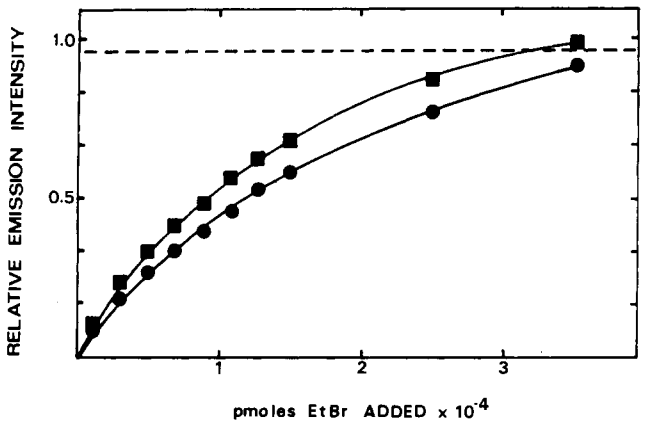

FIGURE 9: Ethidium binding capacity of aa-tRNA and of the ternary complex. Samples containing either aa-tRNA·EF-Tu·GTP (●) or aa-tRNA + EF-Tu·GDP (■) were titrated with ethidium as described under Experimental Procedures. The dashed line indicates the total emission intensity expected if the strong ethidium binding sites in the free aa-tRNA sample were totally occupied. This was calculated from the total picomoles of aa-tRNA in the sample as described under Experimental Procedures.

site even when aa-tRNA is associated with EF-Tu·GTP.

The formation of ternary complexes in samples containing EF-Tu·GTP was confirmed in the experiments of Figure 8 and Figure 9 by both gel filtration chromatography as in Figure 7 and aminoacyl bond protection as in Figure 5 (inset).

Three possible explanations for the reduced ethidium fluorescence in the presence of the ternary complex are the following: (i) EF-Tu·GTP sterically reduces ethidium access to its binding site on aa-tRNA; (ii) EF-Tu·GTP binding changes the conformation of the aa-tRNA site so that its affinity for ethidium is reduced; and/or (iii) EF-Tu·GTP binding alters the site on the aa-tRNA, specifically the environment of the bound ethidium, so that the total fluorescence intensity change per bound ethidium is reduced. Because of the difficulty of obtaining large quantities of pure ternary complex, we have been unable to ascertain whether or not possibility iii occurs. If the reduced ethidium emission is caused by a reduced overall association constant with the EF-Tu-complexed aa-tRNA, our data cannot distinguish between possibilities i and ii. However, in view of the close proximity of the ethidium binding site to the s^4U base (see Discussion), the most likely interpretation of the ethidium data is that the aa-tRNA conformational change detected by s^4U-bound fluorescein (Figure 2) has affected ethidium fluorescence by some combination of possibilities ii and iii.

In any case, it is clear that the ethidium binding site on aa-tRNA is not blocked by EF-Tu in the ternary complex. This rules out the possibility that an aromatic amino acid side chain of EF-Tu is binding to the same site on aa-tRNA that binds ethidium, as has been suggested (Wells & Cantor, 1977).

Discussion

An understanding of ternary complex function and the role of EF-Tu in protein biosynthesis requires an understanding of two different aspects of ternary complex structure: conformational changes and topology. We have investigated ternary complex topology and the possible involvement of conformational changes in ternary complex function by using fluorescence spectroscopy. This approach is especially useful in such studies because fluorescence probes are, in general, extremely sensitive to changes in their environment and can be monitored continuously, selectively, and nondestructively. The last property is particularly advantageous in this case because the macromolecules are not being altered during the experiment. Thus, in contrast to most enzymatic digestion and chemical modification studies, one can demonstrate that the macromolecules were associated throughout the experiment (cf. Figures 5 and 7). This, of course, simplifies the interpretation of the results.

Two different types of extrinsic fluorescent probes were used in this work. Both monitored aa-tRNA structure in the hinge region, near the juncture of the two arms of the tRNA. In one case, site-specific labeling at a known location was obtained by covalently attaching a fluorescein moiety to the s^4U base of $tRNA^{Phe}$ (Johnson et al., 1982). The covalent attachment of the probe to s^4U had little effect upon the function of the macromolecule: $tRNA^{Phe}$–F^8 was nearly as active as the unmodified tRNA in several different assays (Figure 1) (Johnson et al., 1982). In the other case, a noncovalently bound probe, ethidium, was used. Specificity was obtained because ethidium binds to a single site on tRNA with relatively high affinity (Bittman, 1969; Tao et al., 1970). There is, however, disagreement about the precise location of the binding site. X-ray crystallographic data indicate that the ethidium partially stacks on top of the s^4U base (Liebman et al., 1977). Some NMR data indicate that the ethidium intercalates between the base pairs at the end of the acceptor stem (Jones & Kearns, 1975; Jones et al., 1978), but others have disagreed with this assignment (Hurd & Reid, 1979). Either location is consistent with singlet–singlet energy transfer data obtained by using a 3′-dansylated tRNA (Wells & Cantor, 1977). This uncertainty in the location of bound ethidium was counterbalanced in our experiments by the fact that we were able to examine unmodified and unfractionated aa-tRNA by using this approach.

Our results show that the conformation of aa-tRNA changes when it binds to EF-Tu·GTP. This was demonstrated by a change in the fluorescein emission intensity when Phe-$tRNA^{Phe}$–F^8 associated with EF-Tu·GTP (Figure 2). The spectral change was not caused by a direct interaction between the dye and EF-Tu because the solvent accessibility of the dye was not altered significantly by ternary complex formation (see Results; Figure 5). Ternary complex formation also altered the nature of the ethidium binding site on unmodified and unfractionated aa-tRNA (Figures 8 and 9), probably because the conformation of the aa-tRNA changed at or near the ethidium binding site. Thus, the data from two independent methods are consistent with each other and indicate that ternary complex formation causes a conformational change in the aa-tRNA.

The exact nature of the conformational change near s^4U cannot be ascertained by our experiments, but the change in fluorescein emission intensity (Figure 2) was caused primarily by an increase in the molar absorptivity of the dye (Figure 3). Since the molar absorptivity of the dye is sensitive to pH (Mercola et al., 1972) and therefore to the extent of deprotonation of the fluorescein, it appears that a change in the electrostatic environment of the dye led to the fluorescence change. Most likely, a change in the location of negatively charged phosphate groups near the dye resulted in a change in either the local pH or the pK_a of the monoanion form of fluorescein.

The conformational flexibility of tRNA is well established [reviewed by Crothers & Cole (1978)], and the involvement of structural changes in tRNA function has been proposed by many investigators. Particularly relevant to our results is the observation that a point mutation in the dihydrouridine stem alters the codon recognition properties of $tRNA^{Trp}$, creating

a tRNA that can translate both its normal codon, UGG, and the nonsense codon, UGA (Hirsch, 1971; Hirsch & Gold, 1971). Interestingly, the coding properties of this UGA suppressor $tRNA^{Trp}$ can be altered by the photo-cross-linking of s^4U-8 and C-13 (Vacher & Buckingham, 1979). This indicates that the structure of the aa-tRNA near s^4U is involved, directly or indirectly, in the decoding process. In view of the EF-Tu-induced change in aa-tRNA conformation described in this paper, it seems likely that one function of EF-Tu in protein biosynthesis is to ensure that every aa-tRNA is in a particular (probably the same) conformation prior to initiation of the recognition process at the ribosomal complex. In effect, EF-Tu would serve to regulate aa-tRNA conformation. It is possible that this is a primary mechanism by which EF-Tu facilitates protein biosynthesis.

Is the region near s^4U accessible to the ribosome during decoding? Our fluorescence quenching results demonstrate that EF-Tu does not cover the s^4U region upon binding to aa-tRNA (Figure 5). It must be emphasized that these data were obtained under conditions which did not destroy the complex between aa-tRNA and EF-Tu·GTP. Indeed, the complex was shown to be intact even after 4 h of spectral measurements (Figures 5 and 7). EF-Tu also does not block ethidium access to its strong binding site (Figures 8 and 9), which is located either near the s^4U or at the end of the acceptor stem (see above). These results explain why attempts to cross-link EF-Tu to aa-tRNA by using photoaffinity labels attached to s^4U-8 have failed (D. L. Miller and J. Ofengand, unpublished results).

There is disagreement, however, about the extent of exposure of the D stem and the D loop of the aa-tRNA in the ternary complex. Nuclease digestion studies indicate that the D loop is accessible (Jekowsky et al., 1977), while kethoxal modification data have been interpreted as demonstrating that the EF-Tu is in close proximity to the D stem and loop (Bertram & Wagner, 1982). Since the rates of chemical and enzymatic reactions with aa-tRNA depend upon the conformation of the aa-tRNA at or near the reactive site(s), it is necessary to interpret with caution any data comparing chemical or enzyme reactivities in the presence and absence of ternary complex formation. In view of our finding that association with EF-Tu·GTP causes a conformational change in aa-tRNA, it seems likely that the change in kethoxal reactivity observed by Bertram & Wagner (1982) was caused by the change in aa-tRNA conformation, and the D stem and D loop are not covered by EF-Tu in the ternary complex. This interpretation is consistent with all of the data reported.

On the other side of the aa-tRNA, the emission of a fluorescamine fluorophore covalently attached to X-47 of $tRNA^{Phe}$ was not altered upon ternary complex formation (Sprinzl & Faulhammer, 1978). EF-Tu can be cross-linked to the X base in the variable loop of $tRNA^{Phe}$ only if the photoaffinity label is at least 20 Å long (T. H. Kao, D. L. Miller, M. Abo, and J. Ofengand, unpublished results). Thus, neither side of the aa-tRNA appears to be covered by EF-Tu in the ternary complex. In addition, the apex of the tRNA "L" is free because the TΨC and D loops are accessible to digestion with a single-strand-specific nuclease (Jekowsky et al., 1977). Thus, we conclude that EF-Tu binds only to the aminoacyl end of the aa-tRNA and to some portion of its acceptor-TΨC arm and that at least half of the aa-tRNA (its anticodon-containing arm) is available for direct interaction with the ribosome during recognition.

Finally, others have found, using photo-cross-linked (Favre et al., 1979; Holler et al., 1981) and fluorescent-labeled (Rigler et al., 1981) tRNAs, that the conformation of the tRNA near s^4U-8 is altered upon association with the cognate synthetase. The small but reproducible difference in the rate of iodide ion quenching of $tRNA^{Phe}$–F^8 and Phe-$tRNA^{Phe}$–F^8 that we have observed (Figure 4) is also consistent with a structural change near s^4U-8. However, we observed no change in fluorescence emission when $tRNA^{Phe}$–F^8 was aminoacylated (Figure 2). Apparently, the nature of the change is such that it causes no net change in the emission intensity of the fluorescein probe.

Acknowledgments

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Registry No. Ethidium bromide, 1239-45-8.

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Altered Protein Synthesis in Ataxia–Telangiectasia Fibroblasts†

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ABSTRACT: Analysis of protein production in various strains of ataxia–telangiectasia (A–T) fibroblasts demonstrated the overproduction of a group of secreted proteins and variations in the protein characteristics of the extracellular matrix. The most prominent differences involved fibronectin, which was identified by immunochemical analysis. One- and two-dimensional gel electrophoresis demonstrated differences in the production, accumulation, and molecular weight of fibronectin on the cell surface and in the culture medium as compared to normal human fibroblasts. Three other secreted proteins with molecular weights of 185 000, 150 000, and 70 000 were also observed to be produced in excess amounts in some strains of A–T. The finding that extracellular matrix alterations are involved in the abnormal DNA synthesis and reduced cell survival in A–T cells in response to X radiation would be additional evidence for a close association between the extracellular and nuclear architecture.

Ataxia–telangiectasia (A–T) is an autosomal recessive human genetic disease characterized by neurological disorders, immune deficiencies, a high incidence of cancer, and sensitivity to ionizing radiation (Kraemer, 1977; Bridges & Harnden, 1982). The biochemical lesions involved in this disease have yet to be determined, but various lines of evidence indicate that abnormalities in coordination of DNA synthesis are somehow involved. A–T cells have a prolonged S phase (Cohen & Simpson, 1980; Murnane & Painter, 1982) despite their normal rate of DNA chain elongation (Kapp & Painter, 1981) and fail to demonstrate the inhibition of replicon initiation normally occurring in the presence of X-ray-induced DNA damage (Edwards & Taylor, 1980; Houldsworth & Lavin, 1980; Painter & Young, 1980). With the use of these observations, A–T strains have been categorized into at least five complementation groups representing separate gene mutations (Murnane & Painter, 1982; Jaspers & Bootsma, 1982b).

The possible role of chromatin conformation in regulation of DNA synthesis (Blumenthal et al., 1973) has led to the proposal that these mutations may involve structural irregularities in packaging of DNA (Painter & Young, 1980). To gain further insight into the possible relationships of various structural proteins to the A–T phenotypes, we compared protein content and production in fibroblasts from several normal, A–T, and other human genetic disease strains. Although initial experiments centered around investigation of nuclear proteins, these studies demonstrated major differences in the extracellular matrix proteins, which were then further characterized.

Materials and Methods

Cells. Normal human skin fibroblasts HS-1 and HS-27 were established by the Cell Culture Facility, University of California, San Francisco. AT3BI, AT5BI, and AT7BI skin fibroblasts were provided by D. G. Harnden and A. M. R. Taylor, University of Birmingham, England, and AT2SF skin fibroblasts were provided by Diane Wara, Department of Pediatrics, University of California, San Francisco. A–T strains AT3BI and AT5BI represent separate complementation groups (Murnane & Painter, 1982; Jaspers & Bootsma, 1982b); AT5BI and AT7BI have been reported to be proficient

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