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Magnesium Ion Dependent Equilibria, Kinetics, and Thermodynamic Parameters of *Artemia* Ribosome Dissociation and Subunit Association†

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ABSTRACT: The influence of magnesium ion concentration on the equilibrium and kinetics of *Artemia* ribosome dissociation and subunit association has been studied by laser light scattering. Ribosomal aggregation was found to be reduced by addition of 0.1–0.05 mM spermidine and KCl concentrations of 100 mM. The ribosomes were found to be stable at low [Mg^{2+}], and the curves obtained for ribosome–subunit equilibrium were independent of the direction and origin of the magnesium ion titration. Thermodynamic parameters were obtained from the temperature-dependent equilibria and have been compared to those of wheat germ and *Escherichia coli* type A ribosomes. The entropy term calculated for the association of 40S and 60S subunits is small, and the reaction is exothermic. The entropy term is negative, favoring subunit dissociation, and contributes less to the free energy than the enthalpy term. Rate constants for ribosome dissociation and subunit association have been determined. The reaction curves gave no evidence for sequential processes and were homogeneous.

The reversible association and dissociation of ribosomal subunits play a central role in the process of protein biosynthesis in both eucaryotes and procaryotes. The ion-dependent association and dissociation equilibrium and kinetics of procaryotic *Escherichia coli* ribosomal subunits have been extensively studied, but there is less data available for eucaryotic ribosomes. Some procaryotic protein biosynthesis is greater than an order of magnitude faster than eucaryotic protein biosynthesis, kinetic and thermodynamic studies are of particular interest in understanding the differences between protein synthesis in eucaryotes and procaryotes. The effects of a variety of cations on the equilibrium of eucaryotic wheat germ ribosomal subunits with 80S monosomes has been explored (Sperrazza et al., 1980, 1981; Sperrazza & Spremulli, 1983; Moore & Spremulli, 1985), but no kinetic data were reported. Physicochemical studies have been carried out on *Artemia* (brine shrimp) ribosomes (Nieuwenhuysen & Clauwaert, 1981; Nieuwenhuysen et al., 1981; Donceel et al., 1982). Electron microscopy (Lake et al., 1974; Boublik & Hellman, 1978) and other physical techniques (Nieuwenhuysen & Clauwaert, 1981; Nieuwenhuysen et al., 1980) suggested

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general similarity between eucaryotic and procaryotic ribosomes. The composition of eucaryotic and procaryotic ribosomes are similar with eucaryotic ribosomes having larger rRNA (28S, 18S, and 5.8S compared to 23S, 16S, and 5S) and approximately 17 more proteins (Wood & Stoffler, 1974) than *E. coli* ribosomes. Because of these structural similarities the subunit interactions may be quite similar. We have investigated the kinetics and equilibria of *Artemia* ribosomal subunits and 80S monosomes using laser light scattering which does not perturb the system. Centrifugation methods may cause pressure-induced dissociation or aggregation artifacts. These studies will form a sound basis for more detailed investigations of subunit interactions and the effects of initiation factors on these interactions.

We have found that *Artemia* ribosome dissociation and subunit association is qualitatively very similar to *E. coli* and wheat germ ribosomes.

Materials and Methods

Artemia 80S ribosomes were prepared as described by MacRae et al. (1979). The ribosomes were stored in liquid nitrogen in buffer consisting of 20 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl)[1] (pH 7.5), 250 mM sucrose, 100 mM KCl, 0.1 mM EDTA, and 9 mM $MgCl_2$. For some experiments, ribosomes were used within 48 h of preparation. *Artemia* ribosomes were assayed for polypeptide synthesis activity. The assay conditions and activity are the same as reported previously [Table VIII (Woodley et al., 1981)]. Buffers for kinetics and equilibria were either of the following: buffer A—20 mM HEPES, pH 7.6, 1 mM dithiothreitol, 0.1 mM EDTA, and KCl and magnesium acetate as indicated; buffer B—20 mM Tris-HCl, pH 7.6, 1 mM dithiothreitol, 0.1 mM EDTA, and KCl and magnesium acetate as indicated. Tris (Trizma) base and HEPES [*N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid] were products of Sigma Chemical Co. All other chemicals were reagent grade.

Determination of Ribosome Concentrations. Ribosomes were assumed to have an absorbance of 0.121 at 260 nm, for a 0.001% solution (Nieuwenhuysen & Clauwaert, 1981). The molecular weights of the 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, as reported by Nieuwenhuysen et al. (1981).

Determination of A_{260}/A_{280} Ratios. Absorbance measurements were made with a Beckman Varian DMS 90 spectrophotometer on solutions diluted appropriately in 1-cm quartz cuvettes.

Static Light Scattering Experiments. Static light scattering experiments were performed in a specially constructed light-scattering and fluorescence apparatus. The excitation light source was a Liconix 4210 NB helium–cadmium laser with UV optics. The output wavelength was 325 nm. Detection of scattered light was at 90° to the incident light. The scattering light was passed through a Jarrell-Ash 82-000 monochromator set at 325 nm. Quartz fluorescence curvettes with a 4 mm × 1 cm path length were used. A thermostated cell holder regulated temperatures to ±0.2 °C. The temperature in the curvette was determined by an Omega Model 747 digital thermistor thermometer previously calibrated. In all experiments, the transmittance at 325 nm was >98%, and all solutions were filtered through Millipore filters (0.1 m) to remove dust particles. To determine 80S ribosome dissociation equilibria, ribosome stock solution was diluted into buffer containing 0.2 mM $Mg(OAc)_2$. The sample was then diluted into a series of 1-mL samples containing 0.2–9 mM $Mg(OAc)_2$. The samples were incubated at the appropriate temperature for 20–30 min, and relative light scattering intensity was determined. The final ribosome concentration was 0.003–0.020 μM. Final dilution of the stock solution was at least 1:5000.

Kinetic Measurements. Kinetic experiments were performed in the light-scattering spectrophotometer. The dissociation reaction was induced either by adding a small volume of concentrated solution of associated ribosomes to 3 mL of buffer A or B containing 0.2 mM $Mg(OAc)_2$ by means of a special plunger which allowed rapid mixing or by adding EDTA to the 80S ribosome solution to obtain a final [$Mg(OAc)_2$] of 0.2 mM. The ribosome association was measured by addition of $Mg(OAc)_2$ to ribosomal subunits equilibrated at 0.2 mM $Mg(OAc)_2$.

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). Values of ΔV at Mg^{2+} concentrations where easily calculated amounts of 80S, 40S, and 60S ribosomes were present were used to calculate the values for K_{eq}. The values ΔG were calculated from the standard equation $\Delta G = -RT \ln K_{eq}$. ΔH and ΔS were obtained from the slope and intercept, respectively, of a linear least-squares fit of $\ln K$ vs. $1/T$ data. Kinetic data were fit by 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 each fit, a time-series analysis of the residuals was made (Swed & Eisenhardt, 1943).

Results

Characterization of Ribosome Preparation. In order to determine the stability of *Artemia* ribosomes at low Mg^{2+} concentrations, the A_{260}/A_{280} ratio was measured before and during incubation in 0.2 mM Mg^{2+}. The ratio before dilution (80S ribosomes) was 1.93 ± 0.03. The ratio found immediately upon dilution into 0.2 mM Mg^{2+} was 1.90 ± 0.02. The ratio did not change for incubation times up to 60 min. For ribosomes incubated in buffer A or B containing 250 mM KCl the ratio began to increase after 90 min. For lower KCl concentrations (50–200 mM) the ratio began to increase after 120 min. The dilution ratio for these experiments was typically 1:2000 or greater.

The extent of association of ribosomal subunits was determined from the amplitude of the light-scattering signal and ultracentrifugation. The theoretical maximum for the light-scattering signal change can be calculated as described elsewhere (Gorisch et al., 1976; Goss et al., 1980). The molecular weights reported by Nieuwenhuysen et al. (1981) were used to estimate the change in light-scattering signal of the ribosome solutions. The theoretical change in scattering intensity after background is subtracted for completely dissociated ribosomes going to 100% associated 80S ribosomes is $I_{80S} = 1.87 I_{40S+60S}$. Sucrose gradient centrifugation and the light-scattering intensities indicated that 90 ± 5% of the subunits were capable of forming 80S particles.

Under some conditions, aggregation of both the subunits and 80S particles was found to occur. Aggregation by eucaryotic ribosomes has been reported by others (Nieuwenhuysen & Clauwaert, 1981; Nieuwenhuysen et al., 1980). We found this particularly to be a problem if Mg^{2+} was greater than 9 mM (see Figure 1) and at low temperature and low

[1] Abbreviations: Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid.

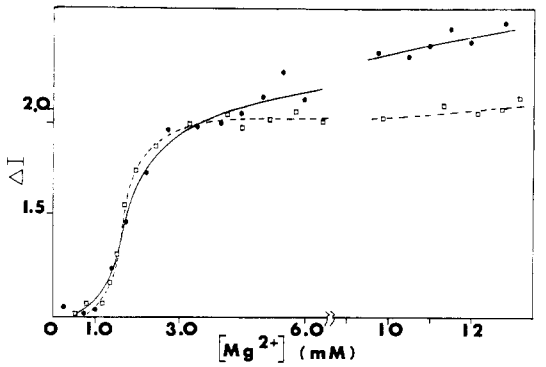

FIGURE 1: Mg^{2+}-induced association equilibria of *Artemia* 80S ribosomes. The left axis is the light-scattering intensity with background subtracted. The intensity is normalized to the intensity for dissociated 40S and 60S subunits. Association to 80S particles then gives a light-scattering intensity of 1.87 (see text for details). The solid circles represent the data for association of ribosomes in buffer A and 100 mM KCl. The open squares represent the data for ribosomes in buffer A, 100 mM KCl, and 0.05 mM spermidine. Ribosome concentration was 0.012 μM, T = 25 °C.

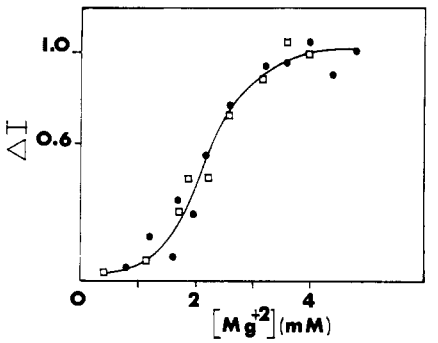

FIGURE 2: Equilibria between 80S ribosomes and subunits. The solid circles represent the data for incubation of ribosomes at 0.2 mM Mg^{2+} and subsequent addition of Mg^{2+} to the indicated concentrations. The open squares show the data for ribosomes diluted from 9 mM Mg^{2+} to give the indicated final Mg^{2+} concentration. The data are normalized so that the left axis gives the fraction 80S ribosomes.

K^+ (less than 100 mM). The aggregation problems at low temperatures were much worse for ribosomes that had been stored in liquid nitrogen for more than 60 days. As shown in Figure 1 addition of 0.05–0.1 mM spermidine reduced the aggregation. For ribosomes that were stored less than 60 days, we were able to work at ribosome and Mg^{2+} concentrations where less than 2% of the ribosomes aggregated. Samples where >2% aggregation occurred were not further analyzed.

Magnesium-Dependent Association of Ribosomal Subunits. It has been well established that ribosomes are in equilibrium with their subunits (Spirin, 1971; Infante & Baierlin, 1971). Association is induced by high Mg^{2+}.

Figure 2 shows the Mg^{2+}-induced association equilibrium of *Artemia* 80S subunits at 22 °C and 100 mM KCl. The equilibrium curve obtained for 80S ribosomes diluted into low concentrations of Mg^{2+} was identical with that obtained when ribosomal subunits were associated by addition of Mg^{2+}.

Studies of *E. coli* ribosomes have used mainly Tris buffer, while many studies of eukaryotic ribosomes have used HEPES–KOH buffer. Titrations were performed in buffer A (20 mM HEPES/KOH) and buffer B (20 mM Tris) both with 100 mM KCl present. The titration curves (data not shown) were identical for both buffers except that at higher Mg^{2+} concentrations slightly less aggregation was observed for ribosomes in buffer A (HEPES).

Monovalent cations have been shown to dissociate ribosomes (Zitomer & Flaks, 1972). The effects of varying KCl concentrations on the Mg^{2+}-dependent equilibria are shown in Figure 3. The Mg^{2+} concentration required for 50% association is shifted from 1.9 mM Mg^{2+} at 50 mM KCl to 2.9 mM Mg^{2+} at 150 mM KCl.

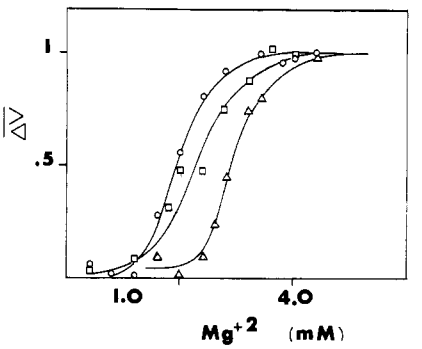

FIGURE 3: Effects of KCl on ribosome equilibria. The left axis gives the fraction 80S ribosomes. The circles, squares, and triangles depict the data for ribosomes in buffer A containing 50, 100, and 150 mM KCl, respectively. T = 37 °C.

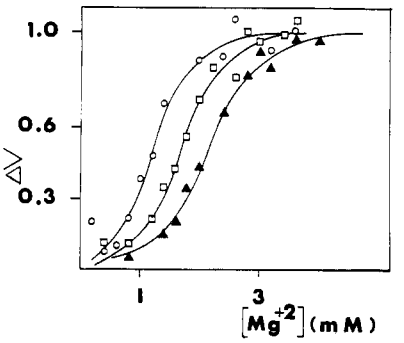

FIGURE 4: Temperature dependence of ribosome equilibria. Ribosomes were incubated in buffer A containing 100 mM KCl at the indicated Mg^{2+} concentrations at (○) 16.9, (□) 27, and (▲) 37 °C. All measurements were taken after equilibrium had been reached. Each point represents the mean of four determinations.

Table I: Thermodynamic Parameters for Ribosomal Subunit Equilibria[a]

temp (°C)	$[Mg^{2+}]_{1/2}$ (mM)	$K_{obsd} \times 10^{-7}$ (M^{-1})	ΔG_{obsd} (kcal/mol)
37	2.1	4.6	−10.9
27	1.7	11.1	−11.0
16.9	1.2	46.9	−11.5

[a] Values were determined at 1.7 mM Mg^{2+}. Errors in the values of K_{obsd} were $1\sigma = \pm 10\%$ of the value of the parameter.

Thermodynamic Parameters. The effects of temperature on the Mg^{2+}-dependent equilibria of *Artemia* ribosomes in 100 mM K^+ are shown in Figure 4. The Mg^{2+} concentration for 50% association shifts from 1.2 mM Mg^{2+} at 16.8 °C to 2.1 mM Mg^{2+} at 37 °C. The equilibrium constants for association (K_{obsd}) were calculated at 1.7 mM Mg^{2+} for all three curves. This Mg^{2+} concentration gave easily measured amounts of 40S, 60S, and 80S ribosomes for all three temperatures. K_{obsd} ranged from 4.6×10^7 M^{-1} at 37 °C to 46.9×10^7 M^{-1} at 16.9 °C. The values obtained for K_{obsd} were used to calculate $\Delta G°$ for subunit association (Table I). Values were obtained for ΔH and ΔS from the van't Hoff plot shown in Figure 5. The slope, $\Delta H_{obsd}/R$, and intercept, $\Delta S_{obsd}/R$, gave values of −25.7 kcal/mol and −49.0 cal/(mol deg) for ΔH_{obsd} and ΔS_{obsd}, respectively. At 20 °C the value for $T\Delta S$ was −14 kcal/mol. The association of subunits is exothermic, and the entropy term is negative and contributes less to the free energy than the enthalpy term.

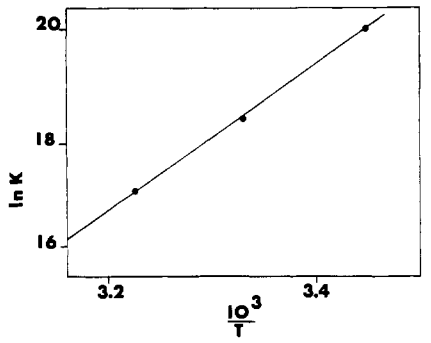

FIGURE 5: van't Hoff plot of ln K_{obsd} vs. $10^3/T$. Equilibrium constants are given in Table I. The magnesium ion concentration was 1.7 mM.

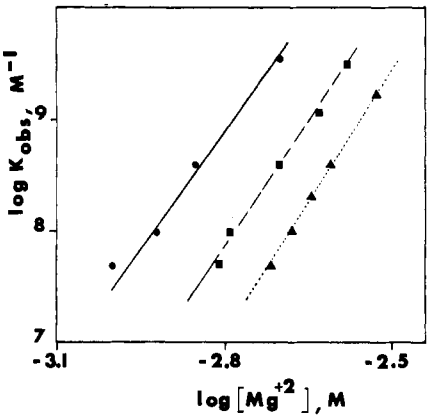

FIGURE 6: Log–log plots for the association equilibrium curves. The values for K_{obsd} were calculated from the data of Figure 4. (●) 16.9, (■) 27, and (▲) 37 °C.

Table II: Rate Constants for 80S Ribosomes Dissociating to 40S and 60S Subunits

T (°C)	k_{-1} (s^{-1})[a]
37	0.039 + 0.007
27	0.022 + 0.001
18	0.0092 + 0.0003

[a] Dissociation was induced by a shift in $[Mg^{2+}]$ from 6 to 0.2 mM.

The Mg^{2+}-dependent equilibrium between 40S and 60S subunits and 80S ribosomes can be represented by

$$40S + 60S + nMg^{2+} \rightleftharpoons 80S(Mg^{2+})_n$$

The equilibrium can then be described by

$$\frac{80S(Mg^{2+})_n}{(40S)(60S)(Mg^{2+})^n} = K_{assoc}$$

and taking the log of both sides gives

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

$$\log K_{obsd} = \log K + n \log [Mg^{2+}]$$

Figure 6 shows the linear portion of the Hill plots taken from the data in Figure 4. The value of n was determined to be ~7.

Kinetic Experiments. The dissociation and association reactions were monitored under conditions where the reactions go to completion. For the dissociation reaction at low ribosome concentrations, the overall voltage change, V_{max}, is proportional to the initial concentration of 80S ribosomes and at any time, t, $(80S)_t$ is proportional to $V_{max} - V_t$. For the association reaction, $V_{max} - V_t$ is proportional to the concentration of dissociated subunits at time t.

Table II shows the rate constants obtained for 80S ribosomes dissociation over the temperature range 18–37 °C. Dissociation was induced by change in Mg^{2+} concentration from 6 to 0.2 mM Mg^{2+}. For these experiments, the Mg^{2+} was reduced by addition of EDTA. The experiment was also performed by dilution of the stock ribosome solution (9 mM Mg^{2+}) into buffer containing Mg^{2+} to give a final Mg^{2+} concentration of 0.2 mM. The data for the dilution experiments showed more noise, probably because due to the small volume added (2 μL), filtering of the ribosome stock solution was difficult. Fitting these data resulted in the same rate constants obtained for addition of EDTA. The errors in the parameters were about twice those for the EDTA data. The addition of EDTA also permitted accurate determination of the initial voltage so that the reaction amplitude was known, and it could be determined if dissociation occurred so rapidly that part of the reaction was not observed. At the highest ribosome concentrations (0.05 mg/mL) a maximum of 10% of the amplitude was missed. This was probably due to dissociation of some aggregated material. Lower ribosome concentrations showed the expected amplitudes ±5% when extrapolated to $t = 0$. The dissociation reactions showed no concentration dependence as expected for the reaction going to completion. Figure 7 shows the dissociation reaction at three

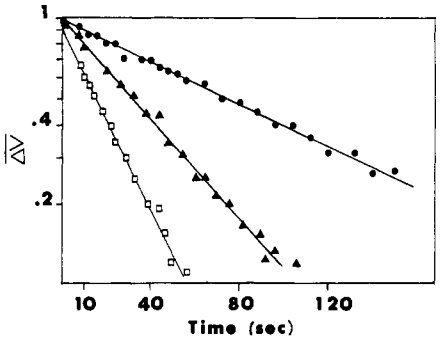

FIGURE 7: Dissociation kinetics of 80S ribosomes. Dissociation was induced by a drop in $[Mg^{2+}]$ from 9 to 0.2 mM. The rate constants are given in Table II. (●) 18, (▲) 27, and (□) 37 °C. The solid lines are the calculated curves for one-exponential fits to the data. ΔV values are plotted on a semilog scale.

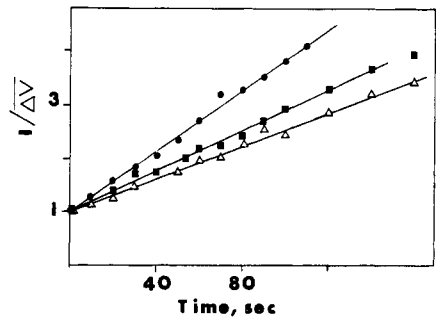

FIGURE 8: Association kinetics of ribosomal subunits. Association was induced by a increase in $[Mg^{2+}]$ from 0.2 to 6 mM. The ribosome concentration ranged from 5.3×10^{-9} to 6.7×10^{-9} M. (△) 16, (■) 24, and (●) 38 °C. The solid lines are the calculated curves for second-order fits to the data.

Table III: Rate Constants for Ribosomal Subunit Association

T (°C)	k_{+1} (M^{-1} s^{-1})[a] × 10^{-6}
37.6	5.95 + 0.05
23.70	3.66 + 0.04
16.20	3.00 + 0.05

[a] Association was induced by a shift in $[Mg^{2+}]$ from 0.2 to 9 mM. The association rate constant varied approximately 15% among different ribosome preparations. The uncertainties reported are for 1σ calculated from fitting the average of four runs. The ribosome concentration was 5.8–6.2 nM.

temperatures. The activation energy for the dissociation reaction was 14 ± 1 kcal/mol.

Figure 8 depicts the association reaction for 40S and 60S subunits forming 80S ribosomes induced by a jump in Mg^{2+} concentration from 0.2 to 9 mM Mg^{2+}. The reaction was second order. Table III gives the calculated values for the association rate constant over the temperature range 16.2–37.6 °C. The activation energy for the association reaction was 5.5 ± 0.2 kcal/mol.

Discussion

In the present work we have examined the Mg^{2+}-induced association and dissociation of *Artemia* ribosomal subunits. We have found that spermidine reduces aggregation of the ribosomes which can be particularly troublesome for biophysical measurements such as light scattering, neutron diffraction, etc. We have also found conditions (low ribosome concentration, high monovalent cation) where equilibrium and kinetic measurements could be obtained to compare our results more directly with those obtained on other systems. The mechanism of the effect of spermidine on ribosome aggregation is unclear since spermidine itself is a powerful promoter of ribosomal subunit association (Gorisch et al., 1976). Spermidine-associated subunits are more susceptible to hydrostatic pressure than are Mg^{2+}-associated subunits (Sperrazza et al., 1981). Sperrazza and Spremulli (1983) have also shown that the total charge neutralization during subunit association by Mg^{2+} and polyamines combined is much less than the charge neutralization by Mg^{2+} alone. This may indicate a different conformation of the 80S ribosome and/or the subunits in the presence of spermidine. A shift in Mg^{2+} concentration of 1 mM can change the subunit association as much as 90%. The magnitude of the shift in midpoint with KCl was less than that reported by Sperrazza et al. (1981) for wheat germ ribosomes. The number obtained from linear portions of the Hill plots was ~7 and did not vary with temperature. The model used for fitting these data does not distinguish between a site-specific model where Mg^{2+} binds to specific sites on the ribosome or an electrostatic model. The Hill number may be taken to represent a minimum number of Mg^{2+} ions bound rather than an exact determination. Our value of 7 can be compared to a value 7–9 (Zitomer & Flaks, 1972) for *E. coli* type B ribosomes and n = 5–10 for *E. coli* type A ribosomes (Noll & Noll, 1976; Debey, 1975). Sperrazza et al. (1980) obtained a value of ~8 for wheat germ ribosomes.

The Mg^{2+}-dependent equilibrium of *Artemia* is in general quite similar to that observed for wheat germ and *E. coli* type A ribosomes. *E. coli* type A ribosomes which associate at about 2.2 mM Mg^{2+} have been found to be converted to type B or "loose" couples or inactivated upon exposure to low Mg^{2+} (1 mM) (Gorisch et al., 1976; Weiss et al., 1973). For this reason, subunit equilibria were determined after a large dilution (typically 1:5000) into the appropriate buffer, rather than after extensive dialysis.

Type B ribosomes display different kinetics (Gorisch et al., 1976; Chaires et al., 1975) and a different midpoint in the Mg^{2+}-induced association curve (Debey et al., 1975) from type A ribosomes. The reversibility of the dissociation curve and our studies of A_{260}/A_{280} ratios suggests that *Artemia* ribosomes are more stable at low Mg^{2+} (0.2 mM) than either type A or type B *E. coli* ribosomes (Weiss et al., 1973) and may be quite similar to wheat germ ribosomes (Sperraza et al., 1980). The midpoint of the Mg^{2+} association curve is intermediate between that obtained for wheat germ and *E. coli* type A ribosomes under similar conditions. At 50 mM KCl, Debey et al. (1975) reported $[Mg^{2+}]_{1/2}$ of 3.5 mM at 35 °C while Sperrazza et al. have reported values of 0.95 mM $[Mg^{2+}]_{1/2}$ at 37 °C compared to our value of 2.2 mM $[Mg^{2+}]_{1/2}$ at 37 °C.

The thermodynamic parameters determined are qualitatively quite similar to those obtained for wheat germ and *E. coli* ribosomes. The $\Delta G°$ values ranged from −10.9 to −11.5 kcal/mol. The $\Delta G°$ values are very similar to those obtained for wheat germ ribosomes (Sperrazza et al., 1981) at 1.5 mM Mg^{2+} and *E. coli* type A ribosomes at 3 mM Mg^{2+} (Hui Bon Hoa et al., 1977). The temperature dependence of $\Delta G°$ was less than that for wheat germ ribosomes where $\Delta G°$ ranged from −8.9 to −10.7 kcal/mol over the temperature range 37–22 °C. Values of ΔH reported for *E. coli* type A ribosomes range from −5.2 (Infante et al. 1982) to −20 kcal/mol (Noll & Noll, 1976) and from −38 to −85 kcal/mol for type B ribosomes (Noll & Noll, 1976; Debey et al., 1975). Sperrazza et al. (1981) reported a value of −46 kcal/mol for wheat germ ribosomes. Our value of −25.7 kcal/mol is intermediate between the values obtained for *E. coli* type A ribosomes and wheat germ ribosomes. The entropy term calculated is almost negligible, and the reaction is exothermic. These results indicate that the subunit interactions are mainly electrostatic.

Rate constants for the association and dissociation reactions have been determined. There are no previous reports of eucaryotic subunit association or dissociation kinetics, although *E. coli* ribosomes have been extensively studied. The association and dissociation reactions were second and first order, respectively, suggesting a prior rapid equilibrium with Mg^{2+}. The rate constant obtained for subunit dissociation at 27 °C is quite close to that obtained for *E. coli* ribosomes. The value reported here of 0.022 s^{-1} is intermediate between that reported for *E. coli* type A ribosomes [0.015 s^{-1} (Goss et al., 1980; Chaires et al., 1977)] and that for type B ribosomes [0.033 s^{-1} (Gorisch et al., 1976)]. The association rate constant is again intermediate between the values obtained for *E. coli* type A and type B ribosomes. Our value of 3.7×10^6 M^{-1} s^{-1} obtained at 23.7 °C compares with values of 2.24×10^7 M^{-1} s^{-1} (Chaires et al., 1977) and 1.7×10^7 M^{-1} s^{-1} (Goss et al., 1980) obtained for type A ribosomes and a value of 1.05×10^6 (Gorisch et al., 1976) obtained for type B ribosomes. Our association and dissociation rate constants may not be directly comparable to those for *E. coli* since different Mg^{2+} concentrations were used. We presently investigating the Mg^{2+} dependence of these rate constants.

Conformational changes have been reported to occur in both *E. coli* and wheat germ ribosomal subunits (Chaires et al., 1977; Nieuwenhuysen et al., 1980; Bonnert et al., 1980; Moore & Spremulli, 1985). We have not considered conformational transitions in fitting these reactions, but since the association process is second order, such transitions cannot be the rate-limiting step for association. The reaction curves give no evidence for sequential processes and are quite homogeneous. These data suggest a simple mechanism for eucaryotic ribosomal subunit association and dissociation. In fact, the details of the mechanism are almost certainly more complex.

In view of the similarities in equilibria and thermodynamic parameters among *Artemia*, wheat germ, and *E. coli* ribosomes as well as the similarities between *Artemia* and *E. coli* in rate constants for subunit association and dissociation, it is tempting to suggest that the mechanism of association may be similar. Nieuwenhuysen et al. (1980) have shown that the molar volume changes resulting from ribosomal subunit association are similar for *Artemia* and *E. coli* type A ribosomes. This large volume change is consistent with either an electrostatic model for association or the binding of ions to specific sites. Base pair recognition between rRNAs alone could not account

for this change. Binding of divalent cations can also cause further aggregation of multisubunit proteins (Chiancone et al., 1976, 1980; David & Daniel, 1974). Without more detailed studies of ribosomal subunit association and dissociation kinetics, further considerations of the mechanism are mere speculation.

The data presented here indicate that *Artemia*, like wheat germ and *E. coli*, ribosomes are present mainly as 80S monomers under physiological conditions. In order to maintain a pool of ribosomal subunits for protein synthesis initiation, some mechanism must exist for dissociation of the ribosomes. In *E. coli*, initiation factor 3, IF3, serves as an allosteric effector to regulate the subunit equilibrium (Goss et al., 1980, 1982). Rabbit reticulocyte initiation factor 3, eIF3, and wheat germ initiation factor 6, eIF6, have both been reported to have ribosome dissociation activity (Trachsel et al., 1977; Russell & Spremulli, 1979). The data reported here form a basis for more detailed studies of the effects of initiation factors on subunit equilibria and kinetics.

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