The Heavy Form of Elongation Factor 1 in *Artemia Salina* Embryos is Functionally Analogous to a Complex of Bacterial Factors EF-Tu and EF-Ts.

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Summary

The purified heavy form of elongation factor 1 (EF-1) from cysts of *Artemia salina* was found to catalyze the exchange of free GTP with a complex of EF-1_L (EF-1_α) and GDP. Furthermore, after heat treatment of EF-1_H in the presence of GTP, the factor, while inactive by itself, stimulated aminoacyl-tRNA binding to ribosomes as well as polyphenylalanine synthesis when combined with EF-1_α. These functional properties are similar to those reported recently for purified EF-1_β from pig liver [Nagata,S., Motoyoshi,K., and Iwasaki,K. (1976) Biochem. Biophys. Res. Comm. 71, 933-938]. We suggest that Artemia EF-1_H consists of a EF-1_α. EF-1_β complex which is functionally analogous to bacterial EF-Tu · EF-Ts.

Introduction

The number of different soluble protein factors required for polypeptide chain elongation in eucaryotes has recently been placed in some doubt. Several laboratories, including our own, have reported that two factors suffice: elongation factor 2 (EF-2), the translocase enzyme, and EF-1, the enzyme which binds aminoacyl-tRNA to the ribosome (1-3). Evidence has accumulated that the multiple forms of EF-1 (MW ranging from 2×10^5 - 10^6) which occur in a wide variety of different cells and are usually referred to as EF-1_H, consists of aggregates of a light form of the enzyme, EF-1_L with a MW of about 50.000 (4-6). However, other laboratories have reported that a third protein component, termed EF-1_β (to distinguish it from EF-1_L which has been designated as EF-1_α) markedly stimulates EF-1_L activity and is also required for polyuridylic acid dependent polyphenylalanine synthesis (7,8). Recently, EF-1_β has been purified from pig liver to apparent homogeneity (9) and been found to catalyze the exchange of free GTP with a preformed EF-1_α. GDP complex (10). As pointed out by Nagata *et al*.(10) the guanine nucleotide exchange activity reported for EF-1_β makes this factor analogous

to the bacterial factor Ts which is required for the regeneration of EF-Tu·GTP from EF-Tu·GDP (11-12).

We have reported elsewhere on the purification and properties of two forms of elongation factor 1 in embryos of Artemia salina: a heavy form, EF-1_H, which is found in cysts (desiccated gastrula) and a light form, EF-1_L, which is present exclusively in the free-swimming naupillus larva (1,14). No significant difference in the ribosome dependent properties of the two forms of the enzyme could be detected. However, in contrast to EF-1_L, EF-1_H showed little affinity for guanine nucleotides at 0^o, although the heavy form of the factor could be inactivated by treatment with GTP at 42^o. In the absence of guanine nucleotides EF-1_H was heat stabile whereas EF-1_L was heat labile. These observations suggested that EF-1_H is dissociated by GTP into EF-1_L. The work of Nagata et al.(10) prompted us to re-examine the possibility that the dissociation of EF-1_H by GTP also releases EF-1_β from the intact enzyme.

In this paper we show that purified EF-1_H after treatment with GTP contains all the activities presently associated with EF-1_β. We conclude that Artemia EF-1_H is functionally analogous to the complex EF-Tu·EF-Ts which is commonly found in bacteria (13).

Materials and Methods

Elongation factors- The heavy form of EF-1 (EF-1_H) was prepared from Artemia cysts (Metaframe, San Fransisco) as described previously (14) except that the initial fractionation of the postribosomal supernatant with dextran and polyethyleneglycol was replaced by an affinity chromatography step using heparin-Sepharose (15). Homogeneous EF-1_L was prepared from developing cysts as described (14). A highly purified preparation of EF-2 was kindly provided by Dr. A.J. Maassen.

Assay for displacement of [^{3}H] GDP bound to EF-1_L by GTP- The assay was performed exactly as described by Nagata et al (10). Briefly, a complex of EF-1_L (15 µg) and 4 mM [^{3}H] GDP (660 CPM/ pmol) was preformed by heating the enzyme with the nucleotide at 37^o for 5 min. in a 60 µl reaction mixture containing 40 mM Tris-HCl buffer, pH 7.5, 0.2 mM dithiothreitol, 10 mM magnesium acetate, 100 mM NH_4Cl, 1 mg/ml bovine serum albumin and 25% (v/v) glycerol. The EF-1_L·[^{3}H] GDP complex, prepared as above, was mixed with 500 µl of exchange buffer (20 mM Tris-HCl, pH 7.5, 10 mM magnesium acetate, 50 mM NH_4Cl, 150 µM GTP and 10% (v/v) glycerol containing an appropriate amount of heat-inactivated EF-1_H. Heat inactivation of EF-1_H was performed as described previously (14)

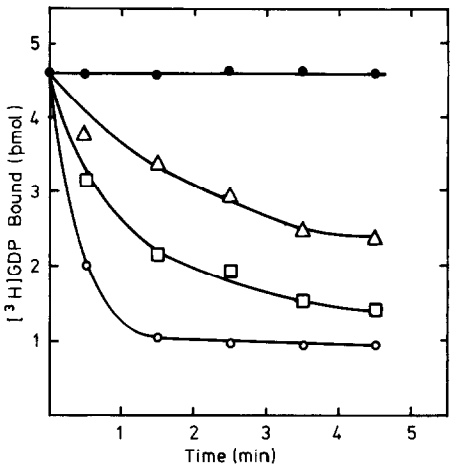

Fig.1 Exchange of [^{3}H] GDP bound to EF-1_α with GTP. Amounts of EF-1_H added per tube were: 0 (●-●), 0.5 µg (Δ-Δ), 1.0 µg (□-□), 5.0 µg (○-○). Other conditions are described in Materials and Methods.

by incubating EF-1_H (10 µg) in a buffer (50 µl) containing 20 mM Tris-HCl, pH 7.5, 100 mM KCl, 1 mM magnesium acetate, 10 mM 2-mercaptoethanol, 0.1 mM EDTA and 20 µM GTP for 10 min. at 42°. After incubating the exchange mixture at 0° for the indicated period, aliquots (100 µl) were diluted with 0.5 ml of dilution buffer (20 mM Tris-HCl, pH 7.5, 10 mM magnesium acetate, 100 mM NH_4Cl, 100 µg/ml bovine serum albumin and 25% v/v glycerol) and the sample was filtered through a nitrocellulose filter. The filter was washed twice with 0.5 ml of the dilution buffer without glycerol, dried and counted with 5 ml Instafluor (Packard) in a liquid scintillation spectrometer.

Assay for exchange of GDP bound to EF-1_L with [^{3}H] GDP- The method was essentially the same as described above except that [^{3}H] GDP was replaced by 5 µM GDP in the first incubation and GTP was replaced by 4 µM [^{3}H] GDP (600 CPM/ pmol) in the second incubation.

Other assays dependent on EF-1. - The binding of [^{3}H] Phe-$tRNA^{Phe}$ (1500 CPM/ pmol) to Artemia 80S ribosomes and the assay for polyphenylalanine synthesis were performed as previously described (1).

Results

Testing for EF-1_β activity in purified EF-1_H was facilitated by the knowledge that EF-1_β should act catalytically in the EF-1_α. GDP-GTP exchange reaction (10). EF-1_H was heated at 42° in the presence of GTP. This treatment served to inactivate EF-1_α activity (1) thereby minimizing the contribution of the EF-1_α present in EF-1_H to the guanine nucleotide binding assays (see Discussion).

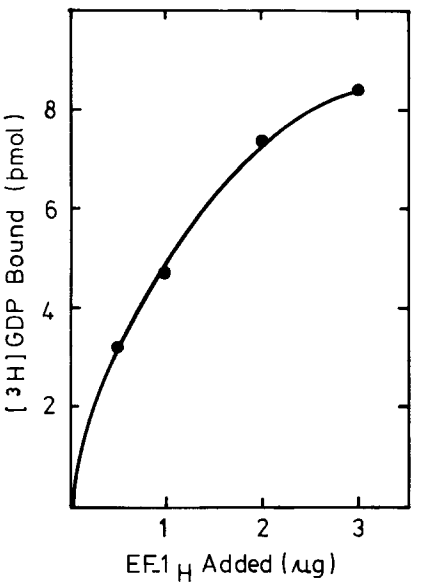

Fig.2 Effect of various amounts of EF-1_H on the exchange of EF-1_α·GDP with [^{3}H] GDP. Aliquots (10 µl) of the EF-1_α·GDP complex containing 2.5 µg EF-1_α were mixed with 100 µl of the exchange buffer containing [^{3}H] GDP and various amounts of EF-1_H as indicated. The radioactivity retained in the nitrocellulose membrane filter was determined after 1 min. incubation. A blank value of 2.0 pmol GDP bound in the absence of factors was subtracted from each point. In addition the amount of [^{3}H] GDP bound to EF-1_H in the absence of EF-1_α was subtracted. These latter values were less than 15 percent of the amount of nucleotide bound when both factors were present simultaneously.

We then tested the heat-treated EF-1_H in the GDP-GTP exchange assay in the presences of EF-1_α as described by Nagata et al (10). As shown in fig.1 [^{3}H] GDP bound to EF-1_α was not displaced by unlabelled GTP at 0° during a five min. exchange period. However, the amount of [^{3}H] GDP bound to the filters was markedly reduced by the addition of increasing amounts of heat-treated EF-1_H. The same result could also be obtained without prior heat treatment of EF-1_H (not shown).

In addition bound unlabelled GDP could be exchanged with free [^{3}H] GDP. The results, illustrated in fig.2, demonstrated that this exchange occurs only in the presence of heat-treated EF-1_H and that, furthermore, the rate of exchange is proportional to the amount of EF-1_H added.

Table 1. The Effect of Heat Inactivated EF-1_H on the EF-1_α Catalyzed Binding of Aminoacyl-tRNA to Ribosomes.

Experiment No	[EF-1_α·GDP] µg	[EF-1_H·GTP] µg	Phe-tRNA bound (pmol)	Stimulation by EF-1_H (fold)
1	0.1	-	1.1	
	-	0.04	0.08	
	0.1	0.04	2.3	2
2	0.54	-	2.5	
	-	0.2	1.1	
	0.54	0.2	5.1	1.6
3	0.027	-	0.26	
	-	0.1	0.43	
	0.027	0.1	1.4	3.8

Each 50 µl reaction mixture contained the following components: 20 mM Tris-HCl, pH 7.5, 100 mM KCl, 5 mM magnesium acetate, 10 mM 2-mercaptoethanol, 1.2 A_{260} units 80S ribosomes, 0.3 mM GTP and 12 pmol purified yeast Phe-$tRNA^{Phe}$. The EF-1_α·GDP complex was preformed as described in Materials and Methods. EF-1_H was treated with GTP at 42° for 10 min. as described. Incubations were for 2 min. at 37°. The reaction mixtures were processed for radioactivity measurements as reported previously (1). The stimulation by EF-1_H was calculated by subtracting the amount of Phe-tRNA bound to ribosomes in the absence of EF-1_α from the amount bound in the presence of both factors and dividing this value by the amount of Phe-tRNA bound in the presence of EF-1_α alone. In experiment 3, EF-1_α was added directly (without preincubation) to the assay mixtures.

Since EF-1_β has been reported to stimulate both the binding of aminoacyl-tRNA to ribosomes as well as polyphenylalanine synthesis (7-9) we also examined the effect of heat-treated EF-1_H on these EF-1_α- dependent reactions. Table 1 presents the results of the Phe-tRNA binding assays. As can be seen from the table, heat-treated EF-1_H has a definite stimulatory effect on EF-1_α activity. This is especially the case at low EF-1_α concentrations and in the absence of GDP (Table 1, experiment 3) where almost a four-fold stimulation of Phe-tRNA binding was observed. The stimularity effect can still be seen even with large amounts of EF-1_α, where the concentration of the factor is about half the concentration of starting ribosomes (Table 1,experiment 2). These results are consistent with the view that the primary effect of EF-1_H in the Phe-tRNA binding assay is to catalyze the exchange of free GTP with an EF-1_L·GDP complex.

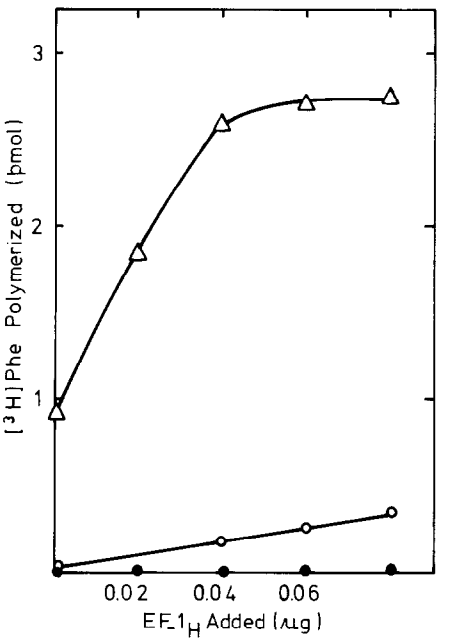

Fig.3 The effect of EF-1_H on EF-1_α and EF-2 dependent polyphenylalanine synthesis. Each 50 µl mixture was the same as described for Phe-tRNA binding, (Table 1) except for the following components: 1 mM GTP and 0.2 A_{260} units ribosomes. In addition each assay mixture contained 0.36 µg of EF-2 and 40 ng of EF-1_α when indicated. EF-1_H was heated with GTP prior to addition as described in Materials and Methods. Incubations were for 10 min. at 37°.
(Δ-Δ), complete system; (o-o), without EF-1_α (●-●), without EF-2.

We next examined the effect of heat-treated EF-1_H on the EF-1_α and EF-2 dependent synthesis of polyphenylalanine. As shown in fig.3, the heat-treated EF-1_H stimulated polyphenylalanine synthesis approximately 3-fold. Thus the presence of heat-treated EF-1_H accelerated the recycling of EF-1_L such that in 2 min. EF-1_α can recycle at least twice while in the absence of the factor only stoichiometric activity of EF-1_α can be seen.

Discussion

The findings in this paper provide strong evidence that EF-1_H purified from A. salina cysts possesses EF-1_β activity. All of the functions presently attributable to EF-1_β- stimulation of guanine nucleotide exchange with EF-1_L (figs. 1 and 2), augmentation of EF-1_L dependent aminoacyl-tRNA

binding to ribosomes (Table 1) and polyphenylalanine synthesis (fig.3)- appear to be associated with EF-1_H. The presence of EF-1_β in EF-1_H is also consistent with the polypeptide composition of EF-1_H. We have found that three different polypeptide chains are detectable in purified EF-1_H (14): an A chain (mw $\simeq$ 53.000) and B chain (mw $\simeq$ 51.000) and a C chain (mw $\simeq$ 26.000). The A chain had a mobility corresponding to EF-1_L (EF-1_α) during electrophoresis on acrylamide gels containing dodecylsulfate. More recent work further confirms the structural relationship between the A chain and EF-1_α (Slobin and Möller, unpublished). The C chain appeared to be acidic (14). Recently Iwasaki et al (9) have characteryzed EF-1_β as a protein consisting of two different polypeptides with mw's of 55.000 and 30.000, one basic and the other acidic. The molecular weight of EF-1_α from pig liver was reported by the same group to be 53.000 (22). The simularity between the B and C chain and the subunits of purified EF-1_β is apparent.

We previously suggested that the basic unit of EF-1_H consists of one A or B chain in combination with the C-polypeptide. This view was predicated in large part on the similarity in molecular weight of the A and B chains as well as preliminary hydrodynamic measurements on the intact enzyme. Based on the studies presented in this paper we now strongly favor a model in which the basic unit of Artemia EF-1_H consists of a 1:1:1 complex of A,B and C polypeptides. Functionally such a complex would correspond to 1:1 complex of EF-1_α (A-chain) and EF-1_β (B+C chain). The properties of EF-1_α clearly resemble those of the bacterial factor EF-Tu whereas EF-1_β possesses activities which are similar to EF-Ts (7-9, 13). We are led to the conclusion that Artemia EF-1_H is functionally analogous to the bacterial complex EF-Tu·EF-Ts. Final proof of this hypothesis will rest upon purification and characterization of EF-1_β from the starting EF-1_H.

A number of additional observations in the literature suggest that EF-1_H from other sources consist of an EF-1_α·EF-1_β complex. It has been reported that the reticulocyte EF-1_H contains two components, one of which

was heat-labile and resembled EF-Tu; the other, heat stabile, was found to be similar to EF-Ts (17). Bollini et al (18) have found that $EF\text{-}1_H$ from wheat embryo is composed of three polypeptide chains which are essentially indistinguishable in molecular weight from those we find in the Artemia factor. Furthermore, the wheat factor could be dissociated by GTP in A chains and B+C chains (19).

Our data (Table 1 and fig.3) confirm those of Iwasaki et al (9) that $EF\text{-}1_\beta$ can be expected to stimulate $EF\text{-}1_\alpha$ activity about three-fold. Such an effect, although real, might easily be missed (20) or ignored (3). We reported previously that purified $EF\text{-}1_L$ ($EF\text{-}1_\alpha$) can recycle in catalyzing the binding of aminoacyl-tRNA to ribosomes. The data presented in this paper raise the possibility that such recycling may have been due to the presence of trace amounts of $EF\text{-}1_\beta$ contaminating our ribosome preparations.

If the suggestion that $EF\text{-}1_\beta$ can accelerate polypeptide chain elongation by a factor of about three is correct, the relative amount of this factor in cells may provide a mechanism for a fine adjustment of the rate of protein synthesis. This may be true under conditions such as hormonal stimulation where EF-1 activity has been reported to increase (21). Increased amounts of $EF\text{-}1_\beta$ may be especially important for poikilotherms which maintain themselves at temperatures where little exchange of nucleotides bound to $EF\text{-}1_\alpha$ can be expected in the absence of $EF\text{-}1_\beta$.

Finally we have observed that $EF\text{-}1_H$ is disaggregated in vivo into $EF\text{-}1_L$ ($EF\text{-}1_\alpha$) during the development of A.salina (1,23). This observation, in view of the findings reported in this communication, suggest that under conditions of rapid growth, $EF\text{-}1_\alpha$ and $EF\text{-}1_\beta$ may not be associated. The association of the two factors may ensure the stability of $EF\text{-}1_\alpha$ during periods of biological dormancy (1) or act as a storage form of the factors prior to their participation in protein synthesis. In this regard it will be important to establish whether $EF\text{-}1_H$ purified from other sources consists of $EF\text{-}1_\alpha \cdot EF\text{-}1_\beta$ complexes or, as usually believed, aggregates of $EF\text{-}1_\alpha$.

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