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Study of the Interaction between Uncharged Yeast tRNA^{Phe} and Elongation Factor Tu from *Bacillus stearothermophilis*†

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ABSTRACT: Proton NMR studies are presented on the interaction of nonaminoacylated yeast tRNA^{Phe} and elongation factor Tu·GTP from *Bacillus stearothermophilis*. From experiments in which transfer of magnetization is observed between proton spins of tRNA and the protein, it is concluded that complex formation takes place. Amino acid residues of the protein come into close contact with the base pair A5U68 and/or U52A62 of the acceptor TΨC limb of the tRNA molecule. From the line broadening of tRNA resonances, associated with complex formation, an association constant of 10^3–10^4 M^{-1} is estimated. The NMR experiments do not monitor a significant conformational change of the tRNA molecule upon interaction with the protein. However, at times long after the onset of complex formation, spectral changes indicate that the upper part of the acceptor helix becomes distorted.

In the elongation cycle of protein synthesis, the specific binding of aminoacyl-tRNA (aa-tRNA) to the programmed ribosome is mediated by elongation factor Tu (EFTu) (Miller & Weissbach, 1977; Kaziro, 1978; Bosch et al., 1983). In this process, the EFTu protein and guanosine 5′-triphosphate (GTP) form a complex (EFTu·GTP) which preferentially binds to aminoacylated noninitiator tRNAs. The thus formed ternary complex then interacts with the ribosome in such a way that the tRNA is positioned in the ribosomal A site. The molecular mechanism underlying the formation of the ternary complex and also the why and wherefore of its existence are still a matter of conjecture. For instance, EFTu·GTP does not appear to discriminate among various aminoacylated elongation tRNAs and therefore must obviously recognize structural features common to all of these tRNAs. However, the characteristic features of the common structural elements that are recognized by the protein are not well-known. Conformational changes both in the tRNA and in the protein as a result of their mutual interactions have been postulated as being necessary for proper ribosome binding and codon recognition, but so far, no definite experimental evidence is available to prove or disprove such proposals.

A variety of experiments have been performed to detect which sites on aa-tRNA interact with EFTu and how this interaction influences the conformation of tRNA. These include (a) probing of tRNA structure in the ternary complex by enzymatic modifications (Jekowsky et al., 1977; Boutorin et al., 1981; Wikman et al., 1982), (b) chemical modification (Bertram & Wagner, 1982; Douthwaite et al., 1983; Riehl et al., 1983), (c) spin-labeling of tRNA (Kruse et al., 1978; Sprinzl et al., 1978; Weygand-Durasevic et al., 1981), (d) cross-linking between EFTu and tRNA (Kao et al., 1983), (c) oligonucleotide binding (Kruse et al., 1980), (f) binding of other tRNAs with a complementary anticodon (Yamane et al., 1981), and (g) measurement of GTPase activity as a function of aa-tRNA fragment binding (Guesnet et al., 1983). The conclusions from these experiments can be summarized as follows: (1) The 3′-aminoacylated end of tRNA is most important for the interaction with EFTu·GTP. (2) EFTu·GTP covers the helix formed by the acceptor and T stem and most of the variable loop. (3) Anticodon stem and loop are not in close contact with the protein. This is probably also true for most parts of the D stem, D loop, and T loop. (4) Structural rearrangements as a result of complex formation occur in several regions of the tRNA molecule, including the anticodon loop and stem. The nature and extent of these changes have remained unclear.

Also, proton NMR studies have been carried out to investigate whether conformational changes occur in tRNA molecules upon complex formation with EFTu·GTP (Shulman et al., 1974). The imino protons in tRNA, of which the majority are involved in hydrogen bonds in the base pairs, resonate in the low-field part of the ^1H NMR spectrum between 9 and 15 ppm (Hilbers, 1979). Because EFTu, like other proteins, hardly contributes any resonance intensity to this spectral region, it is possible to observe the hydrogen-bonding network of tRNA through this spectroscopic window while tRNA interacts with the elongation factor. From this NMR work,

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it was concluded that, within the available experimental accuracy, the secondary and tertiary structure of aminoacylated *Escherichia coli* $tRNA^{Glu}$ and yeast $tRNA^{Phe}$ remains intact in the complex formed between these tRNAs and the EFTu protein from *E. coli* (Shulman et al., 1974). Since that time, substantial progress has been made in the characterization of imino proton spectra of tRNA: on the one hand, the development of new NMR instruments has provided increased resolution and sensitivity, on the other hand, the application of nuclear Overhauser effects has led to a reliable interpretation of the imino proton spectrum (Hilbers et al., 1983). At present, nearly all imino proton resonances in the 500-MHz spectrum of yeast $tRNA^{Phe}$ have been assigned (Heerschap et al., 1982, 1983a,b; Roy & Redfield, 1983), so that it may now be possible to use these spectra to obtain more detailed information about the structure of $tRNA^{Phe}$ present in a complex formed with EFTu. Our first studies on this subject are presented in this paper.

Contrary to the opinion prevailing at that time, the earlier NMR experiments showed that uncharged tRNA may form a complex with EFTu·GTP (Shulman et al., 1974), a finding that was confirmed subsequently with the aid of other techniques (Oesterberg et al., 1981; Pingoud et al., 1982; Van Noort et al., 1982). In addition, it was found that the resonances of the uncharged tRNA broadened somewhat less upon complex formation with the elongation factor than those of the aminoacylated tRNA. Therefore, in the present investigation we studied the complex formation between uncharged yeast $tRNA^{Phe}$ and EFTu·GTP. Elongation factor Tu from *Bacillus stearothermophilis* was selected for these experiments because it is much more stable than the corresponding protein from *E. coli* (Wittinghofer & Leberman, 1976). It will be demonstrated that the EFTu·GTP complex binds to the double-helical domain formed by the acceptor and TΨC stems in tRNA. Apart from the possible disruption of the GU base pair in the acceptor stem of yeast $tRNA^{Phe}$, no gross structural rearrangements of tRNA structure have been observed. An exception must be made for times long after complex formation took place: the experiments suggest that after these long times the upper part of the acceptor helix may be melted out.

Materials and Methods

GTP, ITP, and yeast $tRNA^{Phe}$ were purchased from Boehringer Mannheim. GTP was freed from contaminating GDP and GMP by elution over a DEAE-A25 column (0.5 × 20 cm) with a NaCl gradient [0.05–0.22 M NaCl in 0.1 M tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 8; 2 × 160 mL]. The GTP fractions were desalted over a Sephadex G10 column (0.5 × 80 cm).

The tRNA, which had an amino acid acceptance of at least 1260 pmol/A_{260}, was dissolved in 300 μL of a buffer containing 80 mM NaCl, 20 mM sodium cacodylate, and 5 mM $MgCl_2$, pH 7.2 (buffer A). This solution was extensively dialyzed against the same buffer in a microdialysis cell and subsequently lyophilized.

EFTu·GDP was isolated from *B. stearothermophilis* essentially according to Wittinghofer and Leberman (1976). As an extra purification step, the protein was applied to a column of G150 (5 × 100 cm) equilibrated with a buffer that contained 50 mM Tris-HCl, 10 mM $MgCl_2$, 10 mM 2-mercaptoethanol, and 0.1 mM sodium azide, pH 7. The column was eluted with this same buffer. EFTu·GDP was converted into EFTu·ITP according to Wittinghofer and Leberman (1976). About 15 mg of this complex was dissolved in 300 μL of a buffer containing 50 mM Tris, 1 mM dithioerythritol (DTE), 1 mM ethylenediaminetetraacetic acid (EDTA), and 0.2 M ITP, pH 8. Subsequently, the ITP was removed from EFTu by passing the solution over a Bio-Gel P4 column which was eluted with a buffer of 50 mM Tris, 1 mM DTE, and 1 mM EDTA, pH 7.2. The fractions of EFTu free of nucleotide were pooled (~5 mL) and dialyzed against a buffer of 1 mM sodium cacodylate and 0.1 mM DTE, pH 7.2. The sample was then frozen by dropwise addition to liquid nitrogen and subsequently lyophilized. In this way, several samples were prepared from one batch of EFTu·ITP.

To be able to compare the spectra recorded separately for the tRNA, the EFTu·GTP, and the ternary complex, the NMR experiments were carried out in the following order. The lyophilized tRNA sample was dissolved in 300 μL of a mixture of H_2O/D_2O (95:5 v/v). After spectra were recorded at various temperatures, the sample was lyophilized while it remained in the NMR tube. NMR spectra of EFTu·GTP were obtained by dissolving EFTu free of nucleotide in 300 μL of buffer A which also contained 5% D_2O and GTP in slight excess to the amount of EFTu (concentration of EFTu was ~1 mM). To obtain spectra of the ternary complex, an EFTu·GTP sample identical with the one described above was prepared with the only exception that buffer A was now 10 times diluted (the low-field ^1H NMR spectrum of this sample was found to be identical with that of the other EFTu·GTP sample). The latter solution was then added to the tRNA, which had been lyophilized in the NMR tube, after which NMR spectra of the ternary complex could be recorded.

After the NMR experiments, tRNA was checked for degradation by gel electrophoresis. To this end, tRNA and EFTu were separated by phenol extraction (residual phenol in the water layer was removed with the help of ether). Samples of approximately 5 μg of tRNA were applied to a 12% polyacrylamide gel in 5 M urea and 30 mM Tris-HCl at pH 8.0. After electrophoresis, the gel was stained according to Merril et al. (1981).

The NMR spectra were recorded on a Bruker WM 500 spectrometer interfaced with an Aspect 2000 computer. To avoid, as much as possible, excitation of the proton spins of H_2O, a semiselective observation pulse was used; further suppression of the remaining water signal was achieved by an alternative delayed acquisition (ADA) as described previously (Haasnoot & Hilbers, 1983). Chemical shifts are quoted relative to 4,4-dimethyl-4-silapentane-1-sulfonate (DSS). The NOE measurements were carried out according to the procedure described earlier (Heerschap et al., 1982).

Results

Spectral Changes Induced by EFTu. Proton NMR spectra were recorded separately for three different samples: i.e., yeast $tRNA^{Phe}$, EFTu·GTP from *B. stearothermophilis*, and the complex formed by these molecules after they were added together in a 1:1 ratio. The spectrum of $tRNA^{Phe}$ is shown in Figure 1a; the displayed spectral region includes the imino proton resonances (between 9.5 and 14.5 ppm) and the resonances of amino and aromatic protons (between 6 and 9.5 ppm). The assignments of the imino proton resonances, which are indicated by letters in Figure 1a, are presented in Table I. Figure 1b shows the same spectral region for the proton spectrum of the EFTu·GTP complex. Histidine and tryptophan ring N protons are expected to resonate downfield from 9.5 ppm (Wuethrich, 1976). According to the amino acid composition of this EFTu molecule (Wittinghofer & Leberman, 1976), a maximum of 18 such NH resonances may be observed, but as it is, not all of these are visible probably due to solvent exchange. The huge resonance intensity between 7 and 9.5 ppm mainly arises from amide protons of which

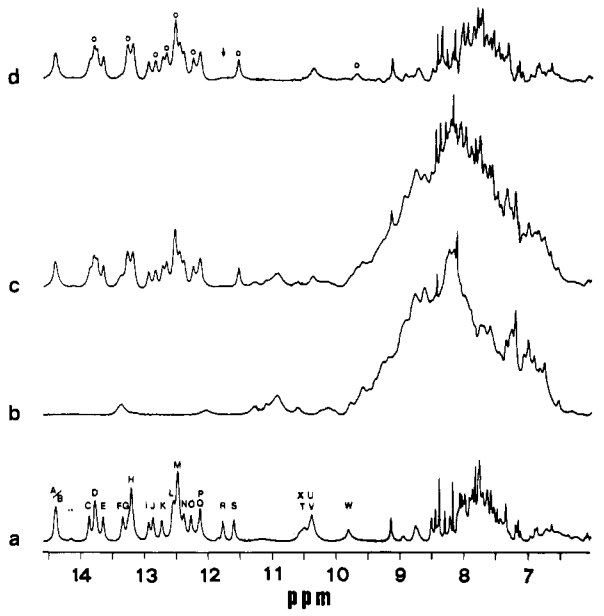

FIGURE 1: 500-MHz ^1H NMR spectra of the spectral region between 6 and 14.6 ppm of EFTu·GTP from *B. stearothermophilis* and yeast $tRNA^{Phe}$ dissolved in buffer A. Temperature was 30 °C. (a) $tRNA^{Phe}$; imino proton resonances are marked by letters (see Table I). (b) EFTu·GTP. (c) $tRNA^{Phe}$ + EFTu·GTP. The spectrum in (d) is obtained after subtracting the spectrum in (b) (EFTu·GTP) from that in (c) (EFTu·GTP + $tRNA^{Phe}$). The arrow indicates the disappearing resonance of residue U69. Imino proton resonances that shift with respect to the spectrum in (a) are indicated by o.

Table I: Spectral Marking of the Imino Proton Resonances of Yeast $tRNA^{Phe}$

part of tRNA structure	base pair	spectral marking
acceptor stem	C1G72[a]	M
	C2G71[a]	J
	G3C70	O
	G4U69	R
		U
	A5U68	C
	U6A67	A
	U7A66	G
D stem	m^2G10C25	K
	C11G24	E
	U12A23	D
	C13G22	I
anticodon stem	C27G43	L
	C28G42	P
	A29U41	H
	G30m^5C40	L
	A31Ψ39H3[b]	H
	A31Ψ39H1[b]	T
T stem	m^5C49G65	M
	U50A64	H
	G51C63	N
	U52A62	D
	G53C61	M/N
tertiary structure	U8A14	B
	G15C48	Q
	G18(H1)	W
	G19C56	M
	m_2^2G26(H1)	V
	m^7G46G22	F
	T54m^1A58	M
	Ψ55(H1)	X
	Ψ55(H3)	S

[a] On the basis of NOESY experiments (Heerschap et al., 1985), the assignments of these two imino protons have been reversed with respect to the one-dimensional NOE experiments (Hilbers et al., 1983). [b] There is still some uncertainty whether the H1 or the H3 proton should be assigned to resonance H or T.

nearly 450 are present in the molecule. However, due to the use of the semiselective observation pulse, the intensity between 6 and 8 ppm is significantly reduced with respect to that between 10 and 15 ppm. The spectrum presented in Figure 1c is from a sample in which $tRNA^{Phe}$ and EFTu·GTP had been added together in a 1:1 ratio. A better impression of the spectrum of the tRNA in the complex is obtained when the resonances of the elongation factor are "removed". This has been done by subtracting the spectrum of EFTu·GTP (Figure 1b) from the spectrum of the ternary complex (Figure 1c); the result is shown in Figure 1d. The overall appearance of the spectra in Figure 1a and 1d is quite similar. This indicates the following: (1) Possible conformational changes in the EFTu molecule are not monitored by the aromatic and amide proton resonances. It is noted in passing that in the high-field part of the spectrum (above 6 ppm) several resonances from EF-Tu protons shift their position upon addition of tRNA to the protein. Therefore, in the high-field part of the spectrum, no useful difference spectrum could be obtained. (2) As far as monitored by the imino proton resonances, no large conformational changes take place in $tRNA^{Phe}$ as a result of EFTu binding. Nevertheless, a more detailed examination of the imino proton spectra of tRNA free in solution and in the complexed state reveals some interesting features.

Although the top spectrum of Figure 1 shows some (30%) increase in line width with respect to the bottom spectrum, the resolution in the top spectrum is retained remarkably well. This indicates that the lifetime of the complex formed between tRNA and EFTu·GTP is relatively short on the NMR time scale. Futhermore, rather surprisingly the resonances of the G4U69 base pair disappear from the spectrum. This is immediately clear for the imino proton resonance of U69 at 11.8 ppm (see arrow in Figure 1d). Because there is a decrease of resonance intensity at 10.4 ppm, where the imino proton of G4 resonates, it is likely that its resonance has disappeared as well. Attempts, by means of NOE measurements (preirradiation of the imino proton resonances of the neighboring base pairs A5U68 and G3C70, vide infra), to trace whether these resonances are possibly shifted to a different position in the spectrum did not yield NOE's for the GU resonances. This is additional support for the interpretation that the resonances are obliterated from the spectrum by EFTu.

In addition to the GU base pair resonances, also the resonances of the "non-hydrogen-bonded" imino protons of Ψ39 and Ψ55 (both at 10.5 ppm) disappear from the spectrum. This is not a result of complex formation between EFTu and tRNA but is due to the presence of Tris buffer which is introduced in the sample after the addition of the EFTu solution which contains residual Tris (≤30 mM); the Tris buffer accelerates the exchange of non-hydrogen-bonded imino protons with H_2O (experiments not shown). At the temperatures employed in the experiments, this concentration of Tris was found to have no influence on the imino proton resonances of the GU base pair.

Other spectral changes induced by the EFTu protein are the relatively small shifts of some imino proton resonances; they are indicated in Figure 1d. Some of these resonances are well resolved, e.g., resonances J, O, S, and F from base pairs C2G71, G3C70, Ψ55H3, and m^7G46C22, respectively (see Figure 1a). These, as well as others which exhibit overlap, have been assigned by NOE experiments. As an example of the latter type of resonances, we consider one of the two signals originally resonating at 13.78 ppm (the resonances from base pairs U12A23 and U52A62 are overlapping at this position)

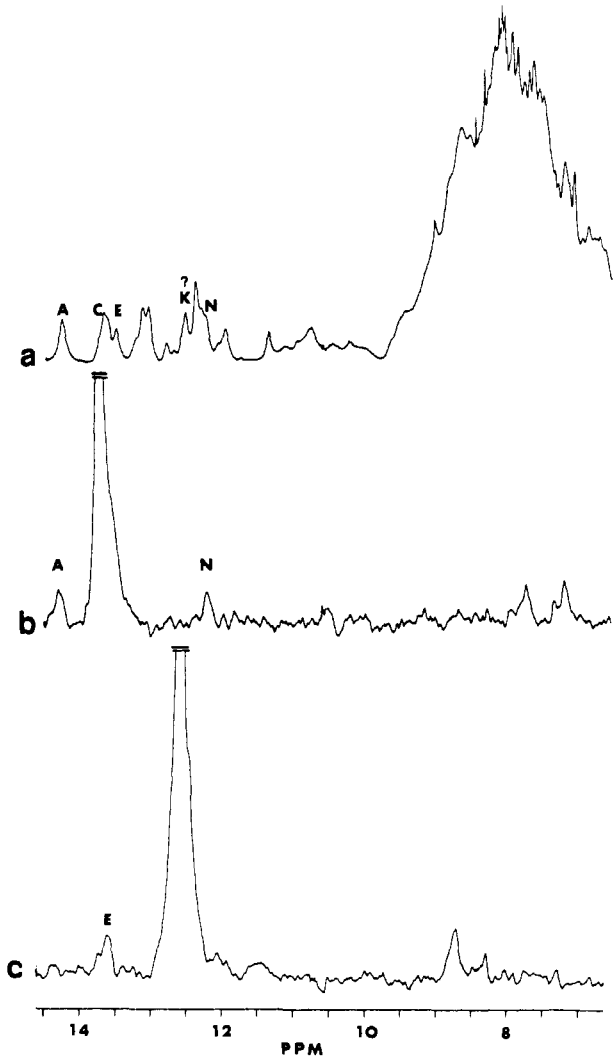

FIGURE 2: NOE measurements on the complex $tRNA^{Phe}$·EFTu·GTP. Preirradiation was for 0.4 s. (a) Reference spectrum at 25 °C; NOE difference spectra are in (b) and (c); (b) preirradiation of peak C; (c) after irradiation of peak K. The lettering in the NOE difference spectra indicates the resonances for which NOE's are observed.

which shifts downfield and overlaps with peak C. It is of interest to know whether this is the resonance from base pair U52A62 or U12A23. This is solved as follows: preirradiation (see Figure 2b) of the signal at 13.86 ppm (peak C) gives NOE's at peak A (U6A67) and peak N (G51C63). One of the signals composing peak C (in Figure 2a) arises from base pair A5U68; saturation of this resonance is expected to give rise to Overhauser effects for the resonances of U6A67 and G4U69 (Heerschap et al., 1982). We have already mentioned (vide supra) that no effects are found for the resonances of the GU base pair because EFTu obliterates its resonances. We do see an effect for U6A67 as expected; in addition, we see an effect for base pair G51C63 so that it is the resonance arising from base pair U52A62 (T stem) and not from U12A23 (D stem) which is shifted downfield. This interpretation is bolstered by the finding that in the aromatic part of the spectrum two NOE's are observed at positions close to where we have previously found the H2 resonances of A5 and A62 (Heerschap et al., 1982, 1983a) (see Figure 2b). Furthermore, we observe a resonance at 12.65 ppm (Figure 1d) that we have never seen before at this position in the course of our studies of the imino proton spectrum of yeast $tRNA^{Phe}$. Most likely it has shifted from the peak at 12.5 ppm. Irradiation of this resonance resulted in NOE's expected for the

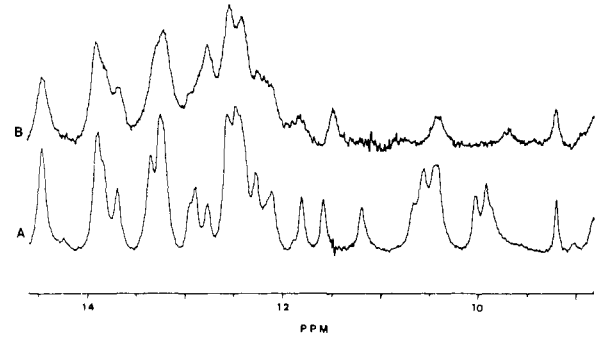

FIGURE 3: Imino proton spectra obtained at 11 °C (A) of yeast $tRNA^{Phe}$ and (B) of yeast $tRNA^{Phe}$ in the complex with EFTu·GTP from *B. stearothermophilis*.

saturation of the imino proton resonance of $m^2G10C25$ which partially overlaps with the resonance at 12.65 ppm (Figure 2c). This indicates that the imino proton resonating at this position is quite isolated from other (imino) protons and we cannot provide a definite assignment.

Interestingly, the resonances of the augmented D stem, including those of the tertiary interactions U8A14 and G15C48, are not affected at all by EFTu binding. Most of the resonances of the anticodon stem overlap with others, but as far as we can see, they do not shift either, as exemplified by the resonance positions of the imino protons of C28G42 at 12.1 ppm and of A29U41 and/or A31Ψ39 at 13.2 ppm (Figure 1a,d).

Experiments have also been performed at lower temperatures as exemplified by the spectra in Figure 3 which were obtained at 11 °C. It is found that at this temperature the imino proton resonances of $tRNA^{Phe}$ in the presence of EFTu·GTP are twice as broad as in the absence of the elongation factor. The fraction of tRNA complexed to EFTu can be estimated from the line-width formula

$$\pi\Delta\nu_{1/2} = f_A/T_{2A} + f_B/T_{2B}$$

applicable in the fast exchange limit (Carrington & McLachlan, 1969). $\Delta\nu_{1/2}$ is the resonance line width at half-height, f_A and f_B are the fractions of the complexed and free tRNA, respectively, and T_2 is the corresponding transverse relaxation time. With $T_{2B} \sim 3T_{2A}$ (the complex has a molecular weight which is about 3 times as high as that of tRNA), we estimate from single proton resonances that about 50% of the tRNA is complexed to EFTu at 11 °C. Assuming that a 1:1 complex is formed, this result means that the association constant is 10^3–10^4 M^{-1} at this temperature.

Magnetization Transfer between Protein and tRNA. Despite the diminished resolution at low temperatures, some important additional information about the tRNA·EFTu complex can be obtained. To this end, the following experiment was performed. Resonances at 0.6 ppm in the aliphatic spectral region of the protein were irradiated with a long (0.8 s) powerful presaturation pulse. The effect on the spectrum of EFTu·GDP dissolved in a D_2O buffer is shown in Figure 4. It is seen that most of the proton resonances of the molecule become saturated, due to strong spin diffusion in the protein. This saturation can be transferred to the resonances of tRNA in the complex provided that protons of the tRNA and of the protein are within certain distances, i.e., ~5 Å, and that during the saturation pulse a large portion of the tRNA in solution is complexed with the protein. An example of the results of such an experiment performed at 8 °C with a sample dissolved in H_2O is presented in Figure 5. The broad NOE's seen at 13.2 and 10.8 ppm are from protons of the EFTu·GTP molecule and arise from magnetization transfer from the aliphatic

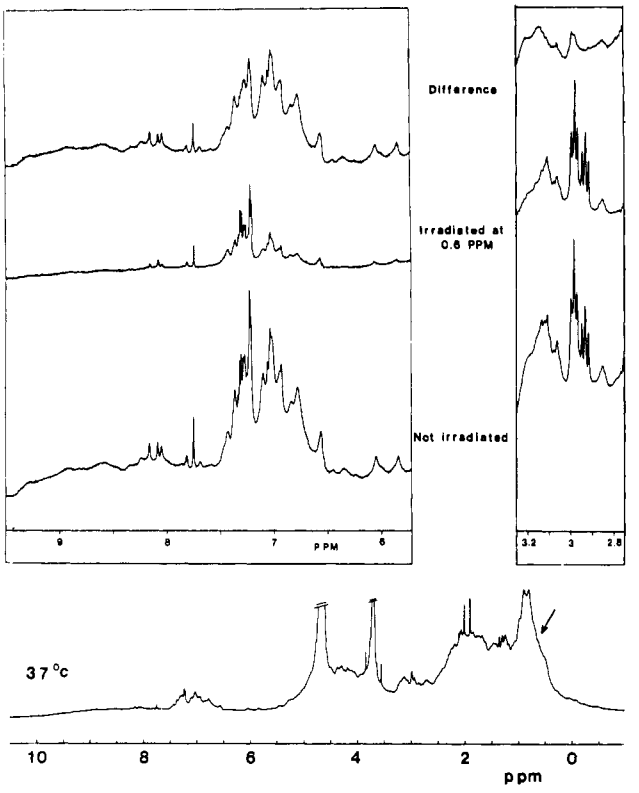

FIGURE 4: 500-MHz ^1H NMR spectrum of EFTu·GDP from *B. stearothermophilis* in D_2O with 50 mM Tris at pD ~7.2 obtained at 37 °C. The arrow in the spectrum indicates the frequency of the preirradiation pulse which lasted for 0.8 s. The insets show the effect of preirradiation for selected parts of the spectrum.

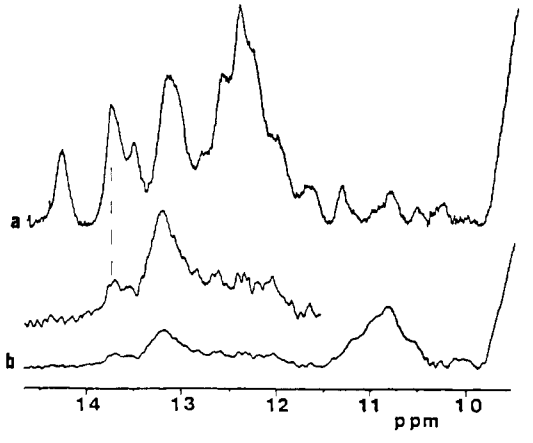

FIGURE 5: Same experiment as shown in Figure 4 but now with $tRNA^{Phe}$ and EFTu·GTP in H_2O (buffer A) at 8 °C. Only the imino part of the spectrum is shown. (a) Reference spectrum; (b) NOE difference spectrum obtained after irradiating at 0.6 ppm for 0.8 s. Middle trace is a blowup of spectrum b.

region to these protons. EFTu does not exhibit any resonance intensity at 13.8 ppm, so we conclude that the NOE seen at this position arises from the transfer of magnetization from protein residues to tRNA. The most likely candidates for the effect seen are the imino protons of A5U68 and/or A62U52 from the acceptor stem and the T stem, respectively. At higher temperatures, the same experiments did not result in an NOE on tRNA resonances. However, it appeared possible to produce transfer of magnetization in the opposite direction. For instance, after preirradiation of the imino proton resonance of A5U68 (and partly that of U52A62), NOE's could be observed in the aliphatic part of the spectrum at about 0.7, 1.2, and 3.0 ppm (see Figure 6b). The latter effect is in a

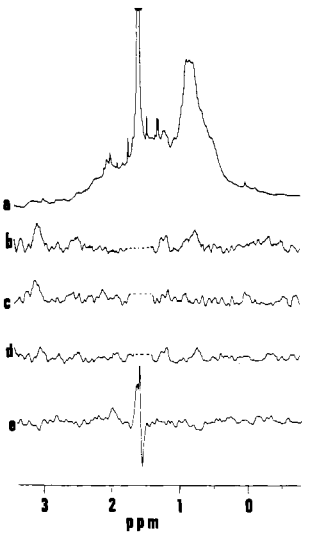

FIGURE 6: NOE measurements on the complex $tRNA^{Phe}$·EFTu·GTP at 25 °C. Preirradiation was for 0.4 s in the imino part of the spectrum. (a) Reference spectrum. NOE difference spectra are in (b) – (c); (b) preirradiation of peak C; (c) preirradiation of peak S; (d) after irradiating peak K; (e) after irradiating peak F. See Figure 1a for the corresponding peaks.

spectral region where lysine ϵ-protons and arginine δ-protons resonate. Irradiation of the resonance of the N3 proton of Ψ55 also results in an effect close to 3 ppm but not at the other positions (see Figure 6c). Irradiation of the unknown imino proton resonance at 12.65 ppm did not reveal any clear effect (see Figure 6d), but for the resonance at 13.3 ppm (indicated by F in Figure 1) a small effect is seen at 2 ppm (see Figure 6e).

In all these experiments, we also see an effect at the position of the cacodylate resonance which is probably an artifact due to a small distortion of the large cacodylate peak. The concentration of protons from the cacodylate buffer, which are all resonating at this position, is nearly 0.3 M.

EFTu May Cause Base Pair Disrupture at the Beginning of the Acceptor Stem. It is interesting to see that after prolonged times after complex formation, additional resonances disappear quite selectively in the spectra of the complex. In Figure 7, the spectrum of yeast $tRNA^{Phe}$ free in solution is presented and compared with the spectra of the complex obtained after 2, 20, and 45 h. In addition to the disappearance of the GU resonances, we see that resonances positioned at 13.8 (from A5U68), 12.8 (C2G71), 12.2 (G3C70), 12.5, and 10.4 ppm diminish in intensity and eventually disappear. [The assignment of C2G71 is based on new NOESY experiments (Heerschap et al., 1985).] The disappearance of these resonances is complete at 28 h; subsequently, after 45 h no additional changes are observed in the spectrum. It is most interesting that the three resolved resonances are from the acceptor stem and probably the resonance at 12.5 ppm as well; the imino proton of G1C72 resonates at the latter position (Heerschap et al., 1985). Because there is no clear effect on the resonance of U6A67, these results indicate that the acceptor stem is disrupted up to the last 2 AU base pairs. The resonance disappearing at 10.4 ppm is (likely) from the imino proton of m_2^2G26.

After these experiments, the tRNA was checked for degradation as described under Materials and Methods. The electrophoresis pattern showed that the tRNA sample was intact for at least 90%.

For the interpretation of the NMR experiments, it is important to note that none of the effects described previously

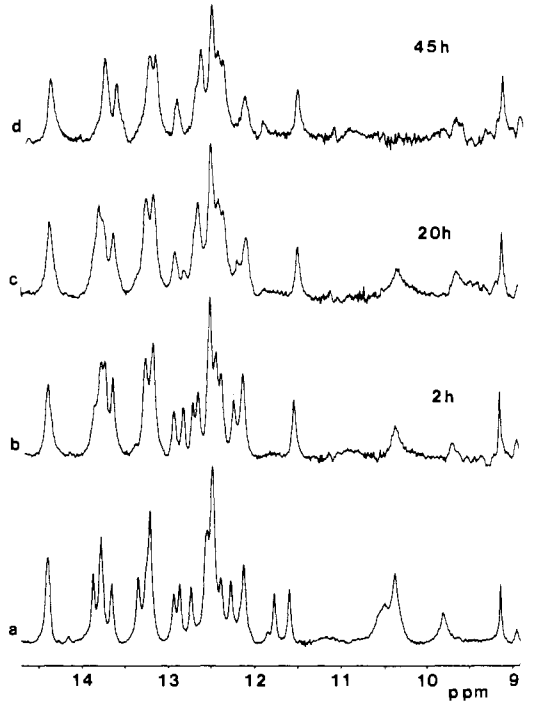

FIGURE 7: Imino proton spectra of yeast $tRNA^{Phe}$ complexed to EFTu·GTP as a function of time (indicated). The spectrum in (a) is of free tRNA.

were observed when EFTu·GDP was used instead of EFTu·GTP. In the experiments with EFTu·GDP, the line broadening of tRNA resonances between 10 and 30 °C is less than 10%, and up till 48 h after addition of EFTu·GDP to tRNA, no shift, broadening, or disappearance of resonances is observed in the imino part of the spectrum. Some of the effects brought about by EFTu·GTP on $tRNA^{Phe}$ could be recovered to a limited extent when the GTP analogue GppCp was used instead of GTP, but not the time-dependent effect of the resonances of the acceptor stem.

Discussion

Direct proof that protein complexes are formed with uncharged tRNA is provided by experiments in which transfer of magnetization was found between protons on the tRNA and on the protein. In this way, it was established that elements of the protein are in close contact with the base pair(s) A5U68 and/or U52A62 (from the acceptor stem and T stem). Consequently, the protein binds to the acceptor/TΨC limb of the L-shaped tRNA molecule.

An additional indication for binding of the protein to the acceptor stem is the disappearance of the imino proton resonances of the GU base pair. We can think of two reasons for this effect. First, it may arise from catalysis of imino proton solvent exchange by some basic residue from EFTu (e.g., lysine or histidine) which comes close to the GU base pair. Indeed, NOE experiments indicate that lysine residues may be involved in the interaction between protein and tRNA (see Figure 6b,c). This situation resembles the influence of the binding of polyamines which, as we have demonstrated, catalyzes the solvent exchange of the GU imino proton (Heerschap et al., 1986). A second possible reason for the disappearance of these imino proton resonances is destabilization (disrupture) of the GU base pair by the EFTu protein. At present, we lack sufficient experimental data to be able to discriminate between these two possibilities.

A number of biochemical experiments have indicated that the EFTu·GTP complex binds at the aminoacylated end of tRNA, and it is generally accepted that this part of the tRNA molecule forms an important recognition feature for the EFTu protein. In addition, it has been concluded from nuclease digestion experiments that the protein binds along the acceptor/TΨC helix (Boutorin et al., 1981; Wikman et al., 1982). This indicates that apart from the aminoacyl moiety other sites on the tRNA (most likely situated in the acceptor and TΨC stems) are also recognized. Indeed, studies by Parmeggiani and his co-workers probing the influence of aa-tRNA and aa-nucleotides on the GTPase activity of EFTu provide strong support for this notion (Guesnet et al., 1983; Parlato et al., 1983). From such studies, it also followed that uncharged tRNA affects the GTPase activity, though weaker than aminoacylated tRNA, and that it competes with the charged tRNA for the same binding site (Picone & Parmeggiani, 1983).

These observations are supported by the present NMR experiments. From the line broadening observed upon complex formation, it is estimated that the association constant is 10^3–10^4 M^{-1}, which is in reasonable agreement with the value of $\sim 10^4$ M^{-1} estimated from small-angle X-ray scattering for the complex of *E. coli* EFTu·GTP and nonaminoacylated $tRNA^{Val}$ (Oesterberg et al., 1981). This association constant is 3–4 orders of magnitude smaller than that obtained for the complex formation of EFTu·GTP and aminoacylated tRNA (Sprinzl et al., 1978; Oesterberg et al., 1981). The NMR experiments also show that recognition sites on tRNA exist other than the aminoacyl group. Upon binding to uncharged tRNA, association takes place to the acceptor stem and most likely also to the T stem, suggesting that EFTu recognition sites are situated in this part of the tRNA molecule. We cannot as yet establish the groups involved in recognition, but the experiments indicate as contact regions the base pairs G4U69 and A5U68 in the acceptor stem and possibly A62U52 in the TΨC stem. It has been suggested that the relative instability of the GU base pair in the acceptor step may be functional and act as a protein recognition site (Rhodes, 1977); GU base pairs are often found in the acceptor/TΨC part of tRNA molecules (Gauss & Sprinzl, 1984). If it is functional in the case of EFTu binding, we would expect such an unstable site to be conserved among various elongator tRNAs.

Complex formation between the tRNA and EFTu·GTP introduces a number of small changes in the positions of some of the imino proton resonances (Figure 1). None of these resonances belong to the anticodon loop and stem or the D stem. This is in contrast with earlier biochemical experiments which suggest that conformational changes in these parts of the molecule do occur (Boutorin et al., 1981; Weygand-Durasevic et al., 1981; Wikman et al., 1982). It might be that these latter changes only occur when a tight complex between EFTu·GTP and charged tRNA is formed.

A rather puzzling observation is the selective disappearance of the imino proton resonances from the acceptor stem (except those of AU6 and AU7) after keeping EFTu·GTP and tRNA together for several hours at temperatures between 10 and 30 °C. In light of our experiments on the imino proton solvent exchange of GC base pairs from the acceptor stem (Heerschap et al., 1986). It seems unlikely that this can be explained by a catalytic enhancement of the imino proton exchange of GC base pairs. Hence, the disappearance of the corresponding resonances seems to represent an active disrupture of the top part of the acceptor stem. Analysis of tRNA after these experiments by gel electrophoresis did not show any significant degradation although the loss of a few bases at the 3′ or 5′ end of the tRNA may go undetected in this analysis. It is

unlikely that such a possible degradation comes from a contaminating nuclease activity because tRNA preparations with EFTu·GDP or EFTu·GppCp did not reveal any of the time-dependent spectral effects observed with EFTu·GTP. The most straightforward explanation for these effects is that EFTu·GTP has a slow "melting" activity for the acceptor end of the tRNA molecule. Very slow reaction rates (on the order of hours) with ligands are not uncommon for EFTu molecules. For example, maximal binding of GDP to nucleotide-free EFTu from *E. coli* takes 0.5 h at 37 °C but 15 h at 0 °C probably due to slow structural rearrangements in the molecule (Fasano et al., 1982). In relation to this temperature dependence, it is noted that EFTu from *B. stearothermophilis* is employed in the cell at higher temperatures than EFTu from *E. coli*. Furthermore, we note that EFTu has been shown to have a second tRNA binding site at particular conditions (Van Noort et al., 1982; Bosch et al., 1983). It cannot be excluded that such a site is involved in the time-dependent experiments.

There appears to be no obvious reasons to postulate a role in protein synthesis for EFTu·GTP binding with uncharged tRNA. However, this binding could reflect a more general property of EFTu·GTP to be able to bind to nucleic acids which might be of importance for other functions of the protein such as participation in phage RNA replication. Initiation of Qβ RNA replication only takes place when the elongation factors Tu and Ts are present as a subunit of Qβ replicase (Blumenthal & Carmichael, 1979). It seems reasonable to suggest that EFTu might be involved in this initiation by interacting with the tRNA-like 3′ end of the phage RNA, but so far, it has not been possible to prove that such an interaction exists (Blumenthal & Carmichael, 1979).

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