EXCHANGEABILITY OF SILK GLAND ELONGATION FACTOR 1b AND PIG LIVER ELONGATION FACTOR 1β IN POLYPEPTIDE CHAIN ELONGATION

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1. Introduction

Three polypeptide chain elongation factors, i.e., EF-Tu, EF-Ts and EF-G, have been purified and their properties studied extensively in prokaryotes. While in eukaryotes only two factors, i.e., EF-1 and EF-2, had been known until EF-1 was resolved into two complementary factors, EF-1a(APase I) and EF-1b (APase II), in the silk gland [1,2]. The latter which corresponds to the smallest subunit (mol. wt 26 000) of EF-1 was found to stimulate 3 reactions:

(i) EF-1a- and EF-2-dependent polyphenylalanine synthesis (polymerization reaction);
(ii) EF-1a-dependent binding of [^{14}C]Phe-tRNA to ribosome (binding reaction);
(iii) Exchange of GDP bound to EF-1a with exogeneous GTP (exchange reaction).

From these results it was concluded that the EF-1b factor corresponds to the EF-Ts [2]. Similar factors were also prepared from pig liver [3], *Artemia salina* [4] and rabbit reticulocyte [5]. However, except for the pig liver EF-1β characterization of their proteins and functions is obscure. Recently pig liver EF-1β (mol. wt 30 000) has been purified to apparent homogeneity and shown to stimulate the three reactions [6]. From these results it seems most likely that the EF-1β factor corresponds to EF-1b.

This paper describes the complete exchangeability of the silk gland EF-1b and the pig liver EF-1β in the polymerization, binding and guanine nucleotide exchange reactions. Similarity of the amino acid compositions of EF-1b, EF-1β and EF-Ts is also noted.

2. Materials and methods

2.1. *Silk gland EF-1a and EF-1b*

Homogeneously purified EF-1a and EF-1b were prepared as in [2].

2.2. *Pig liver EF-1α and EF-1β*

EF-1α was prepared as in [7]. EF-1βγ (mol. wt 90 000) which consists of two subunits, EF-1β (mol. wt 30 000) and EF-1γ (mol. wt 55 000), was prepared as in [8]. It was resolved into each subunit by chromatography on a DEAE-cellulose (DE-32) in the presence of 6 M urea as described for the preparation of the silk gland EF-1b [2].

2.3. *Assay for polymerization reaction*

Poly(U)-dependent polyphenylalanine synthesis in the presence of heterogeneous combinations of EF-1a or EF-1α, EF-1b or EF-1β, and pig liver EF-2, was carried out as in [1].

2.4. *Assay for binding reaction*

Poly(U)-dependent binding of [^{14}C]Phe-tRNA to ribosome in the presence of EF-1a or EF-1α, and EF-1b or EF-1β, was carried out as in [1].

2.5. *Assay for exchange reaction*

Stimulation of the exchange of [^{3}H]GDP bound to EF-1a or EF-1α with exogeneous GTP by EF-1β or EF-1b was assayed as in [2]. The reaction mixture contained, in total vol. 0.1 ml, 50 mM Tris–HCl, pH 7.6, 10 mM $MgCl_2$, 70 mM KCl, 2 mM 2-mercapto-

ethanol, 20% glycerin (v/v), 50 μg bovine serum albumin, 38–60 pmol [^{3}H]GDP, and EF-1a or EF-1α, was incubated for 3 min at 37°C. Then various amounts of EF-1b or EF-1β and 10 nmol GTP were added to the mixture and reacted for 60 s at 0°C. The mixture was spotted on a membrane filter (TM-2, 0.45 μm, Toyo) and washed with three 0.5-ml portions of cold buffer (0.05 M Tris–HCl, (pH 7.6), 50 mM KCl, 10 mM $MgCl_2$, 15% glycerin (v/v) and 2 mM 2-mercaptoethanol). The radioactivity retained on the filter was measured in a liquid scintillation spectrometer.

2.6. *Amino acid analysis of EF-1b*

The purified EF-1b was hydrolyzed in constantly-boiling 6 N HCl in an evacuated and sealed tube for 22 h at 105°C. The hydrolysate was evaporated to dryness under a reduced pressure at 40°C and analyzed with a Hitachi Model KLA-3B amino acid analyzer.

3. Results and discussion

In [2] we have shown the resolution of EF-1_H into three subunits, i.e., EF-1a (α subunit, mol. wt 51 000), EF-1b (γ subunit, mol. wt 26 000) and EF-1c (β subunit, mol. wt 46 000) by chromatographies on a DEAE-cellulose (DE 32) and CM-cellulose (CM 32) in the presence of 6 M urea. It was also shown that the action of EF-1b corresponds to EF-Ts. Pig liver EF-1βγ [8] was also resolved into each subunit by the same methods used for the preparation of the silk gland EF-1b. EF-1γ was not absorbed on the DEAE-cellulose column, while EF-1β was absorbed and eluted at ~0.2 M KCl. Each subunit was dialyzed against a buffer solution which did not contain urea, and assayed for EF-1b activities. Since only EF-1β (mol. wt 30 000) had some activity, it was used in the polymerization, binding, and exchange reactions to know the mutual exchangeability with EF-1b.

To compare the activities of EF-1b and EF-1β in the polymerization reaction, we used them in the protein ratio 5:6 based on mol. wt 26 000 and 30 000, respectively. As shown in fig.1, EF-1b and EF-1β were completely exchangeable in the stimulation of EF-1a- or EF-1α-, and EF-2-dependent polyphenylalanine synthesis. That is, in spite of heterogeneous combinations of EF-1a and EF-1β, or EF-1α and

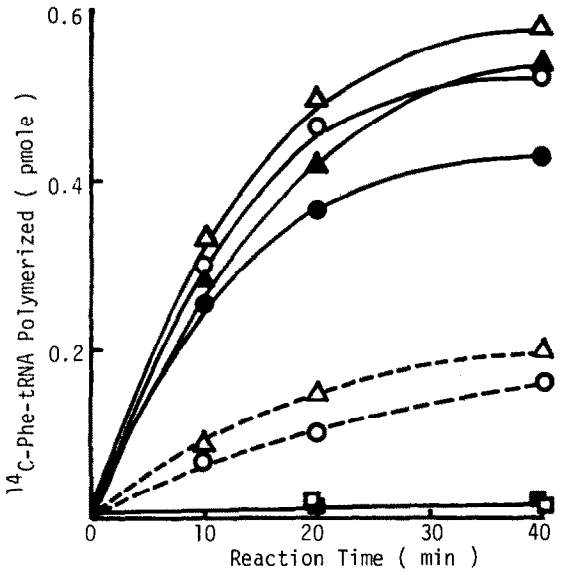

Fig.1. Effect of EF-1b or EF-1β on the EF-1a- or EF-1α-, and EF-2-dependent polyphenylalanine synthesis. Reaction was carried out using 0.45 μg EF-1a and EF-1α, 0.05 μg EF-1b, 0.06 μg EF-1β, 3.5 μg EF-2, in the following combinations. EF-1a, EF-1b and EF-2(–●–); EF-1a, EF-1β and EF-2(–○–); EF-1α, EF-1β and EF-2(–△–); EF-1α, EF-1b and EF-2(–▲–); EF-1a and EF-2 (- -○- -); EF-1α and EF-2 (- -△- -); EF-1b and EF-2(–□–), EF-1β and EF-2(–■–).

EF-1b, they showed nearly the same activities as those of homologous combinations. As there were no activities with the combination of EF-1b and EF-2, of EF-1β and EF-2, EF-1b and EF-1β preparations were not contaminated with EF-1a or EF-1α, respectively. Figure 2 shows that EF-1b stimulated markedly EF-1α-dependent binding of [^{14}C]Phe-tRNA to ribosome. The extent of stimulation was nearly the same as that of homologous combination EF-1a and EF-1b. EF-1β stimulated similarly EF-1a-dependent binding reaction. In the absence of EF-1a or EF-1α, EF-1b or EF-1β had no activity on the binding reaction. Figure 3 shows the amount of [^{3}H]GDP bound to EF-1a was markedly reduced by the addition of increasing amounts of EF-1b or EF-1β. While in the absence of EF-1β [^{3}H]GDP bound to EF-1a was not displaced with unlabelled GTP at 0°C during a 30 s incubation period. Figure 4 shows the effect of EF-1b or EF-1β on the exchange of [^{3}H]GDP bound to EF-1α with exogeneous GTP. Essentially the same

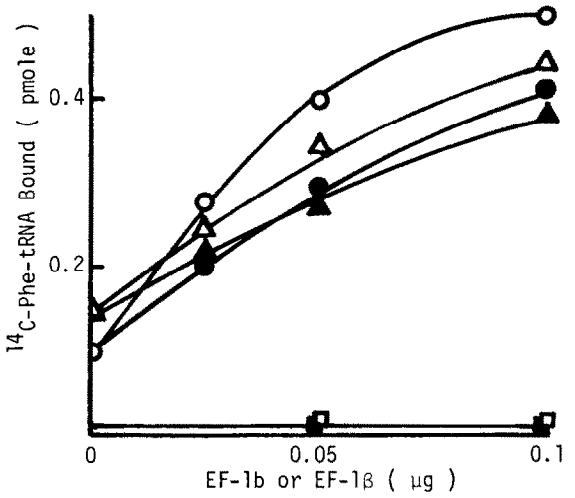

Fig.2. Effect of EF-1b or EF-1β on the EF-1a- or EF-1α-dependent binding of [^{14}C]Phe-tRNA to ribosome. The binding reaction was carried out at 25°C for 30 min in the presence of: 1.3 μg EF-1a and EF-1b(—●—); EF-1a and EF-1β(—○—); 1.3 μg EF-1α and EF-1β(—△—); EF-1α and EF-1b(—▲—); EF-1b(—□—); EF-1β(—■—).

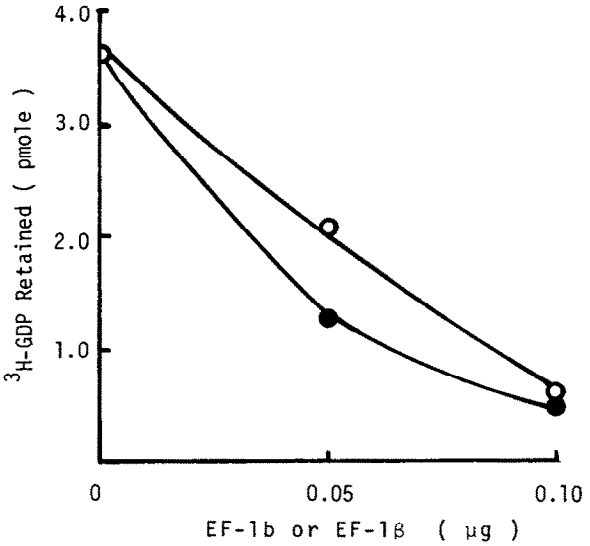

Fig.3. Effect of EF-1b or EF-1β on the exchange of [^{3}H]-GDP bound to EF-1a with exogeneous GTP. The exchange reaction was carried out as in section 2 in the presence of 1.4 μg EF-1a and various amounts of EF-1b(—○—), or 1.4 μg EF-1a and various amounts of EF-1β(—●—).

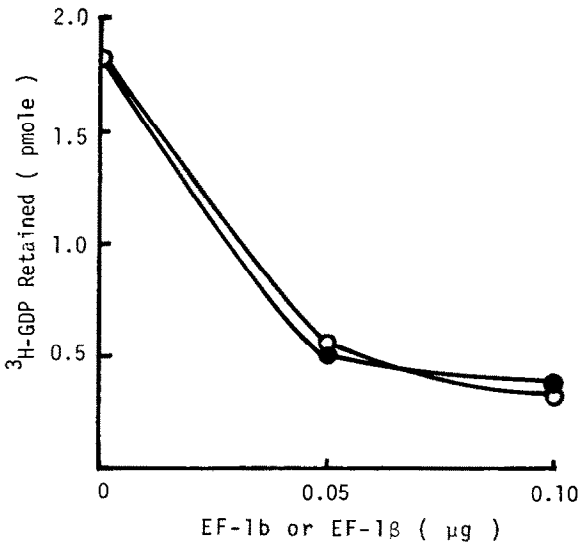

Fig.4. Effect of EF-1b or EF-1β on the exchange of [^{3}H]-GDP bound to EF-1α with exogeneous GTP. The exchange reaction was carried out as in section 2 in the presence of 1.2 μg EF-1α and various amounts of EF-1b(—○—), or 1.2 μg EF-1α and various amounts of EF-1β(—●—).

results as those in fig.3 were obtained in this experiment. The specific activities of EF-1b and EF-1β were nearly equal.

As shown above the silk gland EF-1b was completely replaced by the pig liver EF-1β. These results clearly show that EF-1b and EF-1β are the same factors and correspond to EF-Ts. Although it has been established that EF-1 or EF-2 from various eukaryotes is exchangeable with each other, this is the first case of a demonstration of the exchangeability of EF-1 which had resolved into complementary factors. Thus it is of interest to compare the amino acid compositions of EF-1b and EF-1β. Table 1 shows the amino acid compositions of EF-1b. When these data were compared with those of EF-1β in [6], a close similarity was found between them. An overall similarity was found also between EF-1b and EF-Ts [9]. The ratios of acidic to basic amino acids of EF-1b, EF-1β, and EF-Ts were 2.3, 2.2, and 1.9, respectively. The isoelectric points of EF-1b and EF-1β were both ~5. These data and chromatographic properties of these factors show that the three factors are acidic proteins with similar characters. A similar

Table 1
Amino acid compositions of purified EF-1b

Residue	mol%
Lysine	8.8
Histidine	0.6
Arginine	3.3
Aspartic acid	13.5
Threonine	4.8
Serine	6.2
Glutamic acid	15.4
Proline	5.3
Glycine	6.1
Alanine	9.9
Half-cystine	trace
Valine	7.4
Methionine	1.1
Isoleucine	4.1
Leucine	8.3
Tyrosine	3.1
Phenylalanine	2.3

factor which corresponds to EF-1b has been also obtained from pine pollen EF-1_H (to be published elsewhere). From the above results it should be stressed that the mechanisms of polypeptide elongation are universal throughout all organisms. Since *Artemia salina* [10] and wheat germ [11] EF-1 contain three different subunits as we originally noted in the silk gland [12], the smallest subunits (mol. wt $\simeq$ 30 000) most likely correspond to EF-1b.

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