JOURNAL OF BACTERIOLOGY, Mar. 1983, p. 1266–1271
0021-9193/83/031266-06$02.00/0

Vol. 153, No. 3

# Bacterial Elongation Factor Ts: Isolation and Reactivity with Elongation Factor Tu

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Received 30 August 1982/Accepted 2 December 1982

An improved method for the purification of bacterial polypeptide elongation factor Ts (EF-Ts) from one mesophile (*Escherichia coli*) and two thermophiles (*Bacillus stearothermophilus* and PS3) is described. The improvements are both in the facility of isolation and in increased yields. The purified factors were used for cross-reactivity studies with elongation factor Tu (EF-Tu) obtained from the same bacterial strains. In all combinations studied, the efficiency of EF-Ts in catalyzing the exchange of EF-Tu-bound GDP was proportional to the strength of the protein-protein complex. Whereas the factors from the two thermophiles were interchangeable, the mesophilic EF-Ts formed a very weak complex with thermophilic EF-Tu; however, thermophilic EF-Ts formed very strong complexes with mesophilic EF-Tu. Thus, e.g., EF-Tu from *E. coli* formed a complex with EF-Ts from *B. stearothermophilus* which was 10 times more stable than the corresponding homologous complex.

The polypeptide elongation factor Tu (EF-Tu) catalyzes the enzymatic binding of aminoacyl-tRNA to ribosomes and is one of the most abundant proteins of the bacterial cell (for reviews, see references 13 and 19). Filer and Furano (10) and Filer et al. (9) have shown that the gene coding for EF-Tu is highly conserved even among taxonomically unrelated species of procaryotes, which suggests that structural features of the protein might also be highly conserved. EF-Tu from a number of procaryotes has already been isolated and characterized (3, 18, 21, 23) and found to have similar properties.

Although EF-Tu is mostly present as its GDP complex in the bacterial cell, it forms an almost equally strong complex with the protein elongation factor Ts (EF-Ts) (18). Since the formation of specific protein-protein complexes can be structurally more demanding, a study of the interaction between EF-Tu and EF-Ts from different sources should provide information on the relatedness of the various factors. Studies of this type have been performed with EF-Tu and EF-Ts from *Caulobacter crescentus* (21) and chloroplast elongation factor Ts from *Euglena gracilis* (11).

Two roles have been ascribed to EF-Ts in uninfected cells, and one function has been established in cells infected with a class of RNA bacteriophages (14). In normal cells, EF-Ts is believed to function in protein biosynthesis as a catalyst for the exchange of EF-Tu–bound nucleotides via an EF-Tu–EF-Ts complex (4, 7, 22). In cells under stringent conditions, it has been proposed that the factor might have a control function for RNA polymerase (5). The replication of the RNA of some small bacteriophages in infected cells is catalyzed by an enzyme of four subunits, of which two are the host polypeptide elongation factors EF-Tu and EF-Ts (6).

## MATERIALS AND METHODS

**Abbreviations.** EC, BS, and PS3 denote the factors from *Escherichia coli*, *Bacillus stearothermophilus*, and PS3, respectively. CM denotes carboxymethyl.

**Chemicals.** [$^3$H]GDP (10 Ci/mmol) was from Amersham Buchler; DEAE-Sepharose CL-6B and CM-Sepharose CL-6B were obtained from Pharmacia Fine Chemicals, Inc., Uppsala, Sweden; and AcA44 was obtained from LKB Instruments Inc., Rockville, Md. Elongation factors were isolated from three strains of bacteria: *E. coli* (MRE600), *B. stearothermophilus* (NCA 1503), and PS3. Freshly harvested cells were used immediately for the isolation of EF-Tu and EF-Ts as described earlier (15) and below.

**Buffers.** Buffer A was 0.05 I Tris-hydrochloride (pH 7.6) at 20°C, 10 mM $MgCl_2$, 0.5 mM dithioerythritol, 10 μM phenylmethylsulfonyl fluoride (freshly added), and 1 mM $NaN_3$. Buffer B was 0.1 I sodium acetate (pH 4.6), 1 mM dithioerythritol, and 0.2 mM EDTA.

**Assay.** The assay for EF-Ts is the standard GDP exchange nitrocellulose filter assay (22, 23) using 10 μl

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of appropriately diluted EF-Ts solution, 10 μl of 10 μM EF-Tu (100 pmol), and 200 μl of 10 μM [$^3$H]GDP solution in assay buffer (buffer A without phenylmethylsulfonyl fluoride and $NaN_3$) and incubating the reaction mixture for 5 min at 0°C. One unit is the amount of EF-Ts that catalyzes the exchange of 1 pmol of [$^3$H]GDP under these conditions.

Analytical polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate was performed in slab gels in the Tris-bicine buffer system of Ziegler et al. (24). Protein concentrations were determined by the method of Ehresmann et al. (8).

**Interaction of EF-Ts with EF-Tu.** The reaction between the two factors was tested either under non-equilibrium conditions at 0°C for 5 min with low amounts of EF-Ts or under equilibrium conditions with a mixture of elongation factors and [$^3$H]GDP incubated for 30 min at room temperature.

The concentration of the CM-Sepharose CL-6B fractions was by applying the pooled fractions after threefold dilution to a small (2.5 by 1 cm) CM-Sepharose CL-6B column in buffer B and eluting the protein with 0.8 M NaCl in buffer B. The EF-Ts–containing fractions were pooled (less than 15 ml), brought to pH 7.6 with 1 M Tris-hydrochloride (pH 9.0), and applied to the AcA44 column.

## RESULTS

**Purification of EF-Ts.** We have previously described a procedure for the lysis of the bacteria and, after the removal of cell debris, the application of the lysate to a column of DEAE-Sepharose CL-6B in buffer A (15). The analysis of the column effluent revealed, after the breakthrough fraction, usually one, but sometimes two, peaks of EF-Ts activity. (Fig. 1; 15).

For further purification, all EF-Ts activity was collected and, by a method described earlier (12), acidified to pH 4.6 by the slow addition of 2 N acetic acid. After standing for 1 h at 4°C, the resulting precipitate was removed by centrifugation and discarded. The clear supernatant was applied to a column of CM-Sepharose CL-6B (5 by 22 cm) in buffer B and developed at 220 ml/h with a 4-liter linear gradient of 0.1 to 0.6 M KCl in buffer B. The elution pattern obtained is shown in Fig. 1A for the EF-Ts from *B. stearothermophilus*, and similar patterns were obtained for *E. coli* and PS3. It can be seen that the EF-Ts–containing fractions were well separated from most of the UV-absorbing material. The column procedure shown in Fig. 1A resulted in a 5- to 20-fold purification, depending on the bacterial strain, and EF-Ts was obtained which was 50 to 80% pure and suitable for many biochemical studies and which has been used to prepare EF-Tu–EF-Ts for crystallization (16).

Completely homogeneous EF-Ts was obtained by gel filtration on AcA44. For this, the active CM-Sepharose CL-6B fractions were concentrated as described above, thereby avoiding ammonium sulfate precipitation, which partly destroys the activity, and filtered through AcA44 in buffer A without magnesium at 24 ml/h (Fig. 1B). Figure 1C shows the polyacrylamide gel analysis of the various EF-Ts–containing fractions, and Table 1 is a summary of the purification of the *B. stearothermophilus* factor. EF-Ts purified through the AcA44 step from the three bacterial species listed was used for the following studies.

TABLE 1. Summary of purification of EF-Ts from 250 g of cell paste of *B. stearothermophilus*

| Step | Vol (ml) | Total protein (mg) | Total activity (U × $10^6$) | Sp act (U/mg × $10^6$) | Yield (%) |
|---|---|---|---|---|---|
| Cell extract | 700 | 20,100 | —[a] | — | — |
| DEAE-Sepharose | 430 | 4,030 | 121 | 0.03 | 100 |
| pH 4.7 | 460 | 1,470 | 88 | 0.06 | 73 |
| CM-Sepharose | 170 | 64 | 73 | 1.14 | 60 |
| AcA44 | 30 | 17 | 39 | 2.30 | 32 |

[a] The determination of EF-Ts in the cell extract was unreliable.

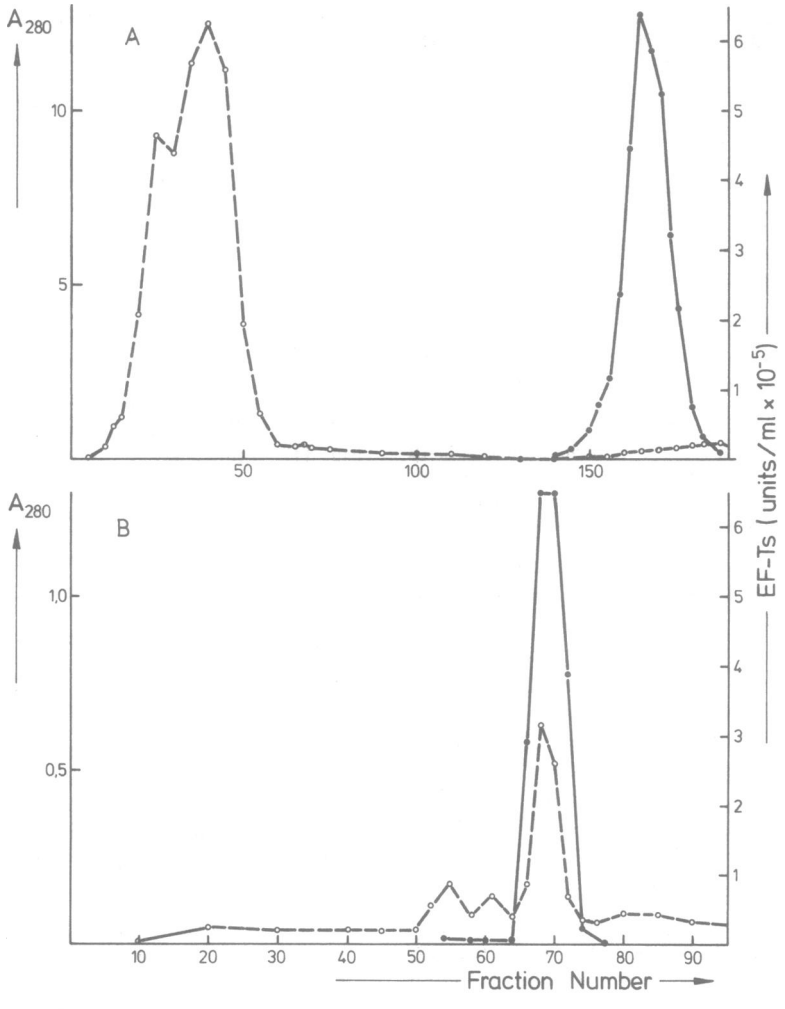
**Interaction with EF-Tu.** We first studied the effect of low amounts of EF-Ts on the GDP exchange reaction under non-equilibrium conditions, i.e., a reaction time of 5 min at 0°C. EF-Ts (BS) catalyzed the exchange reaction with EF-Tu from *E. coli* and *B. stearothermophilus* (Fig. 2B), and the reaction was linearly dependent on the EF-Ts concentration only over a small concentration range of up to about 1 to 2 pmol/as-

TABLE 2. Catalysis of GDP exchange reaction and estimated ratio of binding constants $K_3$ (see the text) for various pairs of elongation factors

| Components | Catalysis | $K_3$[a] |
|---|---|---|
| EF-Tu (EC) | | |
| +EF-Ts (EC) | + | 2.4 |
| +EF-Ts (BS) | + | 33 |
| +EF-Ts (PS3) | + | >100 |
| EF-Tu (BS) | | |
| +EF-Ts (BS) | + | 3.1 |
| +EF-Ts (EC) | − | — |
| +EF-Ts (PS3) | + | 2.9 |
| EF-Tu (PS3) | | |
| +EF-Ts (PS3) | + | 3.2 |
| +EF-Ts (EC) | − | — |
| +EF-Ts (BS) | + | 3.1 |
| EF-Tu (TT)[b] | | |
| +EF-Ts (BS) | + | ND |
| +EF-Ts (EC) | − | ND |

[a] Molecular weight values used for the calculation were 36,000 for EF-Ts (PS3) and 47,000 for EF-Tu (PS3); literature values were used for the other factors. —, None. ND, Not determined.

[b] EF-Tu (TT), Ef-Tu from *Thermus thermophilus*.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A
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1,0
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Fraction Number
EF-Ts ( units / ml × 10-5)
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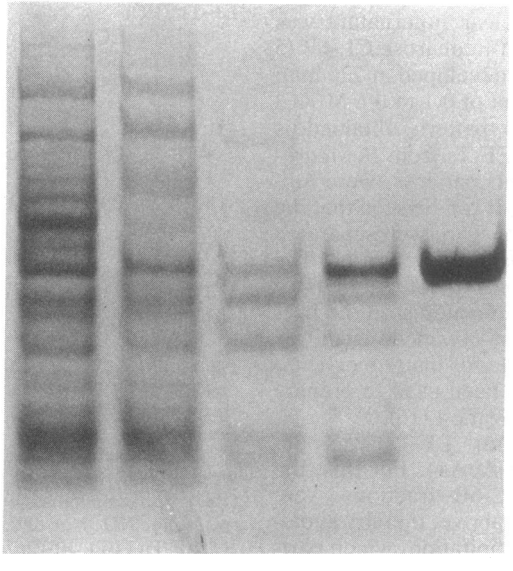

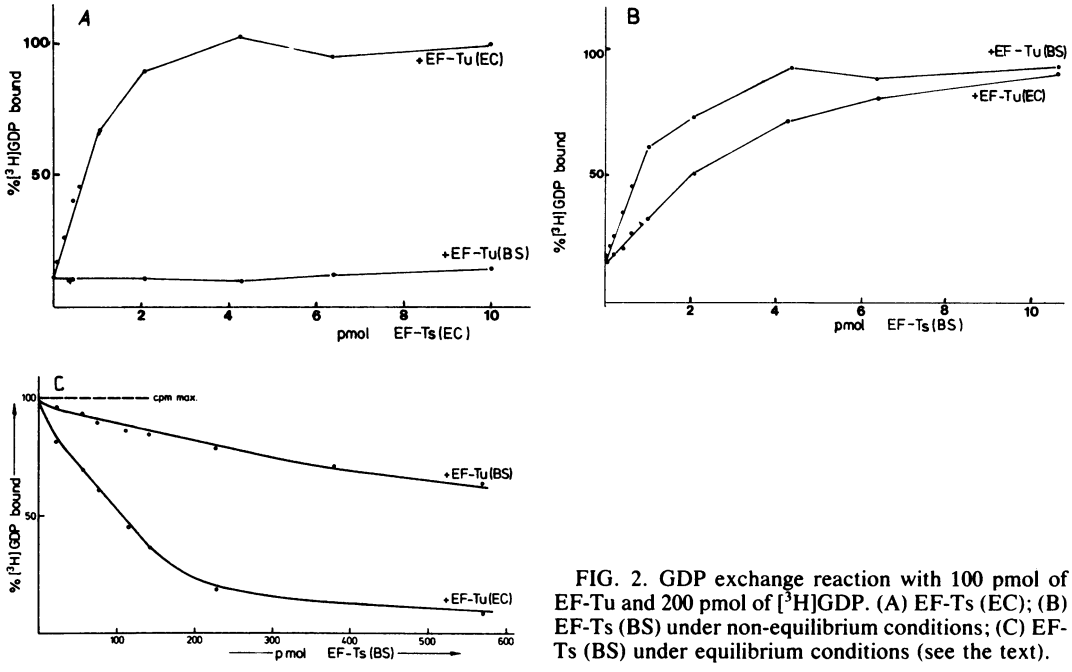


FIG. 2. GDP exchange reaction with 100 pmol of EF-Tu and 200 pmol of [$^3$H]GDP. (A) EF-Ts (EC); (B) EF-Ts (BS) under non-equilibrium conditions; (C) EF-Ts (BS) under equilibrium conditions (see the text).

say, which is the basis of the EF-Ts assay. Surprisingly, EF-Ts (EC) did not catalyze the GDP exchange with EF-Tu (BS) (Fig. 2A). Similar phenomena were observed for the factors from the extreme thermophile PS3 and *E. coli* and also between *T. thermophilus* and *E. coli* (data not shown), whereas all of the thermophilic factors were exchangeable. Table 2 summarizes the results obtained with all possible pairs of elongation factors.

It has been shown that for *E. coli* the binding constant of EF-Tu with EF-Ts is of the same order of magnitude as that for the EF-Tu–GDP complex (2, 18) and that EF-Ts and GDP probably bind at or near the same site on EF-Tu (2, 17). Since the bindings of GDP and EF-Ts are mutually exclusive, one can inhibit the binding of [$^3$H]GDP to EF-Tu by increasing concentrations of EF-Ts. For this, a reaction mixture containing EF-Tu, EF-Ts, and [$^3$H]GDP was incubated for 30 min at 25°C, and the EF-Tu–bound GDP was determined by the nitrocellulose filter method. The results of these experiments are shown in Fig. 2C and Table 2 for factors from *E. coli* and *B. stearothermophilus*. For the homologous pairs, with 300 pmol of EF-Ts in the reaction mixture, approximately 70 to 80% of the GDP remained bound to EF-Tu. As was expected from kinetic data, EF-Ts (EC) did not inhibit GDP binding to either EF-Tu (BS) or EF-Tu (PS3) (data not shown). With EF-Tu (Ec) and EF-Ts (BS) GDP binding, EF-Ts (BS)–GDP binding was reduced to 50% with 90 pmol, indicating that the heterologous complex between mesophilic EF-Tu and thermophilic EF-Ts has a much higher binding constant than any of the respective homologous complexes. The binding constant for the EF-Tu–EF-Ts and EF-

FIG. 1. Purification of EF-Ts from *B. stearothermophilus* on CM-Sepharose CL-6B (A) and AcA44 (B). $A_{280}$, Absorbance at 280 nm. (A) The EF-Ts–containing fractions from DEAE-Sepharose CL-6B (15) were acidified to pH 4.6 as described in the text and applied to a column (5 by 22 cm) of CM-Sepharose CL-6B in buffer B. After being washed, a 4-liter linear gradient from 0.1 to 0.6 M KCl in buffer B was applied; the flow rate was 220 ml/h; the fraction size was 14 ml. EF-Ts was eluted at approximately 0.25 M KCl. (B) The EF-Ts pool from CM-Sepharose CL-6B was concentrated as described in the text and applied to a AcA44 column (2.5 by 140 cm) in buffer A without magnesium in the presence of 0.2 mM EDTA. The flow rate was 24 ml/h; the fraction size was 6 ml. (C) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the EF-Ts–containing fractions obtained during the purification of EF-Ts from *B. stearothermophilus*. The lanes contain, from left to right, with milligrams of protein in parentheses, the following; crude extract (72); DEAE-Sepharose CL-6B (45); DEAE-Sepharose CL-6B after pH 4.6 acidification (32); CM-Sepharose CL-6B (9.5); AcA44 (8.6).

Tu–GDP complexes is given by

$$K_1 = \frac{(\text{EF-Tu–EF-Ts})}{(\text{EF-Tu})\,(\text{EF-Ts})} \text{ and}$$

$$K_2 = \frac{(\text{EF-Tu–GDP})}{(\text{EF-Tu})\,(\text{GDP})}$$

The ratio of these constants was given by

$$K_3 = \frac{K_1}{K_2} = \frac{(\text{EF-Tu–EF-Ts})\,(\text{GDP})}{(\text{EF-Tu–GDP})\,(\text{EF-Ts})}$$

If all EF-Tu is complexed to either EF-Ts or GDP, then $EF\text{-}Tu_T$ = EF-Tu–EF-Ts + EF-Tu–GDP, which is reasonable considering the high binding constants. Values for this ratio can be obtained from measurements shown for two pairs in Fig. 2 and are given in Table 2. The value of 2.4 for the homologous *E. coli* system is higher than the previously reported values of 1.1 (18) and 0.65 (2) found under somewhat different conditions. For the heterologous systems with EF-Ts from the thermophilic strains and EF-Tu from *E. coli*, we found ratios of binding constants $K_3$ to be 10-fold and at least 30-fold higher.

## DISCUSSION

The method presented here for the purification of bacterial elongation factor Ts is coupled with the simplified purification procedure of EF-Tu published earlier (15). Its major advantages are the avoidance of any ammonium sulfate fractionation (1, 12), necessitating subsequent dialysis, and the use of fast-running ion-exchange columns. The high yields of about 40 to 50 mg/200 g of bacterial cell paste for *E. coli* and between 15 and 30 mg for the thermophilic bacteria compare well with the yields obtained earlier for *E. coli* (1, 12). Although the specific activities varied from batch to batch and for the different bacterial strains, only very high specific activities were obtained when rapidly multiplying cells were harvested and used immediately. Nevertheless, we always found specific activities greater than $2 \times 10^6$ U/mg, which compares well with other published values (23).

Our studies with various pairs of elongation factors showed that EF-Ts from any of the three thermophiles is able to catalyze GDP exchange with *E. coli* EF-Tu and two of them are also able to form a strong complex. On the other hand, EF-Ts from *E. coli* showed neither effect with the thermophilic EF-Tus. This is a further indication that both activities are coupled and that catalysis of GDP exchange at low concentrations of EF-Ts proceeds via a transient EF-Ts–EF-Tu–GDP complex (7), whereas at high concentrations of EF-Ts, the binary complex EF-Tu–EF-Ts is the most stable complex of the reaction mixture.

Stringfellow et al. (21) have observed that between the elongation factors of *E. coli* and *C. crescentus*, a pattern of cross-reactivity similar to that between *E. coli* and the thermophilic factors exists. Stringfellow and Blumenthal (20) have also demonstrated that the complex EF-Tu (EC)–EF-Ts (BS) is just as effective as the homologous *E. coli* complex in Q-beta replicase. It is difficult to interpret all of these observations about the interactions between the elongation factors at a structural level. However, with the methods presented here, the study of the interaction between EF-Ts and EF-Tu seems to be useful to establish structural relatedness between bacterial elongation factors and between bacterial and organellar elongation factors and to establish a structural phylogenetic tree for these proteins.

## ACKNOWLEDGMENTS

We thank L. A. Stringfellow and T. Blumenthal for providing a copy of their manuscript before publication and U. Genz for a sample of EF-Tu from *T. thermophilus*.

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