AGGREGATION OF EUCARYOTIC ELONGATION FACTOR eEF-Ts AND ITS ISOLATION BY MEANS OF HYDROPHOBIC ADSORPTION CHROMATOGRAPHY

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1. Introduction

A heavy form of EF-1 ($EF\text{-}1_H$) has been resolved into two complementary factors thought to be analogous to the procaryotic elongation factors EF-Tu and EF-Ts [1–4].

Initially eEF-Ts, the eucaryotic counterpart of bacterial EF-Ts was reported to consist of two polypeptide chains, having est. mol. wt 55 000 and 30 000 [5]. This complex was designated EF-1$\beta\gamma$ for pig liver [6]. However preparations of active eucaryotic eEF-Ts have been isolated, containing one single polypeptide chain of an app. mol. wt 30 000 [7–9].

Such preparations of eEF-Ts stimulate the EF-1-dependent binding of aminoacyl-tRNA to the 80 S ribosome, the poly(U) directed synthesis of polyphenylalanine and the exchange of guanine nucleotides, bound to EF-1 [7–9].

Moreover, evidence has been provided that preparations of *Artemia salina* eEF-Ts which resemble EF-1$\beta\gamma$ from pig liver [6], inhibit the aminoacyl-tRNA-independent binding of EF-1 to the 80 S ribosome and that this inhibition is abolished by an excess of aminoacyl-tRNA [10].

In addition, eEF-Ts from silk glands (mol. wt 26 000) strongly repressed non-specific ribosomal GTP hydrolysis, catalysed by EF-1 in the absence of aminoacyl-tRNA [11]. Addition of aminoacyl-tRNA abolished this repression [11].

Here we present a simple procedure for the isolation of *Artemia* eEF-Ts via hydrophobic adsorption chromatography under non-denaturing conditions. This procedure resolves the 55 000 from the 30 000 mol. wt chains. The 30 000 mol. wt chains exhibit the usual activities ascribed to eEF-Ts and in addition they repress the binding of [^{3}H]eEF-Tu to the ribosome.

Considerations are given which pertain to the observed association of eEF-Ts with itself and other proteins in the extracts of *Artemia*.

2. Materials and methods

2.1. *Preparation of partially purified eEF-Ts*

Elongation factor eEF-Ts was partially purified until the Ultrogel ACA 44 step following [9]. At this stage of purification, analysis by SDS–polyacrylamide gel electrophoresis shows a doublet of app. mol. wt 30 000 and 28 000. In addition, these preparations show two other bands, having mol. wt 57 000 and 50 000, respectively. With respect to its complexity, our eEF-Ts preparation resembles that of $EF1_{\beta\gamma}$, which shows 2 bands of mol. wt 55 000 and 30 000 [6]. In this context we stress that our preparations of eEF-Ts, described here, are free of eEF-Tu activity. eEF-Ts from *Artemia salina* consists of one single polypeptide chain of an app. mol. wt 26 000 according to [9]. However, using this procedure as such, we observed mostly the somewhat more complex pattern, as described here.

2.2. *Polyacrylamide gelelectrophoresis*

SDS gel electrophoresis was done as in [13]. Gel electrophoresis under non-denaturing conditions was done as in [14].

2.3. *Assay for enzymatic activity*

eEF-Tu and eEF-Ts were assayed for activity as in [15], using salt-washed 80 S ribosomes prepared

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following [16]. eEF-Tu was purified to homogeneity as in [12].

2.4. *Chemicals*

[^{3}H]Phenylalanine (11 Ci/mmol) for the preparation of [^{3}H]Phe-tRNA was purchased from the Radiochemical Centre, Amersham. DEAE–Sephadex A50, Sephacyl S200 and Sepharose CL4B were obtained from Pharmacia. Guanidine–HCl was a product of Merck. All other chemicals were reagent grade.

3. Results

Partially purified eEF-Ts elutes under non-denaturing conditions on a Sephacryl S200 column as an aggregate of mol. wt 100 000–150 000 (fig.1c). Judged from SDS–polyacrylamide gel electrophoresis, 4 different proteins are clearly discernable in this aggregate, viz. 2 major proteins having mol. wt 28 000 and 30 000 as well as 2 minor proteins of 50 000 and 57 000, respectively (fig.1C,2C).

Amino acid analysis and peptide mapping of the material, extracted from each of the two lower molecular weight bands as observed on SDS gel electrophoresis indicate that the 2 proteins of 28 000 and 30 000 mol. wt are structurally related (in preparation). To get a better impression of whether the lower and higher molecular weight chains are part of the same or of different aggregates, preparations of eEF-Ts were subjected to varying degrees of denaturation and next chromatographed on Sephacryl S200 columns.

Fig.1A,B show the elution profile of partially purified eEF-Ts on a column of Sephacryl S200 (1 × 200 cm) in the presence of 5 M urea. Before application to the column, the sample was treated with 6 M guanidine–HCl at 20°C (fig.1A) and at 42°C, respectively (fig.1B). Subsequently selected aliquots of the eluates were analyzed by means of SDS gel electrophoresis (fig.2A, 2B). Fig.1A shows that on guanidine–HCl treatment at 20°C, part of the lower molecular weight chains are refractory to dissociation (see fraction 31–34 of fig.1A and the gel at most left of fig.2A). Apparently, even under strongly denaturing conditions, the 28 000 and 30 000 mol. wt chains aggregate strongly with themselves.

On raising the temperature of denaturation to 42°C, such aggregates disappear and elution occurs from the column in the expected order of decreasing molecular weights (see fig.1B,2B). Moreover, under non-denaturing conditions, the chains of 57 000 and 50 000 mol. wt are part of different aggregates and elute from the column in an order reversed with respect to their subunit molecular weights (compare the most left and the most right gel of fig.2C). The combined elution profiles of fig.1 indicate that in aqueous solvents the chains of 28 000 and 30 000 mol. wt are able to aggregate independently of the

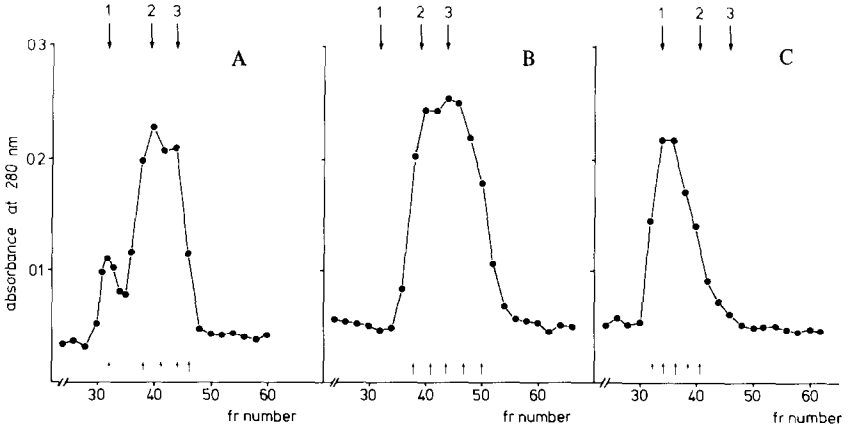
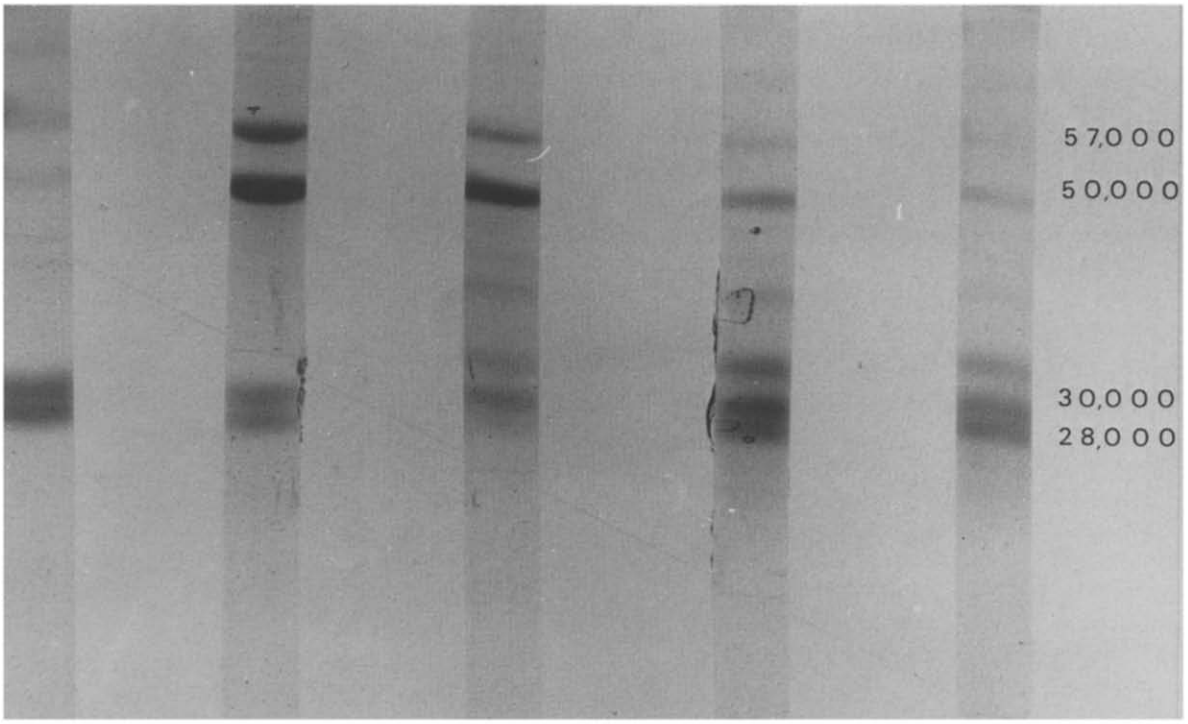


Fig.1. Partially purified eEF-Ts (2 mg) was treated with 6 M guanidine–HCl at 20°C (A) and at 42°C (B) and subsequently dialysed against a buffer containing 20 mM Tris–HCl (pH 7.5); 0.1 mM EDTA; 50 mM 2-mercaptoethanol and 5 M urea. The protein was applied to a Sephacryl S200 column (200 × 1 cm) and eluted with the same buffer. As a control, partially purified eEF-Ts (2 mg) was eluted with a buffer containing 20 mM Tris–HCl (pH 7.5); 0.1 mM EDTA, 1 mM Mg-acetate and 25% (v/v) glycerol without prior treatment with guanidine–HCl (C). The column was pre-equilibrated with the same buffer. The arrows 1, 2 and 3 indicate the positions of protein molecules having app. mol. wt 120 000, 60 000 and 40 000, respectively.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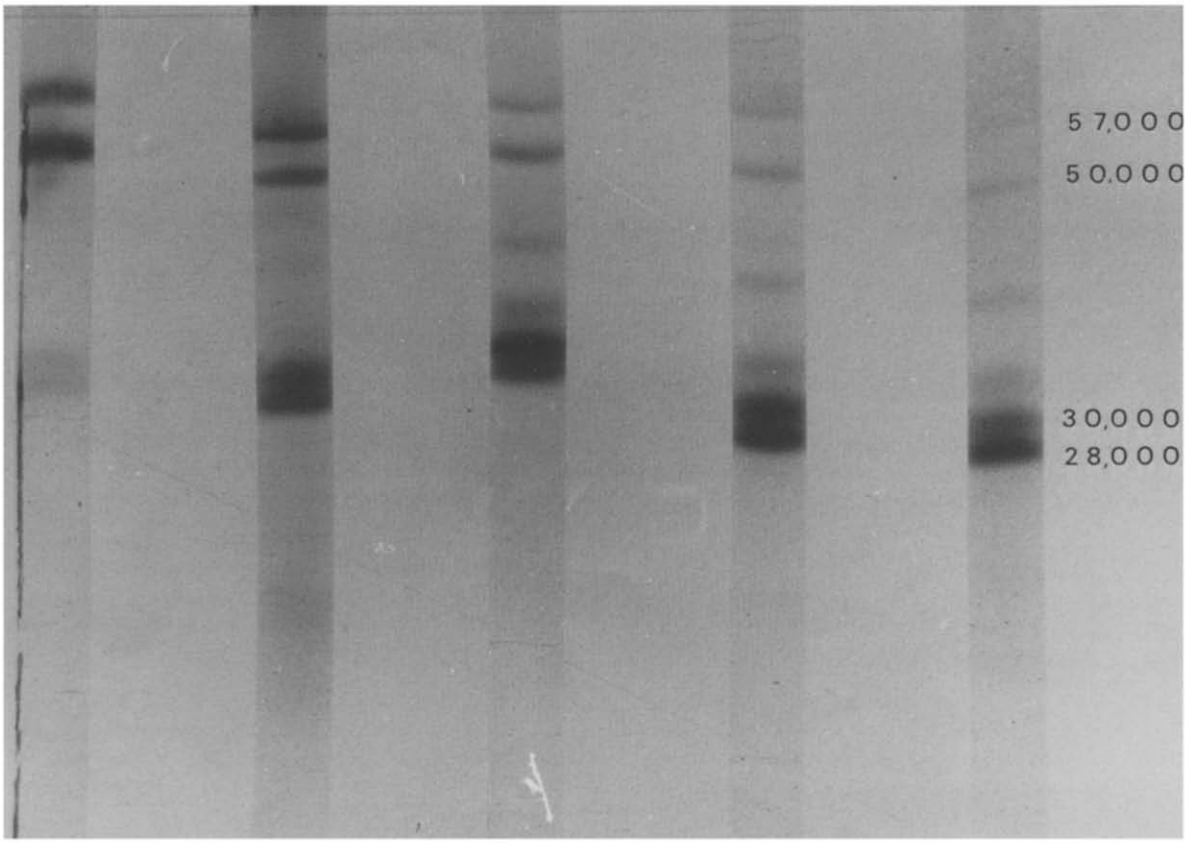
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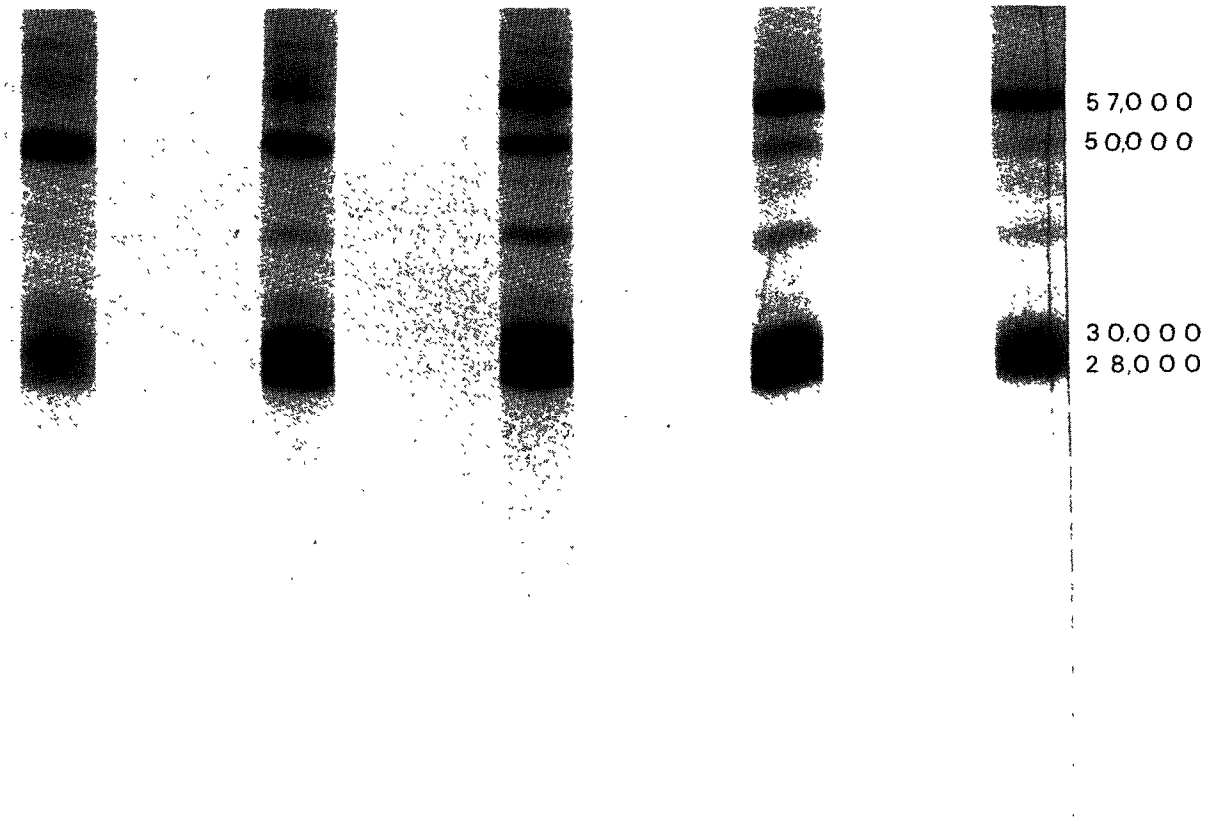


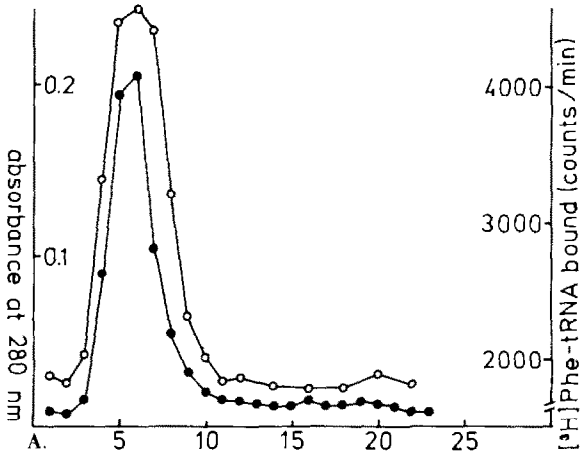
Fig.2. Aliquots (100 μl) from fractions 32-38-41-44-46; 38-41-44-47-50 and 31-33-35-37-39 were taken from the eluates, shown in fig.1A–C (see arrows at the horizontal axis of fig.1) and analysed by means of SDS gel electrophoresis.

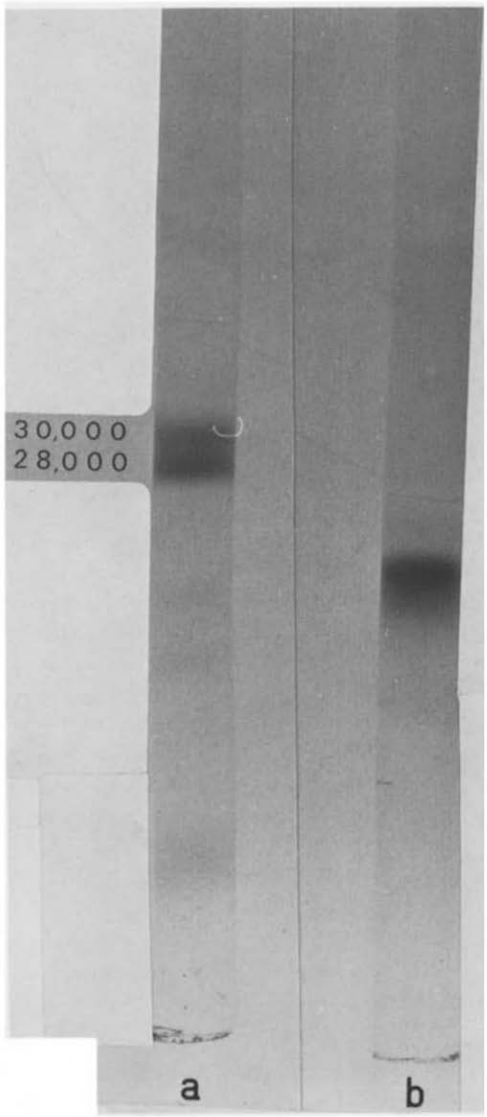
presence of the 50 000 and 57 000 mol. wt species.

To purity eEF-Ts more completely, we subjected partially purified eEF-Ts therefore to hydrophobic absorption chromatography under non-denaturing conditions. The eEF-Ts activity eluted unretarded from the column at a position coinciding precisely with the 28 000/30 000 doublet protein bands (fig.3A,B, left gel). When analysed by means of gel electrophoresis under non-denaturing conditions, the active fractions showed a single band on gel electrophoresis at pH 8.9 (fig.3B, right). After hydrophobic adsorption chromatography, the eEF-Ts activity eluted on Sephacryl S200 at a position corresponding to an app. mol. wt 70 000 (fig.3C).

The chains of 50 000 and 57 000 mol. wt could only be eluted from the phenyl–Sepharose column with a buffer containing 1% (w/v) Triton X-100 and they showed no eEF-Ts activity. Besides the 2 high molecular weight species, only a very weak band in the range of 30 000 mol. wt was observed on SDS gel electrophoresis in this case. Hydrophobic adsorption chromatography confirms therefore that the 50 000/57 000 chains are dispensable for eEF-Ts activity as defined by the stimulation of eEF-Tu-dependent Phe-tRNA binding [7–9]. In addition, eEF-Ts purified by hydrophobic adsorption chromatography also inhibits the permanent binding of tritiated eEF-Tu to the 80 S ribosome as shown in fig.4.

Both types of activity, namely stimulation of eEF-Tu-dependent binding of aminoacyl tRNA and the inhibition [^{3}H]eEF-Tu-binding to the ribosome, reside in the 28 000/30 000 mol. wt doublet. Concerning the stimulation of eEF-Tu-dependent binding of amino acyl tRNA to the ribosome, preliminary experiments indicate that the 30 000 and not the 28 000 mol. wt chain is the active component in the reaction.





We observed consistently that on SDS–polyacrylamide gel electrophoresis the two chains of eEF-Tu move significantly more slowly than both chymotrypsinogen (fig.5) and the *Artemia* RNA binding protein as defined in [17]. An antibody against their RNA binding protein crossreacts immunologically with our highly purified eEF-Ts preparation [17]. On the other hand, their RNA binding protein shows also in our SDS gel systems mol. wt 26 000 and therefore cannot be identical to eEF-Ts consisting of a doublet of 28 000/30 000 mol. wt. The reason for the immunological crossreactivity is therefore not yet clear.

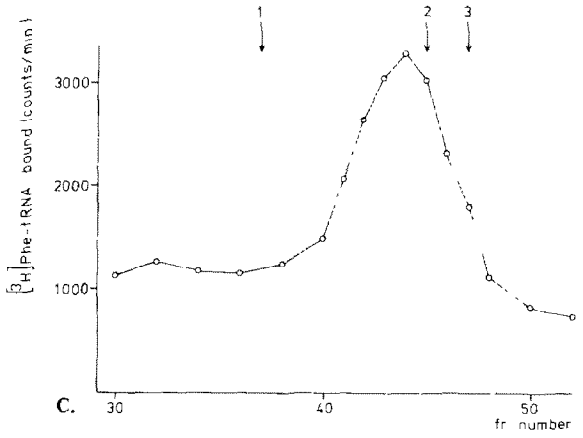


Fig.3. Partial purified eEF-Ts (5 mg) was applied to a phenyl–Sepharose CL 4B column (1.5 × 20 cm) and eluted with a buffer, containing 20 mM Tris–HCl, (pH 7.5); 0.1 mM EDTA; 1 mM Mg-acetate; 10 mM 2-mercaptoethanol and 10% (v/v) 2-propanol. Both the A_{280} (• – •) and the eEF-Ts activity (○ – ○) of the eluate were determined. The active fractions were pooled, concentrated and analysed by means of gel electrophoresis both under denaturing and non-denaturing conditions (fig.3B, a and b, respectively). When analyzed on a calibrated Sephacryl S200 column, purified eEF-Ts shows a single peak corresponding with app. mol. wt 70 000 (fig.3C); the arrows 1, 2 and 3 indicate the positions of immunoglobulin (mol. wt 156 000) bovine serum albumin (mol. wt 68 000) and eEF-Tu (mol. wt 50 000), respectively.

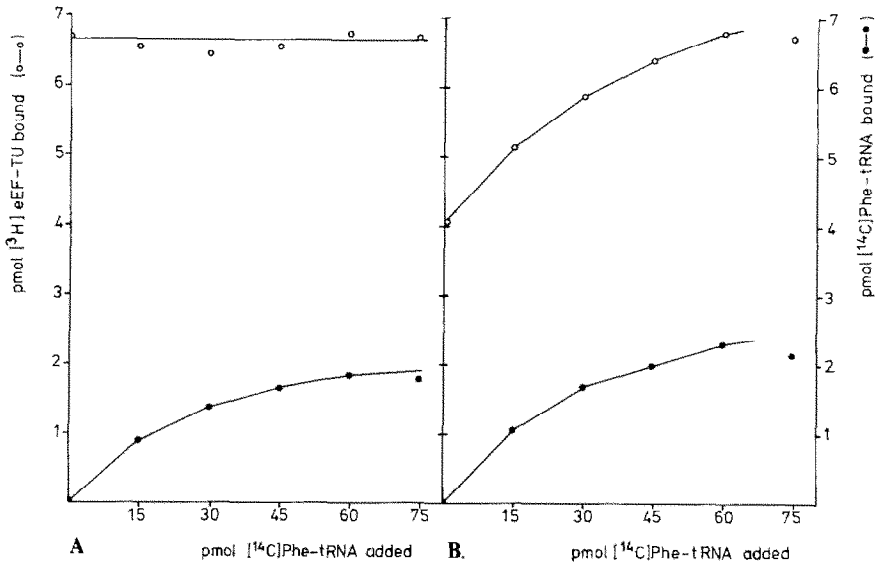


Fig.4. Salt-washed 80 S ribosomes (35 pmol) were incubated in the absence (A) and presence (B) of 2 μg highly purified eEF-Ts, with 50 pmol [^{3}H]eEF-Tu, prepared according to [10], spec. act. 700 cpm/pmol and with varying amounts of [^{14}C]Phe-tRNA, specific activity 200 cpm/pmol. The incubation mixture contained in addition 10 μg poly(U) and 0.25 mM GMPPCP. Other experimental conditions were as in [10]. The amounts of [^{3}H]eEF-Tu (○ – ○) and [^{14}C]Phe-tRNA (• – •), bound to the ribosome were determined.

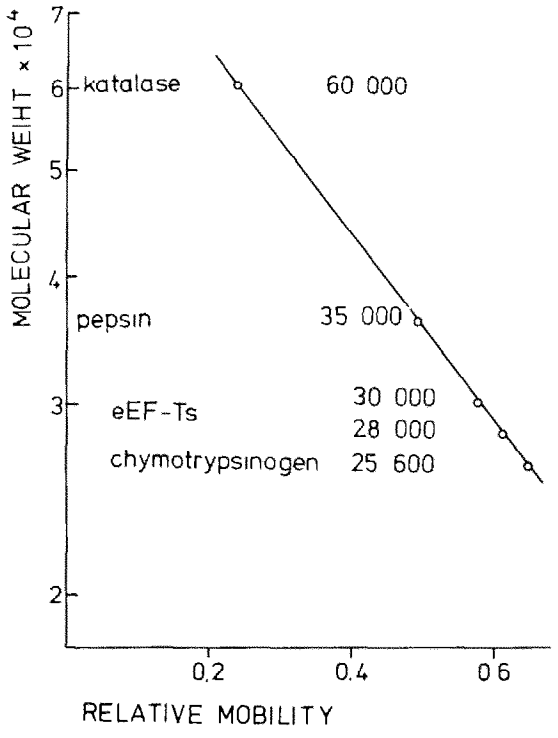


Fig.5. Highly purified eEF-Ts was analyzed by means of SDS-gel electrophoresis and its mobility compared with catalase (MW 60 000), pepsin (MW 35 000) and chymotrypsinogen (MW 25 600).

4. Discussion

The results presented above show that in *Artemia salina* the eEF-Ts activity purified by hydrophobic adsorption chromatography under non-denaturing conditions runs on SDS–polyacrylamide gel electrophoresis as 2 bands of est. mol. wt 28 000 and 30 000.

Unlike with pig liver (cf. [6]), no evidence was obtained that a 55 000 mol. wt chain could only be separated from a 30 000 mol. wt protein by denaturing agents such as guanidine–HCl, in combination with 6.8 M urea.

In view of the strong intrinsic aggregation behaviour of the 30 000 and 28 000 mol. wt chains (presumably a dimer) and the lack of an aggregation behaviour of eEF-Tu itself [15], eEF-Ts rather than eEF-Tu may be responsible for the occurrence of a number

of so-called high molecular weight forms of EF-1 which contain both eEF-Tu and eEF-Ts activity [12].

A new function of eEF-Ts in eucaryotic protein synthesis is that eEF-Tu binding to the ribosome is suppressed by eEF-Ts in the absence of aminoacyl tRNA. In eucaryotes, the binding of [^{3}H]methylated eEF-Tu as such to ribosomes is rather strong and independent of the presence or absence of nucleotides [18]. In procaryotes EF-Tu GDP does not bind at all to the 70 S ribosome [19]. Therefore eEF-Ts may play a physiological role in the cell by modulating the fraction of ribosomes containing bound eEF-Tu.

Such an effect should be especially apparent at low levels of aminoacyl transfer RNA in the cell. A modulating effect of eEF-Ts on the binding of eEF-Tu to the ribosome seems indeed the correct explanation for the inhibition of eEF-Tu-dependent, uncoupled hydrolysis of GTP [11].

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