ELONGATION FACTOR 1 FROM THE SILK GLAND OF SILKWORM

Reconstruction of EF-1_M from its subunits, EF-1a and EF-1bc

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1. Introduction

Elongation factor 1 (EF-1) which catalyzes the binding of aminoacyl-tRNA to ribosomes, exists in multiple forms in a variety of different eukaryotes [1,2]. They can be classified conventionally into three groups, EF-1_H (heavy form), EF-1_M (medium form) and EF-1_L (light form), with molecular weights of $> 3 \times 10^5$, $\sim 1.5 \times 10^5$ and $\sim 5.1 \times 10^4$, respectively. Although EF-1_M is generally grouped into EF-1_H, we distinguished EF-1_H and EF-1_M from each other for the further study of their functional differences. It was observed that EF-1_H and EF-1_M represent aggregates of EF-1_L [3–5]. While we reported that silk gland EF-1_H and EF-1_M consisted of three different subunits, 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) [6]. More recently, it was demonstrated that EF-1a and EF-1b correspond to prokaryotic EF-Tu and EF-Ts, respectively [7,8]. Similar factors were also observed in pig liver [9], *Artemia salina* [10], rabbit reticulocyte [11], and wheat embryo [12]. Since EF-1_H and EF-1_M from these organisms are thought to consist of three different subunits, reconstructions of EF-1_H and EF-1_M from subunits has received much attention. As EF-1b and EF-1c were separated from each other only when a denaturant such as 8 M urea is present [8], we used mainly the complex (EF-1bc).

The present work demonstrates the reconstruction of EF-1_M from its subunits, EF-1a and EF-1bc, or EF-1a, EF-1b and EF-1c. EF-1_M reconstructed from EF-1a and EF-1bc showed 70–80% of the activity of native EF-1_M.

2. Materials and methods

2.1. *Preparation of EF-1_H, EF-1_M and its subunits*

Silk gland EF-1_H, EF-1_M, EF-1a (EF-1_L), EF-1b and EF-1c were prepared as in [8]. EF-1bc which corresponds to pig liver EF-1$\beta\gamma$ was prepared from EF-1_H and EF-1_M with a DEAE-cellulose column in the presence of GTP according to a slight modification of the procedure in [13].

2.2. *Reconstruction of EF-1_M from its subunits*

EF-1a and EF-1bc, or EF-1a, EF-1b and EF-1c, were mixed in a solution containing 50 mM Tris–HCl, pH 7.6, 2 mM 2-mercaptoethanol, 1 mM EDTA, 100 mM KCl, and 15% (v/v) glycerol, and reacted for 30 min at 0°C. The mixture was dialyzed against buffer A (50 mM Tris–HCl, pH 7.6, 2 mM 2-mercaptoethanol, 0.1 mM EDTA, 100 mM KCl, 200 mM sucrose) containing 80% ammonium sulfate. The precipitate that appeared was collected by centrifugation and dissolved in 100 μl buffer A. The solution was applied to a Sephadex G-150 column (0.8 × 30 cm) equilibrated beforehand with buffer A, and developed with the same buffer. About 0.4 ml fractions (4 drops) were collected.

2.3. *Polyacrylamide gel electrophoresis*

Gel electrophoresis of reconstructed EF-1 was carried out on a slab gel (150 × 100 mm, 1.0 mm thick) in the presence of sodium dodecylsulfate (SDS) according to [14]. Concentration of the gel was 12.5%. Proteins were stained with 0.1% Coomassie Brilliant Blue.

2.4. *Assay for EF-1 activity*

EF-1 activity was assayed using the system of GTP- and EF-1-dependent binding of [^{14}C]Phe-tRNA to ribosomes as in [7].

3. Results and discussion

Figure 1A shows the elution profile of EF-1a. It was eluted at fraction number 26 a little earlier than egg albumin (mol. wt 45 000). Figure 1B shows the elution profile of the incubated mixture of EF-1a and EF-1bc. EF-1 activity was eluted at fraction number 15 where native EF-1_M was eluted. In this experiment no

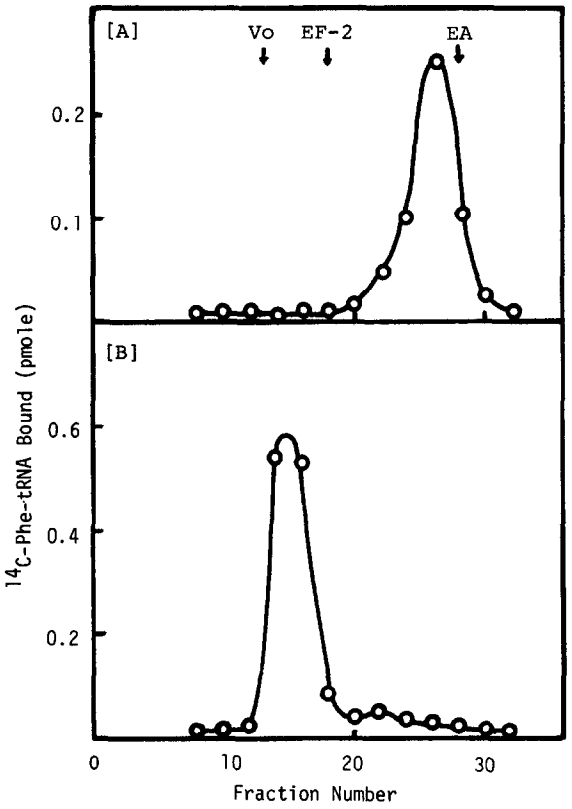

Fig.1. Reconstruction of EF-1_M from EF-1a and EF-1bc. (A) Elution profile of EF-1a (32 μg) on a Sephadex G-150 column. (B) Elution profile of the reacted mixture of EF-1a (160 μg) and EF-1bc (168 μg). Details are described in section 2. Arrows indicate the elution positions of blue dextran (V_O), silk gland EF-2(EF-2) and egg albumin (EA), respectively.

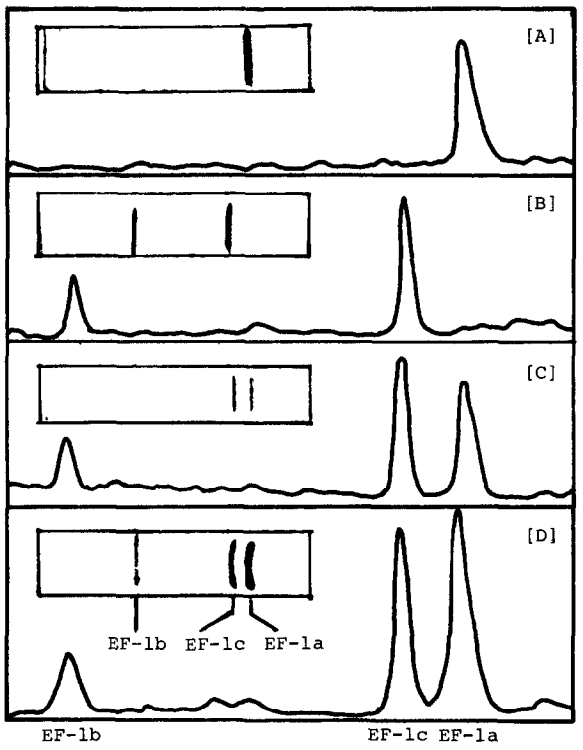

Fig.2. SDS–polyacrylamide gel electrophoresis of native and reconstructed EF-1_M. Electrophoreses were carried out as in section 2; (A) EF-1a, (B) EF-1bc, (C) reconstructed EF-1_M and (D) native EF-1_M.

peak was observed near mol. wt 50 000. From these results it is likely that EF-1_M was reconstructed from EF-1a and EF-1bc. To confirm this, subunit composition of EF-1 eluted at the fraction number 15 was analyzed with polyacrylamide gel electrophoresis in the presence of SDS. As shown in fig.2C the EF-1 eluted at fraction number 15 consisted of three different subunits, EF-1a, EF-1b, and EF-1c. The protein ratio of each subunit was nearly 1:1:1 from the densitometric analysis. These results clearly show that EF-1_M was reconstructed from EF-1a and EF-1bc. Figure 3 shows the comparison of the activities of native EF-1_M and reconstructed EF-1_M. Reconstructed EF-1_M showed 70–80% of the activity of the native factor. Figure 4A shows the elution profile of the incubated mixture of EF-1a, EF-1b and EF-1c. In this experiment a peak and a shoulder of EF-1 activities were observed. Although details are not shown,

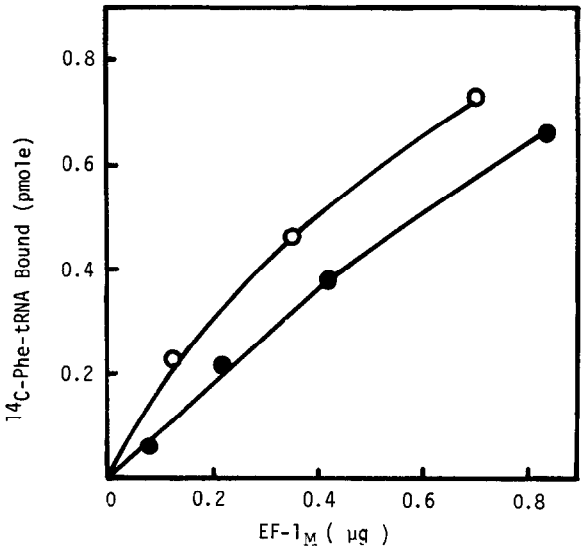

Fig.3. Activities of native and reconstructed EF-1_M. Native EF-1_M (○) and reconstructed EF-1_M (fraction numbers 14–16 of fig.1) (●) were assayed for EF-1 activity as described in section 2.

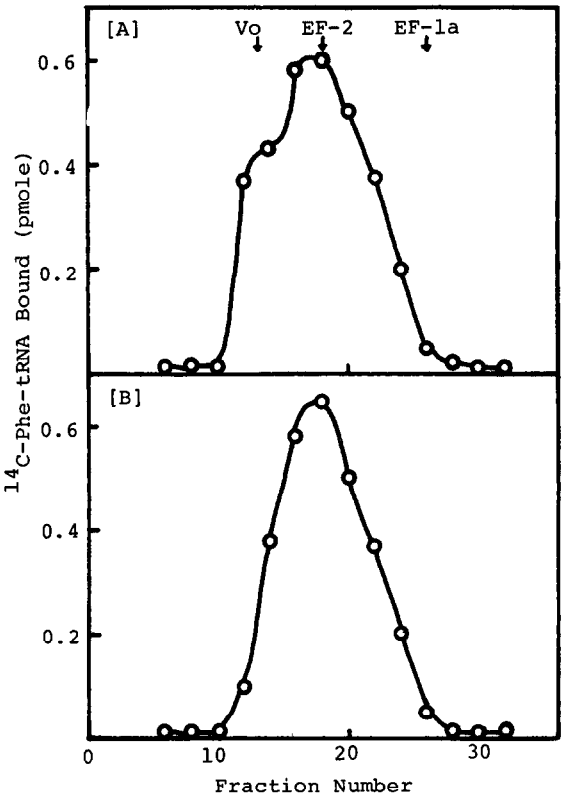

Fig.4. Formation of EF-1_M and EF-1a–EF-1b complexes from EF-1a, EF-1b and EF-1c, or formation of EF-1a–EF-1b complex from EF-1a and EF-1b. (A) Elution profiles of the reacted mixture of EF-1a (160 μg), EF-1b (55 μg) and EF-1c (112 μg) on a Sephadex G-150 column. (B) The same experiment as (A) with EF-1a (240 μg) and EF-1b (82 μg). Details are described in section 2. Arrows indicate the elution positions of blue dextran (V_0), silk gland EF-2(EF-2) and EF-1a, respectively.

the shoulder consisted of EF-1a, EF-1b and EF-1c, while the peak consisted mainly of EF-1a and EF-1b. When the incubated mixture containing EF-1a and EF-1b was analyzed, a single peak of EF-1 activity was observed at mol. wt 80 000 (fig.4B). Since EF-1a and EF-1b have mol. wt 51 000 and 26 000, respectively, EF-1 at the peak may consist of EF-1a and EF-1b in a molar ratio of 1:1. Analysis with polyacrylamide electrophoresis also supported this idea. Since EF-1a and EF-1b correspond functionally to EF-Tu and EF-Ts, respectively [8], the EF-1a–EF-1b complex seems to correspond to EF-Tu–EF-Ts complex. But it would not be the physiological complex for the following reasons:

(i) The complex, free EF-1b, and free EF-1c were not detected in the cell.
(ii) EF-1_H or EF-1_M was resolved into EF-1a and EF-1bc in the presence of GTP.
(iii) EF-1bc was resolved into EF-1b and EF-1c only when a denaturant such as 8 M urea is present.
(iv) The EF-1a–EF-1b complex was not detected when EF-1a was incubated with EF-1bc.

These results also indicate that eukaryotic factor which corresponds to EF-Ts is the complex of EF-1b and EF-1c. The formation of the complexes of EF-1α and EF-1β, or EF-1α and EF-1βγ with a pig liver in vitro system has been reported [15]. Since EF-1α and EF-1a, or EF-1β and EF-1b were exchangeable with each other in polypeptide chain elongation reactions [16], the complexes most likely correspond to the EF-1a–EF-1b and EF-1a–EF-1bc complexes, respectively. Although aggregated forms of EF-1 were detected in some experiments, it is not clear whether it corresponds functionally to native EF-1_H, because native EF-1_H contains fairly large amounts of lipids. Further experiments are required to clarify this

point and the differences between EF-1_H and EF-1_M.

The results described above showing that EF-1_M is formed from EF-1a and EF-1bc indicate that the function of EF-1bc (or EF-1b) is to convert EF-1a-GDP to EF-1a-GTP [8] via EF-1_M which corresponds to the EF-Tu–EF-Ts complex.

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