

Histidine-118 of elongation factor Tu: its role in aminoacyl-tRNA binding and regulation of the GTPase activity

Jiří Jonák^{a,*}, Pieter H. Anborgh^{b,**}, Andrea Parmeggiani^b

^aInstitute of Molecular Genetics, Academy of Sciences of the Czech Republic, Flemingovo n. 2, 16637 Prague 6, Czech Republic

^bLaboratoire de Biochimie, Ecole Polytechnique, 91128 Palaiseau Cedex, France

Received 28 February 1994

Abstract

The function of His¹¹⁸ in elongation factor (EF)-Tu from *Escherichia coli* was investigated by its substitution with glycine. The substitution had a differential effect on individual functions of the protein. The affinity for aminoacyl (aa)-tRNA and the intrinsic GTPase activity of the mutant EF-Tu were decreased whereas the response of its GTPase center to aa-tRNA was strongly increased. These results suggest that the region around His¹¹⁸ is involved in the binding of aa-tRNA and in the transmission of a turn-off signal generated by the interaction with aa-tRNA and directed to the GTPase center of EF-Tu.

Key words: Elongation factor Tu; Histidine 118; Aminoacyl-tRNA; GTPase activity; *Escherichia coli*

1. Introduction

Elongation factor Tu (EF-Tu) is a guanine nucleotide-binding protein, the GTP-bound complex of which transports aminoacyl (aa)-tRNA to the mRNA-programmed ribosome during bacterial protein biosynthesis. EF-Tu displays weak intrinsic GTPase activity that can be enhanced by the antibiotic, kirromycin, aa-tRNA and ribosomes. This stimulation becomes maximal during the decoding of the mRNA-programmed ribosome. The rate of this activity correlates with amino acid incorporation into the polypeptide chain and the accuracy of this process. X-ray diffraction studies have clarified the structural background of the interaction of GDP and GTP with the protein residues forming the specific binding pocket, suggesting possible mechanisms for the catalytic process [1–4]. However, the structural information about the binding of aa-tRNA by EF-Tu · GTP and the regulation of the EF-Tu GTPase by aa-tRNA is still limited. Berchtold et al. [4] have recently suggested that the binding of aa-tRNA takes place in a solvent-filled cleft between the N-terminal and the middle domains of EF-Tu. This cleft is formed during the dramatic conformational rearrangement of EF-Tu induced by the binding of GTP [4,5]. Chemical modifications, protection and cross-linking experiments have enabled the mapping of

a binding site for aa-tRNA around Cys⁸¹, His⁶⁶ and His¹¹⁸ in the N-terminal domain of EF-Tu [6–10]. Studies with the 3'-aminoacylated fragments of aa-tRNA have shown that besides the 3'-aminoacylated extremity, which is of primary importance for the stimulation of the GTPase activity, other regions of the tRNA molecule are also involved in the modulation of the GTPase activity of EF-Tu [11–13]. However, the precise topography of the regulatory regions has yet to be elucidated. His¹¹⁸ is one of the amino acid residues of the N-terminal domain of EF-Tu that was found to be protected by aa-tRNA from photooxidation [8] and to selectively cross-link Phe-tRNA via *trans*-diamine dichloroplatinum (II) in a ternary complex with EF-Tu · GTP [10]. Substitution of histidine by glycine makes it possible to evaluate the net effect of histidine side chain removal on the properties of the protein. In this work we have compared the mutant, EF-TuH118G, with wild-type (wt) EF-Tu in poly(Phe) polymerization, interaction with aa-tRNA and regulation of the GTPase activity.

2. Materials and methods

Analytical grade chemicals were obtained from Merck, Germany. Pyruvate kinase from rabbit muscle, phosphoenolpyruvate, GDP and GTP were from Boehringer, Germany, and [γ -³²P]GTP (5,000 Ci/mmol) and [¹⁴C]phenylalanine (479 mCi/mmol) from Amersham, England.

To obtain EF-TuH118G, site-directed mutagenesis was carried out on pEMBL9-*tufA* as described [14], using the synthetic oligodeoxynucleotide, GACTCGTGAGGGCATCTG. The mutated *tufA* gene was transferred to pTTQ18 [15] under control of the *tac* promoter. Over-

*Corresponding author. Fax: (42) (2) 2431 0955.

**Present address: Laboratory of Cellular Oncology, NCI, Bethesda, MD 20814, USA.

production, isolation and purification of EF-TuH118G was carried out as described in [16,17]. The purified protein stored in 50 mM Tris-HCl, pH 7.5, 50 mM KCl, 5 mM MgCl₂, 7 mM 2-mercaptoethanol, 1 μM GDP and 50% glycerol was stable for at least 9 months at -20°C. EF-TuH118G was pure as determined on SDS-polyacrylamide gel electrophoresis. Wild-type EF-Tu and the other biological components were prepared as described [18].

EF-Tu concentration and dissociation constants (K_d 's) of EF-TuH118G · GDP and EF-TuH118G · GTP complexes were determined according to [16]. Poly(U)-directed poly(Phe) synthesis, protection of aa-tRNA by EF-Tu against non-enzymatic degradation and GTPase activity were examined as reported previously [16]. The formation of stable complexes between EF-Tu · GTP and aa-tRNA was tested by gel filtration on a Sephadex G-25 fine column (12.5 × 0.4 cm), equilibrated in buffer S (50 mM Tris-HCl, pH 7.6, 60 mM NH₄Cl, 10 mM MgCl₂, 7 mM 2-mercaptoethanol) at 4°C [19].

3. Results

3.1. Activity of EF-TuH118G in poly(Phe) synthesis

EF-TuH118G displayed a 50% lower activity in poly(U)-directed poly(Phe) polymerization than wt EF-Tu. This was determined under conditions of linear amino acid incorporation and rate-limiting amounts of the two EF-Tu species (Fig. 1).

3.2. Interaction of EF-TuH118G with GDP and GTP

The mutation affects the interaction of EF-Tu with GDP and GTP only slightly. The K_d 's of EF-TuH118G

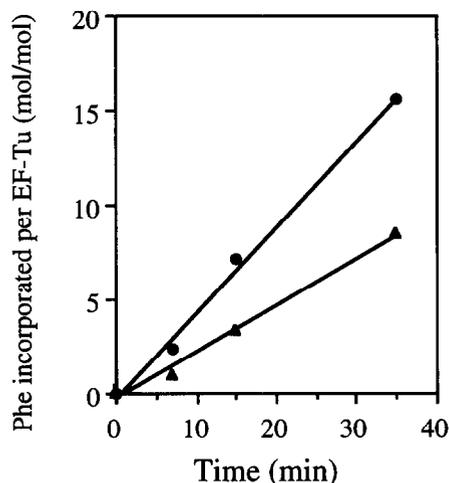


Fig. 1. Activity of wt EF-Tu and EF-TuH118G in poly(Phe) synthesis. Mix I (230 μl), containing 40 mM Tris-HCl, pH 7.8, 8 mM MgCl₂, 16 mM KCl, 8 mM phosphoenolpyruvate, pyruvate kinase (150 μg/ml), 1.8 mg bulk *E. coli* tRNA, 1 mM ATP, 26 μM [¹⁴C]Phe (specific activity 325 cpm/pmol) and saturating amounts of phenylalanyl-tRNA synthetase, was preincubated at 30°C for 20 min, added to Mix II (820 μl) containing 60 mM Tris-HCl, pH 7.8, 50 mM NH₄Cl, 60 mM KCl, 12 mM MgCl₂, 0.6 mM DTT, 1.2 mM GTP, 100 μg/ml poly(U) and 0.4 μM *E. coli* ribosomes and incubation took place at 4°C for 15 min. Then the concentration of ATP was increased to 1.2 mM, EF-G was added to 0.15 μM and EF-Ts to 0.19 μM. The polymerization reaction at 30°C was initiated by adding 170 μl of Mix I and II to 2 μl (2.19 pmol) of either wt EF-Tu (●) or EF-TuH118G (▲). Aliquots of 55 μl were withdrawn at the indicated times and spotted on Whatman 3 MM filters. The poly(Phe) formed was measured as described in [16].

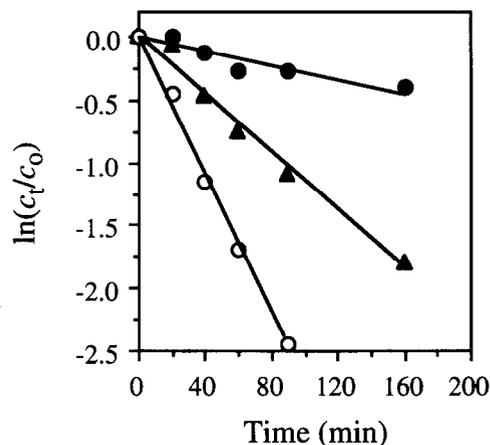


Fig. 2. Protection of Phe-tRNA by wt EF-Tu or EF-TuH118G against spontaneous deacylation. 2.7 μM wt EF-Tu (●) or EF-TuH118G (▲) was converted to EF-Tu · GTP by treatment with 4.5 mM phosphoenolpyruvate and pyruvate kinase (57 μg/ml) at 30°C for 15 min in 90 μl buffer S containing 6% glycerol and cooled on ice. To this mixture 57 pmol of [³H]Phe-tRNA^{Phe} (specific activity 531 cpm/pmol) were added in 16 μl buffer S without glycerol followed by incubation at 27°C. At the indicated times, samples of 15 μl were withdrawn, spotted on Whatman 3MM filters and [³H]Phe-tRNA^{Phe} was measured as in [16]. Note that the absolute rates of Phe-tRNA^{Phe} deacylation both without (○) and with wt EF-Tu (●) are about twice as fast as those described by Pingoud et al. [20]. This is due to the presence of 5% glycerol in all experimental samples [30].

complexes with GDP and GTP at 4°C were found to be 14 nM and 2.5 μM, respectively. In comparison, the K_d 's of the same complexes of wt EF-Tu provided values of 1–2 nM and 0.3–0.5 μM, respectively.

3.3. Interaction between EF-TuH118G and aminoacyl-tRNA

The observation that the efficiency of poly(U)-directed poly(Phe) synthesis with EF-TuH118G is about 2-fold lower than that with wt EF-Tu supports the previous findings that integrity of the protein around His¹¹⁸ might be important for the aa-tRNA-mediated interactions. To clarify this point we have tested the protection, by EF-Tu, of Phe-tRNA^{Phe} against spontaneous deacylation under similar experimental conditions as described in [20]. The rate of the reaction was about twice as fast if wt EF-Tu was replaced by EF-TuH118G (Fig. 2). The results indicate that the orientation of the aminoacylated 3' acceptor end of aa-tRNA with respect to the protein is influenced by the mutation but their interaction is only slightly affected.

A typical property of aa-tRNA is to greatly stabilize the binding of GTP to EF-Tu. Gordon [21,22] has reported that the formation of a complex between GTP and EF-Tu that can be isolated by Sephadex gel filtration is entirely dependent on the presence of EF-Tu and aa-tRNA. Whereas EF-Tu · GTP (half-life 2 min [23]) completely dissociates during the filtration process, the pres-

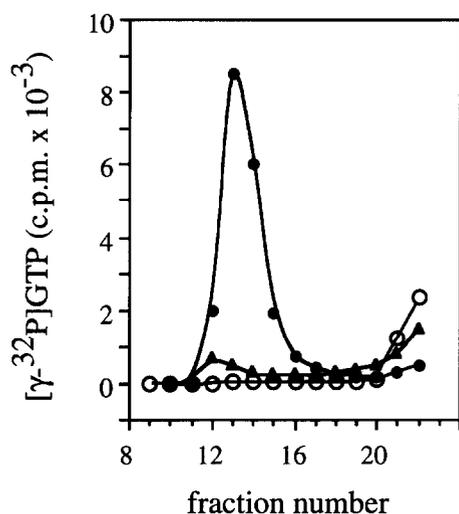


Fig. 3. Ternary complex formation between wt EF-Tu or EF-TuH118G, $[\gamma\text{-}^{32}\text{P}]\text{GTP}$ and aa-tRNA. The reaction mixture (30 μl) containing buffer S, 6 mM phosphoenolpyruvate, 66 $\mu\text{g/ml}$ pyruvate kinase, 10 μM $[\gamma\text{-}^{32}\text{P}]\text{GTP}$ (2,350 cpm/pmol) and 1.2 μM wt EF-Tu (●) or EF-TuH118G (▲) was pre-incubated at 30°C for 15 min. Phe-tRNA^{Phe} (200 pmol) was added where indicated. Incubation took place at 4°C for 30 min and the reaction mixture was applied to a Sephadex G-25 fine column. Fractions of 50 $\mu\text{l}/30$ s were collected and the radioactivity counted. (○) Control, no aa-tRNA added.

ence of aa-tRNA strongly stabilizes this interaction (half-life 4 h [22]). Thus, when using $[\gamma\text{-}^{32}\text{P}]\text{GTP}$, the peak of radioactivity excluded from the column is a measure of ternary complex formation or affinity between EF-Tu · GTP and aa-tRNA. As shown in Fig. 3, the recovery of $[\gamma\text{-}^{32}\text{P}]\text{GTP}$ in the exclusion volume of the Sephadex column in the presence of EF-TuH118G was only about 9% of that recovered in the presence of wt EF-Tu. The substitution of His¹¹⁸ in EF-Tu has a considerably more pronounced effect in this assay than in the hydrolysis protection assay.

3.4. The GTPase of EF-TuH118G and its response to aa-tRNA

Under all conditions tested the intrinsic GTPase activity of EF-TuH118G was lower than that of wt EF-Tu. For instance in Fig. 4, in the presence of 20 mM MgCl₂ and 50 mM NH₄Cl, initial rates of the GTP hydrolysis catalyzed by EF-TuH118G and wt EF-Tu differed by about 4 times. Addition of Val-tRNA^{Val} had no effect on the GTPase activity of wt EF-Tu, in agreement with the results described by Parlato et al. [13]. However, under the same conditions, Val-tRNA^{Val} strongly stimulated the GTP hydrolyzing activity of EF-TuH118G so that the rate of GTP hydrolysis reached the same level as that observed with wt EF-Tu. This effect of aa-tRNA was even more pronounced in the presence of ribosomes. Under these conditions, whereas the GTPase activity of the wt EF-Tu · ribosome complex was increased by aa-

tRNA less than 30%, that of the EF-TuH118G · ribosome complex was increased more than 20 times. The Phe-tRNA^{Phe}-induced EF-TuH118G · ribosome-dependent GTP hydrolysis was almost 4-times higher than the GTPase activity of the corresponding wt EF-Tu complex (Fig. 5). The stimulatory effect of aa-tRNA was not dependent on the nature of the bound amino acid (data not shown). The positive effect of aa-tRNA on the EF-TuH118G-catalyzed GTP hydrolysis was also pronounced at low (5 mM) MgCl₂ (data not shown), i.e. in conditions that are known to inhibit the GTP hydrolyzing activity of wt EF-Tu [12].

4. Discussion

His¹¹⁸ is one of the residues of the Q₁₁₄TRE(K)H₁₁₈ sequence in the α -helix of the N-terminal domain of EF-Tu. This sequence is conserved among prokaryotes, archaeobacteria and eukaryotes. According to the 3D model of EF-Tu · GDP from *E. coli* the residues of this sequence are likely to participate in the hydrogen bonding pattern connecting loops 18–23 and 82–83 which are part of the GDP binding pocket [3].

Removal of the His¹¹⁸ side chain decreased the intrinsic activity of the GTPase center of EF-Tu but strongly increased the response of the center to aa-tRNA. The rate of GTP hydrolysis catalysed by the EF-Tu · H118G · ribosome · aa-tRNA complex was about

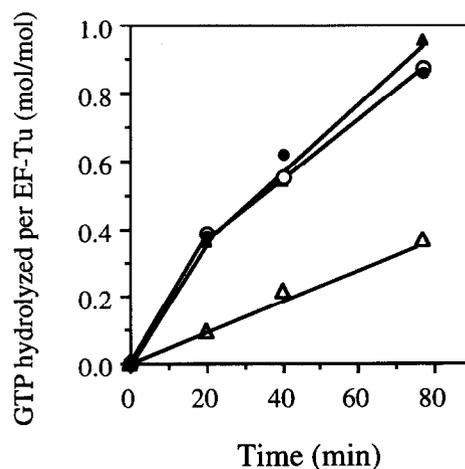


Fig. 4. Kinetics of GTPase activity of wt EF-Tu or EF-TuH118G in the absence and presence of aa-tRNA. The reaction mixture (70 μl) containing 50 mM imidazol acetate, pH 7.7, 20 mM magnesium acetate, 50 mM NH₄Cl, 7 mM 2-mercaptoethanol, 44 μM $[\gamma\text{-}^{32}\text{P}]\text{GTP}$ (1175 cpm/pmol), 2 mM phosphoenolpyruvate, pyruvate kinase (21 $\mu\text{g/ml}$) and 0.65 μM wt EF-Tu (○,●) or EF-TuH118G (△,▲) was pre-incubated at 30°C for 20 min and cooled on ice. Val-tRNA^{Val} to 1.9 μM concentration was added (filled symbols) followed by incubation at 35°C. At the indicated times, samples of 20 μl were withdrawn and ³²P_i liberated was measured [16]. (Open symbols) GTPase activity in the absence of aa-tRNA. Blank values (without EF-Tu) were subtracted.

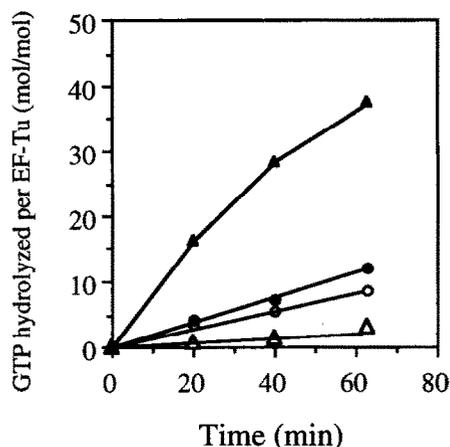


Fig. 5. Kinetics of GTPase activity of wt EF-Tu or EF-TuH118G in the presence of ribosomes. Effect of aa-tRNA. The procedure was the same as described in Fig. 4 except that ribosomes to 1.2 μ M concentration were added to the pre-incubated reaction mixture. (○,●) wt EF-Tu; (△,▲) EF-TuH118G; open symbols, GTPase activity in the absence of aa-tRNA. Blank values (without EF-Tu) were subtracted.

4-times higher than that with the wt EF-Tu · ribosome · aa-tRNA complex. Thus, the effect of aa-tRNA on the EF-TuH118G GTPase appears to be more complex than only compensatory.

Photooxidation and cross-linking experiments [8,10] indicated that His¹¹⁸ comes into close contact with aa-tRNA upon formation of a stable complex between this ligand and EF-Tu · GTP. This may be the result of a conformational change in EF-Tu induced by aa-tRNA binding [5,24]. As shown here, the substitution of His¹¹⁸ perturbs the formation of a stable complex with aa-tRNA to a greater extent than the interaction with the 3' terminal end of aa-tRNA (cf. Figs. 2 and 3). This suggests that the 3' end of aa-tRNA interacts with an area of EF-Tu different from that located around His¹¹⁸. Previous findings favoured binding of the 3' end of aa-tRNA to an area around Cys⁸¹ and His⁶⁶ [6,7,9], although the former residue is not essential for this binding [16]. By contrast, other authors [25,26] have proposed that the 3' terminal end of aa-tRNA (or tRNA) is in the vicinity of residues located in the middle domain. The participation of both the N-terminal and the middle domain in the formation of the cleft proposed to accept aa-tRNA [4,5] may reconcile these differences.

What is uncontested the experimental evidence demonstrating the primary importance of the aminoacylated 3' end of aa-tRNA for the stimulation of the GTPase center of EF-Tu [11,27,28]. The present work suggests that another, as yet unidentified region of the aa-tRNA · EF-Tu complex, including His¹¹⁸, constrains the GTP-binding site of the protein into a catalytically inactive conformation, if aa-tRNA is present. Failure to form a complex with aa-tRNA at this position, as in the case of EF-TuH118G, or in the presence of only a 3' terminal fragment of aa-tRNA [12], will result in the loss

of this regulatory function and in the unblocking of the catalytic center of EF-Tu.

It appears from this work that the His¹¹⁸ region is not directly involved in the structure of the GTPase center of EF-Tu but rather in the regulation of its activity by aa-tRNA, both in the presence and in the absence of the ribosome. One can propose that the α -helical stretch of EF-Tu situated in the proximity of His¹¹⁸ acts as a switch and/or a transducer of a signal for GTPase inhibition generated by the EF-Tu-aa-tRNA interaction. It is worth mentioning in this context that the sequence, 52–60, in EF-Tu from *T. thermophilus* has been suggested to function as a transducer of a signal for GTPase activation [29]. Thus, these two areas could participate in the regulation of the cyclic process of activation and inhibition of the EF-Tu GTPase during the EF-Tu-dependent decoding by aa-tRNA of mRNA codons on the ribosome.

Acknowledgements: This work was supported by the Czechoslovak Academy of Sciences, Grant 55253, and by the Association pour la Recherche sur le Cancer, Grant 6377. P.H.A. received a grant from the Fondation pour la Recherche Medicale.

References

- [1] La Cour, T.F.M., Nyborg, J., Thirup, S. and Clark, B.F.C. (1985) *EMBO J.* 4, 2385–2388.
- [2] Jurnak, F. (1985) *Science* 230, 32–36.
- [3] Kjeldgaard, M. and Nyborg, J. (1992) *J. Mol. Biol.* 223, 721–742.
- [4] Berchtold, H., Reshetnikova, L., Reiser, C.O.A., Schirmer, Schirmer, N.K., Sprinzl, M. and Hilgenfeld, R. (1993) *Nature* 365, 126–132.
- [5] Kjeldgaard, M., Nissen, P., Thirup, S. and Nyborg, J. (1993) *Structure* 1, 35–50.
- [6] Jonák, J., Smrt, J., Holý, A. and Rychlík, I. (1980) *Eur. J. Biochem.* 105, 315–320.
- [7] Jonák, J., Petersen, T.E., Clark, B.F.C. and Rychlík, I. (1982) *FEBS Lett.* 150, 485–488.
- [8] Jonák, J., Petersen, T.E., Meloun, B. and Rychlík, I. (1984) *Eur. J. Biochem.* 144, 295–303.
- [9] Duffy, L., Gerber, L., Johnson, A., Miller, D.L. (1981) *Biochemistry* 20, 4663–4666.
- [10] Metz-Boutigue, M.-H., Reinboldt, J., Ebel, J.-P., Ehresmann, C. and Ehresmann, B. (1989) *FEBS Lett.* 245, 194–199.
- [11] Guesnet, J., Parlato, G. and Parmeggiani, A. (1983) *Eur. J. Biochem.* 133, 499–507.
- [12] Picone, D. and Parmeggiani, A. (1983) *Biochemistry* 22, 4400–4405.
- [13] Parlato, G., Pizzano, R., Picone, D., Guesnet, J., Fasano, O. and Parmeggiani, A. (1983) *J. Biol. Chem.* 258, 955–1000.
- [14] Parmeggiani, A., Swart, G.W.M., Mortensen, K.K., Jensen, M., Clark, B.F.C., Dente, L. and Cortese, R. (1987) *Proc. Natl. Acad. Sci. USA* 84, 3141–3145.
- [15] Stark, M.J. (1987) *Gene* 51, 255–267.
- [16] Anborgh, P.H., Parmeggiani, A. and Jonák, J. (1992) *Eur. J. Biochem.* 208, 251–257.
- [17] Swart, G.W.M., Parmeggiani, A., Kraal, B. and Bosch, L. (1987) *Biochemistry* 26, 2047–2054.
- [18] Chinali, G., Wolf, H. and Parmeggiani, A. (1977) *Eur. J. Biochem.* 75, 55–65.
- [19] Sedláček, J., Rychlík, I. and Jonák, J. (1974) *Biochim. Biophys. Acta* 349, 78–83.

- [20] Pingoud, A., Urbanke, C., Krauss, G., Peters, F. and Maass, G. (1977) *Eur. J. Biochem.* 78, 403–409.
- [21] Gordon, J. (1967) *Proc. Natl. Acad. Sci. USA* 58, 1574–1578.
- [22] Gordon, J. (1968) *Proc. Natl. Acad. Sci. USA* 59, 179–183.
- [23] Fasano, O., Bruns, W., Crechet, J.-B., Sander, G. and Parmeggiani, A. (1978) *Eur. J. Biochem.* 89, 557–565.
- [24] Jonák, J. and Rychlík, I. (1987) *Biochim. Biophys. Acta* 908, 97–102.
- [25] Van Noort, J.M., Kraal, B., Bosch, L., La Cour, T.F.M., Nyborg, J. and Clark, B.F.C. (1984) *Proc. Natl. Acad. Sci. USA* 81, 3969–3972.
- [26] Kinzy, T.G., Freeman, J.P., Johnson, A.E. and Merrick, W.C. (1992) *J. Biol. Chem.* 267, 1623–1632.
- [27] Campuzano, S. and Modolell, J. (1980) *Proc. Natl. Acad. Sci. USA* 77, 905–909.
- [28] Bhuta, P., Kumar, G. and Chládek, S. (1982) *Biochemistry* 21, 899–905.
- [29] Peter, M.E., Schirmer, N.K., Reiser, C.O.A. and Sprinzl, M. (1990) *Biochemistry* 29, 2876–2884.
- [30] Johnson, A.E. and Adkins, H.J. (1984) *Anal. Biochem.* 137, 351–359.