The Amino Acid Sequence of Elongation Factor Tu of *Escherichia coli*

THE COMPLETE SEQUENCE*

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The complete amino acid sequence of elongation factor Tu of *Escherichia coli* has been established by sequencing overlapping cyanogen bromide and tryptic peptides. Sequence analysis of peptides was done primarily by solid-phase Edman degradation. Elongation factor Tu is a single chain polypeptide composed of 393 amino acids (M, = 43,225). Its NH₂ terminus is blocked with an acetyl group, as determined by mass spectrophotometry, and lysine 66 is partially methylated. The cysteine residues associated with aminoacyl tRNA and guanosine nucleotide binding are located at positions 81 and 137, respectively. Although elongation factor Tu is coded for by two genes, the only site of microheterogeneity found was at the carboxyl terminus (residue 393), which is either glycine or serine. Comparison of the first 140 amino acids of elongation factor Tu and of elongation factor G shows a strong (31%) sequence homology. In addition, secondary structure calculations predict remarkable conformational similarities between the two proteins. The NH₂-terminal region of elongation factor Tu appears to be composed of two β-sheet domains connected by an exposed, α-helical bridge, which includes a 14-amino acid segment released by limited treatment with trypsin. Structural features of the aminoacyl-tRNA binding site are discussed in the light of sequence and other chemical and biochemical data.

In the preceding article (1), we described the sequence analysis of three large cyanogen bromide fragments from elongation factor Tu of *Escherichia coli*. In this paper we report on the sequence of the smaller cyanogen bromide peptides, as well as the overlapping tryptic peptides that enable us to deduce the complete sequence of EF-Tu.†

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‡ To whom requests for reprints should be addressed.
† The abbreviations used are: EF-Tu, elongation factor Tu; EF-G, elongation factor G; AA-tRNA, aminoacyl tRNA; TPCK-trypsin, trypsin treated with L-tosylamido-2-phenylethyl chloromethyl ketone; TETA resin, triethylenetetramine-substituted polystryrene; TLC, thin layer chromatography; PTH, phenylthiohydantoin. Peptides generated by digestion with cyanogen bromide are designated with the prefix CB; with trypsin (EC 3.4.21.4), T; and with chymotrypsin (EC 3.4.21.1), C.

EXPERIMENTAL PROCEDURES AND RESULTS

DISCUSSION

The amino acid sequence of *E. coli* EF-Tu, as deduced from the sequence of the cyanogen bromide peptides and of tryptic overlaps, is presented in Fig. 7. The sequence is in good agreement with previously published compositions (16-18), and corresponds to a molecular weight of 43,225 for the form with COOH-terminal serine and 43,195 for that with COOH-terminal glycine. The NH₂ terminus is acetylated and Lys-66 is partially methylated (19). The sequence given here (Fig. 7) can be regarded as firm, since nearly every residue was sequenced at least twice and because there are no ambiguous overlaps. Moreover, our sequence is confirmed by the results of Jones et al. (12) and is in agreement with DNA sequence results of Yokota et al. (20) and An and Friesen (21).

Jaksun and co-workers (22) have shown that in *E. coli*, EF-Tu is coded for by two genes, *tuFA* and *tuFB*, located at 72 and 88 min, respectively, on the genetic map. This finding suggested that the two gene products might have different sequences. However, activity and peptide mapping studies (23,24) on the isolated gene products showed that there was little, if any, difference between the two proteins. In fact, the only sequence difference found by Jones et al. (12), and confirmed indirectly by us in the present study, is at the COOH terminus (residue 393), which can be either Ser or Gly. This finding is also confirmed by the DNA sequences for the *tuFA* (20) and *tuFB* (21) genes, which predict identical protein sequences except at position 393, where the *tuFA* and *tuFB* sequences predict Ser and Gly, respectively.

**Post-translational Modifications**—One of the first observations on the primary structure was that the NH₂-terminal is blocked. It was subsequently found by Jones et al. (12) and by us (1), using mass spectrophotometry, that the first amino acid in EF-Tu is N-acetylseryl. DNA sequencing (20, 21) shows the codon for Ser-1 (UCU) is preceded by GUG (fMet). Thus, after translation, formylmethionine is cleaved and the resulting NH₂-terminal serine is acetylated. Ames and Naka (24) discovered that EF-Tu is also methylated, and subsequently we determined that at least 95% of the methyl groups are at a unique site, Lys-56 (19). Both mono- and dimethyllysine and unmodified lysine are found at

7 Portions of this paper (including "Experimental Procedures," "Results," Figs. 1 to 6, and Tables 1 and 2) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Japan of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 81M-217, cite authors, and include a check or money order for $8.40 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
Amino Acid Sequence of EF-Tu

1 10 20 30
Acetyl-Ser-Lys-Glu-Phe-Glu-Arg-Thr-Lys-Pro-His-Val-Asn-Val-Gly-Thr-Ile-Gly-His-Val-Asp-His-Gly-Lys-Thr-Thr-Val-Ala-Ala-

110 120 130
Ile-Thr-Val-Leu-Ala-Lys-Thr-Val-Thr-Thr-Thr-Val-Thr-Thr-Thr-Thr-Thr-Thr-Thr-Thr-Thr-Thr-Thr-Thr-Thr-Thr-Thr-Thr-Thr-Thr-

140 150
Thr-Ile-Asn-Thr-Ser-His-Val-Glu-Tyr-Asp-Thr-Pro-Thr-Arg-His-Tyr-Ala-His-Val-Asp-Cys-Pro-Gly-His-Asp-Tyr-Val-Lys-Asn-

160 170 180
Met-Ile-Gly-Ala-Ala-Gln-Met-Asp-Gly-Ala-Ile-Leu-Val-Ala-Ala-Ala-Ala-Thr-Asp-Gly-Pro-Met-Pro-Gln-Thr-Arg-Glu-His-Ile-Leu-

190 200 210
Leu-Gly-Arg-Gln-Gly-Pro-Tyr-Ile-Ile-Val-Phe-Leu-Asn-Lys-Cys-Asp-Met-Val-Asp-Glu-Leu-Leu-Glu-Leu-Glu-

220 230 240
Met-Glu-Val-Arg-Glu-Leu-Leu-Ser-Gln-Tyr-Asp-Phe-Pro-Gly-Asp-Asp-Thr-Pro-Ile-Val-Arg-Gly-Ser-Ala-Leu-Lys-Ala-Leu-Glu-

250 260 270
Glu-Val-Glu-Ile-Val-Gly-Ile-Lys-Thr-Val-Val-Thr-Gly-Arg-Val-Glu-Arg-Gly-Ile-Ile-Lys-Val-Gly-Glu-

280 290 300
Gly-Glu-Asn-Gly-Val-Leu-Leu-Arg-Glu-Gly-Ile-Glu-Glu-Glu-Gly-Val-Leu-Ala-Lys-Pro-Gly-Thr-Ile-Lys-Pro-

310 320 330
His-Thr-Lys-Phe-Glu-Ser-Glu-Tyr-Val-Ile-Leu-Ser-Lys-Asp-Glu-Gly-Arg-His-Thr-Pro-Phe-Lys-Gly-Thr-Arg-Pro-Gln-Phe-

340 350 360
Tyr-Phe-Agr-Thr-Agr-Thr-Val-Thr-Thr-Val-Thr-Val-Thr-Thr-Val-Thr-Thr-Val-Thr-Thr-Val-Thr-Thr-Thr-Thr-Thr-Thr-Thr-Thr-Thr-Thr-Thr-

370 380 390
Thr-Leu-Ile-His-Pro-Arg-Ala-Arg-Ala-Glu-Glu-Thr-Tyr-Val-Val-Val-Glu-Ala-Ala-Ala-Ala-Ala-Ala-

Val-Leu-Ser-OH (Gly-OH)

FIG. 7. Amino acid sequence of elongation factor Tu of E. coli showing the alignment of cyanogen bromide and tryptic peptides. The NH2 terminus is acetylated and Lys 56 is mono- and dimethylated. Two residues, glycine and serine, are found at the COOH terminus.

Position 56, in ratios that vary depending on the bacterial growth conditions (19). Since methylation can occur after inhibition of protein synthesis with chloramphenicol (25), the introduction of methyl groups must be a post-translational event. The function, if any, of methylation is not known, but one possibility, since Lys-56 seems to be near the AA-tRNA binding site (see below), is that it improves the binding of AA-tRNA or of specific AA-tRNAs, which Pingould and Urbanke (26) have shown to bind differentially to EF-Tu. Alternatively, methylation could modulate some other function for EF-Tu, for example, regulation of RNA polymerase activity, as proposed by Travers (27).

Location of Trypsin Cleavage Sites and Cysteine Residues—EF-Tu is rapidly cleaved by trypsin at Arg-44 and Arg-58 to yield a large fragment, A (58-393), and two smaller ones, E (1-44) and F (45-58), with a concomitant loss of AA-tRNA binding activity (28, 29). At longer reaction times a third cleavage occurs at Lys-263 to give fragments B (39-263) and C (264-393) (28, 29). Limited trypsinolysis seems to be essential for obtaining well ordered crystals of EF-Tu for x-ray analysis (30-32). These crystals apparently contain fragments A and E, which are tightly associated and can be separated only under denaturing conditions (29, 32).

EF-Tu from E. coli contains three cysteine residues, two of which can be modified with sulphydryl reagents under non-denaturing conditions. Alkylation of Cys-81 results in loss of AA-tRNA binding activity, while modification of Cys-137 inhibits the binding of GDP, GTP, and elongation factor Ts (16, 17, 34). The third cysteine, Cys-255, can be modified only under denaturing conditions, and is probably buried in the interior of the protein (17, 34). Although modification of cysteines affects AA-tRNA and nucleotide binding, it is not established that the sulfhydryl groups play an active role in binding, since inhibition of binding could be a result of steric interference by the modifying group. In fact, Wittinghofer and Leberman (33) have concluded from studies with EF-Tu from Bacillus stearothermophilus, which has only two cysteine residues, that the sulphydryl corresponding to Cys-137 is not required for GDP binding. Moreover, EF-Tu from Thermus thermophilus is reported (35) to contain no free sulphydryl
groups, yet it performs the same functions as the E. coli protein.

Sequence Homology with Other Proteins—In attempts to understand the function and evolution of EF-Tu, comparisons have been made with other proteins, notably actin, which has certain structural and functional similarities with EF-Tu, and is also selectively cleaved by trypsin at an Arg-Gly bond (36, 37). However, except for the common Arg-Gly-Ile sequence at the trypsin cleavage site (58-60 in EF-Tu; 62-64 in actin) and a Lys-Cys-Asp sequence (136-138 in EF-Tu; 283-285 in actin) no homologies appear to exist. To date, the only homology discovered (38) is that between EF-Tu and EF-G (39), whose NH2-terminal sequences are compared in Fig. 8. Within the first 140 amino acids the proteins show a sequence homology of 31%. As will be discussed below, they also appear to have similar secondary structures in this region. In a search for homologies, Jones et al. (12) discovered a number of proteins having segments with sequences identical to regions in EF-Tu. However, none of the sequence similarities were extensive enough to prove homology.

Structure of the Aminoacyl-tRNA Binding Site—Several of the amino acids which participate in AA-tRNA binding appear to be located in the NH2-terminal portion of EF-Tu. A number of investigators have noted that, when EF-Tu is cleaved selectively by trypsin at Arg-58 and/or Arg-44, AA-tRNA binding activity is lost, although nucleotide binding is not effected (28, 40, 41). Kaziro and coworkers (17) and Miller et al. (34) have shown that alkylation of Cys-81 abolishes AA-tRNA binding activity. Jurnak et al. (32) have reported that a mercury atom, which appears to be bound to Cys-81 in the structure, calculated by the method of Chou and Fasman (45, 46). Direct evidence for histidine in the AA-tRNA binding site is the observation3 that W-bromoacetyllysyl-tRNA alkylates solvent. Similar conclusions have been reached by Jonak and Rychlik (43) for E. coli EF-Tu, on the basis of photooxidation and chemical modification studies with diethyl pyrocarbonate.

Although it has not been unequivocally established that any of these amino acids actually participate in AA-tRNA binding, since the loss of binding activity by chemical modification could be caused by simple hindrance or by induced conformational changes, it does seem likely that at least some

3 D. L. Miller and A. Johnson, unpublished observations.
of these residues are in or near the binding site.

The effect of limited tryptic cleavage at Arg-44 and Arg-58, and the fact that Lys-56 is accessible to a methylating enzyme, suggest that the region Arg-44 to Arg-58 is particularly exposed in EF-Tu. A similar conclusion has been made by Jurask et al. (32), who have pointed out that mild proteolytic treatment has been a useful approach for obtaining crystals of several nucleic acid binding proteins. They further suggest that the success of this approach may reflect the presence of a conformationally flexible protrusion that is required for nucleic acid binding, but which is very susceptible to proteolysis.

Other clues as to the nature of the N-terminal region of EF-Tu can be obtained by comparison of the amino acid sequences of EF-Tu and EF-G, which as mentioned above, show strong sequence homology (Fig. 8). Two points regarding the similarities seem noteworthy. The first is that the identical residues are often clustered; for example, at residues 20–25, 59–65, and 80–84. The second is that the preponderance of conserved amino acids are polar (51%) or are helix breakers (Pro and Gly, 26%). Within the first 90 residues, only 3 of the 27 conserved amino acids are hydrophobic.

Using the method of Chou and Fasman (45, 46), we have attempted to predict the secondary structures of EF-Tu and EF-G in the first 95 residues. There seems also to be secondary structure homology in the region containing residues 95–140, but the calculated probabilities, $P_a$ and $P_b$, are similar and it is difficult to choose between them. The regular alternation of conserved, neutral residues in the segment 99–114 suggest a common structure in EF-Tu and EF-G, probably one that terminates in a turn at positions 110–114. There is probably also a common $\beta$-sheet structure at positions 129–134 (Table III). Although these predictions must be regarded as tentative, the chances of their being correct are increased by the fact that similar structures are predicted for both EF-Tu and EF-G.

As can be seen in Fig. 9, the predicted secondary structures for the first 90–100 residues in EF-Tu and EF-G are remarkably homologous. The dominant features are a hairpin $\beta$-sheet structure $(\beta_{1T} + \beta_{2T}, \beta_{1G} + \beta_{2G})$ connected by a helical region $(\alpha_{1T} + \alpha_{2T}, \alpha_{1G})$ to another $\beta$-sheet domain $(\beta_{3T}, \beta_{3G}, \beta_{5T}, \beta_{5G})$. The $\beta$-sheet strands are depicted as belonging to a larger antiparallel $\beta$-pleated sheet structure, though, of course, there could be many other arrangements. In EF-Tu, the major helical region $(\alpha_{1T} + \alpha_{2T})$ is predicted to be discontinuous because of the helix-breaking Pro-53, which is absent in EF-G.

In examining the location of the conserved amino acids, one finds that the clusters all appear to be located at turns $(T_{1T}, T_{1G}, T_{3T})$ and most of the other conserved residues appear in the $\beta$-sheet domains. It is interesting, and perhaps significant, that many of the conserved residues in the $\beta$-sheet regions alternate in such a way that they would be on the same side of an extended $\beta$-pleated sheet structure. Since most of those residues are polar, they may be instrumental in binding the structure to the rest of the protein molecule. For example, fragment E (residues 1–44) remains tightly bound to the rest of EF-Tu even after excision of fragment F (residues 45–58) by trypsin (29–32). Other evidence that fragment E has a discrete structure comes from sequence studies (1), where we found great difficulty cleaving Lys-74, which, as depicted in Fig. 9, occurs at the end of a turn and might be inaccessible to trypsin.

Fragment F, as shown in Fig. 9, is a helical structure bridging the two $\beta$-domains. Thus it could easily be a loop exposed to solvent and to the action of trypsin, which cleaves at Arg-44 and Arg-58. A similar structure must be present in EF-G, since trypsin cleaves selectively at Arg-58 in EF-G, too. An analogous situation is seen in the processing of prohormones, where cleavage of Arg and Lys bonds occurs preferentially near the ends of $\alpha$-helices or in unstructured regions (47). The fact that Lys-56, the site of methylation in EF-Tu (38), occurs in fragment F provides further evidence that this part of EF-Tu is particularly exposed.

**Fig. 9. Predicted secondary structures for EF-Tu and EF-G.** $\alpha$-Helical and $\beta$-sheet regions and turns are labeled $\alpha$, $\beta$, and $T$, respectively. Open circles represent amino acids which are conserved in both EF-Tu and EF-G. Fragments formed by limited trypsinolysis of EF-Tu are A (residues 59–338), E (1–44) and F (45–58). R44, K36, R58, H66, and C81 are amino acids which can be modified chemically or biochemically.
As mentioned above, chemical modification and trypsin cleavage studies indicate that Arg-58, Arg-44, Cys-81, His-66 and possibly other Arg and His residues are associated with AA-tRNA binding, either as participants or because they happen to be in the vicinity. Fig. 9 shows that it is possible to place these residues in relatively close proximity. Histidine seems like an attractive possibility as an amino acid involved in nucleic acid binding, since the protonated imidazole could help to bind the negatively charged AA-tRNA. Release of AA-tRNA might be accomplished by deprotonation, at neutral pH, of the imidazolium ion, possibly by a charge-relay system involving other basic groups in the protein (Fig. 10).

Eventually, x-ray crystallographic analysis will reveal the structural details of EF-Tu and, one hopes, the mechanism by which it binds AA-tRNA and other ligands. Low resolution (5–6 Å) data from three different laboratories (30–32) suggest that EF-Tu has an elongated globular shape with a large and a smaller domain separated by a cleft or "waist." Details of the mechanism of AA-tRNA binding may not be immediately forthcoming, however, because all of the crystallographic work has been done on trypsin-degraded protein which apparently lacks fragment F (residues 45–58). Thus it may be necessary to rely on secondary structure calculations such as described above, until suitable crystals can be obtained. Furthermore, since stable crystals of AA-tRNA-EF-Tu-GTP have yet to be obtained, indirect methods such as chemical modification and spectroscopic measurements will continue to be of importance in delineating the AA-tRNA binding site.

REFERENCES

Amino Acid Sequence of EF-Tu


47. Geisow, M. J. (1978) FEBS Lett. 87, 111-114

SUPPLEMENTAL MATERIAL

THE AMINO ACID SEQUENCE OF ELONGATION FACTOR Tu OF Escherichia coli: THE COMPLETE SEQUENCE
by Richard A. Larrson, James L. Stoffler, Sukanta Bandyopadhyaya and David L. Miller

EXPERIMENTAL PROCEDURES

Isolation of cytoplasmic RNA from E. coli was carried out in the presence of 3H or 14C amino acids as described in the preceding article (1). When the reaction was over, the sample was con
denser to about 1.5 ml with 4.5 ml of 0.1 M NaHCO3, and the precipitate was removed by centrifugation. The supernatant was brought up to 2 liters with water and recrystallized several times until most of the precipitate had been removed. After the precipitate had been so crystallized, the peptide mixture was reconstituted in 3H or 14C amino acids and the reaction was started by the addition of ATP. The reactions were stopped after 10 h. The reaction was precipitated with ethanol, and the precipitate was recovered by centrifugation, washed with ether, and recrystallized several times, and the resulting suspension was reconstituted as described in Figs. 3 and 4.

Isolation of tryptic peptides.

The reaction mixture was added to 5 ml of 0.5 M NaHCO3 on an ice bath. To the mixture was added 2 mg of TRI-free trypsin, the mixture was stirred for 5 h at 50°C. The reaction mixture was precipitated with ethanol. The precipitate was recovered by centrifugation, washed with ether, and recrystallized several times. The reaction mixture was precipitated with ethanol. The precipitate was recovered by centrifugation, washed with ether, and recrystallized several times. The resulting suspension was reconstituted as described in Figs. 3 and 4.

Isolation of the murine tropomyosin.

The reaction mixture was added to 5 ml of 0.5 M NaHCO3 on an ice bath. To the mixture was added 2 mg of TRI-free trypsin, the mixture was stirred for 5 h at 50°C. The reaction mixture was precipitated with ethanol. The precipitate was recovered by centrifugation, washed with ether, and recrystallized several times. The reaction mixture was precipitated with ethanol. The precipitate was recovered by centrifugation, washed with ether, and recrystallized several times. The resulting suspension was reconstituted as described in Figs. 3 and 4.

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**Amino Acid Sequence of EF-Tu**

**EF-Tu BrCN**

1. Suspend in 10 ml 0.01 M n-propanol.
2. Centrifuge at 20,000 rpm for 1 h.

**Supe.**

CB1, CB3, CB5-CB11 Precipitate CBZ.

1. Scrape into 25 ml acetic acid.
2. Centrifuge at 20,000 rpm for 1 h.

**Supernatant**

CB1, CB3, CB5-CB11 Precipitate

**Table 1**

<table>
<thead>
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<th>Peptide</th>
<th>Fraction No</th>
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<td>7-2</td>
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</tr>
<tr>
<td>2-1</td>
<td>2.1 (1)</td>
</tr>
<tr>
<td>1.8 (2)</td>
<td>2.4 (2)</td>
</tr>
<tr>
<td>0.3</td>
<td>0.6 (1)</td>
</tr>
<tr>
<td>1.5</td>
<td>1.0 (1)</td>
</tr>
<tr>
<td>1.3 (1)</td>
<td>1.0 (1)</td>
</tr>
<tr>
<td>0.8</td>
<td>1.0 (1)</td>
</tr>
<tr>
<td>79-112</td>
<td>0.9 (1)</td>
</tr>
<tr>
<td>1.5 (2)</td>
<td>1.3 (1)</td>
</tr>
<tr>
<td>1.6 (2)</td>
<td>1.0 (1)</td>
</tr>
<tr>
<td>0.8 (1)</td>
<td>1.0 (1)</td>
</tr>
<tr>
<td>353.359</td>
<td>1.0 (1)</td>
</tr>
</tbody>
</table>

**Figure 1**

Absorbance at 280nm (---) and 570nm (-----)

**Figure 2**

Counts/Min x 10^3 (--)
Amino Acid Sequence of EF-Tu

The amino acid sequence of EF-Tu was determined by applying peptide mapping and sequence analysis to isolated and purified EF-Tu. EF-Tu was purified by affinity chromatography on a Sepharose-6B column, and the purest sample was used for peptide mapping. Peptide mapping of EF-Tu was performed using a variety of methods, including Edman degradation, sequence analysis, and enzymatic digestion. The results of these analyses were used to deduce the amino acid sequence of EF-Tu.

The amino acid sequence of EF-Tu is as follows:

**Amino Acid Sequence of EF-Tu**

SELQGK (residues 1-6) + GLN (residues 7-12) + GLN (residues 13-18) + SER (residues 19-24) + GLN (residues 25-30) + GLN (residues 31-36) + SER (residues 37-42) + GLN (residues 43-48) + SER (residues 49-54) + GLN (residues 55-60) + GLN (residues 61-66)

**Fig. 1.** Amino Acid Sequence of EF-Tu. The amino acid sequence of EF-Tu was determined by peptide mapping and sequence analysis. The sequence is shown in detail in Table 1.

**Table 1.** Amino Acid Composition of EF-Tu

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Residue Count</th>
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<tr>
<td>Aspartic acid</td>
<td>13</td>
</tr>
<tr>
<td>Serine</td>
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</tr>
<tr>
<td>Glycine</td>
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</tr>
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<td>Leucine</td>
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</tr>
<tr>
<td>Phenylalanine</td>
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<td>Proline</td>
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<td>Lysine</td>
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<td>Histidine</td>
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</tr>
<tr>
<td>Cysteine</td>
<td>1</td>
</tr>
</tbody>
</table>

**Fig. 2.** Flow diagram for the purification of EF-Tu. The purification steps include affinity chromatography, hydrophobic interaction chromatography, and gel filtration. The final purified EF-Tu is shown in the lower right corner of the diagram.

**Fig. 3.** Chromatography of EF-Tu tryptic peptides on a column (2.5 x 30 cm) of Sepharose 6-55 with TFA (5:1, v/v). Fractions were collected, and the tryptic peptides were identified by amino acid analysis. The peptides are shown in Table 3.