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Reversible Dissociation of Wheat Germ Ribosomal Subunits: Cation-Dependent Equilibria and Thermodynamic Parameters[†]

Joan M. Sperrazza, David W. Russell, and Linda L. Spremulli*

ABSTRACT: The influence of magnesium ion concentration upon the equilibrium between the wheat germ ribosome and its subunits has been studied by light scattering. The curves obtained for ribosome dissociation and subunit reassociation were identical and were independent of the origin and direction of the magnesium ion titration, suggesting that the wheat germ ribosomes are behaving as a homogeneous population equivalent to *Escherichia coli* type A ribosomes. Increasing K^+ concentrations in the presence of Mg^{2+} favored ribosome dissociation. Polyamines favored subunit reassociation, with 0.1 mM spermine acting as effectively as 1 mM spermidine. Thermodynamic parameters have been determined from the temperature-dependent equilibria and have been compared to those of *E. coli* type A ribosomes. The association of the 40S and 60S subunits is exothermic. At 1.5 mM Mg^{2+} and 100 mM K^+, the entropy term is negative, favoring ribosome dissociation, and contributes less to the free energy than the enthalpy term. The determination of these thermodynamic parameters was based on molecular weights of 1.2×10^6 and 2.3×10^6 for the 40S and 60S subunits, respectively, calculated from laser light scattering data.

The subunits of both eucaryotic and procaryotic ribosomes are capable of reversible association and dissociation. The importance of this phenomenon became clear when it was observed that the initiation of protein synthesis requires free ribosomal subunits and involves the formation of a complex containing the mRNA and the initiator tRNA on the small subunit of the ribosome prior to the joining of the large subunit (Weissbach & Ochoa, 1976). A thorough investigation of the reversible equilibrium between the ribosome and its subunits and of the factors that influence this equilibrium is important for our understanding of the function of the ribosome in polypeptide synthesis.

Magnesium ions play a major role in the equilibrium between the ribosome and its subunits by promoting subunit association. The nature of the Mg^{2+}-dependent equilibrium of *Escherichia coli* 70S ribosomes has been extensively investigated by several laboratories (Zitomer & Flaks, 1972; Debey et al., 1975; Hui Bon Hoa et al., 1977; Noll & Noll, 1976). Zitomer & Flaks (1972) observed that monovalent cations favored ribosome dissociation, thus increasing the Mg^{2+} requirement for subunit association. The polyamines putrescine and spermidine, on the other hand, favored reassociation and reduced the Mg^{2+} requirement for subunit association. Many investigators have sought to explain the profound effect of Mg^{2+} on the equilibrium between the ribosome and its subunits. Edelman et al. (1960) demonstrated that Mg^{2+} binds exclusively to the rRNA in rabbit reticulocyte ribosomes, and the data of Sissoëff et al. (1976) indicate that Mg^{2+} binds exclusively to the phosphate groups in polynucleotides. Wishnia et al. (1975) and Wishnia & Boussert (1977) have used stopped-flow kinetics combined with light scattering to study the roles of various ions in the association of *E. coli* ribosomal subunits. They suggest that Mg^{2+} plays a non-site-specific role in subunit association by modulating the electrostatic repulsion between the negatively charged phosphate groups in the rRNAs, thereby allowing reassociation.

Few systematic studies of the effects of various cations on the dissociation of eucaryotic ribosomes have been performed, and virtually no thermodynamic data are available on eucaryotic ribosomal subunit interactions. We have investigated the effects of KCl, spermidine, spermine, and temperature on the Mg^{2+}-dependent association and dissociation of wheat germ ribosomal subunits by using light scattering.

Materials and Methods

Materials. Wheat germ, kindly supplied by J. M. de Rosier of International Multifoods Corp., was stored at −20 °C under vacuum in the presence of a desiccant. Hepes[1] was purchased from Sigma. Buffer A contains 20 mM Hepes–KOH, pH 7.6, 6 mM β-mercaptoethanol, and 10% glycerol, with $Mg(OAc)_2$ and KCl as indicated.

[†] From the Department of Biochemistry (J.M.S.) and the Department of Chemistry (D.W.R. and L.L.S.), University of North Carolina, Chapel Hill, North Carolina 27514. *Received June 28, 1979.* This work was supported in part by funds from the Petroleum Research Fund, administered by the American Chemical Society, Research Corporation, The North Carolina Science and Technology Committee, and National Institutes of Health (GM 26731).

[1] Abbreviation used: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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Preparation of Wheat Germ Ribosomes. High-salt-washed ribosomes were obtained from 120 g of raw wheat germ as described previously (Spremulli et al., 1977). They were resuspended to a concentration of ~45 mg/mL in buffer A containing 50 mM KCl and 5 mM $Mg(OAc)_2$, divided into small aliquots, fast frozen in a dry ice–isopropyl alcohol bath, and stored at −70 °C. Concentrations were determined after dilution in water by the absorbance at 260 nm in a Beckman Model 25 spectrophotometer (12 A_{260} units/mL is equivalent to 1 mg of ribosomes per mL).

Preparation of Ribosomal Subunits. Wheat germ 40S and 60S ribosomal subunits were separated by zonal centrifugation as described previously (Spremulli et al., 1977) except that ribosome dissociation and zonal centrifugation were carried out in buffers containing 120 mM KCl and 1 mM $Mg(OAc)_2$. Each subunit preparation had less than 2% cross-contamination by the other subunit.

Laser Light Scattering. All measurements were performed by using a 5-mW helium/neon laser (Metrologic) as described by Carr et al. (1977) except that an IP21 Pacific Photometrics digital ammeter was substituted for the original photomultiplier. Three-milliliter glass cuvettes, cleaned in 1.5 N HCl and 50% EtOH, were used. Solutions were filtered through Millipore filters (0.22 μm) and degassed before use. Subunits were clarified by centrifugation at 20000*g* in a Sorvall SS34 rotor at 0 °C for 1 h. The 40S or 60S subunits were diluted to appropriate concentrations (Table I) in 2 mL of 20 mM Hepes–KOH, pH 7.6, 50 mM KCl, 4.5 mM $Mg(OAc)_2$, and 6 mM β-mercaptoethanol. Intensities (in millivolts) of light scattered at 90° were determined for standard benzene and ribosomal subunit solutions.

Molecular weights were determined from Rayleigh's equation (Tanford, 1961; Van Holde, 1971)

$$R_\theta = \frac{KC}{P_\theta(1/\bar{M}_w + 2bC + 3cC^2 + \ldots)} \qquad (1)$$

where R_θ is the Rayleigh scattering ratio for the species in question, K is a collection of constants defined below, C is the total concentration (g/cm^3), $\bar{M}_w$ is the weight-average molecular weight, P_θ is the particle scattering factor, and b and c are the second and third virial coefficients. The diameter of the eucaryotic ribosome is believed to be less than 300 Å (Van Holde & Hill, 1974). This means that the dimensions of the scattering particles are less than 1/20 of the wavelength of the light being used (633 nm). Hence, there should be little angular dependence of scattering and under these conditions we may take $P_\theta \simeq 1$.

At a wavelength of 633 nm, the intensity of light scattered was linear with respect to concentration (results not shown). The virial coefficients can therefore be set as ~0, and the equation can be written as

$$\bar{M}_w = R_\theta/KC \qquad (2)$$

where

$$K = \frac{2\pi^2 n_0^2 (\mathrm{d}n/\mathrm{d}C)^2}{N\lambda^4} \qquad (3a)$$

and

$$R_\theta = \left(\frac{n_0}{n_b}\right)^2 \left(\frac{i}{i_b}\right) R_b \qquad (3b)$$

R_θ = Rayleigh's ratio for the species in question; R_b = Rayleigh's ratio for benzene = 8.5×10^{-6} (this value yields an accurate molecular weight for fibrin on the instrument used); n_0 = refractive index of solvent = 1.33; n_b = refractive index of pure benzene = 1.50; i = intensity of the scattered signal from the species in question; i_b = intensity of the scattered signal from benzene; $\mathrm{d}n/\mathrm{d}C$ = refractive index increment between solvent and solute = 0.20 mL/g (Reale Scafati et al., 1971); N = Avogadro's number; λ = wavelength = 6.33×10^{-5} cm.

Determinations were performed in duplicate and were quite consistent. The molecular weights determined are accurate within 15%.

Turbidimetric Technique. Association–dissociation equilibrium curves of wheat germ ribosomes were determined by turbidimetric measurements at 310 nm with a Beckman Model 25 spectrophotometer fitted with a jacketed cell holder attached to a thermostatically controlled circulating water bath. Temperatures were determined with a probe from a YSI Model 42 SC Tele-Thermometer which was enclosed in the sample cuvette. Temperatures were controlled to within ±0.1 °C. Solutions were filtered through nitrocellulose (Millipore Type HA, 0.45 μm), and ribosomes were clarified by centrifugation at 20000*g* in a Sorvall SS34 rotor at 0 °C for 1 h.

In a typical experiment, 0.970 mL of buffer A containing the appropriate concentrations of KCl and $Mg(OAc)_2$ was added to an acid-washed quartz cuvette. The ribosome sample (15 A_{260} units in 30 μL) was added, and ~10 min was allowed for temperature equilibration before the A_{310} was determined. There was a linear relationship between turbidity and concentration in the range used (data not shown).

Analysis of Data. Analysis of data was performed basically as described by Hui Bon Hoa et al. (1977). For a constant ribosome concentration

$$\%\ 80\ \mathrm{S} = \frac{\Upsilon_y - \Upsilon_0}{\Upsilon_{100} - \Upsilon_0} \times 100 \qquad (4)$$

where Υ_{100} = turbidity at 310 nm at 100% association, Υ_0 = turbidity at 310 nm at 0% association, and Υ_y is the turbidity of the sample under investigation. The value of Υ_{100} was determined in each experiment by taking the average of all the values after the curve began to plateau. There was no apparent aggregation of the ribosomal particles under the conditions used as determined by a lack of change in Υ from 5 to 15 mM Mg^{2+} and as analyzed by sucrose gradient centrifugation. The value of Υ_0 was determined in each experiment from the average of all the points that could not be distinguished from 0% association (0.15 mM Mg^{2+} and 100 mM KCl). There was no apparent unfolding of the subunits under these conditions, as indicated by a lack of change in their sedimentation pattern on sucrose gradients and by the reversibility of the association.

It was not possible in our case to determine the expected value of $\Upsilon_{100}/\Upsilon_0$ because of several factors, including inaccuracies in the molecular weights and a small amount of absorption of light of this wavelength by the sample. In addition, light with a wavelength of 310 nm is only ~10 times the diameter of the ribosome and P_θ may not be equal to 1. However, we were able to obtain consistent ratios of $\Upsilon_{100}/\Upsilon_0$ between experiments. Therefore, although our exact value for % 80 S may not be absolutely accurate, these values are consistent from one curve to another, thereby allowing direct comparisons and the analysis of trends.

The apparent association constant (K_{obsd}) for the equilibrium between the 80S ribosome and its subunits at any Mg^{2+} concentration is given by

$$K_{\mathrm{obsd}} = X/[C(1 - X)^2] \qquad (5)$$

Table I: Determination of the Molecular Weight of Wheat Germ Ribosomal Subunits

subunit	concn (mg/mL)	i^a (mV)	$i_b{}^b$ (mV)	calcd M_r
40 S	0.322	10.0	1.20	1.2×10^6
40 S	0.322	10.0	1.20	1.2×10^6
60 S	0.423	26.1	1.23	2.3×10^6
60 S	0.423	26.5	1.23	2.3×10^6

[a] Intensity of light scattered from the ribosomal subunit solution. [b] Intensity of light scattered from the benzene standard.

where X is the fraction of the ribosomes present as 80S particles (the 40S and 60S free subunit concentrations are equal) and C is the total molar concentration of ribosomes at 100% association.

Sucrose gradient centrifugation of the high-salt-washed ribosomes used for these studies (Russell & Spremulli, 1978) indicated that 85–90% of the A_{260}-absorbing material was associated with 80S ribosomes. The remaining 10–15% sedimented at 60S. The material in this 60S fraction was unable to associate with excess 40S subunits even in high concentrations of Mg^{2+} (5–15 mM) and will not interfere with the equilibrium measurements reported here. (The contribution to the intensity contributed by an inert species is automatically subtracted as background in the analysis of the turbidity data.) Attempts to purify the 80S species by zonal centrifugation resulted in a severe loss of activity in polymerization.

Results

Molecular Weight Determinations. Calculations of the equilibrium constant and thermodynamic parameters governing ribosomal subunit interactions required knowledge of the molar concentrations of the 40S, 60S, and 80S particles in solution. It was therefore important to determine the approximate molecular weights of the wheat germ ribosomal subunits. Laser light scattering measurements were performed with a benzene standard, and the intensity of the scattered signal was used to calculate the molecular weights of the separated ribosomal subunits from Rayleigh's equation as described under Materials and Methods. The results shown in Table I indicate that the molecular weight of the wheat germ 40S subunit is $\sim 1.2 \times 10^6$ while that of the 60S subunit is $\sim 2.3 \times 10^6$. Using the indicated values for the wheat germ subunits, we calculated a molecular weight of 3.5×10^6 for the 80S ribosome. This value agrees within experimental error with that determined for plant 80S ribosomes (3.9×10^6) from a number of sources by Cammarano et al. (1972). Our value is also similar to the molecular weight of 3.8×10^6 recently determined for *Artemia salina* ribosomes (Nieuwenhuysen & Clauwaert, 1978).

Magnesium-Dependent Association of Ribosomal Subunits. It has been well established that ribosomes are in dynamic equilibrium with their subunits (Spirin, 1971; Infante & Baierlein, 1971) and furthermore that high concentrations of magnesium ion induce subunit association. We have chosen to study the equilibrium between the 80S ribosome and its subunits by a turbidimetric method. This procedure does not disturb the equilibrium as does ultracentrifugation and permits the processing of a larger number of samples.

Figure 1 illustrates the magnesium-dependent association and dissociation of wheat germ ribosomal subunits at 37 °C and 50 mM KCl. The curve obtained when 80S ribosomes were dissociated in buffers containing reduced concentrations of magnesium ions was identical with that obtained when ribosomal subunits were associated by the addition of increasing concentrations of Mg^{2+}, indicating that the dissociation of 80S ribosomes is fully reversible even after the ribosomes have been exposed to very low concentrations of Mg^{2+} (0.15 mM). The lack of hysteresis suggests that we are dealing with a homogeneous population of ribosomes that behave similarly to *E. coli* tight couples or type A ribosomes (Debey et al., 1975).

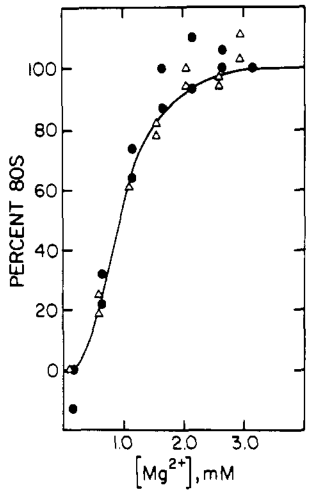

FIGURE 1: Magnesium ion dependent equilibrium between the 80S ribosome and its subunits. (△) 80S ribosomes in buffer A containing 50 mM KCl and 5 mM $Mg(OAc)_2$ were diluted with buffer A containing 50 mM KCl and the indicated concentration of $Mg(OAc)_2$. (●) Ribosomes were dissociated in buffer A containing 50 mM KCl and 0.15 mM $Mg(OAc)_2$ and subsequently reassociated by addition of $Mg(OAc)_2$ to the indicated concentration. Incubations were at 37 °C.

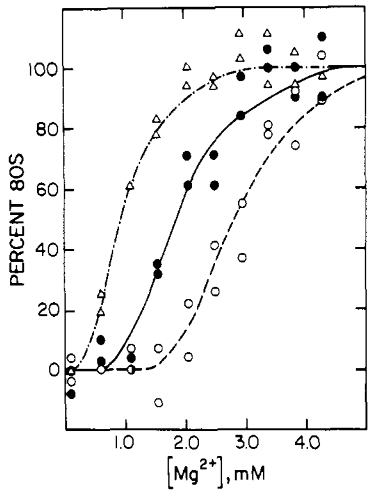

FIGURE 2: Effect of KCl on the magnesium ion dependent dissociation. Ribosomes in buffer A containing 50 mM KCl and 5 mM $Mg(OAc)_2$ were diluted into buffer A containing the indicated $Mg(OAc)_2$ concentration and either 50 mM KCl (△), 100 mM KCl (●), or 150 mM KCl (○). Incubation was at 37 °C, and all measurements were taken after equilibrium had been reached.

E. coli type B ribosomes or loose couples, which are less active than type A ribosomes, tend to dissociate more readily when subjected to hydrostatic pressure (Hapke & Noll, 1976) and display different kinetics for Mg^{2+}-dependent subunit association and dissociation (Chaires et al., 1977). Wheat germ ribosomes are apparently more stable in low concentrations of Mg^{2+} (0.15 mM) than either type A or type B *E. coli* ribosomes (Weiss et al., 1973).

The effect of the K^+ ion concentration on the magnesium-dependent equilibrium is shown in Figure 2. Increasing concentrations of K^+ ion shift the equilibrium toward dissociation. The Mg^{2+} concentrations required to give 50% as-

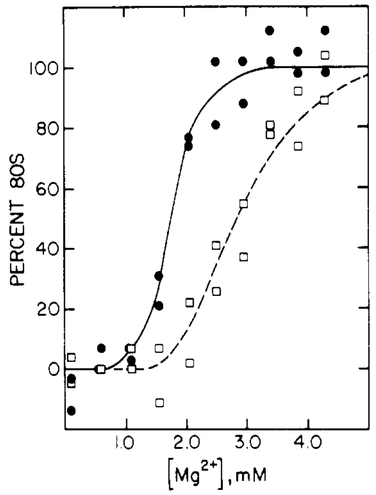

FIGURE 3: Effect of spermine on the dissociation equilibrium. Ribosomes in buffer A containing 50 mM KCl and 5 mM $Mg(OAc)_2$ were diluted into buffer A at 150 mM KCl containing the indicated concentration of $Mg(OAc)_2$ and either no spermine (□) or 50 μM spermine (●). Samples were incubated at 37 °C until equilibrium had been reached.

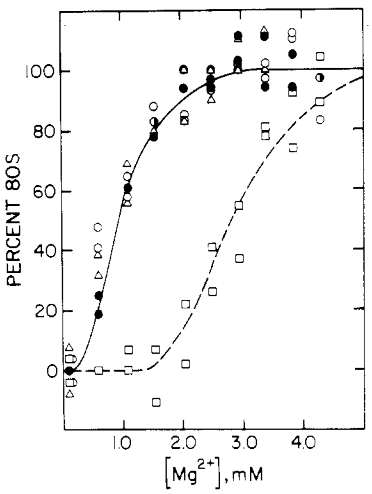

FIGURE 4: Comparison of KCl, spermine, and spermidine effects on the equilibrium. Ribosomes in buffer A containing 50 mM KCl and 5 mM $Mg(OAc)_2$ were diluted into buffer A to give the final indicated concentrations of $Mg(OAc)_2$, KCl, or polyamine. Samples were incubated at 37 °C until equilibrium had been reached before turbidity measurements were made. (□) 150 mM KCl; (○) 150 mM KCl and 0.1 mM spermine; (△) 150 mM KCl and 1.0 mM spermidine; (●) 50 mM KCl. The solid line was drawn to fit the data for 50 mM KCl from Figure 2.

sociation, $[Mg^{2+}]_{1/2}$, were 0.9, 1.9, and 2.8 mM at 50, 100, and 150 mM K^+, respectively. The addition of each 50 mM K^+ increment increased the Mg^{2+} requirement for association by ~1 mM. A similar dissociating effect of monovalent cations was observed by Zitomer & Flaks (1972) for *E. coli* type B ribosomes.

The polyamines spermine and spermidine had the opposite effect; i.e., they shifted the equilibrium toward subunit association. Figure 3 shows the effect of 50 μM spermine on the magnesium-dependent equilibrium at 150 mM K^+. This low concentration of spermine shifted the $[Mg^{2+}]_{1/2}$ from ~2.8 to ~1.8 mM, an effect equivalent to a 50 mM reduction in K^+ concentration. Spermine, a tetravalent polyamine, was ~10 times more effective than spermidine, a trivalent polyamine, at promoting subunit association (Figure 4). The magnesium ion dependent dissociation curves of wheat germ ribosomes in 150 mM K^+ and either 0.1 mM spermine or 1 mM spermidine were almost identical with each other and with the curve obtained with 50 mM K^+ alone. The addition of these spermine or spermidine concentrations had the same effect as a 100 mM reduction in K^+ concentration and shifted the $[Mg^{2+}]_{1/2}$ from ~2.8 to ~0.9 mM. Polyamines have a similar strong associating effect on *E. coli* 30S and 50S ribosomal subunits (Zitomer & Flaks, 1972).

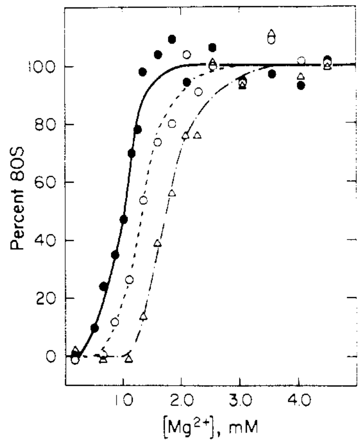

FIGURE 5: Effect of temperature on the magnesium ion dependent equilibrium. Ribosomes were incubated in buffer A containing 100 mM KCl at the indicated concentrations of $Mg(OAc)_2$ at (●) 17, (○) 27, and (△) 37 °C. All measurements were taken after equilibrium had been reached. Each point represents the mean of six independent determinations. The average standard deviation is ±9%.

Table II: Thermodynamic Parameters Governing Ribosomal Subunit Association

temp (°C)	$[Mg^{2+}]_{1/2}$ [a]	K_{obsd} × 10^{-7} (M^{-1}) [b]	$\Delta G°_{obsd}$ (kcal/mol)	n
17	1.05			8.4
22		9.04	−10.7	
27	1.35	2.20	−10.1	7.7
32		0.91	−9.7	
37	1.75	0.18	−8.9	8.2

[a] Concentration of magnesium ion required to give 50% association. [b] Values for 27 and 37 °C were calculated from the data in Figure 5 at 1.5 mM Mg^{2+} and 100 mM K^+ in buffer A. Values for 22 and 32 °C were determined at 1.5 mM Mg^{2+} in six independent determinations.

Thermodynamic Parameters Governing Ribosome Dissociation. Figure 5 shows the effect of various temperatures on the magnesium ion dependent dissociation of wheat germ 80S ribosomes at 100 mM K^+. The equilibrium was clearly affected by the incubation temperature, and the association of the subunits was exothermic. The $[Mg^{2+}]_{1/2}$ at 100 mM K^+ shifted from 1.05 to 1.75 mM as the temperature was raised from 17 to 37 °C. The equilibrium constants for association (K_{obsd}) were calculated for various temperatures at 1.5 mM Mg^{2+}, where there were easily measurable amounts of 40S, 60S and 80S ribosomes at most of the temperatures tested. Due to an increased incidence of scatter above 80% association, this value from the 17 °C curve was clearly unreliable and was not calculated. The percentage of the 80S species was also determined at 1.5 mM Mg^{2+} in six independent trials at both 32 and 22 °C. The K_{obsd} at the various temperatures covered a 100-fold range from 9.0 × 10^7 M^{-1} at 22 °C to 1.8 × 10^6 M^{-1} at 37 °C. The K_{obsd} values were used to calculate $\Delta G°_{obsd}$ for subunit association (Table II). These values ranged from −10.7 kcal/mol at 22 °C to −8.9 kcal/mol at 37 °C. Wheat germ ribosome dissociation at 1.5 mM Mg^{2+} corresponds to that observed at 3 mM Mg^{2+} for *E. coli* type A ribosomes, permitting a comparison of our data with those of Hui Bon Hoa et al. (1977). These numbers are quite comparable to

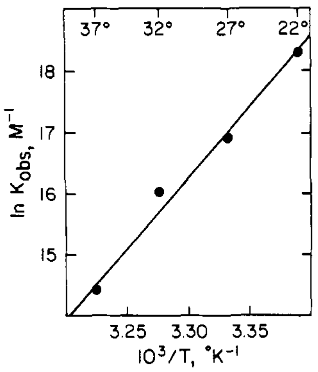

FIGURE 6: van't Hoff plot of ln K_{obsd} vs. $10^3/T$. Magnesium ion concentration was 1.5 mM. The equilibrium constants for association were taken from Table II.

those obtained for *E. coli* ribosomes which vary from −11 to −9.8 kcal/mol between 7.5 and 35 °C (Hui Bon Hoa et al., 1977). A van't Hoff plot of ln K_{obsd} vs. $10^3/T$ at 1.5 mM Mg^{2+} (Figure 6) was linear with a slope of $-\Delta H°_{obsd}/R$ and an intercept of $\Delta S°_{obsd}/R$. This plot allowed the calculation of a standard enthalpy change ($\Delta H°_{obsd}$) of −46 kcal/mol and of a standard entropy change ($\Delta S°_{obsd}$) of −120 cal/(mol deg) for ribosomal subunit association. $T\Delta S°_{obsd}$ was calculated to be −35 kcal/mol at 22 °C.

It should be noted that there are a number of potential sources of error that may affect the actual values of the thermodynamic parameters reported here. These include possible errors in the molecular weight and the percentage of 80S as described previously which will affect K_{obsd}. Because of these potential sources of error, the reported values of $\Delta H°_{obsd}$, $\Delta S°_{obsd}$, and $\Delta G°_{obsd}$ should not be thought of in terms of rigorous quantitative values. However, the association of the 40S and 60S subunits is definitely exothermic and the entropy term is negative and contributes less to the free energy than the enthalpy term.

Another factor affecting K_{obsd} and thus the thermodynamic parameters is the Mg^{2+} ion concentration selected at which to determine them. We have chosen to determine these values at 1.5 mM Mg^{2+} because this concentration provided the most reliable number for K_{obsd} at the temperatures tested and because this concentration of Mg^{2+} yields approximately the same degree of dissociation with wheat germ ribosomes as that observed with *E. coli* ribosomes at 3 mM Mg^{2+} where the thermodynamic parameters have been calculated by Hui Bon Hoa et al. (1977).

If the association of eucaryotic ribosomal subunits involves the binding of a critical number of magnesium ions (n), the equilibrium can be written as

$$40\ \mathrm{S} + 60\ \mathrm{S} + n\mathrm{Mg}^{2+} \rightleftharpoons [80\ \mathrm{S}\cdot n\mathrm{Mg}^{2+}]$$

This equation is valid whether one envisions a model in which Mg^{2+} ions bind to specific sites on the ribosome or whether it acts in a site-independent manner to reduce the electrostatic repulsion between the subunits (Record et al., 1978). The equilibrium constant for such a reaction is given by

$$K_a = \frac{[80\ \mathrm{S}\cdot n\mathrm{Mg}^{2+}]}{[40\ \mathrm{S}][60\ \mathrm{S}][\mathrm{Mg}^{2+}]^n} \qquad (6)$$

Taking the log of both sides of the equation gives the form

$$\log K_a = \frac{[80\ \mathrm{S}\cdot n\mathrm{Mg}^{2+}]}{[40\ \mathrm{S}][60\ \mathrm{S}]} - n \log [\mathrm{Mg}^{2+}] = \log K_{obsd} - n \log [\mathrm{Mg}^{2+}] \qquad (7)$$

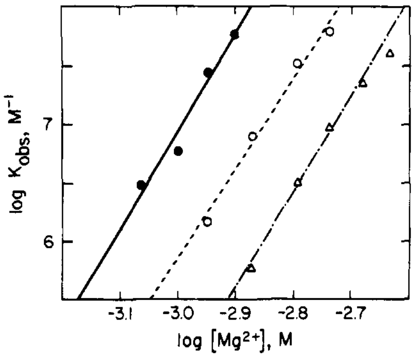

FIGURE 7: Log–log plots for the association equilibrium curves. Values for [80 S]/([40 S][60 S]) were calculated from the data points in Figure 5. (△) 37 °C; (○) 27 °C; (●) 17 °C.

A Hill plot of log K_{obsd} vs. log [Mg^{2+}] gives a curve having a slope which yields n. Figure 7 shows linear portions of Hill plots drawn from the data in Figure 5. The values of n determined from the linear portions of the Hill plots are ~8 at the various temperatures (Table II). Because the full plots are nonlinear (not shown), these values represent a minimum estimate for n rather than the exact number of Mg^{2+} ions involved. The value of n is not significantly affected by possible errors in the determination of the molecular weights, although it is affected by errors in the percentage of 80 S. Although not necessarily accurate when viewed in an absolute sense, it is clear that the value of n does not vary with temperature. Further analysis of the Hill plots is not possible due to the unreliability of the data, especially near 0 and 100%. It is interesting to note that the number, n, determined by our procedure for wheat germ ribosomes is remarkably similar to those determined by Zitomer & Flaks (1972) for *E. coli* type B ribosomes (n = 7–9) and by Debey et al. (1975) and Noll & Noll (1976) for *E. coli* type A ribosomes (n = 5–10).

Discussion

In the present work we have investigated the Mg^{2+}-dependent association of wheat germ ribosomal subunits. The effect of Mg^{2+} on subunit association is a dramatic one, and under some conditions a 1 mM change in the Mg^{2+} concentration can cause a 90% change in the degree of subunit association. Like Mg^{2+}, the trivalent and tetravalent polyamines spermidine and spermine are both effective in promoting association. It is interesting to note that the addition of the extra positive charge on spermine makes this polyamine about 10-fold more effective than spermidine in promoting the interaction of the 40S and 60S subunits. Unlike divalent or polyvalent ions, monovalent cations shift the equilibrium toward dissociation. K$^+$ is about 50-fold less effective on a molar basis in dissociating the ribosome than Mg^{2+} is in promoting subunit association. A much more detailed investigation of the monovalent, divalent, and polyvalent cation effects on ribosome subunit association and on the specificity of these ions will be necessary to provide a more complete understanding of the role of cations in this equilibrium. Such a study is currently in progress in this laboratory.

In general, the qualitative effects of various cations on the equilibrium between the wheat germ ribosome and its subunits are quite similar to those observed for *E. coli* type A ribosomes (Zitomer & Flaks, 1972; Debey et al., 1975; Hui Bon Hoa et al., 1977). However, two major differences are evident. First, wheat germ ribosomal subunits associate at a consistently lower magnesium ion concentration than *E. coli* ribosomes under similar conditions. For example, Debey et al. (1975) found that type A *E. coli* ribosomal subunits associate with a $[Mg^{2+}]_{1/2}$ of 3.6 mM at 50 mM KCl and 35 °C. Under similar conditions (50 mM KCl, 37 °C) wheat germ ribosomes exhibit a $[Mg^{2+}]_{1/2}$ of 0.95 mM. Second, Hui Bon Hoa et al. (1977) reported that n, the slope of the linear portions of their Hill plots, for type A *E. coli* ribosomes increases from 6 to 7.5 as the temperature is raised from 7.5 to 35 °C. Wheat germ ribosomes, on the other hand, exhibited no consistent change in n with temperature between 17 and 37 °C.

However, because of the generally similar characteristics between *E. coli* and wheat germ ribosomal subunit association, it is tempting to speculate that the mechanisms of association may be similar. Sequence analysis of the *E. coli* rRNAs reveals the presence of two complimentary stretches of nucleotides between the 3′ end of the 16S rRNA and the 5′ end of the 23S rRNA. It has been suggested (Grunberg-Manago & Gros, 1977; Kurland, 1974; Branlant et al., 1976) that the interaction between the 30S and 50S ribosomal subunits to form the 70S particle could be facilitated and stabilized by the formation of base pairs between the rRNAs of each subunit. Recently, the formation of a complex between eucaryotic 18S and 5S rRNA has been observed in solution (Oakden et al., 1977). Thus, it might be suggested that the formation of base pairs between the rRNAs of the large and small subunits might also be important in the formation of 80S particles. In this context, it is interesting to note that monovalent and divalent cations generally have a profound effect on the formation of double-stranded polynucleotides (Record, 1975; Krakauer, 1974). These effects are quite complex and depend on the ratio of monovalent and divalent cations to each other and to the polynucleotide. But, in general, Mg^{2+} tends to stabilize the formation of base-paired structures, while K^+ at some concentrations may destabilize such structures when in the presence of Mg^{2+}.

The thermodynamic values we have observed for the equilibrium between the wheat germ ribosome and its subunits are qualitatively quite similar to those observed with *E. coli* ribosomes (Hui Bon Hoa et al., 1977). Interactions between the ribosomal subunits in both procaryotes and eucaryotes are exothermic but the entropy term in both cases is negative, favoring the dissociation of the subunits. The free energy change observed (ΔG_{obsd}) is negative for both types of ribosomes, indicating that the enthalpy term contributes more to the free energy change than the entropy term.

The initiation of protein biosynthesis in eucaryotes involves the formation of a complex containing mRNA, Met-$tRNA_f^{Met}$, GTP, and initiation factors on the 40S ribosomal subunit (Weissbach & Ochoa, 1976). The data presented here indicate that under estimated physiological concentrations of Mg^{2+}, K^+, and polyamines and under in vitro natural mRNA translation conditions [2.5–3.0 mM Mg^{2+}, 90–100 mM K^+, and 0.1 mM spermine (Spremulli et al., 1979)], wheat germ ribosomes are present almost exclusively as 80S monosomes. The eucaryotic cell must, therefore, possess some mechanism for maintaining a pool of ribosomal subunits. It has recently been reported (Trachsel et al., 1977) that rabbit reticulocyte initiation factor eIF-3 has a ribosome dissociation factor activity. In addition, a ribosome dissociation factor distinct from eIF-3 has been purified from extracts of wheat germ (Russell & Spremulli, 1979). The effects of these proteins on the equilibrium between the ribosome and its subunits are currently under investigation.

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