Interaction of EF-Tu with EF-Ts: substitution of His-118 in EF-Tu destabilizes the EF-Tu·EF-Ts complex but does not prevent EF-Ts from stimulating the release of EF-Tu-bound GDP

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Abstract Elongation factor Tu from *Escherichia coli* with His-118 substituted by glycine (EF-TuH118G) was found to be defective in complex formation with EF-Ts. EF-Ts in excess failed to dissociate kirromycin from the EF-TuH118G·kirromycin complex and to form a stable complex with EF-TuH118G on column chromatography. However, the stimulatory effect of EF-Ts on GDP dissociation from EF-TuH118G·GDP and on poly(U)-directed poly(Phe) synthesis catalyzed by EF-TuH118G was only partially influenced. These results indicate that His-118, while very important for the formation of a stable EF-Tu·EF-Ts complex, is not essential for the transmission of the EF-Ts-dependent signal accelerating the release of the EF-Tu-bound GDP.

Key words: Elongation factor Tu; Elongation factor Tu histidine 118; Elongation factor Ts; Elongation factor Tu·elongation factor Ts interaction; *Escherichia coli*

1. Introduction

In the cycle of reactions which binds aminoacyl-tRNA (aa-tRNA) to mRNA-programmed ribosomes engaged in polypeptide chain elongation, the guanine nucleotide exchange protein, called elongation factor Ts (EF-Ts) [1], determines an efficient regeneration of the active, GTP-bound state of aa-tRNA transfer protein, elongation factor Tu (EF-Tu), by accelerating the dissociation of the EF-Tu·GDP complex. This function is essential because the recycling of the GDP complex to the GTP complex prevents EF-Tu from being locked in an inactive state. The regenerated EF-Tu·GTP complex is then stabilized by the interaction with aa-tRNA [2]. Indeed, in the presence of aa-tRNA, EF-Tu binds GTP with a higher affinity than GDP, whereas in the absence of aa-tRNA the affinity of EF-Tu for GTP is more than two orders of magnitude smaller than that for GDP [3].

In 1996, the crystal structure of the EF-Tu·EF-Ts complex from *Escherichia coli* was determined to a resolution of 2.5 Å so that amino acid residues involved in the interaction of the two proteins could be directly identified and a mechanism for the release of EF-Tu-bound GDP proposed [4]. According to this model, the release reaction predominantly involves the disruption of the magnesium binding site in domain I of EF-Tu. This results in a loss of affinity for the magnesium ion of EF-Tu and, consequently, for the nucleotide. It was proposed that the disruption is induced by the insertion into the EF-Tu structure of the EF-Ts side chains D80 (sD80) and F81 (sF81), two residues forming a part of a peptide sequence TDFV conserved in all EF-Ts from different organisms and tissues. The model predicts that one of the most significant interaction partners of sF81 is EF-TuH118 (uH118). The critical interaction between sF81 and uH118 appears to take place between the hydrophobic side chains of both molecules. Recently, on the basis of an X-ray diffraction model of EF-Tu·EF-Ts from *Thermus thermophilus* it has been proposed that the interaction between sF82 (sF81 in *E. coli* EF-Tu) and uH119 (uH118 in *E. coli* EF-Tu) starts a cascade of steric displacements via Q115 (Q114 in *E. coli* EF-Tu), H19 and V20, resulting in a conformational change of the phosphate binding loop (P-loop), residues 18–25 of EF-Tu, leading to an electrostatic and steric displacement of the β-phosphate of GDP (Y. Wang, Y. Jiang, M. Meyering-Voss, M. Sprinzl and P.B. Sigler, personal communication).

In a previous work we described the aa-tRNA binding properties and GTPase activity of mutant EF-TuH118G [5,6] that allowed us to trace the net effect of histidine side chain removal on these functions of the protein. In the present work – as an additional result, particularly important in the light of the three-dimensional structure – we show that the substitution of uH118 markedly impairs a crucial aspect of the interaction between EF-Tu and EF-Ts: the formation of a stable EF-Tu·EF-Ts complex. This provides experimental evidence for the prediction of the crystal model [4] that uHis-118 plays a prominent role in the stabilization of the complex. On the other hand, the substitution of uHis-118 by Gly was found to be accompanied by only a partial loss of sensitivity of EF-TuH118G·GDP to the dissociating effect of EF-Ts.

2. Materials and methods

Kirromycin was a gift from Dr. H. Beukers, Gist-Brocades, Delft, The Netherlands. All other chemicals, methods and procedures as well as preparation of mutated elongation factors EF-TuH118G and EF-TuC81G from *E. coli* were as described previously [3,6,7]. EF-Ts was overproduced from an expression plasmid pTS21 (a gift from Dr. Y. Hwang) and then isolated from a bacterial extract in a complex with EF-Tu by chromatography on a DEAE-Sepharose FF column in the absence of Mg^{2+} ions and a gradient of KCl [8,9]. The EF-Tu·EF-Ts

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Abbreviations: EF-Tu, elongation factor Tu; EF-Ts, elongation factor Ts; aa-tRNA, aminoacyl-tRNA; uH118, EF-TuHis-118; sF81, EF-TsPhe-81; uH84, EF-TuHis-84; sD80, EF-TsAsp-80; wt, wild type; EF-TuH118G, EF-Tu whose His-118 was substituted by glycine

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complex was further dissociated with an excess of GDP and EF-Ts was purified to homogeneity by column chromatography on DEAE-Sepharose FF in the presence of Mg^{2+} ions and a gradient of KCl.

3. Results

3.1. Thermostability of the EF-TuH118G mutant

Fig. 1 shows that the substitution of His-118 with glycine considerably decreased the heat stability of both conformational forms of the EF-Tu mutant, i.e. EF-TuH118G·GDP and EF-TuH118G·GTP, as compared to the corresponding states of the wtEF-Tu. The temperature at which only half of the EF-Tu molecules remained active in nucleotide binding ($\phi_{1/2}$) was about 51°C for EF-TuH118G·GDP and about 43.5°C for EF-TuH118G·GTP. This means that the thermal destabilization of both conformational states of EF-TuH118G was about 6.5°C. Thus, above 35°C the number of EF-TuH118G molecules active in GTP binding after 8 min incubation is reduced. As a consequence, all experiments were carried out at a maximum of 30°C, i.e. at a temperature at which the enzymatic activity of the mutated protein is fully retained.

3.2. EF-Ts is unable to dissociate kirromycin from EF-TuH118G·kirromycin and to form a stable complex with EF-TuH118G

The main step in the isolation of EF-TuH118G mutant protein expressed from a plasmid was the separation from the chromosome-borne, kirromycin-resistant EF-TuAr, constitutively produced in *E. coli* host strain PM1455 [6]. Within certain concentration limits EF-TuAr does not interact with kirromycin [8], in contrast to kirromycin-sensitive EF-Tus whose complex formation with the antibiotic results in an increased net negative charge and a retarded elution from a DEAE-Sepharose column [9]. Application of this observation has already proved useful in the separation from EF-TuAr of seven different EF-Tu species: EF-B_0 [8], EF-TuBs [9], EF-TuV20G [10], EF-TuC81G [7], EF-TuD138N [11], EF-TuH118G [6] and EF-TuH66G (J. Jonák and P.H. Anborgh, unpublished). The next step in the purification procedure was the removal of bound kirromycin from the EF-Tu molecule. This has been routinely achieved by incubation of EF-Tu·kirromycin complexes with an excess of EF-Ts, a natural com-

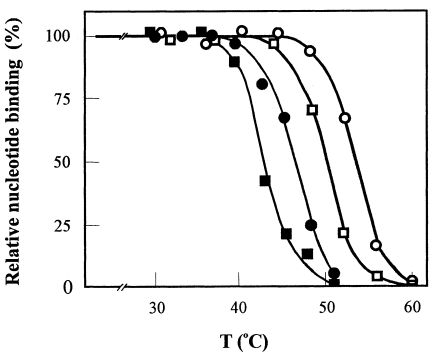

Fig. 1. Thermal stability of wtEF-Tu and EF-TuH118G in the presence of GDP or GTP. EF-Tu[^{3}H]GDP or EF-Tu[γ-^{32}P]GTP was incubated for 8 min at different temperatures and the residual activity in nucleotide binding was determined using the nitrocellulose filter binding assay. Conditions of the assay were as described in [7]. wtEF-Tu·GDP (○), wtEF-Tu·GTP (●), EF-TuH118G·GDP (□), EF-TuH118G·GTP (■).

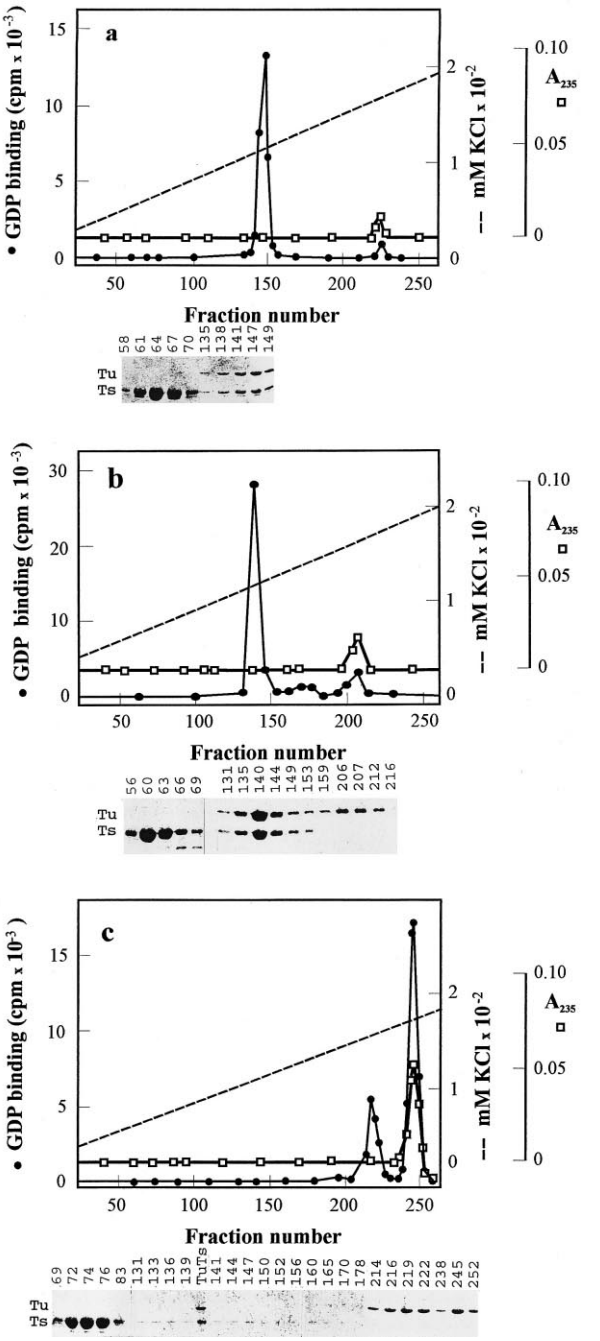

Fig. 2. Formation of EF-Tu·EF-Ts and its separation from EF-Tu·kirromycin. To dissociate kirromycin from EF-Tu, EF-Tu·kirromycin (6.5 mg) was mixed with a 2-fold molar excess of purified EF-Ts (9 mg) and the mixture (20 ml) was extensively dialyzed at 0°C against several liters of 50 mM Tris-HCl, pH 7.7, 50 mM KCl, 7 mM 2-mercaptoethanol and finally against the same buffer but with 10 mM KCl, to remove the dissociated antibiotic [6,7]. The sample was applied on a DEAE-Sepharose FF column (1×50 cm) and eluted with a continuous gradient (total volume 800 ml) of 20–200 mM KCl (a,c) or 40–220 mM KCl (b) in the above buffer at 4°C. Binding of [^{3}H]GDP (●) and kirromycin absorbance at 325 nm (□) were determined and SDS-polyacrylamide gel electrophoretic analysis of some fractions is shown. TuTs, EF-Tu·EF-Ts standard. a: EF-TuC81G mutant. b: EF-TuH66G mutant. c: EF-TuH118G mutant. Under these conditions free EF-Ts, EF-Tu·EF-Ts and EF-Tu·kirromycin were eluted at concentrations around 65 mM KCl, 115 mM KCl and 168 mM KCl, respectively.

petitor of kirromycin [8,9], resulting in the formation of a stable EF-Tu·EF-Ts complex which could be isolated by ion-exchange chromatography and separated from any residual undissociated EF-Tu·kirromycin (see [7] and Fig. 2 for details). While this method has proved entirely successful with six of the above-mentioned EF-Tu species (for EF-TuC81G and EF-TuH66G used as controls see also Fig. 2a,b, respectively), the preincubation of the EF-TuH118G·kirromycin with an excess of EF-Ts failed to result in the formation of a detectable EF-TuH118G·EF-Ts complex (expected to appear around fraction 150, Fig. 2c). In contrast, most of EF-TuH118G·kirromycin was not at all affected by the presence of EF-Ts and retained its original elution characteristics (fractions 239–253). Only a detailed screening of the column fractions revealed the existence of a new, small peak with GDP binding activity (fractions 205–230) preceding the EF-TuH118G·kirromycin fractions. On SDS-polyacrylamide gel electrophoresis a pure EF-TuH118G without EF-Ts was detected in the peak (Fig. 2c). This shows that EF-Ts is very inefficient in dissociating kirromycin from EF-TuH118G and strongly suggests that the interaction of the mutant EF-Tu with EF-Ts displays a transient character so that the complex between EF-TuH118G and EF-Ts is not sufficiently stable to withstand the chromatographic procedure.

3.3. *The uH118G substitution influences only partially the ability of EF-Ts to stimulate GDP dissociation from EF-Tu·GDP and to support poly(Phe) synthesis*

As the physiological role of EF-Ts is to control the interaction between EF-Tu and GDP [3] we tested the effect of EF-Ts on the rate of the [^{3}H]GDP/GDP exchange on EF-TuH118G and compared it with that on wtEF-Tu. Despite the fact that we were unable to detect the formation of a stable complex between EF-TuH118G and EF-Ts as shown in Fig. 2c, we found that the [^{3}H]GDP/GDP exchange rate of the mutant could still be significantly stimulated by EF-Ts (Table 1). Indeed, the exchange on EF-TuH118G reached a level only about 2.3 times lower than that on wtEF-Tu. Due to the increased intrinsic GDP/GDP exchange rate of mutant EF-Tu (Table 1), the stimulation by EF-Ts corresponded to about three times vs. a stimulation of 65 times observed with wtEF-Tu.

Table 1
Effect of EF-Ts on EF-TuH118G·GDP and wtEF-Tu·GDP dissociation

Additions	Apparent rate constants of dissociation $10^4\ k^{-1}\ s^{-1}$ between EF-Tu and GDP	
	EF-TuH118G	wtEF-Tu
GDP	24.3 ± 3.0	2.7 ± 0.5
GDP+EF-Ts	76.5 ± 17.3	176.6 ± 18.2

EF-Tu·GDP/GDP exchange reaction in the presence or absence of EF-Ts was performed at 0°C [3]. The initial mixture contained, in 1 ml standard buffer (50 mM Tris-HCl, pH 7.6, 60 mM NH_4Cl, 10 mM $MgCl_2$, 7 mM 2-mercaptoethanol), 15 pmol EF-TuH118G·[^{3}H]GDP or wtEF-Tu·[^{3}H]GDP, and the reaction was started by the addition of 100 nmol unlabeled GDP and 15 pmol EF-Ts, if present. The radioactivity bound to EF-Tu was determined on aliquots of 1/10 volume of the reaction mixture withdrawn at various time intervals. The apparent first order rate dissociation constant ($k^{-1}\ s^{-1}$) of the EF-TuH118G·GDP complex or wtEF-Tu·GDP complex was calculated according to the equation $\ln(c_t/c_0) = -k^{-1}\ t$, where c_0 is the initial concentration of the EF-Tu·nucleotide complex and c_t the concentration at time t [3].

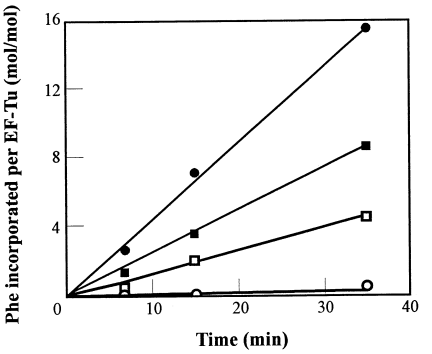

Fig. 3. Activity of EF-TuH118G in poly(Phe) synthesis: effect of EF-Ts. Conditions of the polymerization assay at 30°C were as described [6]. wtEF-Tu (○), wtEF-Tu+EF-Ts (●), EF-TuH118G (□), EF-TuH118G+EF-Ts (■).

Similarly, the susceptibility of poly(U)-directed poly(Phe) synthesis, catalyzed by EF-TuH118G, to the stimulatory effect of EF-Ts was also partially retained (Fig. 3). Indeed, the rate of poly(Phe) synthesis was increased at least twice by EF-Ts and reached about 50% of that obtained with wtEF-Tu.

4. Discussion

In vitro, in the absence of Mg^{2+} ions and nucleotides, elongation factor Tu forms an equimolar, stable complex with EF-Ts which can be quantitatively isolated by ionic chromatographic methods [12]. The recent determination of the crystal structure of the EF-Tu·EF-Ts complex from *E. coli* has provided a schematic representation of intermolecular contacts between these two proteins involving 27 amino acid residues of EF-Tu and 22 residues of EF-Ts, the most extensive contact on EF-Tu taking place on domain 1 [4]. Several secondary structures of EF-Tu domain 1 are involved, such as the phosphate binding loop, the amino terminus of α-helix B – tightly associated with domain 3, and the α-helices C and D. Besides domain 1, only the tip of domain 3 appears to interact directly with EF-Ts. The failure of the EF-TuH118G mutant to form a tangible complex with EF-Ts and the insensitivity of the EF-TuH118G·kirromycin complex to the usual dissociating effect of EF-Ts described here provide the first experimental evidence for the participation of uH118 in the EF-Tu·EF-Ts interaction. In good agreement with the crystal model [4] our results clearly show that the substitution uH118G strongly affects the stability of the EF-Tu·EF-Ts complex. Interestingly, however, the level of the EF-Ts-stimulated GDP/GDP exchange on EF-Tu is affected by the substitution only partially.

In the 3D model of Kawashima et al. [4], uH118 is one of the three amino acid residues of EF-Tu interacting with sF81. The sF81 protrudes into EF-Tu·GDP between uH84 of α-helix B, part of which changes to a β-conformation in the GTP state [13,14], and uH118, situated in α-helix C. Also the recently elucidated EF-Tu·EF-Ts model from *T. thermophilus* (Y. Wang, Y. Jiang, M. Meyering-Voss, M. Sprinzl and P.B. Sigler, personal communication) suggests that the homologous residue sF82 plays an important role in the transmission of the EF-Ts signal to EF-Tu. In this context, uH119 (uH118 in *E. coli* EF-Tu) has been proposed to be the receptor residue of the EF-Ts signal inducing the GDP release from EF-Tu·GDP (see Section 1). Experimental evidence for the

functional involvement of sF81 in the EF-Ts signal has recently been reported [15]. Mutation of sF81, like the sD80 mutation, was found to decrease partially (2–3 times) the rate of the EF-Ts-dependent EF-Tu·GDP/GDP exchange. Mutation of both sF81 and sD80 reduced the EF-Ts stimulation to 10% when EF-Ts was present at lower concentrations but at higher concentrations the stimulation became more significant, showing that these two residues are important but not essential for the EF-Ts signal. Similarly, our observation that the rate of GDP/GDP exchange on EF-TuH118G in the presence of EF-Ts is about 43% of that on wtEF-Tu under the same conditions is difficult to reconcile with the proposal that uH118 is the major receptor residue of the EF-Ts signal. Our results indicate that the transmission of the EF-Ts signal involves a more complex network of interactions than the pathway dependent on uH118. It is likely that besides the bonds interacting with the phosphate groups, also those fixing the guanine base and the ribose of the nucleotide are involved, as suggested by Kawashima et al. [4].

The results in Fig. 3 and Table 1 reveal that in the absence of EF-Ts the rates of poly(Phe) synthesis and GDP exchange are several times faster for EF-TuH118G than for wtEF-Tu. Thus, an accelerated nucleotide exchange is an intrinsic characteristic of this mutant. The low but reproducible stimulatory effect of EF-Ts on the poly(Phe) synthesis supported by EF-TuH118G (Fig. 3) could be a consequence of the ability of EF-Ts to increase the rate of the EF-TuH118G·GDP dissociation (Table 1). Nevertheless, the rate of poly(Phe) synthesis with the EF-TuH118G mutant only reaches about 50% of that obtained with wtEF-Tu suggesting that the mutation uH118G involves other basic aspects of poly(Phe) synthesis and not only the interaction with EF-Ts. A considerably lower rate of poly(U)-directed poly(Phe) synthesis upon EF-Ts stimulation was also observed with two other uHis-118 mutants: EF-TuH118A and EF-TuH118E [19]. Certainly, the lower efficiency of poly(Phe) synthesis is at least partially due to a strongly impaired binding of the EF-TuH118 mutants to aa-tRNA, an interaction shown to be deeply affected by the mutation or uH118 photooxidation [5,6,16,20]. The observation that aa-tRNA (and analogs of its 3′ terminus) can selectively protect this residue and uH66 from photooxidation contributed to mapping out the binding site for the acceptor end of aa-tRNA on EF-Tu [21,22]. The nearness of uH118 and the aa-tRNA binding site within the ternary complex was further indicated by successful cross-linking [23]. Besides, experiments with EF-TuH118G suggested that the His-118 region is also involved in the regulation by aa-tRNA of the GTPase center of EF-Tu [5,6]. However, in the crystal model of the ternary complex, uH118 is situated about 1.6 nm from tRNA [24]. To explain this discrepancy between the indication of the crystal structure and the results of biochemical experiments a transient exposure of uH118 has been proposed to take place during transformation of EF-Tu from the GDP form to the GTP form [24].

In conclusion, our experiments show that the substitution H118G, though preventing the formation of a stable EF-Tu·EF-Ts complex, only partially influences the rate of GDP/GDP exchange on EF-Tu in the presence of EF-Ts. In all available 3D models of EF-Tu (EF-Tu·GDP, EF-Tu·GTP and EF-Tu·EF-Ts [4,13,14,16–18]), uH118 is buried in the interface between domains 1 and 3, practically inaccessible to the solvent. Its location in α-helix C with the side chain pointing to the turn preceding α-helix B, particularly in the GDP-bound state, suggests that its substitution can affect the relative reorientation of α-helices B and C and induce long-range effects. Altogether, the results of this work and of Jonák et al. [6] emphasize an interesting, multifunctional role of residue uH118 as well as the complexity of interactions between EF-Tu and EF-Ts. Both these phenomena should be taken into consideration when building new functional models of EF-Tu. Further mutagenic work will be required to create a more dynamic and flexible picture of the EF-Tu molecule enabling a detailed analysis of the structural modifications induced by its macromolecular ligands in order to reconcile functional and structural data at the molecular level.

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