# THE PRIMARY STRUCTURE OF ELONGATION FACTOR G FROM *ESCHERICHIA COLI*

## Amino acid sequence of the region containing the GTP-binding center

Yu. B. ALAKHOV, L. P. MOTUZ, O. A. STENGREVICS and Yu. A. OVCHINNIKOV
*Institute of Protein Research, Academy of Sciences of the USSR, 142292 Poustchino, Moscow Region, USSR*

Received 18 November 1977

### 1. Introduction

The protein biosynthesis elongation factor EF-G has an exposed SH-group, the modification of which results in the inhibition of the uncoupled GTPase reaction [1,2]. This SH-group seems to be contained in the GTP-binding region of EF-G since it was shown in our laboratory that the region including the exposed SH-group is labelled by the photoactivated GTP analogs with a high specificity [3]. Moreover it is known that guanyl nucleotides protect this group from thiol reagents [4].

EF-G incubation with trypsin results in the formation of several fragments comparatively stable to further trypsin treatment [5]. A detailed study of the fragments formed by limited trypsinolysis of EF-G modified by [$^{14}$C]-labeled $ICH_2CONH_2$ at the exposed SH-group has shown that this SH-group is located in the N-terminal region of EF-G [6].

In this paper we present the amino acid sequence of the fragment containing the exposed SH-group and apparently participating in the formation of the GTP-binding center. The fragment consists of 69 amino acid residues, the only cysteine residue being in position 55.

### 2. Materials and methods

EF-G and the fragment containing the SH-group essential for the uncoupled GTPase reaction were isolated by the method described [6]. The protein and fragment homogeneities were checked by SDS–electrophoresis in polyacrylamide gel and by determining N-terminal amino acid sequences by automatic Edman degradation. 1.5 μmol of the fragment modified by $^{14}$C-labeled $ICH_2CONH_2$ was cleaved by trypsin (TPCK-treated, Worthington) and the hydrolysate was acidified to pH 3. The soluble part of the hydrolysate was separated by high-voltage paper electrophoresis (4500 V, pH 3.5) and the insoluble part was separated by chromatography on a QAE-Sephadex A-25 column in 0.01 M Tris–HCl buffer, pH 8.3, 6 M urea using a KCl gradient (0.01–0.4 M). The peptides obtained after cyanogen bromide cleavage of 0.5 μmol fragment were also separated on a QAE-Sephadex A-25 column under analogous conditions and desalted on a Sephadex G-15 column equilibrated with ammonia water, pH 9. The cyanogen bromide peptide CB-2 containing 28 residues was subjected to a limited acid hydrolysis and held in 0.01 N HCl for 48 h at room temperature. The hydrolysates were separated by high-voltage paper electrophoresis (4500 V, pH 3.5). The tryptic C-terminal 24-membered peptide was digested by thermolysin and staphylococcal protease (Miles Lab). The digests were separated by peptide mapping on cellulose thin-layer plates (Merck). The first dimension was electrophoresis for 1 h, 800 V, in the solvent HCOOH–$CH_3COOH$–$H_2O$ (20:80:900), pH 1.9; the second dimension was chromatography in the butanol–pyridine acetate–$H_2O$ system (1:1:1), pH 5.4. The peptides were localized with a 0.001% fluorescamine solution in acetone and eluted by 50% aqueous pyridine.

The C-terminal amino acid residues of the fragment and those of the peptides T-5 and CB-4 were determined using carboxypeptidases A and B [7].

The amino acid composition was assessed on the D-500 analyzer (Durrum). Cysteine was estimated in the form of carboxymethylcysteine, and the amount of valine and isoleucine by the results of a 72 h hydrolysis. The tryptophan content was measured after hydrolysis of the samples with 4 N methane–sulfonic acid containing 0.2% 3-(2-aminoethyl) indole (Pierce).

Automatic determination of the amino acid sequence was done on a sequencer (model 890 C, Beckman) using the dimethylallylamine program (102974 according to the company handbook). PTH-derevative amino acids were identified on a gas chromatographer (model 5710, Hewlett-Packard), on a liquid high pressure chromatographer (model 8500, Varian) with a LiChrosorb Si 60 column, by thin-layer chromatography and on an amino acid analyzer after back hydrolysis of PTH-derivatives by 5.6 N HCl.

The amino acid sequence of the isolated peptides was determined by the dansyl-Edman method [8]. Amino acid dansyl derivatives were identified by thin-layer chromatography on silica gel plates. Asparagine and glutamine were determined according to [9].

## 3. Results and discussion

According to the data of SDS–electrophoresis in polyacrylamide gel the fragment containing the exposed SH-group has approx. wt 8000 and the following amino acid composition (in mol/%): Cys-1.63; Asp-5.86; Thr-8.74; Ser-4.66; Glu-10.62; Pro-4.50; Gly-9.14; Ala-8.73; Val-11.84; Met-4.38; Ile-8.12; Leu-2.00; Tyr-3.32; Phe-3.12; His-3.68; Lys-1.66; Trp-1.76; Arg-6.24.

We have found that the N-terminal residue of the fragment is glycine and that the C-terminal sequence is –Val–Trp–Arg.

Four fragments were isolated after cyanogen bromide cleavage of the molecule. The structure of the N-terminal fragment CB-1 and that of the fragment CB-3 were completely determined by the dansyl-Edman method. The structure of fragments CB-2 and CB-4 was determined up to position 12 and 14, respectively. The fragment CB-2 was subjected to a limited acid hydrolysis with diluted HCl to determine its complete amino acid sequence. Four peptides composing the complete amino acid sequence of this fragment were isolated and their structure was determined by the dansyl-Edman method.

To obtain overlapping peptides a fragment molecule was digested by trypsin and 5 peptides were isolated. The structure of peptides T-1–T-4 was completely determined by the dansyl-Edman method and that of peptide T-5 up to position 12. The result was the overlapping of all cyanogen bromide fragments including the peptides of fragment CB-2 limited acid hydrolysis. Peptide T-5 was submitted to a digestion with thermolysine and staphylococcal protease to determine the structure of its C-terminal region. The resulting peptides composed the amino acid sequence of T-5.

Determination of the amino acid sequence on a whole molecule by automatic Edman degradation corroborated the results obtained up to the 40th amino acid residue inclusively.

Thus structure studies of peptides isolated after digestion of the molecule or its fragments by cyanogen bromide, trypsin, thermolysine, staphylococcal protease and limited acid hydrolysis gave the amino acid sequence of the G-factor fragment containing the GTP-binding site (see scheme 1).

The precise molecular weight of this EF-G polypeptide chain region is 7511.64 and has the following amino acid composition: $Cys_1$, $Asp_3$, $Asn_1$, $Thr_7$, $Ser_4$, $Glu_4$, $Gln_3$, $Pro_3$, $Gly_6$, $Ala_6$, $Val_8$, $Met_3$, $Ile_6$, $Leu_1$, $Tyr_2$, $Phe_2$, $His_2$, $Lys_1$, $Trp_2$, $Arg_4$.

It is interesting to note that there is no homology in the amino acid sequence of the GTP-binding site of EF-Tu [10] and that of EF-G.

## References

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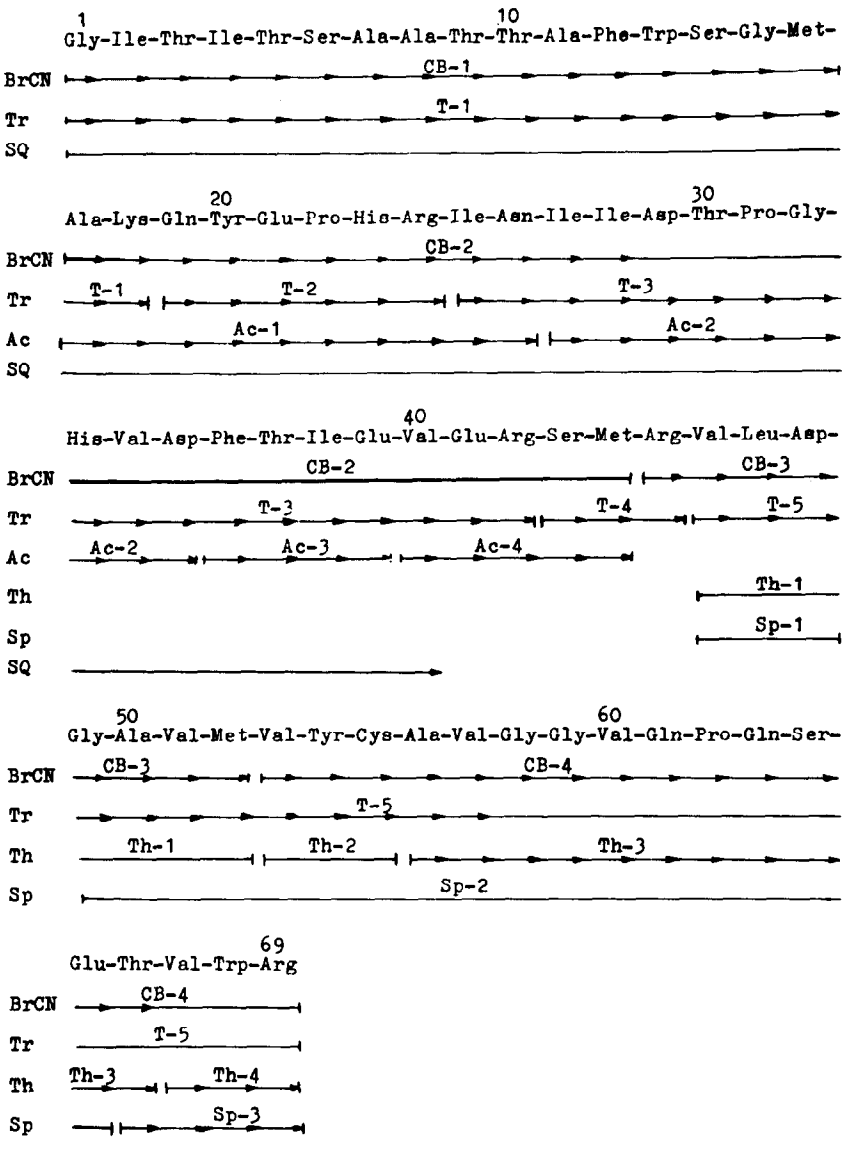


BrCN: peptides resulting from cleavage by cyanogen bromide;
Tr: peptides resulting from digestion with trypsin;
Ac: peptides resulting from limited acid hydrolysis of fragment CB-2;
Th: peptides resulting from digestion with thermolysine of fragment T-5;
Sp: peptides resulting from digestion of fragment T-5 with staphylococcal protease;
SQ - automatic Edman degradation;
→→ - manual Edman degradation;
←← - carboxypeptidase A+B digestion.

Scheme 1

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