Influence of Various Cations on the Equilibria between Wheat Germ Ribosomes and Their Subunits†

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ABSTRACT: The influence of alkaline earth metal ions, alkali metal ions, ammonium ions, and polyamines upon the equilibria between high-salt-washed wheat germ ribosomes and their subunits was investigated by light scattering. The Mg^{2+}-dependent, reversible association of ribosomal subunits was studied with buffers containing 100 mM concentrations of the chloride salts of various monovalent cations. These studies indicate that K^+ and Na^+ are most effective in dissociating the ribosomes into subunits, followed by Rb^+ and then NH_4^+. The dissociation curves determined with buffers containing K^+, Na^+, Rb^+, and NH_4^+ ions exhibit $[Mg^{2+}]_{1/2}$ values of 1.4, 1.4, 1.2, and 0.7 mM, respectively. The divalent cations, in general, strongly promote subunit association but differ in their abilities to do so. Ca^{2+} associates the ribosomal subunits more effectively than Sr^{2+}, which is more effective than Mg^{2+}. In the presence of 100 mM KCl and 0.15 mM $MgCl_2$, Ca^{2+}-, Sr^{2+}-, and Mg^{2+}-dependent association curves exhibit $[M^{2+}]_{1/2}$ values of 0.9, 1.1, and 1.4 mM, respectively. The abilities of the polyamines to associate ribosomal subunits in buffers containing 100 mM KCl and 0.20 mM $MgCl_2$ were investigated. Both spermine ($[spm]_{1/2}$ = 0.09 mM) and spermidine ($[spd]_{1/2}$ = 0.25 mM) can fully associate wheat germ ribosomal subunits. Furthermore, increased concentrations of Mg^{2+} facilitate the association of the ribosomal subunits by these polyamines, suggesting that these cations act additively. Analysis by ultracentrifugation on sucrose gradients indicates that subunits associated by spermine or spermidine are more sensitive to dissociation by hydrostatic pressure than those associated solely by Mg^{2+}. In contrast to the other divalent cations, putrescine cannot associate wheat germ ribosomal subunits. Our data indicate that Mg^{2+} may facilitate ribosomal subunit association by binding to specific sites on the subunits in addition to reducing the electrostatic repulsion between them.

The subunits of both eucaryotic and procaryotic ribosomes are capable of reversible association and dissociation. The types and concentrations of various cations present play a major role in determining the position of the equilibrium between the intact ribosome and its subunits. Kinetic, thermodynamic, and ion-specificity studies have been performed with *Escherichia coli* ribosomes in an effort to explain the roles of Mg^{2+}, K^+, and polyamines in the association of the subunits. Conflicting mechanisms have been proposed as a result of these studies. One model suggests that Mg^{2+} binds to specific sites on the subunits and thereby promotes subunit association (Petermann, 1964; Goldberg, 1966). This model is supported by the data of Zitomer & Flaks (1972), who found that Mg^{2+}, Ca^{2+}, and polyamines promote ribosomal subunit association while K^+ promotes subunit dissociation. Ca^{2+}, however, was less effective than Mg^{2+} in promoting subunit association, suggesting that the ions were acting at specific sites. In contrast, the electrostatic model suggests that Mg^{2+} neutralizes the charged groups on the ribosomal subunits. This reduction in the electrostatic repulsion between the subunits would allow association. The studies of Walters & Van Os (1970, 1971) on yeast ribosomes and Wishnia & Boussert (1977) on *E. coli* ribosomes support the electrostatic model. Calculations by these groups also showed that the reduction of the electrostatic repulsion by Mg^{2+} theoretically is sufficient to promote subunit association.

Nieuwenhuysen et al. (1980), using *Artemia salina* ribosomes, have shown that the molar volume changes produced by ribosomal subunit association are similar to those observed with *E. coli* type A ribosomes. Furthermore, they have suggested that this large volume change is consistent with two mechanisms for association, either ion-pair formation or the binding of ions to specific sites. The condensation of Mg^{2+} as described by Wishnia et al. (1975) and/or base pair recognition between rRNAs alone could not account for this change.

In a previous report (Sperrazza et al., 1980), we showed that the Mg^{2+}-dependent equilibria between the high-salt-washed wheat germ ribosome and its subunits resemble those for *E. coli* type A ribosomes (Debey et al., 1975) in that dissociation at low Mg^{2+} concentrations is fully reversible and the slopes of the subunit reassociation curves are steep. In an attempt to clarify the role of specific sites and electrostatic effects in the Mg^{2+}-dependent association of ribosomal subunits, we have carried out the first systematic study of the effects of various cations on the equilibria between eucaryotic ribosomes and their subunits using light scattering. We have chosen to use the alkaline earth metal ions, alkali metal ions, NH_4^+ ions, and polyamines for our studies because these, like Mg^{2+} and K^+, have been shown to interact primarily with the phosphate backbone of nucleic acids (Sissoëf et al., 1976; Record et al., 1978). Our results indicate that the recognition of specific sites and binding of Mg^{2+} to these sites are indeed likely in ribosomal subunit association.

Materials and Methods

Materials. Wheat germ, kindly supplied by J. M deRosier of International Multifoods Corp., was stored at −20 °C under vacuum in the presence of a desiccant. Buffer A contains 20 mM Hepes[1]–KOH, pH 7.6, 6 mM β-mercaptoethanol, 10% glycerol, and salts as indicated. Spermine hydrochloride, spermidine hydrochloride, putrescine hydrochloride, and Hepes were purchased from Sigma. NH_4Cl, KCl, NaCl, $SrCl_2$, and $MgCl_2$ were purchased from Fisher. $CaCl_2$ was purchased

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[1] Abbreviations used: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; spm, spermine; spd, spermidine.

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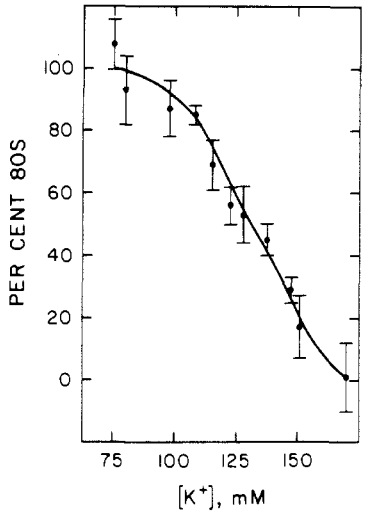

FIGURE 1: K^+-dependent dissociation of wheat germ ribosomes. Ribosomes in buffer A containing 50 mM KCl and 5 mM $MgCl_2$ were diluted into buffer A to yield 1.5 mM $MgCl_2$ and the indicated KCl concentrations.

from Mallinckrodt. $RbCl_2$ was purchased from Alfa. In each case, the highest grade available was obtained.

Preparation of Wheat Germ Ribosomes. The preparation of high-salt-washed wheat germ ribosomes was described previously (Spremulli et al., 1977). Ribosomes were fast frozen in a dry ice–isopropyl alcohol bath and stored at −70 °C in buffer A containing 5 mM $MgCl_2$ and 50 mM KCl.

Light-Scattering Technique and Data Analysis. All techniques and analyses have been described previously (Sperrazza et al., 1980). Briefly, turbidity was determined at 310 nm by using a Beckman Model 25 spectrophotometer fitted with a jacketed cell holder attached to a thermostatically controlled circulating water bath. All incubations were performed at 27 °C until equilibrium had been reached. Approximately 15 A_{260} units of ribosomes were used per mL of buffer. It had been shown previously (Sperrazza et al., 1980) that the ribosomes do not aggregate at this concentration.

For a constant ribosome concentration

$$\% \ 80 \ S = \frac{\Upsilon_y - \Upsilon_0}{\Upsilon_{100} - \Upsilon_0} \times 100 \qquad (1)$$

where Υ_{100} is the turbidity at 100% association, Υ_0 is the turbidity at 0% association, and Υ_y is the turbidity of the sample under investigation. Rayleigh's ratios were constant between experiments, thereby allowing the direct comparison of different curves. However, for reasons described previously (Sperrazza et al., 1980), the quantitative analysis of a particular curve was not appropriate. Each point reported is the mean of six determinations ±1 standard deviation. Identical curves have been obtained with different ribosome preparations, with fresh ribosomes, and with ribosomes stored 6 months at −70 °C as well as with ribosomes incubated in buffers containing $MgCl_2$ or $Mg(OAc)_2$.

Sucrose Gradient Centrifugation. Sucrose gradient centrifugation was performed as described previously (Russell & Spremulli, 1978). Where used, the method for glutaraldehyde fixation was that described by Trachsel et al. (1977). Conditions for specific experiments are given in the figure legends. As shown in Figures 6, 8, and 10, approximately 15% of the 260-nm-absorbing material appears in the 60S peak when the ribosomes are fully associated. This material does not associate with excess 40S subunits, even at 15 mM Mg^{2+}, and presumably consists of inactive ribosomes or subunits, which do not interfere with our calculations.

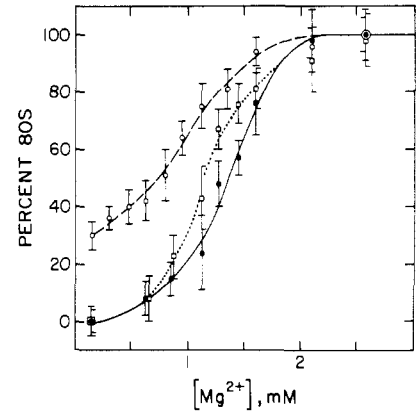

FIGURE 2: Effect of NH_4^+, Rb^+, and K^+ on the Mg^{2+}-dependent association of ribosomal subunits. Ribosomes in buffer A containing 50 mM KCl and 5 mM $MgCl_2$ were diluted into buffer A to yield the indicated $MgCl_2$ concentrations and either 100 mM KCl (●), 98 mM RbCl and 2 mM KCl (□), or 98 mM NH_4Cl and 2 mM KCl (○). The value for 0% association in the NH_4Cl curve was determined by using 100 mM KCl and 0.15 mM $MgCl_2$.

Results

Alkali Metals and NH_4^+ Ions. We have examined the relative abilities of various alkali metal ions and NH_4^+ ions to dissociate wheat germ ribosomes. Figure 1 shows the K^+-dependent dissociation of wheat germ ribosomes at 1.5 mM Mg^{2+} and 27 °C. The percentage of 80S ribosomes falls gradually from close to 100% at 75 mM K^+ to less than 5% as the K^+ concentration is increased to 170 mM. The ribosomes are 50% dissociated at 130 mM K^+ at this Mg^{2+} concentration. The turbidity falls below the value obtained for 0% association above 170 mM K^+ under these conditions, possibly indicating that the structure of the ribosomes is being altered. The turbidity rises above the value obtained for 100% association at 50 mM K^+ (it is difficult to distinguish this effect from normal scatter) and is significant by 25 mM K^+ at 1.5 mM Mg^{2+} and 27 °C, possibly indicating that the ribosomes are aggregating.

Because of problems mentioned above in the K^+-dependent curve, we have compared the effects of various monovalent cations on the equilibria between the ribosome and its subunits by adjusting monovalent cation concentrations to 100 mM and examining the association of the subunits by Mg^{2+}. Figure 2 shows the association curves (M^+ curves) obtained with buffers containing 100 mM K^+, Rb^+, or NH_4^+. (Values for all monovalent ion concentrations include 2 mM K^+ contributed by the buffer in which the ribosomes were stored.) NH_4^+ dissociates ribosomal subunits less effectively than Rb^+, which is less effective than K^+, as indicated by the lower Mg^{2+} concentrations required to associate the subunits. Similar data obtained with buffers containing 100 mM Na^+ and plotted with the K^+ curve are shown in Figure 3. These two sets of data points do not differ significantly (that is, the two sets of standard deviation bars overlap). However, the data indicate that Na^+ may dissociate ribosomal subunits slightly more effectively than K^+ at higher Mg^{2+} concentrations. The Mg^{2+} concentrations at which the ribosomes are 50% associated ($[Mg^{2+}]_{1/2}$) for the K^+, Na^+, Rb^+, and NH_4^+ curves are 1.4, 1.4, 1.2, and 0.7 mM, respectively (Table I).

The data discussed above were obtained by dissociating ribosomes stored in 5 mM Mg^{2+}. To demonstrate that we are studying true, i.e., fully reversible, thermodynamic equilibria, we obtained curves identical with those shown in Figures 2 and 3 by reassociating ribosomes dissociated by incubation at 0.165 mM Mg^{2+} and the monovalent cation under investigation

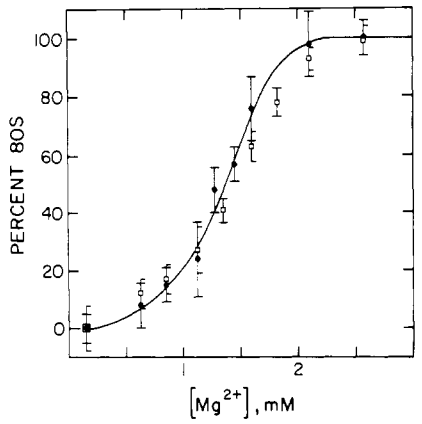

FIGURE 3: Effect of K^+ and Na^+ on the Mg^{2+}-dependent association. Ribosomes in buffer A containing 50 mM KCl and 5 mM $MgCl_2$ were diluted into buffer A to yield the indicated $MgCl_2$ concentrations and either 100 mM KCl (●) or 98 mM NaCl and 2 mM KCl (□). The curve drawn is identical with the curve drawn for 100 mM KCl in Figure 2.

Table I: Effects of Monovalent Cations on Mg^{2+}-Dependent Association of Ribosomal Subunits[a]

monovalent ion	$[Mg^{2+}]_{1/2}$ (mM)
K^+	1.4
Na^+	1.4
Rb^+	1.2
NH_4^+	0.7

[a] All experiments were performed at 27 °C with buffer A containing 100 mM monovalent cations.

(data not shown). There is no evidence of hysteresis (a curve shifted to the right due to irreversible dissociation of the ribosomes) for any of the ions discussed above. Unfortunately, Mg^{2+}-dependent subunit association curves obtained in the presence of 100 mM Li^+ show hysteresis, suggesting that the ribosomal subunits were altered by incubation at 100 mM Li^+ and 0.15–0.35 mM Mg^{2+}. Preliminary work indicated that results obtained in the presence of Li^+ would resemble those obtained in the presence of NH_4^+. Liautard et al. (1973) examined the effects of various monovalent cations on the equilibrium between HeLa cell ribosomes and their subunits fixed by glutaraldehyde using ultracentrifugation in sucrose gradients. They found that Li^+ ions dissociate the ribosomes most effectively, followed by Na^+, K^+, and finally NH_4^+. However, several investigators (Trachsel et al., 1977; Noll & Noll, 1976) question whether glutaraldehyde truly freezes the equilibrium.

Finally, we performed linear regression analyses on the linear portions of Hill plots taken from Figures 2 and 3 (not shown). If the association of eucaryotic ribosomal subunits involves the binding of a critical number of magnesium ions (n), the equilibrium can be written as

$$40S\ \text{subunit} + 60S\ \text{subunit} + n Mg^{2+} \rightleftarrows [80S\ \text{ribosome}\cdot n Mg^{2+}] \quad (2)$$

$$K_{obsd} = \frac{[80S\ \text{ribosome}]}{[40S\ \text{subunit}][60S\ \text{subunit}]} \quad (3)$$

We plotted log K_{obsd} (calculated from % 80S ribosome and the molar concentrations of the wheat germ ribosomes) vs. log $[Mg^{2+}]$. Slopes of nonlinear Hill plots yield minimal estimates for n rather than its actual value. The only value of n that differs significantly from that calculated from the K^+ curve (>95% confidence) is that calculated from the NH_4^+ curve. The slope of the K^+ Hill plot is approximately 9, while that

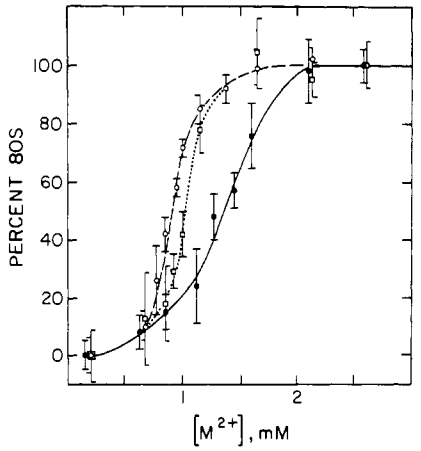

FIGURE 4: Effects of Mg^{2+}, Ca^{2+}, and Sr^{2+} on the equilibria between ribosomes and their subunits. Ribosomes in buffer A containing 50 mM KCl and 5 mM $MgCl_2$ were diluted into buffer A to yield 100 mM KCl and the indicated concentrations of $MgCl_2$ (●), $CaCl_2$ (○), or $SrCl_2$ (□). Divalent ion concentrations include 0.15 mM $MgCl_2$.

Table II: Effects of Divalent Cations and Polyamines on the Equilibrium between Ribosomes and Their Subunits[a]

M^{n+} [b]	$[Mg^{2+}]$ (mM)	$[M^{n+}]_{1/2}$ [c] (mM)
Mg^{2+}		1.4
Sr^{2+}	0.15	1.1
Ca^{2+}	0.15	0.9
Ca^{2+}	1.00	1.1
spd^{3+}	0.20	0.25
spm^{4+}	0.20	0.09

[a] All experiments were performed at 27 °C with buffer A containing 100 mM KCl. [b] M^{n+} refers to the ion used to associate the ribosomes. [c] Values for $[Ca^{2+}]_{1/2}$ and $[Sr^{2+}]_{1/2}$ include background $[Mg^{2+}]$, while those for $[spm^{4+}]_{1/2}$ and $[spd^{3+}]_{1/2}$ do not.

of the NH_4^+ Hill plot equals 4. However, given the scatter inherent in the data, a smaller, but very real, difference between n for the K^+ Hill plot and n obtained for the other M^+ Hill plots may not appear significant by this analysis. For example, the slope of the Hill plot calculated from the Rb^+ data is approximately 7. The confidence level for the difference between this slope and that for the K^+ Hill plot is slightly less than 90% and is, therefore, not highly significant.

Alkaline Earth Metal Ions. We have compared the abilities of Mg^{2+}, Ca^{2+}, and Sr^{2+} to associate wheat germ ribosomal subunits. We have confined our studies to the alkaline earth metal ions since only these ions have been shown to interact exclusively with the phosphate backbone of nucleic acids (Sissoëf et al., 1976). ($BaCl_2$ precipitates out of our buffer, so we were unable to study its effect.)

Figure 4 shows the association of wheat germ ribosomal subunits by Mg^{2+}, Ca^{2+}, and Sr^{2+} in the presence of 100 mM K^+, and Table II lists the corresponding $[M^{2+}]_{1/2}$ values. (All the values of $[M^{2+}]_{1/2}$ include the 0.15 mM Mg^{2+} contributed by the ribosome storage buffer.) Ca^{2+} reassociates the ribosomes more effectively than Sr^{2+}, which is more effective than Mg^{2+}. The $[M^{2+}]_{1/2}$ values are 0.9, 1.1, and 1.4 mM, respectively. The standard deviation bars on the Ca^{2+}- and Mg^{2+}-dependent curves are clearly widely separated. The Sr^{2+} curve is in an intermediate position, the standard deviation bars overlapping those of the Mg^{2+} curve at lower concentrations of M^{2+} and those of the Ca^{2+} curve at higher concentrations of M^{2+}. We have performed linear regression analyses on Hill plots of data points from these experiments. The slopes of the Hill plots differ slightly, but not significantly (data not shown).

Wishnia & Boussert (1977) analyzed the effects of a number of divalent cations on the equilibria between *E. coli* ri-

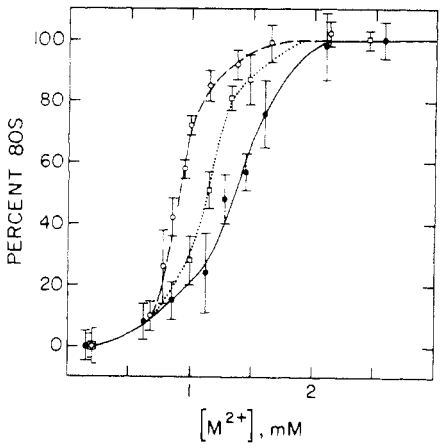

FIGURE 5: Effects of Mg^{2+} and Ca^{2+} on the association equilibria. Ribosomes in buffer A containing 50 mM KCl and 5 mM $MgCl_2$ were diluted in buffer A to yield 100 mM KCl and the indicated concentrations of $MgCl_2$ (●), $CaCl_2$ and 1.0 mM $MgCl_2$ (□), or $CaCl_2$ and 0.15 mM $MgCl_2$, (○).

bosomes and their subunits. They found no significant differences among the effects of Mg^{2+}, Sr^{2+}, Ba^{2+}, and Ca^{2+}. However, because *E. coli* ribosomes are sensitive to low concentrations of Mg^{2+} in the absence of cosolvents such as glycerol, all of their studies were performed with solutions containing 1.18 mM Mg^{2+}. These conditions resulted in a high proportion of Mg^{2+} compared to the other divalent ions in all of their buffers. If the data of Wishnia & Boussert (1977) (Figure 1) are examined closely, it can be seen that there is a trend wherein Ca^{2+} associates *E. coli* ribosomes more effectively than Sr^{2+}, which is more effective than Mg^{2+}, in agreement with our results. However, these differences are less than one standard deviation in their curves.

To determine whether the high Mg^{2+} concentrations in the buffers used by Wishnia & Bousssert could account for the failure to observe differences in the abilities of the alkaline earth metals to promote subunit association, we obtained a second Ca^{2+}-dependent association curve produced with buffers containing 1 mM Mg^{2+}. At 1 mM Mg^{2+} and 100 mM K^+, approximately 25% of the wheat germ ribosomes are associated, as are *E. coli* ribosomes at 1.18 mM Mg^{2+} and 50 mM NH_4^+ [conditions used by Wishnia & Boussert (1977)]. As expected (Figure 5), this Ca^{2+}-dependent association curve is shifted significantly toward the Mg^{2+}-dependent curve. We therefore suspect that under conditions similar to ours, *E. coli* ribosomal subunit association would show a similar specificity for the divalent cations. At this point, one could argue that the 10% glycerol in our buffers, by changing the hydration sphere of these ions, is changing their relative binding affinities toward the ribosomes. However, Hui Bon Hoa et al. (1980) have shown that cosolvents do not affect the binding of Mg^{2+} or Ca^{2+} to either the nucleotide triphosphates or RNA.

Polyamines. Zitomer & Flaks (1972) showed that both the trivalent polyamine spermidine and the divalent polyamine putrescine, which are found in vivo in procaryotes, promote the Mg^{2+}-dependent association of *E. coli* ribsomes. Most recently, Sperrazza et al. (1980) have shown that spermidine and the tetravalent polyamine spermine promote the Mg^{2+}-dependent association of wheat germ ribosomal subunits. In this study, we have explored further the abilities of the polyamines to associate wheat germ ribosomes in low [Mg^{2+}].

Putrescine-dependent ribosomal subunit association at 0.20 mM Mg^{2+} and 100 mM K^+ as studied by light scattering yielded a totally atypical association curve (not shown). The curve plateaus at a Rayleigh's ratio corresponding to approximately 50% association at 10–12 mM putrescine and begins to fall again at about 16 mM putrescine. For further investigation of this phenomenon, an incubation mixture containing ribosomes placed in 0.2 mM Mg^{2+} and 12 mM putrescine was analyzed following centrifugation in sucrose gradients. As indicated in Figure 6b, there appears to be a small peak in the 80S region, but the vast majority of the A_{260}-absorbing material appears in the 40S and 60S regions of the gradient. Furthermore, these peaks are much broader than in the normal ribosome pattern (Figure 6a). When ribosomes incubated under these conditions were fixed with glutaraldehyde before centrifugation, A_{260}-absorbing material was found in the 40S, 60S, and 80S regions of the gradient. These results indicate that the unfixed ribosomes examined under the same conditions (Figure 6b) consisted of loose 80S couples in equilibrium with free 40S and 60S subunits. Because hydrostatic pressure resulting from centrifugation has been shown to dissociate ribosomes (van Holde & Hill, 1974), we raised the concentration of Mg^{2+} in another gradient to 0.5 mM to compensate for this hydrostatic effect. Under these conditions (Figure 6c), only a broad peak in the 60S region and a shoulder are present. Ribosomes incubated in 0.2 mM Mg^{2+} and 26 mM putrescine were also analyzed following centrifugation in sucrose gradients (not shown). Species which sedimented at greater than 80S were observed, indicating that the ribosomes aggregate at this high ionic strength. Therefore, we conclude that at low Mg^{2+} concentrations and 100 mM

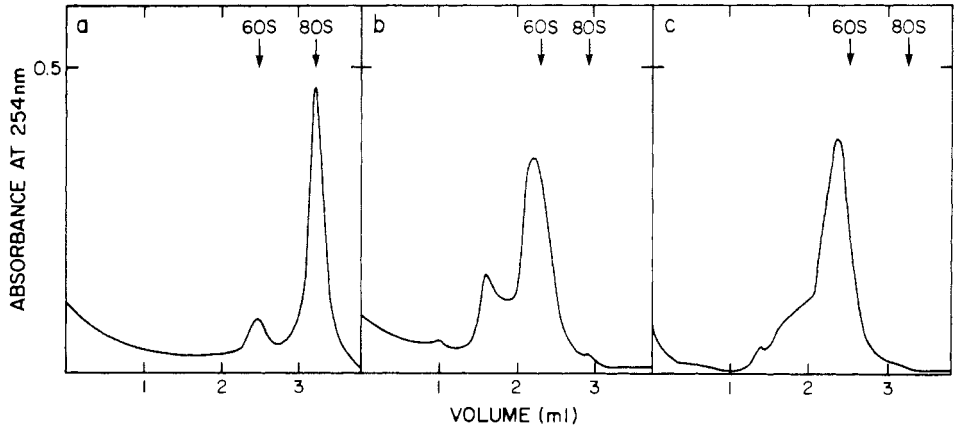

FIGURE 6: Effects of putrescine on ribosomal subunit association. Ribosomes in buffer A containing 50 mM KCl and 5 mM $MgCl_2$ were diluted into buffer A to yield 100 mM KCl and Mg^{2+} and putrescine concentration identical with those in the corresponding gradients. They were then subjected to centrifugation on 5%–30% linear sucrose gradients containing 20 mM Hepes–KOH, pH 7.6, 100 mM KCl, 6 mM β-mercaptoethanol, and either (a) 5 mM $MgCl_2$, (b) 0.2 mM $MgCl_2$ and 12 mM putrescine, or (c) 0.5 mM $MgCl_2$ and 12 mM putrescine.

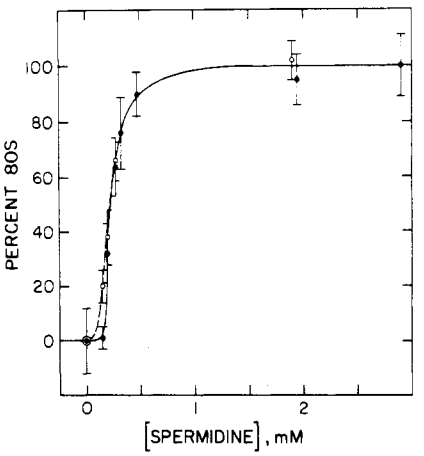

FIGURE 7: Effects of spermidine on the association of ribosomal subunits. Ribosomes in buffer A containing 50 mM KCl and 5 mM $MgCl_2$ were diluted into buffer A to yield 100 mM KCl, the indicated concentrations of spermidine, and either 0.2 mM $MgCl_2$ (●) or 0.4 mM $MgCl_2$ (○).

K^+, where wheat germ ribosomes are completely dissociated, putrescine alone is unable to associate them normally.

Spermidine and spermine, on the other hand, are able to associate wheat germ ribosomes completely at low concentrations of Mg^{2+}. The spermine- and spermidine-dependent association data yield normal Rayleigh's ratios (Figures 7 and 9). The spermidine-dependent curve (Figure 7) is extremely steep and exhibits a $[spermidine]_{1/2}$ value of 0.25 mM at 0.2 mM Mg^{2+} (Table II). The ribosomes are 90% associated over a range of 0.35 mM spermidine. At 0.4 mM Mg^{2+}, the spermidine-dependent association curve is shifted very slightly to the left, indicating that Mg^{2+} and spermidine act together. Figure 8b shows the pattern obtained when an incubation mixture containing ribosomes placed in 0.2 mM Mg^{2+} and 2 mM spermidine was analyzed following sucrose density gradient centrifugation. Under these conditions, A_{260}-absorbing material is observed in the 40S, 60S, and 80S regions of the gradient, but all of the peaks are very broad, indicating that the ribosomal subunits dissociate as they move into the gradient. In contrast, Figure 8a shows the pattern obtained from gradients which contained 5 mM Mg^{2+} and no spermidine. The peaks in these gradients are much sharper. The pattern of ribosomes incubated in 0.2 mM Mg^{2+} and 2 mM spermidine and fixed before centrifugation (not shown) has a sharp peak in the 80S region, indicating that although 80S couples are formed by spermidine, they are very sensitive to hydrostatic pressure induced dissociation. In an effort to counteract this hydrostatic pressure induced dissociation, the pattern of ribosomes obtained by sucrose gradient centrifugation at 0.5 mM Mg^{2+} and 2 mM spermidine (Figure 8c) was analyzed and appears typical of fully associated ribosomes.

The spermine-dependent subunit association curve (Figure 9) is extremely steep. The ribosomes are 80% associated over a range of 0.035 mM spermine, approximately $^1/_{10}$ that of the spermidine range, and $^1/_{50}$ that of the Mg^{2+} range. The $[spermine]_{1/2}$ (Table II) is 0.09 mM. At 0.4 mM Mg^{2+} (Figure 9), the association curve is shifted significantly to the left, indicating that Mg^{2+} and spermine act together. The sucrose density gradient pattern obtained with ribosomes incubated in buffer containing 0.25 mM spermine and 0.2 mM Mg^{2+} (Figure 10b) is very similar to that just described for 0.2 mM Mg^{2+} and 2 mM spermidine. The peaks are broad, and few 80S ribosomes are evident. When ribosomes incubated under these conditions are fixed prior to centrifugation (not shown), the subunits appear associated in the gradient, indicating that 80S couples are formed by spermine but are very sensitive to hydrostatic pressure induced dissociation. As to the case of spermidine, at 0.5 mM Mg^{2+} and 0.25 mM spermine (Figure 10c), the ribosomes appear completely associated in the gradient.

Discussion

We have shown that Mg^{2+}, Ca^{2+}, and Sr^{2+} exhibit significantly different effects upon the equilibria between wheat germ ribosomes and their subunits. The monovalent ions also show different effects on these equilibria. Either spermine or spermidine (but not putrescine) can completely associate wheat germ ribosomal subunits in buffers containing 0.20 mM Mg^{2+} and 100 mM K^+.

As described in the introduction, investigators have proposed two conflicting models to explain the role of Mg^{2+} in the association of ribosomal subunits, the electrostatic and site-specific models. Although there is considerable evidence to support the electrostatic model and we do believe that electrostatic interactions play a major role in ribosomal subunit association, our data support the idea that the binding of Mg^{2+} to specific sites may also play a role. We, therefore, support the combined model (Grunberg-Manago, 1979) in which Mg^{2+} would reduce the electrostatic repulsion between the subunits but would also bind to sites specific for Mg^{2+} and thereby promote association of the subunits.

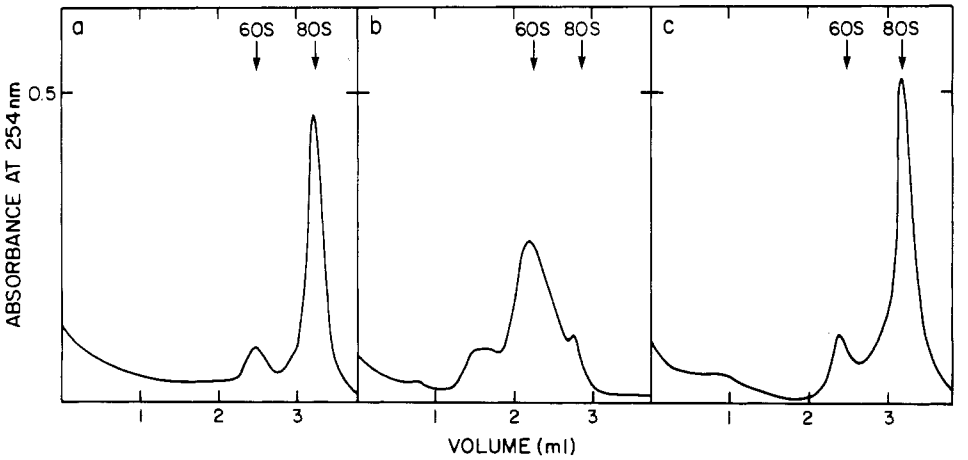

FIGURE 8: Effects of spermidine on ribosomal subunit association. Ribosomes in buffer A containing 50 mM KCl and 5 mM $MgCl_2$ were diluted into buffer A to yield 100 mM KCl and Mg^{2+} and spermidine concentrations identical with those in the corresponding gradients. They were then subjected to centrifugation on 5%–30% linear sucrose gradients containing 20 mM Hepes–KOH, pH 7.6, 100 mM KCl, 6 mM β-mercaptoethanol, and either (a) 5 mM $MgCl_2$, (b) 0.2 mM $MgCl_2$ and 2 mM spermidine, or (c) 0.5 mM $MgCl_2$ and 2 mM spermidine.

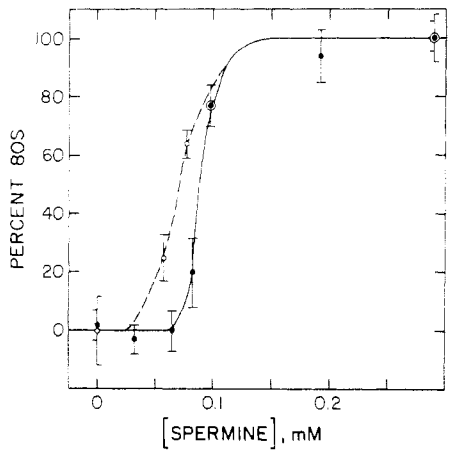

FIGURE 9: Effect of spermine on the equilibrium between the ribosome and its subunits. Ribosomes in buffer A containing 50 mM KCl and 5 mM $MgCl_2$ were diluted into buffer A to yield 100 mM KCl, the indicated concentrations of spermine, and either 0.2 mM $MgCl_2$ (●) or 0.4 mM $MgCl_2$ (○).

At this point, a general summary of the specific sites model and the electrostatic model seems appropriate. According to the elecrostatic theory, (Wishnia & Boussert, 1977), Mg^{2+} binds only the rRNA, and this binding is territorial, i.e., the ions with hydration spheres intact are mobile rather than bound to one site on the polyelectrolyte. These ions associate ribosomal subunits by reducing the electrostatic repulsion between them. Monovalent ions, in the presence of ions having higher valences, oppose this effect by noncovalent and nonspecific electrostatic screening of the ribosomal subunits from condensation (Record et al., 1978). This model involves two major assumptions. First, the extent of binding by a given ion is determined by and increases with its valence. Second, any differences in the effects of ions having the same valence should be very small.

In contrast with the electrostatic model, the specific sites model argues that Mg^{2+} promotes ribosomal subunit association by binding to specific sites on either the ribosomal proteins or RNA. For example, the divalent ions could act as bridges between negative charges at specific sites within or between ribosomal subunits to promote association. Monovalent ions, by competing for binding at these sites, could prevent association because they are unable to act as bridges. Alternatively, electrostatic screening by monovalent ions could prevent the subunit from assuming a conformation that would allow specific site binding by divalent ions. The specific sites model predicts that different ions having the same valence would exhibit very different binding constants. Furthermore, the relative binding affinities of ions having the same valence would not necessarily follow any particular order; it would depend entirely upon the nature of the ligand and the geometry of the site.

Our data show that the alkaline earth cations do exhibit significantly different effects upon the equilibria between wheat germ ribosomes and their subunits. Ca^{2+} associates the ribosomal subunits more effectively than Sr^{2+}, which is more effective than Mg^{2+}. These results, unlike those of Wishnia & Boussert (1977), imply that the divalent ions bind specific sites on the ribosomal subunits. As discussed under Results, it is likely that the 1.18 mM background Mg^{2+} included in the buffers used by Wishnia & Boussert (1977) masked similar results.

Two recent studies reinforce our conclusions. Hui Bon Hoa et al. (1980) have shown that cosolvents can partially replace Mg^{2+} to promote *E. coli* ribosomal subunit association. These studies indicate that Mg^{2+} probably binds specific sites on ribosomal subunits (Grunberg-Manago, 1979). Mg^{2+} had been shown to bind DNA territorially (Reimarsson et al., 1979), and these data had been the basis for predictions concerning the electrostatic theory. However, Rose et al. (1980) have now shown that a significant percentage of Mg^{2+} actually binds specific sites on DNA. These data also lend support to the combined model for the interactions of Mg^{2+} with ribosomal subunits.

Our studies with the alkali metal cations and NH_4^+ indicate that there is some specificity in their interactions with the wheat germ ribosomal subunits. Na^+ and K^+ dissociate the subunits more effectively than Rb^+, which is more effective than NH_4^+. In contrast with the observations reported for the divalent cations, the effects of the alkali metals are inversely related to their atomic radii. The strength of the interactions between these ions and the subunits follows that observed with R17 RNA (Gordon, 1965). These observations are consistent with the Hoffmeister series (Von Hippel & Wong, 1964) predicted by Daune (1974) for ion–solvent interactions and are therefore consistent with the electrostatic model. Here again, more sensitive binding experiments are required, as for the alkaline earth metal cations, to investigate whether these ions are binding specific sites. NH_4^+ is far less effective than the alkali metal cations at dissociating ribosomal subunits. This ion can hydrogen bond with the solvents or the carboxyl

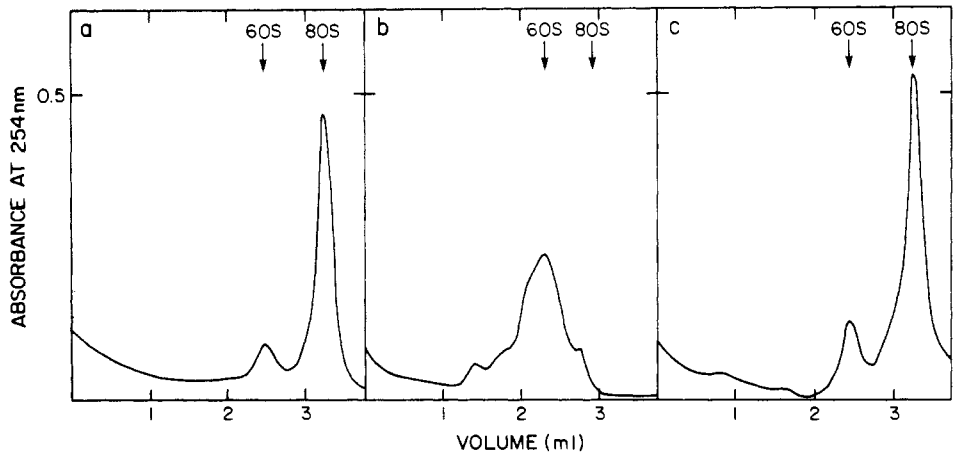

FIGURE 10: Effects of spermine on ribosomal subunit association. Ribosomes in buffer A containing 50 mM KCl and 5 mM $MgCl_2$ were diluted into buffer A to yield 100 mM KCl and Mg^{2+} and spermine concentrations identical with those in the corresponding gradients. They were then subjected to centrifugation on 5%–30% linear sucrose gradients containing 20 mM Hepes–KOH, pH 7.6, 100 mM KCl, 5 mM β-mercaptoethanol, and either (a) 5 mM $MgCl_2$, (b) 0.2 mM $MgCl_2$ and 0.25 mM spermine, or (c) 0.5 mM $MgCl_2$ and 0.25 mM spermine.

groups on the proteins, and so its interactions with the ribosomes could be expected to deviate from those of the alkali metal cations.

The mechanism by which polyamines promote ribosomal subunit association also remains unclear. Zitomer & Flaks (1972) using *E. coli* ribosomes demonstrated that putrescine and spermidine act additively to promote Mg^{2+}-dependent ribosomal subunit association. We find that of the two, only the trivalent polyamine spermidine can promote complete association of wheat germ ribosomal subunits at low magnesium ion concentrations. The tetravalent polyamine spermine is even more efficacious. These ions may interact with specific sites, probably different from those which recognize Mg^{2+}, or they may increase the affinity of the specific sites for Mg^{2+} by affecting the conformation of the ribosome via counterion condensation.

The reactivities of the polyamines are consistent with the electrostatic model. Lower concentrations of spermine ($[spm^{4+}]_{1/2}$ = 0.09 mM) than spermidine ($[spd^{3+}]_{1/2}$ = 0.25 mM) promote ribosomal subunit association. The spermine-dependent association curve is approximately 10-fold steeper than the spermidine-dependent association curve, approximately 50-fold steeper than the Mg^{2+}-dependent curve, and approximately 2500-fold steeper than the K^+-dependent dissociation curve. The fact that putrescine, which is divalent and geometrically very different from the alkaline earth metal cations, fails to associate these ribosomes is not inconsistent with either model.

Our results are consistent with previous studies of the effects of the polyamines on *E. coli* ribosomes. Goss et al. (1980) have shown that spermidine alone can associate *E. coli* ribosomal subunits. Weiss & Morris (1973), however, showed that while either spermidine or putrescine could replace Mg^{2+} in 30S ribosomal subunits, the subunits lost their structural and functional integrity when incubated in low Mg^{2+} and high putrescine concentrations.

Ribosomes associated by either spermine or spermidine are more extensively dissociated by hydrostatic pressure in sucrose gradients than those associated by Mg^{2+} (Figures 8 and 10). The effect of hydrostatic pressure is explained by Le Chatelier's principle (van Holde & Hill, 1974). Because the molar volume of the monosome is greater than the sum of the molar volumes of the two subunits, pressure applied to monosomes leads to a net dissociation. The increased sensitivity to pressure of ribosomes associated by the polyamines would therefore imply that there is a larger molar volume change on going from the dissociated to the polyamine-associated state in these ribosomes than there is in ribosomes associated by Mg^{2+} alone.

Much work needs to be done before exact mechanisms for the interaction of ribosomal subunits can be proposed. The results obtained here imply that Mg^{2+} may facilitate subunit association by binding to specific sites on the subunits in addition to reducing the electrostatic repulsion between them. We are currently involved in determining the number of various ions which interacts with the wheat germ ribosome or its subunits and are looking for possible conformational changes in the subunits caused by Mg^{2+}.

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