AMINO ACID SEQUENCE OF ELONGATION FACTOR Tu. CHARACTERIZATION AND ALIGNMENT OF THE CYANOGEN BROMIDE FRAGMENTS AND LOCATION OF THE CYSTEINE RESIDUES

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1. Introduction

Elongation factor Tu (EF-Tu) of *E. coli* possesses a remarkable variety of functions and properties. In addition to having a role in protein biosynthesis [1], EF-Tu is a component of Qβ RNA replicase [2] and a regulator of RNA polymerase activity [3], and is coded for by two genes [4]. EF-Tu is also reported to be associated with membranes [5] and to have actin-like properties [6]. In conjunction with X-ray crystallographic studies currently in progress [7,8], we have undertaken the amino acid sequence analysis of EF-Tu. In this report we describe the sequence of nearly half the molecule, and discuss some tentative conclusions that can be made about its structure.

2. Materials and methods

EF-Tu was purified from *E. coli* B as described by Miller and Weissbach [9].

A 400-mg sample of [^{14}C]carboxymethylated EF-Tu was cleaved with 1.1 g of CNBr in 70% HCOOH for 22 h. The solvent was evaporated, and the mixture was suspended in 25% HOAc and centrifuged to remove undissolved material (mostly CB-3 and CB-7; see fig.1); the supernatant was then chromatographed on Sephadex G-50 in 25% HOAc. Five fractions were obtained. Peptides CB-2 and CB-4 were isolated as precipitates by adjusting the pH of fraction 1 to pH 6 and 3 to pH 7.8, respectively. The remaining peptides were separated by chromatography on DEAE Biogel and QAE Sephadex using NH_4HCO_3 buffers.

Overlapping peptides were obtained by digestion of [^{14}C]carboxymethylated EF-Tu with 2% (w/w) of trypsin in 0.1 M NH_4HCO_3, pH 7.8. Peptides were separated on Sephadex G-25, DEAE Biogel and QAE Sephadex in NH_4HCO_3 buffers. Cysteine peptides were located by radioactivity and methionine peptides by spraying thin-layer chromatograms of fractions with $PdCl_2$ [10].

Cleavage of unmodified EF-Tu by 2-[^{14}C]nitro-5-thiocyanatobenzoic acid (NTCB) in 6 M guanidine HCl was performed according to Jacobson et al. [11]. The NTCB peptides were separated by chromatography on Sephadex G-75 in 10% HOAc and on DEAE Biogel. Purity and size were assessed by SDS-urea gel electrophoresis [12].

Peptides were analyzed on a Beckman Model 119C amino acid analyzer and were sequenced by solid-phase Edman degradation [13,14] on a Sequemat Model 12 sequencer or manually [15]. Phenylthiohydantoins were identified by thin-layer chromatography and by HI hydrolysis to amino acids.

3. Results

Cyanogen bromide cleavage produced eleven peptides ranging in length from 2 to about 110 residues. The eight smaller peptides, except for CB-4 and CB-5 (see fig.1 and 2), which had been reported [16] earlier, were sequenced entirely. The C-terminal regions of the three large peptides, CB-1, CB-2 and CB-3, were sequenced by solid-phase Edman degradation after tryptic digestion and selective attachment [17,18] of the C-terminal homoserine moiety to the sequencing resin. The N-terminal regions of these peptides, except for CB-1 which is blocked, were obtained by manual Edman degradation.

Alignment of the cyanogen bromide peptides was accomplished by isolation and characterization of the overlapping tryptic peptides containing methionine (fig.1). It is a pecularity of EF-Tu that all of the sulfur-containing amino acids (3 Cys and 10 Met) are located in only six tryptic peptides. Additional confirmation of the sequence was obtained by cleaving the protein at cysteine with 2-nitro-5-thiocyanatobenzoic acid and isolating three of the theoretical four NTCB fragments. The cleavage yields at Cys-1, -2, and -3 (numbering from the N-terminus) were calculated to be approximately 18%, 25% and 80%, respectively. Further fragmentation of the NTCB peptides by cyanogen bromide, followed by peptide mapping, gave the peptides expected from the proposed sequence (figs.1 and 2).

4. Discussion

The sequence shown in fig.2 accounts for 180 of the approximately 400 amino acid residues in EF-Tu. EF-Tu contains three cysteines, one of which (Cys-1, fig.1) is associated with tRNA binding and a second (Cys-2) with GTP binding; the third cysteine has no known function [19,20]. The sequence of the tryptic peptide containing Cys-2 (T2) was reported earlier by Wade et al. [16] and those containing Cys-1 (T6) and Cys-3 (T1) by Nakamura et al. [21]. Our sequence data for T1 and T6 agree with those of Nakamura et al. [21], except that we find methionine in T1, whereas the other investigators did not. There can be no doubt that methionine is present, since T1 can be cleaved with CNBr.

The present work shows that the cysteine at the tRNA site (Cys-1) is about 100 residues from the N-terminus and that at the GTP site (Cys-2) is at about position 156. Arai et al. [22] and Gast et al. [7] have shown that limited trypsin digestion of EF-Tu removes a fragment of 6–10 000 daltons from the N-terminus, and that such treatment abolishes tRNA, but not nucleotide, binding. Thus the trypsin cleavage point must be at least 10 residues ahead of Cys-1 in the polypeptide chain. Either portions of the tRNA binding site reside in the N-terminal tryptic fragment or loss of the fragment disrupts the conformation of the binding site at Cys-1.

Using the parameters of Chou and Fasman [23],

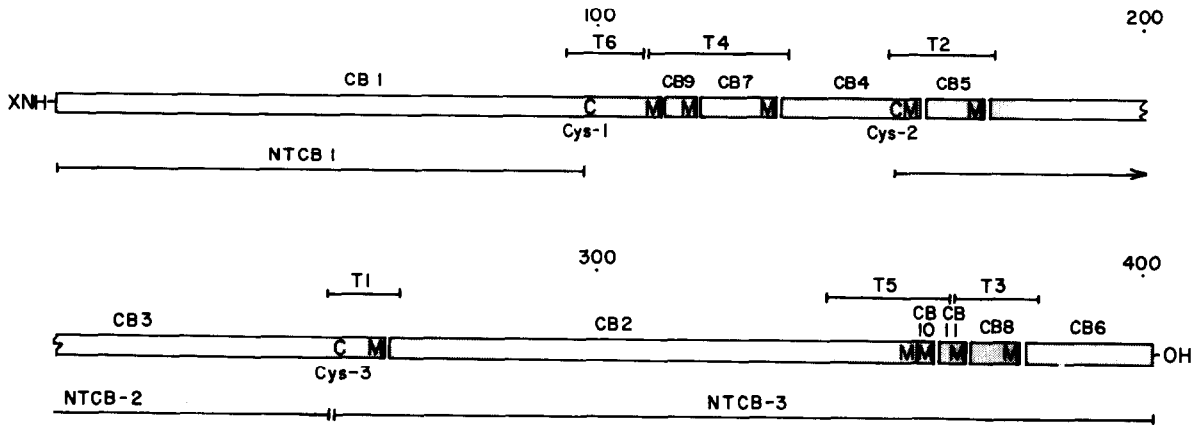

Fig.1. Alignment of cyanogen bromide peptides of EF-Tu with overlapping tryptic and NTCB peptides. Cys-1 and Cys-2 are cysteines at the tRNA and GTP binding sites, respectively. The shaded areas indicate regions that have been sequenced.

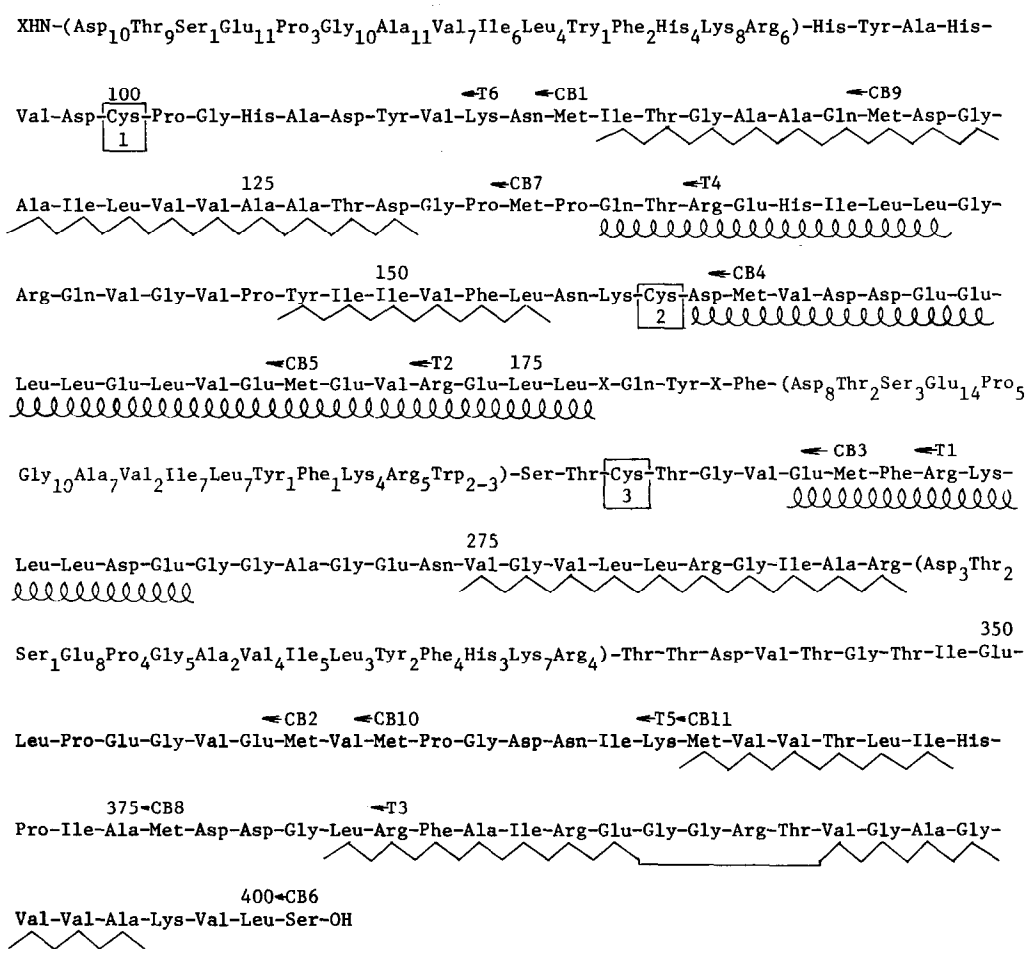

Fig.2. Partial amino acid sequence of EF-Tu. Tryptic and cyanogen bromide peptides are indicated by the peptide number over the C-terminal amino acid of each peptide. An overlapping chymotryptic peptide, Val-Lys-Asn-Hse, from CB1 established the position of T6. Coiled lines designate predicted α-helical regions and zigzag lines β-sheet; the two C-terminal β-regions appear to be linked by β-turn.

we have calculated that the sequenced portion of EF-Tu contains about 20% α-helix and 35% β-sheet. The predicted conformations are indicated schematically in fig.2. Particularly noteworthy are the β-structure and β-turn near the C-terminus and an extended α-helical stretch following Cys-2. This region comprises most of peptide T2, which was found earlier [15] to crystallize spontaneously at about pH 4.

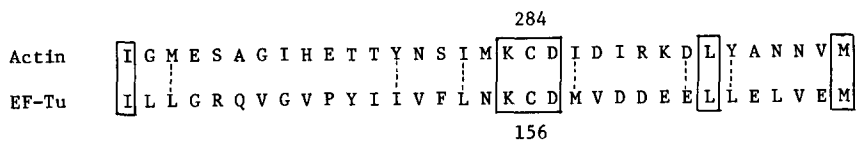

Fig.3. Possibly analogous sequences in EF-Tu and rabbit muscle actin. No other similarities have yet been detected. Dashed lines indicate residues of similar type (acidic or hydrophobic).

Beck et al. [6] have noted that EF-Tu and the contractile protein, actin, have a number of similarities in terms of size, amino acid composition and a variety of reactions and functions, and have suggested that the two proteins may be evolutionarily related. We have compared our sequence data with that for rabbit muscle actin and find that there is indeed some similarity in the sequence around Cys-156 (Cys-2) of EF-Tu and Cys-284 [24] of actin (fig.3). However, the similarities do not extent beyond this region. Furthermore the two cysteine residues occupy quite different positions in their respective polypeptide chains, which are of similar lengths. We conclude therefore that the proteins are not homologous; the observed similarities may be characteristic of a nucleotide binding site (for GTP or ATP) which arose by convergent evoluation.

Escherichia coli possesses two genes that code for EF-Tu [4], which suggests the possibility that there may be two forms of the protein. Thus far, however, we have found no evidence of allotypes. In addition, peptide mapping studies in our laboratory and in Furano's [25] on the isolated *tufA* and *tufB* gene products have shown that the two proteins are very similar, if not identical.

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