Volume 319, number 1,2, 185–188 FEBS 12241 March 1993

Participation of the overproduced elongation factor Tu from *Thermus thermophilus* in protein biosynthesis of *Escherichia coli*

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Accepted 18 January 1993

The influence of the overproduced elongation factor Tu (EF-Tu) from *Thermus thermophilus* on the protein biosynthesis in *Escherichia coli* was investigated both in vivo and in vitro. A kirromycin-resistant *E coli* strain became sensitive to this antibiotic upon the expression of the *tufA*-gene of *T. thermophilus* present on a plasmid. In in vitro translation with components of the kirromycin-resistant *E. coli* strain the poly(Phe) synthesis stopped when minute amounts of the EF-Tu from *T. thermophilus* were added. Both results indicate the sensitivity of the *T thermophilus* EF-Tu to kirromycin and its participation in the polypeptide synthesis of *E. coli*.

Kirromycin; *tuf*-gene expression; Polysome block; GTPase activity

1. INTRODUCTION

The elongation factor Tu (EF-Tu), which mediates the binding of aminoacyl-tRNA to the ribosome, is encoded by two almost identical genes (*tufA* and *tufB*) in *Escherichia coli* [1], *Thermus thermophilus* [2–4] and *Salmonella typhimurium* [5]. It is one of the most abundant cellular proteins [6]. In *E. coli* and *T. thermophilus* the *tufA*-gene is arranged as the most distal gene in the polycistronic *str*-operon comprising the genes for the ribosomal proteins S12, S7 and the elongation factor G. In another unlinked operon *tufB* is preceded by and co-transcribed with four tRNA genes [7–9]. The expression of the *str*-operon in *E. coli* involves a major promoter upstream of the *rpsL*-gene [10] and two secondary promoters located within the *fus*-gene [11]. The expression of individual genes of this operon is controlled by autogenous regulation on the translational level [12]. The expression of the *tufB* operon is under the positive control of the trans-activating protein FIS [13] and another yet unidentified protein [14]. In addition, the involvement of stringent control has been reported [15]. However, little is known about the expression regulation of the *tuf*-genes in *T. thermophilus*. The expression of the *T. thermophilus str*-operon using its own upstream region in *E. coli* was very poor [3]. In order to obtain large amounts of the thermostable EF-Tu, we used regulatory elements of *E. coli* for the overexpression of the *T. thermophilus tufA*-gene [16].

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The overproduction of the active *T. thermophilus* EF-Tu in *E. coli* could potentially disturb the polypeptide synthesis of *E. coli* in vivo, because this protein is functionally and structurally homologous to the corresponding host protein but has presumably different thermodynamic properties [16]. It has previously been demonstrated that the *T. thermophilus* EF-Tu is active in vitro in poly(U)-dependent poly(Phe) synthesis [17]. To study the possible participation of the *T. thermophilus* EF-Tu in the protein biosynthesis of *E. coli* in vivo we made use of a kirromycin-resistant host strain. Kirromycin (mocimycin) belongs to the group of kirromycin-like antibiotics from *Streptomyces* species with a narrow antibacterial spectrum against Gram-positive and certain Gram-negative bacteria [18]. In wild-type *E. coli* cells these antibiotics inhibit protein synthesis by preventing the release of EF-Tu·GDP from the ribosome during the elongation cycle, thus blocking the ribosomal translocation on the mRNA, which leads to a polysome jam. Bacterial protein synthesis stops when only a few EF-Tu molecules sensitive to kirromycin are present in the cell. This explains why kirromycin sensitivity is dominant. Accordingly, the kirromycin-resistant *E. coli* strain LBE2012 contains alterations in both *tuf*-genes [19,20], corresponding to the change of arginine-375 to threonine in *tufA* [21] and glycine-222 to aspartic acid in *tufB* [22]. The dominance of kirromycin sensitivity has been experimentally established by expressing the wild-type *E. coli tufA*-gene in LBE2012 [20]. The kirromycin-resistant host strain became sensitive to the antibiotic and the bacterial growth stopped. In the strain LBE2012, the gene product of the mutated *tufA*-gene, EF-TuA_R, does not bind kirromycin and participates in the polypeptide elongation cycle. The

product of the mutated *tufB*-gene, EF-TuB_0, is inactivated by the binding of the antibiotic.

In this report we demonstrate both in vivo and in vitro, that the wild-type EF-Tu from *T. thermophilus* confers kirromycin sensitivity to a kirromycin-resistant *E. coli* strain. It thus can be concluded that it participates in the host's polypeptide synthesis. The consequences for bacterial growth and protein biosynthesis by the expression of the native or a mutated *tufA*-gene from *T. thermophilus* are discussed.

2. MATERIALS AND METHODS

2.1. *Bacterial strains, growth conditions, and transformation*

The bacterial strains used in this work are *T. thermophilus* HB8 (ATCC27634), *E. coli* LBE1001 (F^-, su^-), earlier designated as KMBL1001, and LBE2012 (F^-, su^-, *tufA375, tufB222*) [19]. *E. coli* was grown at 37°C in Luria-Bertani medium [23]. The antibiotics ampicillin and a 50% pure preparation of kirromycin were used in concentrations of 400 μg/ml and 140 μg/ml, respectively. For the induction of the *tac* promoter, IPTG was added to a final concentration of 1 mM. For determining the growth rates, 5 ml of liquid medium were inoculated; the absorbance of the culture was measured by a Klett-Summerson photometer (Klett Mfg. Co., New York, USA) in intervals of one hour. Competent *E. coli* cells were obtained by $CaCl_2$ treatment as specified in Sambrook et al. [24]. The plasmids used for transformations were pKK223-3 (Pharmacia LKB, Uppsala, Sweden) and pEFTu-10 [16].

2.2. *Protein purification and in vitro translation*

The *T. thermophilus* EF-Tu was purified from the overproducing *E coli* strain JM109(pEFTu-10) according to Ahmadian et al. [16], and the *E coli* EF-Tu was purified as previously reported [3]. For the preparation of crude cellular extracts from *E. coli* LBE1001 and LBE2012 the bacteria were grown in 1 litre cultures to an A_{600} of 0.8 without antibiotics. The cells were collected by centrifugation and frozen. The frozen bacteria (1.5 g) were disintegrated by grinding with 3 g of Al_2O_3 for 20 min at 4°C. The mixture was resuspended in 50 mM Tris, pH 7.5, 10 mM $MgCl_2$, 60 mM NH_4Cl, 0.5 mM EDTA, 0.5 mM DTT, and homogenized by stirring for 20 min. To remove cell debris and Al_2O_3 the suspension was centrifuged at 16 000 × *g* for 30 min at 4°C. Centrifugation was repeated with the supernatant at 30 000 ×*g* for 40 min. The resulting supernatant (S30) was frozen with 10% glycerol.

For the in vitro poly(Phe) synthesis 3 mM ATP, 0.2 mM GTP, 8.8 μM [^{14}C]phenylalanine (475 Ci/mol), 150 μg poly(U), and 0.1 A_{260} bulk-tRNA from *E. coli* were added to a buffer containing 20 mM Tris, pH 7.5, 20 mM $MgCl_2$, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT in a total volume of 150 μl and preincubated for 1 min at 37°C. The poly(Phe) synthesis was started by the addition of 24 μg crude cellular extract. To study the influence of kirromycin and extra added EF-Tu, these components were used in concentrations of 20 μM and 2.2 μM, respectively. Aliquots of 15 μl were withdrawn at certain time intervals and pipetted onto Whatman 3MM filters. The proteins, peptides and aminoacyl-tRNA were precipitated on the filter by 10% trichloroacetic acid; the aminoacyl-tRNA was hydrolyzed by boiling for 10 min in the same solution; the filters were rinsed successively with cold 10% trichloroacetic acid, ethanol, diethyl ether, and dried; the filter-bound radioactivity, corresponding to poly(Phe), was counted.

3. RESULTS AND DISCUSSION

3.1. *In vivo studies of growth rates*

During the overproduction of the elongation factor Tu of *T. thermophilus* in *E. coli* from a plasmid-encoded *tufA*-gene [16], the induction of the *tac*-promoter by IPTG in the logarithmic growth phase resulted in a reduction of the proliferation rate, as compared to the non-induced control or a strain with the expression vector lacking the *tufA*-gene (Fig. 1a). This can be explained by growth retardation, when the synthesis of an additional protein begins, and is usually observed when overproducing a protein which is not toxic for the cells. However, growth continued, which indicates that either the foreign elongation factor remains inactive or it participates properly in the host's protein biosynthesis, implying that its structural features and kinetic properties are similar to those of the *E. coli* EF-Tu under these experimental conditions.

To determine whether the overproduced *T. thermophilus* EF-Tu is involved in the translation of *E. coli*, we transformed the kirromycin-resistant *E. coli* strain LBE2012 with the EF-Tu-overproducing plasmid pEFTu-10 and the expression vector pKK223-3, respectively, and inoculated liquid medium with the transformed bacteria. The control strain LBE2012(pKK223-3), which harbors the vector but lacks the inserted *tufA*-gene, grew well both in the absence and presence of kirromycin (Fig. 1a, b). However, no significant growth was observed with LBE2012(pEFTu-10) in the presence of kirromycin, whereas in its absence the bacteria grew well. These results show that the presence of the foreign *tufA*-gene makes the otherwise kirromycin-resistant bacteria sensitive to this antibiotic. Growth of LBE2012(pEFTu-10) was inhibited in the presence of kirromycin even when the *tac*-promoter was not induced. This indicates that minute amounts of the *T. thermophilus* EF-Tu are sufficient to recover kirromycin sensitivity. The leakage of the *tac*-promoter in the absence of the inducer IPTG leading to a residual expression of the *tufA*-gene has previously been established by detecting small amounts of EF-Tu by rocket-immunoelectrophoresis in non-induced cells [16]. If the thermostable EF-Tu, however, remained inactive in the cells, an interference with the kirromycin resistance mechanism would be unlikely. In the absence of kirromycin the growth of LBE1001 was slightly faster than that of LBE2012 (Fig. 1c). This can be explained by the lower amount of active EF-Tu in the latter strain, since EF-TuB_0 may not be functional in vivo.

3.2. *In vitro translation assays*

To prove that the purified EF-Tu from *T. thermophilus* is capable of interfering with the kirromycin-resistant translational system of *E. coli* LBE2012, we performed in vitro translation experiments using cellular extracts from this *E. coli* strain and poly(U) as an external template. In the absence of kirromycin the poly(Phe) synthesis with LBE2012 extracts occurred, indicating that the kirromycin-resistant translational system is active in vitro (Fig. 2). Poly(Phe) synthesis increased upon addition of purified wild-type EF-Tu from *E. coli*

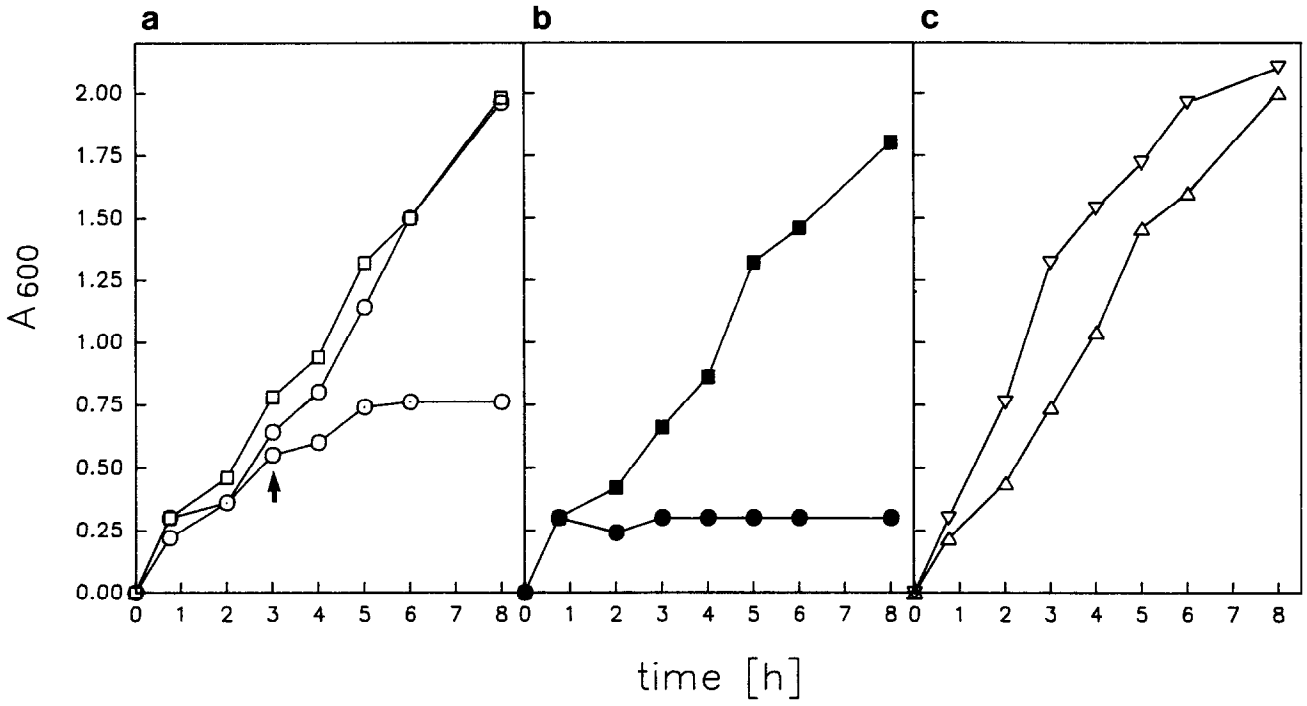

Fig. 1. Growth rates of kirromycin-resistant and sensitive *E. coli* strains with dependance on kirromycin and the expression of the *tufA*-gene from *T. thermophilus*. Open symbols = no kirromycin added to the medium; filled symbols = kirromycin added. □, LBE2012(pKK223-3); ○, LBE2012(pEFTu-10); ⊙, LBE2012(pEFTu-10), *tac* promoter induced; △, LBE2012; ▽, LBE1001. (a) Growth inhibition upon the expression of *tufA*. The arrow indicates the induction of the *tac* promoter. (b) Influence of kirromycin. (c) Strain-specific growth differences.

LBE1001 to the cellular extracts of LBE2012. The addition of purified EF-Tu from *T. thermophilus* advanced the poly(Phe) synthesis somewhat less. This may be attributed to the reduced ribosome-induced GTPase activity of the thermostable EF-Tu at 37°C, a temperature which is 30–40°C below its optimum. On the other hand it again indicates that the thermostable EF-Tu participates in the polypeptide synthesis of *E. coli*, which is consistent with previous results [17].

LBE2012 extracts were less productive in poly(Phe) synthesis in the presence of kirromycin than in its absence (Fig. 2), a finding which is in accordance to previously published results [6,25]. In contrast, no poly(Phe) synthesis was detected with LBE1001 extracts in the presence of kirromycin. By the addition of 2.2 μM purified EF-Tu from either *E. coli* LBE1001 or *T. thermophilus* to the kirromycin-resistant translational system, no poly(Phe) synthesis occurred. It thus can be concluded that, like the wild-type *E. coli* EF-Tu, the *T. thermophilus* EF-Tu is able to bind kirromycin and consequently blocks the *E. coli* ribosomes. This presumes that the *T. thermophilus* $EFTuB_0$·GDP·aatRNA·kirromycin complex interacts with the *E. coli* ribosomes in a similar manner as the homologous protein of *E. coli*.

It can be expected that, like the native EF-Tu from *T. thermophilus*, mutationally changed EF-Tu species are also able to participate in the protein biosynthesis of *E. coli*. Alterations of amino acid residues essential for the functioning of the EF-Tu in the elongation cycle, however, will lead to an inhibition of the protein synthesis. We have recently attempted to overproduce a *T. thermophilus* EF-Tu species altered in its GTPase center. This protein is probably unable to hydrolyze GTP and, as a consequence, cannot dissociate from the ribosome. In such a case the translocation of the involved ribosome and all ribosomes succeeding on the same mRNA molecule will be hindered. Similar to the effect of kirromycin, only a few EF-Tu molecules with reduced GTPase activity are sufficient to halt bacterial growth. This, indeed, was found with the *T. thermophilus* EF-Tu with the substitution of histidine-85 by glycine.

The possibility of detecting even a minor expression of the *T. thermophilus tuf*-genes in the kirromycin-resistant *E. coli* strain by attaining kirromycin sensitivity can be used for the crude localization of promoters in the *str*- and *tufB*-operons of *T. thermophilus* via deletion analyses. In preliminary experiments we found evidence for a secondary *str*-operon promoter located in the *fus*-gene, a situation which is similar to that found in *E. coli*. This approach could contribute important results to our less advanced knowledge of the regulation of these operons from *T. thermophilus*.

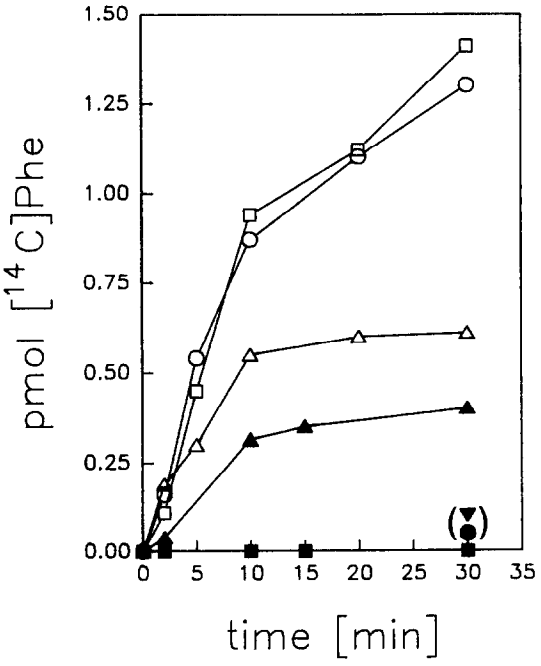

Fig. 2. In vitro poly(Phe) synthesis with dependence on kirromycin and EF-Tu. Open symbols = in the absence of kirromycin; filled symbols = in the presence of kirromycin. △, LBE2012; ▼, LBE1001; ○, LBE2012+EF-Tu$_{T\ thermophilus}$; □, LBE2012+EF-Tu$_{E\ coli}$.

Acknowledgements: This work was supported by the Deutsche Forschungsgemeinschaft (SFB 213/D5). We thank Lothar Seidler for stimulating discussions, Leo Zeef of the group of Prof. L. Bosch, Leiden, for kindly providing us the *E coli* strains LBE1001 and LBE2012, Prof. A. Pingoud for providing the kirromycin, and M. Beikman for critically reading the manuscript.

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