Conformational Changes in *Escherichia coli* Ribosomes at Low Magnesium Ion Concentrations*

David L. Weller,† Yael Shechter, David Musgrave, Malcolm Rougvie, and Jack Horowitz‡

ABSTRACT: As Mg^{2+} is removed from *Escherichia coli* ribosomes by dialysis against 0.005 M EDTA, both the 50S and 30S subunits unfold to produce slower sedimenting components. The 50S ribosome undergoes a series of alterations the first step of which is reversible and results in the formation of a 40S (38–42S) particle. This is converted into a 29S component, on removal of additional Mg^{2+}, in a transformation that cannot be reversed. Lowering the magnesium ion concentration still further brings about a gradual and reversible reduction in the sedimentation coefficient from 29 to 19–20 S.

Analogous changes are observed in the sedimentation behavior of the 30S ribosome. At low magnesium concentrations this subunit is converted into a 17–19S particle with a 27S component as intermediate in the conversion. These alterations occur with little or no loss of protein or ribonucleic acid and the ribonucleic acid remains intact as judged by analytical ultracentrifugation and viscosity studies. Interpretation of the changes as an expansion of the compactly folded ribonucleoprotein particle is supported by evidence that the magnesium-depleted ribosomes have a higher intrinsic viscosity and an increased susceptibility to digestion by pancreatic ribonuclease. The conformational changes in ribosomes at low Mg^{2+} do not appear to involve changes in the secondary structure of ribosomal ribonucleic acid.

Ribosomes play an important role in the synthesis of proteins; however, little is yet known about the details of their function in this process nor is much known concerning the steps involved in the biosynthesis of these cellular components. To a large degree this is due to a lack of precise information about the internal organization of the RNP[1] particle, of the way in which proteins and RNA are arranged in the compact structure of the completed ribosome. One method for gaining some insight into this structure is to partially disrupt it without at the same time degrading the rRNA. Several years ago we reported briefly on our observation that following removal of Mg^{2+} by chelation with EDTA, *Escherichia coli* ribosomes underwent structural changes which resulted in a decrease in their sedimentation coefficients (Horowitz and Weller, 1963; Weller and Horowitz, 1964). Extensive depletion of Mg^{2+} led to an irreversible transformation of the 50S subunit to a particle sedimenting at about 30 S (Weller and Horowitz, 1964). Degradation of RNA was avoided by working in the cold and using bentonite to inhibit the latent RNase I (Elson, 1959; Tal and Elson, 1963). The apparent intactness of the rRNA and the absence of appreciable protein loss led to the conclusion that the observed alterations in ribosome structure were the result of conformational changes leading to a less compact folding, an expansion, of the ribonucleoprotein particle (Weller and Horowitz, 1964).

In this report we deal with a continuation and extension of our previous studies with magnesium-depleted ribosomes. The changes in sedimentation behavior on removal of Mg^{2+} are examined in greater detail and additional physical-chemical measurements have been made in order to characterize the altered ribosomes and their RNA components more fully. The reversibility of the structural changes has also been investigated and some of the early stages in the transformation were found to be reversed by the restoration of Mg^{2+}.

A number of others have also made observations regarding what has been termed the "unfolding" (Spirin *et al.*, 1963) of ribosomes at low divalent cation concentrations. High concentrations of monovalent cations have the same effect and probably act by displacing Mg^{2+} or other divalent cations from the ribosome (Gavrilova *et al.*, 1966). Elson (1961) noted the conversion of the 50S ribosomal subunit of *E. coli* into a 43S component at NaCl concentrations above 0.1 M; the 30S ribosome appeared stable under these conditions. Similar observations were made by Morgan (1962) when yeast ribosomes were dialyzed against buffers containing low concentrations of magnesium ions. Spirin and coworkers (Spirin *et al.*, 1963; Gavrilova *et al.*, 1966), Rodgers (1964), Gesteland (1966), and Cammack and

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† Present address: Department of Agricultural Biochemistry, The University of Vermont, Burlington, Vt. 05401.

‡ To whom requests for reprints should be addressed.

[1] Abbreviations used that are not listed in *Biochemistry 5*, 1445 (1966), are: RNP, ribonucleoprotein; SDS, sodium dodecyl sulfate.

Wade (1965) have investigated these transformations in some detail using ribosomes from mutants deficient in RNase I or RNP particles washed with 0.5 N NH_4Cl to remove the enzyme, thus avoding ribosome degradation. The results from different laboratories generally agree although there are some differences especially in connection with the reversibility of the changes observed at low Mg^{2+} concentrations (compare Weller and Horowitz, 1964; Gesteland, 1966; Gavrilova *et al.*, 1966).

Methods and Materials

Preparation of Ribosomes and RNA. E. coli strain B was used in these studies. For the most part cells were purchased as a frozen paste from Grain Processing Corp., Muscatine, Iowa, although some of the earlier experiments were carried out with cells grown at 37° in Penassay broth, under forced aeration and harvested during exponential growth. Purified ribosomes were prepared essentially as described by Tissières *et al.* (1959). The ribosomes were washed three to four times with standard buffer (0.001 M Tris-HCl (pH 7.4)–10^{-4} M magnesium acetate), dissolved in this buffer, frozen, and stored at −20°. Purified 50S and 30S subunits were prepared either by differential centrifugation (Tissières *et al.*, 1959) or by sucrose density gradient centrifugation (Britten and Roberts, 1960) in the SW 25.1 or 25.2 rotors of the Spinco Model L-2 ultracentrifuge. Concentration of ribosomes was calculated from the absorbance at 260 mμ, assuming a value of $A_{260}^{0.1\%} = 16$.

RNA was extracted from ribosomes by the phenol–SDS method described by Kurland (1960), modified only by the use of bentonite (Fraenkel-Conrat *et al.*, 1961), at a final concentration of 0.5 mg/ml. The RNA was dissolved in 0.001 M Tris-HCl buffer (pH 7.4) containing 0.01 M magnesium acetate and 0.15 M NaCl and stored at −20°.

Removal of Magnesium Ions. In most experiments ribosomes were depleted of magnesium by dialysis, at 4°, against 0.005 M EDTA in 0.001 M Tris-HCl buffer (pH 7.4). The ribosome preparations contained bentonite, usually at a concentration of 0.5 mg/ml, to inhibit RNase. After dialysis against EDTA for the indicated time, a portion of the ribosome sample was taken for examination in the analytical ultracentrifuge. The remainder of the sample was maintained at 4° until the first photo was taken in the ultracentrifuge run (30–45 min), at which time it was dialyzed against standard buffer to remove the EDTA (18–24 hr). The sedimentation pattern at a given EDTA concentration was found to change with time, even in the cold, and the start of dialysis against Mg^{2+} was delayed so that the sample being assayed in the ultracentrifuge would be as nearly as possible identical with the one being dialyzed back into 10^{-4} M Mg^{2+}.

All dialysis tubing was boiled in 0.1 M sodium bicarbonate, washed extensively with deionized water, and stored at 4° in water.

Analytical Methods. Protein and RNA analyses were carried out on samples heated in 7% trichloroacetic acid at 95–100° for 10 min. The amount of RNA in the supernatant was measured by the orcinol procedure (Mejbaum, 1939), and the method of Lowry *et al.* (1951) was used to determine protein in the pellet. Phosphorus was determined as described by King (1932).

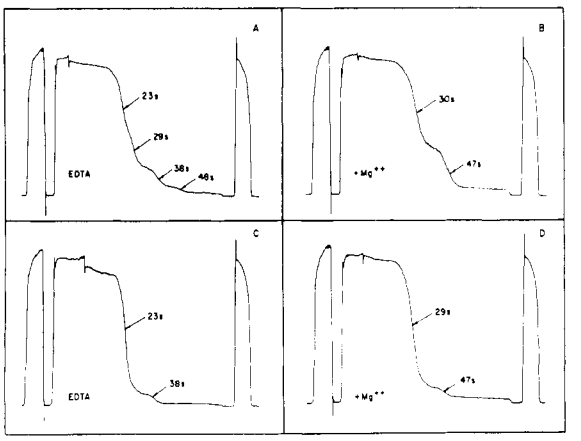

FIGURE 1: Sedimentation patterns of EDTA-treated ribosomes. Duplicate ribosome samples (60–90 μg/ml), in standard buffer containing 0.1 mg of bentonite/ml, were dialyzed against 0.005 M EDTA for 75 min (A and B) or 90 min (C and D). One of each pair was examined directly in the ultracentrifuge (A and C), while the other was first dialyzed against standard buffer (10^{-4} M Mg^{2+}) for 24 hr (B and D) (see Methods for details). Centrifugation was carried out at 3–4°, at 31,410 rpm.

Digestion of ribonucleoprotein particles by pancreatic RNase (1 μg/ml) was followed by incubating the samples at 4° for the indicated time intervals and then stopping the reaction by adding perchloric acid to a final concentration of 0.25 N. The precipitate was removed by centrifugation and the absorbancy of the supernatant at 260 mμ was determined. Results are expressed as the percentage of total A_{260}-absorbing material released in acid-soluble form.

Physical Methods. Ultracentrifuge analyses were made with a Spinco Model E ultracentrifuge equipped with schlieren and ultraviolet optics. All runs were made at 2 to 6°. The films obtained with the ultraviolet optics were scanned with a Beckman Model RB analytrol. Values for sedimentation coefficients were corrected to water at 20°.

The viscosity of nucleoprotein solutions was measured with an Ubbelohde viscometer, with a solvent flow time of 580 sec at 5°. Measurements were made in a refrigerated water bath at 5 ± 0.03°. Viscosity of RNA solutions was determined at temperatures between 27 and 72° with RNA extracted by the SDS method (Shakulov *et al.*, 1962), in an Ostwald-type viscometer with a flow time for water of 60 sec at 25°.

Ultraviolet optical rotatory dispersion measurements were made with a Jasco Model ORD/UV-5 automatic recording spectropolarimeter.

Materials. Pancreatic RNase and DNase were obtained from Worthington Biochemical Corp. as the crystalline enzymes. Alumina (bacteriological grade, A-305) was the gift of the Aluminum Co. of America. Bentonite (U.S.P. grade) was purchased from Fisher Scientific Co. All other reagents used were either primary standard or analytical grade.

TABLE I: Effect of Dialysis against EDTA on the Sedimentation Behavior of Ribosomes.[a]

Expt	Conditions of Ultracentrifugation	Time of Dialysis against EDTA (min)					
		0	45	60	75	90	120
A	EDTA	32 (37)	23 (30)		23 (37)	19 (25)	
		49 (63)	28 (40)		29 (35)	22 (65)	
			38 (15)		40 (15)	36 (10)	
			47 (15)		47 (15)		
	Mg^{2+}		30 (66)		29 (68)	27 (91)	
			48 (34)		47 (32)	45 (9)	
B	EDTA	31 (42)		29 (74)			20 (100)
		49 (52)		42 (11)			
		67 (6)		47 (15)			
	Mg^{2+}			30 (72)			28 (100)
				48 (28)			

[a] Conditions were the same as those in Figure 1. Values in parentheses indicate the percentage of each component based on the height of the trace of the boundary in scans of the photographic plate. Values outside parentheses are S values.

Results

Sedimentation Behavior of Magnesium-Depleted Ribosomes. Removal of Mg^{2+} from ribosomes by dialysis against 0.005 M EDTA resulted in the gradual conversion of the 50S and 30S subunits into more slowly sedimenting nucleoprotein particles (Figure 1 and Table I). After 20–40 min new peaks sedimenting at 38–42 and 19–23 S appeared and an increased amount of the 29S particle was also apparent (Table I). The slower sedimenting components became increasingly more prominent on longer exposure to EDTA until eventually the 50S subunit completely disappeared and only a 19–20S particle could be observed (Table I). Occasionally a peak sedimenting somewhat more slowly, at 17 S, was also seen. No components with sedimentation coefficients less than 17–20 S were observed in these experiments, although nucleoprotein particles sedimenting at 12 S or lower were found at higher ribosome concentrations, in experiments using the schlieren optics of the ultracentrifuge. These low S values were probably the result of charge effects.

Little or no material was lost during the EDTA treatment, as judged from the area under the peaks or by the ultraviolet absorbance of the samples. Although the observations were readily reproducible, the rate at which the changes occurred varied and depended upon, among other things, the bentonite concentration and the concentration of ribosomes and their past history, seemingly reflecting variations in the endogenous level of Mg^{2+}.

It was possible to classify the newly formed RNP particles into two groups largely on the basis of the reversibility of the observed changes following readdition of Mg^{2+} (see below): one with a sedimentation coefficient of about 40 S (38–42 S) and another which consisted of a number of components with sedimentation coefficients in the range between 19 and 29 S. The 40S ribonucleoprotein particle was certainly derived from the 50S ribosome (see experiments with purified subunits in Table II). Although the source of the 19–29S components is more difficult to define because of the presence of normal 30S subunits, the large amount of material present in these particles makes it clear that they were derived at least in part from the 50S ribosomal subunit. The sedimentation coefficient of this slower sedimenting group of nucleoprotein particles varied over wide limits between 19 and 29 S, depending, evi-

TABLE II: Effect of Dialysis against EDTA on the Sedimentation Behavior of 50S and 30S Ribosomal Subunits.[a]

Ribosome Prepn	Expt	Dialysis Time *vs.* EDTA (hr)	Conditions of Ultracentrifugation: EDTA, S (%)	Conditions of Ultracentrifugation: Mg^{2+}, S (%)
50 S	1	0		52
		0.5	19 (78)	33 (31)
			38 (22)	45 (69)
	2	4	23	33
30 S	1	0		32
		1	21	27
		2	17	28
		0		31
	2	0.5	27	32
		2	20	27

[a] Conditions were the same as those described in Figure 1.

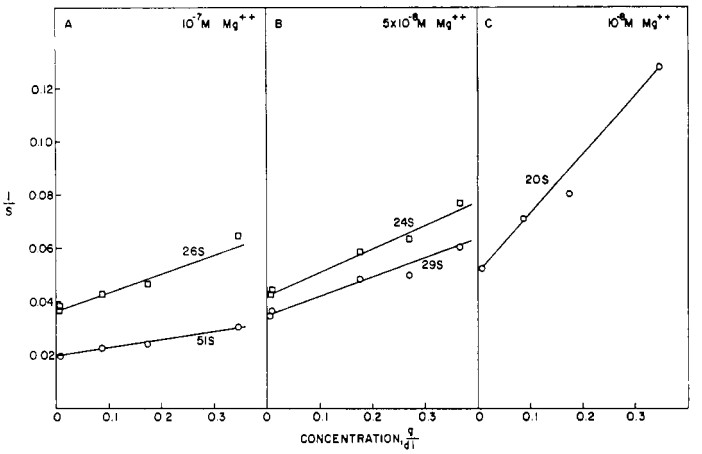

FIGURE 2: Concentration dependence of the sedimentation coefficients of ribosomes dialyzed against low magnesium ion concentrations. Ribosomes, 50S plus 30S subunits, in standard buffer (10^{-4} M Mg^{2+}) containing 0.7 mg/ml of bentonite, were dialyzed against 0.001 M Tris-HCl buffer (pH 7.4) containing: (A) 10^{-7} M magnesium acetate, (B) 5×10^{-8} M magnesium acetate, and (C) 10^{-8} M magnesium acetate, until equilibrium was established (5 days).

dently, upon the extent to which the ribosomes were depleted of Mg^{2+}. This can be seen in a series of experiments in which magnesium ions were removed from ribosomes by dialysis to equilibrium against different low levels of Mg^{2+}, 10^{-7}–10^{-8} M (Figure 2). Two peaks were observed at each magnesium ion concentration, with the slower becoming predominant as the divalent cation concentration was lowered. At 10^{-8} M Mg^{2+} the faster component was apparently converted into the slower on dilution and only the results for the slower particle are presented. Figure 2 shows that the extrapolated value of the sedimentation coefficient of the slower boundary at each magnesium ion concentration, which undoubtedly corresponds to the slower sedimenting component observed in EDTA, decreased as the level of Mg^{2+} decreased, from 26 S at 10^{-7} M Mg^{2+} to 24 S at 5×10^{-8} M Mg^{2+} and 20 S at 10^{-8} M Mg^{2+}.

Reversal by Magnesium. The changes in sedimentation behavior brought about by magnesium ion depletion could be at least partly reversed by Mg^{2+}. Figure 1 and Table I show that dialysis back into standard buffer (10^{-4} M Mg^{2+}) resulted in the reappearance of two peaks sedimenting at approximately the same rate as the original ribosome subunits, 28–30 and 47–49 S. However, the sedimentation patterns were altered in that the slower 28–30S, RNP particle now predominated. The 40S (38–42S) component vanished, accompanied by a corresponding increase in the proportion of the 50S (47–48S) subunit, an indication that the conversion of the 50S ribosome into the 38–42S component is reversible. Occasionally a particle with a sedimentation coefficient of 34–36 S was produced by treatment with EDTA, *e.g.*, the 90-min sample of experiment A in Table I. After the addition of Mg^{2+}, this component sedimented at 43–45 S (Table I), somewhat slower than the original 50S ribosome, and this may represent only a partial or incomplete reversal.

Restoration of Mg^{2+} also resulted in the disappearance of particles with sedimentation coefficients lower than 29 S, with a simultaneous increase in the 30S (29–30S) component (Table I and Figure 1). Since the ribosomal components sedimenting between 19 and 29 S contain, in part, material derived from the 50S subunit, it is clear that some of the 50S ribosomes were altered on dialysis against EDTA in a manner not entirely reversible by Mg^{2+}. This altered 50S ribosome sedimented at approximately 30 S at 10^{-4} M Mg^{2+} and will be referred to as the 30*S particle to differentiate it from the normal 30S subunit.

EDTA Treatment of Purified 50S and 30S Subunits. To clarify the origins of the various altered ribosomal components, experiments were conducted with purified 50S and 30S subunits (also see Weller and Horowitz, 1964). The results shown in the first part of Table II make it evident that the 50S subunit is converted into both a 38S and 19–23S component at low Mg^{2+}. In a few experiments three components, all derived from the 50S subunit, could be observed together (Figure 3): the unaltered 50S ribosome and two slower components sedimenting at 34 and 20 S. Restoration of Mg^{2+} led to a partial reversal of the transformations caused by EDTA. Results essentially identical with those already described with mixtures of 30S and 50S ribosomes were obtained (Table II). The less drastic change to a 38S particle was reversed by Mg^{2+}; however, the more extensive transformation to a slower 19–23S particle could not be completely reversed; instead addition of Mg^{2+} resulted in the appearance of the 30*S component.

Results with the 30S subunit were not as clear-cut, partly because the changes in sedimentation coefficients were smaller and their significance more difficult to evaluate. The data in Table II show that short times of dialysis against EDTA produced a 27S particle that could be reversibly converted into the 30S subunit (32 S). More extensive removal of Mg^{2+} resulted in the formation of a 17–20S component, which was converted into a particle sedimenting at 27 S after dialysis back into standard buffer (10^{-4} M Mg^{2+}). The sedimentation coefficient of this component was consistently lower than that of the original 30S ribosome (31–32 S), an indica-

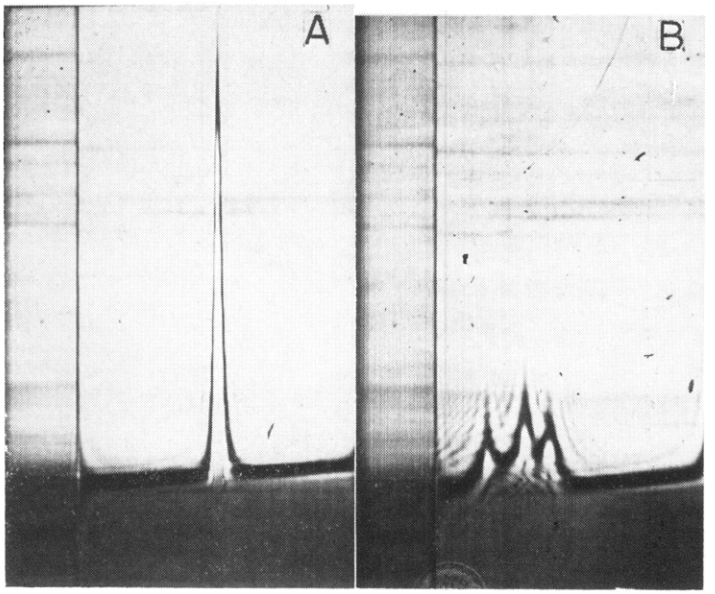

FIGURE 3: Intermediates in the conversion of 50S ribosomes into slower sedimenting components. Magnesium was removed from a preparation of purified 50S ribosomes in standard buffer (10^{-4} M Mg^{2+}) by the addition of EDTA to a final concentration of 0.001 M. The sample was examined in the ultracentrifuge within 30 min after the EDTA was added. (A) Original 50S ribosomes; $s_{20,w}$ = 46 S. (B) EDTA-treated ribosomes; $s_{20,w}$ = 45, 34, and 20 S. Centrifugation was carried out at 31,410 rpm at room temperature.

tion that, as in the case of the 50S ribosome, the more extensive changes in the 30S subunit may not be completely reversible.

Characterization of Mg^{2+}-Depleted Ribosomes. Ultracentrifugal studies of magnesium-deficient ribonucleoprotein particles cannot provide all the information necessary for an understanding of the structural changes in ribosomal particles which occur at low Mg^{2+} concentrations. A further characterization of these components was therefore undertaken.

Ribonucleic Acid and Protein Content. Ribonucleic acid isolated from magnesium-depleted ribosomes had the same sedimentation characteristics as that from normal ribosomes. The RNA obtained from mixtures of 30S plus 50S ribosomes, treated with EDTA, contained the usual 16S and 23S components in about the same ratio as normal (Figure 4) while that isolated from 30*S particles obtained from purified 50S ribosomes consisted almost entirely of 23S RNA (see Weller and Horowitz, 1964).

The RNA isolated from 30* S was essentially intact and contained few, if any, breaks in the polynucleotide chain. This was determined by examining the reduced viscosity–temperature profile of this RNA (Figure 5). Bogdanova *et al.* (1962) had shown that intact RNA exhibits a marked increase in reduced viscosity on being heated to 70°, while RNA containing "hidden" damage will show little or no such increase. Our results, Figure 5, indicate that the RNAs from both normal and EDTA-treated ribosomes exhibit increases in reduced viscosity as the temperature is increased from 27 to 72°. These results make it unlikely that the changes in sedimentation behavior observed at low Mg^{2+} are due to a dissociation of the ribosomal particle into subunits since it is known that the 23S RNA component of 50S ribosomes consists of a single polynucleotide chain (Kurland, 1960; Iwabuchi *et al.*, 1965).

The changes in ribosome sedimentation could not be accounted for by a loss of protein. Analysis of the RNA to protein ratio showed that the magnesium-depleted particles contained 65–67% RNA and 33–35% protein, as did normal ribosomes.

Viscosity Properties. The results thus far indicate that the alterations in sedimentation behavior observed at low Mg^{2+} are due to changes in the structure of the ribosomal particle, an expansion or unfolding, *i.e.*, an increase in frictional coefficient. This is supported by the evidence on the viscosity of the various nucleoprotein particles. Figure 6 shows that the intrinsic viscosity of magnesium-depleted RNP particles is considerably higher than that of the original ribosomes. The latter gave a value of 0.044 dl/g at 10^{-4} M Mg^{2+}, while the intrinsic viscosity of 30* S was 0.23 dl/g at 10^{-4} M Mg^{2+} and 0.38 dl/g in 5 mM EDTA. The values are those for 50S subunits and the Mg-deficient particles derived from them; mixtures of 50S and 30S subunits behaved in a similar fashion. Estimates of the molecular weight of the RNP particles from $s^0_{20,w}$ and $[\eta]$, using the Scheraga–Mandelkern (1953) equation, give values of 1.7×10^6 for the original 50S subunit and $1.8 \times$ and 1.6×10^6 for the 30*S particle in 10^{-4} M Mg^{2+} and 5 mM EDTA, respectively (assuming a value of 2.12×10^6 for β).

Degradation by Pancreatic Ribonuclease. Another indication that ribosomes take on a less compactly folded structure at low divalent cation concentrations is the increased sensitivity of the magnesium-depleted particles to pancreatic ribonuclease (Figure 7). Addition of the enzyme to a preparation of 30* S rendered more than one-third the nucleic acid in the particles soluble in 0.25 M perchloric acid in 30 min at 4°; at room

temperature more than 50% of the RNA was degraded within 5 min. This is in contrast to the behavior of normal ribosomes, which were relatively resistant to attack by the RNase (Figure 7).

Optical Properties. The structural changes in the RNP particles at low Mg^{2+} do not seem to involve significant changes in the secondary structure of the RNA. This was demonstrated by comparing the ϵ(P) of the 50S ribosome and the 30* S derived from it. The values obtained, 7420 and 7870 l. per mole of P × cm, respectively, are in good agreement, indicating that little disruption in the ordering of bases in the rRNA with its accompanying hyperchromicity had occurred. Similar conclusions could be drawn from ultraviolet–optical rotatory dispersion studies. The curves obtained for the Mg-deficient RNP particles and for ribosomes, both in standard buffer, were quite similar, a broad peak at 280 mμ, crossover at 265 mμ, and a trough at 250 mμ. For the 30* S, $[\alpha]_{280}$ +2800° and $[\alpha]_{250}$ −4000°; the 50S subunit gave values of $[\alpha]_{280}$ +2500° and $[\alpha]_{250}$ −4750°.

Discussion

The results presented here and those reported earlier make it evident that the compact structure of the ribosome undergoes alterations at low divalent cation concentrations, which lead to an expansion or unfolding of the nucleoprotein particle. In the case of the 50S subunit the effects of magnesium depletion may be summarized as follows

$$50\text{ S} \rightleftharpoons \underset{(38\text{–}42\text{ S})}{40\text{ S}} \longrightarrow \longrightarrow \underset{(30^*\text{ S})}{29\text{ S}} \rightleftharpoons \rightleftharpoons \rightleftharpoons 19\text{ S}$$

Detailed study of the kinetics of Mg^{2+} removal has revealed that the first stage of this transformation, the formation of the 40S (38–42S) component, is reversible by magnesium.[2] Only a single step appears to be involved in this change since no intermediates were noted in a large number of experiments. Our results, therefore, agree with those of Gesteland (1966) who has described the reversible formation of a 36S particle as the first step in the unfolding of the 50S ribosomal subunit. On the other hand Spirin and his coworkers, after first reporting the complete reversibility of the transformations observed at low Mg^{2+} (Spirin *et al.*, 1963), later presented evidence showing that the conversion of the 50S subunit into a 35S particle could not be reversed (Gavrilova *et al.*, 1966). The reasons for this discrepancy are not clear although Gavrilova *et al.* (1966) may have been observing components resembling our 34–36S particle (Table I) which also would not revert to the 50S ribosome.

More extensive removal of Mg^{2+} leads to further changes in the 50S subunit, producing next a 29S particle (30* S). Unlike the first step, this second stage in the alteration of the 50S ribosomal particle involving the conversion of a 38–42S component into one sedimenting at 29 S could not be reversed by magnesium.[2] One or more intermediates may be involved in this transformation since a nucleoprotein particle sedimenting at 34–36 S, whose sedimentation coefficient was not reversed to 50 S with magnesium ions, was observed on occasion. The value of the 29S particle was gradually, and reversibly, decreased to 19 S as additional Mg^{2+} was removed.

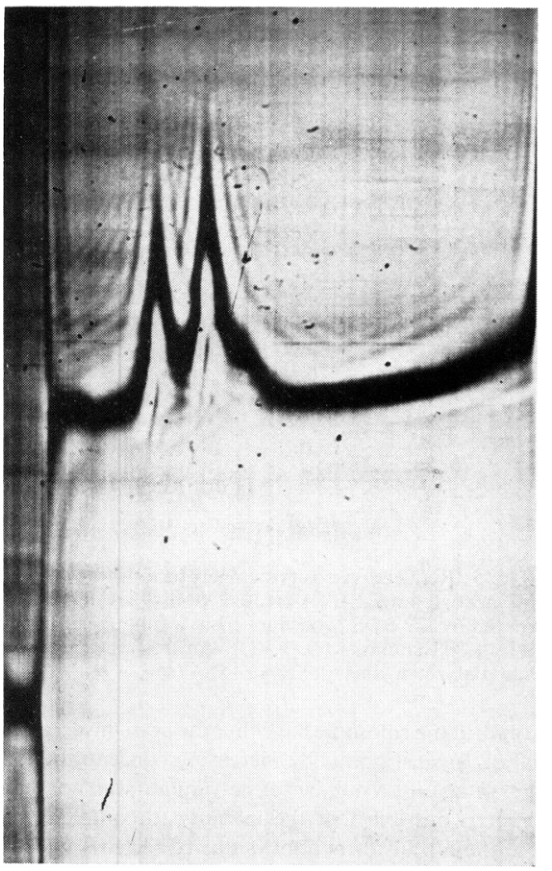

FIGURE 4: Sedimentation pattern of RNA isolated from EDTA-treated ribosomes. A mixture of 50S and 30S ribosomes was dialyzed against 0.005 M EDTA and the magnesium ion concentration restored to 10^{-4} M by dialysis against standard buffer; all the 50S subunits were converted into the 30*S component by this treatment. RNA was isolated as described under Methods. Centrifugation was carried out at 50,740 rpm at 4°; $s_{20,w}$ = 22 and 16 S.

Results with the 30S subunit, while not as clear-cut, also indicate a series of transformations, ultimately resulting in a 17–19S component, the first step of which is reversible (Table II).

Some of the reported differences in the occurrence and extent of reversal of ribosome unfolding may be due to differences in the ionic strength of the medium used. We have noted that after restoring the Mg^{2+} concentration to 10^{-4} M, the value of the slow-sedimenting ribosomal particle derived from the 50S subunit depended on the buffer concentration, increasing from 26 S to over 30 S as the concentration of Tris-HCl buffer (pH 7.4) was decreased from 10^{-2} to 10^{-3} M. Whether still lower ionic strengths will permit a more complete reversal is an open question.

Attempts to completely reverse the more extensive

[2] It should be pointed out that reversibility in this context refers only to changes in sedimentation behavior and not to biological activity.

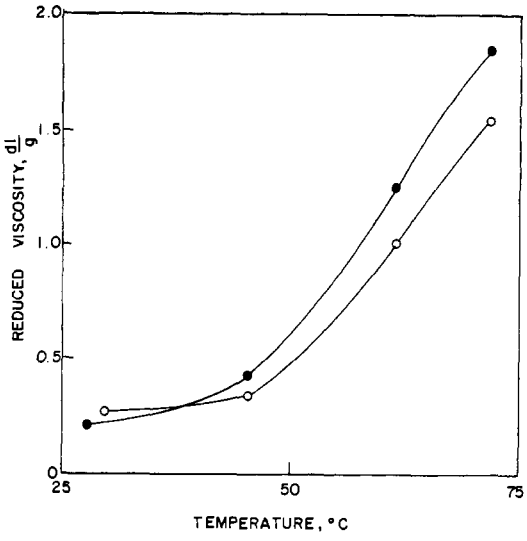

FIGURE 5: Reduced viscosity–temperature profile of RNA from normal and EDTA-treated ribosomes. RNA was prepared by the SDS procedure from a mixture of normal 50S and 30S subunits (O---O) and from the same ribosomes dialyzed against 0.005 M EDTA (●---●).

changes in the ribosome have thus far been unsuccessful. Dialysis against gradually increasing concentrations of Mg^{2+} or against a number of polyamines was not effective. It is possible that the inability to completely reverse these transformations is due to the loss of small

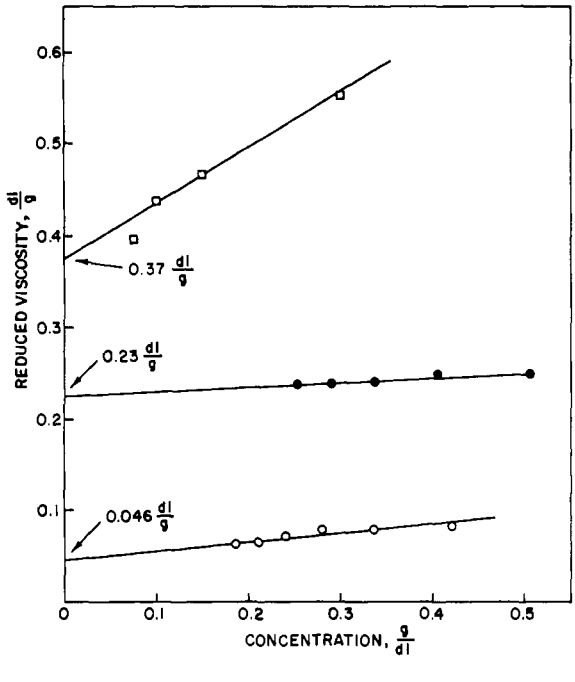

FIGURE 6: Concentration dependence of the reduced viscosity of normal and EDTA-treated ribosomes. Viscosity measurements were made at 4–5° on purified 50S ribosomal subunits in standard buffer (O---O); on these ribosomes in 0.005 M EDTA (□---□) and on the EDTA-treated ribosomes after the magnesium ion concentration was restored to 10^{-4} M by dialysis against standard buffer (●---●). The intrinsic viscosity of each preparation, determined by extrapolation of the reduced viscosity to infinite dilution, is indicated on the ordinate.

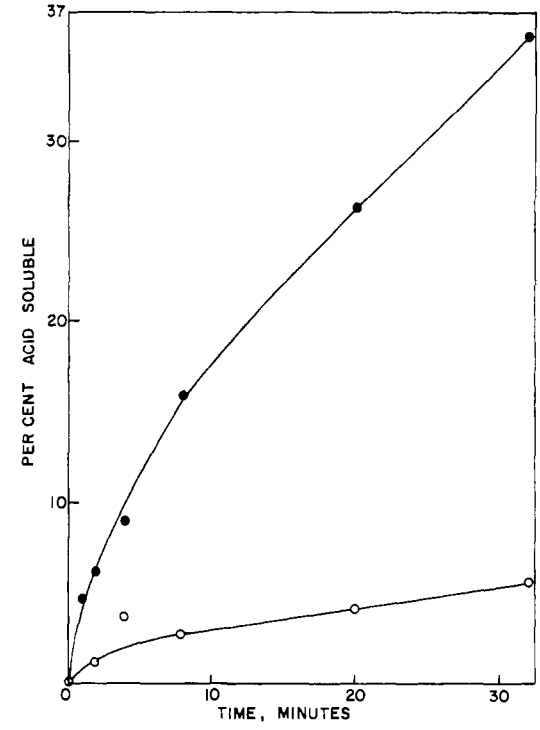

FIGURE 7: Digestion of normal and EDTA-treated ribosomes by pancreatic RNase. The release of 260-mμ-absorbing material, soluble in 0.25 N $HClO_4$, was determined at various times after the addition of 1 μg/ml of enzyme to the ribosome preparations suspended in standard buffer. Temperature 4°. (O---O) Normal 50S ribosomal subunits; (●---●) 30*S particles derived from them by dialysis against 0.005 M EDTA. The results are reported as the per cent of total A_{260}-absorbing material rendered soluble in $HClO_4$.

amounts of RNA or protein. Although our experiments and those of others (Gesteland, 1966; Gavrilova *et al.*, 1966) do not show such losses, the methods used were generally not sensitive enough to detect the release of one or two protein subunits or a small fraction of the RNA. Indeed, a number of the ultracentrifuge patterns we obtained did show a small amount of slow-sedimenting material released by EDTA treatment. In this connection it would be interesting to examine the fate of the 5S rRNA associated with the 50S subunit (Rosset *et al.*, 1964). Preliminary experiments (K. R. Yamamoto and J. Horowitz, unpublished observations) have indicated it is removed during exposure to EDTA. Comb and Sarkar (1967) have reported the loss of 5S RNA from the ribosomes of the aquatic fungus *Blastocladiella emersonii* after EDTA treatment and Morell and Marmur (1968) have obtained similar results with *E. coli* ribosomes. It is possible that the function of this RNA species, which is as yet unknown, is concerned with the maintenance of ribosome structure and that loss of this component accounts for the nonreversible transformation of the 50S subunit to the 30*S particle at low Mg^{2+}.

All of the physical-chemical characteristics of the magnesium-depleted particles as well as their increased susceptibility to digestion by pancreatic RNase indicate a loosening of the normally compact ribosome structure. These conformational changes do not seem to in-

volve changes in the secondary structure of the rRNA. Comparison of the $\epsilon(p)$ and the optical rotatory dispersion profiles of 50S subunits and the 30*S particles derived from them, both in 10^{-4} M Mg^{2+}, shows that these parameters do not change as the result of the structural changes in the ribonucleoprotein particle. Both hyperchromicity (Schlessinger, 1960) and optical rotatory dispersion (Sarkar *et al.*, 1967; McPhie and Gratzer, 1966; Bush and Scheraga, 1967) are quite sensitive to conformational change and any significant alteration in the secondary structure of the RNA would have been detected. At lower levels of Mg^{2+} (*i.e.*, in EDTA) where the loosening of ribosomal structure is more extensive, changes in the secondary structure of RNA are also unlikely for the following reasons. (a) There is no increase in the ultraviolet absorbancy of ribosome solutions on addition of EDTA, and indeed a slight decrease, which can be reversed with Mg^{2+}, is generally noted (these results are, however, complicated by changes in the absorption spectrum of EDTA below 265 mμ following chelation of divalent cations). (b) McPhie and Gratzer (1966) looked at the optical rotatory dispersion curve of yeast ribosomes in 0.01 M EDTA and found it to be no different from that of ribosomes in Mg^{2+}.

One final point which might be mentioned is that the gradual conversion of the 50S ribosome into a 30*S particle, which can be separated from each other by sucrose density gradient centrifugation, offers the possibility of fractionating the 50S subunit and examining the question of the heterogeneity of the ribosome population in *E. coli*. Examination of the nucleotide composition of the various fractions has thus far revealed no differences among them.

References

Bogdanova, Ye. S., Gavrilova, L. P., Dvorkin, G. A., Kisselev, N. A., and Spirin, A. S. (1962), *Biokhimiya 27*, 387.

Britten, R. J., and Roberts, R. B. (1960), *Science 131*, 32.

Bush, C. A., and Scheraga, H. A. (1967), *Biochemistry 6*, 3036.

Cammack, K. A., and Wade, H. E. (1965), *Biochem. J. 96*, 671.

Comb, D. G., and Sarkar, N. (1967), *J. Mol. Biol. 25*, 317.

Elson, D. (1959), *Biochim. Biophys. Acta 36*, 372.

Elson, D. (1961), *Biochim. Biophys. Acta 53*, 232.

Fraenkel-Conrat, H., Singer B., and Tsugita, A. (1961), *Virology 14*, 54.

Gavrilova, L. P., Ivanov, D. A., and Spirin, A. S. (1966), *J. Mol. Biol. 16*, 473.

Gesteland, R. F. (1966), *J. Mol. Biol. 18*, 356.

Horowitz, J., and Weller, D. L. (1963), *Federation Proc. 22*, 301.

Iwabuchi, M., Kono, M., Oumi, T., and Osawa, S. (1965), *Biochim. Biophys. Acta 108*, 211.

King, E. J. (1932), *Biochem. J. 26*, 292.

Kurland, C. G. (1960), *J. Mol. Biol. 2*, 83.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.

McPhie, P., and Gratzer, W. B. (1966), *Biochemistry 5*, 1310.

Mejbaum, W. (1939), *Z. Physiol. Chem. 258*, 117.

Morell, P., and Marmur, J. (1968), *Biochemistry 7*, 1141.

Morgan, R. S. (1962), *J. Mol. Biol. 4*, 115.

Rodgers, A. (1964), *Biochem. J. 90*, 548.

Rosset, R., Monier, R., and Julien, J. (1964), *Bull. Soc. Chim. Biol. 46*, 87.

Sarkar, P. K., Yang, J. T., and Doty, P. (1967), *Biopolymers 5*, 1.

Scheraga, H. A., and Mandelkern, L. (1953), *J. Am. Chem. Soc. 75*, 179.

Schlessinger, D. (1960), *J. Mol. Biol. 2*, 92.

Shakulov, R. S., Aitkhozhin, M. A., and Spirin, A. S. (1962), *Biokhimiya 27*, 577.

Spirin, A. S., Kisselev, N. A., Shakulov, R. S., and Bogdanov, A. A. (1963), *Biokhimiya 28*, 920.

Tal, M., and Elson, D. (1963), *Biochim. Biophys. Acta 76*, 40.

Tissières, A., Watson, J. D., Schlessinger, D., and Hollingworth, B. R. (1959), *J. Mol. Biol. 1*, 221.

Weller, D. L., and Horowitz, J. (1964), *Biochim. Biophys. Acta 87*, 361.