Primary structure of elongation factor 1β from *Artemia*

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cDNA complementary to mRNA coding for the elongation factor EF-1β has been cloned. A λgt 11 cDNA library has been screened with an antiserum against EF-1β which exchanges GDP bound to EF-1α with exogenous GTP during protein synthesis. The derived amino acid sequence corresponds to 208 amino acids including the N-terminal methionine which is absent in the mature protein. About sixty percent of the protein was sequenced by direct protein sequence analysis. Comparison of *Artemia salina* EF-1β with *Escherichia coli* EF-Ts shows no evident homology.

(Artemia salina) *Protein synthesis* *Elongation factor 1β* *Guanine nucleotide exchange protein* *Nucleotide sequence* *cDNA cloning* *λgt 11 phage library*

1. INTRODUCTION

Besides elongation factor EF-1α, which mediates the GTP-dependent binding of aminoacyl tRNA to the 80 S ribosome [1], eukaryotes possess a second elongation factor EF-1$\beta\gamma$, which is functionally analogous to prokaryotic elongation factor EF-Ts. This factor is composed of two subunits β and γ, and catalyzes the exchange of GDP bound to EF-1α for GTP via an intermediary, ternary EF-1α·EF-1$\beta\gamma$ nucleotide complex [2–4]. It has been shown that the actual exchange activity resides in EF-1β whereas the function of EF-1γ is still unknown [4–7].

This paper reports on the successful cloning of *A. salina* EF-1β and the nucleotide sequence of its cDNA. Most of the cDNA-predicted amino acid sequence was confirmed independently by direct protein sequencing of a number of mainly tryptic peptides. Comparison of the primary structure of *A. salina* EF-1β with the deduced amino acid sequence of *E. coli* EF-Ts [8] revealed low, if any homology, suggesting less restriction with respect to changes in primary structure than in the corresponding case of *A. salina* EF-1α and *E. coli* EF-Tu [9].

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2. MATERIALS AND METHODS

2.1. *Materials*

A. salina cysts were obtained from Metaframe (San Francisco), peroxidase-conjugated goat anti-rabbit antibodies were obtained from Miles and Freund's adjuvant was purchased from CalBiochem. ^{32}P-labelled nucleotides and nick-translation kits were purchased from Amersham. Enzymes were from Boehringer or Promega. The other chemicals were of the highest grade commercially available.

2.2. *Preparations*

EF-1β was prepared according to a new method the details of which will appear elsewhere. The following steps were used: DEAE-cellulose chromatography, phenyl-Sepharose chromatography and DEAE-cellulose (6 M urea) chromatography to separate the protein chains β and γ [3]. Occasionally, the $\beta\gamma$-complex was separated on a Sephacryl S300 column in the presence of 0.1% SDS and 50 mM sodium bicarbonate, pH 9.0 [10].

During the final purification step of EF-1β (dialysis against 5 mM sodium pyrophosphate, pH 6.0, 2.5 mM $MgCl_2$), a distinct fragment of the protein was gradually formed, which had an M_r on

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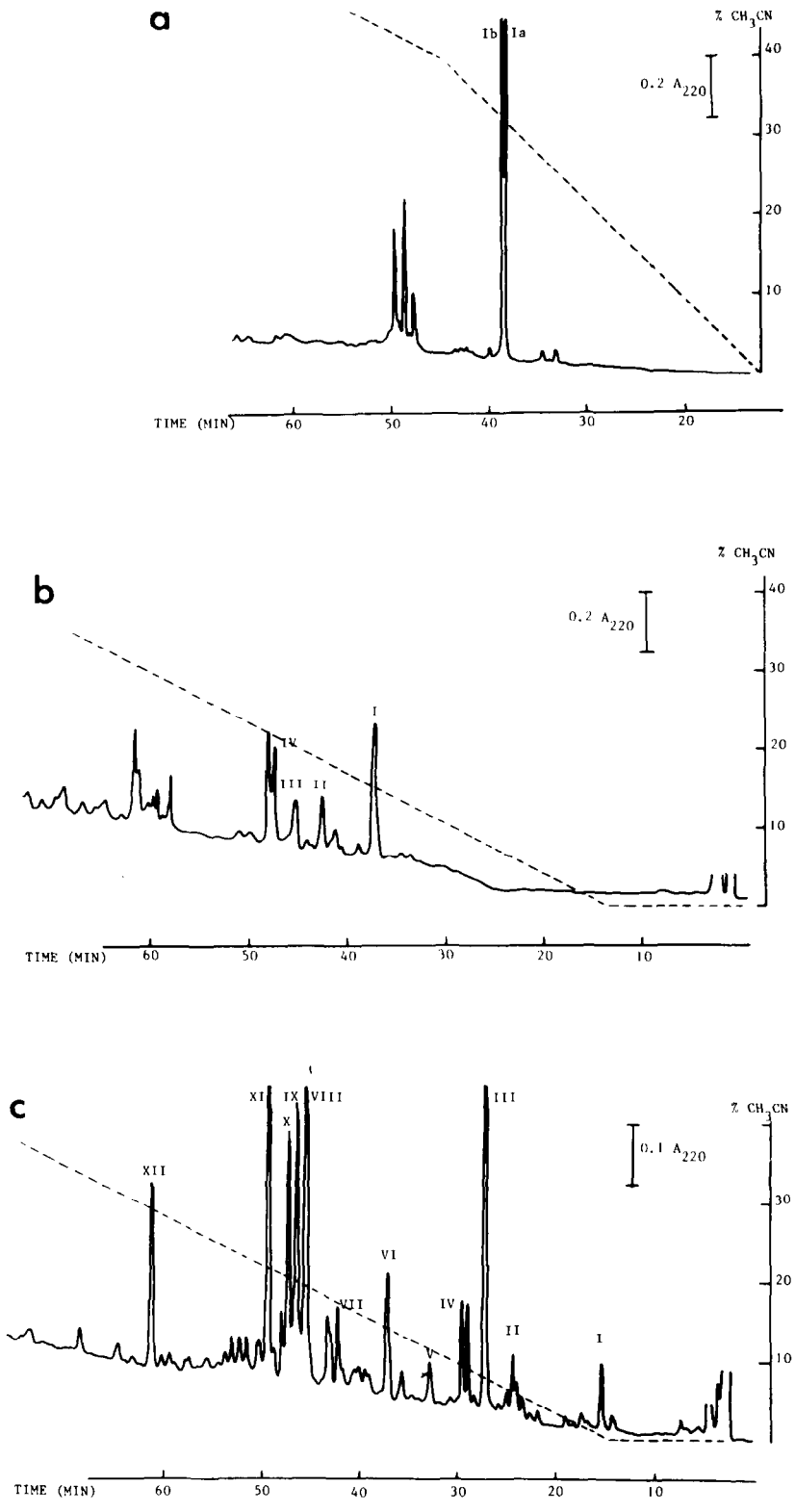
a
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SDS-polyacrylamide gels of about 14000. This fragment was purified on reverse-phase HPLC, as described in fig.1a, and used for sequence analysis as well.

2.3. *Protein sequence analysis*

S-Pyridylethylation of EF-1β and its 14000 Da fragment (see above) was performed as described in [11]. The intact protein was also *S*-carboxymethylated according to a procedure suitable for small amounts of protein [10].

Electroelution from polyacrylamide gels of a BrCN-generated fragment of EF-1β was performed as described [10]. Tryptic peptides were separated on a Waters μBondapak C18 column (see the legends of fig.1b and c). Typically 4–5 nmol peptide were used for sequencing in a Beckman model 890 C sequencer. Polybrene (3 mg) was added to the peptide, and a 0.25 M Quadrol programme was used. PTH-amino acids were identified by reverse-phase HPLC as described [12].

2.4. *Construction of a cDNA library in λgt 11*

Total RNA was extracted from *A. salina* cysts, which had been developed for 16 h at 27°C in artificial sea water, by the urea/LiCl procedure [13]. Poly(A)-rich RNA was isolated as in [14]. Total poly(A)-rich RNA was used for cDNA synthesis according to [15]. *Eco*RI sites in cDNA were methylated, and the cDNA was ligated with *Eco*RI linkers (Boehringer) and digested with *Eco*RI as described [16]. The modified cDNA was separated according to size on a Sepharose CL 2B column. A cDNA library was constructed in the arms of phage λgt 11 and subsequently packaged in vitro using the Promega and Amersham kits, respectively. Starting from 1 μg of poly(A)-rich RNA a library of approx. 2.5×10^5 recombinants was obtained.

2.5. *Immunological screening*

Purified EF-1β was used to raise an antiserum in a rabbit. 1 mg of the protein was injected in complete Freund's adjuvant followed by a booster of 0.5 mg after 30 days. The serum was collected 10 days later.

The library was plated out onto *E. coli* strain Y 1090 at 10^5 plaque-forming units and incubated for 4 h at 37°C. A filter soaked in 10 mM IPTG (*β*-D-thiogalactopyranoside) was placed on the top agar and the plates were incubated overnight. The filters were removed, washed in 6 × STE, 1% NP-40 (Nonidet P-40) during 5 min and preincubated in 1 × STE, 1% BSA (1 × STE = 10 mM Tris-HCl, pH 7.4, 100 mM NaCl, 0.1 mM EDTA). The filters were incubated for 16 h at 4°C in the antiserum buffer: 6 × STE, 1% NP-40, polyclonal rabbit anti-EF-1β (1/100 dilution) and Y 1090 lysate (1/40 dilution). The filters were washed twice for 20 min at 37°C with each of the following solutions: 3 × STE, 1% BSA; 1 × STE,

Fig.1. (a) Purification of the large (proteolytic) fragment of EF-1β by reverse-phase HPLC. A sample of about 5 nmol was injected onto a wide-pore C 18-column (Baker no.RP 7104-0; 4.6 × 250 mm). The column was developed with a gradient of acetonitrile in 0.1% (v/v) trifluoroacetic acid as indicated, at a speed of 1 ml/min. The largest fragment appeared for unknown reasons as a doublet, indicated as Ia/Ib. (b) Tryptic peptide mapping of the peptides obtained from the 14 kDa fragment of EF-1β. A sample of about 8 nmol (previously *S*-alkylated with 4-vinylpyridine and tributyl phosphine, see [11]) was digested with trypsin in 1 ml of 0.2 M ammonium carbonate (pH 8.5) for 1 h at 37°C with 10 μl TPCK-trypsin (Worthington), and subsequently injected onto a μBondapak C 18 column. I, WYK*; II, APSDKFPY*; III, GQEQLNELLANK*; IV, SYLQGYEPSQEDVAAFNQLNK*. *, The peptide was sequenced to the end; underlining indicates a correction for this residue with respect to a previous report [3]. (c) Tryptic peptide mapping of the peptides obtained from *S*-carboxymethylated EF-1β (see legend of fig.1b). A sample of about 10 nmol was digested with trypsin and chromatographed as described above. I, AYSDKK*; II, skkPAIVAK*; III, WYK*; IV, HISSF*; V, N-terminal blocked peptide (see text); VI, APSDKFPY*; VII, a mixture of two peptides, GQEQLNELLANK* and AEKGQEQLNEL---; VIII, SVQMDGLVWGAAK*; IX and X, both peaks were mixtures of the peptides GFPGIPTSAA-EE--- and SYLQGYEPS---; XI, SSVILDIKPWDDETDMAEMEK*; XII, ISEFEDFVQSVDIAAFNK*. The sequence following the methionine residue in VIII was also confirmed by analysis of a BrCN-generated peptide. The peptide mixtures IX and X were, prior to their sequencing, resolved into their constituent peptides by rechromatography on the same reverse-phase column and with the same gradient system, but in the presence of 10 mM *t*-butylammonium acetate, pH 6.0, instead of 0.1% (v/v) trifluoroacetic acid (not shown). * and underlining are as in (b).

1% NP-40; and 1 × STE, 1% BSA. Then, the filters were incubated for 1 h in 4.5 × STE, 1% NP-40, peroxidase-conjugated goat anti-rabbit IgG (1/100 dilution) and the Y 1090 lysate (1/40 dilution). The filters were washed twice for 20 min at 37°C with each of the following solutions: 3 × STE, 1% BSA; 1 × STE, 1% NP-40; and phosphate-buffered saline. α-Chloronaphthol was used as a substrate for the peroxidase reaction.

Immunologically positive clones were subcloned in PUC 8 and these subclones were also screened with a synthetic oligonucleotide probe 5′-dTG(A,T,C,G)ACGAAGTC(T,C)TCAAG-(T,C)TC-3′ which was synthesized on the basis of the underlined part of the tryptic peptide IS-EFEDFVQSVDIAAFNK of EF-1β. Conditions of hybridization and washing were as in [17].

2.6. *DNA sequencing analysis*

Sequencing was performed according to the sequencing technique of Maxam and Gilbert [18].

2.7. *RNA and DNA blot analysis*

RNA samples were electrophoresed on a 1.5% agarose gel containing 2.2 M formaldehyde [19] and subsequently blotted onto nitrocellulose and hybridized as described [20].

Restriction fragments of digested DNA were separated on a 0.8% agarose gel by electrophoresis and transferred to gene screen plus [NEN] by alkaline blotting [21]. Hybridization and washing of the sheets were as in [20].

3. RESULTS AND DISCUSSION

Fig.1a shows the chromatographic purification of the 14 kDa fragment of EF-1β; fig.1b and c shows the separation of the tryptic peptides, derived from the 14 kDa fragment and the intact protein, respectively. The amino acid sequences of the peptides analyzed are indicated in the legends. One of the peptides (XII in fig.1c) was used to synthesize an oligonucleotide probe (see section 2). This probe was used for rescreening the recombinants that were subcloned into PUC 8. These recombinants were previously selected immunologically from 10^5 recombinant phages. In this way clone B4 was picked up. Using this clone as probe, three of the four immunological positive

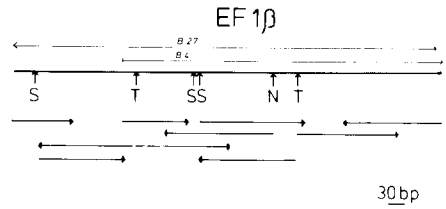

Fig.2. Sequencing strategy of the overlapping cDNA clones B4 and B27. Relevant restriction sites are indicated. Arrows indicate sequencing from a given site. N, *Nco*I; S, *Sau*3AI; T, *Taq*I. Both strands of the cDNA were sequenced.

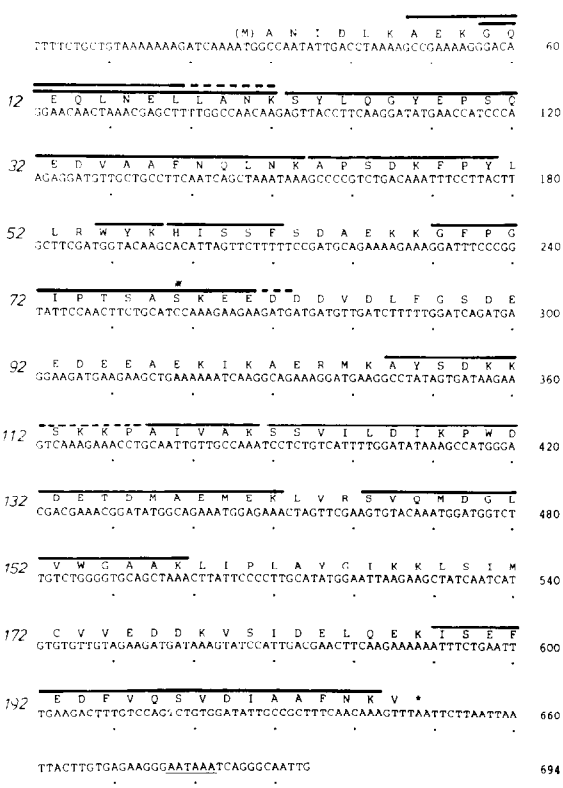

Fig.3. The combined sequences of the cDNA clones B4 and B27 coding for *A. salina* EF-1β. The derived amino acid sequence is shown in the one letter code above the cDNA sequence. Peptides found by direct protein sequencing are marked by —— and cover some 60% of the total sequence; – – –, indicates uncertain residues. The polyadenylation signal is underlined. The only difference between sequences derived from the cDNA and direct protein sequencing is indicated by *.

clones appeared to be an EF-1β clone. On rescreening the phage library with clone B4 subsequently another clone B27 was picked up, which had an overlapping sequence with B4. Together these clones contained the complete coding sequence for EF-1β.

The sequencing strategy for EF-1β is given in fig.2. Fig.3 depicts the cDNA sequence and the derived amino acid sequence coding for EF-1β of *A. salina*. Fig.4 shows the mRNA corresponding to EF-1β as revealed by Northern blot hybridization.

The translational initiation site was assigned to the methionine codon at nucleotide position 26–28. It is the first ATG triplet that appears after a nonsense codon TAA at position 11–13 in the same reading frame. The initiation sequence AAAATGG at position 23–29 resembles the optimal Kozak initiation sequence ACCATGG [22]. The reading frame terminates with the codon TAA at position 650–652. The polyadenylation signal AATAAA [23] is found at position 680–685. The clone B4 has a long poly(A) stretch.

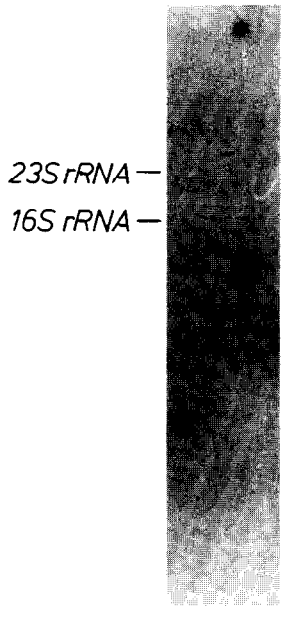

Fig.4. Northern blot analysis of EF-1β mRNA from *A. salina*. The amount of poly(A)-rich RNA used was 3 μg. The blot was hybridized with nick-translated clone B4. The positions of 16 S and 23 S rRNA of *E. coli* are indicated as markers.

Fig.5. Maximal 'homology' between EF-1β and EF-Ts allowing all possible shifts of the chains with respect to each other.

The open reading frame predicts an amino acid sequence for the β-subunit of *A. salina* EF-1 of 208 amino acids including the initiator methionine. The polypeptide has a calculated M_r of 23300 which is smaller than the M_r of 26000 estimated on the basis of SDS-polyacrylamide gel electrophoresis [5].

Both EF-1β and the fragment were found to be blocked at their amino-terminal end. The initiator methionine residue must have been removed during the in vivo processing of the protein, because an N-terminal blocked tryptic peptide (V in fig.1c) with an amino acid composition corresponding to the first six residues following the initiator methionine has been found. This is in line with a recent report [24] that after removal of the N-terminal methionine the next amino acid, if alanine, methionine or serine, is frequently acetylated.

Given the functional analogy between the complex of EF-1α·EF-1$\beta\gamma$ in eukaryotes and of EF-Tu·EF-Ts in prokaryotes on one hand and the structural homology of EF-1α and EF-Tu on the other, it is of interest to compare the structure of EF-1β and EF-Ts. Although the size, isoelectric point and hydrophobicity of EF-1β and EF-Ts are comparable and the functional homology is obvious, there is little if any primary structural homology (see fig.5). There is also hardly any resemblance in hydrophilicity pattern (see fig.6).

Although EF-1α is a very conserved protein, EF-1β has almost no homology with its prokaryotic counterpart EF-Ts. It is not yet clear to what extent EF-1β is conserved in eukaryotes.

Interestingly, unlike the γ-component, the β-component of EF-1$\beta\gamma$ can be phosphorylated in vitro, using ATP as a phosphate donor; on isoelectric focusing part of EF-1β, it turned out to be already phosphorylated as such (unpublished results). The site and possible functional significance of this modification are under investigation.

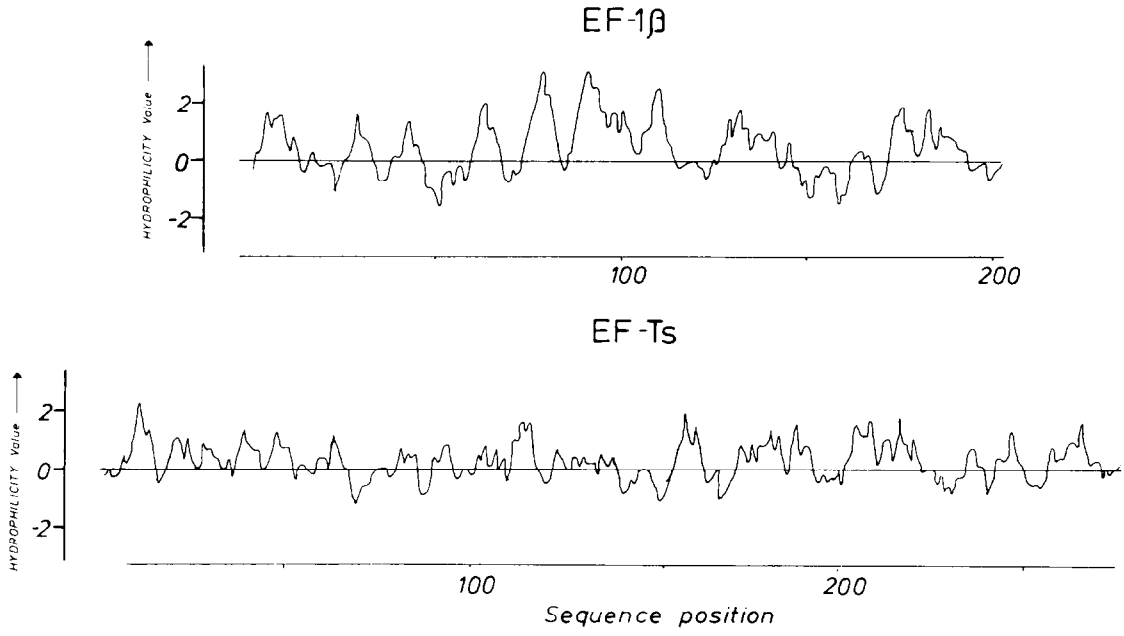

Fig.6. Hydrophilicity pattern of EF-1β and EF-Ts calculated according to Hopp and Woods [25]. Each point represents an average value over six amino acid residues. Positive values indicate hydrophilic regions and negative values hydrophobic areas.

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