Gehron Robey, P., Fisher, L. W., Stubbs, J. T., & Termine, J. D. (1987) *UCLA Symp. Mol. Cell. Biol., New Ser. 46*, 115–126.
Geliebter, J., Zeff, R. A., Melvoid, R. W., & Nathenson, S. G. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 3371–3375.
Gilbert, W. (1985) *Science (Washington, D.C.) 235*, 823–824.
Hewick, R. M., Hunkapiller, M. W., Hood, L. E., & Dreyer, W. J. (1981) *J. Biol. Chem. 256*, 7990–7997.
Hunkapiller, M. W., & Hood, L. E. (1983) *Methods Enzymol. 91*, 486–493.
Lonberg, N., & Gilbert, W. (1985) *Cell (Cambridge, Mass.) 40*, 81–90.
Maniatis, T., Fritsch, E. F., & Sambrook, J. (1982) *Molecular Cloning*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Mason, I. J., Taylor, A., Williams, J. G., Sage, H., & Hogan, B. L. M. (1986a) *EMBO J. 5*, 1465–1472.
Mason, I. J., Murphy, D., Munke, M., Franke, U., Elliot, R. W., & Hogan, B. L. M. (1986b) *EMBO J. 5*, 1831–1837.
Miyada, C. G., Klofelt, C., Reyes, A. A., McLaughlin-Taylor, E., & Wallace, R. B. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 2890–2894.
Odermatt, E., Tamkun, J. W., & Hynes, R. O. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 6571–6575.
Peralta, E. G., Winslow, J. W., Peterson, G. L., Smith, P. H., Ashkenazi, A., Ramachandran, J., Shimerlik, M. I., & Capon, D. J. (1987) *Science (Washington, D.C.) 236*, 600–605.
Romberg, R. W., Werness, P. G., Lollar, P., Riggs, B. L., & Mann, K. G. (1985) *J. Biol. Chem. 260*, 2728–2736.
Romberg, R. W., Werness, P. G., Riggs, B. L., & Mann, K. G. (1986) *Biochemistry 25*, 1176–1180.
Sage, H., Tupper, J., & Bramson, R. (1986) *J. Cell. Physiol. 127*, 373–387.
Sanger, F., Nicklen, S., & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463–5467.
Smith, B. D., Martin, G. R., Miller, E. J., Dorfman, A., & Swarm, R. (1975) *Arch. Biochem. Biophys. 166*, 181–186.
Stenner, D. D., Tracy, R. P., Riggs, B. L., & Mann, K. G. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 6892–6896.
Sudhof, T. C., Goldstein, J. L., Brown, M. S., & Russell, D. W. (1985) *Science (Washington, D.C.) 228*, 815–822.
Termine, J. D., Belcourt, A. B., Conn, K. M., & Kleinman, H. K. (1981a) *J. Biol. Chem. 256*, 10403–10408.
Termine, J. D., Kleinman, H. K., Whitson, S. W., Conn, K. M., McGarvey, M. L., & Martin, G. R. (1981b) *Cell (Cambridge, Mass.) 26*, 99–105.
Tilgham, S. M., Curtis, P. J., Tiemeier, D. C., Leder, P., & Weissman, C. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 1309–1313.
Wieringa, B., Meyer, F., Reisser, J., & Weissman, C. (1983) *Nature (London) 301*, 38–43.
Yamada, Y., Avvedimento, V., Mudryji, M., Ohkubo, H., Vogeli, G., Irani, M., Pastan, I., & deCrombrugghe, B. (1980) *Cell (Cambridge, Mass.) 22*, 887–892.
Yang, J.-Q., Remmers, E. F., & Marcu, K. B. (1986) *EMBO J. 5*, 3553–3562.
Young, M. F., Bolander, M. E., Day, A. A., Ramis, C. I., Gehron Robey, P., Yamada, Y., & Termine, J. D. (1986) *Nucleic Acids Res. 14*, 4483–4497.

Effects of Eucaryotic Initiation Factor 3 on Eucaryotic Ribosomal Subunit Equilibrium and Kinetics†

Dixie J. Goss,*,‡ Donna Rounds,‡ Teresa Harrigan,‡ Charles L. Woodley,§ and Albert J. Wahba§
Department of Chemistry, Hunter College of the City University of New York, New York, New York 10021, and Department of Biochemistry, The University of Mississippi Medical Center, Jackson, Mississippi 39216
Received April 22, 1987; Revised Manuscript Received October 8, 1987

ABSTRACT: In order to understand the possible role of eucaryotic initiator factor 3 (eIF-3) in maintaining a pool of eucaryotic subunits, we have measured the effects of eIF-3 on the equilibria and kinetics of ribosomal subunit association and dissociation. The ribosomal subunit interactions have been studied by laser light scattering, which does not perturb the system. We find that eIF-3 reduces the apparent association rate of reticulocyte, wheat germ, and *Artemia* ribosomes. The kinetics of the reassociation for a shift in [Mg^{2+}] from 0.5 to 6 mM are best explained by a model where eIF-3 dissociates from the 40S subunits prior to association of the 40S and 60S subunits. Static titrations indicate there is some binding of eIF-3 to 80S ribosomes at lower [Mg^{2+}].

The Mg^{2+}-dependent association and dissociation of ribosomal subunits play a key role in the initiation of protein synthesis in both eucaryotes and procaryotes. This process is regulated by initiation factor 3 in procaryotic systems [see Maitra et al. (1982) for a review]. The procaryotic initiation factor 3 is a single polypeptide of about M_r 23 000.

Eucaryotic initiation factor 3 (eIF-3) has been isolated by several groups from different sources (Schreier & Staehelin, 1973; Safer et al., 1976; Benne & Hershey, 1976; Trachsel et al., 1977; Thompson et al., 1977; Brown-Luedi et al., 1982; Nygard & Westermann, 1983; Spremulli et al., 1979; Seal et al., 1983). In all cases, eIF-3 was shown to be a large multicomponent protein complex composed of at least nine different polypeptides. Benne and Hershey (1976) reported that rabbit reticulocyte eIF-3 bound to the 40S and not the 60S ribosomal subunits. Trachsel and Staehlin (1979), using glutaraldehyde fixation, found eIF-3 bound to the 40S and not the 60S ribosomal subunit. Behlke et al. (1986) have demonstrated by electron microscopy the binding of eIF-3 to

† Supported in part by grants from Research Corp., a PSC-CUNY Faculty Research award, an American Heart Association-NYC Affiliate Investigator award (D.J.G.), and NSF DMB 86-007070 and NIH GM 24451.
* Address correspondence to this author.
‡ Hunter College of the City University of New York.
§ The University of Mississippi Medical Center.

0006-2960/88/0427-1489$01.50/0

the 40S subunits. It has been reported that eIF-3 stimulated the binding of the ternary complex (eIF-3·GTP-Met-tRNA$_f$) to the 40S subunit (Schreier & Staehelin, 1973) and is essential for the binding of mRNA to the 40S subunit (Trachsel et al., 1977). eIF-3 has been shown in some cases to shift the ribosome subunit equilibrium toward dissociation (Thompson et al., 1977; Trachsel & Staehelin, 1979). Checkley et al. (1981) have reported that wheat germ eIF-3 does not prevent the association of wheat germ 40S and 60S ribosomal subunits nor does it significantly enhance the dissociation of 80S subunits.

In order to understand the possible role of eIF-3 in maintaining a pool of eucaryotic ribosomal subunits, we have measured the effect of eIF-3 on the equilibria and kinetics of ribosomal subunit association and dissociation. We have studied ribosomal subunit interactions using laser light scattering, which does not perturb the system. The only previous study (Trachsel & Staehelin, 1979) of ribosome–eIF-3 kinetics used glutaraldehyde fixation followed by ultracentrifugation. Centrifugation may cause pressure-induced dissociation artifacts. We find that eIF-3 reduces the apparent association rate of reticulocyte, wheat germ, and *Artemia* ribosomes. The kinetics of the reassociation for a shift in [Mg^{2+}] from 0.5 to 6 mM are best explained by a model where eIF-3 dissociates from the 40S subunit prior to recombination of the 40S and 60S subunits. At lower [Mg^{2+}] there is some binding of eIF-3 to the 80S ribosomes.

Materials and Methods

Artemia 80S ribosomes were prepared as described by MacRae et al. (1979). Rabbit reticulocyte 80S ribosomes were prepared by incubation of polysomes with ATP, GTP, creatine phosphate, and unlabeled amino acids as described (Gupta, 1968), chilled and made 0.5 M in NH_4Cl and 9 mM in $MgCl_2$, and pelleted through a 50% sucrose cushion containing 20 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) (pH 7.5), 500 mM NH_4Cl, 1 mM dithiothreitol, and 9 mM Mg^{2+} (Mehta et al., 1983). Wheat germ was generously supplied by J. M. de Rosier (International Multifoods, Minneapolis, MN) and stored at −20 °C. Wheat germ ribosomes were isolated by the method of Sperrazza and Spremulli (1983). Ribosomes were stored under liquid nitrogen in buffer consisting of 20 mM Tris-HCl (pH 7.5), 250 mM sucrose, 100 mM KCl, 0.1 mM ethylenediaminetetraacetic acid (EDTA), and 9 mM $MgCl_2$. Buffer B consisting of 20 mM Tris-HCl, pH 7.6, 1 mM dithiothreitol, 0.1 mM EDTA, and KCl and $MgCl_2$ as indicated was used for kinetics and equilibria measurements. Tris (Trizma) base was a product of Sigma Chemical Co. All other chemicals were of reagent grade.

Eucaryotic initiation factor 3 (eIF-3) was purified from the ribosomal salt wash of rabbit reticulocyte ribosomes by batch elution from DEAE-cellulose and phosphocellulose, by sucrose gradient fractionation (Merrick, 1979), and followed by chromatography on Mono S (the factor did not absorb to the column at 100 mM KCl) and gradient elution from Mono Q (eIF-3 elutes at 400 mM KCl). Mono S and Mono Q are products of Pharmacia. Wheat germ eIF-3 was the generous gift of Professor J. Ravel.

Determination of Ribosome Concentrations. Ribosomes were assumed to have an absorbance of 0.121 at 260 nm for a 0.001% solution (Niewenhuysen & Clauwaert, 1981). The molecular weights of the *Artemia* 80S ribosomes and the 40S and 60S subunits were assumed to be 3.8×10^6, 1.4×10^6, and 2.4×10^6, respectively (Nieuwenhuysen et al., 1981). Due to the slightly larger contribution of the RNA components of mammalian 60S ribosomal subunits, the reticulocyte 60S subunit has a M_r of 2.6×10^6 and the 80S ribosome a M_r of 4.0×10^6. The molecular weight of eIF-3 was taken to be 660 000 (Behlke et al., 1986).

Static Light Scattering Experiments. Static light scattering experiments were performed as previously described (Goss & Harrigan, 1986) with slight modifications. Data were collected by a Zenith Z-100 minicomputer with 768K of memory. Software for data acquisition was designed by OLIS, Inc. For static experiments, data for a sample were collected over 60 s and averaged to a single point. Multiple samples were read for each titration curve.

Sucrose Gradient Analysis. Ribosomes (0.25 A_{260}) were incubated 5–10 min at 37 °C in a reaction mixture (0.5 mL) containing 1.0 mM Mg^{2+}. Separate samples containing eIF-3 and ribosomes (1:1 molar ratio) were also prepared. The samples were overlayed on a 12-mL, 10–30% sucrose gradient with a bufer containing 20 mM Tris, pH 7.5, 100 mM KCl, 2 mM dithiothreitol, 0.05 mM EDTA, and 1 mM $MgCl_2$. After centrifugation at 4 °C for 5 h at 32000 rpm in a Beckman SW 40 rotor, 0.25-mL fractions were collected. The Mg^{2+} concentration was raised to 6 mM Mg^{2+} by addition of 1 mL of 6 mM Mg^{2+} to each fraction collected. The absorbance profile at 260 nm was obtained by the use of a Varian 634 spectrophotometer. For some experiments, the gradients were pumped through an ISCO UA-5 optical monitor with 260-nm filter and the profiles recorded.

Kinetic Measurements. Kinetic measurements were performed on a Hi-Tech SF-51 stopped-flow device interfaced to a Zenith Z-100 computer with 768K memory and dual disk drives. The light source for the stopped flow was a Liconix 4210 NB helium–cadmium laser with UV optics. The ribosome association reaction was measured by flowing buffer B containing 7.5 or 11.5 mM $MgCl_2$ and 100 mM KCl against ribosomal subunits in buffer B with 0.5 mM $MgCl_2$ and 100 mM KCl. For experiments comparing the effect of eIF-3 on ribosomal subunit association, the ribosome stock solution was diluted into the flow buffer (low [Mg^{2+}]) and allowed to equilibrate.

Control reactions without eIF-3 were monitored. The eIF-3 was then added to the flow syringe and allowed to equilibrate, and the association reaction was again monitored. For some experiments, eIF-3 was diluted into the high-Mg^{2+} buffer and flowed against ribosomal subunits.

Data Fitting. The values obtained for ΔV, the normalized voltage change for a light scattering experiment, are directly proportional to the fraction of 80S ribosomes (Gorisch et al., 1976). For static titrations in the absence of eIF-3, the fraction of 80S ribosomes was easily calculated (Goss & Harrigan, 1986). Titrations in the presence of eIF-3 required taking into account the light scattering contributions from eIF-3. The curves are not presented as a direct fraction of 80S since this would require some assumptions about the amount, if any, eIF-3 bound to the 80S ribosomes (see Results and Discussion). Kinetic data were fit with a Fletcher–Powell nonlinear minimization algorithm (Fletcher & Powell, 1963). For each apparent minimum, an estimate of the standard errors in the parameters was obtained from the variance–covariance matrix (Draper & Smith, 1966). For kinetic models where eIF-3 was present, it was not convenient to integrate the rate expressions, and a Runge-Kutta (Margenau & Murphy, 1956) numerical integration program was used.

Results

Magnesium-Dependent Association of Ribosomal Subunits. We have previously shown that *Artemia* ribosomes are 50% associated at 1.5 mM Mg^{2+} (100 mM KCl, T = 22 °C).

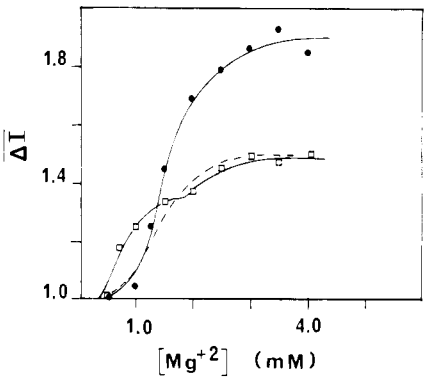

FIGURE 1: Equilibria between *Artemia* 80S ribosomes and subunits. The left axis is the light scattering intensity with background subtracted. The intensity is normalized to the intensity of 0.2 mM Mg^{2+}. The solid circles represent the data for association of ribosomes in buffer B and 100 mM KCl. Association to 80S particles then gives a light scattering intensity of 1.87 (see text for details). The open squares represent the data for ribosomes and equimolar eIF-3 in buffer B and 100 mM KCl. Ribosome concentration was 0.012 μM, T = 25 °C. eIF-3 concentration (open squares) was 0.011 μM. The solid lines represent calculated curves for the best fitting models (see text). The dotted line represents the best fitting curve for a model where eIF-3 does not bind to 80S ribosomes.

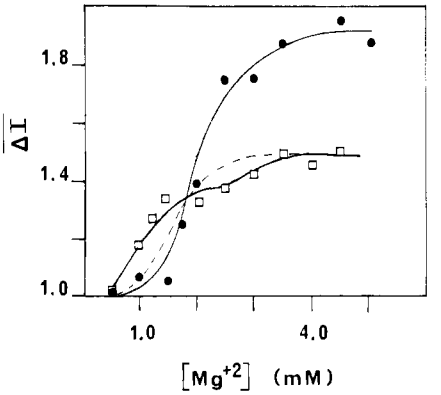

FIGURE 2: Equilibria between reticulocyte 80S ribosomes and subunits. The left axis is the same as for Figure 1. The solid circles represent the data for association of ribosomes in buffer B and 100 mM KCl. The open squares depict the data for ribosomes and equimolar eIF-3 in buffer B and 100 mM KCl. Ribosome concentration was 0.013 μM, and eIF-3 concentration (open squares) was 0.013 μM. The solid and dotted lines represent calculated curves (see text and Figure 1 caption).

Figure 1 shows the equilibria between *Artemia* 80S ribosomes and subunits at 22 °C and 100 mM KCl. The open circles depict the equilibria in the presence of 0.011 μM eIF-3. Ribosome concentration was 0.012 μM. The left axis is the light scattering intensity with background subtracted. The intensity is normalized to the intensity for dissociated 40S and 60S subunits or for 40S and 60S subunits and eIF-3. In the absence of eIF-3 the association of 40S and 60S subunits to 80S ribosomes gives a light scattering intensity of 1.87 on the basis of the molecular weights of the particles (Goss & Harrigan, 1986). In the presence of eIF-3, the calculation is somewhat more complex, since the molecular weight of eIF-3 suggests that scattering due to eIF-3 cannot be ignored. In order to determine the theoretical amplitude for 80S association, we have considered two models. In the first model, most of the eIF-3 is bound to 40S subunits initially. At the final $[Mg^{2+}]$, no eIF-3 is bound to 80S ribosomes. This leads to a scattering intensity of $I_{80S+eIF\text{-}3} = 1.47I_{40S\cdot eIF\text{-}3+60S}$. The second model is the case where eIF-3 is bound to the 40S subunit initially but remains on the 80S ribosome. In this case $I_{80S-eIF\text{-}3} = 1.99I_{40S\cdot eIF\text{-}3+60S}$. The curves shown in Figure 1 are normalized to the initial low (0.5 mM) Mg^{2+} value with background subtracted in each case. The initial value for the curve with eIF-3 (open circles) was about 20% higher than that for the curve of the intensity for 40S and 60S subunits without eIF-3. A comparison of the scattering intensity of the samples at 0.5 and 4.0 mM Mg^{2+} showed that in the presence of eIF-3 the difference in scattering intensity was 1.48, in close agreement with the predicted value of 1.47 from calculations based on the molecular weights. However, at $[Mg^{2+}]$ between about 1.0 and 2.0–2.5 mM, the scattering intensity is higher than would be expected for the simple equilibrium between subunits and Mg^{2+} where eIF-3 is not bound to the 80S ribosome. The titration curves do not appear to fit a smooth sigmoidal curve as would be expected for a simple equilibrium reaction. The curves in fact suggest at least two equilibrium processes. One interpretation of these data is that there are two types of ribosomes interacting. However, curves in the absence of eIF-3 appear homogeneous, and the kinetics of subunit association are homogeneous. Further, the presence of eIF-3 would appear to induce an association of ribosomal subunits in contrast to other findings. A more reasonable explanation is that eIF-3 binds to 80S ribosomes at low $[Mg^{2+}]$. In this case, the light scattering signal will be

$$I = 1.99X + 1.47([80S]_T - X)$$

where X is the fraction of 80S ribosomes with eIF-3 bound. This equation involves two unknowns, the amount of 80S ribosomes as a function of $[Mg^{2+}]$ and the fraction of these 80S ribosomes with eIF-3 bound. We have used the points above 2.5 mM Mg^{2+} to estimate the equilibrium constant for 80S association described by

$$K = \frac{[80S\cdot(Mg^{2+})_n]}{[40S][60S][Mg^{2+}]^n}$$

With this approximation and the above equation for ΔI, then at 1.0 mM $[Mg^{2+}]$ 30% of the 80S ribosomes would have eIF-3 bound (10% of the total ribosomes).

This is admittedly an approximation, and until we have detailed data concerning the Mg^{2+} dependence of eIF-3 binding to 40S and 80S subunits, more precise analyses are not meaningful. The solid line in Figure 1 gives the calculated value for I from the above equation. The dotted line shows the best fitting values for a model where no eIF-3 bound to the 80S ribosomes. Data for reticulocyte ribosomes are shown in Figure 2. The solid and dotted lines represent the calculated curves as described above.

Sucrose Gradient Analysis. The light scattering data show that eIF-3 shifts the subunit–80S equilibrium toward dissociation of the 80S ribosomes. Sucrose gradient analysis was performed to confirm these results and to compare the values obtained by the two techniques. Sucrose gradient analysis at 1.0 mM Mg^{2+} showed an increase in subunits when eIF-3 was present. All of the gradients show a substantial increase in the amount of subunits in the presence of eIF-3. The 60S peak does at times show some trailing in both the presence and absence of eIF-3. If this trailing were due to dimerization of the 40S subunits, it would be apparent in the light scattering experiments. A few preparations did show evidence of aggregated ribosomes and were not used for eIF-3 studies. We did not determine K_{eq} values from these results. However, it appears that there is slightly more dissociation when the results are analyzed by sucrose gradients as compared to light scattering. This may be due to pressure-induced dissociation of the ribosomes. Figure 3 shows the results of these studies with *Artemia* ribosomes. Similar results were obtained with reticulocyte ribosomes.

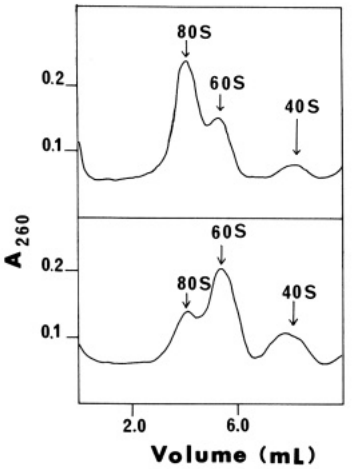

FIGURE 3: Sucrose gradient analysis. The lower panel shows the sucrose gradient profile for equimolar *Artemia* ribosomes and reticulocyte eIF-3 (5.4 nmol). The upper panel shows the sucrose gradient profile on *Artemia* ribosomes in the absence of eIF-3. The [Mg^{2+}] was 1.0 mM in both cases. See text for details.

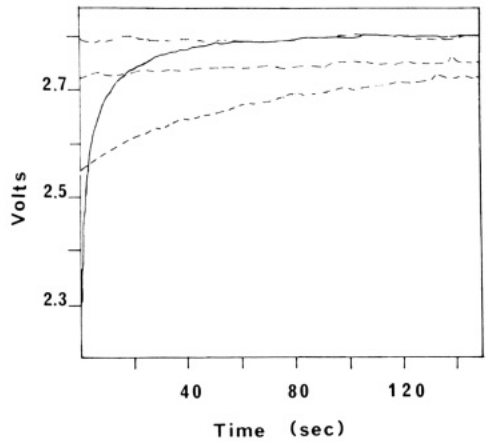

FIGURE 4: Effects of eIF-3 on the Mg^{2+}-induced association of *Artemia* ribosomal subunits. The curves represent the association of *Artemia* ribosomal subunits caused by a shift in [Mg^{2+}] from 0.5 to 6 mM. The solid line represents the data for the reaction in the absence of eIF-3. The dashed line depicts the data for the same reaction with reticulocyte eIF-3. The eIF-3 concentration was 0.021 μM and was added to the ribosomal subunits prior to association. The curves represent averages of five to seven runs. T = 24 °C; buffer B with 100 mM KCl was used. The top traces represent the voltage at the end of the reaction.

Subunit Association Kinetics. The rate constants for *Artemia* ribosome subunit association have been reported previously (Goss & Harrigan, 1986). The presence of equimolar eIF-3 reduces the apparent association rate. The amplitude of the curves and the static titration data strongly suggest that little or no eIF-3 is bound to the 80S ribosomes at 4 mM Mg^{2+}. Figure 4 shows the reaction curves for *Artemia* ribosome association in the presence of reticulocyte eIF-3. The curves in the presence of eIF-3 were fit to a model where eIF-3 did not have appreciable affinity for 80S particles, even though at lower [Mg^{2+}] it apparently does bind to 80S ribosomes. This model can be written as

$$40S + \text{eIF-3} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} 40S\cdot\text{eIF-3}$$

$$40S + 60S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} 80S$$

A detailed discussion of the equations and data fitting for such a model is presented for the procaryotic IF-3 binding to *Escherichia coli* ribosomes (Goss et al., 1980, 1982). According to this model, eIF-3 reduces the apparent rate of ribosomal subunit association by reducing the pool of reactive 40S subunits. The second-order rate of 40S and 60S association depends on the initial concentration of reactants.

Table I: Rate Constants for Mg^{2+}-Induced Ribosomal Subunit Association[a]

reactants	k_1 (M^{-1} s^{-1})	k_1' (M^{-1} s^{-1})
Artemia ribosomes	2.1×10^7	
reticulocyte eIF-3		8.1×10^5
reticulocyte ribosomes	5.0×10^6	
reticulocyte eIF-3		2.1×10^5
wheat germ ribosomes	2.1×10^7	
reticulocyte eIF-3		1.0×10^7
wheat germ eIF-3		3.0×10^5

[a] Rate constants are for a change in Mg^{2+} from 0.5 to 6 mM. The k' values are the apparent second-order rate constants for equimolar eIF-3. Concentrations are given in the captions for Figures 4–6. The errors in the rate constants for fitting data from a single set of experiments were 4% of the values given (1σ); however, for different sets of experiments and different preparations, the variation in rate constants were approximately 20%.

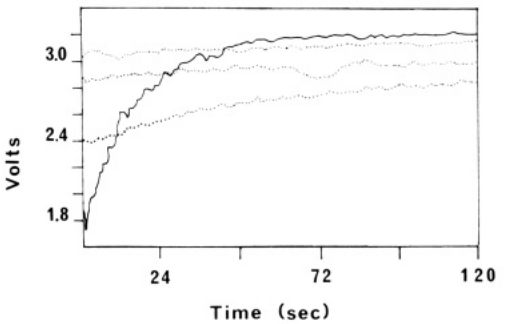

FIGURE 5: Effect of eIF-3 on the Mg^{2+}-induced association of reticulocyte ribosomes. The solid curve shows the data for association of reticulocyte ribosomes induced by a jump in [Mg^{2+}] from 0.5 to 6 mM. The dotted lines depict the data for the same reaction with reticulocyte eIF-3 added to the ribosomes prior to association. Ribosome concentration was 0.011 μM, [eIF-3] was 0.010 μM, T = 22 °C, and buffer B with 100 mM KCl was used for the reaction. The top lines represent the observed voltage at the end of the reaction.

This model will yield second-order kinetics if the equilibration of 40S and eIF-3 is rapid and if most of the 40S subunit is in the form of a 40S·eIF-3 complex. The data were second order. The apparent rate constant (k_1') is approximately k_1K_2, where

$$K_2 = \frac{k_{-2}}{k_2} = \frac{[40S][\text{eIF-3}]}{[40S\cdot\text{eIF-3}]}$$

The rate constants are presented in Table I. At these concentrations, eIF-3 reduces the apparent rate of subunit association almost 25-fold.

We have also examined the effects of eIF-3 on reticulocyte ribosome association. Again, eIF-3 reduces the apparent rate of subunit association. The concentrations are very close to those used for *Artemia* association. The effect of reticulocyte eIF-3 on reticulocyte ribosome subunit association was to reduce the apparent association rate by 25-fold (Figure 5). In a crossover experiment, where the effects of reticulocyte eIF-3 on *Artemia* ribosome subunit association were measured, it was found that the reticulocyte factor reduced the *Artemia* ribosome association rate by 25-fold, the same effect as with the cognate ribosomes.

It has been reported (Russell & Spremulli, 1979) that eIF-3 does not affect the wheat germ 80S ribosome–subunit equilibrium. We have examined the effects of both wheat germ eIF-3 and reticulocyte eIF-3 on wheat germ association. Reaction curves for the association of wheat germ ribosomal

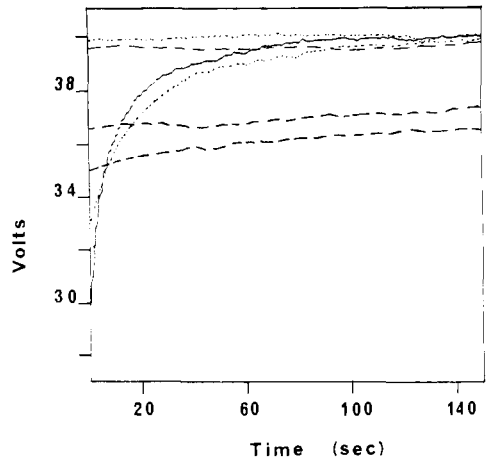

FIGURE 6: Effect of eIF-3 on the Mg^{2+}-induced association of wheat germ ribosomal subunits. The solid curve shows the data for association of wheat germ ribosomes (0.011 μM) induced by a jump in [Mg^{2+}] from 0.5 to 6 mM. The dotted and dashed lines depict the same reaction in the presence of 0.014 μM reticulocyte and wheat germ eIF-3, respectively. Buffer B with 50 mM KCl was used. T = 23 °C. The top lines represent the observed voltage at the end of the reaction.

subunits in the absence of eIF-3 and in the presence of reticulocyte or wheat germ eIF-3 are shown in Figure 6. The wheat germ factor has a pronounced effect on subunit association. The apparent association rate is reduced 100-fold under these conditions.

For all of these studies, it was found that eIF-3 quickly lost its ability to affect ribosomal subunit association. Freezing and thawing a few times or storage at 4 °C for several hours caused eIF-3 to be inactivated. We do not know whether there are irreversible conformational changes in eIF-3. Interestingly, eIF-3 that has lost the ability to inhibit ribosomal subunit association is still able to increase mRNA binding, suggesting that very subtle changes are involved.

Discussion

Structurally, eucaryotic eIF-3 bears little resemblance to the procaryotic IF-3. The eucaryotic factor consists of 9–11 different polypeptides and has a mass of 660 000–724 000 daltons (Thompson et al., 1977; Checkley et al., 1982; Brown-Luedi et al., 1982; Safer et al., 1976; Benne & Hershey, 1976; Behlke et al., 1986). In contrast, procaryotic IF-3 is a single polypeptide of 24 000 daltons. Interestingly, our studies show that at least some of the functional properties of the two proteins are quite similar.

There have been conflicting reports about the function of eIF-3. Most reports seems to agree that eIF-3 enhances the binding of Met-tRNA to 40S subunits and that it is an absolute requirement for mRNA binding to 40S subunits. There has been disagreement over the ribosome dissociation activity of eIF-3. Ribosome dissociation activity has been reported by Trachsel and Staehelin (1979) for eIF-3 isolated from rabbit reticulocyte lysate. Dissociation activity has also been reported for rat liver eIF-3 by Thompson et al. (1977), as well as by Jones et al. (1980). However, no dissociation activity has been found for wheat germ eIF-3 (Russell & Spremulli, 1979) nor for calf liver eIF-3 (Maitra et al., 1982). Our data are in agreement with the finding that eIF-3 shifts the 80S–subunit equilibrium toward dissociation. In fact, our results show a larger effect than the previous studies. These studies have relied on sucrose gradient analysis, usually with glutaraldehyde fixation. Glutaraldehyde may reduce the protein–nucleic acid interaction. Glutaraldehyde has been observed to reduce the proportion of smaller particles in eIF-3 preparations even at low concentrations of glutaraldehyde (<0.5%) and short fixation times (1 min) (Behlke et al., 1986). Further, it has not been shown that glutaraldehyde fixation gives satisfactory stabilization (Behlke et al., 1986). Centrifugation may also cause pressure-induced artifacts such as aggregation of subunits or dissociation of the eIF-3–ribosome complex.

The fact that reticulocyte eIF-3 shifts the subunit equilibrium toward dissociation for both *Artemia* and reticulocyte ribosomes indicates that these two systems must be very similar. While some structural variations may exist in the ribosomal proteins and nucleic acids, it has been shown that ribosomes from different, divergent systems are functionally interchangeable in vitro (Tuite et al., 1980; Warner et al., 1977). Wheat germ ribosomes and eIF-3 must interact somewhat differently since reticulocyte eIF-3 has a much smaller effect on the wheat germ ribosomes.

There is no evidence yet to suggest why eIF-3 is so large or to implicate specific polypeptides in different functional roles. The binding mechanism to the 40S subunit also remains to be clarified. Clearly much further work is needed to fully understand the multiple functions of eIF-3 and its interactions with other components of the protein synthesis system.

References

Behlke, J., Bommer, U.-A., Lutsch, G., Henske, A., & Bielka, H. (1986) *Eur. J. Biochem. 157*, 523–530.

Benne, R., & Hershey, J. W. B. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 3005–3009.

Brown-Luedi, M. L., Meyer, L. J., Milburn, S. C., Yau, P. M.-P., Corbett, S., & Hershey, J. W. B. (1982) *Biochemistry 21*, 4202–4206.

Checkley, J. W., Cooley, L. L., & Ravel, J. M. (1981) *J. Biol. Chem. 256*, 1582–1586.

Draper, N. A., & Smith, H. (1966) *Applied Regression Analysis*, Wiley, New York.

Fletcher, R., & Powell, M. J. D. (1963) *Comput. J. 6*, 163.

Gorisch, H., Goss, D. J., & Parkhurst, L. J. (1976) *Biochemistry 15*, 5743–5753.

Goss, D. J., & Harrigan, T. (1986) *Biochemistry 25*, 3690–3695.

Goss, D. J., Parkhust, L. J., & Wahba, A. J. (1980) *J. Biol. Chem. 255*, 225–229.

Goss, D. J., Parkhust, L. J., & Wahba, A. J. (1982) *J. Biol. Chem. 257*, 10119–10127.

Gupta, N. K. (1968) *J. Biol. Chem. 243*, 4959–4065.

Jones, R. L., Sadnik, I., Thompson, H. A., & Moldave, K. (1980) *Arch. Biochem. Biophys. 199*, 277–285.

MacRae, T. H., Roychowdhury, M., Houston, K. J., Woodley, C. L., & Wabha, A. J. (1979) *Eur. J. Biochem. 100*, 66–76.

Maitra, U., Stringer, E. A., & Chaudhuri, A. (1982) *Annu. Rev. Biochem. 51*, 869–900.

Margenau, H., & Murphy, G. M. (1956) *The Mathematics of Physics and Chemistry*, p 486, Van Nostrand, Princeton, NJ.

Mehta, H. B., Woodley, C. L., & Wahba, A. J. (1983) *J. Biol. Chem. 258*, 3438–3441.

Merrick, W. C. (1979) *Methods Enzymol. 60*, 101–108.

Nieuwenhuysen, P., & Clauwaert, J. (1981) *J. Biol. Chem. 256*, 9623–9626.

Nieuwenhuysen, P., De Voeght, F., & Clauwaert, J. (1981) *Biochem. J. 197*, 689–693.

Nygard, O., & Westermann, P. (1983) *Biochim. Biophys. Acta 697*, 1582–1586.

Russell, D. W., & Spremulli, L. L. (1979) *J. Biol. Chem. 254*, 8796–8800.

Safer, B., Adams, S. L., Kemper, W. K., Berry, K. W., Lloyd,

M., & Merrick, W. C. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 2584-2588.
Schreier, M. H., & Staehelin, T. (1973) *Nature (London), New Biol. 242*, 35-38.
Seal, S. N., Schmidt, A., & Marcus, A. (1983) *J. Biol. Chem. 258*, 866-871.
Sperrazza, J. M., & Spremulli, L. L. (1983) *Nucleic Acids Res. 11*, 2655-2679.
Spremulli, L. L., Walthall, B. J., Lax, S. R., & Ravel, J. M. (1977) *Arch. Biochem. Biophys. 178*, 565-576.
Thompson, H. A., Sadnik, I., Scheinbuks, J., & Moldave, K. (1977) *Biochemistry 16*, 2221-2230.
Trachsel, H., & Staehelin, T. (1979) *Biochim. Biophys. Acta 565*, 305-314.
Trachsel, H., Erni, B., Schreier, M., & Staehelin, T. (1977) *J. Mol. Biol. 116*, 755-767.
Tuite, M. F., Plesset, J., Moldave, K., & McLaughlin, C. S. (1980) *J. Biol. Chem. 255*, 8761-8766.
Warner, J. R., & Gorenstein, C. (1977) *Cell (Cambridge, Mass.) 11*, 201-212.

Functional Role of Vitamin K_1 in Photosystem I of the Cyanobacterium *Synechocystis 6803*†

John Biggins*,‡ and Paul Mathis
Service de Biophysique, Département de Biologie, Centre d'Etudes Nucléaires de Saclay, 91191 Gif-sur-Yvette Cedex, France
Received October 6, 1987; Revised Manuscript Received November 9, 1987

ABSTRACT: The function of vitamin K_1 in the primary electron-transfer processes of photosystem I (PS I) was investigated in the cyanobacterium *Synechocystis 6803*. A preparation of purified PS I was found to contain two vitamin K_1's per reaction center. One vitamin K_1 was removed by extraction with hexane, and further extraction using hexane including 0.3% methanol resulted in a preparation devoid of vitamin K_1. The hexane-extracted PS I was functional in the photoreduction of $NADP^+$, but the PS I after extraction using hexane–methanol was totally inactive. Activity was restored by using exogenous vitamin K_1 plus the hexane extract. Vitamin K_3 would not substitute. The room temperature recombination kinetics of the PS I extracted with hexane were not significantly modified. However, following the removal of both vitamin K_1's, the 20-ms recombination between $P\text{-}700^+$ and $P\text{-}430^-$ was replaced by a dominant relaxation ($t_{1/2}$ = 30 ns) due to recombination of the primary biradical $P\text{-}700^+$ A_0^- and a slower component originating from the P-700 triplet. This kinetic behavior was consistent with an interruption of forward electron transfer to the acceptor A_1. Addition of either vitamin K_1 or vitamin K_3 to such preparations resulted in restoration of the slow kinetic phase (>2 ms), indicating significant competition by the two exogenous quinones for electron transfer from A_0^-. In the case of vitamin K_3, this change in the kinetics was completely reversed by the addition of dithionite. A similar addition of reductant did not alter the kinetics induced by vitamin K_1, suggesting successful reconstitution of the acceptor site A_1. These data support the hypothesis that acceptor A_1 is vitamin K_1 and is a component of the electron-transfer pathway for $NADP^+$ reduction.

The reaction center of photosystem I (PS I)[1] in higher plants, algae, and cyanobacteria is known to comprise the primary donor (P-700) and five acceptors. The acceptors F_X, F_B, and F_A are iron–sulfur centers thought to engage in the terminal stages of electron transfer during the reduction of $NADP^+$ (Golbeck, 1987; Rutherford & Heathcote, 1985; Sétif & Mathis, 1986). The acceptor A_0 is presumably a chlorophyll *a* monomer (Bonnerjea & Evans, 1982; Gast et al., 1983; Shuvalov et al., 1986) and forms the primary biradical with P-700, and A_1 is a transient intermediate operating between A_0 and F_X (Bonnerjea & Evans, 1982; Gast et al., 1983). It has been suggested that A_1 is a quinone on the basis of ESR experiments (Gast et al., 1983; Thurnauer & Gast, 1985), and because of the exclusive localization of vitamin K_1 in PS I, it has been suggested that the acceptor A_1 might be vitamin K_1 (Schoeder & Lockau, 1986; Takahashi et al., 1985).

Flash-induced absorption experiments have shown that the recombination of $P\text{-}700^+$ A_1^- occurs with a $t_{1/2} \simeq 120$ μs at low temperature (Mathis & Conjeaud, 1979; Sétif et al., 1984), and a spectrum of this signal corrected for the contribution in absorbance by $P\text{-}700^+$ provides strong evidence that vitamin K_1 is the acceptor A_1 (Brettel et al., 1986).

The concentration of vitamin K_1 in the PS I of higher plants and cyanobacteria was shown to be 2 mol/mol of P-700 (Malkin, 1986; Palace et al., 1987; Schoeder & Lockau, 1986; Takahashi et al., 1985). Malkin (1986) has recently shown that 1 mol of vitamin K_1 per P-700 was readily extracted from PS I by dry organic solvent. The extracted preparation containing one vitamin K_1 per P-700 was not modified in primary electron transfer as judged by low-temperature ESR. This suggests that the vitamin K_1 which was readily extracted may not function in charge separation.

This paper reports that extraction of the second vitamin K_1 per P-700 requires a slightly more hydrophilic solvent. The

† This work was supported by the Competitive Research Grants Office of the USDA (81-CRCR-1-0767), the National Science Foundation (PCM-8302983), and a Senior International Fellowship sponsored by the Fogarty International Center, NIH.
* Correspondence should be addressed to this author.
‡ Permanent address: Section of Biochemistry, Division of Biology and Medicine, Brown University, Providence, RI 02912.

[1] Abbreviations: PS I, photosystem I; ΔA, absorption change; DPIP, dichlorophenolindophenol; ESR, electron spin resonance; Tricine, *N*-[tris(hydroxymethyl)methyl]glycine; HPLC, high-performance liquid chromatography.

0006-2960/88/0427-1494$01.50/0