FEBS Letters 391 (1996) 330–332 FEBS 17396

Role of the conserved aspartate and phenylalanine residues in prokaryotic and mitochondrial elongation factor Ts in guanine nucleotide exchange

Yuelin Zhang[a], Xin Li[a], Linda L. Spremulli[a,b,*]

[a]*Department of Chemistry, Campus Box No. 3290, University of North Carolina, Chapel Hill, NC 27599-3290, USA*
[b]*Lineberger Comprehensive Cancer Research Center, Campus Box No. 3290, University of North Carolina, Chapel Hill, NC 27599-3290, USA*

Received 25 June 1996

Abstract The guanine nucleotide exchange reaction catalyzed by elongation factor Ts is proposed to arise from the intrusion of the side chains of D80 and F81 near the Mg^{2+} binding site in EF-Tu. D80A and F81A mutants of *E. coli* EF-Ts were 2–3-fold less active in promoting GDP exchange with *E. coli* EF-Tu while the D80AF81A mutant was nearly 10-fold less active. The D84 and F85 mutants of EF-Ts_{mt} were 5–10-fold less active in stimulating the activity of EF-Tu_{mt}. The double mutation completely abolished the activity of EF-Ts_{mt}.

Key words: Protein synthesis; Elongation factor; Mitochondria; Elongation factor Tu; Elongation factor Ts

1. Introduction

During the process of protein biosynthesis, EF-Tu promotes the binding of aminoacyl-tRNA (aa-tRNA) to the A-site of the ribosome [1]. This reaction is mediated through a ternary complex [EF-Tu:GTP:aa-tRNA]. Following ribosome binding, the GTP is hydrolyzed and EF-Tu is released from the ribosome as an EF-Tu:GDP complex. GDP is then exchanged for GTP in a process mediated by EF-Ts [2]. The structure of EF-Tu is known in both the GDP- and GTP-bound forms [3,4]. This protein folds into three domains. Domain I, encompassing the first 200 residues, contains the guanine nucleotide binding site, while all three domains are involved in binding aa-tRNA [5]. The structure of the *E. coli* EF-Tu·Ts complex has recently been determined [6]. Examination of this structure suggests that nucleotide exchange arises in part because the side chains of D80 and F81 of EF-Ts intrude near the site on EF-Tu where the Mg^{2+} ion interacting with GDP is normally located. The resulting disruption of the Mg^{2+} ion binding site is believed to reduce the affinity of EF-Tu for GDP.

The mammalian mitochondrial factors equivalent to EF-Tu and EF-Ts have been purified from bovine liver mitochondria as a tightly associated complex (EF-Tu·Ts_{mt}) [7,8]. This complex, unlike the corresponding complex from *E. coli*, is not dissociated by either GDP or GTP even at high concentrations of the guanine nucleotides. The cDNAs for both of these proteins have been cloned and sequenced [9,10]. EF-Tu_{mt} has 55–60% identity to prokaryotic EF-Tu. In contrast, EF-Ts_{mt} is less than 30% identical to the bacterial factors. Mammalian EF-Ts_{mt} has residues corresponding to D80 and F81 postulated to be involved in the guanine nucleotide exchange reaction with *E. coli* EF-Ts. When EF-Ts_{mt} is expressed in *E. coli*, it forms a tight complex with *E. coli* EF-Tu [10]. The properties of this heterologous complex are similar to those of the mitochondrial EF-Tu·Ts_{mt} complex and, unlike the *E. coli* EF-Tu·Ts complex, the heterologous complex formed with EF-Ts_{mt} is not dissociated detectably by guanine nucleotides. These observations suggest that EF-Ts_{mt} confers several unusual properties upon the EF-Tu·Ts complex. In the current manuscript, we have examined the roles of the conserved Asp and Phe residues in both *E. coli* EF-Ts and EF-Ts_{mt} that are thought to facilitate the guanine nucleotide exchange reaction.

2. Materials and methods

2.1. Construction of clones and mutants

The *E. coli* EF-Tu and EF-Ts genes were amplified from *E. coli* chromosomal DNA by PCR and cloned into pET24C(+) (Novogen). This vector provides a His-tag to facilitate purification of the expressed proteins on Ni-NTA resins. Site-directed mutagenesis of the *E. coli* EF-Ts and EF-Ts_{mt} genes was performed using a PCR-based 'link scanning' method [11].

2.2. Expression and purification of EF-Tu and EF-Ts

The His-tagged form of *E. coli* EF-Tu was expressed and purified as described previously [9] except that 10 mM $MgCl_2$ and 10 μM GDP were included. *E. coli* EF-Ts was purified under three different conditions. (1) Extracts were prepared as described [9] in buffer containing $MgCl_2$; (2) 10 μM GDP was also included in the isolation buffer. This condition was used for the large-scale preparation of EF-Ts and its mutants; (3) no $MgCl_2$ was added to the extraction buffers and the cell extract was dialyzed twice against a 100-fold excess of buffer containing 10 mM Tris-HCl, pH 7.6, 40 mM KCl and 10% glycerol prior to purification of EF-Ts. Expression of EF-Ts_{mt} was carried out as described [10]. When cell extracts were prepared under native conditions, EF-Ts_{mt} was isolated as a 1:1 complex with *E. coli* EF-Tu (EF-Tu_{Eco}·Ts_{mt}). To purify EF-Ts_{mt} free of *E. coli* EF-Tu, the EF-Tu_{Eco}·Ts_{mt} complexes were denatured. EF-Ts_{mt} was then purified through a Ni-NTA column and renatured (Xin and Spremulli, in preparation).

2.3. Assays

The activities of *E. coli* EF-Ts and its mutated forms were determined by measuring their ability to promote guanine nucleotide exchange with *E. coli* EF-Tu:GDP and to stimulate the activity of EF-Tu in the poly(U)-directed polymerization of phenylalanine on *E. coli* ribosomes [12,13]. The activity of EF-Ts_{mt} was determined by the ability to stimulate the activity of EF-Tu_{mt} (Xin and Spremulli, in preparation).

3. Results and discussion

3.1. Conserved D and F residues in E. coli EF-Ts

Three mutants of *E. coli* EF-Ts (D80A, F81A and D80AF81A) were made by site-directed mutagenesis. When

*Corresponding author. Fax: (1) (919) 962-2388.
E-mail: Linda_Spremulli@unc.edu

PII S0014-5793(96)00789-2

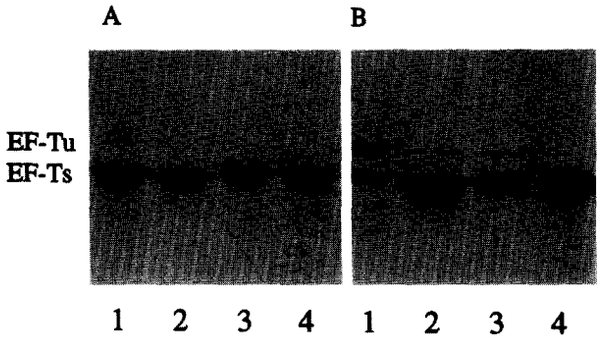

Fig. 1. SDS-PAGE analysis of the expressed *E. coli* EF-Ts and its mutants. (A) *E. coli* EF-Ts and its mutants were purified using method (1). Lanes: 1, wild-type; 2, D80A; 3, F81A; 4, D80AF81A. (B) Extracts were prepared using method (3). Lanes: 1, wild type; 2, D80A; 3, F81A; 4, D80AF81A.

a His-tagged derivative of wild-type EF-Ts is prepared from *E. coli* in buffer containing Mg^{2+} and purified on Ni-NTA resin, a small amount of EF-Tu can be observed in the purified preparation (Fig. 1A, lane 1). This observation suggests that most of the EF-Tu in the extract is complexed with GDP or in the ternary complex. Under similar conditions, no EF-Tu could be observed associated with the D80A, F81A or the D80AF81A mutated forms of EF-Ts (Fig. 1A, lanes 2–4). This observation suggests that the mutation of the conserved D and F residues reduces the affinity of EF-Ts for EF-Tu

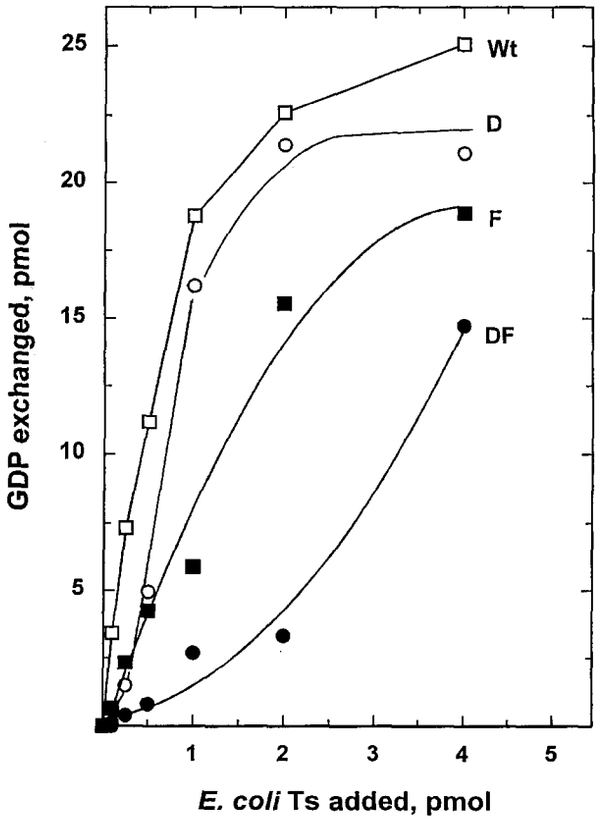

Fig. 2. Stimulation of GDP exchange by *E. coli* EF-Ts and its mutants. Nucleotide binding assays contained 3.6 μg of expressed *E. coli* EF-Tu and the indicated amount of wild-type or mutated forms of *E. coli* EF-Ts. Wt, wild-type; D, D80A; F, F81A; DF, D80AF81A. Incubation was at 0°C for 5 min.

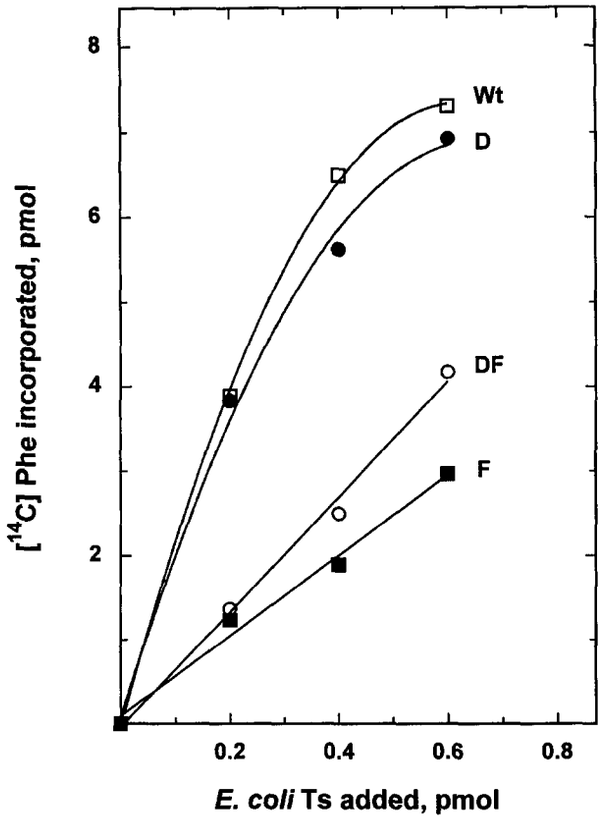

Fig. 3. Stimulation of *E. coli* EF-Tu in poly(U)-directed polymerization: Reaction mixtures contained 0.75 pmol of *E. coli* EF-Tu and the indicated level of *E. coli* EF-Ts. Wt, wild-type; D, D80A; F, F81A; DF, D80AF81A.

significantly. When extracts were prepared in buffers lacking Mg^{2+} and dialyzed prior to purification on Ni-NTA, wild-type EF-Ts is purified as a 1:1 complex with EF-Tu (Fig. 1B, lane 1). In contrast, the ratio of *E. coli* EF-Tu to the D80A and F81A derivatives of EF-Ts is less than 1:10 (Fig. 1B, lanes 2,3). The ratio of *E. coli* EF-Tu to the D80AF81A form of EF-Ts is even lower and EF-Tu could barely be detected in these preparations (Fig. 1B, lane 4). These data again suggest that all three mutated derivatives of *E. coli* EF-Ts can interact with *E. coli* EF-Tu, but that the interactions are much weaker than that observed with wild-type EF-Ts.

EF-Ts is very active in stimulating nucleotide exchange with *E. coli* EF-Tu:GDP (Fig. 2). The D80A mutant has about 2-fold lower activity than the wild-type EF-Ts at limiting concentrations of EF-Ts while the F81A mutant has about 3-fold lower activity. The double mutant shows a somewhat sigmoidal response characteristic of a defect in the interaction of two proteins in a 2-component system. At lower concentrations, it is about 10-fold less active than the wild-type EF-Ts but has significant activity at higher concentrations. These observations suggest that both D80 and F81 are important but not essential for the function of *E. coli* EF-Ts.

The D80A mutant is almost as active as wild-type EF-Ts in stimulating the poly(U)-directed polymerization of phenylalanine (Fig. 3). The F81A and the double mutant are about 4-fold less active than wild-type EF-Ts in this assay. The polymerization assay appears to be somewhat less sensitive than the GDP-exchange assay. The availability of aa-tRNA in this assay most likely pulls the nucleotide exchange reaction in the

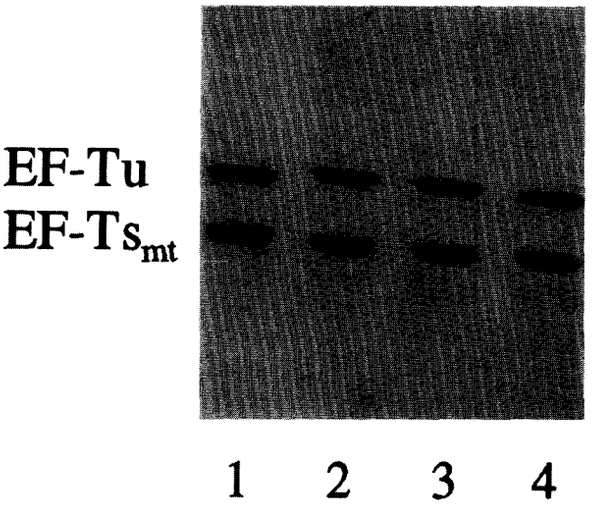

Fig. 4. SDS-PAGE analysis of purified EF-Tu$_{Eco}$·Ts$_{mt}$ complexes. Extracts were prepared under native conditions from cells expressing wild-type and mutated forms of EF-Ts$_{mt}$. Lanes: 1, wild-type EF-Ts$_{mt}$; 2, D84A; 3, F85A; 4, D84AF85A.

forward direction by the formation of the ternary complex and subsequent ribosome binding. Hence, the reduced ability of the mutated forms of EF-Ts to promote guanine nucleotide exchange may not be as apparent when coupled to the favorable formation of the ternary complex.

3.2. *Role of D84 and F85 in EF-Ts*$_{mt}$

The corresponding mutants (D84A, F85A and D84AF85A) were prepared in bovine EF-Ts$_{mt}$. When wild-type EF-Ts$_{mt}$ is prepared from *E. coli* under non-denaturing conditions, it is present as a 1:1 complex with *E. coli* EF-Tu (Fig. 4, lane 1 and [10]). All three of the mutated forms of EF-Ts$_{mt}$ are able to form tight complexes with *E. coli* EF-Tu (Fig. 4, lanes 2–4). This observation indicates that neither D84 nor F85 plays a crucial role in allowing the interaction of EF-Ts$_{mt}$ with EF-Tu. This observation is in contrast with those made with *E. coli* EF-Ts where mutation of either of these residues significantly weakens the interaction between EF-Tu and EF-Ts.

The ability of EF-Ts$_{mt}$ to stimulate GDP exchange with EF-Tu$_{mt}$ cannot be tested directly since no direct GDP binding to this factor can be demonstrated. The ability of wild-type EF-Ts$_{mt}$ and its mutated derivatives to stimulate the activity of EF-Tu$_{mt}$ in poly(U)-directed polymerization was, therefore, tested. Both D84A and F85A derivatives of EF-Ts$_{mt}$ show significantly lower activity than wild-type EF-Ts$_{mt}$ (Fig. 5). The dose responses are sigmoidal, suggesting some defect in the interaction between these two proteins that was not apparent by the SDS-PAGE analysis. The D84AF85A variant is completely inactive. The combination of these residues appears to play a more essential role in EF-Ts$_{mt}$ than in *E. coli* EF-Ts.

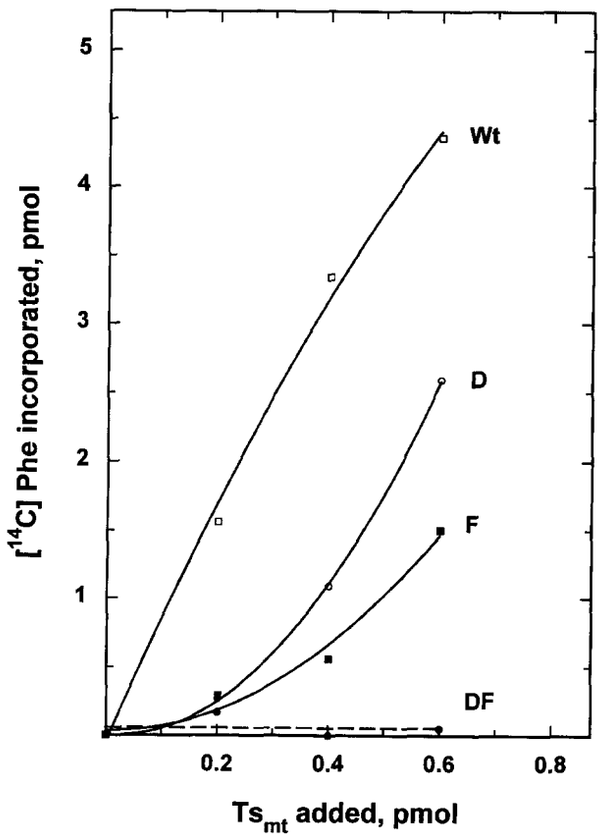

Fig. 5. Stimulation of the activity of EF-Tu$_{mt}$ in poly(U)-directed polymerization: reaction mixtures contained 2 pmol of EF-Tu$_{mt}$ and the indicated amount of EF-Ts$_{mt}$. Wt, wild-type; D, D84A; F, F85A; DF D84AF85A.

References

[1] Bosch, L., Kraal, B., Van der Meide, P. and Van Noort, J. (1983) Prog. Nucl. Acid Res. Mol. Biol. 30, 91–126.
[2] Miller, D. and Weissbach, H. (1970) Biochem. Biophys. Res. Commun.38, 1016–1022.
[3] Kjeldgaard, M. and Nyborg, J. (1992) J. Mol. Biol. 223, 721–742.
[4] Kjeldgaard, M., Nissen, P., Thirup, S. and Nyborg, J. (1993) Structure 1, 35–49.
[5] Nissen, P., Kjeldgaard, M., Thirup, S., Polekhina, G., Reshetnikova, L., Clark, B. and Nyborg, J. (1995) Science 270, 1464–1472.
[6] Kawashima, T., Berthet-Colominas, C., Wulff, M., Cusack, S. and Leberman, R. (1996) Nature 379, 511–518.
[7] Schwartzbach, C. and Spremulli, L. (1989) J. Biol. Chem. 264, 19125–19131.
[8] Schwartzbach, C. and Spremulli, L. (1991) J. Biol. Chem. 266, 16324–16330.
[9] Woriax, V., Burkhart, W. and Spremulli, L. (1995) Biochim. Biophys. Acta 1264, 347–356.
[10] Xin, H., Burkhart, W. and Spremulli, L. (1995) J. Biol. Chem. 270, 17243–17249.
[11] Li, X. and Shapiro, L. (1993) Nucl. Acids Res. 21, 3745–3748.
[12] Fox, L., Erion, J., Tarnowski, J., Spremulli, L., Brot, N. and Weissbach, H. (1980) J. Biol. Chem. 255, 6018–6019.
[13] Schwartzbach, C., Farwell, M., Liao, H. and Spremulli, L. (1996) Methods Enzymol. 264, 248–261.