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Quantitation of cation binding to wheat germ ribosomes: influences on subunit association equilibria and ribosome activity

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Abstract

The binding of Mg^{2+}, spermine, and spermidine to wheat germ ribosomes was quantitated following equilibrium dialysis. The Mg^{2+} binding data demonstrate that Mg^{2+} and K^{+} compete for binding to the ribosomes. Mg^{2+} binding saturates at approximately 0.56 positive charges per phosphate (+/P). The Mg^{2+}, spermine and spermidine binding data indicate that either polyamine replaces Mg^{2+} upon binding to the ribosomes. Mg^{2+} and polyamine binding combined saturates at approximately 0.29 +/P under the conditions reported. When a critical number of Mg^{2+} ions are replaced by either polyamine, the activity of the ribosomes falls dramatically. Ribosomal subunit association increases with the degree of phosphate charge neutralization due to the binding of Mg^{2+}. Total charge neutralization during subunit association by Mg^{2+} and polyamine binding combined, is much less than that achieved by Mg^{2+} alone.

INTRODUCTION

The reversible association and dissociation of ribosomal subunits plays a central role in the process of protein synthesis in both eukaryotes and prokaryotes. The importance of cations in controlling the position of the equilibrium between the prokaryotic ribosome and its subunits has been well established (1). In recent studies we have used light scattering to study the effects of various monovalent, divalent, and polyvalent cations on the interaction of eukaryotic ribosomal subunits (2,3). These studies have shown that monovalent cations such as K^{+} promote wheat germ ribosomal subunit dissociation while divalent cations such as Mg^{2+} and the polyvalent cations spermine and spermidine promote subunit association. Physiologically, these ions play important roles in the equilibrium between the ribosome and its subunits and in protein biosynthesis. The effects of Mg^{2+} and the polyamines

are particularly dramatic. However, their precise roles in ribosomal subunit association and protein synthesis remain to be clarified.

Before the exact roles of cations in the association of ribosomal subunits and in protein synthesis can be determined, many questions about the precise nature of their binding to ribosomes and the effects of their binding on ribosome activity must be answered. Although a number of preliminary studies have been performed with E. coli ribosomes, (4-8) essentially no systematic information is available for eukaryotic ribosomes from any source. Furthermore, disagreements between the results of a number of previous studies on E. coli ribosomes suggest that some of the techniques used in those studies require further analysis.

In this report we describe a systematic study, using equilibrium dialysis, of the binding of Mg^{2+}, spermidine and spermine to eukaryotic ribosomes, and their effects on the polyphenylalanine synthesizing ability and association equilibria of these ribosomes.

MATERIALS AND METHODS

Materials. Wheat germ, kindly supplied by J.M. deRosier of International Multifoods Corporation, was stored at $4^{o}C$ in the presence of a dessicant. [^{3}H]-spermine and [^{3}H]-spermidine were purchased from NEN. Each preparation was greater than 97% pure (determined by NEN). MgO (Johnson Matthey Chemicals, Ltd., lab. no. S.8495, catalog no. JMC 130) was kindly supplied by Dr. Miles Crenshaw of the Dental Research Center at UNC. Ammonium molybdate and Scintiverse were purchased from Fisher Scientific Co. and aminonaphthol sulfonic acid was purchased from Aldrich Chemical Company. Buffer A contains 20 mM Hepes-KOH, pH 7.6, 10% glycerol, 1 mM dithiothreitol and salts as described. Dialysis tubing was prepared by boiling twice for 1 hour each time in the following solutions: (a) 50% ethanol (b) 10 mM $NaHCO_3$ (c) 1 mM EDTA and (d) H_2O.

Preparation of Wheat Germ Ribosomes. The wheat germ ribosome purification procedure was based on the method described by Spremulli et al. (9). Approximately 15% of the

A_{260} absorbing material in ribosome preparations obtained by this method sediments at 60 S on sucrose gradients and does not associate with excess 40 S subunits. In order to minimize this contamination which would interfere with binding studies, we eliminated the high-salt-wash step from the ribosome preparation procedure and were able to obtain ribosome preparations in which less than 3% of the A_{260} absorbing material sedimented at 60 S. The purified ribosome preparation was clarified by centrifugation at 20,000 g in a Sorvall SS34 rotor at $0^{o}C$ for 1 hour. Ribosomes prepared from wheat germ by this procedure which used 120 mM KCl are virtually free of all of the initiation factors even without a subsequent high-salt-wash step (9).

Polyphenylalanine Synthesis. The assays for poly(U)-directed polyphenylalanine synthesis were performed as described previously (10). The solution in which the ribosomes were dialyzed does not significantly affect the ionic conditions used for the assay since the ribosomes are diluted substantially on addition.

Assay for the Determination of Moles Phosphorus/A_{260} Ribosomes. The ribosomes (5.4 A_{260} in 0.2 ml) and standard phosphorus solution controls were hydrolyzed in 2 mls of 6 N HCl *in vacuo* at $110^{o}C$ for 48 hrs. The HCl was then evaporated and the hydrolysate resuspended in H_2O. The phosphorus content of each sample was determined by the procedure of Fiske and Subbarow (11). In an extensive series of control experiments performed with *E. coli* ribosomes, we found that the Fiske and Subbarow assay yielded consistently low estimates of the phosphorus contents of the samples unless the ribosomes were first hydrolyzed. This trend may have resulted in overestimates of Mg^{2+} binding in studies by Choi and Carr (5) and Walters and Van Os (12,13). We also obtained low estimates of the nucleotide contents of samples when we tried the alkaline hydrolysis procedure used by Elson *et al.* (8).

Our results indicate that there are $1.44 \pm .04 \times 10^{-7}$ moles phosphate per A_{260} of wheat germ ribosomes. Using 12 A_{260} per mg of ribosomes and an estimated molecular weight of 3.5×10^{6} (2) we calculate that there are 6060 $\pm$ 180 phosphate

groups per wheat germ ribosome. This number is consistent with that calculated from the molecular weight and the reported ratio of protein to RNA in plant ribosomes (49%) (14) which predicts that there are approximately 6000 phosphate groups per wheat germ ribosome.

As a control, the number of phosphate groups per E. coli ribosome was determined in parallel, since this number (4565 RNA phosphate groups) is known exactly from nucleic acid sequence data (15). Our assay yields $1.21 \pm 0.04 \times 10^{-7}$ moles phosphate per A_{260}. Using an extinction coefficient of 15 A_{260} per mg and a molecular weight of 2.6×10^6 for E. coli ribosomes (6), we calculate 4720 ± 170 phosphate groups per E. coli ribosome.

Equilibrium Dialysis and Determination of Cation Binding. Wheat germ ribosomes were diluted to a concentration of 15 to 66 A_{260}/ml in Buffer A containing the cation concentrations under investigation. Samples were dialyzed at 27°C twice against 100 volumes each time of the appropriate buffer initially for 1 hr and then until equilibrium had been reached. Control experiments of Mg^{2+} and spermidine binding at low ion concentrations (not shown) demonstrated that there was no detectable Donnan Effect up to at least 66 A_{260} units of ribosomes/ml. Both Mg^{2+} binding and the activity of the ribosomes in polyphenylalanine synthesis were assayed at 2 hour intervals during the dialysis period to establish (a) whether equilibrium had been reached and (b) whether the ribosomes had been inactivated by dialysis under the ionic conditions being tested. Although this approach had not been used in previous studies (4-8,12,13) we found that it was essential since the time required to reach equilibrium and the stability of the ribosomes varied with different ionic conditions. For example, at 150 mM K^+ and 0.5 mM Mg^{2+}, equilibrium is reached by 5 hours of dialysis and the ribosomes are still fully active. However, they lose 40% of their activity after 7 hours of dialysis under these same conditions. In contrast, at 50 mM K^+ and 7 mM Mg^{2+}, equilibrium is not reached until the ribosomes have been incubated for 9 hours but they retain 100% of their activity at this time. In all of the cases where Mg^{2+} binding alone was

investigated, the ribosomes were still fully active after dialysis.

Mg^{2+} concentrations were determined using a Perkin-Elmer Atomic Absorbance Spectrophotometer, model 305A. The stock $MgCl_2$ concentration was adjusted to 1 M by calibration against the standard MgO solutions. Standard Mg^{2+} solutions were in Buffer A containing the same cation concentrations as the experimental solutions. The amount of Mg^{2+} bound per ribosomal phosphate group was determined by subtracting the Mg^{2+} concentration outside of the dialysis bag from that inside the bag. The molar phosphorus content of the sample was calculated from the absorbance at 260 nm and the ratio of moles phosphorus per A_{260} of wheat germ ribosomes determined as described above.

Early studies of Mg^{2+} binding to ribosomes employed adaptations on the eriochrome T black assay (4,5) to determine Mg^{2+} concentrations. We attempted to adopt this assay using variations described by Sobel and Hanok (16) and Cantor and Hearst (17) which are useful for Mg^{2+} determinations in the absence of polynucleotides. However, we found problems in an extensive series of control experiments that measured Mg^{2+} bound to both hydrolyzed and unhydrolyzed ribosomes under a variety of conditions. In general, false, high values for bound Mg^{2+} were obtained under some conditions when unhydrolyzed ribosomes were used, and other conditions caused artifacts when hydrolyzed ribosomes were used.

Solutions for the spermine and spermidine binding experiments contained 33 μCi [^{3}H]-spermine or [^{3}H]-spermidine per 100 mls of buffer. 50 μl of solution from inside or outside the dialysis sack were added to 10 ml Scintiverse, and the tip of the pipetter was rinsed 3 times with distilled, deionized H_2O and the rinse added to the Scintiverse each time. The amount of radioactivity present in each sample was measured in a scintillation counter. The concentration of polyamine outside of the dialysis sack was subtracted from that inside the sack, permitting a calculation of the amount of polyamine bound per ribosomal phosphate group. Mg^{2+} and polyamine binding is reported as the mean of 4 determinations $\pm$1 standard deviation.

Light Scattering Technique. Light scattering was performed as described previously (2) except that ribosomes were first dialyzed to equilibrium in the solution under investigation. The changes in the concentrations of ribosomes due to dialysis were normalized.

RESULTS

Magnesium Binding by Wheat Germ Ribosomes. In order to assess the degree of charge neutralization due to Mg^{2+} binding we have examined the amount of Mg^{2+} bound to ribosomes under various ionic conditions. Because a number of studies have led to the conclusion that Mg^{2+} and K^+ interact primarily if not exclusively with the phosphate groups of the RNA in ribosomes, (18,19) we have expressed cation binding in terms of the number of ions bound per ribosomal phosphate group (M^{n+}/P). Mg^{2+} binding was determined by atomic absorption spectroscopy on ribosomes dialyzed to equilibrium against buffers containing various concentrations of Mg^{2+} and K^+. As indicated in Figure 1, at both 50 mM and 150 mM K^+, the ribosomes bind a maximum of 0.28 Mg^{2+} per phosphate when sufficient Mg^{2+} is present, resulting in a charge neutralization of 0.56 positive charges per ribosomal phosphate (+/P). Ribosomes bind less Mg^{2+} at 150 mM K^+ than at 50 mM K^+ when less than 5 mM Mg^{2+} is present. At 50 mM K^+, a Mg^{2+} concentration of 3 mM or greater is required for saturation, while at 150 mM K^+, a Mg^{2+} concentration of 5 mM is required. These results indicate that Mg^{2+} and K^+ directly compete for binding to the ribosomes. A similar observation has been made for E. coli ribosomes by Goldberg (4).

Spermidine and Spermine Binding to Wheat Germ Ribosomes. Both spermidine and spermine have been shown to promote the association of wheat germ ribosomal subunits (2,3). Because the polyamines like Mg^{2+} bind to the phosphate groups of the rRNA (5-7), studies were performed to determine the degree of charge neutralization that could be achieved in the presence of various concentrations of these polyamines and to determine whether the polyamines compete with Mg^{2+} for binding to ribosomes. These experiments were performed by equilibrium

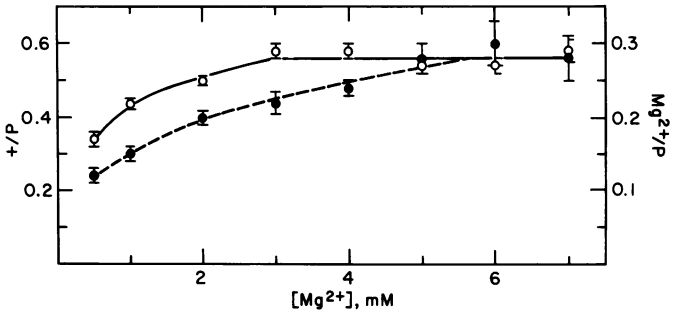

Figure 1: Magnesium binding to wheat germ ribosomes. Mg^{2+} binding was determined on wheat germ ribosomes dialyzed to equilibrium at 27° in Buffer A containing either 150 mM K^+(-●-) or 50 mM K^+ (-o-) and the indicated Mg^{2+} concentrations.

dialysis with buffers containing [^{3}H]-spermidine or [^{3}H]-spermine of known specific activities. Mg^{2+} binding at various polyamine concentrations was determined in parallel by atomic absorption.

Figure 2 demonstrates the effect of various spd^{3+} concentrations on the amount of this polyamine and Mg^{2+} bound to wheat germ ribosomes. The data indicate that this trivalent polyamine binds to ribosomes and, in so doing, displaces Mg^{2+} from them. Above 1.5 mM spd^{3+}, the slope of the spd^{3+} binding curve begins to level off and there is a corresponding decrease in the release of Mg^{2+} from the ribosome. This result probably indicates that the remaining Mg^{2+} ions have a greater affinity for the ribosome and are more difficult to displace.

As in the case of spd^{3+} binding, spm^{4+} binding to wheat germ ribosomes results in the release of Mg^{2+} (Figure 3). Again the initial Mg^{2+} are released more readily than the subsequent ones suggesting the presence of certain sites from which Mg^{2+} is more difficult to displace.

Figure 4 shows the total charge neutralization caused by the sum of Mg^{2+} and spd^{3+} or Mg^{2+} and spm^{4+} binding as a function of polyamine concentration. The total charge neutralization by spd^{3+} and Mg^{2+} remains constant within experimental error at 0.29 +/P over the concentration range from 0.2 to 4 mM spd^{3+}. The constant charge neutralization

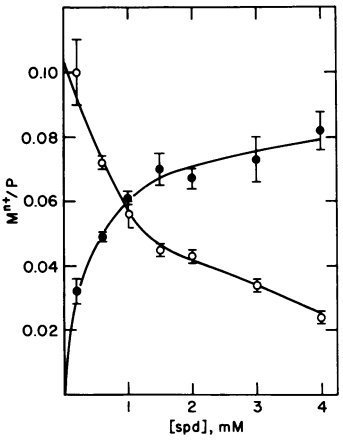

Figure 2: Spermidine and magnesium binding to wheat germ ribosomes . Spd^{3+} (- ● -) and Mg^{2+} (- o -) binding were determined on wheat germ ribosomes dialyzed to equilibrium at 27°C in Buffer A containing 150 mM K^+, and 0.5 mM Mg^{2+}, and the indicated spd^{3+} concentrations

suggests the spd^{3+} exchanges virtually exclusively with Mg^{2+} rather than K^+ for binding to the ribosomes. This result is in contrast to the increase in charge neutralization caused by increasing Mg^{2+} binding alone (Figure 1) and is consistent with results reported by Weiss and Morris (6) and Kimes and Morris (7) for spd^{3+} binding to E. coli ribosomal subunits. Total charge neutralization by both spm^{4+} and Mg^{2+} binding also remains constant at 0.29 +/P. There appears to be a slight increase in the overall charge neutralization in this case, but the only data points between which this change is significant are between 0.02 and 0.5 mM spm^{4+}. These data indicate that,

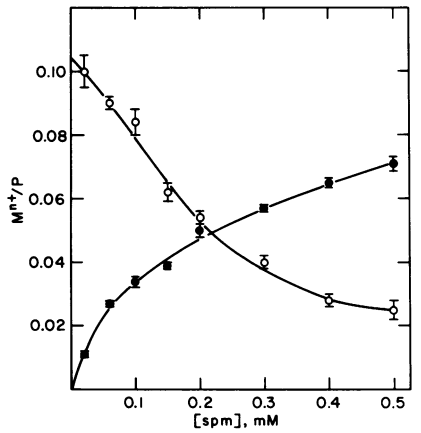

Figure 3: Spermine and magnesium binding to wheat germ ribosomes. Spm^{4+} (- ● -) and Mg^{2+} (- o -) binding were determined on wheat germ ribosomes dialyzed to equilibrium at 27°C in Buffer A containing 150 mM K+, 0.5 mM Mg^{2+}, and the indicated concentrations of spm^{4+}.

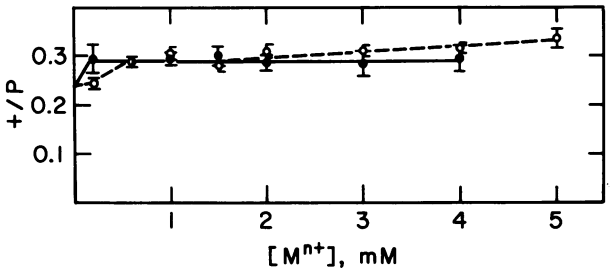

Figure 4: Charge neutralization by magnesium and polyamine binding to wheat germ ribosomes. Data from Figures 2 and 3 were treated as follows: at every spd^{3+} concentration investigated, the number of spd^{3+}/P times 3 and the number of Mg^{2+}/P times 2 were added (-●-) and at every spm^{4+} concentration investigated the number of spm^{4+}/P times 4 and the number of Mg^{2+}/P times 2 were added (-o-). The ordinate represents the spd^{3+} concentration or 10 times the spm^{4+} concentration.

for the most part, spm^{4+} also exchanges with Mg^{2+} when binding to the ribosomes with the net charge neutralization remaining essentially constant.

The 10-fold lower concentration of spm^{4+} than spd^{3+} required to achieve the same degree of replacement of Mg^{2+} implies that spm^{4+} binds with a higher affinity than spd^{3+} to the ribosomes, in agreement with studies of their abilities to associate ribosomal subunits (3).

Effect of Magnesium Replacement by Polyamines on the Ability of Wheat Germ Ribosomes to Synthesize Polyphenylalanine. To examine whether the replacement of Mg^{2+} by either spd^{3+} or spm^{4+} interferes with the structural integrity of the ribosomes, we measured polyphenylalanine synthesizing activity of ribosomes from which various amounts of Mg^{2+} had been displaced by either of these polyamines. Activity was assayed as soon as polyamine binding reached equilibrium since there was a subsequent time-dependent loss in activity. As indicated in Figure 5, wheat germ ribosomes maintain essentially 100% of their activity in poly(U)-directed polyphenylalanine synthesis as long as about 0.1 Mg^{2+}/P group are present. When the number of Mg^{2+} bound to the ribosome drops below this point, there is a corresponding decrease in

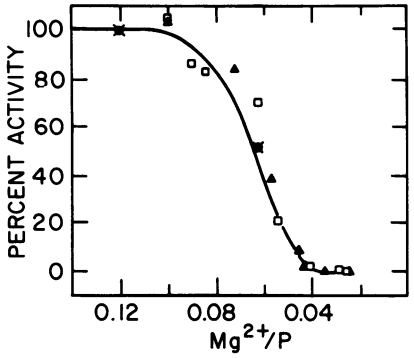

Figure 5: Polyphenylalanine synthesizing activity of wheat germ ribosomes as a function of Mg^{2+}/P. Wheat germ ribosomes were dialyzed to equilibrium against Buffer A containing 150 mM K^+ with 0.2 or 0.5 mM Mg^{2+} and no polyamine (-✖-), with 0.5 mM Mg^{2+} and 0.02 to 0.5 mM spm^{4+} (-□-), or with 0.5 mM Mg^{2+} and 0.2 to 4 mM spd^{3+} (-▲-). Polyphenylalanine synthesizing activity was then determined as described in Materials and Methods and plotted as a function of the number of Mg^{2+}/P shown in Figures 2 and 3.

the activity of the ribosomes. Approximately 50% of the activity remains when there are 0.065 Mg^{2+}/P group and activity is essentially abolished when the Mg^{2+}/P group drops below 0.04. We cannot distinguish whether this loss in activity is due to loss of Mg^{2+}, or to distortion of the subunits due to polyamine binding. However, it is interesting to note that a similar loss in activity is observed when Mg^{2+} binding to the ribosome is reduced by lowering the Mg^{2+} concentration directly in solution rather than by displacement of the Mg^{2+} by polyamines (Figure 5). These data suggest that a critical number of bound Mg^{2+} ions are required to maintain the structural and functional integrity of the ribosome. The inactivation of wheat germ ribosomes by incubation in spd^{3+} correlates well with that reported for E. coli 30 S subunits by Weiss and Morris (6) who found a reduction in activity when the number of Mg^{2+}/P fell below 0.1.

Association Equilibria of Wheat Germ Ribosomal Subunits as a Function of Bound Cations. We have examined the relationship between the degree of ribosomal subunit association and the degree of charge neutralization by the multivalent cations.

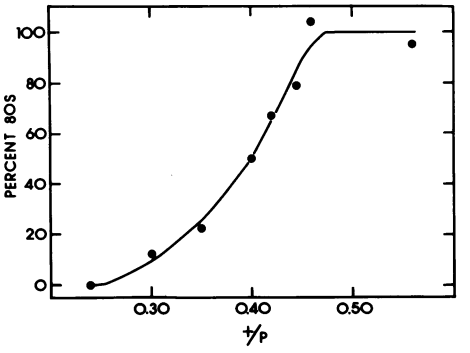

Figure 6: Association of wheat germ ribosomal subunits as a function of charge neutralization. The degree of association of ribosomal subunits (% 80 S) was determined by light scattering on ribosomes dialyzed to equilibrium at 27°C in Buffer A containing 150 mM K^+ and the indicated Mg^{2+} concentrations and plotted as a function of charge neutralization (+/P) by bound Mg^{2+}.

The degree of association of ribosomal subunits was determined by light scattering which does not perturb the equilibrium and confirmed by sucrose gradient centrifugation of fixed and unfixed ribosomes. Figure 6 shows the relationship between phosphate charge neutralization by Mg^{2+} and the degree of ribosomal subunit association in ribosomes dialyzed against solutions containing 150 mM K^+ and various concentrations of Mg^{2+}. The bulk of ribosomal subunit association occurs over a narrow range of charge neutralization with 50% association occurring at approximately 0.4 + /P. Within experimental error, the subunits are 100% associated before Mg^{2+} binding is saturated. It should be noted, however, that the built-in experimental error equals approximately $\pm$10% (2,3). This error prohibits the assignment of an exact degree of charge neutralization to a percent association.

At 50 mM K^+, subunits are 50% associated at approximately 0.8 mM Mg^{2+}, and are completely associated before Mg^{2+} binding reaches saturation. As at 150 mM K^+, the subunits are 50% associated at a charge neutralization of about 0.4 +/P. However, the experimental error built into this assay is too great to make a direct, quantitative comparison between subunit association at these two K^+ concentrations.

The ribosomal subunits are 50% associated at approximately 0.45 mM spd^{3+} or 0.05 mM spm^{4+} when dialyzed against Buffer A containing 150 mM K^+ and 0.5 mM Mg^{2+}. Under these ionic conditions the ribosomes remain fully active. Charge neutralization by Mg^{2+} and these polyamines together equals approximately 0.29 +/P as opposed to 0.40 +/P when the subunits are 50% associated by Mg^{2+} alone. Furthermore, charge neutralization is still 0.29 +/P as subunit association approaches 100%. These data imply that, in contrast to the suggestion of Walters and Van Os (12), charge neutralization by the multivalent cations is not the only important factor in subunit association.

When subunits are associated at relatively low concentrations of polyamines (for example 0.45 mM spd^{3+} or 0.05 mM spm^{4+}) the particles formed remain fully active suggesting that there is no gross alteration in their conformation. They sediment as 80 S particles when fixed with glutaraldehyde prior to sucrose density gradient centrifugation (not shown). Unfixed particles, are, however more sensitive to hydrostatic pressure-induced dissociation than are subunits associated by Mg^{2+} alone. When incubated for brief periods at high polyamine concentrations (for example 2.5 mM spd^{3+} or 0.25 mM spd^{4+}) ribosomal subunits also form 80 S particles which are sensitive to hydrostatic pressure-induced dissociation (3 and this study, not shown). On the other hand, ribosomes dialyzed to equilibrium against 2.5 mM spd^{3+} or 0.25 mM spm^{4+} were no longer active in poly(U)-directed polyphenylalanine synthesis and no longer formed normal "80 S" particles, even when fixed with glutaraldehyde before being examined by sucrose density gradient ultracentrifugation (not shown). This result implies that while brief exposure to high polyamine concentrations can promote subunit association, replacement of a critical number of Mg^{2+} ions by long exposure to these same polyamine concentrations results in considerable alteration in the structure of the ribosome.

DISCUSSION

In summary, we have shown that Mg^{2+} and K^+ compete for binding to wheat germ ribosomes when less than 0.28 Mg^{2+}/P

group are bound and that the polyamines spd^{3+} and spm^{4+} replace Mg^{2+} upon binding to ribosomes. When Mg^{2+} displacement results in less than 0.1 Mg^{2+}/P, the ribosomes begin to lose activity in poly(U)-directed polyphenylalanine synthesis and are unable to form normal 80 S particles. Finally, the degree of association of the ribosomal subunits increases with the charge neutralization of the ribosomal phosphate groups due to Mg^{2+} binding in the absence of the polyamines. There is a much lower degree of charge neutralization during subunit association in the presence of both Mg^{2+} and either spd^{3+} or spm^{4+} than in the presence of Mg^{2+} alone.

A variety of values have been reported for the number of Mg^{2+} ions bound per phosphate group on E. coli ribosomes (4,5,8,18,19). The reader is referred to Grunberg-Manago et al. (1) for a comprehensive review of this material. We have carefully investigated some of the methods used in these reports and have found that there may have been several sources of systematic error present in them (see Materials and Methods). Despite the differences, these reports all indicate that E. coli ribosomes bind more Mg^{2+} per phosphate group than do wheat germ ribosomes under similar conditions. We believe that the higher percentages of protein to RNA found in wheat germ ribosomes (49%) (14) as opposed to E. coli ribosomes (29%) (20) may result in decreased availability of RNA for cation binding in the former.

Most of the work to date on cation-dependent ribosomal subunit association has focused on the role of Mg^{2+} rather than that of the polyamines. In vivo, however, Mg^{2+}, K^{+}, and polyamines are all present, and in vitro protein synthesizing systems require all of these for optimal activity. Hence, a complete picture of the roles of cations in ribosomal subunit association must include the polyamines. The effects of polyamines on wheat germ ribosomes certainly differ from those of Mg^{2+} both quantitatively and qualitatively and deserve careful study. Our previous work (3) showed that the equilibrium between the wheat germ ribosome and its subunits is extremely sensitive to small increases in spd^{3+} or spm^{4+} concentrations as well as to Mg^{2+}. Furthermore, ribosomal

subunits associated by the polyamines are more sensitive to hydrostatic pressure than are normal 80 S particles.

Although both Mg^{2+} and the polyamines bind to the rRNA, the work presented here shows that the polyamines are unable to replace Mg^{2+} in maintaining the structural and functional integrity of the ribosome. While Mg^{2+} and K^{+} compete for binding to the ribosome, the polyamines compete primarily with Mg^{2+} as opposed to K^{+} for binding to ribosomes even under conditions in which Mg^{2+} binding is not saturating and K^{+} is also presumably bound. Furthermore, the charge neutralization that can be achieved by the binding of the polyamines and Mg^{2+} is significantly less than that which can be obtained in the presence of Mg^{2+} alone. These observations imply that the factors which govern the binding of these polyamines to ribosomes may differ from those which govern Mg^{2+} binding. The lower degree of charge neutralization observed in the presence of the polyamines may reflect a greater specificity of polyamine binding and/or that polyamine binding tightens the rRNA structure. In fact, we have found that, as Mg^{2+}, spermine, or spermidine concentrations are increased, longer times are required for cation binding to reach equilibrium. This trend probably reflects changes in ribosomal structure which result in decreased accessibility of internal regions to these cations due to a tightening of the RNA secondary structure. A tighter structure in the ribosome may prevent entry of the larger, more bulky polyamines into the interior resulting in a lower net charge neutralization by the polyamines.

The studies reported here point out the importance of monitoring the time of dialysis, ribosomal activity, and subunit association in addition to cation binding and represent the most extensive study to date on the binding of multivalent cations to eukaryotic ribosomes. These studies will provide a framework for a much more detailed investigation of cation binding to RNA and ribosomes.

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REFERENCES

1. Grunberg-Manago, M., Hui Bon Hoa, G., Douzou, P., & Wishnia, A. (1981) Advances in Inorganic Biochemistry 3, 193-232.
2. Sperrazza, J.M., Russell, D.W. & Spremulli, L.L. (1980) Biochemistry 19 1053-1058.
3. Sperrazza, J.M., Moore, M.N., & Spremulli, L.L. (1981) Biochemistry 20 5073-5079.
4. Goldberg, A. (1966) J. Mol. Biol. 15, 663-673.
5. Choi, Y.S. & Carr, C.W. (1967) J. Mol. Biol. 25,331-345.
6. Weiss, R.L. & Morris, D.R. (1973) Biochemistry 12, 435-441.
7. Kimes, B.W. & Morris, D.R. (1973) Biochemistry 12, 442-449.
8. Elson, D., Spitnik-Elson, P., Avital, S., & Abramowitz, R. (1979) Nucleic Acids Res. 7, 465-480.
9. Spremulli, L.L., Walthall, B.J., Lax, S.R., & Ravel, J.M. (1977) Arch. Biochem. Biophys. 178, 565-575.
10. Spremulli, L.L., Walthall, B., Lax, S., & Ravel, J.M. (1979) J. Biol. Chem. 254, 143-148.
11. Fiske, C.H. & Subbarow, Y. (1925) J. Biol. Chem. 66, 375-400.
12. Walters, J.A.L.I. & Van Os, G.A.J. (1970) Biochim. Biophys. Acta 199, 453-463.
13. Walters, J.A.L.I. & Van Os, G.A.J. (1971) Biopolymers 10, 11-20.
14. Cammarano, P., Romeo, A., Gentile, M., Felsani, A., & Gualerzi, C. (1972) Biochim. Biophys. Acta 281, 597-624.
15. Noller, H.F. (1979) Ribosomes Structure, Function and Genetics (Chambliss, G., Craven, G., Davies, J., Davis, K., Kahan, L. & Nomura, M. eds.) pp. 4-8, University Park Press, Baltimore.
16. Sobel, A.E. & Hanok, A. (1951) Proc. Soc. Exp. Biol. Med. 77, 737-740.
17. Cantor, K.P. & Hearst, J.E. (1970) J. Mol. Biol. 49, 213-229.
18. Edelman, I.S., Ts'o, P. & Vinograd, J. (1960) Biochim. Biophys. Acta 43, 393-403.
19. Wishnia, A., & Boussert, A.S. (1977) J. Mol. Biol. 116, 577-591.
20. Van Holde, K.E. & Hill, W.E. (1974) in Ribosomes (Nomura, M., Tissieres, A., & Lengyel, P., eds.) pp. 53-91, Cold Spring Laboratory, Cold Spring Harbor, New York.