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Primary structure of elongation factor Tu from *Escherichia coli*

(sequence heterogeneity/active sites/protein homology/posttranslational modification/prediction of secondary structure)

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ABSTRACT **The amino acid sequence of elongation factor Tu (EF-Tu) from *Escherichia coli* has been determined. EF-Tu is a single-chain polypeptide composed of 393 amino acids (M_r 43,225 for the species bearing COOH-terminal serine). The NH_2-terminal serine is acetylated, and lysine-56 is partially methylated. The sites of facile tryptic cleavage are at arginines 44 and 58 and at lysine-263. The cysteinyl residues associated with aminoacyl-tRNA and guanosine nucleotide binding activities are residues 81 and 137, respectively. The COOH-terminal amino acid is heterogeneous in that analyses of the COOH-terminal peptides isolated from different EF-Tu preparations gave position 393 as glycine and serine in ratios (Gly/Ser) ranging from about 0.7 to 3.**

Elongation Factor Tu (EF-Tu) promotes the binding of aminoacyl-tRNA (AA-tRNA) to ribosomes during protein biosynthesis in *Escherichia coli* (1–3). The protein consists of a single polypeptide chain of M_r 43,225 (for the species bearing COOH-terminal serine). Proteins of similar size and function are essential constituents of other prokaryotes and also of eukaryotes (4, 5). The protein is interesting not only because of this important biological function but also because of the number and diversity of the substances with which it interacts. In addition to AA-tRNA, the protein binds GDP, GTP, EF-Ts, the antibiotics kirromycin (6), Aurodox (X5108) (7), pulvomycin (8), puromycin (P. Grant and B. Cooperman, personal communication), and certain as-yet-undefined components of the ribosome. In addition to promoting peptide chain elongation, EF-Tu is a subunit of bacteriophage $Q\beta$ RNA polymerase (9).

During peptide chain elongation EF-Tu binds GTP before combining with AA-tRNA to form the ternary complex AA-tRNA·EF-Tu·GTP. The ternary complex will bind to ribosomes containing the appropriate codon in the A (AA-tRNA binding) site; then GTP is hydrolyzed, EF-Tu·GDP is released, and AA-tRNA is oriented on the ribosome so that peptide bond formation can occur. The role of GTP may be described as that of an allosteric effector, which alters the protein's tertiary structure to expose AA-tRNA binding sites (3, 10). Conversely, the binding of GDP may mask these sites, because the affinity constant of the EF-Tu·GDP for AA-tRNA differs from that of EF-Tu·GTP by a factor of about 10^5 (11). This cyclic opening and closing of the AA-tRNA binding sites dependent upon the state of phosphorylation may be regarded as a simple energy transduction system and is reminiscent of contractile proteins.

As the foundation for detailed studies of the structure and function of EF-Tu and its complexes, we have determined its primary structure. This report describes the sequence determined independently in three laboratories. Because the experimental approaches employed by our laboratories differed substantially, full details will be reported elsewhere.

MATERIALS AND METHODS

EF-Tu was purified as the GDP complex from *E. coli* B, *E. coli* Q13, or *E. coli* MRE 600, according to published procedures (12, 13). So far we have detected no differences in the sequences of EF-Tu isolated from these strains. Peptides were generated by cleavage with cyanogen bromide or limited trypsinolysis as described (14–17); large peptides were further fragmented with trypsin, *Staphylococcus aureus* protease, or chymotrypsin. Amino acid sequences were determined either by the manual dansyl-Edman procedure (18–23) or by automated solid-phase Edman degradation (24, 25). Phenylthiohydantoins were identified by thin-layer chromatography and high-performance liquid chromatography, or by back hydrolysis to amino acids.

RESULTS AND DISCUSSION

The amino acid sequence of EF-Tu is shown in Fig. 1. The amino acid composition calculated from the sequence and listed in Table 1 is in good agreement with the compositions previously published (26, 27), if corrections are made for the differing molecular weights. The protein contains two modifications: Lys-56 is methylated (28), and Ser-1 is acetylated.**

The amino acid composition of EF-Tu differs in few respects from the average for 108 protein families compiled by Dayhoff and Hunt (29). EF-Tu contains about 20% more charged residues and 30% more aliphatic hydrophobic residues than the average. The uncharged hydrophilic residues are correspondingly reduced, there being 51% fewer Asn + Gln and 61% fewer Ser than expected. The ratio of Thr to Ser is 2.7, whereas the average ratio is 0.85. These residues are generally interchangeable, and the Ser deficit is partially compensated for by a Thr excess. Acidic residues outnumber basic ones. Assuming half the His residues are protonated, the protein has a net negative charge at neutral pH of about −10, befitting a protein whose isoelectric point is 5.5.

The distribution of residues along the peptide chain (Fig. 2) shows some patterns possibly related to the protein's structure and function. The first 119 residues form a region that is highly hydrophilic and basic; 8 of the 11 His residues lie here. Histidine has been implicated in AA-tRNA and GDP binding (30); furthermore, this region contains not only the Cys residues whose

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Abbreviations: EF, elongation factor: AA-tRNA, aminoacyl-tRNA.

¶ To whom reprint requests should be addressed.

** M. D. Jones, T. E. Petersen, and K. M. Nielsen, unpublished data.

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

                                    40                                  50                  (Me)            60
Ile-Thr-Thr-Val-Leu-Ala-Lys-Thr-Tyr-Gly-Gly-Ala-Ala-Arg-Ala-Phe-Asp-Gln-Ile-Asp-Asn-Ala-Pro-Glu-Glu-Lys-Ala-Arg-Gly-Ile-

                                    70                                  80                                  90
Thr-Ile-Asn-Thr-Ser-His-Val-Glu-Tyr-Asp-Thr-Pro-Thr-Arg-His-Tyr-Ala-His-Val-Asp-Cys-Pro-Gly-His-Ala-Asp-Tyr-Val-Lys-Asn-

                                   100                                 110                                 120
Met-Ile-Thr-Gly-Ala-Ala-Gln-Met-Asp-Gly-Ala-Ile-Leu-Val-Val-Ala-Ala-Thr-Asp-Gly-Pro-Met-Pro-Gln-Thr-Arg-Glu-His-Ile-Leu-

                                   130                                 140                                 150
Leu-Gly-Arg-Gln-Val-Gly-Val-Pro-Tyr-Ile-Ile-Val-Phe-Leu-Asn-Lys-Cys-Asp-Met-Val-Asp-Asp-Glu-Glu-Leu-Leu-Glu-Leu-Val-Glu-

                                   160                                 170                                 180
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-Gly-

                                   190                                 200                                 210
Asp-Ala-Glu-Trp-Glu-Ala-Lys-Ile-Leu-Glu-Leu-Ala-Gly-Phe-Leu-Asp-Ser-Tyr-Ile-Pro-Glu-Pro-Glu-Arg-Ala-Ile-Asp-Lys-Pro-Phe-

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

                                   250                                 260                                 270
Glu-Val-Glu-Ile-Val-Gly-Ile-Lys-Glu-Thr-Gln-Lys-Ser-Thr-Cys-Thr-Gly-Val-Glu-Met-Phe-Arg-Lys-Leu-Leu-Asp-Glu-Gly-Arg-Ala-

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

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

                                   340                                 350                                 360
Tyr-Phe-Arg-Thr-Thr-Asp-Val-Thr-Gly-Thr-Ile-Glu-Leu-Pro-Glu-Gly-Val-Glu-Met-Val-Met-Pro-Gly-Asp-Asn-Ile-Lys-Met-Val-Val-

                                   370                                 380                                 390
Thr-Leu-Ile-His-Pro-Ile-Ala-Met-Asp-Asp-Gly-Leu-Arg-Phe-Ala-Ile-Arg-Glu-Gly-Gly-Arg-Thr-Val-Gly-Ala-Gly-Val-Val-Ala-Lys-

Val-Leu-Ser-OH-(Gly-OH)
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FIG. 1. Amino acid sequence of EF-Tu from *E. coli*. The COOH-terminal residue is heterogeneous, both Ser and Gly being found.

modification inhibits the interaction with AA-tRNA, guanosine nucleotides, and EF-Ts, but also the sites of facile tryptic cleavage that affect AA-tRNA binding.

The central region, residues 119–250, contains the highest densities of both aliphatic hydrophobic and acidic residues in the protein: 42% of the hydrophobic residues and 46% of the acidic residues are located here. Together these six amino acids account for 55% of the residues in this region. The ratio of acidic to basic residues is 2.0.

The COOH-terminal region, residues 251–393, like the NH_2-terminal region, is rich in basic residues but in contrast has a compensating number of acidic residues. The fraction of hydrophobic residues in this region is 10% lower than that of total protein.

Table 1. Amino acid composition of EF-Tu

Residue	Residues per molecule	
	From sequence	From analysis
Lys	23	21–25
Arg	23	24
His	11	10–11
Asp	24	34–36
Asn	7	
Glu	37	49–50
Gln	8	
Ser	11	11
Thr	30	30–31
Cys	3	3
Pro	20	19–20
Gly	40	40–43
Ala	27	28
Val	37	33–34.5
Leu	28	29–31
Ile	29	25–28
Met	10	7.4–9.1
Phe	14	14
Tyr	10	9
Trp	1	2–3

The sequence composition refers to the species bearing COOH-terminal Ser. The analyses for Asp or Glu include Asn or Gln. Analytical data from refs. 15 and 16 were corrected to a M_r of 43,225.

Sequence Heterogeneity. Because there are two genes for EF-Tu in the *E. coli* chromosome (31), the protein preparation whose sequence was determined may consist of two gene products differing in primary structure. It is not likely, however, that these gene products differ extensively in sequence. Previous studies showed that the two gene products differed only slightly in their activities and in their tryptic peptide maps (32, 33), and the DNA from the genes hybridized completely under stringent conditions (34). No sequence heterogeneity has yet been clearly identified except for the COOH-terminal residue 393, where variable amounts of glycine and serine have been found in different digests.

The COOH-terminal CNBr fragment from *E. coli* B was found to contain about 0.4 Ser residues, and from a tryptic digest of this fragment, two peptides, Val-Leu-Gly and Val-Leu-Ser, were isolated in a molar ratio of 4:1. In other preparations the ratio of Gly to Ser in mixtures of COOH-terminal tryptic peptides has varied from 0.7 to 2. Hydrazinolysis of intact EF-Tu preparations released both Gly and Ser in ratios of 2.3 and 2.9 (Gly/Ser). On the other hand, carboxypeptidase Y digestion of EF-Tu isolated from the MRE 600 strain released only Ser in significant quantities (17). Whether this heterogeneity results from the two gene products will only be known when their DNA sequences are determined.

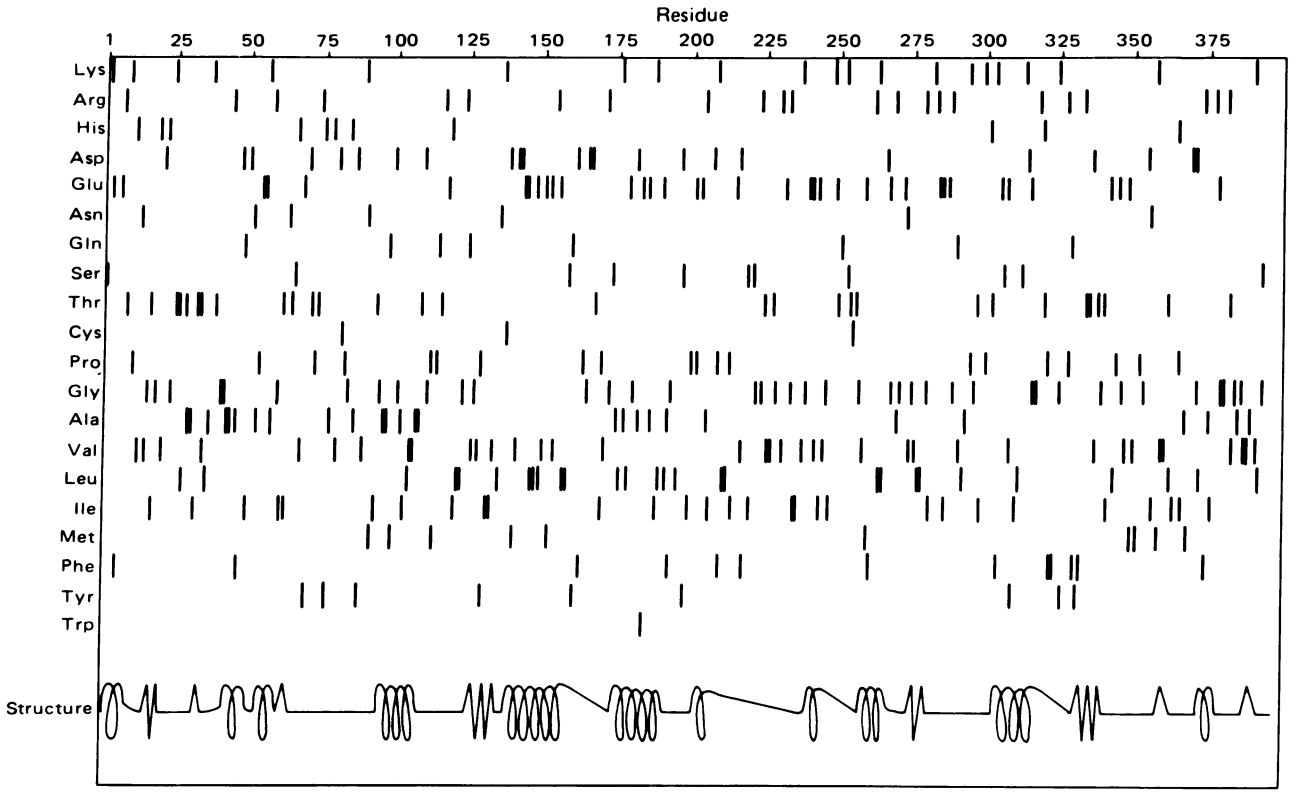

FIG. 2. Amino acid distribution in EF-Tu. Vertical bars indicate sequence position of each amino acid. Predicted regions of secondary structure are shown by helices (α helix) and zig-zags (β structure).

Active Sites and Trypsin Cleavage Sites. Residues that had previously been identified by chemical modification can now be located in the complete sequence. The Cys that has been implicated in the AA-tRNA binding function (35, 36) is located at position 81, and the Cys whose modification by *N*-ethylmaleimide inhibits the binding of GDP, GTP, and EF-Ts is at position 137. Cys-255 has not been modified in the native protein and is probably buried in the three-dimensional structure.

Trypsin rapidly cleaves EF-Tu at Arg-44 and Arg-58 to give a large fragment (A) and two small fragments (E and F) with a concomitant loss of AA-tRNA binding activity (16, 37). Intermediates (A′ and D) formed by cleavage at either residue have also been observed (38). In a longer incubation trypsin cleaves fragment A at Lys-263, forming fragments B and C (16, 17). It has been observed that brief trypsin treatment enhances the formation of well-ordered crystals suitable for x-ray diffraction (39–41). These crystals contain fragments A and D, or A and E, the latter crystals presumably lacking fragment F. The structures and sizes of these fragments are listed in Table 2.

Table 2. Sizes of tryptic fragments of EF-Tu

Fragment designation	Residues	M_r
A′	45–393	37,849
A	59–393	35,971
B	59–263	19,324
C	264–393	16,647
D	1–58	7,254
E	1–44	5,376
F	45–58	1,878

Fragments are produced by brief treatment of EF-Tu with trypsin. The nomenclature for fragments A–D is that of Nakamura *et al.* (17).

At present, no direct chemical information about the guanosine nucleotide binding site is available. An analysis of the electron density maps obtained by x-ray crystallography reveals a GDP binding site associated with a region of the molecule containing a high proportion of secondary structure (41). Currently, a chain tracing based on the sequence in this report is being pursued, but it is not yet clear which amino acid residues are involved in GDP binding (T. LaCour, J. Nyborg, J. Rubin, and B. F. C. Clark, unpublished).

Posttranslational Modifications. One of the first observations on the primary structure of EF-Tu was that the NH_2-terminal residue was blocked. This terminal residue has been identified as N-acetylserine.** The sequence of the *tufA* DNA coding for this region of the protein reveals that the serine codon (UCU) is preceded by GUG (T. Yokota, H. Sugisaki, M. Takanami, and Y. Kaziro, unpublished data); thus translation initiates with formylmethionine, which then is cleaved and the resulting NH_2-terminal serine is acetylated.

Ames and Nikaido (42) found that EF-Tu is one of a very few prominent proteins in *E. coli* cytosol methylated by methionine. Both monomethyl- and dimethyllysine were formed. It has been found that essentially all of the methylation occurs at Lys-56 (28). In the protein preparations used for sequence determination, 43% of Lys-56 is monomethylated and 7% is dimethylated. This region of the protein is probably exposed because here both trypsin cleavage and methylation occur preferentially.

Structural Relationships Between EF-Tu and Other Proteins. In an attempt to understand the function and evolution of EF-Tu, comparisons have been made to other proteins, notably actin (43). Actin is also selectively cleaved by trypsin at an Arg-Gly bond (44). Nevertheless, aside from 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 have been detected.

Table 3. Predicted regions of secondary structure

α helix			β sheet		
Residues	P_α	P_β	Residues	P_α	P_β
2–9	1.18	0.81	14–20	0.88	1.22
42–50	1.17	0.95	31–35	1.00	1.39
52–57	1.26	0.61	60–64	0.90	1.29
95–107*	1.20	1.08	102–108*	1.15	1.31
138–157	1.26	0.92	124–134	0.96	1.35
174–192	1.26	0.87	274–281	0.97	1.26
203–208	1.19	0.84	329–341	0.99	1.20
240–245	1.29	1.02	358–363	1.11	1.40
258–267	1.22	0.97	387–392*	1.16	1.33
303–315	1.12	0.93			
372–378	1.19	1.02			

* Region of similar α and β probabilities.

A much stronger homology has been discovered between EF-Tu and EF-G (45), proteins which in addition to having related functional roles are located adjacent to one another in the *E. coli* chromosome (46). Like EF-Tu, EF-G contains a site of preferential trypsin cleavage (47). If the major fragments are aligned at their cleavage sites, seven out of the first eight amino acids are identical; for the first 60 amino acids, the degree of identity is 33%. A thorough assessment of the homology must await the determination of the remaining 80% of the EF-G sequence. The functional resemblances among initiation factor 2, EF-Tu, EF-G, and release factor suggest that they may have descended from a common ancestor or have acquired some sequence similarity through convergent evolution. Other likely relatives of EF-Tu are the eukaryotic, mitochondrial, and chloroplast elongation factors.

Confirmation by DNA Nucleotide Sequence Determination. Much of the protein sequence has now been confirmed by determining the sequence of the DNA of the *Sma* I restriction fragment containing the *tufA* gene, which lies adjacent to the gene for EF-G in the *E. coli* chromosome (46). The DNA sequence, to be reported elsewhere (T. Yokota, H. Sugisaki, M. Takanami, and Y. Kaziro, unpublished data), confirms the protein sequence between residues 1–18 and 28–393.†† The agreement between the protein sequence and the *tufA* DNA sequence reinforces our conclusion that the protein preparation has a homogenous sequence despite the possible presence of two gene products.

Secondary Structure Predictions. Extending and updating an earlier report (15), we estimated the regions of α helix and β sheet according to the method of Chou and Fasman (Table 3) (48). The average conformation parameters (P_α and P_β) were calculated by using the revised single-residue parameters based upon 29 proteins (49). EF-Tu was calculated to contain 30% α helix, in good agreement with the value of 24–32% obtained by circular dichroism measurements (2, 50). The β sheet content was calculated to be 16%. The α and β structures are not evenly distributed throughout the protein. The central region of the molecule, trypsin fragment B, is composed of 34% α helix and 8% β sheet, whereas the COOH-terminal third of the molecule is 15% α helix and 25% β sheet. The relationship of the secondary structure to the protein's function is not yet apparent. The region 44–57, which is thought to be involved in AA-tRNA binding, contains two helical segments; however, because the binding of GDP or GTP does not alter the circular dichroism of the protein, neither nucleotide is likely to induce a major change in helix content.

†† Since submission of this manuscript we have found that the codon for the COOH-terminal amino acid is GGC (glycine).

In an overall view of the protein based upon its primary structure and the predictions of its secondary structure, EF-Tu is composed of a basic, hydrophilic, NH_2-terminal region composing one-third of the molecule and containing all of the residues thus far implicated in its function. The central region of the molecule is rich in acidic and hydrophobic residues arranged in α helices. This region might interact with basic ribosomal proteins. The COOH-terminal region has no known function but may well contain part of the AA-tRNA and GDP binding sites. With the aid of the primary structure, many of the gaps in our understanding of the function of EF-Tu can now be filled by using x-ray crystallography and chemical modification.

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