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Leucine-zipper motif is responsible for self-association of translation elongation factor 1Bβ

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Translation elongation factor 1Bβ (eEF1Bβ) is a metazoan-specific protein catalyzing the guanine nucleotide exchange on translation elongation factor 1A (eEF1A). eEF1Bβ was reported to form oligomers. **Aim.** To define the structural region of human eEF1Bβ that mediates its self-association. In addition, the various truncated forms of this protein were tested in the guanine nucleotide exchange assay with two isoforms of mammalian eEF1A. **Methods.** The truncated forms of eEF1Bβ were generated by PCR, cloned, expressed in *Escherichia coli* and purified to homogeneity. Their apparent molecular masses were determined by analytical gel filtration and their guanine nucleotide exchange activities were assessed by filter binding assay. **Results.** Complete deletion of the N-terminal domain of eEF1Bβ does not affect its oligomerization propensity while deletion of the leucine-zipper motif drastically decreases the apparent molecular mass of the truncated form compared to the full-length protein. Also, the leucine-zipper motif of eEF1Bβ fused to glutathione S-transferase causes oligomerization of the chimeric protein. It was demonstrated that all N-terminally truncated forms of eEF1Bβ displayed similar catalytic activity to that of the full-length protein. Weak inhibitory effect on the catalytic activity was observed only for the truncated form with partially deleted central acidic region. **Conclusions.** The leucine-zipper motif facilitates oligomerization of recombinant eEF1Bβ. Stepwise deletion of the eEF1Bβ N-terminal domain does not significantly affect the guanine nucleotide exchange activity of the truncated proteins.

Keywords: translation elongation factor 1, guanine nucleotide exchange, protein structural domains.

Introduction

Guanine-nucleotide exchange factors (GEFs) play an important role during the elongation cycle of protein biosynthesis in eukaryotes catalyzing the GDP/GTP exchange on translation elongation factor 1A (eEF1A) and, thus, restoring its active GTP bound conformation. In turn, the eEF1A*GTP molecules form the complexes with various aminoacylated tRNAs and deliver them to the ribosome ensuring the process of protein biosynthesis [1]. In mammals, there are two isoforms of eEF1A (designated eEF1A1 and eEF1A2) that share 97 % amino acid homology but are encoded by different genes [2]. Both eEF1A1 and eEF1A2 have different non-canonical functions, but their role in translation is believed to be identical [2–4].

In higher eukaryotes, GEFs that participate in translation elongation are organized in a stable macromolecular complex, eEF1B, containing three subunits designated eEF1Bα, eEF1Bβ and eEF1Bγ. We use the unifying nomenclature proposed by Merrick [5]. Both eEF1Bα and eEF1Bβ catalyze the GDP/GTP exchange on eEF1A, while a functional role of eEF1Bγ is still poorly understood [3]. eEF1Bγ may be considered as a scaffold component of the eEF1B complex since it contains the binding sites for both eEF1Bα and eEF1Bβ [6–8]. It has been reported that eEF1Bγ

binding enhances the activity of eEF1Bα but not eEF1Bβ [7]. Recently, we have shown that the N-terminal domain negatively influences the catalytic activity of full-length eEF1Bα. Deletion of the eEF1Bα N-terminal domain as well as formation of a tight complex with the eEF1Bγ N-terminal domain eliminated this inhibitory effect [9].

eEF1Bβ is a guanine-nucleotide exchange factor specific for metazoans. Its C-terminal domain is highly homologous to the C-terminal domain of eEF1Bα that is responsible for guanine nucleotide exchange activity [7, 10]. Recently, the structure of human eEF1Bβ C-terminal fragment (residues 153–281), comprising a central acidic region (termed the CAR domain) and the GEF domain, was solved by NMR. Both human eEF1Bα and eEF1Bβ GEF domains superimpose with an RMSD of 1.6Å, revealing a highly conserved overall fold [11]. The CAR domain was described as a new structural element of eEF1Bβ that binds translationally controlled tumor protein (TCTP) [12]. The CAR domain is conserved in all eEF1B guanine nucleotide exchange factors and is structurally independent from the GEF domain [11]. The less conserved N-terminal domain of eEF1Bβ interacts with the N-terminal part of eEF1Bγ [7, 8, 13] and the N-terminus of valyl-tRNA synthetase (ValRS) [7]. Another specific feature of eEF1Bβ is the presence of relatively long leucine-zipper motif that was suggested to be involved in the eEF1Bβ self-association [7, 14, 15]. Mansilla and co-workers demonstrated the direct interaction between eEF1Bβ molecules using yeast two-hybrid system [13]. However, the eEF1Bβ N-terminal fragment including the leucine-zipper motive was, surprisingly, not sufficient to sustain its self-association [13]. Thus, the oligomeric structure of eEF1Bβ and domain facilitating its self-association is still unidentified.

In this study, we attempted to delineate the structural region of human eEF1Bβ responsible for its self-association. For this purpose, several truncated forms of the protein were designed, purified to homogeneity and characterized by size-exclusion chromatography. In order to determine the significance of the eEF1Bβ N-terminal domain and the leucine-zipper motif for the catalytic activity of the GEF domain, the rate of GDP/GDP exchange on eEF1A1 and eEF1A2 in the presence of all eEF1Bβ N-terminally truncated forms were measured and compared to that of the full-length protein.

Materials and Methods

The conservative domains in the primary structure of eEF1Bβ were defined by multiple sequences alignment using CLUSTAL W [16]. The following eEF1Bβ amino acid sequences from Genbank database were used: *Homo sapiens*, NCBI reference sequence NP_001951.2; *Mus musculus*, GenBank accession number AAG17466.1; *Xenopus laevis*, NCBI reference sequence NP_001081523.1; *Drosophila melanogaster*, GenBank accession number Q9VL18.1; *Bombyx mori*, GenBank accession number BAB21109.1; *Hydra vulgaris*, NCBI reference sequence XP_002156180.1; *Salmo salar,* GenBank accession number ACN12466.1; *Caligus clemensi*, GenBank accession number ACO15230.1.

Plasmid construction

The cDNA fragments encoding the N-terminally truncated forms of human eEF1Bβ were produced by PCR using forward primers (as listed below) containing *EcoRI* restriction site and the reverse primer 5'- TTCTCGAGTCAAATCTTGTTGAAAGCTGC TATATCTACACTCT, containing an *XhoI* restriction site. The forward primer for the eEF1Bβ(43-281) fragment was 5'-AAGAATTCGCCAGCGTGATC CTCCGTGACATT, that for eEF1Bβ(78–281) was 5'-TTGAATTCGGTGAACTCGTCGTTCGTAT TGCCA, that for eEF1Bβ(117–281) was 5'-AAGA ATTCAAGAGTTCGCCTGGACACCGAGCCA, that for eEF1Bβ(136-281) was 5'-AAAGAATTC ATGCGCCAAGTGGAGCCCCCA and that for eEF1Bβ(183-281) – 5'-AAAGAATTCGCGGAGA AGAAGGCCAAGAA.

The cDNA encoding the leucine-zipper motif of eEF1Bβ comprising the residues 78-118 was amplified using the forward primer 5'-TTGAATTCGGTGA ACTCGTCGTTCGTATTGCCA and the reverse primer 5'-AAACTCGAGGCTCTTCTCCAGCACGT,

containing an *EcoRI* and an *XhoI* restriction sites, respectively. PCR was performed with DreamTaq DNA Polymerase (Thermo Fisher Scientific, USA) using as a template pGEX6P-1/eEF1Bβ plasmid encoding full-length human eEF1Bβ [17]. The PCR pro-ducts were purified and digested by *XhoI* and *EcoRI* (Thermo Fisher Scientific, USA), and ligated into pGEX6P-1 (GE Healthcare, UK) expression vector digested by the same enzymes. All obtained constructs were verified by sequencing. The resulting recombinant plasmids encoded the truncated forms of eEF1Bβ in-frame with the GST sequence.

Expression and purification of recombinant proteins

Full-length eEF1Bβ, also referred as to eEF1Bβ(FL), and its truncated forms were expressed in *Escherichia coli* BL21Gold strain (Stratagene, USA) grown on LB medium supplemented with 100 μg/ml ampicillin. All target proteins except for GST-eEF1Bβ(183–281) and GST-eEF1Bβ(78–118) were purified according to the procedure described below. The bacterial culture was grown at 37 °C and the expression of protein was induced for 3 hours with 1 mM isopropyl-β-D-thiogalactopyranoside when the absorbance at 600 nm reached 0.5. Then, the cells were harvested by centrifugation (3220 g for 10 min at 4 °C), washed twice (10 min at 4 °C) with 40 ml of ice-cold extraction buffer containing PBS (1.47 mM KH_2PO_4, 10 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl pH 7,4), 10 % glycerol and 5 mM 2-mercaptoethanol, followed by centrifugation (6000 g for 5 min at 4 °C). The cell pellet was dissolved in 8 ml of extraction buffer per 1 mg of cells and sonicated. All subsequent steps were performed at 4 °C. After centrifugation at 18500 g for 30 min, the clear supernatant was recovered. This solution was mixed with 4–5 ml of a 50 % slurry of Glutathione sepharose™ 4B (GE Healthcare, Sweden), pre-equilibrated with the same buffer, and incubated on the orbital shaker overnight. The resin was washed three times for 20 min in a tube with 40 ml of the extraction buffer, followed by centrifugation at 3000 g for 5 min. After the last wash Glutathione sepharose™ 4B was packed into a column and washed extensively to remove unbound material. The target protein was stepwise eluted using 20 mM glutathione, pH 8.0, in the extraction buffer. Fractions were collected and analyzed by SDS-PAGE. The purest fractions were combined and dialyzed against the cleavage buffer (30 mM Tris-HCl, pH7.5, 150 mM NaCl, 10 % glycerol, 5 mM 2-mercaptoethanol and 0.01 % Tween 20). The GST-tag was removed by incubation with PreScission protease (GE Healthcare, Germany) according to manufacturer's instruction. Then, the protein mixture was applied onto a HiTrap Q sepharose (1 ml) column (GE Healthcare, Sweden), equilibrated with cleavage buffer. The column was washed with 250 mM NaCl in buffer containing 30 mM Tris-HCl, pH7.5, 10 % glycerol, 5 mM 2-mercaptoethanol and 0.01 % Tween 20. eEF1Bβ and its N-terminally truncated forms were eluted from the column using a 250–450 mM NaCl gradient in the same buffer. Fractions were collected and analyzed by SDS-PAGE. The purest fractions were combined and dialyzed against the storage buffer (30 mM Tris-HCl, pH7.5, 150 mM NaCl, 55 % glycerol and 5 mM 2-mercaptoethanol).

GST-eEF1Bβ(183–281) was purified on a Glutathione sepharose™ 4B column only, as described above. The eEF1Bβ(183–281) fragment precipitated after incubation with PreScission protease, and therefore, the GST-eEF1Bβ(183–281) fusion protein was used for the experiments. The purest fractions of the fusion protein after a Glutathione sepharose™ 4B column were combined and dialyzed against the storage buffer.

GST-eEF1Bβ(78–118) was purified on a Glutathione sepharose™ 4B column, as described above. Then, the purest and most concentrated fractions were combined and applied onto a HiLoad 16/600 Superdex 200 per grade (16 × 600 mm, 120 ml) column (GE Healthcare, Sweden), equilibrated with buffer containing 25 mM Tris-HCl, pH7.5, 150 mM NaCl, 5 % glycerol and 5 mM 2-mercaptoethanol. Fractions were collected and analyzed by SDS-PAGE. The purest fractions of the major protein peak were combined and dialyzed against the storage buffer.

The proteins were stored at – 20 °C. The protein concentrations were determined using the following calculated absorption coefficients (A_{280} units·mg^{-1}·cm^2): 0.72 for eEF1Bβ(FL), 0.52 for eEF1Bβ(43–281), 0.60 for eEF1Bβ(78–281), 0.74 for eEF1Bβ(117–281), 0.81 for eEF1Bβ(136–281), 1.46 for GST-eEF1Bβ(183–281) and 1.36 for GST-eEF1Bβ (78–118).

Analytical gel filtration of proteins

Analytical gel filtration of all the purified proteins was performed on a Superose 6 HR 10/30 (24 ml) column (GE Healthcare, Sweden). The column was equilibrated with 25 mM imidazole HCl, pH 7.5, 150 mM NaCl, 5 % glycerol, 5 mM 2-mercaptoethanol. The sample (0.1 ml) was loaded onto the column and eluted at a flow rate of 0.4 ml/min. The column calibration and molecular mass determination of the protein of interest have been described previously [18].

Preparation of the eEF1Bβγ complex

Human recombinant eEF1Bγ was purified as described earlier [18]. For formation of the eEF1Bβγ complex, purified eEF1Bγ and eEF1Bβ(FL) were mixed in the buffer containing 25 mM imidazole HCl, pH 7.5, 150 mM NaCl, 5 % glycerol, 5 mM 2-mercaptoethanol and incubated during 5 min at 37 ºC. The final concentration of both eEF1Bγ and eEF1Bβ(FL) was 20 μM. Then, the eEF1Bβγ complex was purified on a Superose 6 HR 10/30 column (24 ml), equilibrated with the same buffer. The most concentrated fractions of the eEF1Bβγ complex were combined, dialyzed and concentrated against the storage buffer (30 mM Tris-HCl, pH7.5, 150 mM NaCl, 55 % glycerol and 5 mM 2-mercaptoethanol), and used to measure the rate of guanine nucleotide exchange on eEF1A1 and eEF1A2. The protein concentration of the eEF1Bβγ complex was determined using the calculated molar absorption coefficient 109820 M^{-1}·cm^{-1} assuming that the stoichiometry of the subunits in the complex is 1:1.

[^{3}H]GDP/GDP exchange

eEF1A2 and eEF1A1 were purified as previously described [19, 20]. The kinetics of guanine nucleotide exchange on eEF1A2 in the absence or presence of 4 nM concentration of eEF1Bβ(FL), its truncated forms or the eEF1Bβγ complex were performed at 25 °C as described in detail previously [21]. For eEF1A1, the same conditions were used except the concentration of exchange factors and the temperature of reaction. The concentration of eEF1Bβ(FL), its truncated forms or the eEF1Bβγ complex was 20 nM, and the exchange reaction was carried out at 0 °C. The reaction mixtures contained eEF1A1*[^{3}H]GDP or eEF1A2*[^{3}H]GDP complexes in a final concentration of 692 nM and 150 μM GDP.

First order rate constants of the GDP/GDP exchange reaction in the absence (k_{-1}) and in the presence (k_{app}) of exchange factors were obtained by fitting to a single exponential function ($y=A_1\exp(-x/t_1)+y_0$) using OriginPro 8 software (OriginLab, USA).

Results and Discussion

Domain structure of eEF1Bβ

By multiple sequence alignment four conservative domains can be delineated in the primary structure of eEF1Bβ from different species (Fig. 1A): the N-terminal domain (residues 1~82) contains highly conservative (residues 1~33) and low conservative (residues 33~82) regions, the leucine-zipper motive (residues 82~124) [14, 15], the central acidic region (residues ~165–210) [12] and the C-terminal domain (residues ~210–281) responsible for GEF activity [7]. The leucine-zipper motive and the CAR domain are connected by a linker region that possesses low homology among the different species. By contrast, the leucine-zipper motive is strongly conserved (Fig. 1A) implying an important function for this structure region. To explore the significance of the eEF1Bβ structural domains for its self-association and catalytic activity, several truncated forms of human eEF1Bβ were designed (Fig. 1B). First deletion mutant, eEF1Bβ(43–281), lacks the most conservative part of the N-terminal domain, whereas eEF1Bβ(78–281) is deprived of the entire N-terminal domain. Then we removed the leucine-zipper motive to generate the eEF1Bβ(117–281) deletion form.

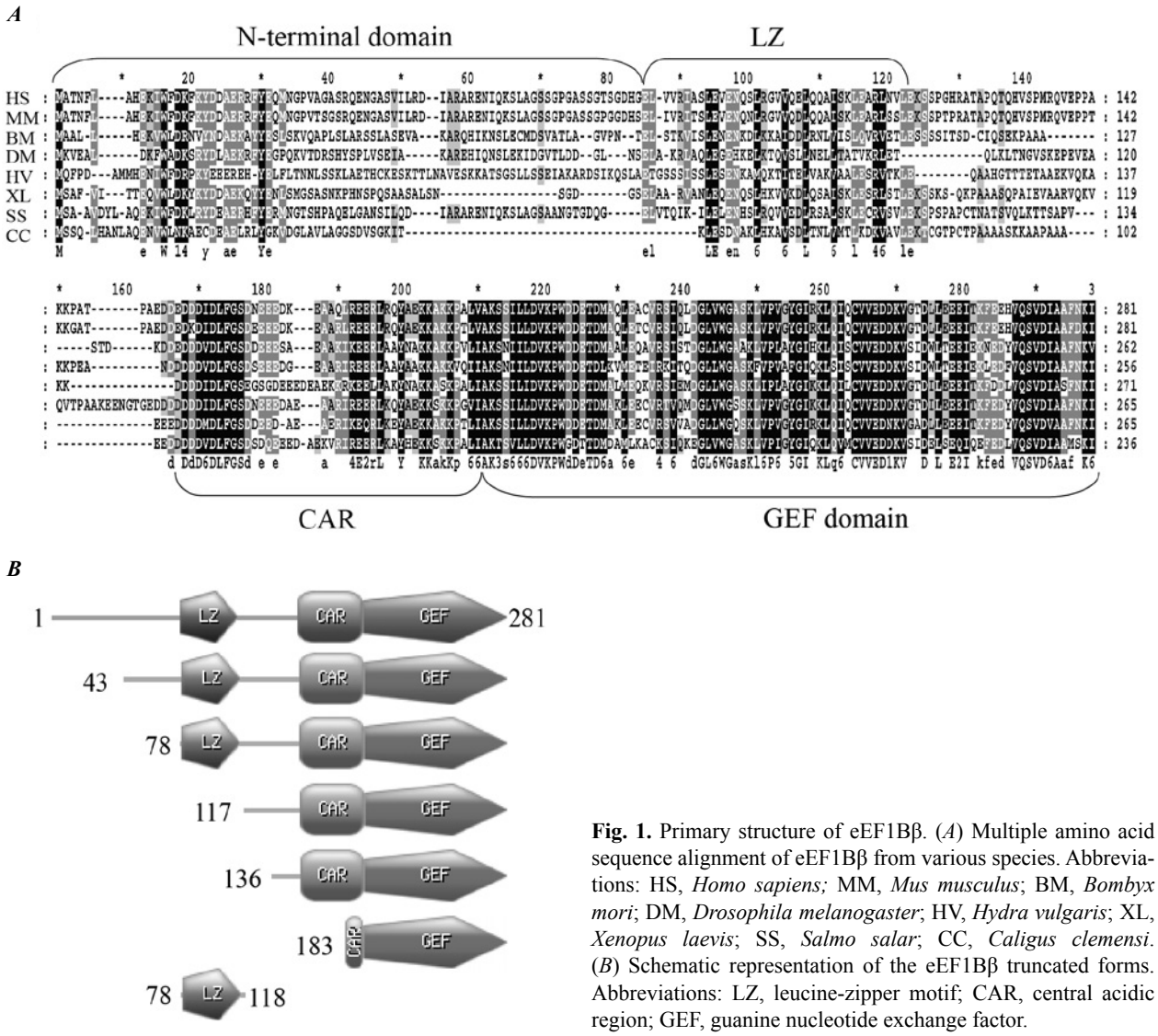


Fig. 1. Primary structure of eEF1Bβ. (*A*) Multiple amino acid sequence alignment of eEF1Bβ from various species. Abbreviations: HS, *Homo sapiens;* MM, *Mus musculus*; BM, *Bombyx mori*; DM, *Drosophila melanogaster*; HV, *Hydra vulgaris*; XL, *Xenopus laevis*; SS, *Salmo salar*; CC, *Caligus clemensi*. (*B*) Schematic representation of the eEF1Bβ truncated forms. Abbreviations: LZ, leucine-zipper motif; CAR, central acidic region; GEF, guanine nucleotide exchange factor.

eEF1Bβ(136–281) and eEF1Bβ(183–281) possess partly truncated linker and partly truncated central acidic region, respectively. The leucine-zipper motive comprising amino acids 78–118 fused to GST was also used in this study.

Purification of full-length eEF1Bβ and its truncated forms

Purification of eEF1Bβ(FL) and its several truncated forms, except for GST- eEF1Bβ(183–281) and GST-eEF1Bβ(78–118), was performed by two consecutive Glutathione sepharose™ 4B and HiTrap Q sepharose column chromatographies. The GST fusion proteins obtained after the first purification step were treated by PreScission protease. Further, the target proteins were separated from GST on a HiTrap Q sepharose column. eEF1Bβ(FL) and its truncated forms eEF1Bβ(43–281), eEF1Bβ(78–281), eEF1Bβ(117–281) and eEF1Bβ(136–281) bind HiTrap Q sepharose at 200 mM NaCl whereas GST does not. Finally, highly purified proteins were obtained after their elution from the column by a salt

gradient as jugged by SDS-PAGE (Fig. 2). The yield of eEF1Bβ(FL) was ~ 2 mg, that of eEF1Bβ(43–281) was ~ 7 mg, eEF1Bβ(78–281) was ~ 5 mg, that of eEF1Bβ(117–281) was ~ 5 mg, eEF1Bβ(136–281) was ~ 5 mg, per 1 L of BL21Gold bacterial culture.

GST-eEF1Bβ(183–281) was purified on a Glutathione sepharose™ 4B column only, because treatment with PreScission protease resulted in precipitation of the eEF1Bβ(183–281) fragment. About 22 mg of GST-eEF1Bβ(183–281) can be obtained from 1 liter of bacterial culture and its purity was more than 90 % as jugged by SDS-PAGE (Fig. 2).

The GST-eEF1Bβ(78–118) deletion form comprising the eEF1Bβ leucine-zipper motif was also purified by affinity chromatography and additionally on a HiLoad 16/600 Superdex 200 per grade column (Fig. 2). The yield of the GST-eEF1Bβ(78–118) protein was estimated to be ~ 4 mg per 1 L of BL21Gold bacterial culture.

Leucine-zipper motif of eEF1Bβ facilitates the formation of oligomers

The molecular masses of eEF1Bβ(FL) and its truncated variants were assessed by size-exclusion chromatography on a Superose 6 HR column (Fig. 3). eEF1Bβ(FL) was eluted from the column as a 350 kDa protein. This value is about 11 times greater than the value of its monomer molecular mass (Table 1), strongly suggesting the oligomerization of eEF1Bβ(FL). Interestingly, partial or complete deletion of the N-terminal domain only slightly changed the elution volume of the proteins: the molecular masses of eEF1Bβ(43–281) and eEF1Bβ(78–281) were estimated to be 340 and 305 kDa, respectively. These results clearly indicate that the N-terminal domain of eEF1Bβ may be considered as a distinct structural unit that is not involved into oligomerization of the full-length protein. Remarkably, deletion of the leucine-zipper motif (~5 kDa) causes considerable 5-fold decrease of the protein apparent molecular mass as estimated by gel filtration. The truncated form eEF1Bβ(117–281), lacking the leucine-zipper motif, eluted from the column as a 60 kDa protein (Fig. 3). Further shortening of the linker region resulted only in a slight decrease of the eEF1Bβ(136–281) molecular mass (58 kDa) compared to eEF1Bβ(117–281). The apparent molecular masses of GST-eEF1Bβ(183–281) and GST-eEF1Bβ(78–118) were estimated to be 175 and 380 kDa, respectively, suggesting that the self-association of these proteins occurs.

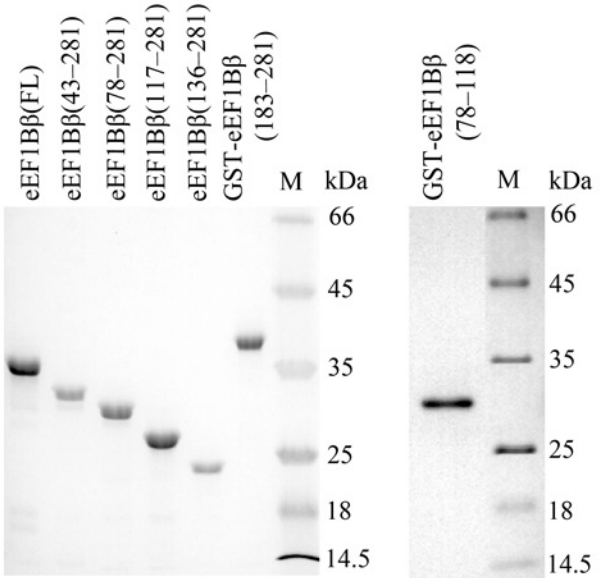
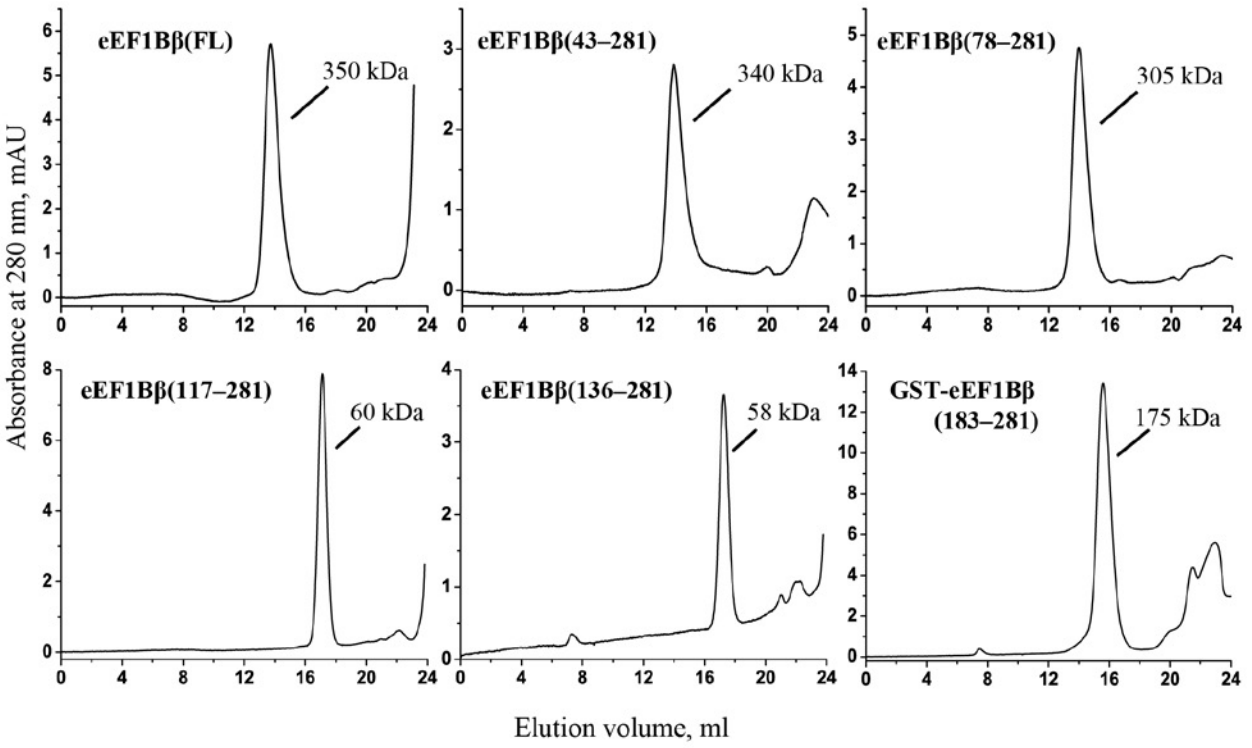


Fig. 2. SDS-PAGE of eEF1Bβ(FL) and its truncated forms. The indicated proteins (3 μg of each) were loaded onto a 13 % acrylamide gel.

Recently, the structure of the eEF1Bβ C-terminal fragment (residues 153–281), comprising the central acidic region and the GEF domain has been reported [11]. The GEF domain of eEF1Bβ has an elongated shape similarly to the GEF domain of the human and yeast eEF1Bα proteins [22, 23]. The CAR domain is folded into loop-α-helix-loop structure and is connected to the GEF domain by a flexible linker [11, 12]. Thus, the eEF1Bβ C-terminal fragment is a non-globular protein with the extended overall shape. This three-fold discrepancy between the experimental and theoretical molecular masses of the eEF1Bβ(117–281) and eEF1Bβ(136–281) deletion mutants may be rationally explained by the non-globular nature of both proteins (Table 1).

The difference between the theoretical and experimentally determined molecular masses of the GST-



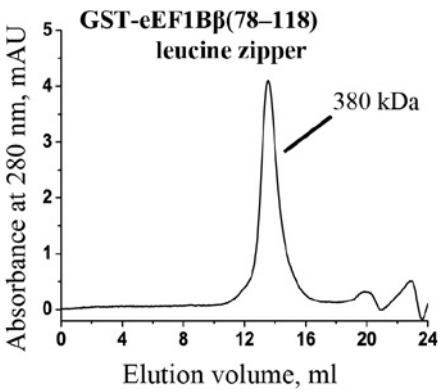


Fig. 3. Analysis of eEF1Bβ(FL) and its truncated variants by gel filtration: 100 μl of 10 μM eEF1Bβ(FL), eEF1Bβ(43–281), eEF1Bβ(77–281), eEF1Bβ(117–281) or eEF1Bβ(136–281), 17 μM GST-eEF1Bβ(183–281) or 5.3 μM GST-eEF1Bβ (77–118) were injected into a Superose 6 HR column.

eEF1Bβ(183–281) deletion form (Table 1) may be explained not only by an elongated shape of the GEF domain but also by homo-dimerization of GST. Noteworthy, GST-eEF1Bβ(183–281) is highly homologous to the GST-eEF1Bα(91–225) truncated form, which was shown to behave as a stable dimer in solution due to its GST moiety[9].

The GST-eEF1Bβ(78–118) fusion protein is composed of the eEF1Bβ leucine-zipper motif (~ 5 kDa) and GST (26 kDa). As mentioned above, GST-

Table 1. **Molecular masses of full-length eEF1Bβ and its truncated forms**

Protein	Molecular mass, kDa	
	Estimated by gel filtration	Theoretical (monomer)
eEF1Bβ(FL)	350	31.9
eEF1Bβ(43–281)	340	26.9
eEF1Bβ(78–281)	305	23.5
eEF1Bβ(117–281)	60	19.2
eEF1Bβ(136–281)	58	17.3
GST-eEF1Bβ(183–281)	175	38.2
GST-eEF1Bβ(78–118)	380	31.7

eEF1Bβ(78–118) was eluted from a Superose 6 HR column as a 380 kDa protein (Fig. 3, Table 1). This value is more than 11 times greater than the theoretical value of the GST-eEF1Bβ(78–118) monomer (Table 1). Taking into account that GST may dimerize, there is still more than five-fold difference between the experimental and the theoretical molecular masses. This result strongly suggests that oligomerization of this fusion protein is caused by the eEF1Bβ leucine-zipper motif. A leucine-zipper tends to fold into the α-helical structure that may self-associate to form a coiled coil dimer or oligomer [24]. Noticeably, the length of the eEF1Bβ leucine-zipper motif is twice that of other leucine-zipper proteins [15]. Consequently, it was hypothesized that such a long leucine-zipper motif may form a coiled coil trimer structure [25]. Taking into consideration possible trimerization capacity of the leucine-zipper motif and dimerization of GST, the existence of higher order oligomers like the GST-eEF1Bβ(77–118) hexamers may be expected. However, additional analytical ultracentrifugation experiments are required to confirm this assumption.

As mentioned above, the self-association between the eEF1Bβ molecules was shown by yeast two-hybrid system [13]. However, in the same study the N-terminal part of eEF1Bβ including leucine-zipper motif (residues 1–150) fused to the GAL4 binding domain was not able to sustain the interaction with eEF1Bβ(FL) fused to the GAL4 activating domain, which contradicts to our results. We believe that the absence of the interaction reported in [13] resulted from improper folding of the eEF1Bβ N-terminal fragment in yeast. In our experiments, similar in length N-terminal fragment of eEF1Bβ showed a high aggregation propensity (data not shown).

Hence, deletion of the leucine-zipper motif drastically decreased the apparent molecular mass of the truncated protein compared to full-length eEF1Bβ as judged by its elution profile on analytical gel filtration chromatography. On the other hand, GST fused with the leucine-zipper motif was eluted from the column as an oligomeric protein similar in size to eEF1Bβ(FL) (Table 1, Fig. 3). Thus, the obtained results favor the notion that the leucine-zipper motif is responsible for the eEF1Bβ self-association.

N-terminally truncated forms of eEF1Bβ display the catalytic activities similar to that of full-length protein

The kinetics of guanine nucleotide exchange on eEF1A1 and eEF1A2 in the presence of eEF1Bβ(FL) and its truncated forms were measured as described previously [9, 21]. As shown in Fig. 4A,B, the partial (42 amino acids) and complete (77 amino acids) deletion of the eEF1Bβ N-terminal domain only slightly increased (27 %) the rate of GDP/GDP exchange on eEF1A2. Further truncation of eEF1Bβ, namely deletion of the leucine-zipper motif and the part of linker region, did not change the rate of guanine nucleotide exchange, while partial truncation of the CAR region resulted in 19 % decrease of the rate of guanine nucleotide exchange on eEF1A2. eEF1Bγ did not influence the catalytic activity of eEF1Bβ: the apparent rate constants of GDP dissociation from eEF1A2 catalyzed by eEF1Bβγ complex or eEF1Bβ alone were similar.

Measurements of the rate of GDP/GDP exchange on eEF1A1 (Fig. 4C, D) in the presence of the eEF1Bβ N-terminally truncated forms, lacking 42, 77 and 116 amino acids, revealed that their catalytic activity is very similar to that of the full-length protein. In the presence of two other deletion mutants, eEF1Bβ(136–281) and GST-eEF1Bβ(183–281), the rate of nucleotide exchange decreased by 16 and 34 %, respectively, as compared to that of the full-length protein (Fig. 4C, D). Similarly to eEF1A2, the rate of GDP dissociation from eEF1A1 in the presence of the eEF1Bβγ complex was found to be the same as in the presence of eEF1Bβ alone (Fig. 4C, D).

Thus, stepwise truncation of the eEF1Bβ N-terminal region including the leucine-zipper motif does not significantly affect the catalytic activity of the GEF domain. However, partial deletion of the central acidic region decreased the rate of guanine nucleotide exchange, especially on eEF1A1 (Fig. 4). One may expect the CAR domain to be involved in

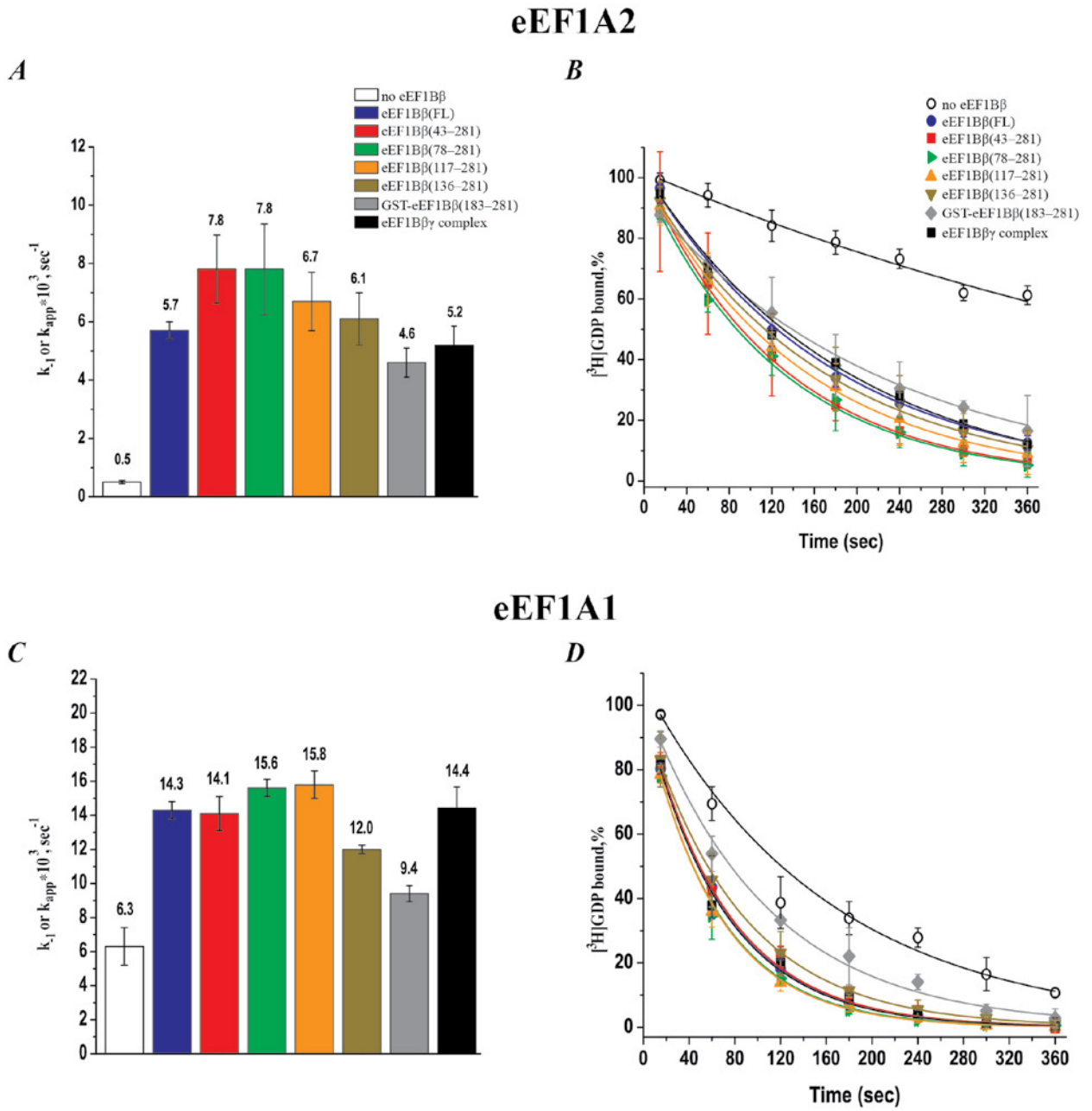


Fig. 4. Guanine nucleotide exchange rates on eEF1A1 and eEF1A2 in the absence and presence of full length eEF1Bβ, its truncated forms and the eEF1Bβγ complex. (*A, C*) First order rate constants of the [^{3}H]GDP/GDP exchange reaction on eEF1A2 (*A*) and eEF1A1 (*C*) in the absence (k_{-1}) and in the presence (k_{app}) of indicated exchange factors. The numbers above the bars indicate the mean values; the error bars represent standard deviations. (*B, D*) Time course of the [^{3}H]GDP/GDP exchange reactions on eEF1A2 (*B*) and eEF1A1 (*D*) without or with the indicated exchange factors. The time courses were obtained by averaging two independent kinetics experiments; the error bars represent standard deviations. Experimental data (symbols) were fitted to a single exponential function (curves). The first order rate constant (k_{-1}) of a spontaneous guanine nucleotide exchange on eEF1A2 was calculated from the kinetic curve obtained for the time period of 70 min (data not shown).

the interaction with eEF1A, however, steric hindrance of the eEF1A binding site by the GST moiety in the GST-eEF1Bβ(183–281) fusion protein cannot be completely excluded. Further experiments are required to verify this assumption.

eEF1Bγ stimulates the guanine nucleotide exchange activity of eEF1Bα but not eEF1Bβ [7, 9]. Recently, the mechanism for eEF1Bγ-mediated stimulation of eEF1Bα activity has been proposed. The N-terminal domain of free full-length eEF1Bα possesses the conformation that impairs eEF1A binding to the GEF domain and in such a way inhibits its catalytic activity. The formation of the stable complex between the eEF1Bγ and the eEF1Bα N-terminal domains may change the conformation of the latter and eliminate this inhibitory effect [9]. The N-terminal parts of eEF1Bα and eEF1Bβ are not homologous. Unlike eEF1Bα, eEF1Bβ has a leucine-zipper motif located in the central part of the molecule between the N-terminal and CAR domains (Fig. 1). Here, we demonstrated that the leucine-zipper motif mediates the self-association of the eEF1Bβ molecules (Fig. 3). As mentioned above, a leucine-zipper is usually folded into the α-helical region that may form the rigid coiled coil homo-dimer or homo-oligomer structures [24]. In contrast to eEF1Bα, such a rigid conformation of the eEF1Bβ N-terminal domain most likely does not interfere with eEF1A binding to the GEF domain. This may explain the reported earlier different effects of eEF1Bγ on the eEF1Bα and eEF1Bβ catalytic activities [7].

Conclusions

The obtained results favor the notion that full-length recombinant eEF1Bβ forms stable oligomers in solution and its leucine-zipper motif facilitates this oligomerization. As eEF1Bβ most probably belongs to a family of non-globular proteins, the exact type of its oligomerization will be determined in the future analytical ultracentrifugation experiments.

Deletion of the eEF1Bβ N-terminal domain does not significantly affect the guanine nucleotide exchange reaction catalyzed by the GEF domain, except for the case when the part of the CAR domain was removed. The inhibition of the GDP/GDP exchange rate after partial deletion of CAR suggests that this region might be involved into eEF1A binding to the catalytic domain. This assumption may be a topic for future studies.

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Структурний мотив типу «лейцинова застібка» відповідає за асоціацію молекул фактора елонгації трансляції 1Bβ

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Фактор елонгації трансляції 1Bβ (eEF1Bβ) – це білок, наявний лише у багатоклітинних організмів. Він каталізує обмін гуанінових нуклеотидів на факторі елонгації трансляції 1A (eEF1A). Відомо, що eEF1Bβ утворює олігомери. **Мета.** Визначити структурний домен eEF1Bβ людини, який опосередковує його олігомеризацію. Для встановлення ймовірного впливу N-кінцевої ділянки eEF1Bβ на каталітичну активність цього білка, було перевірено здатність різних вкорочених форм eEF1Bβ каталізувати обмін гуанінових нуклеотидів на обох ізоформах eEF1A ссавців. **Методи.** Фрагменти кДНК, які кодують вкорочені форми, eEF1Bβ отримували шляхом ПЛР ампліфікації, потім клонували у відповідні вектори, які експресували в клітинах *Escherichia coli*. Рекомбінантні білки очищали і їхні молекулярні маси визначали аналітичною гель-фільтрацією. Активність вкорочених форм eEF1Bβ перевіряли в реакції обміну гуанінових нуклеотидів. **Результати.** Видалення N-кінцевого домену eEF1Bβ не вплинуло на його олігомерізацію, в той час як видалення мотиву типу «лейцинова застібка» значно зменшувало молекулярну масу вкороченої форми білка в порівнянні з повнорозмірною. Також, амінокислотний мотив eEF1Bβ типу «лейцинова застібка» злитий з глутатіон S-трансферазою спричиняв олігомерізацію цього химерного білка. Показано, що всі вкорочені з N-кінця форми eEF1Bβ проявляли каталітичну активність, подібну до повнорозмірного білка. Інгібіторний ефект на каталітичну активність спостерігався лише для вкороченої форми білка, в якої була відсутня частина центрального негативно зарядженого регіону. **Висновки.** Амінокислотний мотив типу «лейцинова

застібка» сприяє олігомерізації рекомбінантного eEF1Bβ. Поступове вкорочення N-кінцевого домену не має значного впливу на каталітичну активність eEF1Bβ.

Ключові слова: фактор елонгації трансляції 1, обмін гуанінових нуклеотидів, структурні домени білків.

Структурный мотив типа «лейциновая застёжка» отвечает за ассоциацию молекул фактора элонгации трансляции 1Bβ.

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Фактор элонгации трансляции 1Bβ (eEF1Bβ) – белок, присутствующий только в многоклеточных организмах. Он катализирует обмен гуаниновых нуклеотидов на факторе элонгации трансляции 1A (eEF1A). Известно, что eEF1Bβ образует олигомеры. **Цель.** Определить структурный домен eEF1Bβ человека, который вызывает его олигомеризацию. Для исследования возможного влияния N-концевого участка eEF1Bβ на каталитическую активность этого белка, мы проверили способность различных укороченных форм eEF1Bβ катализировать обмен гуаниновых нуклеотидов на обеих изоформах eEF1A млекопитающих. **Методы.** Фрагменты кДНК, кодирующие укороченные формы eEF1Bβ, получали при помощи ПЦР амплификации, потом клонировали в соответствующие вектора, которые экспрессировали в клетках *Escherichia coli.* Рекомбинантные белки очищали и их молекулярные массы определяли аналитической гель-фильтрацией. Активность укороченных форм eEF1Bβ проверяли в реакции обмена гуаниновых нуклеотидов. **Результаты.** Полное удаление N-концевого домена не влияло на олигомеризацию eEF1Bβ, однако удаление мотива типа «лейцинова застёжка» значительно уменьшало молекулярную массу укороченной формы белка по сравнению с полноразмерной. Также, присоединение аминокислотного мотива eEF1Bβ типа «лейциновая застёжка» к глутатион S-трансферазе вызвало олигомеризацию этого химерного белка. Показано, что все укороченные с N-конца формы eEF1Bβ проявляли такую же каталитическую активность, как и полноразмерный белок. Слабый ингибирующий эффект на каталитическую активность наблюдался лишь для укороченной формы белка с отсутствующей частью центрального негативно заряженного региона. **Выводы.** Аминокислотный мотив типа «лейциновая застёжка» способствует олигомеризации рекомбинантного eEF1Bβ. Постепенное укорочение N-концевого домена не оказывает значительного влияния на каталитическую активность eEF1Bβ.

Ключевые слова: фактор элонгации трансляции 1, обмен гуаниновых нуклеотидов, структурные домены белков.

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