THE BINDING OF MAGNESIUM TO MICROSOMAL NUCLEOPROTEIN AND RIBONUCLEIC ACID

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(Received February 9th, 1960)

SUMMARY

^{28}Mg was used as a tracer in studying the Mg binding by microsomal RNAP (ribonucleoprotein) and RNA isolated from rabbit reticulocytes. Methods for following the exchange of Mg by dialysis with ^{28}Mg, for precipitating RNA or RNAP with its complement of bound Mg, and for extracting the bound Mg are described in this report. The combination of these methods provides appropriate material for chemical and radioisotopic studies of the binding of Mg to polynucleotides.

Data has been presented to indicate that almost all, if not all, the Mg of RNAP is bound by the RNA moiety. Removal of the protein component from RNAP uncovers about 30% more Mg binding sites.

Preparations of RNAP isolated from buffer of low K:Mg ratio contain mostly intact 80 S particles and have a Mg:P molar ratio of 0.3, similar to the ratio in intact pea microsomal particles. Dissociation of the 80 S particles occurs when the Mg:P molar ratio of RNAP is lowered to about 0.2 by isolating RNAP in buffer of high K:Mg ratio.

INTRODUCTION

A substantial binding affinity between divalent cations and RNA, DNA, RNAP, DNAP and synthetic polynucleotides has been demonstrated in this and other laboratories[1-4]. There is evidence which indicates that the structural integrity of microsomal nucleoprotein particles extracted from pea seedlings is critically dependent on the attachment of an optimal number of magnesium ions in the particles[1]. Similar results have been obtained in ultracentrifugal studies on RNAP isolated from rat liver[5].

In view of the fundamental role of divalent cations in nucleoprotein structure, and presumably in nucleoprotein function as well, we undertook (a) to study the rate of exchange of Mg bound to RNA and RNAP with Mg free in solution, (b) to determine

Abbreviations: P, phosphorus; RNAP, ribonucleoprotein; DNAP, deoxyribonucleoprotein; RNA, ribonucleic acid; DNA, deoxyribonucleic acid; Tris, tris(hydroxymethyl)aminomethane; TCA, trichloracetic acid; EDTA, ethylenediamine tetraacetate; ATP, adenosine triphosphate; ADP, adenosine diphosphate; Poly A, polyadenylic acid; Poly U, polyuridylic acid.

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** Contribution No. 2530.

the chemical Mg content of RNA and RNAP as a function of free Mg concentration, (c) to characterize the distribution of bound Mg in the microsomal particles, and (d) to extend previous observations on the dependence of microsomal particle size and homogeneity on bound Mg.

MATERIALS AND METHODS

Preparation of microsomal particles

Microsomal particles were isolated from rabbit reticulocytes by differential centrifugation as described previously[6,7] with minor modifications. The reticulocytes were harvested in quantity from rabbits made anemic by daily injections of phenylhydrazine[8]. After one week of injections the reticulocytes constitute 80–90 % of the erythrocyte population[6]. Earlier studies demonstrated that these cells synthesize hemoglobin actively and that the isolated microsomal particles take up radioactive amino acids rapidly [6,8–10]. The reticulocytes were washed three times with Na–K–Mg buffer, frozen and stored until used. Leukocytes were removed during the washing process. To prepare microsomal particles, the reticulocytes were thawed and suspended in 2 volumes of cold solution A′ (0.14 *M* KCl, 0.0015 *M* $MgCl_2$ and 0.01 *M* Tris buffer, pH 7.2). Three cycles of differential centrifugation in solution A′ were used to isolate the microsomal particles. The final dilution with solution A′ was adjusted to give a RNAP concentration of 12–17 mg/ml.

RNA was prepared from microsomal RNAP by the method of Ts'o *et al.*[11]. The method consists of diluting the RNAP with solution A′ to a concentration of 5 mg/ml and adding 0.10 g of Na dodecyl sulfate (recrystallized twice from Dupanol-C, Du Pont Co.) and 0.5 ml *n*-butanol to 10 ml of the diluted RNAP solution. The reaction mixture is incubated at room temperature for 15 min. Two volumes of chloroform–amyl alcohol (3:1 v/v) are added to 1 volume of the reaction mixture and homogenized to form a uniform suspension. The homogenate is incubated at 38° for 15 min and then is centrifuged at 10,000 rev./min for 15 min at room temperature in a Servall centrifuge. The upper aqueous layer is aspirated with a syringe and needle from the aggregated protein layer which separates the lower organic phase from the upper aqueous phase. The RNA in the aqueous solution is then precipitated with 2 volumes of ethanol at 0°. The final concentration of RNA in solution A′ was adjusted to 4–7 mg/ml. These solutions were stored at — 60° until used.

Ultracentrifuge techniques

A Model L Spinco was used for preparative ultracentrifugation. A Model E instrument with schlieren or u.v. absorption optics was utilized for analytical ultracentrifugation, as described previously[1]. The films were traced by a Double-Beam Recording Microdensitometer, Joyce Lobel Co., Newcastle upon Tyne, England.

Chemical analyses

Assays for RNAP and RNA were performed by u.v. absorption spectra with a Beckman DU spectrophotometer or a DK-2 ratio recording spectrophotometer and by phosphorus analyses according to the method of ALLEN[12]. A factor of 11 was employed for the conversion of weight of P to the weight of RNA. Protein content was determined by the biuret method of CORNELL *et al.*[13], employing bovine serum

albumin as the standard. Dry weight was determined by dehydration at 60–65° *in vacuo* to constant weight. A modification of the method of ROBINSON AND RATHBUN[14] was used to measure Mg content. All samples to be analyzed for Mg were adjusted to a pH of 8.5 with NH_4OH and calcium was removed with 4 % $NH_4C_2O_4$. The samples were mixed with micro-magnetic stirrers during titration with EDTA in the cuvettes. Data collected to validate the Mg method are presented under RESULTS.

Radioactive tracer methods

Magnesium exchange studies were carried out with $^{28}Mg^*$ using dialysis chambers in one set of experiments and serial ethanol precipitation in a second set. Three-compartment Lucite chambers, with sheets of Visking sausage casing interposed between each compartment, were used for the dialysis studies. The capacity of the center compartment was 10 ml and of the two outer compartments 5 ml each. 10 ml of solution A′ labeled with ^{28}Mg was added to the center chamber and 5 ml of either RNAP or RNA suspended in cold solution A′ to each of the outer chambers. The solutions were mixed by means of separate micromagnets in each chamber. The entire assembly was placed on a magnetic stirrer in the cold room at 3°. Successive 0.1-ml samples were withdrawn at intervals of 15, 30 and 45 min and 1, 2, 3, 5 and 8 h after filling the chambers. The experimental samples and triplicate 0.1-ml aliquots of the standards were plated on aluminum planchets, dried under an infrared lamp and assayed for radioactivity in a gas flow counter, Model 181, with a D 47 Micromil window detector equipped with an automatic sample changer (Nuclear-Chicago Corporation, Chicago).

Calculations

The fractional exchange of bound Mg with radioactive Mg was obtained from the residual radioactivity in the center chamber and the known volumes and concentrations of RNAP or RNA. This system conformed to the kinetics of a two-compartment or two-phase system which may be represented by:

$$N^*_1 = N^*_0 \left(\frac{N_2}{N_1 + N_2}\right) e^{-\lambda t} + N^*_0 \left(\frac{N_1}{N_1 + N_2}\right) \quad (1)$$

$$\lambda = n_x \left(\frac{N_1 + N_2}{N_1 - N_2}\right) \quad (2)$$

where N^*_1 = amount of ^{28}Mg in the center compartment at any time t; N^*_0 = amount of ^{28}Mg introduced in the center compartment at time 0; N_1 = moles of Mg in the center compartment; N_2 = moles of Mg in the outer compartments; n_x = unidirectional flux of Mg in moles/min. The flux values were obtained from the slope of a plot of $Ln\ (N^*_1 - N^*_{1-8})$ *vs.* time, where N^*_{1-8} was the total ^{28}Mg content of the center compartment after 8 h of equilibration. The 8-h exchangeable Mg content of RNAP or RNA was obtained from:

$$Mg^*_e = \left[N_1 \left(\frac{N^*_0}{N^*_1} - 1\right) - N_s\right] \frac{1}{W} \quad (3)$$

* ^{28}Mg is a β, γ emitter with a 21.3 h half-life which decays to ^{28}Al; the latter is a strong β, γ emitter with a 2.3 min half-life. The isotope was supplied as $MgCl_2$:HCl solution by the Brookhaven National Laboratory.

where Mg^*_e = 8-h exchangeable Mg content in moles/mg dry wt. of particle; N_s = moles of free Mg in solution A′ in the outer compartments; W = dry wt. in mg of RNAP or RNA in the outer compartments.

RESULTS

Ultracentrifugal patterns of RNAP and RNA

The reticulocyte RNAP isolated in solution A (0.14 *M* KCl, 0.001 *M* $MgCl_2$, 0.001 *M* Tris, pH = 7.5) invariably contained 79 S particles, as well as a 58 S component which comprised about 30–40 % of the total nucleoprotein. Earlier studies[15] suggested that the 58 S component is one of the sub-units liberated from the primary 79 S particles because solution A is deficient in Mg. As shown in Fig. 1a, microsomal particles isolated in solution A′, which had a Mg content of 0.0015 *M*, also consisted of three components, 58 S, 77 S and aggregates. Reduction of the KCl concentration to 0.05 *M* (solution C, 0.05 *M* KCl, 0.0015 *M* $MgCl_2$, 0.001 *M* Tris, pH 7.5), however, markedly altered the pattern, as seen in Figs. 1c and d. The 58 S component was no longer present, and the preparation consisted solely of 79 S particles, its dimer (114 S) and other aggregates. Moreover, considerable evidence has been obtained to indicate that the 114 S component is formed from the 80 S species[15].

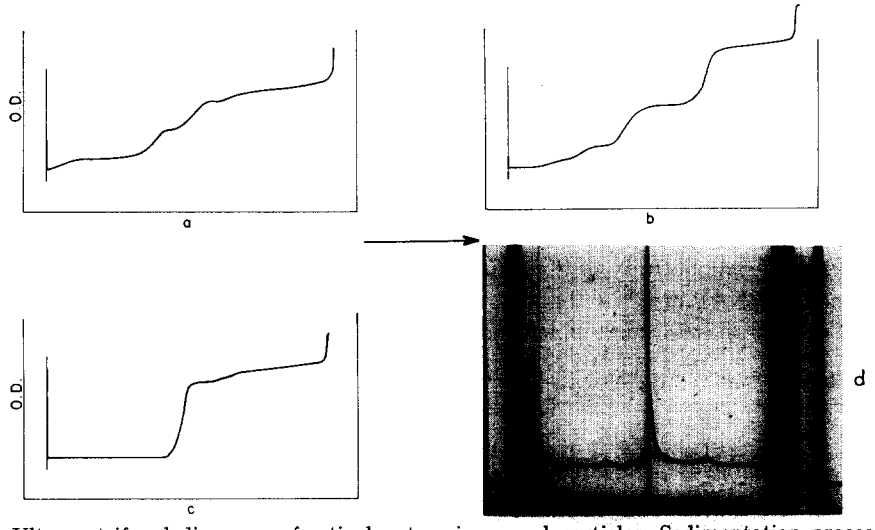

Fig. 1. Ultracentrifugal diagrams of reticulocyte microsomal particles. Sedimentation proceeds from right to left. 1a, b, and c are u.v. absorption diagrams; 1d is the schlieren diagram. a, particles prepared and centrifuged in 0.14 *M* KCl, 0.0015 *M* $MgCl_2$, and 0.001 *M* Tris (pH 7.5) (solution A) at 24.6°; b, microsomal RNA in 0.1 *M* Na-acetate, 0.001 *M* $MgCl_2$ (pH 5.5) at 9.2°. RNA was isolated from particles prepared in solution A; c, particles prepared and centrifuged in 0.05 *M* KCl, 0.0015 *M* $MgCl_2$, and 0.001 *M* Tris (pH 7.5) at 27.8°; d, same material as 1c at 25.5°.

The RNA pattern illustrated in Fig. 1b is similar to those described previously, except that the 13 S component is absent[11]. Most of the material sediments as 18 S and 30 S species, which appear to be reasonably homogeneous.

RNA : Protein ratio of the reticulocyte microsomal particles

The ratio of the weight of RNA to the weight of RNAP as estimated by determi-

nation of the P content and the dry weight of the microsomal particles was 0.495. The ratio of RNA:RNAP calculated from measurement of the P and protein contents was 0.481–0.510. In all subsequent calculations, therefore, we assumed a RNA:protein ratio of 1.00 as characteristic of reticulocyte ribosomes. This ratio agrees closely with the previously reported estimate[6].

Exchange of Mg bound to RNAP and RNA with free ^{28}Mg

The exchange of ^{28}Mg across the dialysis membrane in the three-chamber system at 3° averaged 0.12 μmole/min or $8 \cdot 10^{-3}$ μmole/min/μmole of Mg in the solution A′ in the outer chambers (Table I). The apparent flux of bound Mg from the RNAP

TABLE I

EXCHANGE OF ^{28}Mg WITH ^{24}Mg OF RETICULOCYTE RNAP AND RNA AT 3°

Sample	*8-h exchangeable Mg μmole/mg*	*Flux of bound Mg with solution A′ Mg μmole/min*	*Fractional Mg exchange × 10^{-3} μmole/min/μmole*
RNAP	0.15	0.202	6.73
RNAP	0.15	0.197	6.31
RNA	0.36	0.235	6.87
RNA	0.40	0.210	6.60
Control solution A′	—	0.113	7.69
Control solution A′	—	0.137	9.07

particles with solution A′ Mg averaged 0.20 μmole/min or 0.65 μmole/min/μmole of RNAP Mg. The flux of RNA Mg was about 10% faster. The 8-h exchangeable Mg pool of RNA averaged about 2.5 times the RNAP pool size. Taken together, these data suggest that removal of the protein unblocks additional binding sites for Mg in the polynucleotide since the RNA:protein ratio is close to 1:1 in RNAP. The similarity in the flux rate constant of the dialysis membrane and of the particles suggested that the calculated particle fluxes reflect hindrance to the passage of Mg across the membrane. Consequently, we eliminated the membrane from the system and used a system of serial ethanol precipitation as an alternative method for estimation of RNAP and RNA Mg exchange rates.

Ethanol isolation of reticulocyte RNAP and RNA

The validity of ethanol precipitation in the recovery of RNAP and RNA with an unmodified Mg content was evaluated as follows: The measured ^{28}Mg content of the precipitates obtained by treatment of RNAP or RNA solution with absolute ethanol (1 volume to 3 volumes) at 3°, followed by three ethanol washes, was compared with the calculated ^{28}Mg content of these constituents after equilibration for 8 h in the dialysis chambers. As shown by the data listed in Table II, this procedure results in the recovery of particles substantially free of adsorbed Mg and no loss of intrinsic Mg content. This technique, therefore, makes it possible to study the flux of RNAP and RNA Mg, as well as the exchangeable Mg content, by direct incubation in solution A′ labeled with ^{28}Mg. The exchange of RNAP and RNA Mg was determined by dividing these solutions into seven 1-ml aliquots, adding ^{28}Mg at 3° and precipitating the polynucleotides with 3.0 ml of absolute ethanol serially at 30 min and 1,

TABLE II

^{28}Mg BOUND TO RNA OR RNAP AS MEASURED BY THE ETHANOL PRECIPITATION METHOD AND THE DIALYSIS EXCHANGE METHOD

Sample	*Specific activity*		*Ratio of S.A. of precipitate to S.A. calculated by exchange*
	By ETOH precipitation Counts/min/mg	*Calculated by exchange Counts/min/mg*	
Reticulocyte RNAP	544	502	1.08
Reticulocyte RNA	821	792	1.04
Reticulocyte RNA	1324	1254	1.05

TABLE III

^{28}Mg CONTENT OF RNAP AND RNA BY SERIAL ETHYL ALCOHOL PRECIPITATION

Sample	*Equilibration time (h)*	*^{28}Mg content*	
		Mean	*Range*
		μmole/mg	
RNAP	0.5	0.16	
RNAP	1–10	0.23	0.22–0.25
RNA	0.5	0.76	
RNA	1–10	0.80	0.75–0.90

2, 3, 6 and 10 h. The precipitates were packed by centrifugation and washed 3 times with absolute ethanol. The ethanol was decanted and the last traces were evaporated in a gentle air stream. The precipitates were redissolved in 1.0 ml of distilled water; 0.4-ml aliquots were then plated, dried under an infrared lamp and assayed for radioactivity as detailed above. The results in Table III show that there is almost 100 % exchange after incubation for 30 min. The mean exchangeable Mg content was 0.23 and 0.80 μmole/mg for RNAP and RNA, respectively, or 1.5 to 2.0 times greater than the Mg^*_e values obtained with the chamber dialysis technique. To determine whether hindrance to Mg transport by the dialysis membrane results in incomplete equilibration, we then measured the bound Mg content of RNAP and RNA by a direct chemical method. The cold ethanol precipitation method offers a technique for isolating these substances from the buffer Mg without artifacts. The next step, however, required release of 100 % of the Mg from the bound state and away from interfering phosphate ions. Earlier studies suggested that this might be achieved with either 8 % TCA or 0.05 *M* Na EDTA[1].

Recovery of ^{28}Mg in TCA or EDTA supernatants

Either RNAP or RNA was equilibrated with ^{28}Mg-labeled solution A′ in the dialysis chamber for 10 h. The labeled RNAP or RNA was recovered by ethanol precipitation as outlined previously and resuspended in distilled water; aliquots were then taken for radioassay or treatment with TCA or EDTA. The TCA method consisted of adding 1.0 ml of 16 % TCA to 1.0 ml of RNAP or RNA solution, incubating at 3° for 30 min, centrifuging to remove the precipitate and plating 0.5-ml aliquots of the supernatants for radioassay. The EDTA method consisted of adding 4 ml of

0.05 *M* EDTA (pH 7.65) to 1.0 ml of RNAP or RNA solution, incubating at 3° for 10 h, centrifuging at 100,000 × *g* for 8 h and plating 1.0 ml aliquots of the supernatants for radioassay. As shown in Tables IV and V, approx. 100 % of the RNAP or RNA ^{28}Mg was recovered in the supernatants. The TCA precipitates and EDTA-treated pellets had negligible radioactivity when assayed directly. Because of its convenience we used the TCA method for subsequent chemical measurements of Mg content.

TABLE IV

RECOVERY OF ^{28}Mg IN TRICHLORACETIC ACID SUPERNATANTS

Sample	*^{28}Mg activity in RNAP or RNA*	*^{28}Mg activity in TCA supernatant*	*Fractional recovery*
	Counts/min		
Reticulocyte RNA	3951	3970	1.00
Reticulocyte RNA	6705	7350	1.09
Reticulocyte RNAP	4014	4108	1.02

TABLE V

RECOVERY OF ^{28}Mg IN ETHYLENEDIAMINE TETRAACETATE SUPERNATANTS

Sample	*^{28}Mg activity*	*^{28}Mg activity in EDTA supernatants*	*Fractional recovery*
	Counts/min		
Reticulocyte RNA	2743	2656	0.97
Reticulocyte RNA	7766	7286	0.94

Mg content of reticulocyte RNAP and RNA

To validate the ROBINSON AND RATHBUN[10] method for Mg analysis, we established that (a) the relation between the EDTA titration volume and the Mg concentration of standard solutions is linear and goes through the origin, (b) the titration volume is independent of the anion, yielding the same values for $MgSO_4$ and $MgCl_2$ at equal molarities, (c) addition of up to 25 % Ca or Ba or Sr to the Mg solutions has no detectable effect on the final titration volume, (d) addition of TCA to a final concentration of 8 % and neutralizing with NH_4OH has no effect on the titration volume, and (e) the reproducibility of measurements of both standards and unknown samples of biological material (human plasma) is ± 2.8 % (standard deviation expressed as % of the mean). Aliquots of RNAP or RNA in solution A′ and RNAP in solution C (0.05 *M* KCl, 0.0015 *M* $MgCl_2$, 0.001 *M* Tris, pH 7.5) were subjected to ultracentrifugal analysis as described above. RNAP or RNA was isolated from these solutions by cold ethanol precipitation and the washed precipitates were resuspended in distilled water. The nucleotide content of the samples was estimated from measurements of u.v. absorbancy and P analysis. Additional aliquots were treated with TCA and the supernatants were analyzed for Mg content as described above. The results of chemical analysis of distilled water solutions of reticulocyte RNA and RNAP are summarized in Table VI. RNA bound from 2.8–3.4 times as much Mg as RNAP per unit weight

of particle, and about 30 % more than the RNA in ribosomes. These data agree closely with values obtained in the experiments on ^{28}Mg exchange (see Table III). The fact that similar results were obtained in the dialysis experiments (Table II) supports the view that removal of the protein uncovers additional binding sites in the polynucleotide and justifies the supposition that at physiologic Mg concentrations, all or nearly all of the Mg is bound to the RNA moiety. To evaluate the latter possibility we measured the recovery of ^{28}Mg bound to RNAP in RNA isolated on fractionation

TABLE VI

CHEMICAL ANALYSIS OF RETICULOCYTE RNA AND RNAP

Sample	*Concentration* mg/ml*	*Phosphorus µmole/mg*	*Magnesium µmole/mg*	*Mg/P Mole ratio*	*mean*
RNA (solution A)	2.5	3.00	0.72	0.24	0.23
RNA (solution A)	3.3	3.00	0.67	0.22	
RNAP (solution A)	12.4	1.40	0.23	0.17	0.18
RNAP (solution A)	6.0	1.40	0.27	0.19	
RNAP (solution C)	5.7	1.38	0.43	0.31	

* Determined by u.v. absorption spectra.

of the RNAP and also compared the dependence of RNAP and of RNA bound Mg on free Mg concentration.

Reticulocyte microsomal particles suspended in solution A′ were equilibrated with ^{28}Mg-labeled solution A′ in the dialysis chamber for 8 h as described above. The ^{28}Mg content of the RNAP was calculated from the residual activity of the solution A′ in the center chamber, and the RNAP solution was recovered from the outer chambers. The nucleoproteins were split into an RNA and a protein fraction by the method of Ts'o *et al.*[11] as detailed above and the isolated RNA was counted. All of the RNAP-bound ^{28}Mg was recovered in the RNA fraction within the limits of the error of the methods (Table VII).

To test whether RNA could account for all the Mg bound to RNAP over a wide concentration range, the following reaction mixtures were prepared:

ml of 1.5 M $MgCl_2$	*ml of solution A′ ^{28}Mg*	*ml of RNAP or RNA*	*Total volume in ml*	*Mg concentration in µmole/ml*
0	0.5	0.5	1.00	1.5
0.005	0.5	0.5	1.005	9.0
0.010	0.5	0.5	1.010	16.4
0.025	0.5	0.5	1.025	38.8
0.050	0.5	0.5	1.050	76.0

At Mg concentrations greater than 25 µmoles/ml the solutions invariably were cloudy.

The reaction mixtures were incubated at 3° for 11 h, at which time 3 ml of cold absolute ethanol was added to each tube; RNAP and RNA were then recovered in distilled water as described above. The radioactivity of each sample was assayed; the phosphorus content and u.v. absorption were measured; the results are plotted

TABLE VII

ASSOCIATION OF ^{28}Mg WITH RNA OF RETICULOCYTE RIBOSOMES

8-h exchangeable Mg of RNAP μmole/mg	*Specific activity of RNAP*	*Measured specific activity of RNA*	*Calculated specific activity of RNA*	*Ratio of measured to calculated specific activity*
	Counts/min/mg			
0.12	185	390	370	1.05
0.15	502	903	1004	0.90

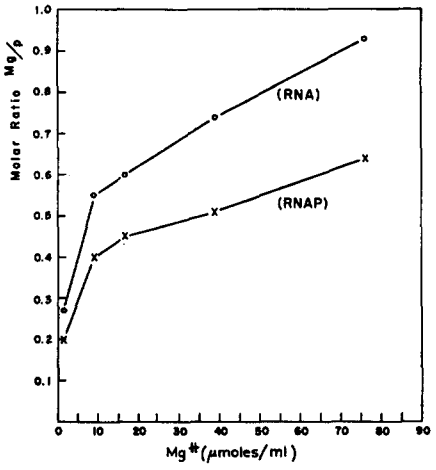

Fig. 2. The molar ratio of Mg:P bound to RNA (O—O) or to RNAP (×—×) as a function of free Mg concentration. For conditions of incubation with ^{28}Mg and details of separation of labeled particles from the incubating medium by ethanol precipitation, see text.

in Fig. 2. The similarity in the shapes of the curves supports the idea that RNA provides all the binding sites for Mg in the nucleoprotein. These data also confirm the greater binding capacity of RNA when freed of protein; at a Mg ion concentration of 0.076 *M*, RNA bound 0.93 moles of Mg/mole of P, whereas RNAP bound 0.64 mole/mole.

DISCUSSION

The suggestion in a previous report[1] that virtually all the Mg in RNAP is bound by RNA with little or no binding by protein is now supported by four lines of evidence: (a) the binding capacity of free microsomal RNA for Mg can account for all the binding capacity of RNAP, (b) the kinetics of the exchange of Mg in both RNA and RNAP is very similar, (c) the ^{28}Mg bound by RNAP can be quantitatively recovered in the RNA moiety, and (d) that Mg:P molar ratios of RNA and RNAP show a parallel dependence on the Mg concentration of the buffer. Moreover, the data (Tables II, III, VI and Fig. 2) indicate that free RNA binds about 30% more Mg than the RNA in RNAP. Thus, the protein in RNAP appears to block about 30% of the sites which are available for Mg binding in RNA. Although the binding sites for Mg in RNA have not been definitely identified, previous investigators have inferred[2,3] that

Mg is bound to the negatively charged phosphate groups. There are indications, however, that in other nucleotides, namely DNA, ATP and ADP, the nitrogen bases may participate in Mg binding by a chelation mechanism[16,17]. If the phosphate group in RNA is the only major binding site for Mg, it may be inferred that the basic groups of the protein in RNAP combine with the phosphate groups of RNA to form the nucleoprotein complex of the intact microsomal particle[18].

The Mg:P molar ratio of RNA and RNAP exceeds 0.5 when the Mg concentration of the medium is greater than 0.01 *M* (see Fig. 2). WIBERG AND NEUMAN[3] assumed that the maximum Mg:P molar ratio would be 0.5, based on the data on divalent cation binding to DNA reported by KATZ[19] and SHACK *et al.*[20]. Our data also indicate that native RNA differs from synthetic polynucleotides in this respect, since FELSENFELD AND HUANG[2] found that Mg binding to Poly A and Poly U is limited to 0.5 moles/mole of polymer phosphate. If the phosphate groups constitute the major binding sites for Mg, then simultaneous binding of anion may also occur in ribosomal particles or native RNA.

The assumption that the method employed for RNA separation yields reproducible and intact species is supported by the observations of HALL AND DOTY[21]. Our method of RNA preparation yielded homogeneous 18 S and 30 S components (Fig. 1b), while the latter authors with the use of Na lauryl SO_4 and phenol obtained 18 S and 28 S components.

The key role of Mg in holding the sub-units of the native ribosomal particle together is confirmed by our results[1,5]. It is likely that the binding of Mg to RNAP, as well as the relationship between RNAP particle size and Mg concentration, is dependent on pH and ionic strength[5,22]. Furthermore, the K:Mg ratio is critical in determining the magnitude of particle dissociation at neutral pH. In solution A' (K:Mg molar ratio of 95), RNAP has a Mg:P ratio of 0.18, and a substantial amount of the 60 S sub-unit is present in the solution (Fig. 1a). Our unpublished data show a dissociation of 80 S particles in solution A' and preferential disintegration of the smaller sub-units (30-40 S). In solution C (K:Mg molar ratio of 33), there is no dissociation of 80 S particles into sub-units, and the Mg:P ratio of RNAP is 0.3, a value similar to that reported for intact pea ribosomal particles[1]. Apparently both reticulocyte and pea ribosomes require a Mg:P ratio of 0.3 to stabilize the 80 S particles.

ACKNOWLEDGEMENTS

We are grateful to Professor L. PAULING and Professor J. BONNER for their interest, support and encouragement. E. VON GAL and A. LUBELL provided valuable technical assistance.

This investigation was supported in part by Grants No. RG-3977 and No. RG-5143 from the National Institutes of Health, United States Public Health Service. This study was carried out during the tenure by I.S.E. of a Special Fellowship from the National Heart Institute of the United States Public Health Service.

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