Effect of elongation factor Tu on the conformation of phenylalanyl-tRNA^{Phe}

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Structural features of the tRNA^{Phe} molecule upon ternary complex formation with the bacterial elongation factor Tu were investigated. Phosphodiester bonds at positions 18 and 34 were found to be labilized in bound tRNA. Conversely, a higher stability of the phosphodiester links at positions 20, 21 and 36 was detected. Using ethylnitrosourea as a chemical probe a conformational change occurring at phosphate position 53 was observed in complexed tRNA. These results are interpreted by a structural rearrangement of the nucleic acid induced by complex formation.

Elongation factor Tu *Aminoacyl-tRNA* *Ethylnitrosourea* *Protein biosynthesis*

1. INTRODUCTION

Elongation factor Tu promotes the A-site specific binding of the aminoacyl-tRNAs (aa-tRNA) to the ribosome via a ternary aa-tRNA·EF-Tu·GTP complex. In the presence of GTP, the protein binds acylated forms of non-initiator tRNAs [1,2] for which it has a high affinity, but it discriminates against initiator tRNA [3], non-acylated tRNAs [3,4] and aminoacyl-tRNAs modified in their 3′-end [3,5,6]. Although the aminoacylated 3′-end of tRNA is an important recognition feature, there are several lines of evidence that EF-Tu not only binds the 3′-end of tRNA, but also the amino-acid acceptor limb of the L-shaped tRNA structure [7,8].

Here, we report the effect of EF-Tu on the conformation of aminoacylated tRNA^{Phe} upon ternary complex formation. For this purpose we investigated the chemical stability of tRNA and the accessibility of phosphates towards ethylnitrosourea, in complexed Phe-tRNA^{Phe} and in free tRNA^{Phe} using end-labelling and rapid sequencing gel methodologies. Ethylnitrosourea is an alkylating reagent which attacks phosphates in nucleic acids and can be efficiently used to probe the accessibility of these residues in tRNA [9]. Taking advantage of this property, we used ethylnitrosourea to monitor the reactivity of phosphate groups in the ternary Phe-tRNA^{Phe}·EF-Tu·GTP complex. We found an EF-Tu-induced labilization of the tRNA structure after residues 18 and 34, and an increased reactivity towards ethylnitrosourea of phosphate 53. These results will be discussed in terms of a factor-dependent conformational change of the tRNA molecule.

2. MATERIALS AND METHODS

Purified yeast tRNA^{Phe} was prepared by counter-current distribution of bulk tRNA (Boehringer, Mannheim) according to [10] followed by a BD-cellulose column chromatography, the elution being performed by a combined gradient of NaCl (0.85–1.2 M) and dimethylformamide (0–4%) in 5 mM sodium formate (pH 4.0), 20 mM $MgCl_2$ [11]. Yeast phenylalanyl-tRNA synthetase and yeast tRNA nucleotidyltransferase were purified using established procedures [12–14]. Purified *Escherichia coli* elongation factor Tu was a generous gift from Dr A. Parmeggiani (Ecole Polytechnique, Palaiseau). Ethylnitrosourea was

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purchased from Fluka AG. For autoradiography, Fuji RX X-ray films (30 ×40 cm) and Ilford intensifying screens were used.

The labelling of tRNA at its 3′- or 5′-end was done as in [15]. Labelled tRNA was purified by electrophoresis on 15% polyacrylamide gels (30 × 40 × 0.05 cm), eluted by the technique in [16] and precipitated with ethanol.

Aminoacylation of labelled $tRNA^{Phe}$ was done as in [12]; unlabelled $tRNA^{Phe}$ was used to verify the extent of charging. The reaction was stopped by addition of 1/10 vol. 2 M sodium acetate (pH 4.5) and the mixture was extracted with an equal volume of water-saturated phenol. Finally the tRNA was precipitated with 3 vol. ethanol.

Ternary complex formation between 3′- or 5′-labelled Phe-$tRNA^{Phe}$ and Tu factor was done as in [17]. EF-Tu·GDP was first converted to EF-Tu·GTP by incubation at 37°C for 10 min in the reaction mixture: 50 mM Tris–HCl (pH 7.5), 50 mM NH_4Cl, 10 mM $MgCl_2$, 5 mM dithiothreitol, 5 mM phosphoenolpyruvate, 0.25 mg pyruvate kinase/ml, 5×10^{-5} M GTP and 1 nmol EF-Tu·GDP in 20 μl total vol. The reaction was then cooled to 0°C and supplemented with 5 μl Phe-$tRNA^{Phe}$ (200 pmol). The incubation was continued for 5 min at 0°C to allow ternary complex formation.

Alkylation of tRNA has been done essentially as in [9]. The reaction was performed in conditions either stabilizing or unfolding the native structure of tRNA. In the first instance, tRNA was alkylated at 20°C for 3 h in a 30 mM sodium acetate buffer (pH 8.0) containing 20 mM $MgCl_2$, 0.2 mM EDTA and 100 mM NaCl. Ethylnitrosourea was added as a saturated ethanolic solution (5 μl) to 20 μl aqueous solution containing either free $tRNA^{Phe}$ or Phe-$tRNA^{Phe}$·EF-Tu·GTP complex. Alkylation of tRNA under denaturing conditions was performed for 1 min at 80°C in a 30 mM sodium acetate buffer (pH 8.0) containing only 2 mM EDTA. In control experiments, ethanol was substituted for the ethylnitrosourea solution. After modification the reaction mixtures were treated as in [9]; the tRNA was split at the alkylated positions and analysed by electrophoresis on 15% and 20% acrylamide gels (30 × 50 × 0.05 cm).

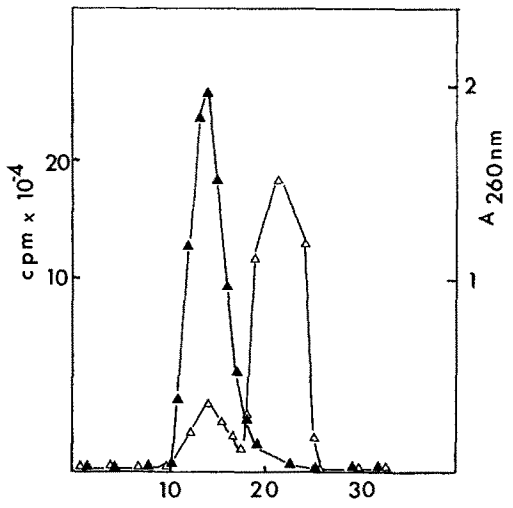

Fig.1. Gel filtration of Phe-[^{32}P]$tRNA^{Phe}$·EF-Tu·GTP complex on a Sephadex G-100 column (0.6 × 60 cm) equilibrated with 50 mM Tris–HCl buffer (pH 7.5) containing 50 mM NH_4Cl, 80 mM KCl, 10 mM $MgCl_2$ and 1 mM DTT. The column was eluted with the same buffer; 300 μl fractions were collected; (△—△) A_{260}; (▲—▲) ^{32}P radioactivity.

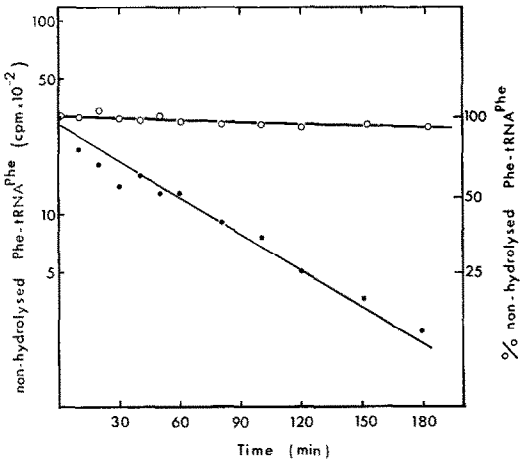

Fig.2. Stability of the ternary [^{14}C]Phe-$tRNA^{Phe}$·EF-Tu·GTP complex as monitored by the non-enzymatic deacylation of Phe-$tRNA^{Phe}$ at 20°C: 800 pmol [^{14}C]Phe-$tRNA^{Phe}$ (●—●) or [^{14}C]Phe-$tRNA^{Phe}$·EF-Tu·GTP (○—○), (each 75 cpm/pmol) were incubated in 120 μl of the reaction mixture used for alkylation (as in section 2). Aliquots of 10 μl were applied on Whatmann 3 MM filter disks, precipitated with 5% trichloroacetic acid, washed twice with 5% trichloroacetic acid and finally with ethanol. Filters were dried and the retained radioactivity was counted in a liquid scintillation spectrometer.

3. RESULTS AND DISCUSSION

3.1. *Stability of the ternary Phe-tRNA^{Phe}·EF-Tu·GTP complex*

As a prerequisite to our experiments, conditions were worked out allowing complexation of all aminoacylated tRNA^{Phe} with the EF-Tu·GTP complex. Total binding was verified by gel filtration of the ternary complex on Sephadex G-100. Only one peak of ^{32}P radioactive tRNA was obtained (fig.1) revealing that all the tRNA was charged and bound to EF-Tu.

The alkylation reaction being carried out over 3 h it was also necessary to investigate the stability of the ternary complex throughout the incubation period (fig.2). This control experiment was based on the protective effect of EF-Tu·GTP against non-enzymatic deacylation of Phe-tRNA^{Phe}. The Phe-tRNA^{Phe}·EF-Tu·GTP complex is not disrupted by the chemical reaction (fig.2); > 85% of the complex is recovered at the end of the incubation time, whereas free Phe-tRNA^{Phe} is rapidly hydrolysed, the deacylation half-lives being 15 h and 1 h, respectively.

3.2. *Chemical stability of tRNA^{Phe}*

Fig.3 and 4 show the chemical stabilities of (Phe)-tRNA^{Phe} either free or in the presence of EF-Tu·GTP under the experimental conditions used in phosphate alkylation. Typical degradations appeared under all these conditions, the strongest splits occurring after phosphate residues 9, 16, 29 (fig.3), 62 and 64 (fig.4). We verified that they were not induced by contaminating nucleases. This kind of cuts often corresponds to pyrimidine–adenine sequences and has been observed for other RNAs [19–21]. They are relevant to a peculiar flexibility of the ribose-phosphate backbone at these positions, and more precisely to the relative positioning of the 2′-OH group of the ribose and the phosphate residue [22]. Additional cuts are observed at positions 18 and 34 in EF-Tu·GTP-complexed tRNA^{Phe}. Conversely, cuts of different

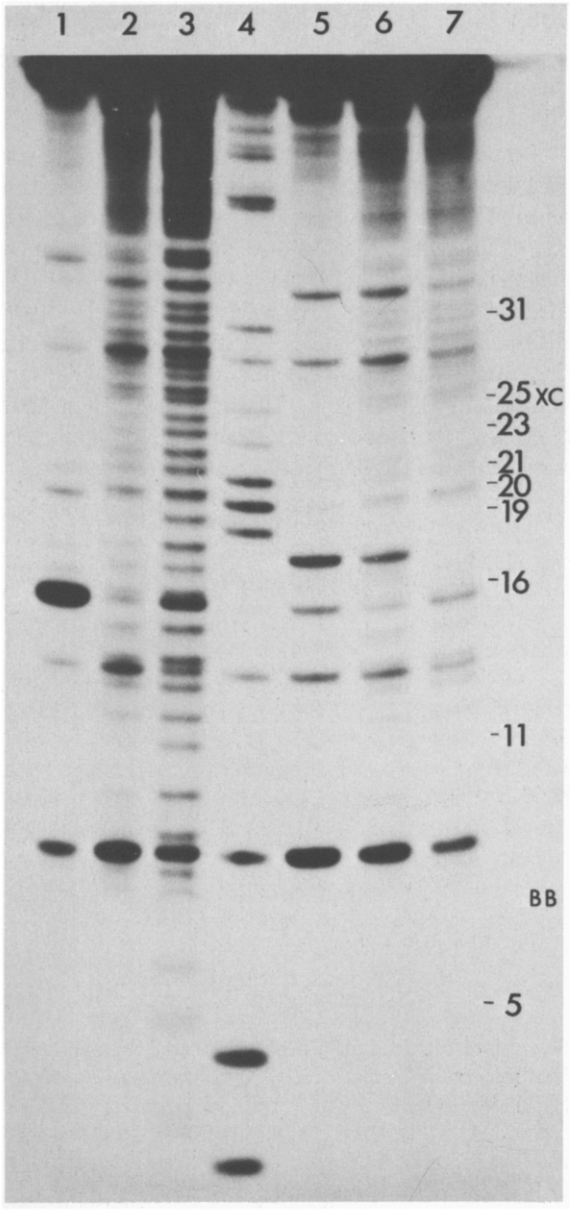

Fig.3.

Fig.3. Alkylation experiments with ethylnitrosourea on 5′-labelled (Phe)-tRNA^{Phe} in the presence of EF-Tu·GTP – autoradiogram of a 20% acrylamide gel: (1) Control incubation of free tRNA under conditions stabilizing the tertiary structure in the absence of reagent; (2,3) alkylation reaction on free tRNA under conditions stabilizing (2), or unfolding (3) the tRNA structure; (4) partial ribonuclease T_1 hydrolysis as in [18]; (5) control incubation of EF-Tu-bound Phe-tRNA^{Phe} under stabilizing conditions in the absence of reagent; (6,7) alkylation reaction on charged (6) and uncharged (7) tRNA^{Phe} in the presence of EF-Tu·GTP. 50000 Cerenkov cpm were layered on each lane of the gel; bands were revealed by fluorography at −80°C for 4 days. The numbers indicated on the figure correspond to those phosphates adjacent to the guanosines on the 3′-side (for further explanations see [9]).

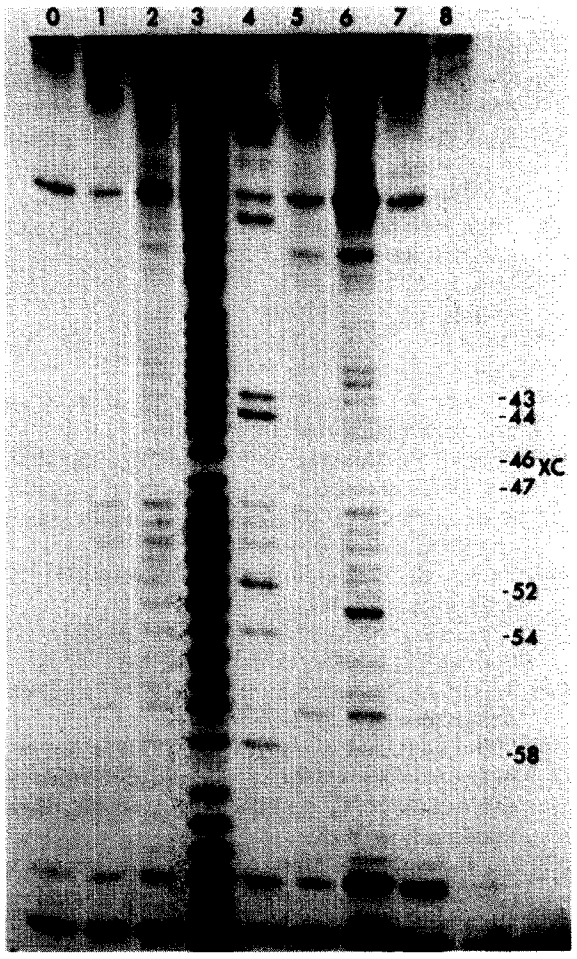

Fig.4. Alkylation experiments with ethylnitrosourea on 3′-labelled (Phe)-tRNA^{Phe} in the presence of EF-Tu·GTP – autoradiogram of a 15% acrylamide gel: (0) untreated tRNA; (1) control incubation of free tRNA under conditions stabilizing the tertiary structure in the absence of reagent; (2,3) alkylation reaction on free tRNA under conditions stabilizing (2) or unfolding (3) the tRNA structure; (4) partial ribonuclease T_1 hydrolysis as in [18]; (5) control incubation of EF-Tu-bound Phe-tRNA^{Phe} under stabilizing conditions in the absence of reagent; (6,7) alkylation reaction on charged (6) and uncharged (7) tRNA^{Phe} in the presence of EF-Tu·GTP (8) untreated Phe-tRNA^{Phe}·EF-Tu·GTP complex; other data as in fig.3.

intensities occurring at positions 20, 21 and 36, which are present in free tRNA^{Phe} are absent when tRNA^{Phe} is bound to the elongation factor (cf. lanes 1 and 5 in fig.3). This behaviour is interpreted in terms of conformational changes in the tRNA molecule upon ternary complex formation.

3.3. *Chemical modification of tRNA^{Phe} by ethylnitrosourea*

The alkylation reaction by ethylnitrosourea of free tRNA^{Phe} confirms results showing the protection of phosphate residues 9,10,11,19,49,58–60 (fig.3 and 4, lane 2); this protection is afforded by the tertiary structure of the nucleic acid [9]. The alkylation pattern of tRNA remains essentially unmodified upon ternary complex formation, except for position 53 where the intensity of the band is significantly increased (fig.4, lane 6). This enhancement is specifically related to the ternary complex since it does not occur when uncharged tRNA^{Phe} is in the presence of Tu factor (fig.4, lane 7). Furthermore, it does not correspond to a degradation of the tRNA since no splits are observed in the different controls (fig.4, lanes 1 and 8). Two possible interpretations can account for this observation:

(i) Phe-tRNA^{Phe} undergoes a structural rearrangement upon binding with EF-Tu·GTP, which leads to a greater accessibility of phosphate 53 to the solvent and thus to a higher reactivity of this residue with ethylnitrosourea;

(ii) The complexed state of tRNA^{Phe} favours a higher concentration of reagent in this region of tRNA; as a consequence, this accumulation would give rise to an enhanced rate of alkylation. Such an effect might be due to local changes in the solvent structure around the complex molecule. In that case, the concept of conformational change in complexed tRNA should be enlarged to the tRNA with its surrounding solvent and counter-ions shell.

4. CONCLUSION

According to previous studies, only part of the tRNA molecule interacts with the elongation factor Tu [7,8,23,24] (reviews [25,26]). In particular, nuclease mapping experiments showed only the amino-acid acceptor limb of the L-shaped structure of tRNA is covered by the factor and on the side that exposes the extra-loop [24]. This region contains $< 50\%$ of the total residues of the molecule. The mapping of the complexed tRNA using ethylnitrosourea as a probe unexpectedly did not allow to define contact points between EF-Tu and the phosphate groups of the aminoacylated tRNA. This result, however, is not contradictory

to the nuclease mapping experiments. Because of the small size of ethylnitrosourea, phosphate residues could be readily more accessible to this reagent, even when they are covered by the Tu factor, than to nucleases which are much more bulky. In addition, phosphates at positions 9,10,11,19,49 and especially 58–60 are already protected by the tertiary structure of the tRNA [9], and phosphates located near the 3′- and 5′-termini could not be tested because of technical limitations [9].

This study reports evidence about 6 discrete conformational changes in tRNA upon ternary complex formation. One of them, occurring at position 53, takes place in the T-stem, a region which has been shown to be covered by EF-Tu [7,24]. This change could be visualized due to the small size of the chemical probe which is not hindered by the factor in reaching this phosphate. The 5 other structural alterations occur in the D- and anticodon (ac)-loops at positions 18,20,21 and 34,36, respectively. In that case, the appearance or disappearance of cuts in complexed tRNA reflects a changed flexibility in its ribose-phosphate backbone. These results clearly indicate that the T region, the D- and ac-loops undergo a conformational rearrangement upon ternary complex formation. In the particular case of the D-loop, the occurrence of a conformational change would explain the contradictory results obtained in ribonuclease T_1 accessibilty studies and in kethoxal modification experiments [8,27]. Concerning the ac-loop, these results agree well with spectroscopic [23,28] and accessibility mapping [7,24] experiments.

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