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Elongation factor 1 from the archaeobacterium *Halobacterium halobium* can be separated in three functionally different proteins

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Peptide elongation factor 1 (aEF-1) from an extremely halophilic archaeobacterium *Halobacterium halobium* could be separated in three components, i. e., aEF-1(I), aEF-1(IIA) and aEF-1(IIB) by the hydrophobic interaction chromatography. aEF-1(IIA) and aEF-1(IIB) made a complex and each of them was separated by addition of GDP. Both aEF-1(I) and aEF-1(IIA) bound GTP and promoted poly(Phe) synthesis in the presence of translocation factor aEF-2, whereas aEF-1(IIB) stimulated the GDP exchange of aEF-1(IIA) but not that of aEF-1(I). We conclude that most likely aEF-1(IIA) corresponds to EF-1 α (EF-Tu) and aEF-1(IIB) to EF-1 β (EF-Ts).

The basic mechanisms of the ribosomal elongation cycle are probably the same for the translational apparatus in all three kingdoms, *viz.* eubacteria, archaeobacteria, and eukaryotes. In fact, common features concerning tRNA binding capabilities of ribosomes and fundamental reactions of the elongation cycle could be demonstrated for both eubacteria (*Escherichia coli*) and archaeobacteria (*Halobacterium halobium*) (NIERHAUS *et al.*, 1986; SARUYAMA and NIERHAUS, 1986; SARUYAMA *et al.*, 1986). The relative evolutionary distance between archaeobacteria and eukaryotes seems to be much shorter than that between archaeobacteria and eubacteria as deduced from structure comparisons of 5S rRNA sequences (HORI and OSAWA, 1986) and RNA polymerases (GROPP *et al.*, 1986). Archaeobacterial translocating factor 2 (aEF-2) can be ADP-ribosylated by diphtheria toxin (KLINK, 1985), a characteristic trait of the corresponding eukaryotic factor EF-2 in contrast to eubacterial EF-G. Furthermore, an archaeobacterial gene (*Methanococcus vannielii*) cloned with a *tufB* gene probe from *E. coli* showed much closer similarity to eukaryotic EF-1 than to the eubacterial EF-Tu (LECHNER and BÖCK, 1987). However, the results of a comparison of 16S rRNA structures do not neatly fit in this picture. They indicated the two prokaryotic kingdoms, although clearly distinct, are relatively close as compared with the eukaryotes (WOESE, 1987). A characteristic feature of the eukaryotic EF-1 is its composition of three proteins, the functions of two of which could be

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assigned (KAZIRO, 1978). The EF-1 α corresponds to the aminoacyl-tRNA binding factor EF-Tu, and EF-1 β to the GDP-recycling factor EF-Ts. Such a recycling factor has not yet been found in archaeobacteria, even though aminoacyl-tRNA binding factor 1 (aEF-1) has been isolated from various archaeobacterial strains (KLINK, 1985).

In this paper, we reported the isolation of three functionally different aEF-1 from an extremely halophilic archaeobacterium *H. halobium*.

Materials and Methods

Buffers

Buffer A: 10 mM Tris-HCl, pH 7.6, 2.2 M (NH₄)₂SO₄, 60 mM Mg acetate, and 7 mM 2-mercaptoethanol. Buffer B: 50 mM Tris-HCl, pH 8.0, 60 mM NH₄Cl, 6 mM Mg acetate, and 7 mM 2-mercaptoethanol. Washing buffer: 10 mM Tris-HCl, pH 7.6, 2.7 M KCl, 0.4 M NH₄Cl, 60 mM Mg acetate, and 7 mM 2-mercaptoethanol.

Materials

L-[U-¹⁴C]Phenylalanine (504 Ci/mol) and [8-³H]GDP (11.7 Ci/mmol) were purchased from Amersham. Poly(U), tRNA^{Phe} derived from brewer's yeast, phosphoenolpyruvate, pyruvate kinase, ATP•2Na and GTP•2Li were obtained from Boehringer Mannheim. Fractogel TSK (HW-55F) was from Tosoh Corporation (Japan).

Preparation of ribosomal subunits and S-100 fraction

Ribosomal subunits and 100,000 \times g supernatant (S-100) fraction from an extremely halophilic archaeobacterium *Halobacterium halobium* S9 were prepared as described (SARUYAMA, 1986). 1 A₂₆₀ unit of 50 S and 30 S subunits was taken to be equivalent to 36 pmol and 72 pmol respectively.

Assay systems

aEF-1 activity was measured by binding with [³H]GDP in the optimized ionic condition for poly(Phe) synthesis (SARUYAMA and NIERHAUS, 1985). 40 μ l reaction mixture contains 10 mM Tris-HCl, pH 7.6, 2.0 M KCl, 2.0 M (NH₄)₂SO₄, 0.4 M NH₄Cl, 60 mM Mg acetate, 7 mM 2-mercaptoethanol, and [³H]GDP (1.1 μ M). After the incubation at 0°C for 20 min, the reaction mixture was filtrated through a nitrocellulose filter, washed three times with 2 ml of washing buffer and the radioactivity was measured.

aEF-1 activity was also measured by poly(U)-dependent poly(Phe) synthesis in the presence of aEF-2 purified from *H. halobium* (SARUYAMA and SASAKI, 1988) in the optimized ionic conditions as above. 100 μ l reaction mixture contains 10 mM Tris-HCl, pH 7.6, 2.0 M KCl, 2.0 M (NH₄)₂SO₄, 0.4 M

NH_4Cl , 60 mM Mg acetate, 7 mM 2-mercaptoethanol, 2.1 mM ATP, 0.5 mM GTP, 5 mM phosphoenolpyruvate, 2 μg pyruvate kinase, 20 μg tRNA^{Phe} from brewer's yeast, 100 μg poly(U), 100 μM [^{14}C] phenylalanine (13 Ci/mol), 0.45 A_{260} unit 30 S and 0.8 unit 50 S ribosomal subunits, 4.0 μg aEF-2, partially purified 4.6 μg phenylalanyl-tRNA synthetase and appropriate amounts of aEF-1. After 1 h incubation at 40°C, radioactivities in hot trichloroacetic acid-insoluble material were measured.

Results and Discussion

During an investigation of archaeobacterial elongation factors, we isolated first an aEF-1 activity from the extreme halophile archaeobacterium *H. halobium*. The activity was tested as GDP binding and migrated as a single fraction during Sepharose 4B, Sephadex G-100 and DEAE-cellulose chro-

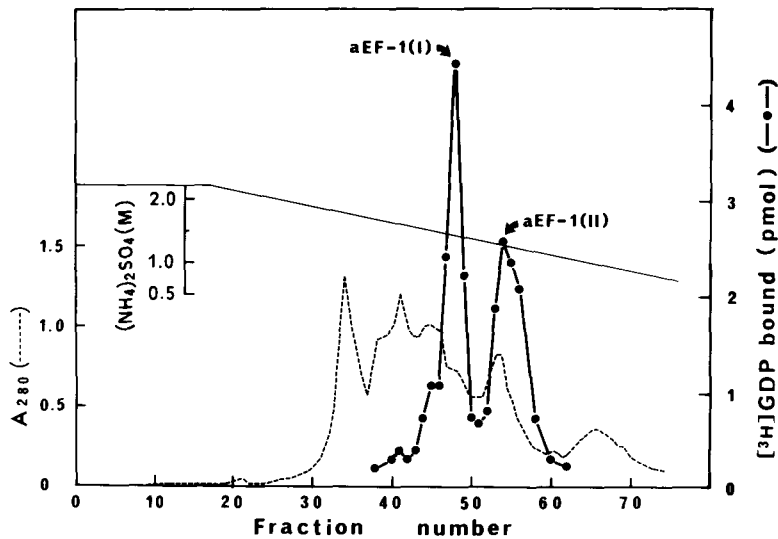


Fig. 1. Isolation of aEF-1 by Fractogel TSK. aEF-1 partially purified from S-100 fraction by two successive Sepharose 4B columns (SARUYAMA and SASAKI, 1988) was applied on a Fractogel TSK (HW-55F) column (1.5 × 45 cm) equilibrated with buffer A. The proteins were eluted by a linear concentration of $(\text{NH}_4)_2\text{SO}_4$ from 2.2 M to 0.5 M (2 × 300 ml).

Table 1. Poly(U)-dependent poly(Phe) synthesis promoted by elongation factors from *H. halobium*

aEF-2	aEF-1(I)	aEF-1(II)	poly(Phe) synthesis (p mol)
—	—	—	9.3
+	—	—	34.0
—	+	—	12.3
—	—	+	11.0
+	+	—	61.0
+	—	+	355.8

Poly(U)-dependent poly(Phe) synthesis promoted by aEF-1(I) (13.6 μg), aEF-1(II) (20.8 μg) and aEF-2 (4.0 μg) were measured in the optimized poly(Phe) system described in the **Materials and Methods**.

Table 2. Complementation of aEF-1(IIA) and aEF-1(IIB) in two assay systems, GDP-binding and poly(U)-dependent poly(Phe) synthesis

Protein	[³ H]GDP bound p mol (%)	poly(Phe) synthesis p mol (%)
aEF-1(IIA)	1.7	16.0
aEF-1(IIB)	0.2	5.0
aEF-1(IIA)+aEF-1(IIB)	4.2 (221)	94.5 (445)

aEF-1(IIA) (16.5 μg), aEF-1(IIB) (2.6 μg) or the both factors were incubated with [³H]GDP (1.1 μM) at 0°C for 1 min, then [³H]GDP-binding activity was measured. Poly(U)-dependent poly(Phe) synthesis was promoted in the presence of aEF-1(IIA) (3.3 μg), aEF-1(IIB) (2.1 μg) or the both factors.

matographies (data not shown) as like as other archaeobacterial strains (KLINK, 1985). However, interestingly after the partial purification of aEF-1 by two successive Sepharose 4B columns (SARUYAMA and SASAKI, 1988) the activity separated in two fractions using the hydrophobic interaction column chromatography (Fractogel TSK, HW-55F; Fig. 1). Both factors referred to as aEF-1(I) and aEF-1(II) promoted poly(U) dependent poly(Phe) synthesis in the presence of the translocation factor aEF-2 (Table 1). aEF-1(I) and aEF-1(II) eluted at 1.4 M and 1.2 M (NH₄)₂SO₄, respectively suggesting that these factors differ in their hydrophobicities. Furthermore, these factors were clearly different with respect to their kinetics for GDP-binding, i. e.,

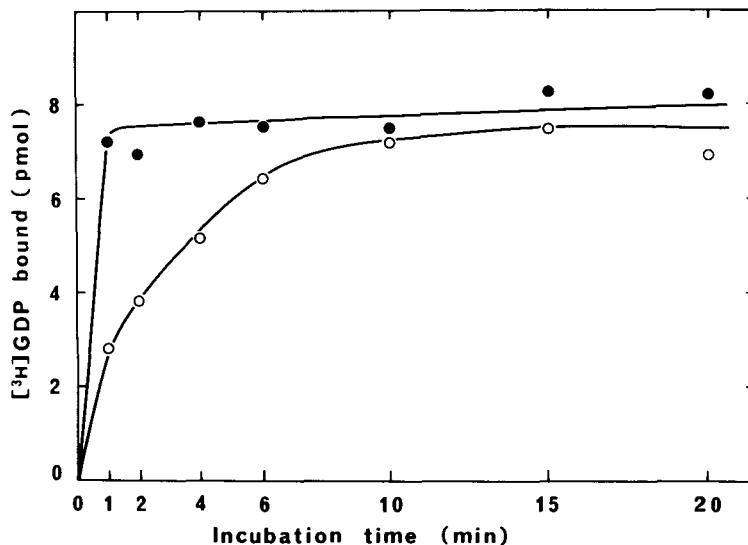


Fig. 2. Kinetics of [³H]GDP-binding of aEF-1(I) and aEF-1(II). 360 μ l of reaction mixture containing aEF-1(I) (378 μ g) or aEF-1(II) (603 μ g) and [³H]GDP (1.1 μ M) was incubated at 0°C in the optimized ionic condition. At the chosen time intervals 50 μ l of aliquot was withdrawn, and the bound [³H]GDP was measured. (—○—), aEF-1(I); (—●—), aEF-1(II).

aEF-1(I) required more than 10 min for saturation in contrast to aEF-1(II) showing the quick completion within 2 min (Fig. 2). Obviously, the factors are different, and one possibility is that aEF-1(II) represents a complex of aEF-1(I) and a GDP-recycling factor known in eubacteria (EF-Ts) (MILLER and WEISSBACH, 1977) and eukaryotes (EF-1 β) ((MOLDAVE, 1985).

Next, in order to isolate the presumed EF-Ts-like factor in aEF-1(II), aEF-1(II) was incubated with 500 μ M GDP at 37°C for 30 min, dialyzed against buffer A contained 50 μ M GDP, and rechromatographed on Fractogel TSK but now in the presence of 50 μ M GDP. The proteins were eluted by a linear concentration gradient (NH₄)₂SO₄ from 2.2 M to 0 M. First [³H]GDP-binding fraction (aEF-1(IIA)) was eluted at around 1.2 M (NH₄)₂SO₄, the second one (aEF-1(IIB)) was eluted with buffer B. Table 2 demonstrates that aEF-1(IIA) clearly binds GDP and promoted poly(Phe) synthesis in the presence of aEF-2, whereas aEF-1(IIB) showed very low activities in both assay systems. Interestingly, the addition of aEF-1(IIA) to aEF-1(IIB) stimulated both GDP binding and poly(Phe) synthesis more than two and four times,

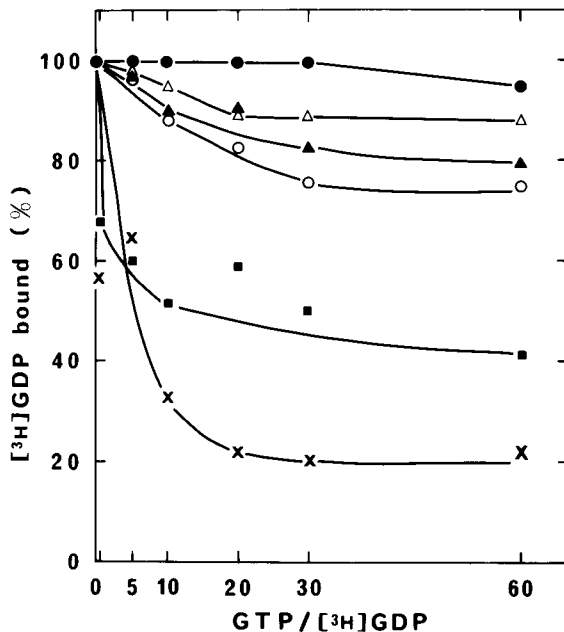


Fig. 3. Effect of aEF-1(IIB) on exchange of bound [^3H]GDP with free GTP. [^3H]GDP ($1.1\ \mu\text{M}$) and the indicated factors were incubated at 0°C for 5 min in the optimized ionic condition, and then various amounts of non-labeled GTP were added. After 1 min incubation at 0°C the bound [^3H]GDP was measured. (—○—), aEF-1(I); (—●—), aEF-1(IIA); (—△—), aEF-1(I) plus aEF-1(IIA); (—▲—), aEF-1(I) plus aEF-1(IIB); (—■—), aEF-1(IIA) plus aEF-1(IIB); and (—×—), S-100.

respectively as compared to the additive activities of both factors (Table 2). In contrast, aEF-1(IIB) stimulated neither activities of aEF-1(I) (data not shown). SDS-PAGE analysis revealed molecular mass of 56 kDa and 54 kDa for aEF-1(I) and aEF-1(IIB), respectively (data not shown), underlining that both factors represent different proteins.

It has been reported that GDP bound to aEF-1 from *Halobacterium cutirubrum* can be easily replaced by GTP indicating an intermediate affinity to GDP as compared to the corresponding factors from eubacteria (high affinity) and eukaryotes (low affinity) (KLINK, 1985). In contrast, our chasing experiment (Fig. 3), where bound [^3H]GDP is chased by non-labeled GTP indicates that this view has to be modified. aEF-1(I) and aEF-1(IIA) showed

an exchange of only 20 and 5% of the bound GDP, respectively, suggesting high affinities to GDP of both factors. However, a striking increase of the exchange (up to 60% of bound GDP) was observed when aEF-1(IIA) and aEF-1(IIB) were combined. This titration curve is very similar to that obtained with unfractionated enzymes (S-100, Fig. 3) and that reported for aEF-1 of *H. cutirubrum* (KLINK, 1985). Neither aEF-1(IIA) nor aEF-1(IIB) stimulated the releases of bound GDP from aEF-1(I)

In conclusion, elongation factor aEF-1 from *H. halobium* can be separated in three proteins of aEF-1(I), aEF-1(IIA) and aEF-1(IIB). The similarity to eukaryotic EF-1 is evident. Most probably, aEF-1(IIA) corresponds to the aminoacyl-tRNA binding factor EF-1 α , and aEF-1(IIB) to the GDP-recycling factor EF-1 β . The importance of aEF-1(I) is not yet clear, its molecular mass of 56 kDa is similar to that of EF-1 γ (MOLDAVE, 1985). These results together with the eukaryotic features of aEF-2 from extreme halophiles (KESSEL and KLINK, 1980; SARUYAMA and SASAKI, 1988) strongly suggest that the archaeobacterial peptide elongation factors are of the eukaryotic type.

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