Roles of Residues in Mammalian Mitochondrial Elongation Factor Ts in the Interaction with Mitochondrial and Bacterial Elongation Factor Tu*

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The crystal structure of the complex between Escherichia coli elongation factors Tu and Ts (EF-TuTs) and subsequent mutagenesis work have provided insights into the roles of a number of residues in E. coli EF-Ts in its interaction with EF-Tu. The corresponding residues in bovine mitochondrial EF-Ts (EF-Tsmt) have been mutated. The abilities of the resulting EF-Tsmt derivatives to stimulate the activities of both E. coli and mitochondrial EF-Tu have been tested. Mutation of several residues in EF-Tsmt corresponding to amino acids important for the activity of E. coli EF-Ts has little or no effect on the activity of the mitochondrial factor, suggesting that these factors may use somewhat different mechanisms to promote guanine nucleotide exchange. In general, mutations that reduce the strength of the interaction between EF-Tsmt and E. coli EF-Tu increase the ability of EF-Tsmt to stimulate the activity of the bacterial factor. In contrast, these mutations tend to reduce the ability of EF-Tsmt to stimulate the activity of the bacterial factor. However, these mutations significantly decrease the ability of EF-Tsmt to stimulate EF-Tu.

During the process of polypeptide chain elongation, elongation factor (EF) Tu promotes the binding of aminoacyl-tRNA (aa-tRNA) to the A-site of the ribosome (1). This process requires the formation of a ternary complex consisting of EF-Tu-GTP-aa-tRNA which interacts at the A-site after stable codon-anticodon interactions. Once the cognate ternary complex has been selected, EF-Tu is released from the ribosome as the inactive EF-Tu-GDP complex. The catalytic use of EF-Tu is facilitated by a second elongation factor EF-Ts, which promotes the exchange of GDP for GTP through the intermediate formation of an EF-Tu-GDP complex (2).

X-ray structural analysis indicates that EF-Tu folds into three domains (3–5). Domain I encompasses the first 200 residues and includes the guanine nucleotide binding site. Domains II and III are each about 100 residues in length. All three domains are required for binding the aa-tRNA (6). The structure of the Escherichia coli EF-Tu-Ts complex has also been determined (7). EF-Ts can be divided into four structural units: the NH2-terminal domain, the core domain, the dimerization domain, and the COOH-terminal module. The core domain is divided into subdomain N and subdomain C. There are extensive regions of contact between E. coli EF-Tu and EF-Ts (7). The NH2-terminal domain, subdomain N, and the COOH-terminal module all interact with domain I of EF-Tu, whereas subdomain C interacts with domain III. Extensive mutational analysis of the surface residues of EF-Ts interacting with EF-Tu has lead to the proposal that the interaction of EF-Ts with EF-Tu causes multiple small conformational changes that give rise to a significant cumulative rearrangement of the guanine nucleotide binding domain of EF-Tu, promoting GDP release (8).

Bacterial EF-Tu and its cytoplasmic equivalent EF-1α have been studied widely. The organellar equivalents have also been identified. Bovine mitochondrial EF-Tu (EF-Tumt) has been purified as a complex with its recycling factor EF-Tsmt (9, 10). Unlike the E. coli complex, the EF-Tumt-EF-Tsmt complex is not readily dissociated by guanine nucleotides (9, 10). Further, no significant amounts of intermediates equivalent to EF-Tu-GTP or EF-Tu-GDP can be readily detected in the bovine mitochondrial system. However, EF-Tumt forms a ternary complex with GTP and aa-tRNA (11) which binds to the A-site of the ribosome. Mammalian mitochondrial protein synthesis thus appears to follow the same basic steps observed in the bacterial elongation cycle. However, the equilibrium constants governing the interaction of EF-Tu with EF-Ts and guanine nucleotides appear to be significantly different.

The cDNAs for both mammalian EF-Tumt and EF-Tsmt have been cloned and sequenced (12, 13). EF-Tumt has 56% identity to E. coli EF-Tu, whereas EF-Tsmt is less than 30% identical to E. coli EF-Ts. EF-Tsmt forms a complex with E. coli EF-Tu which is 100-fold tighter than the homologous EF-TuTs complex (14). The strong binding observed arises primarily from interactions occurring between subdomain N of the core of EF-Tsmt and domain I of EF-Tu (14). Sequence alignment indicates that many of the residues important for the interaction of E. coli EF-Ts with EF-Tu have been conserved in the mitochondrial factor. In the present study a number of these residues have been mutated. The effects of these mutations on the ability of EF-Tsmt to interact with both mitochondrial and E. coli EF-Tu are examined.

MATERIALS AND METHODS

Construction of EF-Tsmt Mutants—E. coli EF-Tu, EF-Tuα, and EF-Tsmt were cloned into pET24c (+) as described previously (12, 13, 15). Site-directed mutagenesis of the EF-Tsmt gene was performed using a polymerase chain reaction-based method (16). The sequences of all of the mutated genes were verified by DNA sequencing.

Expression and Purification of EF-Tsmt Mutants—His-tagged forms
of E. coli EF-Tu and EF-Tsmt were expressed and purified as described previously (15). His-tagged mutants of EF-Tsmt were purified in the absence of GDP to obtain an estimate of the amount of E. coli EF-Tu copurifying with the EF-Tsmt derivative (15). Two different approaches were used to purify mutants of EF-Tsmt free of E. coli EF-Tu. Mutants F19A/I20A, H176A, and L247V/L248M were purified free of EF-Tu by the addition of 100 μM GDP to the purification buffers following the procedure described previously (15). Mutants M9K, R12A/R13A/K14A, F193/203D, M231A/231K, D244A, D244A/F245A, R212A/R213A, and L247/VL248M were purified free of EF-Tu by denaturation of the heterologous E. coli EF-Tu-EF-Tsmt complexes followed by renaturation of EF-Tsmt (17).

Measurement of Binding Constants and Assays—The binding constants governing the interaction of EF-Tsmt or its mutated derivatives with E. coli EF-Tu were determined as described previously (14). The activities of the mutated derivatives of EF-Tsmt in promoting the exchange of guanine nucleotides with E. coli EF-Tu-GDP were determined basically as described (18, 19). Reaction mixtures contained 14 μg of expressed EF-Tu (about 150 pmol of active factor) and the indicated amounts of EF-Ts or mutant EF-Tx. Blanks representing the amount of GDP exchange carried out by EF-Tu alone during this incubation period (about 10 pmol) have been subtracted from each value. No GDP binding could be detected in the absence of EF-Tu, indicating that the preparations of EF-Ts us used here were free of EF-Tu. The activities of the mutated derivatives of EF-Tsmt in stimulating the activity of E. coli EF-Tu in poly(U)-directed polymerization of phenylalanine were determined by the procedure described previously (17). Reaction mixtures contained 1 pmol of expressed EF-Tu and the indicated amounts of EF-Tsmt or its mutated derivatives. Blanks representing the amount of polymerization catalyzed by E. coli EF-Tu alone (about 5−6 pmol) have been subtracted from each value. Stimulation of the activity of EF-Tu in poly(U)-directed polymerization by EF-Tsmt or its mutated derivatives was assayed as described previously (17). Reaction mixtures contained 3 pmol of expressed EF-Tsmt and the indicated amount of EF-Tsmt or the variant shown. Blanks representing the amount of polymerization catalyzed by EF-Tsmt alone (about 2 pmol) have been subtracted from each value.

RESULTS AND DISCUSSION

Previous work was carried out to examine the roles of specific residues in E. coli EF-Ts on its interaction with EF-Tu and on its ability to catalyze guanine nucleotide exchange (8). Many of the residues in EF-Ts which are in contact with EF-Tu in the bacterial EF-Tu-Ts complex are conserved in the mitochondrial factor. However, EF-Tsmt has additional residues in subdomain N of the core which increase the strength of its interaction with EF-Tu (14). In addition, the mitochondrial factor lacks much of the dimerization domain and the COOH-terminal module observed in E. coli EF-Ts (Fig. 1). In this study, we have examined the question of whether residues important for the function of E. coli EF-Tsmt which have been conserved in EF-Tsmt play an important role in the properties of the mitochondrial factor. The underlying hypothesis is that, despite significant differences in portions of these nucleotide exchange factors, the mechanism of nucleotide exchange catalyzed by them will involve similar contacts with EF-Tu. To test this hypothesis, a number of mutated variants of EF-Tsmt were prepared. Several properties of these mutants are described including (i) their binding to E. coli EF-Tu, (ii) their abilities to promote guanine nucleotide exchange with E. coli EF-Tu and to stimulate poly(U)-directed polymerization with the bacterial factor, and (iii) their abilities to stimulate the activity of EF-Tu

Mutational Analysis of the Role of Residues in the NH2-terminal Domain of EF-Tsmt on Interactions with Domain I of EF-Tu—The NH2-terminal domain is essential for the binding of either E. coli EF-Ts or EF-Tsmt to EF-Tu (14, 20). This region of E. coli EF-Ts consists of three helical segments (h1, h2, and h3) and can be aligned readily with the NH2-terminal domain of EF-Tsmt (Fig. 1A). Lys-9, Arg-12, Met-19, Met-20, and Lys-23 of E. coli EF-Ts interact with domain I of E. coli EF-Tu. The analogous residues in EF-Tsmt are Met-9, Arg-12, Phe-19, Ile-20, and Lys-23 (Fig. 1A).

The side chain CH2 of Lys-9 interacts with EF-Tu primarily through a hydrophobic contact with Leu-148. Arg-12 in the NH2-terminal domain of E. coli EF-Ts makes an electrostatic contact with Glu-152 and a hydrogen bond with the backbone of Asp-109. Arg-12 appears to play an essential role in the interaction of E. coli EF-Ts with EF-Tu (Table I). EF-Tsmt has two adjacent Arg residues at positions 12 and 13 and a Lys at position 14. Two mutated derivatives of EF-Tsmt were prepared to assess the potential roles of these residues in the interaction between EF-Tu and EF-Tsmt. The first derivative was based on the idea that the presence of the Met residue might enhance hydrophobic interactions contributing to the overall strength of binding observed with EF-Tsmt. To examine this idea, Met-9 was converted to a Lys residue corresponding to the residue observed in the E. coli factor (Fig. 1A). The second derivative (R12A/R13A/K14A) focused on the role of Arg-12. An indication...
of the effects of these mutations was obtained initially by examining the amount of EF-Tu that copurifies with the variants. When cell extracts are prepared in buffers containing Mg\(^{2+}\) but lacking GDP, an equimolar amount of E. coli EF-Tu copurifies with wild-type EF-Ts\(_{\text{mt}}\) (Fig. 2, lane 2). In contrast, only about 1 mol of EF-Tu is present per 10–20 mol of E. coli EF-Ts (Fig. 2, lane 1). The lower amount of EF-Tu observed copurifying with E. coli EF-Ts reflects the relative ability of this factor to compete for binding to EF-Tu with the guanine nucleotides and aa-tRNA present in the cell extract. This difference is a reflection of the 100-fold tighter binding observed with EF-Ts\(_{\text{mt}}\) compared with E. coli EF-Ts (14). When the M9K and R12A/R13A/K14A derivatives of EF-Ts\(_{\text{mt}}\) are purified from extracts of E. coli, equimolar amounts of EF-Tu are observed copurifying with them (Fig. 2, lanes 3 and 4). These observations indicate that the marker Met-9 nor Arg-12 plays a critical role in the binding of EF-Ts\(_{\text{mt}}\) to the bacterial factor. EF-Ts\(_{\text{mt}}\) binds E. coli EF-Tu quite tightly, but it is significantly less active than E. coli EF-Ts in stimulating the activity of E. coli EF-Tu (Ref. 14 and Fig. 3A). E. coli EF-Ts is recycled by the dissociation of the E. coli-EF-Tu complex by GDP or GTP. When EF-Ts\(_{\text{mt}}\) forms a tight complex with E. coli EF-Tu, the dissociation of the complex by GDP or GTP is very slow, making the turnover number of EF-Ts\(_{\text{mt}}\) in the reaction low. The M9K and R12A/R13A/K14A derivatives of EF-Ts\(_{\text{mt}}\) are purified from extracts of E. coli, equimolar amounts of EF-Tu are observed copurifying with them (Fig. 2, lanes 3 and 4). These observations indicate that the marker Met-9 nor Arg-12 plays a critical role in the binding of EF-Ts\(_{\text{mt}}\) to the bacterial factor.

E. coli EF-Ts

\[ \text{Mutant} \quad \text{Activity in} \quad \text{Activity in} \quad \text{Activity in} \quad \text{Activity in} \]
\[ \text{GDP exchange} \quad \text{polymerization} \]
\[ \text{EF-Ts} \]

| Mutant   | Activity in GDP exchange | Activity in polymerization |
|----------|--------------------------|---------------------------|
| K9A R12A | Inactive                 | Inactive                  |
| M19A M20A| Down 2-fold              | Down 3-fold               |
| M19E M20E| Inactive                 | Inactive                  |
| K23A     | No change                | Down 2-fold               |
| D80A     | Down 2-fold              | No change                 |
| F81A     | Down 3-fold              | Down 4-fold               |
| D80A F81A| Down 10-fold             | Down 4-fold               |
| H147A    | Down 15-fold             | Down 10-fold              |
| K166A H167A| Down 3-fold            | Down 6-fold               |
| V234A M235A| No change              | Down 2-fold               |
| V234E M235E| Inactive                | Inactive                  |
| M9K      | NA                       | No change                 |
| R12A R13A R14A| NA               | No change                 |
| F189A I20A| Down 100-fold            | Up 8-fold                 |
| F19M I20M| Down 4-fold              | Up 3-fold                 |
| R12A K12A| Down 2-fold              | Up 2-fold                 |
| F85A     | No change                | No change                 |
| D84A F85A| Down 70-fold             | Up 8-fold                 |
| H176A    | Down 70-fold             | Up 4-fold                 |
| R212A R213A| Down 6-fold            | Up 3-fold                 |
| L246A L247A| No change              | NA                        |
| L246E L247E| Down 30-fold            | Down 7-fold               |

\* Measured with E. coli EF-Tu.

FIG. 2 SDS-polyacrylamide gel electrophoretic analysis of the interaction EF-Ts\(_{\text{mt}}\) and the NH\(_2\)-terminal domain mutants with E. coli EF-Tu in cell extracts. Proteins were purified as described under "Materials and Methods" under non-denaturing conditions. About 8 µg of each protein was analyzed to detect low levels of EF-Tu copurifying with the His-tagged EF-Ts\(_{\text{mt}}\) mutants in the samples. Lane 1, E. coli EF-Ts; lane 2, wild-type EF-Ts\(_{\text{mt}}\); lane 3, M9K; lane 4, R12A/R13A/K14A; lane 5, F189A/I20A; lane 6, F19M/I20M; lane 7, F189E/I20E; lane 8, K233/K244.

Table I

| Mutant   | Activity in GDP exchange | Activity in polymerization |
|----------|--------------------------|---------------------------|
| K9A      | NA                       | No change                 |
| R12A     | No change                | No change                 |
| M19A     | Down 100-fold            | Up 8-fold                 |
| M19E     | Down 4-fold              | Up 3-fold                 |
| K23A     | No change                | No change                 |
| D80A     | Down 2-fold              | No change                 |
| F81A     | Down 4-fold              | No change                 |
| D80A F81A| Down 4-fold              | No change                 |
| H147A    | Down 10-fold             | No change                 |
| K166A H167A| Down 3-fold            | Down 6-fold               |
| V234A M235A| No change              | Down 2-fold               |
| V234E M235E| Inactive                | Inactive                  |
| M9K      | NA                       | No change                 |
| R12A R13A R14A| NA               | No change                 |
| F189A I20A| Down 100-fold            | Up 8-fold                 |
| F19M I20M| Down 4-fold              | Up 3-fold                 |
| R12A K12A| Down 2-fold              | Up 2-fold                 |
| F85A     | No change                | No change                 |
| D84A F85A| Down 70-fold             | Up 8-fold                 |
| H176A    | Down 70-fold             | Up 4-fold                 |
| R212A R213A| Down 6-fold            | Up 3-fold                 |
| L246A L247A| No change              | NA                        |
| L246E L247E| Down 30-fold            | Down 7-fold               |
with *E. coli* EF-Ts. The most straightforward interpretation of these results is that residues Phe-19 and Ile-20, as opposed to Met-19 and Met-20, contribute about 3-fold to the tighter binding observed with the mitochondrial factor (14). These residues thus appear to be the basis for the contribution of the NH$_2$-terminal domain of EF-Ts mt on the stronger interaction between the mitochondrial factor and *E. coli* EF-Tu. The F19E/I20E variant protein bound very little EF-Tu when purified from cell extracts (Fig. 2, lane 7).

The reduction in binding observed with the F19A/I20A and F19M/I20M derivatives results in an increase in the ability to promote guanine nucleotide exchange with *E. coli* EF-Tu (Fig. 4, A and B). The increased activity in the nucleotide exchange assay suggests that within limits, weaker binding between EF-Ts and EF-Tu allows more catalytic use of the exchange factor. The F19A/I20A derivative is also more active than wild-type EF-Ts mt in promoting poly(U)-directed polymerization with *E. coli* EF-Tu, whereas F19M/I20M is slightly more active in promoting polymerization with the bacterial factor (Fig. 4, B and C). In contrast, the F19A/I20A derivative is about 2-fold less active than wild-type EF-Ts mt in promoting poly(U)-directed polymerization with EF-Tu mt (Fig. 4D). This difference suggests that EF-Tu mt has evolved a structure that is more dependent on a strong interaction with EF-Ts than found with *E. coli* EF-Tu. The F19M/I20M variant is essentially identical to the wild-type factor when tested for the ability to stimulate EF-Tu mt in polymerization (Fig. 4D).

**TABLE II**

| EF-Ts Form          | $K_{Ts}$ ($\times 10^8$ M$^{-1}$) |
|---------------------|----------------------------------|
| *E. coli* EF-Ts     | 8.9 ± 1.3                        |
| Wild-type EF-Ts$_{mt}$ | 860 ± 100                      |
| F19A I20A           | 5.0 ± 1.3                        |
| F19M I20M           | 220 ± 40                         |
| K23A K24A           | 350 ± 40                         |
| H176A               | 13 ± 2                           |
| R212A R213A         | 140 ± 10                         |
| L247E L248E         | 28 ± 3                           |

$K_{Ts}$ is the binding constant for the formation of the EF-Tu-Ts complex.

Finally, Lys-23 in the NH$_2$-terminal domain of *E. coli* EF-Ts forms an electrostatic contact with Asp-141 in EF-Tu. Although this residue is conserved in EF-Ts, it does not appear to be important in *E. coli* EF-Ts (Table I and Ref. 8). Bovine EF-Ts$_{mt}$ has two adjacent Lys residues at positions 23 and 24. A K23A/K24A derivative of this factor binds to *E. coli* EF-Tu about 2-fold less tightly than does the wild-type EF-Ts$_{mt}$ derivative (Fig. 2, lane 7).
EF-Ts mt or its mitochondrial factor contains an insertion in this region (Fig. 1). The side chains of the conserved residues Asp-80 and Phe-81 are inserted into domain I of EF-Tu near the site where the Mg$^{2+}$ ion coordinated to GDP is normally located (Fig. 1B). Mutations in these two residues in E. coli EF-Tu play a surprisingly modest role in the binding of E. coli EF-Ts to EF-Tu (Table I) (15, 18). Mutations in the corresponding residues in EF-Ts mt all bind to E. coli EF-Tu as well as the wild-type factor. The activities of D84A and F85A are lower than EF-Ts mt, and D84A/F85A has no activity in stimulating the activity of EF-Ts mt in polymerization (Ref. 15 and Table I). The activities of the D84A, F85A, and D84A/F85A derivatives are within 2-fold of the activity of wild-type EF-Ts mt in promoting GDP exchange and polymerization with E. coli EF-Tu (Fig. 5, A and B). Asp-80 and Phe-81 are clearly not essential for the activity of either E. coli EF-Ts or EF-Ts mt with E. coli EF-Tu, although these residues appear to be important for the ability of EF-Ts mt to stimulate the activity of its homologous factor. Thus, despite the strong conservation in primary sequence of EF-Tu, subdomain N of the core of EF-Ts mt appears to interact differently with domain I of E. coli EF-Tu compared with EF-Ts mt.

Backbone atoms in subdomain N of EF-Ts and side chains in domain I of EF-Tu also make several contacts (7). One of these contacts involves the carbonyl oxygen of Gly-126, which makes a hydrogen bond with an imidazole nitrogen in His-19. An H19A mutant of EF-Tu shows only a small reduction (about 3-fold) in its binding constant for E. coli EF-Tu and nucleotide exchange with this derivative of EF-Tu is still stimulated to a significant extent by EF-Ts (8). To assess the possible importance of the contact between EF-Ts mt and His-19 in domain I of E. coli EF-Tu, the ability of wild-type EF-Ts mt to stimulate the activity of E. coli EF-Tu was tested (Fig. 6). Surprisingly, EF-Ts mt is not active in promoting GDP exchange with the H19A derivative of E. coli EF-Tu. Furthermore, EF-Ts mt fails to compete with GDP for binding to the H19A derivative of EF-Tu. These observations suggest that the contact between EF-Ts mt and the region around His-19 of E. coli EF-Tu is significantly more important than the corresponding interaction with E. coli EF-Ts. It is possible that the distance between His-19 of E. coli EF-Tu and Gly-152 in EF-Ts mt is shorter than the distance between His-19 and Gly-126 in E. coli EF-Ts, creating a stronger hydrogen-bonded contact between EF-Tu and EF-Ts mt at this point.

**Mutational Analysis of EF-Ts mt Residues Involved in the Interactions between Subdomain C of EF-Ts and Domain III of EF-Tu**—Subdomain C of the core is formed by two segments of EF-Ts interrupted by the dimerization domain. EF-Ts md does not contain much of the region corresponding to the dimerization domain of E. coli EF-Ts but has regions corresponding to most of subdomain C of the core (Fig. 1C). Structural analysis of the E. coli EF-Tu-Ts complex shows that several residues
including His-147, Lys-166, His-167, Val-234, and Met-235 of
E. coli EF-Ts interact with domain III of
E. coli
EF-Tu. The

corresponding residues in EF-Ts mt are His-176, Arg-212, Arg-
213, Leu-246, and Leu-247 (Fig. 1
C).

His-147 of EF-Ts interacts with Asp-354 of EF-Tu through
an ion pair and makes a van der Waals contact with Met-351. Analysis of a H147A mutant of
E. coli
EF-Ts indicates that this

residue plays a crucial role in the interaction between these

two proteins (Table I). Lys-166 forms an ion pair with the side
chain of Glu-348 in domain III, whereas His-167 contacts this

residue through a bridging water molecule. Although Lys-166
is usually a basic residue in the EF-Ts from prokaryotes, it is

not conserved in the mitochondrial factors. His-167 is not a

highly conserved residue. A H166A/K167A variant of
E. coli
EF-Ts has about a 10-fold lower binding affinity for EF-Tu

compared with the normal factor, suggesting that these resi-
dues play a moderate role in the interaction between EF-Tu
and EF-Ts. However, mutation of the corresponding residue
Glu-348 in domain III of EF-Tu to Ala does not affect the
interaction between EF-Tu and EF-Ts significantly. Two residues,
Val-234 and Met-235 of
E. coli
EF-Ts, form part of a hydrophobic
core in the interaction between EF-Ts and domain III of EF-Tu.

Mutation of these residues to Ala reduces the interaction be-
tween the two
E. coli
factors about 3-fold (Table I) (8).

The H176A derivative of EF-Ts mt copurifies with about 10-
fold less
E. coli
EF-Tu than is bound to wild-type EF-Ts mt (Fig.
7, lane 3). The binding constant governing the interaction be-
tween the H176A derivative of EF-Ts mt and
E. coli
EF-Tu is
70-fold lower than that observed with wild-type EF-Ts mt (Table
II). The amount of
E. coli
EF-Tu bound to an R212A/R213A
variant is about 3-fold lower than that bound to the wild-type
factor (Fig. 7, lane 4). Determination of the binding constant
governing the interaction between R212/R213 and EF-Tu indi-
cated that there is a 2.5-fold reduction in this value (Table II).

Three variants in positions corresponding to Val-234 and
Met-235 (Leu-247 and Leu-248) (Fig. 1
C) were prepared. The
first variant (L247A/L248A) reduces the potential hydrophobic
interactions; the second (L247V/L248M) converts the sequence
in the mitochondrial factor to that in
E. coli
EF-Ts; the third
(L247E/L248E) introduces charged residues into this normally
hydrophobic portion of the interface. The L246A/L247A and
L246E/L247E derivatives have about 10-fold reduced binding
affinities for EF-Tu compared with the normal factor, suggesting that these resi-
dues play a moderate role in the interaction between EF-Tu

and EF-Ts. However, mutation of the corresponding residue
Glu-348 in domain III of EF-Tu to Ala does not affect the
interaction between EF-Tu and EF-Ts significantly. Two residues,
Val-234 and Met-235 of
E. coli
EF-Ts, form part of a hydrophobic
core in the interaction between EF-Ts and domain III of EF-Tu.

Mutation of these residues to Ala reduces the interaction be-
tween the two
E. coli
factors about 3-fold (Table I) (8).

The H176A derivative of EF-Ts mt copurifies with about 10-
fold less
E. coli
EF-Tu than is bound to wild-type EF-Ts mt (Fig.
7, lane 3). The binding constant governing the interaction be-
tween the H176A derivative of EF-Ts mt and
E. coli
EF-Tu is
70-fold lower than that observed with wild-type EF-Ts mt (Table
II). The amount of
E. coli
EF-Tu bound to an R212A/R213A
variant is about 3-fold lower than that bound to the wild-type
factor (Fig. 7, lane 4). Determination of the binding constant
governing the interaction between R212/R213 and EF-Tu indi-
cated that there is a 2.5-fold reduction in this value (Table II).

Three variants in positions corresponding to Val-234 and
Met-235 (Leu-247 and Leu-248) (Fig. 1
C) were prepared. The
first variant (L247A/L2478A) reduces the potential hydrophobic
interactions; the second (L247V/L248M) converts the sequence
in the mitochondrial factor to that in
E. coli
EF-Ts; the third
(L247E/L248E) introduces charged residues into this normally
hydrophobic portion of the interface. The L247A/L2478A and
L247V/L248M variants bind to
E. coli
EF-Tu with the 1:1
stoichiometry observed with wild-type EF-Ts mt (Fig. 7, lanes 5
and 6). The amount of
E. coli
EF-Tu bound to the L247E/L248E
derivative is about 5–10-fold less than the amount of EF-Tu
bound to wild-type EF-Tu⁰ (Fig. 7, lane 7). The binding constants for the interaction of the L247E/L248E derivative with E. coli EF-Tu is about 30-fold lower than that of wild-type EF-Tu⁰ and 3–4-fold higher than the binding constant governing the interaction between E. coli EF-Tu and EF-Ts (Table II). This result suggests that disruption of the hydrophobic core of the interaction between subdomain C of EF-Tu⁰ and domain III of E. coli EF-Tu significantly weakens the interaction between these two factors. However, other interactions between EF-Tu⁰ and E. coli EF-Tu are sufficiently strong to allow a substantial interaction between these two factors.

The mutated derivatives in subdomain C of the core were also tested for their activities. The H176A derivative of EF-Tu⁰ is significantly more active than wild-type EF-Tu⁰ and as active as E. coli EF-Ts in these assays (Fig. 8A and C). In contrast, the H176A derivative is 5–6-fold less active in stimulating the activity of EF-Tu⁰ in polymerization (Fig. 8D). The R212A/R213A variant is about 3-fold more active than EF-Tu⁰ in promoting guanine nucleotide exchange and polymerization with E. coli EF-Tu (Fig. 8, B and C). All of these observations suggest that contacts made by Arg-212 and Arg-213 have a small role in promoting the interaction between EF-Tu⁰ and E. coli EF-Tu. The R212A/R213A derivative is comparable or slightly more active in stimulating the activity of EF-Tu⁰ than the wild-type factor (Fig. 8D).

Arg-212 and Arg-213 make contact with Glu-348 in E. coli EF-Tu. To confirm the idea that these residues play a minor role in the interaction of EF-Tu⁰ and E. coli EF-Tu, a E348A derivative of EF-Tu was tested for its ability to be stimulated by wild-type EF-Tu⁰. As shown in Fig. 6, EF-Tu⁰ is about 2-fold more active in stimulating GDP exchange with the E348A mutant of E. coli EF-Tu than with wild-type EF-Tu. This observation suggests that EF-Tu⁰ may interact slightly less tightly with the E348A variant of EF-Tu and that Arg-212 and Arg-213 do not play a major role in the nucleotide exchange reaction.

The L247A/L248A variant of EF-Tu⁰ has about the same activity as that of wild-type EF-Tu⁰ in promoting GDP exchange with E. coli EF-Tu. It is quite difficult to separate this derivative completely from E. coli EF-Tu⁰, making it hard to assess its ability to stimulate the activity of EF-Tu⁰ in polymerization. The L247VL248M variant is about 3-fold less active than wild-type EF-Tu⁰ in stimulating guanine nucleotide exchange with E. coli EF-Tu (Fig. 9A). This reduced activity might arise if the interaction between the L247VL248M variant bound to E. coli EF-Tu more tightly than the wild-type EF-Tu⁰. Attempts to measure the binding constant governing the interaction between these two factors suggested that this was possible, but the strong interaction made it difficult to obtain an accurate value for the actual binding constant. The L247VL248M variant of EF-Tu⁰ has little activity in stimulating E. coli EF-Tu in polymerization, again suggesting that it interacts too strongly with EF-Tu⁰ to function catalytically (data not shown). The activity of the L247VL248E derivative is substantially higher than that of EF-Tu⁰ and is about 2-fold lower than the activity of E. coli EF-Tu (Fig. 9B). This observation was expected since the acidic variant binds E. coli EF-Tu about as tightly as the E. coli factor does. As shown in Fig. 9C, the L247VL248E mutant is about 3-fold more active than wild-type EF-Tu⁰ in stimulating E. coli EF-Tu in polymerization, again reflecting the ability of weaker binding variants to function more catalytically. These observations suggest that hydrophobic contacts between subdomain C of the core of EF-Tu⁰ affect the binding constant governing the interaction of EF-Tu⁰ and E. coli EF-Tu but are not important for the nucleotide exchange catalyzed by this factor.

These EF-Tu⁰ mutants were tested further for their activities in stimulating the activity of EF-Tu⁰ in a polymerization assay. As shown in Fig. 9D, replacing the Leu-Leu sequence of EF-Tu⁰ with the Val-Met sequence found in E. coli EF-Ts has no effect on the ability of this factor to stimulate the activity of EF-Tu⁰. In contrast, the L247E/L248E derivative has at least 5-fold lower activity than the activity of wild-type EF-Tu⁰ (Fig. 9D).

Summary—One theme that emerges from this work is that mutations that weaken the interaction between EF-Tu⁰ and E. coli EF-Tu generally increase the ability of this factor to stimulate the activity of the bacterial factor (Table I). As indicated in a previous study (14), this observation suggests that the strong interaction between EF-Tu⁰ and E. coli EF-Tu makes it difficult for guanine nucleotides to compete for interaction with EF-Tu, reducing the catalytic use of EF-Tu⁰. Clearly, E. coli EF-Tu has evolved a structure that is optimally suited for a nucleotide exchange factor that has a binding constant similar to that of GDP. In contrast, most of the mutations that increase the activity of EF-Tu⁰ with E. coli EF-Tu actually reduce the activity of this factor with EF-Tu⁰. These same residues also appear to be important for the interaction of E. coli EF-Ts with E. coli EF-Tu. Thus, there appears to be conservation of important contacts when the effects of mutations in EF-Ts are examined with the EF-Tu from the corresponding system. However, EF-Tu⁰ interacts with E. coli EF-Tu in a significantly different manner. EF-Tu⁰ and EF-Tu⁰ form a tight complex. EF-Tu⁰ does not bind guanine nucleotides tightly, and the EF-Tu⁰EF-Tu⁰ complex is not disassociated readily by either GDP or GTP (10). A similar situation is observed with the Thermus thermophilus (EF-TuTs) complex (21, 22). In contrast, the E. coli EF-TuTs complex is easily dissociated by GDP. Subtle variations in the conformation of domain I of EF-Tu and in the interactions among the three domains of this factor must account for these differences. Such small conformational differences also apparently give rise to the need for an EF-Ts that binds EF-Tu tightly compared with guanine nucleotides in the mitochondrial and T. thermophilus systems. Structural studies on EF-Tu⁰ and EF-Tu⁰, currently under way, should help shed light on these interesting differences.

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