ELONGATION FACTOR 1 FROM THE SILK GLAND OF SILKWORM

Purification and some properties of its γ subunit having EF-1b activity

Shin-ichiro EJIRI, Kazuyuki MURAKAMI and Teizo KATSUMATA
Department of Agricultural Chemistry, Iwate University, Morioka 020, Japan

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1. Introduction

Elongation factor 1 (EF-1), the factor responsible for binding aminoacyl-tRNA to ribosomes, exists in multiple forms in a variety of different eukaryotes [1,2]. The heavier forms ($EF\text{-}1_H$, mol. wt > 150 000) represent aggregates of a lighter form($EF\text{-}1_L$, mol. wt approx. 60 000) [3–5]. On the other hand, we previously noted that silk gland EF-1(APase I) consisted of three different subunits (α, β and γ) [6]. Their molecular weights were estimated to be about 63 000, 60 000 and 30 000, respectively. Furthermore $EF\text{-}1_H$ was resolved into two complementary factors, EF-1a(APase I) and EF-1b(APase II), which were thought to correspond to prokaryotic EF-Tu and EF-Ts, respectively [7]. Similar factors were also observed in pig liver [8], rabbit reticulocyte [9], wheat embryo [10] and *Altemia salina* cysts [11]. From these results it is of interest and important to compare the properties of each subunit in detail. However, for this purpose the recovery of each subunit of silk gland EF-1 was low in the former procedure [7] because only a part of it was dissociated under the undenaturing conditions. Thus, we tried to dissociate and purify each subunit under denaturing conditions. In this paper we describe the procedures for the purification and some properties of the γ subunit which showed EF-1b(EF-TS-like) activities.

2. Materials and methods

2.1. *Preparation and purification of EF-1 and its subunits*

Silk gland EF-1 was prepared from the postmitochondrial fraction by 0.4–0.65 saturation with ammonium sulfate and hydroxylapatite column chromatography as described previously [7]. The active fractions were pooled and precipitated with 0.65 saturation of ammonium sulfate. The precipitate was dissolved in 5 ml buffer A (0.05 M Tris–HCl, pH 7.6, 50 mM KCl, 15% (w/v) glycerine and 2 mM 2-mercaptoethanol) and applied to a Sepharose 6B column (1.6 × 100 cm) equilibrated beforehand with buffer A. The flow rate was 0.25 g/min and 4 g portions were collected. An aliquot was used for the binding assay. Three peaks of EF-1($EF\text{-}1_H$, $EF\text{-}1_M$ and $EF\text{-}1_L$, mol. wt approx. 600 000, 170 000 and 60 000, respectively) were obtained. Since $EF\text{-}1_H$ and $EF\text{-}1_M$ contained α, β and γ subunits, they were used for the preparation of each subunit. $EF\text{-}1_H$ (fractions 17–23) and $EF\text{-}1_M$ (fractions 24–32) were combined (about 10 mg protein) and dialized against buffer B (8 M urea, 0.05 M Tris–HCl, pH 8.0, 50 mM KCl and 2 mM 2-mercaptoethanol) overnight at 4°C. It was chromatographed on a DEAE–Sephadex column (1.0 × 10 cm) equilibrated beforehand with buffer B containing 6 M urea. The column was eluted with 0.05–0.35 M KCl gradient in buffer B containing 6 M urea. α and β subunits were passed through the column (about 6 mg) and the γ subunit was eluted at about 0.2 M KCl (about 2 mg). Resolution of the α and β subunits was done with CM–Sephadex column chromatography (details will be published elsewhere).

2.2. *Assay of EF-1b activities of the γ subunit*

EF-1b (EF-Ts-like) activities of the isolated subunits were assayed by three methods:

(i) The stimulation of polyphenylalanine synthesis in the presence of a limited amount of EF-1a and a saturating amount of EF-2(polymerization assay) [7].
(ii) The stimulation of [^{14}C]Phe-tRNA binding to ribosomes in the presence of a limited amount of EF-1a (binding assay) [7].
(iii) The stimulation of the exchange of GDP bound to EF-1a with exogeneous GTP (exchange assay).

The polymerization and binding assays were carried out as described previously [7] in the presence of 1.8 μg EF-1a(EF-1_L obtained by the Sepharose 6B column chromatography), 2.0 μg EF-2, and each subunit. The exchange assay was as follows. The reaction mixture containing, in total vol. 0.1 ml, 50 mM Tris–HCl, pH 7.6, 10 mM $MgCl_2$, 50 mM KCl, 2 mM 2-mercaptoethanol, 200 mM sucrose, 50 μg bovine serum albumin, 200 pmol GDP and 0.6 μg EF-1a, was incubated for 1 min at 0°C. Then, 1.4 μg γ subunit and 2.64 pmol [^{3}H]GTP were added to the mixture and reacted at 0°C for the time indicated in fig.3. The mixtures were spotted onto a membrane filter (TM-2, 0.45 μm, Tóyó) and washed with two, 1 ml portions of cold buffer A containing 10 mM $MgCl_2$. The radioactivity retained on the filter was measured in a liquid scintillation spectrometer. Assay for exchange of [^{3}H]GDP bound to EF-1a with GTP was essentially the same as above, except that 57.4 pmol [^{3}H]GDP was used instead of GTP in the first incubation and [^{3}H]GTP was replaced by 250 pmol GDP in the second incubation.

2.3. *Sodium deoxycholate treatment and Sephadex G-75 gel chromatography of γ subunit*

The γ subunit prepared as described above was treated at 0°C or 25°C for 15 min with 1% sodium deoxycholate (DOC). It was chromatographed on Sephadex G-75 column (0.8 × 30 cm) equilibrated beforehand with buffer C (20 mM potassium phosphate buffer, pH 7.6, 2 mM 2-mercaptoethanol, 15% (w/v) glycerin and 1% (w/v) DOC. Four drop (300 μl) portions were collected and assayed for EF-1b activity and protein.

2.4. *Polyacrylamide gel electrophoresis*

SDS polyacrylamide gel electrophoresis of EF-1_H and γ subunit was carried out according to Weber and Osborn [12], 7.5% gel and 1% SDS being used.

3. Results and discussion

As shown in fig.1, the γ subunit of silk gland EF-1 prepared as described above was homogeneous on SDS–polyacrylamide gel electrophoresis. It corresponde to the smallest subunit of EF-1_H and its mol. wt was 30 000.

As shown in fig.2, γ subunit stimulated the binding of [^{14}C]Phe-tRNA to ribosomes in the presence of EF-1a and GTP. This stimulation was not observed when GTP was substituted for GMP-PCP. Since γ subunit itself showed no activity in the binding reaction, it was concluded that the γ subunit preparation was not contaminated with EF-1a(α subunit). γ subunit stimulated also polyphenylalanine synthesis in the presence of EF-1a, EF-2 and GTP. Both binding and polymerization reactions were stimulated by γ subunit only when a limited amount of EF-1a was used.

Since EF-1b(APase II) stimulated the binding of GTP to EF-1a(APase I) as noted previously [7], γ subunit was tested for this activity. As shown in fig.3, γ subunit stimulated the binding of [^{3}H]GTP to EF-1a previously bound with GDP. It stimulated also the exchange of [^{3}H]GDP bound to EF-1a with exogeneous

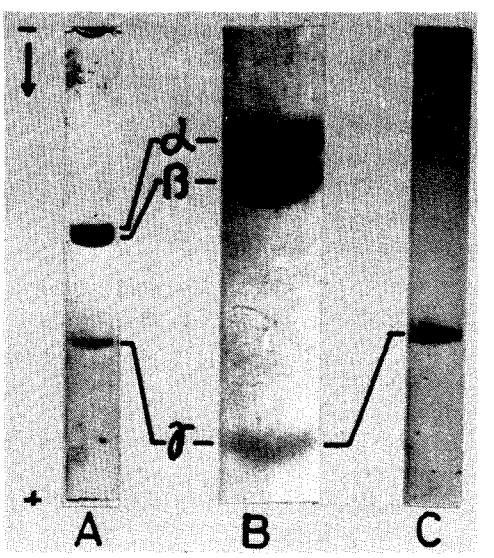

Fig.1. SDS–polyacrylamide gel electrophoresis of EF-1_H and γ subunit. The gel electrophoresis was carried out as described under Materials and methods. (A) EF-1_H (25 μg); (B) enlarged photograph of EF-1_H; (C) purified γ subunit (10 μg).

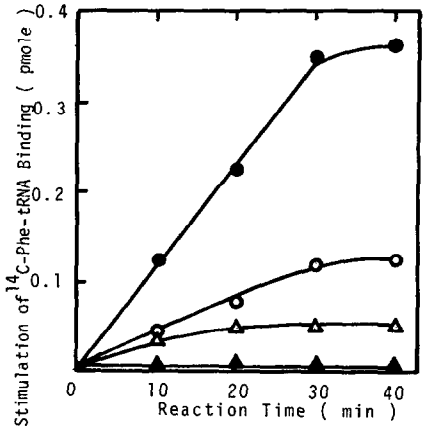

Fig.2. Effect of γ subunit on the EF-1a-dependent binding of [^{14}C]Phe-tRNA to ribosomes. The binding of [^{14}C]Phe-tRNA to ribosomes was assayed as described under Materials and methods. The amounts of factors were 1.8 μg EF-1a and 0.35 μg γ subunit: (–●–) EF-1a, γ subunit and GTP; (–○–) EF-1a and GTP; (–▲–) γ subunit and GTP; (–△–) EF-1a, γ subunit and GMP-PCP.

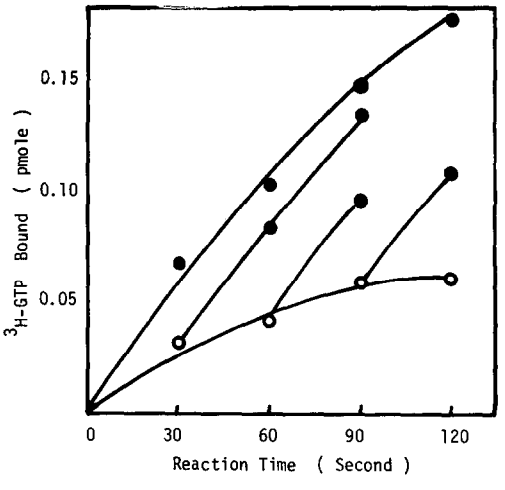

Fig.3. Effect of γ subunit on the exchange of GDP bound to EF-1a with exogeneous [^{3}H]GTP. The exchange of GDP bound to EF-1a with [^{3}H]GTP was carried out as described under Materials and methods. (–●–) γ subunit was added at different times during the second incubation (0 s, 30 s, 60 s and 90 s); (–○–) no addition of γ subunit.

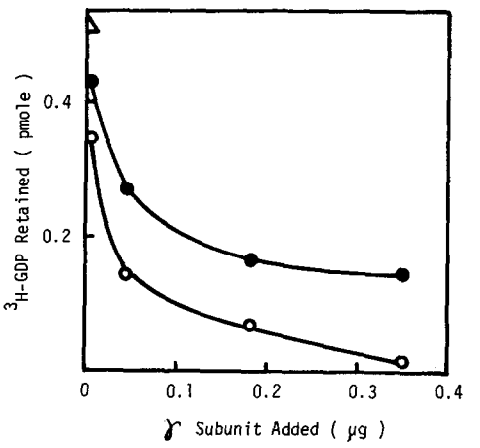

Fig.4. Effect of γ subunit on the exchange of [^{3}H]GDP bound to EF-1a with exogeneous GTP. The exchange reaction was carried out by use of [^{3}H]GDP bound EF-1a as described under Materials and methods. The second incubation was 15 s (–●–) or 30 s (–○–) in the presence of various amounts of γ subunit; (△) initial amounts of [^{3}H]GDP bound to EF-1a.

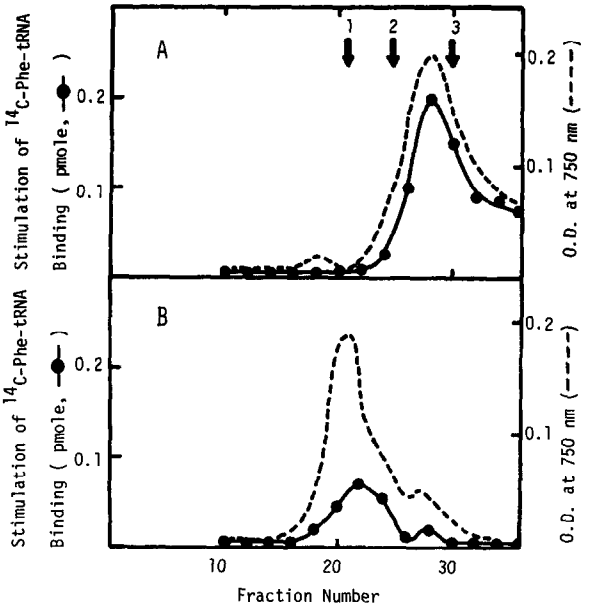

Fig.5. Sephadex G-75 gel chromatography of γ subunit. γ subunit treated with 1% DOC for 15 min at 25°C (A) and 0°C (B) was applied to a Sephadex G-75 column as described under Materials and methods. (–●–) stimulation of [^{14}C]-Phe-tRNA binding to ribosomes; (- - -) protein measured by the method of Lowry, A_{750} nm. The arrows (1), (2) and (3) show the positions where bovine serum albumin (67 000), ovalbumin (45 000) and chymotrypsinogen A (25 000) were eluted, respective).

GTP (fig.4). These results strongly indicate that γ subunit corresponds to EF-Ts.

To exclude the possibility that the γ subunit preparation was contaminated with β subunit, which may show EF-1b(EF-Ts-like) activity in combination with γ subunit, the γ subunit preparation was further subjected to Sephadex G-75 gel filtration. In this experiment γ subunit was treated with DOC to disaggregate it, since Iwasaki et al. [13] hinted that sodium cholate acted to prevent aggregation of pig liver EF-1β which correspond to EF-1b. As shown in fig.5(A) the stimulating activity of the binding reaction and the protein were eluted at the position corresponding to mol. wt. 30 000. Since α and β subunits have mol.wts 63 000 and 60 000, respectively, it can be said that of the three subunits, the γ subunit is responsible for the EF-1b activity. Iwasaki et al. [13] showed that pig liver EF-1β is composed of two subunits (mol.wts 55 000 and 30 000). These may respectively correspond to silk gland β and γ subunits for the following reasons:

(1) Silk gland EF-1b prepared according to them was composed of an equimolar amount of β and γ subunits.

(2) EF-1β [14], EF-1b and γ subunit stimulated similarly the exchange of GDP and GTP.

It should be repeated that γ subunit shows a similar activity to the EF-Ts of *E.coli*. The results strongly suggest that the mechanisms of polypeptide elongation are universal throughout prokaryotes and eukaryotes.

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