AMINO ACID SEQUENCE OF ELONGATION FACTOR Tu. SEQUENCE OF A REGION CONTAINING THE THIOL GROUP ESSENTIAL FOR GTP BINDING

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1. Introduction

Elongation factor Tu (EF-Tu) of *E. coli* contains three thiol groups, each of which can be labelled differentially with *N*-ethyl-maleimide (NEM). One thiol appears to be essential for binding aminoacyl t-RNA, while a second is necessary for GDP and GTP binding [1,2]. The third group reacts only when the protein is denatured, and has no defined function at present [2]. In order to further characterize the thiol groups of EF-Tu, we have determined the amino acid sequence of a 42-residue segment containing the cysteine essential for GTP binding. In the course of this work we have also encountered an unusual reaction of NEM-labelled cysteine.

2. Materials and methods

Tu-GDP was purified from *E. coli* as previously described [3]. The thiol essential for AA-t-RNA binding was blocked by treating 180 mg of Tu-GDP with 5×10^{-4} M [^{14}C]NEM for 2 hr at 0°C. Excess NEM and GDP were removed by dialysis against 4 litre of 10^{-3} M EDTA in pH 8.1 Tris–HCl buffer. The thiol essential for GTP binding was then labelled by treatment with 5×10^{-4} M [^{3}H]NEM at 20°C for 4 hr, followed by dialysis.

The NEM-labelled protein was digested with 10 mg of trypsin, and the ^{3}H-labelled peptide, T-I, containing the GDP binding thiol was isolated by gel filtration on Sephadex G-25, precipitation at pH 4 and chromatography on columns of DEAE and CM cellulose.

Tu was carboxymethylated in 6 M guanidine HCl with [^{14}C]iodoacetate according to Hirs [4].

A 100-mg sample of [^{14}C]carboxymethylated Tu was cleaved with 335 mg of BrCN in 70% HCOOH for 22 hr. The digest was fractionated on

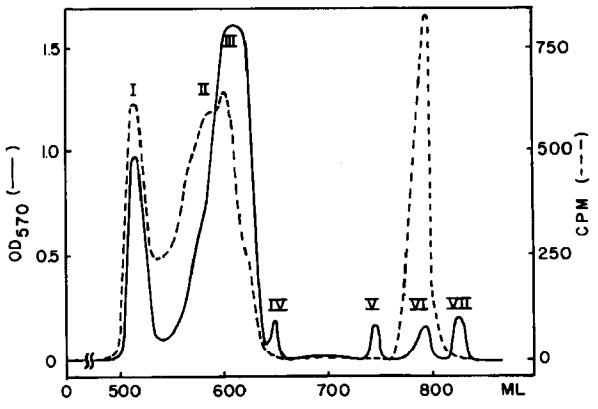


Fig.1. Fractionation of cyanogen bromide digest of [^{14}C]-carboxymethylated Tu on Sephadex G-50. Absorbance at 570 nm was measured after alkaline hydrolysis and reaction with ninhydrin reagent. See text for details.

two 2.5 X 200 cm columns of Sephadex G-50 linked in series and equilibrated with 25% acetic acid. The fraction designated CB-VI (fig.1) was lyophilized and treated with 0.1 M NH_4HCO_3, which dissolved some contaminating soluble material, but not the radioactive peptide CB-VI. A sample of CB-VI was fragmented with chymotrypsin, and a radioactive peptide, CB-VIA, was isolated by chromatography on DEAE cellulose.

Amino acid analyses were carried out on a BioCal Model 200 amino acid analyzer after hydrolysis at 110°C in 6 M HCl for 20 hr. Partial acid hydrolysis was performed in evacuated tubes for 4 or 20 hr at 110°C in 0.03 M HCl, 0.001 M in 2-mercaptoethanol.

Peptides were sequenced by solid-phase Edman degradation [5] after attachment to sequencing resin by the homoserine lactone method [6]. Phenylthiohydantoins were identified by thin-layer chromatography [5] of by HI hydrolysis [7].

3. Results and discussion

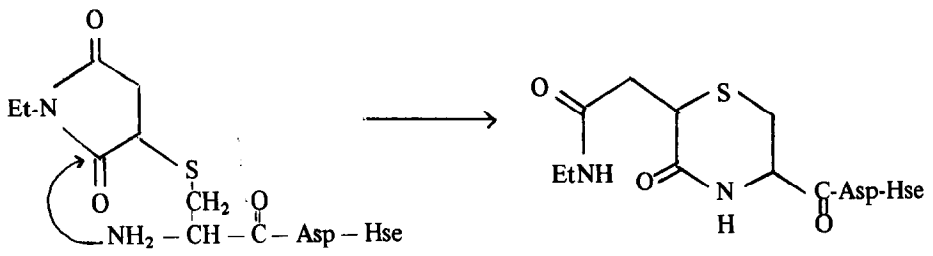
Amino acid analyses of the cyanogen bromide and tryptic peptides are listed in table 1. No N-terminal amino acid could be detected for peptide T-I by Edman degradation, dansylation of aminopeptidase M digestion. This suggested that T-I might be the N-terminal peptide of Tu, since the N-terminal of Tu is blocked (unpublished), or that cyclization to pyroglutamate had occurred.

Peptides T-IA, T-IB and T-IC were obtained by cyanogen bromide fragmentation of T-I. T-IB and T-IC were sequenced directly by solid-phase Edman degradation; T-IA, however, was blocked, indicating that it was the N-terminal peptide of T-I. Limited acid hydrolysis [8] of T-IA for 4 hr gave one residue each of homoserine and aspartic acid upon amino acid analysis. After 20 hr, approx. one residue each of homoserine, aspartic acid and succinyl cysteine

Table 1
Amino acid compositions of peptides

	Peptide					
	T-I	T-IA	T-IB	T-IC	CB-VI	CB-VIA
CM–Cys[a]					(1)	(1)
Succ–Cys[a]	(1)	(1)				
Asp	3.0 (3)	(1)	1.9 (2)		2.1 (2)	1.8 (2)
Thr					0.7 (1)	
Ser						
Glu	4.9 (5)		4.1 (4)	1.0 (1)	3.1 (3)	
Hse[a]		(1)	(1)		(1)	0.8 (1)
Pro					2.2 (2)	
Gly					2.1 (2)	
Ala						
Val	2.7 (3)		2.0 (2)	1.0 (1)	2.6 (3)	
Met	1.8 (2)					
Ile					2.5 (3)	
Leu	2.7 (3)		3.0 (3)		3.0 (3)	1.0 (1)
Tyr					0.8 (1)	
Phe					1.1 (1)	
Lys					1.0 (1)	1.0 (1)
His					1.0 (1)	
Arg	1.1 (1)			1.2 (1)	2.1 (2)	
Total	18	3	12	3	27	6

[a]The yields of homoserine and the cysteine derivatives were variable due to decomposition. Since they occur only once in a peptide, these residues are indicated by the integral value (in parentheses).



I II

was obtained. These results, confirmed as described below, suggest the sequence, X–Cys–Asp–Hse for T-IA. Blockage of the N-terminal could arise by the reaction, I → II.

The initial appearance of only aspartic acid and homoserine on limited acid hydrolysis can be explained by first cleavage at aspartic acid bonds [8] and subsequent hydrolysis of the cyclic cysteine derivate (II) to succinyl cysteine at longer times.

Peptide T-I, which has an unusual distribution of acidic and hydrophobic residues, evidently possesses considerable secondary structure, since it readily forms crystals (approx. 1 mm long) at pH 4–5.

Fig.1 shows the elution pattern of the cyanogen bromide fragments of carboxymethylated Tu on Sephadex G-50. The amino acid composition of CB-VI is listed in table 1. This peptide was sequenced in its entirety by solid-phase Edman degradation [5,6], although there were some ambiguities near the C-terminus. These were resolved by sequencing the C-terminal chymotryptic peptide CB-VIA (fig.2).

As can be seen in fig.2, the peptides CB-VI and T-I overlap, thus T-I cannot be the N-terminal peptide of Tu, nor can CB-VI, since its N-terminal is free while that of Tu is blocked. These two peptides determine a sequence of 42 residues including the thiol essential of GDP or GTP binding. Knowledge of the sequence of this region should prove valuable in interpreting the results of X-ray diffraction studies of Tu, and for comparing Tu with other proteins for homology. Work on determining the entire sequence and identifying the thiol essential for AA-t-RNA binding is in progress.

Acknowledgements

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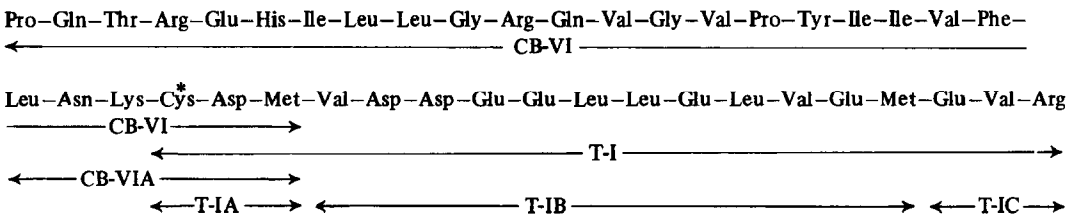

Fig.2. Amino acid sequence of the region containing the thiol essential for GDP binding.