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Primary structure of elongation factor 1γ from *Artemia*

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Complementary DNA corresponding to elongation factor 1γ, which forms a complex with EF-1β, has been cloned. A λgt11 cDNA library has been screened with an antiserum against EF-1$\beta\gamma$. The derived amino acid sequence of EF-1γ corresponds to 429 amino acids excluding the initiator methionine, which is absent in the mature protein. About half of the protein was sequenced by direct protein sequence analysis. No clear homology with any other protein was found.

Protein synthesis; Elongation factor 1γ; cDNA cloning; Nucleotide sequence; *(Artemia)*

1. INTRODUCTION

Elongation factor 1 participates in protein synthesis by mediating the transport of aminoacyl-tRNA to 80 S ribosomes [1]. The protein consists of three subunits EF-1α, EF-1β and EF-1γ which in *Artemia* possess molecular masses of 49, 26 and 46 kDa, respectively [2]. The EF-1$\alpha\cdot$GTP form binds aminoacyl-tRNA and transports it to the ribosome. After releasing the aminoacyl-tRNA and hydrolysis of GTP, EF-1$\alpha\cdot$GDP leaves the ribosome. EF-1$\alpha\cdot$GDP is then converted into EF-1$\alpha\cdot$GTP with the aid of EF-1$\beta\gamma$ and exogenous GTP. The actual nucleotide exchange activity resides in EF-1β [3–6]; EF-1γ is an EF-1β associated protein, which is typical for eucaryotes and exerts a stimulatory effect on EF-1β; its actual function seems related to facilitate EF-1β activity at different locations in the cell [7].

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The nucleotide sequence presented here has been submitted to the EMBL/GenBank database under the accession number Y00700

Recently, we determined the primary structure of EF-1β of *Artemia* from its cDNA sequence as well as from partial protein sequence analysis [8]. Here we report the primary structure of EF-1γ, using the same approach.

2. MATERIALS AND METHODS

2.1. *Materials*

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

2.2. *Preparations*

EF-1γ was isolated as described before [8]. During the dialysis of the $\beta\gamma$-complex against 5 mM pyrophosphate, pH 6.0, 2.5 mM $MgCl_2$, EF-1γ was occasionally split by an endogenous protease. A distinct fragment of EF-1γ with a molecular mass of 24 kDa was isolated on a DEAE-cellulose column. This fragment was also used for direct sequence analysis.

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2.3. *Preparation and separation of peptides*

S-Pyridylethylation or *S*-carboxymethylation of EF-1γ and its 24 kDa fragment (see above) were performed as described in [9]. Peptides from the 24 kDa fragment were obtained by digestion of about 10–25 nmol polypeptide with TPCK trypsin or *Staphylococcus aureus* V8 protease according to standard procedures [10]. On the other hand, intact *S*-pyridylethylated EF-1γ proved to be rather refractory to tryptic digestion. Satisfactory results were obtained by using 2–4 nmol of heat denatured, finely suspended protein in 0.2 M $(NH_4)_2CO_3$, pH 8.5, which was digested with 10 μg TPCK trypsin. After 3 h at 37°C, another 10 μg enzyme was added, and incubation was continued for 3 h at 37°C (see fig.1c). Alternatively, 2–4 nmol protein were dissolved in 0.2 M $(NH_4)_2CO_3$, pH 8.0, and 6.5 M urea, and 10 μg TPCK trypsin was added. After 3 h at 37°C, an equal amount of enzyme was added, and incubation was continued for 15 h at 37°C (see fig.1d).

Peptides were separated by reverse-phase high-performance liquid chromatography on a Waters μBondapak C18 column (4 × 300 mm) which was developed with a linear gradient of 0 to 75% (v/v) acetonitrile in 0.1% (v/v) trifluoroacetic acid over a period of 60 or 120 min, and with a flow rate of 1.0 ml/min. In cases where the peaks of the chromatogram represented peptide mixtures, subsequent separations were performed on the same column, but with another electrolyte, e.g. 10 mM triethylammoniumphosphate (pH 5.8) instead of 0.1% (v/v) trifluoroacetic acid (not shown).

2.4. *Protein sequence analysis*

Typically, 4–5 nmol peptide were used for sequencing on a Beckman model 890 C sequencer. With the Applied Biosystems gas-phase sequencer model 470A, on-line equipped with a model 120A PTH-amino acid analyser, amounts of 100–200 pmol peptide were used.

2.5. *Screening of an* Artemia *cDNA library*

An *Artemia* cDNA library was constructed in λgt11 and screened with a polyclonal rabbit antiserum as described before [8]. Rabbit anti-EF-1$\beta\gamma$ raised against EF-1$\beta\gamma$ was used as antiserum. Possible EF-1γ clones were subjected to an epitope selection according to [11]. In short the method is as follows. The purified immunopositive recombinants were plated out. As a control an immunopositive insulin reecptor clone was used which is not recognized by anti-EF-1$\beta\gamma$. A filter soaked in 10 mM IPTG(β-D-thiogalactopyranoside) was placed on the top agar and the plates were incubated overnight at 37°C. The filters were removed and incubated with rabbit anti-EF-1$\beta\gamma$. After washing, the remaining antibody which had recognized the fusion protein, was eluted from the filter with a glycine-HCl, pH 2.3, buffer. The antibody thus purified, was allowed to react with EF-1γ on a protein blot of an *Artemia* S100 extract as described in [12] and thus proved to be indeed anti-EF-1γ. The immunopositive EF-1γ recombinant was subcloned in PUC8. This EF-1γ cDNA clone was used to screen an *Artemia* PUC9 cDNA library.

2.6. *DNA sequencing analysis*

The immunological cDNA clone was subcloned in PUC8. The cDNA clones were sequenced using the sequencing technique of Maxam and Gilbert [13].

2.7. *RNA blot analysis*

Poly(A)-rich RNA was isolated, separated and blotted as described [7]. Northern blot hybridization was carried out as in [14]. The blot was reprobed with an EF-1α clone (2E3) [15].

3. RESULTS AND DISCUSSION

Fig.1 shows the chromatographic separation of the peptides used for direct sequence analysis. The amino acid sequences are indicated in the legends. Together with the N-terminal sequences obtained for intact EF-1γ (28 residues) and its 24 kDa fragment (16 residues), they comprise more than half of the total sequence (see fig.3).

After screening the λgt11 cDNA library with rabbit anti-$\beta\gamma$, four positive clones out of 100000 recombinants were selected. Three of the four clones were also positive with an EF-1β cDNA probe. The remaining immunologically positive clone was submitted to an epitope selection. In this way the immunological clone was identified as an EF-1γ cDNA clone and subsequently subcloned in PUC8. This subclone was designated ASEF1c2 and from its sequence, it was clear that it did not

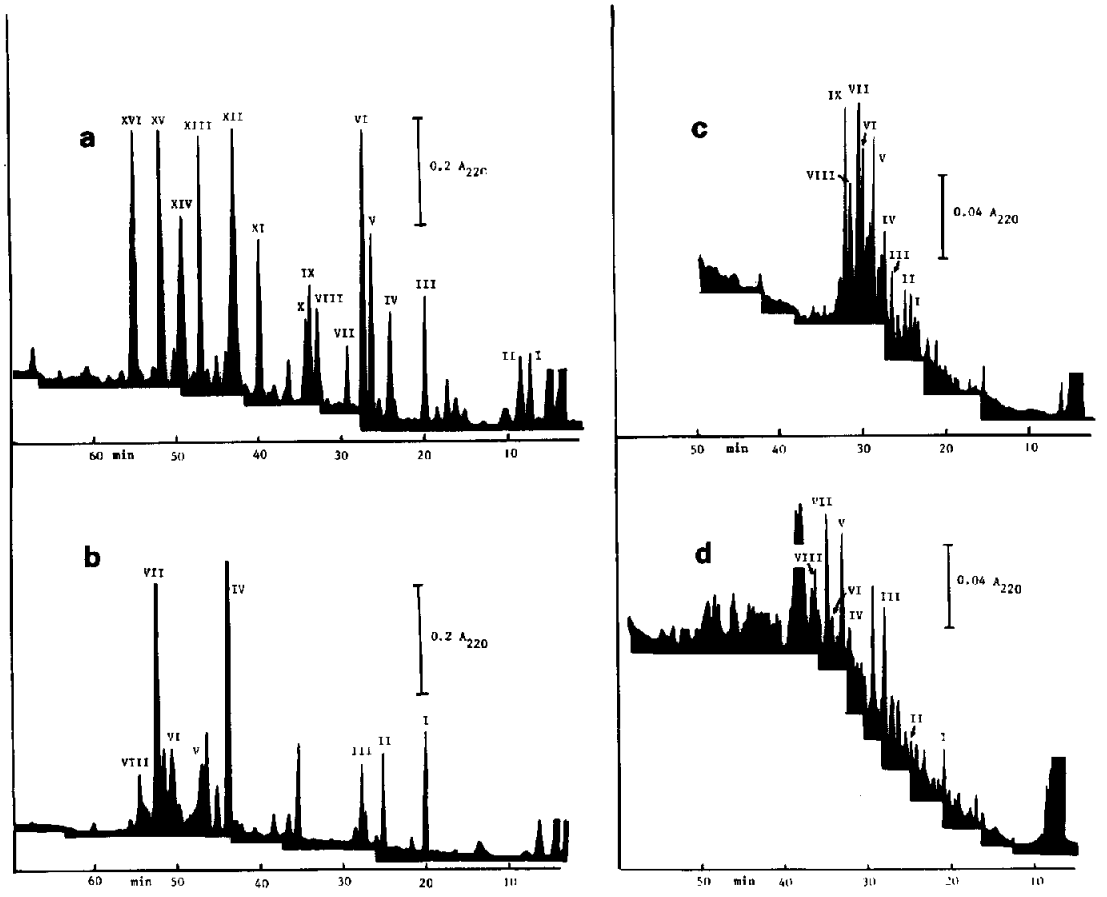

Fig.1. (a) Tryptic peptides from the *S*-carboxymethylated 24 kDa fragment of EF-1γ. I, AKPEKK*; II, AKPEK*; III, KFNQGK*; IV, IFK*; V, KLDPDAQETK*; VI, FYSNNEETK*; VII, YQDELAK*; VIII, SKDPFDE- + GQDLA-; IX, SKDPFDEMPK* + GQDLAFK*; X, GTFNMDDFK*; XI, SKDPFDEMPK*; XII, GTFNMDDFK* + EQEEPADAAEEALAAEPK*; XIII, FDKENYSIWYSEYK*; XIV, SIPYFWEK*; XV, LSPDWQIDYESYDWK*; XVI, DLVTQYFTWTGTDK*. (b) Peptides from the *S*-carboxymethylated 24 kDa fragment of EF-1γ, generated with *Staphylococcus aureus* V8 protease. I, KKEVPKKE*; II, QEEPADAAEE*; III, KKEVPKKEQEEPADAAEE*; IV, SYDWKKLDPDAQE*; V, ALAAEPKSDPF-; VI, TKSIPYFWE*; VII, TKDLVTQYFTWTGTDKQGRKFNQGKif-; VIII, ALAAEPKSKDPFDE*. (c) Tryptic peptides from *S*-pyridylethyl EF-1γ (heat-denatured, see section 2). The gradient was developed in 60 min instead of 120 min. I, SDAFLK* + GQDLAFK*; II, AVIGDF-; III, SFVFGETNK* + LYTYPENFR*; IV, ALEALDDHLLTR*; V, FDKENYSIWYSEYK*; VI, SIPYFWEK*; VII, LSPDWQIDYESYDWKK*; VIII, WFMTLINQK* + VYM--NLITGM-; IX, DLVTQYFSWTGTDK* + VPAFESADG--IAESNAI-. (d) Tryptic peptides from *S*-pyridylethyl EF-1γ (digestion in urea solution, see section 2). I, FYSNNEETK*; II, ALIAAQY-GA-; III, LYTYPENFr*; IV, SKDPFDEMPK* + SIPYF*; V, L-PDWQIDYE-; VI, DLVTQ--S-TG-; VII, DLVTQYFS-TGTD-; VIII, VPA-ESADG-. The long digestion time caused the formation of many autolysis products of trypsin. * The peptide was sequenced to the end. Underlining indicates a difference between direct protein sequencing and cDNA derived protein sequencing.

harbour a full length copy of cDNA for EF-1γ. Therefore an *Artemia* PUC9 cDNA library was screened by hybridization with the ASEF1c2 cDNA probe and in this way ASEF1c3 was picked up. The complete cDNA sequencing strategy is shown in fig.2. On Northern blot a single band was found at the position roughly expected for the size of an mRNA corresponding to EF-1γ. The complete nucleotide sequence of EF-1γ cDNA and the cDNA derived amino acid sequence are shown in

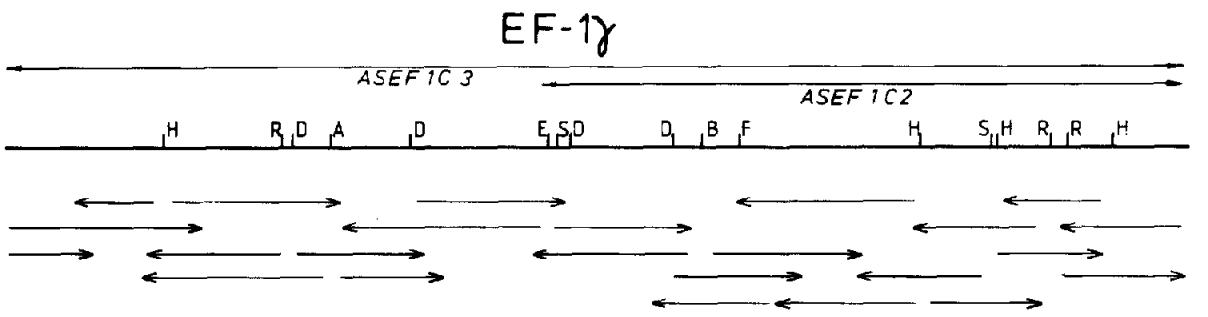

Fig.2. Sequencing strategy of the overlapping cDNA clones ASEF1c2 and ASEF1c3. Both strands of the cDNA were sequenced. Only relevant sites are indicated: A, *Aval*; B, *Bam*HI; D, *Dde*I; E, *Eco*RI; F, *Fok*I; H, *Hin*dIII; R, *Rsa*I; S, *Sau*3AI.

fig.3 together with partial sequences obtained by direct protein sequence analysis.

The translational initiation codon ATG starts at position 13 and the open reading frame terminates with stop codon TAA at position 1302. The initiation sequence AAGAUGG resembles more the initiation sequence AA(A/C)AUGG found in *Drosophila* [16] than the initiation sequence AC-

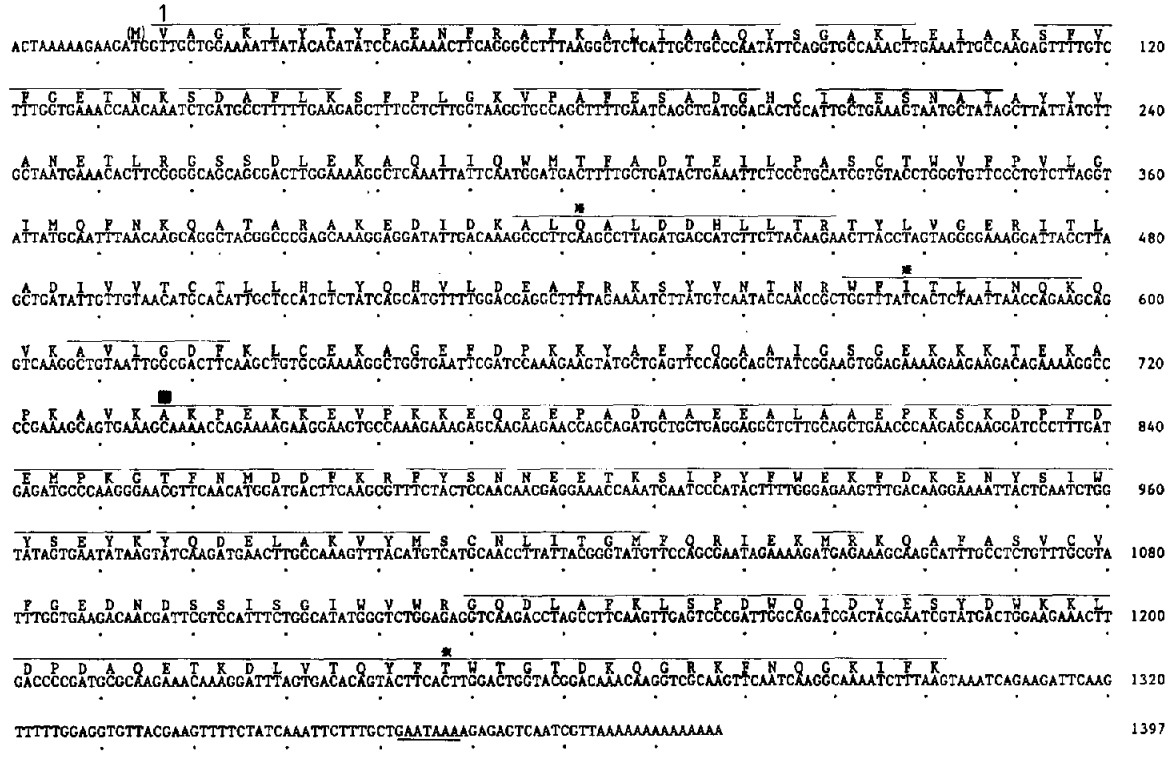

Fig.3. The cDNA sequence and the nucleotide derived amino acid sequence of EF-1γ. The amino acid sequence is shown in the one letter code. Segments found by direct protein sequencing are marked by ——, and covers about half of the total sequence (see also fig.1). An asterisk marks the positions where direct protein sequencing differs from the primary structure derived from cDNA: Q-136 (cDNA) corresponds to E-136 (protein); I-188 (cDNA) corresponds to M-188 (protein); T-411 (cDNA) corresponds to S-411 or T-411 (protein, depending on the batch used). See also section 3. (■) Start of the 24 kDa fragment, which comprises the complete C-terminal moiety of the original protein. The polyadenylation signal is underlined.

CAUGG found by Kozak [17] in vertebrates. The polyadenylate signal AATAAA [18] was located at position 1363 to 1368. Both clones ASEF1c2 and ASEF1c3 have a poly(A) stretch as shown in fig.3.

The predicted EF-1γ protein sequence consists of 429 amino acids, excluding the initiator methionine. The polypeptide chain has a calculated molecular mass of 49200 Da. The isolated protein lacks the initiator methionine at its amino terminus, and is not amino-terminally blocked as is the case with EF-1α and EF-1β. This mode of processing is in line with the rule that when the residue next to the initiator methionine is a valine, the methionine will be removed and the amino-terminus will not be acetylated [19]. The protein is relatively rich in aromatic residues (13%) and contains a highly polar central region, which is amenable to endogenous proteolytic attack. This leaves a fragment which comprises the C-terminal part of the protein, starting from residue 241. There are no hydrophobic domains of sufficient length in EF-1γ to span the plasma membrane repeatedly. The length of the largest hydrophobic, uncharged stretch is 19 residues long (amino acid position 102–120). Judged from the hydrophobicity plot as shown in fig.4 we observed a remarkable alternation of hydrophobic and hydrophilic regions bordering a large hydrophilic region in the middle of the sequence. The EF-1γ sequence does not contain a consensus sequence for ATP or GTP binding [21,22].

A short internal repeat VFGE-N-S is present at two sites in the protein at the positions 35–42 and 355–362; at the nucleotide level this sequence homology is even more evident.

Comparison of the derived and the directly determined amino acid sequence, revealed three differences. One of them at position 411 also showed microheterogeneity at the protein level (see fig.3). In the other two cases the differences may be due to errors made by the reverse transcriptase enzyme [23] or cloning of a minor EF-1γ mRNA.

When two protein data banks NBRF and Swissplot were screened, no significant homology of EF-1γ with any known protein was found.

In conclusion the amino acid sequence of EF-1γ reported here, should fit the findings that the overall negatively charged EF-1β protein forms a

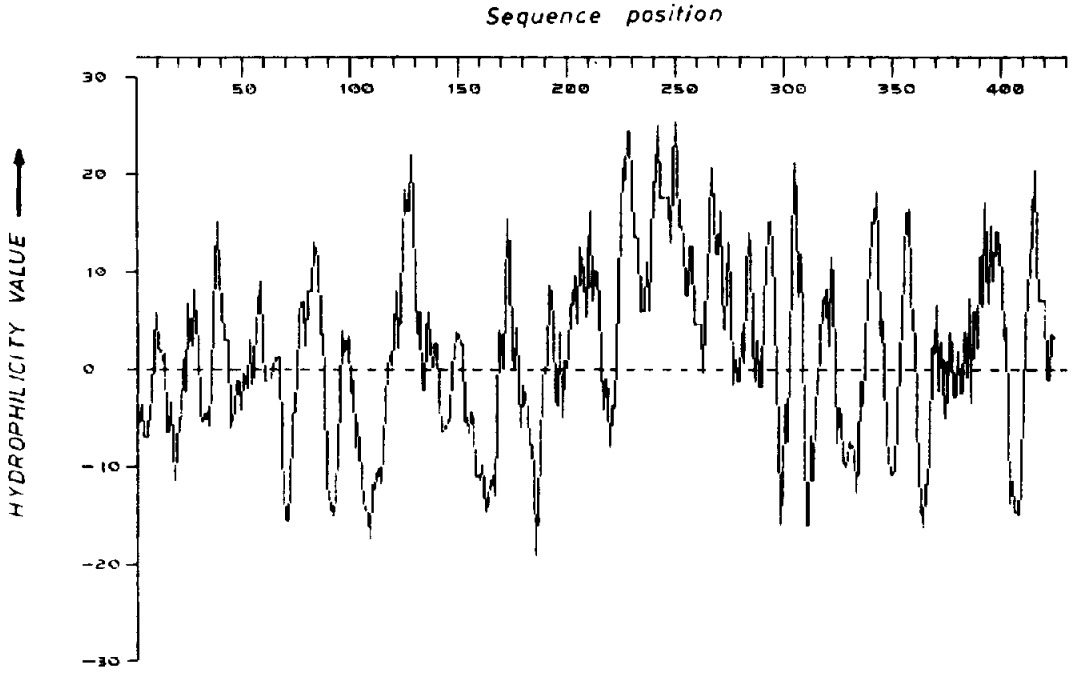

Fig.4. Hydrophobicity pattern of EF-1γ according to Hopp and Woods [20]. Each point represents an average value over six amino acid residues. Negative values represent hydrophobic regions and positive values represent hydrophilic regions.

firm complex with EF-1γ, which seems instrumental in anchoring the nucleotide exchange enzyme to certain structures in the cell [7]. In addition it is interesting that an extremely purified EF-1$\beta\gamma$ preparation displays a protein kinase activity which phosphorylates EF-1β but not EF-1γ (unpublished; and [24]).

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