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The interaction between aminoacyl-tRNA and the mutant elongation factors Tu A_R and B_0

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The binding of Tyr-[AEDANS-s^2C]$tRNA^{Tyr}$ ($Tyr\text{-}tRNA^{Tyr}$ modified at the penultimate cytidine residue with a thio group at position 2 of the pyrimidine ring, to which an *N*-(acetylaminoethyl)-5-naphthylamine-1-sulfonic acid fluorescence group is attached) to mutant elongation factor (EF)-Tu species from *E. coli*, $EF\text{-}TuA_R$ (Ala-375 → Thr) and $EF\text{-}TuB_O$ (Gly-222 → Asp), both complexed to GTP, was investigated in absence of kirromycin by measuring the change in fluorescence of the modified tRNA induced by complex formation. The calculated dissociation constant in the case of $EF\text{-}TuA_R$ is about 4 nM and in the case of $EF\text{-}TuB_0$, about 1 nM. These values are higher than that of wild-type EF-Tu, which was 0.24 nM measured with the same system. The affinity between either $EF\text{-}TuB_0$ · kirromycin · GDP or $EF\text{-}TuB_0$ · kirromycin · GTP on the one hand, and a mixture of aminoacyl-tRNAs on the other, was measured with zone-interference gel electrophoresis. The dissociation constants are 20 μM and 7 μM, respectively, a factor of about two higher than in the case of wild-type EF-Tu · kirromycin. These findings provide a clue for the observed increase in translational errors in strains carrying the mutations. Furthermore, the experiments with $EF\text{-}TuB_0$ · kirromycin deepen our understanding of the effects of the B_0 mutation on the kirromycin phenotype of the mutant cells concerned.

Introduction

The prokaryotic elongation factor Tu (EF-Tu) can form a complex with GTP and aminoacyl-tRNA (aa-tRNA). This complex binds to the ribosomal A-site and after initial recognition of the tRNA anticodon, the GTP bound to EF-Tu is hydrolyzed to GDP. Then the factor leaves the ribosome, making way for peptide bond formation. EF-Tu is known to increase the accuracy of protein synthesis: the longer it is in complex with the tRNA at the A-site, the smaller is the chance that a wrong amino acid is incorporated. For a review, see Ref. 1.

Kirromycin is a potent antibiotic: by binding EF-Tu it prevents the release of this factor from a translating ribosome, which then becomes a deadlock for other ribosomes downstream on the mRNA [2]. Over the years several mutants of EF-Tu have been found [3–6], some of which are resistant to kirromycin. The best characterized mutants have alanine-375 replaced by threonine or glycine-222 replaced by aspartic acid. They are named $EF\text{-}TuA_R$ and $EF\text{-}TuB_0$, respectively.

$EF\text{-}TuA_R$ is resistant to kirromycin because its affinity for the antibiotic is 100-fold lower than that of wild-type EF-Tu [7]. The K_d of the complex of $EF\text{-}TuA_R \cdot GTP \cdot Tyr\text{-}tRNA^{Tyr}$ was reported to be 5- to 6-times higher than the K_d of the wild-type complex [8]. When $Tyr\text{-}tRNA^{Tyr}$ in these complexes is replaced by Phe-tRNAPhe, the wild type complex is 2- to 5-fold more stable than the $EF\text{-}TuA_R$ containing complex, as was reported in Refs. 8 and 9. One must keep in mind that the actual values for the dissociation constants of the wild-type complexes reported in the cited papers are two [8] to three [9] orders of magnitude higher than those reported in more recent papers [10–12].

Abbreviations: EF-Tu, elongation factor Tu; [γ-S]GTP, guanosine-5′-*O*-(3-thiotriphosphate); K_d, dissociation constant; aa-tRNA, aminoacyl-tRNA; Tyr-[AEDANS-s^2C]$tRNA^{Tyr}$, $Tyr\text{-}tRNA^{Tyr}$ modified at the penultimate cytidine residue with a thio group at position 2 of the pyrimidine ring, to which an *N*-(acetylaminoethyl)-5-naphthylamine-1-sulfonic acid fluorescence group is attached.

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Although EF-TuB_0 can bind kirromycin, it does leave the ribosome when in complex with the antibiotic [13]. EF-TuB_0 binds Phe-tRNA^{Phe} less tightly than wild-type EF-Tu does [14]. The K_d of the EF-TuB_0-containing complex was claimed to be 4- to 5-times higher than that of wild-type complex [9]. From a rough calculation of data presented in [15], it can be estimated that the affinity of EF-TuA_R for aminoacyl-tRNA is some 3- to 5-times lower than that of EF-TuB_0. In the ternary complex, EF-TuB_0 is as active in promoting the binding of tRNA to the ribosomal A-site as wild-type EF-Tu is [14]. In the absence of aa-tRNA the GTPase activity of EF-TuB_0 is not stimulated by ribosomes [14], which is in vast contrast to wild-type EF-Tu [16]. EF-TuB_0 is unable to sustain poly-U translation at magnesium concentrations up to 6 mM [9,14], while it is active at higher magnesium concentrations [17,14].

The presence of the A_R and B_0 mutations causes an increase in translational errors, especially when both are expressed simultaneously in the cell [18,6,9].

In this paper we report the K_d values of the mutants EF-TuA_R · GTP and EF-TuB_0 · GTP for Tyr-[AEDANS-s^{2}C]tRNA^{Tyr}, a Tyr-tRNA^{Tyr} from yeast modified with a fluorescence label at the penultimate nucleotide of its 3′ end. Both modified and unmodified tRNA were shown to have an almost identical affinity for wild-type EF-Tu [10]. Furthermore, we determined the affinity of EF-TuB_0 · kirromycin · GDP/GTP for aa-tRNA using zone-interference gel electrophoresis [19,20]. We preferred this method in view of the susceptibility of kirromycin to ultraviolet light. The results are discussed in the light in the anomalous behaviour of both EF-Tu species in the support of translational accuracy and with respect to the kirromycin phenotype of the mutant cells involved.

A preliminary report of raw fluorescence data has already been given in Ref. 21]. The present data are refined by correction for the active fraction in the EF-Tu preparations used.

Materials and Methods

Fluorescence titrations of Tyr-[AEDANS-s^{2}C]-tRNA^{Tyr} with mutant EF-Tu · GTP were carried out as in Ref. 10. Zone-interference gel electrophoresis was performed as described in Ref. 19. EF-TuA_R was isolated according to Ref. 22 from a strain harbouring only the mutated *tufA* gene. EF-TuB_0 was isolated as described previously [23]. Ef-TuB_0 · kirromycin was isolated as described in Ref. 14. All other chemicals and procedures were as indicated in the original papers.

Results

Fluorescence titrations of Tyr-[AEDANS-s^{2}C]-tRNA^{Tyr} with either EF-TuA_R · GTP or EF-TuB_0 · GTP yielded the saturation curves shown in Fig. 1A. In order to determine the dissociation constants of the various complexes, it is necessary to known the maximum change in fluorescence or, in other words, the fluorescence of the tRNA when complexed with EF-Tu. This value was determined using the graphical iterative extrapolation discussed in Ref. 20. A Scatchard analysis of the data presented above is shown in fig. 1B. Two sets of data were collected in the case of EF-TuB_0. Because the measured K_D of the EF-TuB_0 · GTP · Tyr-[AEDANS-s^{2}C]tRNA^{Tyr} complex is relatively low, it is more susceptible to small experimental variations in the concentrations of the ligands. This explains the difference between the two curves of the titration with

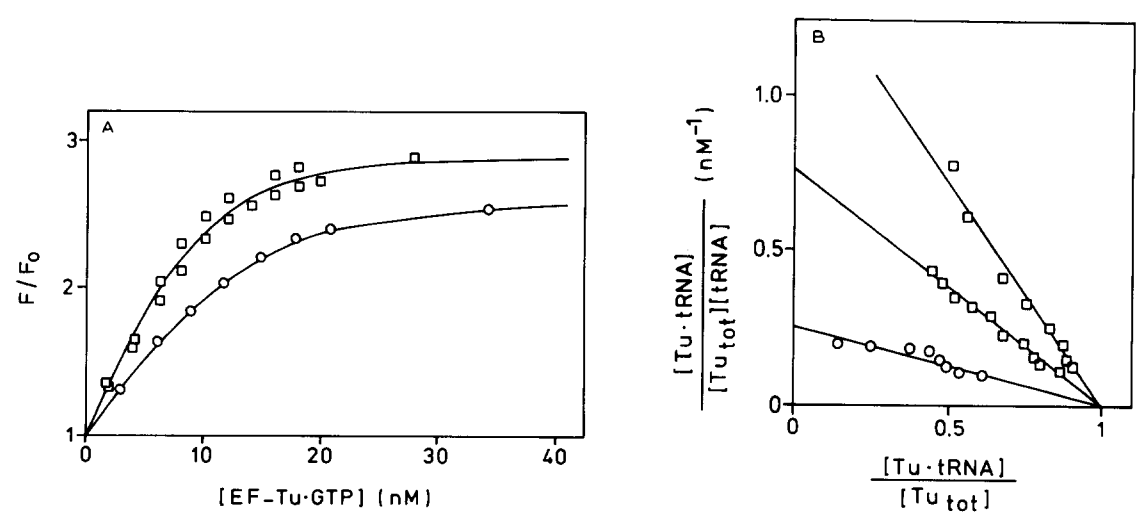

Fig. 1. (A) Titration at 4°C of 10 nM Tyr-[AEDANS-s^{2}C]tRNA^{Tyr} with EF-Tua$_R$·GTP (○ or with EF-TuB_0·GTP (□, two experiments). The buffer contained 50 mM sodium borate (pH 7.0), 50 mM NH_4Cl, 10 mM $MgSO_4$, 0.1 mM GTP, 0.167 mM phospho*enol*pyruvate, 3 μg/ml pyruvate kinase. Excitation of the fluorophore was at 335 nm, emission was measured at 480 nm. (B) Scatchard plot of the data in A. The calculated K_d values are 4 nM for EF-TuA_R and 0.7 or 1.3 nM for EF-TuB_0.

TABLE I

The dissociation constants of complexes of mutant EF-Tu species and aa-tRNA

EF-Tu species	+GTP	+[γ-S]GTP+ kirromycin	+GDP+ kirromycin
EF-TuA_R	4 nm [a]	–	–
EF-TuB_0	1 nM [a]	7 μM [c]	20 μM [c]
EF-Tu (w.t.)	0.24 nM [a,b]	3 μM [c,d]	10 μM [c,d]

[a] Measured in 50 mM sodium borate (pH 7.0), 50 mM NH_4Cl, 10 mM $MgCl_2$, 1 mM GTP at 4°C. The affinity for Tyr-[AEDANS-s^2C]tRNA^{Tyr} is listed.
[b] Data taken from Ref. 10.
[c] Measured in 20 mM Tris.HCl (pH 7.6), 3.5 mM $MgCl_2$, 10 μM [γ-S]GTP or GDP at 9°C. The affinity for a mixture of aa-tRNAs is listed.
[d] Data taken from Ref. 20.

EF-TuB_0. While the K_d of the complex of wild-type EF-Tu · GTP with Tyr-[AEDANS-s^2C]tRNA^{Tyr} was reported to be 0.24 nM [10], this value is about 1 nM for EF-TuB_0 · GTP and about 4 nM for EF-TuA_R · GTP when measured in exactly the same system.

We also determined the affinity of aa-tRNA for EF-TuB_0 · kirromycin complexed with either GDP or [γ-S]GTP. Because addition of kirromycin induces EF-Tu to hydrolyze GTP, the slowly hydrolyzing analogue [γ-S]GTP was used instead. Since EF-TuA_R has a markedly lower affinity for kirromycin, we omitted comparable experiments with this mutant. The above-mentioned affinities were measured with zone-interference gel electrophoresis, a reliable technique for determining weak binding constants [19,20]. As can be seen in Fig. 2, the dissociation constant of the complex EF-TuB_0 · kirromycin · [γ-S]GTP · aa-tRNA is about 7 μM, while substituting the nucleotide by GDP causes a 3-fold increase in the K_d to about 20 μM.

As can be seen from a summary of the present values in Table I, both mutants have a reduced affinity for aa-tRNA when compared to wild-type EF-Tu. In the case of EF-TuB_0 the increase in K_d is about 4-fold, in the case of EF-TuA_R the increase is about 15- to 20-fold. When kirromycin is added to EF-TuB_0 · GTP or to EF-Tub_0 · GDP, the increase in K_d when compared to wild-type EF-Tu · kirromycin is about 2-fold in both cases.

Discussion and Conclusions

The fact that both mutants bind aa-tRNA more poorly than wild-type EF-Tu provides a clue for the observed increase in translational errors in strains carrying the mutations. It could very well be that also when present at the ribosomal A-site, the EF-Tu mutants show a decreased affinity for the bound tRNA, and thus tend to leave this site more quickly (either before or after GTP hydrolysis) than wild-type EF-Tu. This speed is thought to be of high importance for translational accuracy: a high speed of dissociation goes with a poor accuracy [9,24–26] (for a review see Ref. 1). The data presented in this report do not solve the riddle of the synergistic behaviour witnessed when both EF-Tua_R and EF-TuB_0 are present in the cell: strains carrying both mutations show an unproportional increase in mistranslation [18,6]. What the results do imply is that when EF-Tu is present in saturating concentrations with respect to aa-tRNA, the competion of wild-type EF-Tu will defeat both EF-TuB_0 and EF-TuA_R. EF-TuB_0 will compete successfully with EF-TuA_R for aa-tRNA under similar conditions. Especially when ribosomes are present, complicated results can be obtained depending on the concentration levels of all ligands. The affinities of the EF-Tu mutants for aa-tRNA differ, and the affinities of the ternary complexes of these mutants for the ribosome will probably also do so in the light of the reduced translational accuracy observed in cells harbouring these mutations.

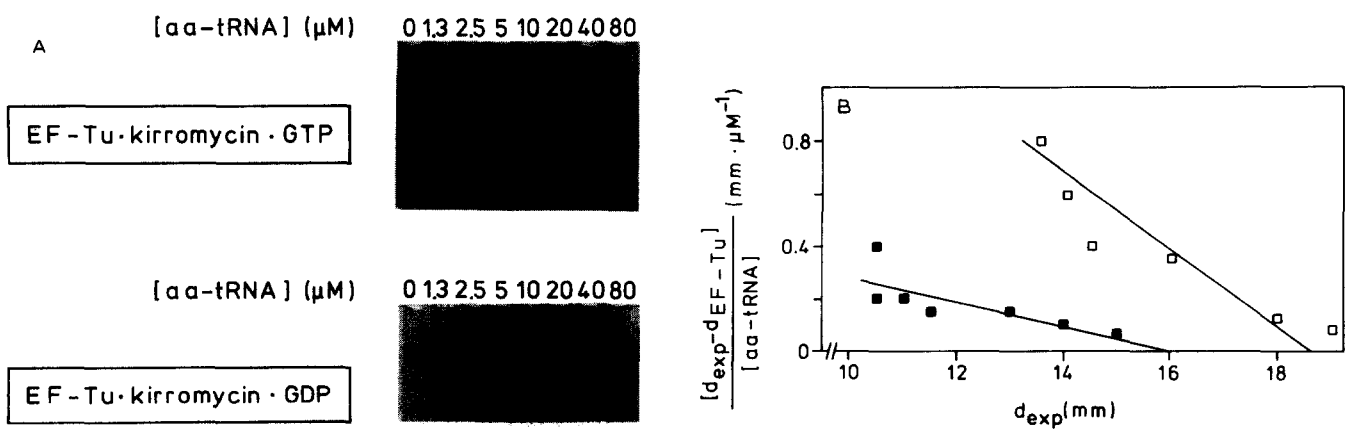

Fig. 2. (A) Zone-interference gel electrophoresis of EF-TuB_0·kirromycin·GDP (■, symbol in B) and EF-TuB_0·kirromycin·GTP (□, symbol in B). The zones contained a mixture of aa-tRNAs at the concentrations indicated. (B) Linearization of the data according to Ref. 19. The lines do not extrapolate to the same maximum migration distance because of a different electrophoresis time in the experiments.

The relative drop in affinity of EF-Tu for aa-tRNA caused by the B_0 mutation is about the same in the presence as in the absence of kirromycin. Therefore, the allosteric change of EF-TuB_0 induced by kirromycin which becomes apparent upon tRNA binding, is probably very much like that induced to wild-type EF-Tu. This last observation also sheds new light on the nature of the behaviour of the EF-TuB_0 mutant species. The fact that the latter leaves the ribosome even when it is in complex with kirromycin is probably not caused by a decreased affinity for the tRNA at the A-site, since the affinity of EF-TuB_0 · kirromycin · GDP does not differ not differ much from that of the wild-type-containing complex (see also Ref. 13). We have thus provided additional, albeit indirect support for the results presented in Ref. 14, which show that especially the interaction between EF-TuB_0 and ribosomes is perturbed.

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