

Sequence homology between EF-1 α , the α -chain of elongation factor 1 from *Artemia salina* and elongation factor EF-Tu from *Escherichia coli*

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In the course of a structural analysis of the α -chain of elongation factor 1 from *Artemia salina* cysts, we present four amino acid sequences comprising together half of the polypeptide chain. A comparison of these sequences with the primary structure of elongation factor EF-Tu from *Escherichia coli* reveals a clear correspondence between the eukaryotic and prokaryotic protein throughout their polypeptide chains. The results support a basic conservation of the structure of the aminoacyl-tRNA carrying enzyme in evolution.

The occurrence, in the eukaryotic factor, of several ϵ -trimethyllysine residues, is remarkable.

Eukaryotic elongation factor *Primary structure* *Sequence homology* *Protein evolution*

1. INTRODUCTION

The elongation factor which binds aminoacyl-tRNA to the ribosome plays a crucial role in protein biosynthesis [1]. The bacterial factor EF-Tu, as found in *Escherichia coli*, has a M_r -value of 43 000 and consists of a single polypeptide chain with a length of 393 amino acid residues. Its primary structure has been elucidated [2–4], and studies concerning its three-dimensional structure are in progress [5–7].

The eukaryotic elongation factor EF-1 occurs in a high- M_r and low- M_r form. In the brine shrimp *Artemia salina*, the high- M_r form is found predominantly in dormant cysts [8], and consists of a complex of three different polypeptide chains [9]. One of these chains, EF-1 α , binds aminoacyl tRNA and is identical to the low- M_r form, which is present as such in free-swimming nauplii [9]. In *A. salina*, this chain has an M_r of ~53 000 and corresponds to bacterial EF-Tu. In contrast to the bacterial factor, it is a basic protein with an isoelectric point of 8.5.

Here we present the amino acid sequences of 4 polypeptide fragments, obtained from EF-1 α from *A. salina*, which clearly are homologous to distinct

regions in EF-Tu from *E. coli*. The sequence of one of these peptides matches exactly with an amino acid sequence deduced from copy DNA of mRNA, isolated from cysts of *A. salina*.

2. MATERIALS AND METHODS

Elongation factor EF-1 α from *A. salina* was isolated from cysts, essentially as in [9]; the two-phase separation step with polyethyleneglycol, however, was omitted and instead a 30–80% ammonium sulphate cut of the postribosomal supernatant was used for the carboxymethyl-Sephadex C50 chromatography.

2.1. Limited digestion with trypsin

Typically, 6.5 mg EF-1 α , in 10 ml Tris-HCl (pH 7.5), 100 mM KCl, 1 mM magnesium acetate, 0.1 mM EDTA, 10 mM 2-mercaptoethanol and 25% (v/v) glycerol, was digested with 150 μ g TPCK-treated trypsin (Worthington) for 2 h at 30°C. Under these conditions, a 45 000 M_r tryptic fragment was generated in high yield, like in the case of EF-Tu from *E. coli* [10,11]. This fragment was purified by gel filtration over Sephadex G75 superfine, in 1% (v/v) HCOOH and 20 mM

2-mercaptoethanol, and lyophilized. The yield was 3.4 mg.

2.2. Isolation and purification procedures of the other peptides

Peptides were purified by gel filtration over Sephadex G75 superfine, in 0.5% (v/v) trifluoroacetic acid. Most peptides proved to be soluble in this solvent, which at the same time permits an absorbance recording at 220 nm. When necessary, a second purification step was accomplished by reversed-phase high-performance liquid chromatography on octadecyl (μ Bondapak C18, Waters) or cyanopropyl (Zorbax CN) columns. Two systems were used: (A) a linear gradient from 0.2% (v/v) trifluoroacetic acid to 0.1% trifluoroacetic acid and 45% (v/v) propan-1-ol, in

90 min (1.0 ml/min); (B) a procedure adapted from that of R. van der Zee (Groningen) – it consists of a gradient from 0.1% (v/v) trifluoroacetic acid, 10% (v/v) 2-methoxyethanol, 3% (v/v) butan-1-ol to 0.04% (v/v) trifluoroacetic acid, 10% (v/v) 2-methoxyethanol, 45% (v/v) ethanol, 15% (v/v) butan-1-ol, in 90 min (1.0 ml/min) (see also [12]).

2.3. BrCN-generated peptides

EF-1 α was treated in 8 M urea, with either 4-vinyl pyridine (redistilled) [13] or iodoacetic acid [14] in order to block its cysteine residues. Subsequently, the dialyzed and lyophilized modified protein was dissolved in 70% (v/v) HCOOH, containing 1% 2-mercaptoethanol, and digested with 3 times its weight of BrCN, for 24 h in the dark

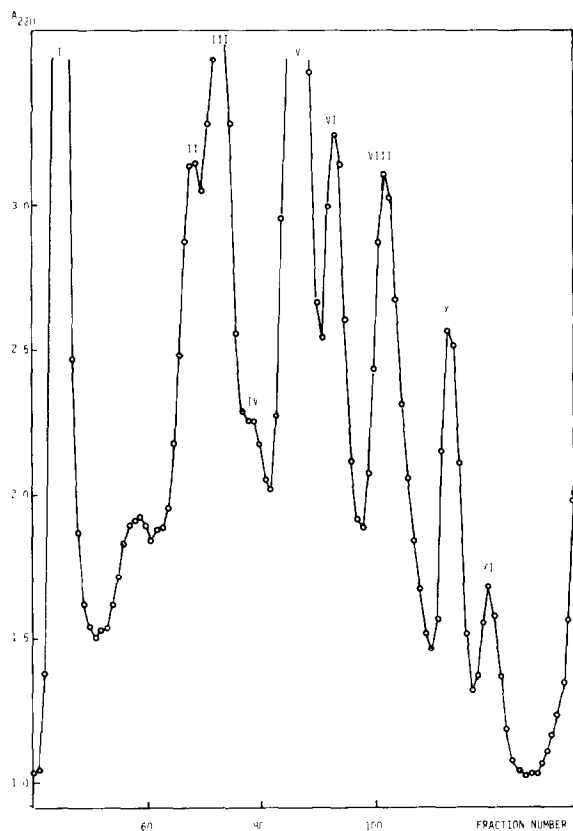


Fig. 1. Elution profile of the BrCN-generated peptides from S-pyridylethyl-EF-1 α , soluble in 0.5% (v/v) trifluoroacetic acid. The column of Sephadex G75 superfine (1.6 cm $\times$ 110 cm) was eluted at $\sim$ 4 ml/h. Fractions of 40 drops were collected.

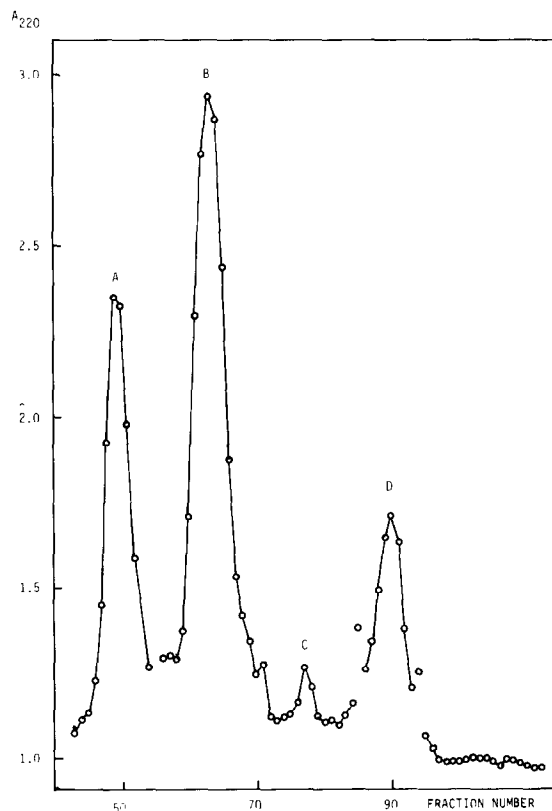


Fig. 2. Elution profile of BrCN-peptide III, digested with hydroxylamine. The desalted peptide was chromatographed on a column of Sephadex G75 superfine (1.0 $\times$ 110 cm) at $\sim$ 2 ml/h. Fractions of 20 drops were collected. Peak A contains the original peptide; peak B a mixture of the two large digestion products.

under nitrogen at room temperature. After rotatory evaporation and lyophilization, most peptides were obtained in pure form by gel filtration (fig.1).

2.4. Citraconylation and digestion with trypsin; digestion with clostripain

S-Pyridylethyl EF-1 α and BrCN-generated peptides were citraconylated [15] and digested with trypsin [16]. A first separation was obtained by gel filtration over Sephadex G75 superfine (section 2.2); cuts from the eluate were subsequently chromatographed using system A (section 2.2).

Alternatively, BrCN-generated peptides were further degraded by clostripain (Boehringer), in 0.1 M sodium phosphate (pH 8.0), 0.5 mM CaCl₂ and 7 mM dithiothreitol, at 30°C for 2 h with a

peptide:enzyme weight ratio of ~25:1. Peptides were purified as described above.

2.5. Treatment with hydroxylamine

Hydrolysis of BrCN-peptide III at its Asn-Gly bonds was performed according to [17]. The peptide mixture was desalted [18], chromatographed over Sephadex G75 superfine (section 2.2; fig.2) and further purified by high-performance liquid chromatography using system B (section 2.2; fig.3).

2.6. Amino acid analysis

This was performed with a Beckman Multichrom M amino acid analyzer. Hydrolysis was either in 6 M HCl containing 0.05% 2-mercaptoethanol for 20 h at 110°C, or in pro-

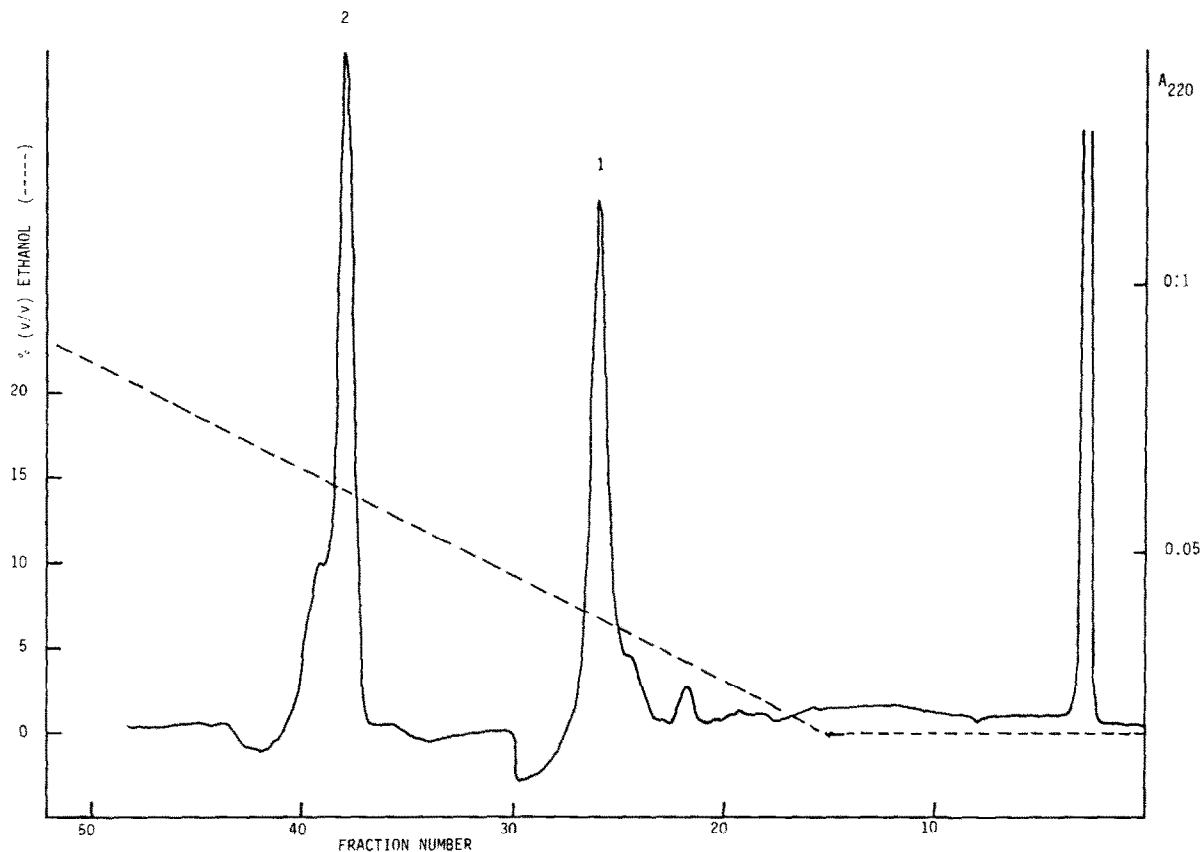


Fig.3. Elution profile of hydroxylamine-generated peptides, obtained from BrCN-peptide III (μ Bondapak C18-column). Peak B from fig.2 was rechromatographed in system B (section 2.2). The elution profile shown was obtained with ~1/30 of the total sample; peak 1 contains the peptide starting at Gly-325; peak 2 the peptide starting at His-261.

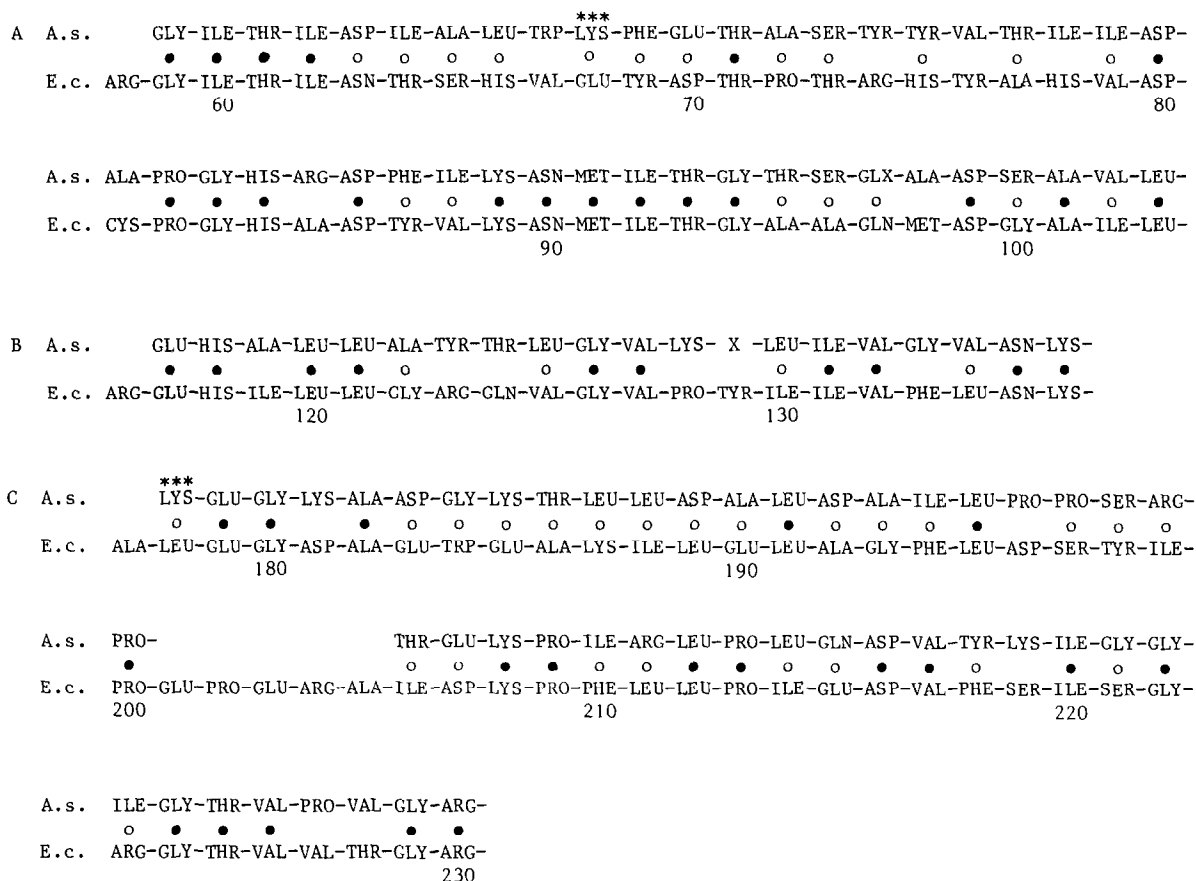
amino acid analysis after back hydrolysis [25,26]. ϵ -Trimethyllysine, found at three positions in the peptides shown (see fig.4), could be identified by means of an authentic sample of this amino acid.

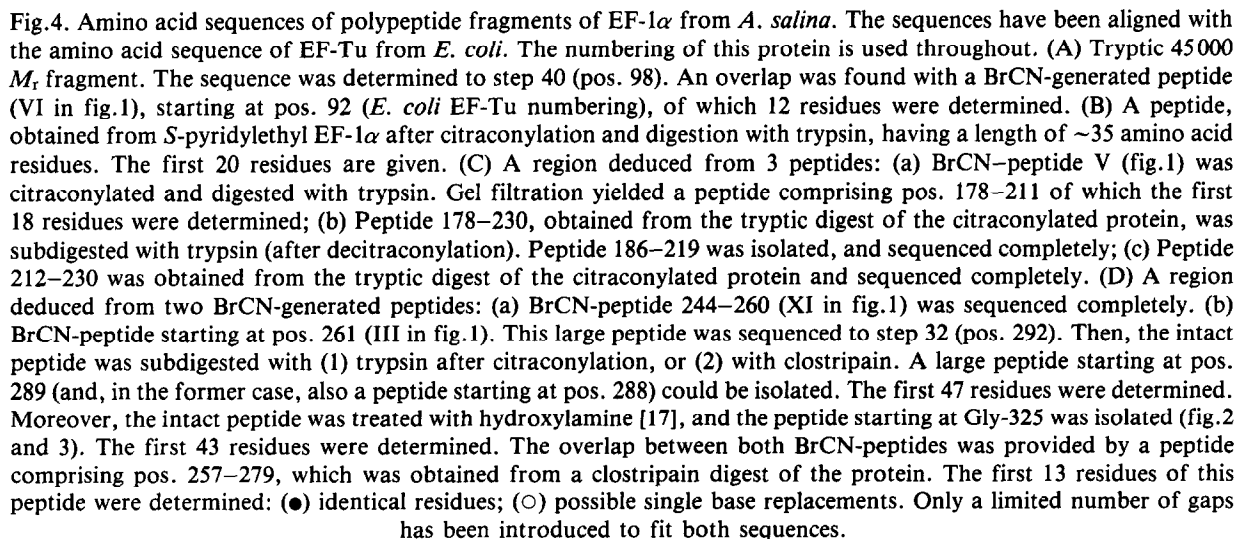
3. RESULTS AND DISCUSSION

At least 4 regions of EF-1 α from *A. salina* have sequences, which are remarkably similar to certain regions of EF-Tu from *E. coli* (fig.4).

Sequence A represents the first 45 residues of a 45000 M_r tryptic fragment of EF-1 α , obtained under limited digestion conditions (section 2.4). The homology with the first 45 residues of the corresponding 37000 M_r fragment of EF-Tu from *E. coli* is obvious. After this observation was made, we learned that a comparable homology was found between the first 12 residues of a similar tryptic fragment of rabbit reticulocyte EF-1 α and the 37000 M_r bacterial fragment [27].

PTH amino acids were identified by high-performance liquid chromatography [24] and by





The total result clearly points to an evolutionary relationship between the prokaryotic and eukaryotic elongation factor, and conservation of their spatial structure as well. The first 180 residues of the bacterial fragment comprise the so-called tight domain [28], which includes sequences homologous to the regions B and C of EF-1 α from *A. salina*.

Region D is composed from the two consecutive BrCN-peptides XI and III, and shows a clear similarity with the region starting at pos. 261 in EF-Tu from *E. coli*. This region contains an amino acid sequence which is identical to that deduced from a cDNA sequence. This cDNA was synthesized using mRNA for EF-1 α from *A. salina* as a template [29].

Interestingly, the protein is post-translationally methylated in at least 3 sites (fig.4), with the formation of exclusively ϵ -trimethyllysine (ϵ -monomethyllysine or ϵ -dimethyllysine were found to be absent at these positions). The significance of this modification is not known. Methylation of EF-1 α from the fungus *Mucor* was reported in [30].

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