DISAGGREGATION OF ELONGATION FACTOR 1 BY EXTRACTS OF ARTEMIA SALINA

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SUMMARY

Extracts of 40 hr Artemia salina nauplii can convert a heavy form of elongation factor 1 ($EF\text{-}1_H$) to a light species ($EF\text{-}1_L$). The data indicate that a protease in the extracts is responsible for this reaction, and these findings may explain the observation that extracts from Artemia salina nauplii have only $EF\text{-}1_L$ whereas before hatching of the Artemia salina embryos $EF\text{-}1_H$ is the predominant species (Slobin and Moller [1975] Nature 258, 452-454).

In most tissues, elongation factor 1 (EF-1) is present in multiple species which have now been shown to be aggregates ($EF\text{-}1_H$) of a polypeptide chain of molecular weight of about 50,000 ($EF\text{-}1_L$) (1-12). The latter form ($EF\text{-}1_L$) has been isolated from liver (4,6) and brain (3) although in general $EF\text{-}1_H$ is the more stable species and the one which has been routinely purified. The size of $EF\text{-}1_H$ varies depending on the tissue. In tissues such as reticulocytes, ascites cells, wheat germ, yeast and A. salina, the predominant form is an aggregate of molecular weight of about $2\text{-}3 \times 10^5$ (2,7,8,9,11,12). However, in liver and brain larger aggregates, some having molecular weights well over 1×10^6, have been observed (3,4). The indications are that $EF\text{-}1_L$ is the biologically active species (12-16). Although both $EF\text{-}1_H$ and $EF\text{-}1_L$ function in aminoacyl-tRNA binding to ribosomes, the ability of GTP to cause disaggregation of $EF\text{-}1_H$ suggests that in the initial interaction of $EF\text{-}1_H$ with GTP, an $EF\text{-}1_L \cdot GTP$ complex is formed (12,15). This can interact with aminoacyl-tRNA to form a ternary complex (16,17) which interacts with the ribosome (17).

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There have been reports that EF-1 is in limiting quantities relative to EF-2 in cell extracts, suggesting that any alteration in the EF-1 level could have an effect on the overall rate of elongation (18-21). In addition, it has been found in two cases that the form of EF-1 changes during the aging or development of an organism. In the nematode, T. aceti, $EF\text{-}1_H$ is present in younger organisms but as the nematode ages, there is a shift to $EF\text{-}1_L$ (22). Another example is in the development of A. salina. Slobin and Möller (23) have recently shown that before hatching, the EF-1 in the embryos is predominantly $EF\text{-}1_H$, but after hatching the nauplii contained almost exclusively $EF\text{-}1_L$. These results suggest that there is a mechanism in the tissues to convert $EF\text{-}1_H$ to $EF\text{-}1_L$. Previous results from our own laboratory have shown that in addition to GTP, which causes disaggregation of $EF\text{-}1_H$ (12,15), incubation of $EF\text{-}1_H$ with partially purified phospholipase C preparations (10, 12,24) as well as the protease elastase cause disaggregation of the enzyme (10,12). In the present study, evidence is provided that a specific protease is present in A. salina extracts after hatching that can convert $EF\text{-}1_H$ to $EF\text{-}1_L$.

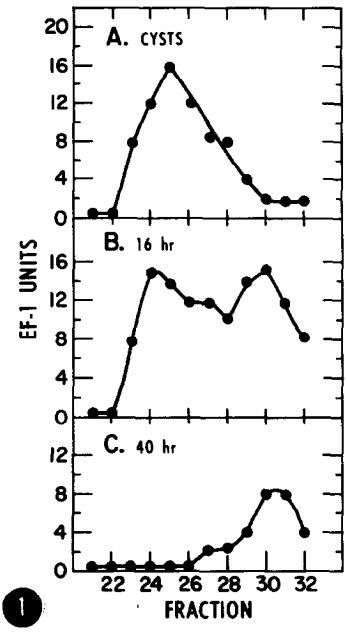
MATERIALS AND METHODS

All the materials used in the present study were obtained from commercial sources as described previously (3,4,14,17,24). EF-1 was purified from rabbit reticulocytes as described previously (10). One unit of EF-1 activity is defined as the amount of enzyme needed to bind one pmole of [^{14}C]-Phe-tRNA (E. coli) to ribosomes in five minutes at 37°C. The specific activity of the enzyme used was about 10,000 units/mg. The development of dry cysts of A. salina as well as the preparation of a 0-75% $(NH_4)_2SO_4$ fraction of the extracts was done as described previously (25). This fraction was used in the present studies and will be referred to as the extract. When necessary, the EF-1 in the extracts was inactivated by heating at 60° for 5 min. Under the conditions employed at 16 hr of development, there was a mixture of embryos and hatched nauplii; but by 40 hr, there was almost complete hatching of the embryos. Sucrose gradient analysis of EF-1 was performed on a 5-20% sucrose gradient which readily separated $EF\text{-}1_H$ from $EF\text{-}1_L$. The details have been described elsewhere (3,10,24) and are presented in the legend to Figure 1. Polyacrylamide gel electrophoresis analysis was done according to the procedure of King and Laemmli (26). EF-1 was reductively methylated with [^{14}C]-formaldehyde by the procedure of Rice and Means (27). Further details are in the legend of Figure 4. The specific activity of the radioactive protein was 10^6 cpm/mg.

RESULTS AND DISCUSSION

Slobin and Möller (23) have reported changes in the form of EF-1 during development of A. salina and we have confirmed their results as shown in Figure 1. As reported previously (12,23), the enzyme is present primarily as an aggregate ($EF\text{-}1_H$) in extracts of A. salina cysts. After 16 hr of development when some hatching has occurred, there is a mixture of $EF\text{-}1_H$ and $EF\text{-}1_L$ and after 40 hr of development, EF-1 is predominantly found as a low molecular weight form.

In order to investigate whether a factor in the 40 hr extract was responsible for this disaggregation, purified reticulocyte $EF\text{-}1_H$ (10) was incubated with A. salina extracts from different stages of development. Sucrose gradient analysis was then used to assay for disaggregation of the reticulocyte enzyme. Typical results are shown in Figure 2. There was no observed disaggregation of reticulocyte $EF\text{-}1_H$ after incubation of the factor with an extract (3 µg protein) from dry cysts. Using similar amounts of extract prepared from 16 hr embryos, significant disaggregation is observed, which might be related to the extent to which hatching has occurred. With extracts prepared from swimming nauplii (40 hr), $EF\text{-}1_L$ was found exclusively. These results indicated that a component was present in the nauplii extract which converted the aggregate form of reticulocyte $EF\text{-}1_H$ to $EF\text{-}1_L$. Figure 3 shows the effect of different concentrations of the 40 hr extract on the gradient profile of reticulocyte EF-1. With 1.4 µg of protein, there was complete conversion of $EF\text{-}1_H$ to $EF\text{-}1_L$ (Fig. 3B). With higher levels of the extract (2.4 µg protein) $EF\text{-}1_L$ is still the predominant species, but the recovery of EF-1 activity was somewhat lower (Fig. 3C) and when 21 µg of protein were used, no EF-1 activity was recovered on the gradient (Fig. 3D). These results suggested that a protease might be responsible for the disaggregation as well as inactivation of $EF\text{-}1_H$. This was confirmed by showing that preincubation of the 40 hr extract with phenylmethylsulfonylfluoride



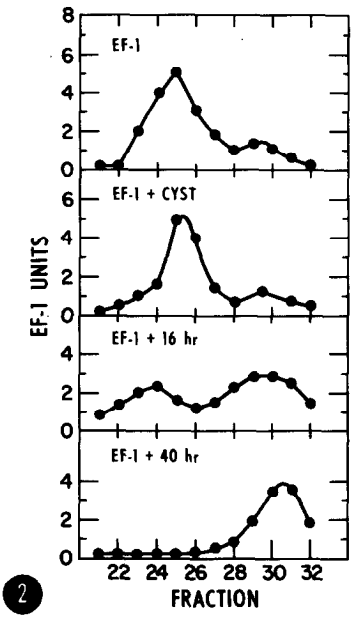


Fig. 1: Sucrose gradient centrifugation profiles of EF-1 activity in 100,000 x g supernatants of Artemia salina. The 100,000 x g supernatant from dry cysts, embryos (16 hr) and nauplii (40 hr), were prepared as described elsewhere (25). The dry cysts were developed as described previously (25) except that after 16 hr of development, the medium was changed from 4% sodium chloride to artificial sea water. To obtain nauplii, the growth was continued for an additional 24 hr (total development time 40 hr). For gradients A and B approximately 150 units, and for C about 75 units of EF-1 were used. The samples (100 µl) were placed on 4.3 ml of a 5-20% sucrose gradient (in 50 mM Tris HCl pH 7.4, 1 mM DTT) and the centrifugations performed in a Spinco SW 56 rotor at 50,000 rpm for 2 hr. The tubes were then punctured and approximately 32 fractions were collected. Each fraction was assayed for EF-1 activity based on Phe-tRNA binding to ribosomes (17).

(A) dry cysts,
(B) 16 hr of development,
(C) 40 hr of development (swimming nauplii).

Fig. 2: Sucrose gradient centrifugation profiles of rabbit reticulocyte EF-1 after incubation with A. salina extracts at different stages of development. EF-1 (about 60 units) was incubated for 40 min at 37° in a total volume of 110 µl containing 50 mM Tris-HCl buffer pH 7.4, and 1 mM DTT. Where indicated, about 3 µg of protein of each extract were added. 100 µl of each reaction mixture were placed on the gradient.

(PMSF) prevented the disaggregation of EF-1_H (Fig. 3E), as well as the inactivation of the factor seen with higher amounts of the 40 hr extract (data not shown).

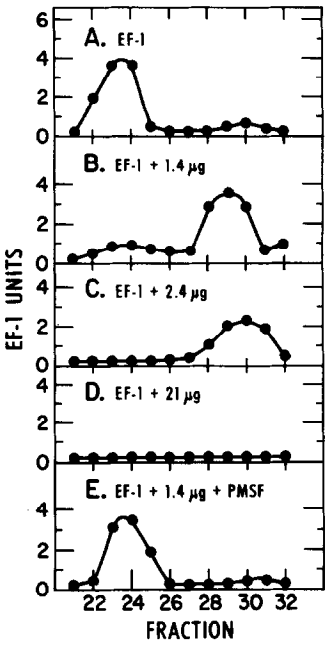


Fig. 3: The effect of different concentrations of 40 hr A. salina extracts on the sucrose gradient profiles of rabbit reticulocyte EF-1. Approximately 60 units of rabbit reticulocyte EF-1 were incubated in a 110 µl solution containing 50 mM Tris-HCl pH 7.4, 1 mM DTT and various amounts of 40 hr A. salina extract for 40 min at 37°C. 100 µl of the incubation mixture were placed on the gradient.

(A) no extract,
(B) 1.4 µg of protein,
(C) 2.4 µg of protein,
(D) 21 µg of protein,
(E) 1.4 µg of protein plus PMSF. The A. salina extract was pre-incubated for 5 min at 37°C in 20 µl of a solution containing 50 mM Tris-HCl pH 7.4, 1 mM DTT and 7.5 mM PMSF.

Sodium dodecylsulfate (SDS) disc gel electrophoresis provided further evidence that a protease was involved in the disaggregation of $EF\text{-}1_H$ (Fig. 4). As shown in Figure 4B, incubation of reticulocyte [^{14}C]-EF-1 with a 40 hr A. salina extract (1.4 µg protein) results in the disappearance of the major EF-1 peak and appearance of polypeptides in the molecular weight range of 10 - 40,000. With larger amounts of the extract (35 µg), the EF-1 peak is lost and fewer polypeptide products were detected on the gel (Fig. 4C). As shown in Figure 4D, PMSF again inhibits the ability of the 40 hr extract to disaggregate $EF\text{-}1_H$ to smaller polypeptides.

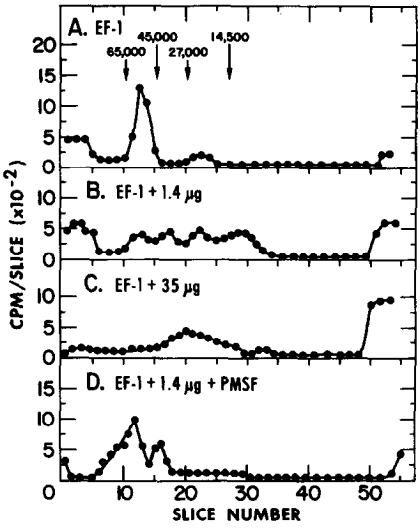


Fig. 4: SDS polyacrylamide gel electrophoresis of [^{14}C]-labeled EF-1 incubated with a 40 hr A. salina extract. To label EF-1, 1 mg of the factor was dialyzed overnight against three 1 liter changes of 25 mM sodium borate pH 8.9. 10 µl of 0.3 M [^{14}C]-formaldehyde (S.A. 10 Ci/mol) were added to the cooled solution. After 2 min, three additions of 10 µl of [^{14}C]-formaldehyde were added. After 10 min of incubation at 0°C, 200 µl of sodium borohydride (5 mg/ml) were added in five 40 µl additions 30 sec apart. The labeled protein was dialyzed (24 hr) against five 1 liter changes of 25 mM sodium borate pH 8.9 followed by dialysis for 12 hr against three 1 liter changes of 50 mM Tris-HCl buffer pH 7.4 containing 1 mM DTT. The [^{14}C]-EF-1 (15,000 cpm) was incubated with the following amounts of 40 hr extract:

(A) alone,
(B) 1.4 µg of protein,
(C) 35 µg of protein,
(D) 1.4 µg of protein. The A. salina extract was preincubated for 5 min at 37°C with 7.5 mM PMSF before the addition of EF-1. (See legend to Fig. 3.)

After incubation, the gels were sectioned into 1 mm slices using a Gilson automatic gel slicer. The slices were suspended in 0.5 ml of 1% SDS and radioactivity determined (29) in a liquid scintillation spectrometer.

Recent studies on the mechanism of elastase disaggregation of EF-1_H showed that two major polypeptide products with molecular weights of about 30,000 and 15,000 were obtained by disc gel analysis after incubating EF-1_H with elastase (28). Since elastase did not inactivate EF-1, it was postulated that these two large polypeptides remained associated, and, therefore, maintained enzymatic activity. The data in Figure 4 suggest that with low levels

of the 40 hr extract, a similar degradation of EF-1_H occurs. However, there is evidence that the size of the products of EF-1 obtained with the A. salina 40 hr extract are not identical to those seen with elastase (data not shown). The protease in the A. salina extract responsible for EF-1 disaggregation was rather specific with regard to EF-1. Several other proteins were examined by SDS gel analysis and found to be inactive as substrates; these included EF-Tu, albumin, lysozyme, elastase and ovalbumin. However, chymotrypsinogen was degraded by the extract although it is not clear whether the activity towards chymotrypsinogen and EF-1 are mediated by the same protein.

The present results demonstrate the presence of proteolytic activity in 40 hr extracts from A. salina nauplii which can disaggregate EF-1_H to EF-1_L. In some respects, the disaggregation of EF-1_H is similar to that observed previously with elastase (10,11,28). Although the role of the aggregate forms of EF-1 is still not clear, the fact that in some tissues the ratio of EF-1_H to EF-1_L changes as a function of age or development (22,23) suggests that EF-1 may be under some sort of regulation. It will be of interest to determine whether proteolysis represents a general mechanism in tissues for the conversion of EF-1_H to EF-1_L.

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