EXCHANGE OF FREE GTP WITH EF-1α·GDP COMPLEX PROMOTED BY A FACTOR EF-1β FROM PIG LIVER

Shigekazu Nagata, Kazuo Motoyoshi,

and Kentaro Iwasaki

Institute of Medical Science, University of Tokyo,

Takanawa, Minatoku, Tokyo

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Summary: Eukaryotic polypeptide chain elongation factor 1 (EF-1) has been resolved into two complementary factors, EF-1α and EF-1β, both of which were purified. Recently, we find that [^{3}H]GDP bound to purified EF-1α is replaced by exogenous GTP rather slowly when the reaction is carried out at ionic strength optimal for polyphenylalanine synthesis. EF-1β stimulates the exchange of free GTP with EF-1α·GDP, indicating that the function of EF-1β is, at least in part, similar to that of bacterial EF-Ts.

We have previously shown that EF-1[1] from pig liver could be resolved into two complementary factors, EF-1α and EF-1β (2), and purified them separately to a homogeneous state (3, 4). Purified EF-1α has a molecular weight of 53,000, and its function is analogous to that of bacterial EF-Tu in that it forms not only a binary complex with guanine nucleotides such as GTP, GDP, GMPP(CH_2)P, but also a ternary complex containing GTP and aminoacyl-tRNA, and promotes the poly(U)-dependent binding of Phe-tRNA to ribosomes (3, 5). Purified EF-1β has a molecular weight of about 90,000, and consists of two unequal subunits of molecular weight of 55,000 and 30,000. It stimulates the poly(U)-dependent polyphenylalanine synthesis in the presence of both EF-2 and EF-1α, as well as the EF-1α-dependent binding of aminoacyl-tRNA to ribosomes (4).

[1] A uniform nomenclature for elongation factors, EF-1, EF-2, EF-Tu and EF-Ts is used in this paper (see Ref. 1). Abbreviation used is: GMPP(CH_2)P for guanyl-5'-yl methylene diphosphonate.

Although we have previously reported that EF-1α·GDP is readily converted to EF-1α·GTP by incubation with GTP (5), more recent experiments revealed that this conversion takes place rather slowly when the reaction is carried out at ionic strength optimal for polyphenylalanine synthesis (Nagata et al. manuscript in preparation). Thus, it is of interest to examine the effect of EF-1β on this exchange reaction, since the role of bacterial EF-Ts is to stimulate the conversion of EF-Tu·GDP to EF-Tu·GTP (6-8). The results to be described in this paper clearly show that EF-1β stimulates the exchange of free GTP with EF-1α·GDP, indicating that the function of EF-1β is, at least in part, similar to that of bacterial EF-Ts.

Materials and Methods

EF-1α and EF-1β —— Homogeneously purified EF-1α and EF-1β were prepared as described previously (3, 4).

Assay for displacement of [^{3}H]GDP bound to EF-1α by GTP —— The reaction mixture for preparing the EF-1α·[^{3}H]GDP complex contained, in a final volume of 60 µl, 40 mM Tris-HCl buffer, pH 7.5, 0.2 mM dithiothreitol, 10 mM magnesium acetate, 100 mM NH_4Cl, 4 µM [^{3}H]GDP (500 Ci/mole), 1 mg/ml bovine serum albumin, 25% (v/v) glycerol and 10 µg of EF-1α. The mixture was incubated at 37° for 5 min, cooled in an ice and used as the EF-1α·[^{3}H]GDP complex. For measuring the displacement of EF-1α·[^{3}H]GDP, 50 µl of the EF-1α·[^{3}H]GDP complex prepared as above were mixed with 500 µl of the exchange buffer consisting of 20 mM Tris-HCl buffer, pH 7.5, 10 mM magnesium acetate, 10% (v/v) glycerol, 50 mM NH_4Cl, 150 µM GTP and an appropriate amount of EF-1β. After incubating the mixture at 0° for the indicated period, a 100 µl-portion of the mixture was diluted with 0.5 ml of the ice-cold dilution buffer containing 20 mM Tris-HCl buffer, pH 7.5, 10 mM magnesium acetate, 100 mM NH_4Cl, 100 µg/ml bovine serum albumin and 25% (v/v) glycerol to stop the reaction. The diluted sample was filtered through a nitrocellulose membrane filter, which was washed twice with 0.5 ml of the dilution buffer without glycerol. The filter was then dried and the radioactivity retained on the filter was measured in a liquid scintillation spectrometer.

Assay for exchange of GDP bound to EF-1α with [^{3}H]GDP —— The method was essentially the same as above, except that [^{3}H]GDP was replaced by 5 µM GDP in the first incubation and GTP was replaced by 4.8 µM [^{3}H]GDP (500 Ci/mole) in the second incubation.

Results and Discussion

In the previous report (5) we have shown that the interaction of EF-1α with GTP was stronger than with GDP, since [^{3}H]GDP

bound to EF-1α was rapidly displaced by unlabeled GTP in a solution containing 0.5 M NH_4Cl. However, more recent investigation indicated that the dissociation constants of EF-1α·GDP complex as well as that of EF-1α·GTP complex change in a great deal depending on the ionic strength of the solution, and that EF-1α binds GDP more tightly than GTP at concentrations of NH_4Cl below 0.1 M (Nagata et al. manuscript in preparation).

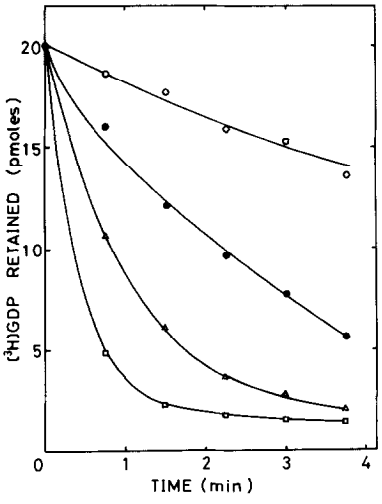

Fig. 1. Displacement of [^{3}H]GDP bound to EF-1α with GTP. Amounts of EF-1β added per tube were; 0 (—○—), 0.36 (—●—), 0.91 (—△—) and 1.8 (—□—) μg, respectively. Other conditions are described in Materials and Methods.

As shown in Fig. 1, [^{3}H]GDP bound to EF-1α was only slowly displaced by unlabeled GTP at 0° in a solution containing 70 mM NH_4Cl in the absence of EF-1β. On the other hand, the radioactivity retained on the filter was significantly reduced by the addition of increasing amounts of EF-1β, indicating that EF-1β stimulated the release of [^{3}H]GDP bound to EF-1α. A similar effect of EF-1β could also be found when EF-1α·[^{3}H]GTP was used

as a binary complex. Furthermore, unlabeled GDP was as effective as GTP in releasing labeled bound guanine nucleotides from EF-1α.

The next experiment was performed to show that the decrease in the amount of the bound nucleotide was due to the exchange of free with bound guanine nucleotides. As shown in Fig. 2A, EF-1α which was preincubated with unlabeled GDP bound [^{3}H]GDP very slowly in the absence of EF-1β, while in the presence of EF-1β, more than 60% of EF-1α bound [^{3}H]GDP within 1 min at 0°. Furthermore, the rate of this reaction was proportional to the amount of EF-1β as shown in Fig. 2B.

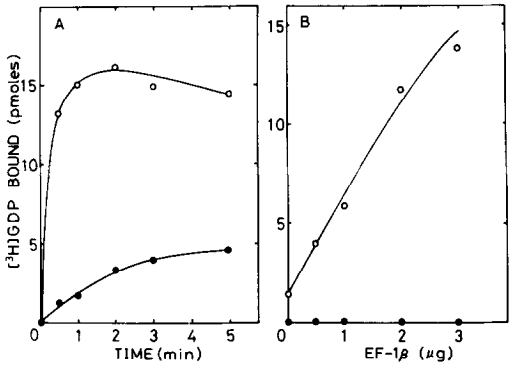

Fig. 2. Exchange of GDP bound to EF-1α with [^{3}H]GDP. (A) Kinetic studies of the reaction with 1.8 μg of EF-1β per tube (—○—) or without EF-1β (—●—). Incubations were carried out as described in Materials and Methods. (B) Effect of the various amounts of EF-1β on the exchange reaction. Ten μl of the EF-1α·GDP complex were mixed with 100 μl of the exchange buffer containing [^{3}H]GDP and various amounts of EF-1β as indicated. After incubation at 0° for 1 min, the radioactivity retained on the nitrocellulose membrane filter was determined. —○—; with EF-1α, and —●—; without EF-1α.

From these results we can conclude the conversion of EF-1α·GDP to EF-1α·GTP proceeds slowly in the solution with a low ionic strength, and EF-1β is required for its rapid conversion, which is accomplished by the following reaction:

$$EF\text{-}1\alpha \cdot GDP + GTP \overset{EF\text{-}1\beta}{\rightleftharpoons} EF\text{-}1\alpha \cdot GTP + GDP$$

In prokaryotic systems it has been well established that the affinity of EF-Tu to GDP is about 100 times greater than to GTP (9, 10) and another factor, EF-Ts, is required for the regeneration of EF-Tu·GTP from EF-Tu·GDP and GTP (6-8). To our knowledge, this is the first report to show the presence of the factor similar to prokaryotic EF-Ts in eukaryotic tissues catalyzing a distinct guanine nucleotide exchange reaction. It seems of interest to examine the occurrence of this factor in other eukaryotic tissues, and a preliminary experiment indicates that 100,000 x g supernatant from Artemia salina cysts had the similar effects as EF-1β from pig liver on the exchange of EF-1α·[^{3}H]GDP with GTP.

There are a few reports which indicate the presence of heterologous components in EF-1 preparations from other tissues. Bollini et al. (11) have reported that the high molecular weight species of EF-1 from wheat embryo is composed of three distinct polypeptides. It was also found that the reticulocyte EF-1 contains two components, one of which is heat-labile and the other heat-stable (12), and that EF-1 from silk gland may contain more than one component (13). Although their significance has not been investigated in detail, some of these components may correspond to pig liver EF-1β and may be functioning in a similar manner as described here.

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