ELONGATION FACTOR 1 FROM THE SILK GLAND OF SILKWORM

Effect of EF-1b on EF-1a- and ribosome-dependent GTPase activity

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

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1. Introduction

Elongation factor 1 (EF-1), which catalyzes the binding of aa-tRNA to ribosome with the concomitant hydrolysis of GTP has been shown to occur in multiple forms with several different molecular weights in a variety of eukaryotic cells [1]. However, their individual functions are unknown. Silk gland EF-1_H (mol. wt $> 3 \times 10^5$) and EF-1_M (mol. wt $\simeq 1.5 \times 10^5$) consisting of three different subunits (α,β and γ) [2,4] were resolved into complementary factors EF-1a (α subunit, APase I) and EF-1b (γ subunit, APase II) [3,4]. They correspond to EF-Tu and EF-Ts, respectively [4]. Although for prokaryotes [5] and for eukaryotes [6] the stoichiometry of the amount of aa-tRNA bound to ribosome and the amount of GTP cleaved in the binding reaction was shown, no data were obtained about the aa-tRNA-dependent GTPase of EF-1 which was resolved into complementary factors (EF-1a and EF-1b). We describe here EF-1a- and ribosome-dependent GTPase (nonspecific GTPase) which is observed in the absence of aa-tRNA. Repression of the nonspecific GTPase by EF-1b is also described.

2. Materials and methods

2.1. *Purification of EF-1 and EF-2*

EF-1a (α subunit) was purified from silk gland by steps including ammonium sulfate fractionation, calcium phosphate gel fractionation, and three successive column chromatographies on hydroxylapatite, Sepharose 6B and CM–Sephadex C-50 (details will be given elsewhere). EF-1c (β subunit) and EF-1b (γ subunit) was purified from EF-1_H by ion exchange chromatography in the presence of 6 M urea as in [4]. EF-1bc (complex of γ and β subunits) was purified from EF-1_H according to the method in [7] with slight modifications. EF-2 was purified according to the method in [8]. These factors were all purified to an apparent homogeneity on polyacrylamide gel electrophoresis.

2.2. *Preparation of ribosomes*

Salt-washed silk gland ribosomes were prepared as in [9].

2.3. *Ribosome-dependent GTPase assay*

The reaction mixture containing in total vol. 250 μl, 50 mM Tris–HCl (pH 7.6), 5 mM $MgCl_2$, 75 mM KCl, 2 mM dithiothreitol, 15% glycerol, 10 μg poly(U), 1.28 nmol [γ-^{32}P]GTP, 1.4 A_{260} units of ribosome, and other components (as indicated in the figure legends and table), was incubated for 30 min at 28°C. The reaction was stopped by adding 0.25 ml cold solution containing 1 mM NaH_2PO_4, 4% perchloric acid, and 0.1 ml 5% (w/v) activated charcoal. The mixture was centrifuged to remove [γ-^{32}P]GTP bound to charcoal and 0.3 ml supernatant was assayed for ^{32}P in a liquid scintillation spectrometer in the absence of scintillator.

3. Results and discussion

Table 1 shows that EF-1a catalyzed the release of P_i from GTP in the presence of ribosomes. Since GTP

Table 1
Ribosome-dependent GTPase activity of each subunits of EF-1

Additions	[γ-^{32}P]GTP hydrolyzed (pmol)
EF-1a	117
EF-1b	1.6
EF-1c	0
EF-1bc	2.9

Reaction was carried out as described in section 2 in the presence of 4 μg EF-1a, 4.4 μg EF-1b, 4 μg EF-1c and 4.8 μg EF-1bc

was hydrolyzed in the absence of aa-tRNA, we named this GTPase 'nonspecific GTPase' in contrast with the 'specific GTPase' which is observed in the presence of aa-tRNA. EF-1b, EF-1bc or EF-1c did not show ribosome-dependent GTPase activity. Figure 1 shows the effect of EF-1b or EF-1bc on the nonspecific GTPase activity. To investigate the differences between EF-1b and EF-1bc activities, both factors

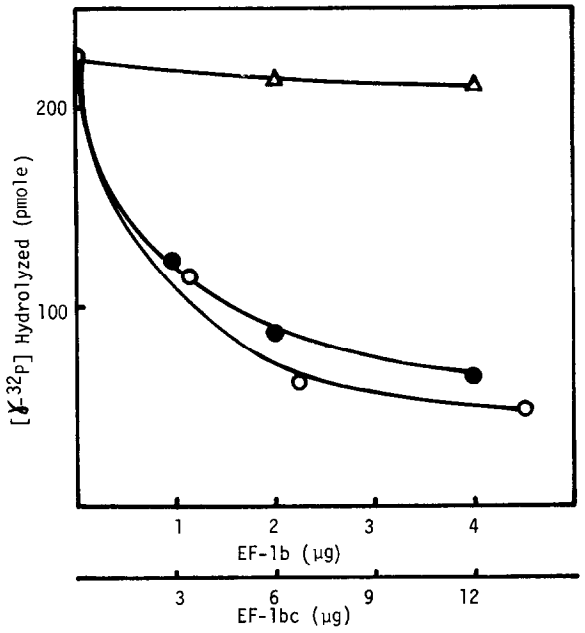

Fig.1. Effect of EF-1b or EF-1bc on EF-1a- and ribosome-dependent GTPase. Reaction was carried out as described in section 2 in the presence of 4 μg EF-1a and various amounts of the following factors: EF-1b (–○–); EF-1bc (–●–); and EF-1c (–△–).

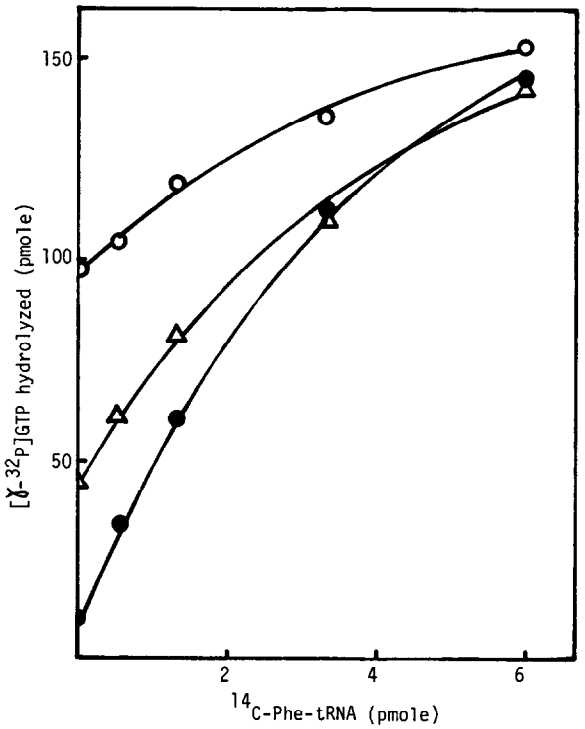

Fig.2. Effect of aa-tRNA and EF-1b on EF-1a-dependent GTPase. Reaction was carried out as described in section 2 in the presence of 1 μg EF-1a, different amounts of [^{14}C]Phe-tRNA and EF-1b. Amounts of EF-1b added per tube were; 0 μg (–○–), 2.2 μg (–△–), and 4.4 μg (–●–), respectively.

were used in the protein ratio 1:3 (calculated from their molecular weights of 26 000 and 72 000, respectively). EF-1b and EF-1bc strongly repressed the nonspecific GTPase. No difference was observed between EF-1b and EF-1bc in this repression. EF-1c had no effect on the nonspecific GTPase activity. Figure 2 shows the effect of Phe-tRNA and EF-1b on the nonspecific GTPase activity. In the absence of Phe-tRNA the nonspecific GTPase was repressed by EF-1b as shown in fig.1. The repression was reduced in proportion to the added Phe-tRNA (fig.2). An inhibitor of EF-G- and ribosome-dependent uncoupled GTPase activity was isolated from *Escherichia coli* [10]; however, EF-1b (EF-1bc) had no effect on the EF-2- and ribosome-dependent GTPase activity. These results indicate that EF-1b (EF-1bc) is a significant factor that not only catalyzes the exchange of GDP bound to EF-1a with exogeneous GTP [4],

but also regulates the hydrolysis of GTP in the aa-tRNA binding reaction. Although EF-1a forms a ternary complex with aa-tRNA and GTP, and the GTP in the complex is hydrolyzed in the presence of ribosomes, free EF-1a also hydrolyzes GTP directly in the presence of ribosomes as described above. Therefore, a mechanism is necessary to prevent EF-1a from interacting with ribosomes directly. In fact, the equilibrium of $EF\text{-}1_M \rightleftharpoons$ EF-1a + EF-1bc is far over to the left side and free EF-1a, which hydrolyzes GTP nonspecifically, may scarcely exist in the cell. As $EF\text{-}1_H$ and $EF\text{-}1_M$ showed little nonspecific GTPase activity, they may have little affinity to ribosomes, in contrast to free EF-1a or the aa-tRNA–EF-1a–GTP complex.

The results described above show that EF-1b (EF-1bc) represses the nonspecific GTPase of EF-1a and help to prevent hydrolysis of GTP unnecessarily. Regulation of the hydrolysis of GTP by $EF\text{-}1_H$ and $EF\text{-}1_M$ is one of their important functions.

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