DISSOCIATION OF EUKARYOTIC RIBOSOMES BY PURIFIED INITIATION FACTOR EIF-3

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SUMMARY

Purified eukaryotic initiation factor, EIF-3, prepared from ascites cells dissociated rat liver 80S ribosomes into subunits. Ribosomes bearing endogenous mRNA and nascent peptide were not dissociated by EIF-3. When 80S ribosomes reconstituted from subunits were used as substrate the reaction had the following characteristics: Dissociation was rapid--the reaction being completed within 2 min at 30°. The extent of dissociation was directly proportional to the amount of EIF-3; with 21 μg of EIF-3 about 70% (or 10.5 μg) of the 80S monomers were dissociated. The dissociation of 80S monomers by EIF-3 decreased with increasing concentrations of magnesium. The reaction was not catalytic: 28 moles of EIF-3 were required to dissociate 1 mole of 80S ribosomes. The characteristic of the dissociation reaction promoted by EIF-3 and by E. coli initiation factor IF-3 are remarkably similar. The dissociation reaction provides a practical assay for EIF-3 independent of complimentation of other initiating factors.

INTRODUCTION

Three factors which are required for the initiation of protein synthesis are found in ascites cell cytosol and in the extract prepared from ribosomes with 1 M potassium chloride (1-3). The factors are similar to those which are found in reticulocytes (4-12), in mouse fibroblasts (13, 14), in the brine shrimp Artemia salina (15), and presumably other animal cells. Two of the initiation factors, EIF-1 and EIF-2, catalyze the binding of met-$tRNA_f$ to ribosomes (4) and lower the concentration of magnesium at which poly (U) directed polyphenylalanine synthesis is maximal (5, 6). The third factor, EIF-3, is needed for the translation of natural mRNA (7).

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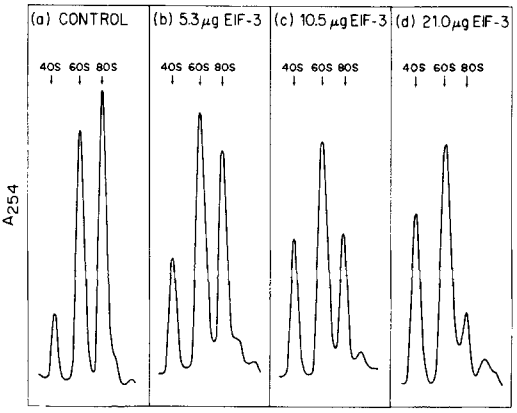


Figure 1. Dissociation of 80S ribosomes by initiation factor EIF-3. Ribosomal subunits (4.28 μg of 40S and 10.71 μg of 60S) were incubated for 15 min at 30° in 100 μl of medium A containing 0.29 mg of bovine serum albumin. Various amounts of EIF-3 were added to the mixture and incubation was continued for another 5 min. The samples were chilled on ice and fixed with 25 μl of glutaraldehyde solution (5%). An aliquot (80 μl) was layered on a 10 to 30% sucrose gradient in medium A. The gradients were centrifuged at 4° for 100 min at 45,000 rpm in a SW 50.1 rotor and the sedimentation of the particles was determined with an ISCO gradient fractionator and UV analyzer.

Dissociation of ribosome monomers into subparticles is a requisite for initiation of protein synthesis. A factor with that activity was found in E. coli by Subramanian et al. (16). Subsequently dissociating activity was demonstrated to be present in Bacillus stearothermophilus (17), Saccharomyces cerevisiae (18), rat liver (19), and rabbit reticulocytes (11, 20). Dissociating activity in prokaryotic cells is known now to be a property of the initiation factor IF-3 (21-27). Thus it is to be expected that one of the eukaryotic initiation factors is also responsible for dissociation. We have found that to be the case: partially purified EIF-3 from ascites cells (resolved free of initiation

factors EIF-1 and EIF-2) dissociates liver 80S ribosomes into 40S and 60S subunits.

EXPERIMENTAL PROCEDURES

Rat liver ribosomal subunits were prepared as described before (28, 29). Just prior to the experiments the suspension of subunits was clarified by centrifugation at 10, 000 g for 5 min at 4°. The concentration of ribosomal subunits was calculated from the absorbance at 260 nm: 1 A_{260} unit was taken to be equivalent to 45 μg of rRNA (30).

The 80S monomers that were used as substrate in the dissociation reaction were prepared by reassociating ribosomal subunits (4. 28 μg of rRNA of 40S and 10. 17 μg of 60S) by incubating them for 15 min at 30° in 100 μl of medium A (10 mM Tris-HCl, pH 7. 6; 120 mM KCl; 3. 5 mM $MgCl_2$) containing 0. 29 mg of bovine serum albumin. The albumin was added to prevent loss of ribosomal particles during glutaraldehyde fixation (31). Dissociation was assayed by adding EIF-3 to the reassociation mixture and continuing the incubation for 5 min. The dissociation reaction was terminated by cooling the sample on ice and fixing the particles with glutaraldehyde; the procedure is a modification (Nakaya and Wool, submitted for publication) of the method of Subramanian (31). An aqueous solution of 50% glutaraldehyde was mixed with an equal volume of 1 M Tris solution in an ice bath and the pH was adjusted to 7. 2 to 7. 6 with a few drops of 0. 5 N KOH. The solution was diluted five-fold with medium A; 25 μl of the freshly prepared cold glutaraldehyde solution was added to each sample (the final concentration of glutaraldehyde was 1%). The fixed sample was kept at 0° for 5 min, then 80 μl was layered onto a 5. 2 ml linear 10 to 30% sucrose gradient in medium A. Centrifugation was in a SW 50. 1 rotor at 45, 000 rpm for 100 min at 4°. The distribution of ribosomal particles in the gradient was deter-

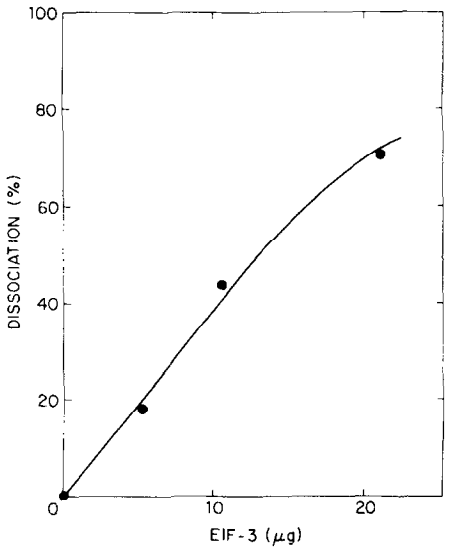


Figure 2. Effect of the amount of EIF-3 on the dissociation of 80S monomers. The data are from Fig. 1. The percentage of dissociation was calculated from the areas under the 80S peaks.

mined with an ISCO density gradient fractionator and ultraviolet analyzer (29). The extent of dissociation was estimated by cutting out and weighing the 80S peak of the optical density tracing.

The initiation factor EIF-3 was extracted from mouse ascites cell ribosomes with 0.25 M KCl and partially purified by chromatography on DEAE-Sephadex A-50 and hydroxyapatite. The factor was resolved free of contamination with EIF-1 and EIF-2. The details of the purification and the characteristics of EIF-3 will be reported in detail later (Ranu and Wool, manuscript in preparation).

RESULTS AND DISCUSSION

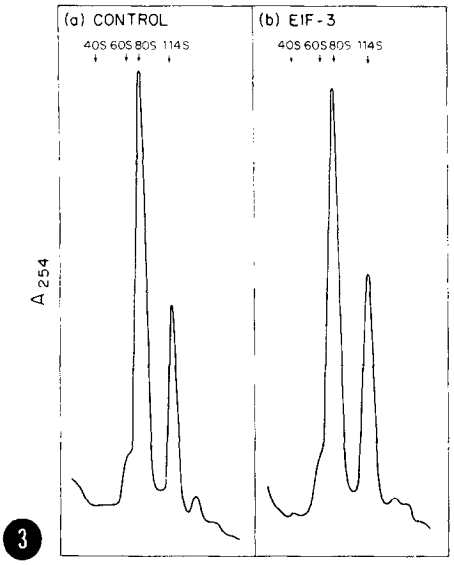
Rat liver ribosomes dissociate to 40S and 60S subparticles when treated with puromycin and high concentrations (0.8 M) of potassium (28); the subunits have a great affinity and will reassociate if the potassium concentration is lowered to 120 mM (Fig. 1a). The 80S monomers so formed are free of pep-

tidyl-tRNA and mRNA (32) and thus are excellent substrate for assaying dissociating activity. Addition of purified EIF-3 from ascites cells to such 80S ribosomes decreased their numbers and pari passu the numbers of 40S and 60S subunits (Fig. 1b-d). While it is convenient to refer to the reaction as dissociation, we cannot tell from our experiments whether the factor prevented association of subparticles or caused dissociation of ribosome monomers.

Dissociation was proportional to the amount of EIF-3 added (Fig. 1b-d; the results are replotted in Fig. 2). With 21 μg of EIF-3, about 70% (or 10.5 μg) of the 80S monomers were dissociated (Fig. 2). We calculate that 28 moles of EIF-3 preparation protein were required to dissociate 1 mole of 80S ribosomes. For the calculation we assumed the molecular weight of EIF-3 to be 139,000 (Ranu and Wool, manuscript in preparation) and that of 18S RNA to be 6.5×10^5. Since the EIF-3 preparation is not pure, and in as much as we do not know what portion of the EIF-3 molecules are active, we cannot be certain how the factor is working. We can say, however, that we have no evidence that it acts catalytically. E. coli initiation factor IF-3 also seems to act stoichiometrically in the dissociation reaction (21, 23).

E. coli IF-3 dissociates only vacant 70S ribosomes (23, 33, 34). To determine if the same was true for EIF-3, single ribosomes bearing endogenous mRNA and nascent peptide (80S monosomes) were prepared from rat liver polysomes by treatment with 0.5% deoxycholate in the absence of RNase inhibitor (35). The 80S monosomes were not dissociated even by a large amount (21 μg) of EIF-3 (Fig. 3).

Dissociation was rapid: the reaction was completed in 2 min at 30° (Fig. 4). The kinetics of dissociation of E. coli ribosomes by IF-3 is similar (16). The kinetics (Fig. 4) and the stoichiometry (Fig. 2) suggest dissociation



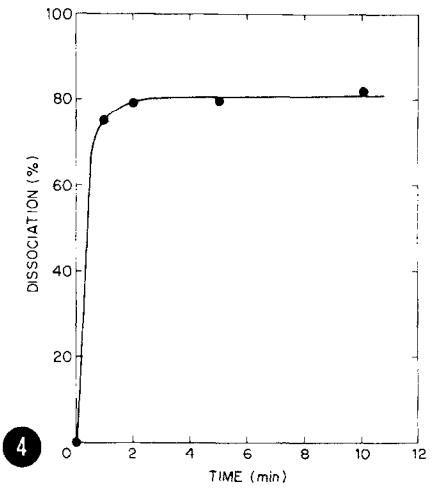


Figure 3. Effect of EIF-3 on ribosomes bearing nascent peptide and endogenous mRNA. (a) Sedimentation profile of 15.8 μg (rRNA) of rat liver monosomes and disomes prepared from polysomes by treatment with 0.5% of deoxycholate in the absence of RNase inhibitor. (b) 21 μg of EIF-3 were added to 15.8 μg (rRNA) of the rat liver monosomes in medium A and the mixture was incubated for 5 min at 30°. Samples were fixed with glutaraldehyde and centrifuged at 58,500 rpm for 25 min at 4° in a SW 65 rotor.

Figure 4. The time course of dissociation of 80S monomers by EIF-3. Ribosomal subunits (4.28 μg of 40S and 10.71 μg of 60S) were incubated for 15 min at 30° in 100 μl of medium A containing 0.29 mg of bovine serum albumin. EIF-3 (21 μg) was added and incubation was continued for the time indicated. Samples were taken at intervals, fixed with glutaraldehyde, and analyzed as in Fig. 1.

is the result of the formation of a complex between EIF-3 and the ribosome or ribosomal subparticle.

Dissociation of ribosome monomers by EIF-3 was not affected by GTP

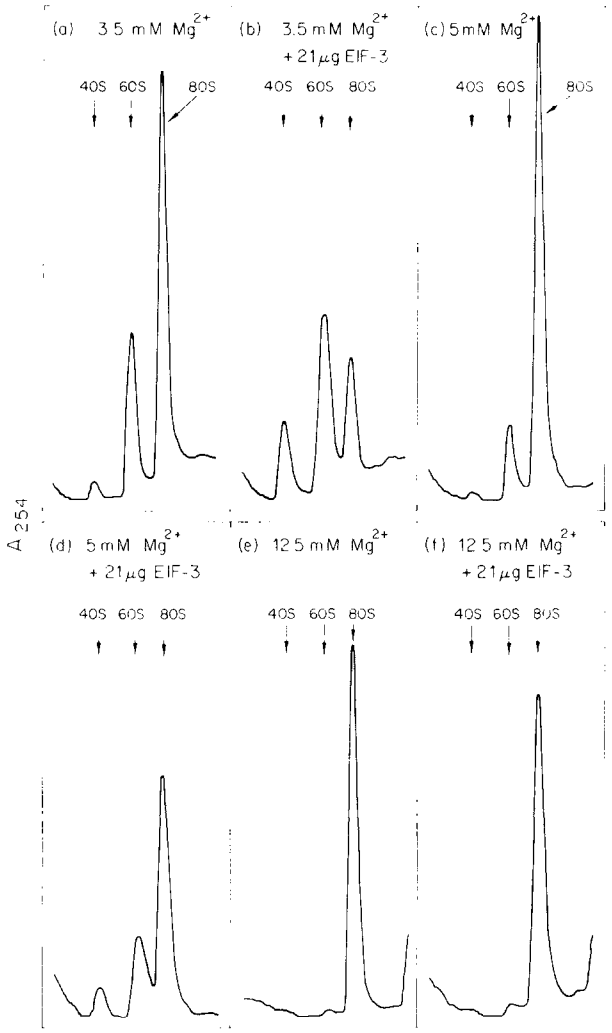


Figure 5. Effect of magnesium concentration on dissociation of 80S monomers by EIF-3. Ribosomal subunits (4.28 μg of 40S and 10.71 μg of 60S) were incubated for 15 min at 30° in media containing 125 mM potassium and the concentration of magnesium indicated. EIF-3 (21 μg) was added to the mixtures and incubation continued for another 5 min. These samples were fixed with glutaraldehyde and analyzed as in Fig. 1.

(results not shown). The original observation that GTP stimulated dissociation of E. coli ribosomes by IF-3 (16, 36) was later shown to be the result of chelation of magnesium by the nucleoside triphosphate (21).

Ribosomal subunits have a greater tendency to reassociate if the concentration of magnesium is raised or that of potassium is lowered (28). We studied the effect of magnesium concentration on the dissociating activity of EIF-3 (Fig. 5); the potassium concentration was kept at 125 mM. When the concentration of magnesium was 3.5 mM, 21 μg of EIF-3 caused dissociation of 64% of the 80S monomers (compare Fig. 5a and 5b). In 5 mM magnesium there were very few free subparticles (Fig. 5c), but 21 μg of EIF-3 caused only 48% of the monomers to dissociate (Fig. 5d). EIF-3 caused little or no dissociation when the magnesium concentration was 12.5 mM.

The characteristics of the dissociation of eukaryotic ribosomes by EIF-3 are very similar to those of the reaction promoted by E. coli IF-3. Thus EIF-3 dissociates only 80S monomers free of mRNA and nascent peptide; dissociation is proportional to the amount of EIF-3; the reaction is rapid, being completed in 2 min at 30°; GTP is not required; and dissociation by EIF-3 is inhibited by increasing the concentration of magnesium. The dissociating properties of EIF-3, and the observation that it is required for translation of natural mRNA, e.g. encephalomyocarditis virus RNA (Ranu and Wool, manuacript in preparation), suggest that in these respects at least eukaryotic EIF-3 and prokaryotic IF-3 are functionally homologous.

Schreier and Staehelin (8) have reported that an unresolved preparation of initiation factors from rabbit reticulocytes has dissociating activity. Mizuno and Rabinovitz (10) also prepared an initiation factor fraction from rabbit reticulocytes and showed that it has dissociating activity when added to cell lysates. Finally, Kaempfer and Kaufman (11) demonstrated that a fraction from reticulocyte ribosomes with initiation factor activity prevented association of 40S and 60S subunits. While the identity of the dissociating activity is not certain from those experiments, since the preparations were not shown to contain a single initiation

factor, it would seem likely now that dissociation is due to the presence of EIF-3. It should be noted, however, that Lubsen and Davis (20) had suggested, on the basis of elution of dissociating activity from DEAE-cellulose with 0.2 and 0.3 M KCl, that the activity was a property of EIF-2. Moreover, they stated (although no data was given) that a preparation of EIF-2 had dissociating activity whereas a preparation containing EIF-1 and EIF-3 did not. We have not had an opportunity to test the dissociating activity of ascites cell EIF-2, but the preparation of EIF-3 we used was free of EIF-2.

The mechanism by which prokaryotic IF-3 causes dissociation is moot. Subramanian et al. (16) have postulated that IF-3 causes dissociation by binding to the 30S subunit of E. coli 70S ribosomes. Sabol and Ochoa (24) have shown that ^{35}S labeled IF-3 binds to 30S but not to the 50S subunit of 70S ribosomes and that binding of IF-3 is followed by dissociation. Kaempfer (37), on the other hand, finds that IF-3 prevents formation of 70S ribosomal monomers from subunits without causing dissociation when the assay is carried out under the ionic conditions which are optimal for protein synthesis in vitro. Kaempfer's results lead him to postulate that IF-3 acts to shift the equilibrium between 70S ribosomes and subunits in favor of the latter by binding to free 30S subparticles and thereby preventing association. He has suggested (11) that the homologous eukaryotic initiation factor has a similar mechanism of action. We are unable, from our experiments, to determine if EIF-3 causes dissociation or is an anti-association factor.

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