TRANSIENT INTERACTION BETWEEN ELONGATION FACTOR 1 FROM *ARTEMIA SALINA* AND THE 80 S RIBOSOME

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1. Introduction

Elongation factor 1 (EF-1) from *Artemia salina* exists in multiple forms [1]. Dehydrated cysts of *A. salina* contain a high molecular weight form of EF-1 (EF-1_H), composed of three different polypeptide chains having est. mol. wt 53 000 (A-chain), 51 000 (B-chain) and 26 000 (C-chain) [2].

The A-chain, which is responsible for enzymatic binding of aminoacyl-tRNA to the 80 S ribosome [3] and ternary complex formation [4], corresponds to the low molecular weight form of EF-1 (EF-1_L) as found in free-swimmin nauplii [1]. The C-chain exhibits stimulatory properties comparable to the bacterial EF-Ts [5], whereas up to now no clear function has been detected for the B-chain.

We report here that a protein factor from *Artemia salina* having structural and functional properties resembling EF-$1_{\beta\gamma}$ from pig liver [6], partially inhibits the binding of EF-1_L, to the 80 S ribosome. Addition of aminoacyl-tRNA abolishes this inhibition.

Furthermore, in the presence of this *Artemia* factor a non-hydrolysable GTP-analogue the additional binding of EF-1_L and aminoacyl-tRNA is mutually coupled.

Coupling does not take place in the presence of GTP, presumably due to the release of EF-1_L from the ribosome, following the binding of aminoacyl-tRNA and GTP-hydrolysis.

Definition: eEF-Ts is defined as the eucaryotic counterpart of the bacterial EF-Ts. Its stimulatory activities in protein synthesis correspond with those of the C-chain of the high molecular weight form of elongation factor 1 (EF-1_H) of *Artemia salina*

2. Materials and methods

Salt-washed 80 S ribosomes were prepared as in [7].

The low molecular weight form of elongation factor 1 (EF-1_L) was purified to homogeneity as in [2]; elongation factor eEF-Ts was partially purified by the method in [8] and showed in SDS–gel electrophoresis mainly two bands, having mol. wt ~30 000 and ~50 000, respectively. The 30 000 band corresponds to the C-chain from EF-1_H [8] while the 50 000 band may be homologous with the γ-chain from EF-$1_{\beta\gamma}$ as in [6].

2.1. *EF-1_L tritiation*

EF-1_L was tritiated by the method in [9] with the exception that the glycerol which is required to preserve enzymatic activity was replaced by 25% (v/v) diethyleneglycol. No reductive methylation occurs in the presence of 25% glycerol, presumably due to acetal formation between formaldehyde and glycerol. The specific activity of the tritiated EF-1_L was 4500 cpm.pmol^{-1}. No loss of enzymatic activity was observed for at least 4 months.

2.2. *EF-1_L–80 S ribosome binding*

Binding of [^{3}H]EF-1_L to 80 S ribosomes was measured by the following standard method: 35 pmol 80 S ribosomes were incubated with 50 pmol [^{3}H]-EF-1_L in 100 μl buffer containing 20 mM Tris–HCl (pH 7.5), 5 mM Mg-acetate, 100 mM KCl, 0.1 mM EDTA, 10 mM 2-mercaptoethanol and 0.25 mM GuoPP(CH_2)P.

After 10 min incubation at 37°C, glutardialdehyde

was added to final conc. 0.2% (w/v) and the ribosomes were pelleted through a sucrose cushion containing 20 mM Tris–HCl (pH 7.5), 100 mM KCl, 5 mM Mg-acetate, 0.1 mM EDTA, 10 mM 2-mercaptoethanol and 1 M sucrose for 3 h at 44 000 rev./min. The pellet was resuspended in 0.5% SDS and its radioactivity determined.

2.3. *GTP hydrolysis*

Hydrolysis of [γ-^{32}P]GTP was measured by the method in [10], using 35 pmol 80 S ribosomes, 250 pmol [γ-^{32}P]GTP, 20 μg poly(U) and varying amounts of EF-1_L.

3. Results

The effect of an eEF-Ts preparation on the binding of [^{3}H]EF-1_L to 80 S ribosomes under the influence of a non hydrolysable analogue of GTP is shown in fig.1. In the absence of eEF-Ts the level of EF-1_L-binding is maximal and independent of the presence or absence of aminoacyl-tRNA (panel A). When a preparation of eEF-Ts is added, a pronounced and significant reduction of the binding of [^{3}H]-EF-1_L to the ribosome takes place (panel B). On addition of [^{14}C]Phe-tRNA in this case, the ratio of extra ribosome bound EF-1_L and aminoacyl-tRNA is close to one. This additional EF-1_L binding, referred to as coupled EF-1_L - aa-tRNA binding, approaches the same level as is observed in the absence of eEF-Ts.

In contrast to the uncoupled EF-1_L binding,

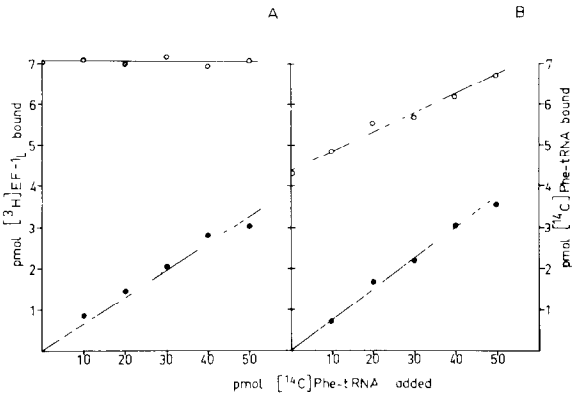

Fig.1. Coupled binding of [^{3}H]EF-1_L and [^{14}C]Phe-tRNA in the presence and absence of eEF-Ts. 35 pmol 80 S ribosomes were incubated in the absence (panel A) and presence (panel B) of 0.8 μg partially purified eEF-Ts with 50 pmol [^{3}H]-EF-1_L (○—○) and various amounts of [^{14}C]Phe-tRNA (·—·) by the method in section 2: In panel B, the amount of [^{3}H]-EF-1_L bound to the ribosome is maximal at 50 pmol [^{14}C]-Phe-tRNA added (results not shown).

aminoacyl-tRNA-dependent binding of EF-1_L to the 80 S ribosome is relatively weak and can only be observed after treatment of the ribosome with glutardialdehyde prior to ultracentrifugation (results not shown).

In addition, the coupling between binding of EF-1_L and aminoacyl-tRNA can only be observed in the presence of a non-hydrolysable GTP analogue like GuoPP(CH$_2$)P and GuoPP(NH)P and not in the presence of GTP, as shown in table 1. Apparently,

Table 1
The effect of guanine nucleotides on the coupled binding of [^{3}H]EF-1_L to the 80 S ribosome

Exp.	Guanine nucleotide	Coupled binding of [^{3}H]EF-1_L	Amount of ribosome-bound [^{14}C]Phe-tRNA
1	GuoPP(CH$_2$)P	1.64	2.54
2	GuoPP(NH)P	3.74	4.68
3	GTP	0.45	5.25

35 pmol 80 S ribosomes were incubated with 50 pmol [^{3}H]EF-1_L and 0.8 μg partially-purified eEF-Ts in the presence and absence of 100 pmol [^{14}C]Phe-tRNA, as in section 2. The guanine nucleotides used were, respectively, GuoPP(CH$_2$)P, GuoPP(NH)P and GTP. Coupled binding of [^{3}H]EF-1_L is the difference in the amount of [^{3}H]EF-1_L (pmol) bound to the ribosome in the presence and absence of 100 pmol [^{14}C]Phe-tRNA (left hand column). The right hand column represents the amount of ribosome bound [^{14}C]Phe-tRNA (pmol)

after hydrolysis of GTP, EF-1_L is released from the ribosome. It seems that this release cannot take place in the presence of a non-hydrolysable GTP-analogue.

The experiments presented show that coupled binding of [^{3}H]EF-1_L and aminoacyl-tRNA exists, provided that eEF-Ts is added and a non-hydrolysable GTP-analogue is used. In order to determine whether a comparable coupling of the GTP-cleavage occurs, hydrolysis of [γ-^{32}P]GTP was measured at varying concentrations of EF-1_L in the presence and absence of phenylalanine-tRNA.

As shown in fig.2, each molecule of EF-1_L added gives rise to 1 molecule of GTP hydrolysed (panel A), provided that aminoacyl-tRNA is present.

Moreover, under similar conditions there is a 1:1 stoichiometry between the amount of aminoacyl-tRNA bound and the amount of EF-1_L added (panel B). We therefore conclude that each molecule of aminoacyl-tRNA, bound to the ribosome under influence of EF-1_L gives rise to the hydrolysis of one molecule of GTP.

4. Discussion

A salient aspect of this paper is the demonstration that a preparation of eEF-Ts, resembling the EF-$1_{\beta\gamma}$ from pig liver [6] inhibits the uncoupled binding of EF-1_L to the 80 S ribosome. This inhibition can be distinguished from the stimulation of eEF-Ts on the nucleotide exchange and the recycling of EF-1_L. Presently we cannot ascribe this inhibitory effect to the 50 000 band or the 30 000 band or both. A reasonable assumption is that the inhibition is caused by the formation of a eucaryotic EF-Tu·EF-Ts complex. In this context eEF-Ts may have another regulatory effect on the rate of protein synthesis.

Equally important to us is the observation that in the presence of a non-hydrolysable GTP analogue the binding of EF-1_L and aminoacyl-tRNA to the ribosome occurs as a 1:1 complex. A coupled binding of EF-1_L and aminoacyl-tRNA cannot be demonstrated in the presence of GTP.

Our results support a transient attachment of EF-1_L to the ribosome and a modulating effect of eEF-Ts on this interaction.

In conclusion, our results also support the notion that the mechanism of enzymatic binding of aminoacyl-tRNA to the ribosome is universal in procaryotes and eucaryotes [11–15].

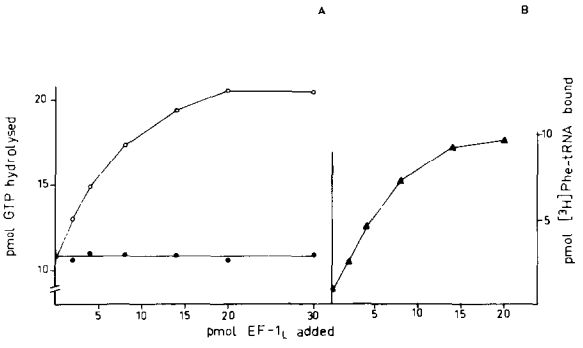

Fig.2. EF-1_L dependency of GTP-hydrolysis and aminoacyl-tRNA binding. Hydrolysis of [γ-^{32}P]GTP was measured as in section 2 in the absence (·—·) and presence (○—○) of 50 pmol unlabeled Phe-tRNA, using various amounts of EF-1_L (panel A). Binding of [^{3}H]Phe-tRNA to the 80 S ribosome was measured under identical conditions using unlabeled GTP (panel B).

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