The Hydrodynamic and Spectroscopic Properties of 16 S RNA from *Escherichia coli* in Reconstitution Buffer*

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The hydrodynamic shape, conformation, and thermal stability of ribosomal 16 S RNA in reconstitution buffer have been studied by sedimentation velocity, intrinsic viscosity, circular dichroism, and difference ultraviolet absorption spectroscopy. These results are compared with the corresponding properties of the 30 S ribosomal subunit in order to understand the role of the RNA molecule in the assembly of the 30 S subunit. Phenol-extracted 16 S RNA in reconstitution buffer was found to have a sedimentation coefficient, $s_{20w} = 21.1$ S, a frictional coefficient ratio, $f/f_{rot} = 3.15$, and an intrinsic viscosity, $[\eta] = 14.3$ ml/g. These results are significantly different from those obtained in other buffers. The corresponding effective hydrodynamic radius is approximately 115 Å which is significantly lower than results previously reported. Similar studies were conducted on 16 S RNA prepared by the acetic acid/urea method of Craven and co-workers. The 16 S RNA prepared by this method was reported to have six or seven new protein-binding sites. No differences in conformation and hydrodynamic shape were detected between the two 16 S RNA preparations and only a small difference in thermal stability was observed. A comparison of these results with those obtained for the 30 S subunit conducted under identical conditions indicates that the effective hydrodynamic radii of the 30 S subunit and the 16 S RNA are similar. Furthermore, CD studies indicate only subtle differences between the conformation of 16 S RNA free in solution and that within the 30 S subunit. Melting studies indicate that the proteins stabilize the RNA since the denaturation profile is less cooperative for the 16 S RNA and the melting temperature for the 16 S RNA is lower than that for the 30 S subunit. These results suggest that the 16 S RNA free in solution is already folded into a unique three-dimensional structure before the ribosomal proteins bind to it to assemble into a functional 30 S subunit.

The elucidation of the shape and conformation of ribosomal RNA is a prerequisite to an understanding of the detailed molecular mechanism of assembly of the ribosome and in deciphering the three-dimensional structure of this protein-synthesizing organelle. Considerable efforts have been made to determine the primary and secondary structures of the 16 S RNA of the 30 S subunit. The nucleotide sequence of *Escherichia coli* 16 S RNA has been almost completely determined (1), and the prediction of the secondary structure of the 16 S RNA from its nucleotide sequence (2) using Tinoco's method (3) suggests that 63% of the bases are paired. This is in agreement with hyperchromic studies (4–7), optical rotatory dispersion measurements (8–10), and infrared spectral analyses (11, 12) of 16 S RNA which indicate that 60 to 70% of the bases are paired. The secondary structure appears to consist of many short double helical regions or "hairpin loops" which result from complementary base pairing of adjacent sections of the nucleotide chain. Purine nucleotides predominate in the short single-stranded regions which join the hairpin loops.

The question still remains unanswered as to whether the overall shape and conformation of the 16 S RNA free in solution is the same as that in the ribosome. A comparison of some physical chemical properties of *E. coli* 16 S RNA and 30 S subunit suggests that the free 16 S RNA is not as compact as the 30 S subunit and is probably unfolded. The radius of gyration of the free 16 S RNA, 176 Å, as determined by small angle x-ray scattering (13), is greater than twice the 69 to 72 Å values obtained for the 30 S subunit (14, 15). These measurements correspond to models equivalent to the following dimensions: 20 Å x 350 Å x 620 Å for the 16 S RNA (13), and 55 Å x 220 Å x 220 Å for the 30 S subunit (14). The intrinsic viscosity of the 16 S RNA (16) is 3 times greater than the value for the 30 S subunit under similar conditions (17). Miall and Walker (7) have concluded from sedimentation and viscosity studies that the hydrodynamic volume of the 16 S RNA is greater than twice that of the 30 S subunit. In addition, because ribosomal protein S4 is one of the major RNA-binding proteins (18) and has been shown to enter the *in vivo* assembly process early (19), the 16 S RNA-protein S4 complex has been used as a model to study RNA-protein interaction in the ribosome. Electron microscopic studies of the free 16 S RNA and the 16 S RNA-protein S4 complex (20) indicate that free 16 S RNA exists in an extended and unfolded conformation, but a much less extended and partially folded form is seen in the 16 S RNA-protein S4 complex. This led to the suggestion that protein S4 organizes approximately one-third of the 16 S RNA. All of these studies suggest that the shape and conformation of 16 S RNA free in solution are drastically different from those of 16 S RNA bound in the ribosome. These observations thus led to the proposal that ribosomal proteins "organize" the conformation of the 16 S RNA from a rather open and unfolded state to its compact conformation during assembly (21).

However, small angle x-ray scattering studies of the 16 S RNA and 16 S RNA-protein S4 complex (13) indicate that the radius of gyration of the complex is identical with that of the free 16 S RNA. This study suggests that the role of 16 S RNA in assembly may be to provide a folded three-dimensional matrix for the binding of the ribosomal proteins. Fur-
Conformation of Ribosomal 16 S RNA in Reconstitution Buffer

thermore, recent evidence indicates that the conformation and hydrodynamic behavior of ribosomal RNA appears to be sensitive to environmental factors such as Mg$^{2+}$ concentration (22, 23) and ionic strength (24, 25). Since the above mentioned studies were not conducted in the presence of Mg$^{2+}$ or in the optimal ionic strength required for reconstitution or functional activity but rather in conditions which are likely to render the RNA unfolded or partially unfolded, the interpretation of the results with regard to structure-function relationship, RNA-protein interaction, or the mechanism of assembly should be made with caution.

The three-dimensional structure of 16 S RNA is complicated further by the observation of Hochkeppel et al. (26) that 16 S RNA extracted by acetic acid/urea differs widely from the phenol-extracted 16 S RNA in its protein-binding capacity. Six or seven new protein-binding sites were reported for the acetic acid/urea-extracted 16 S RNA. These workers proposed that this RNA may have a more "open" or extended conformation.

We have characterized the hydrodynamic shape and conformation of the 16 S RNA in reconstituent buffer (TMK$_{600}$) buffer: 20 mM Tris, 20 mM MgCl$_2$, 360 mM KCl, pH 7.6) at 37°C, i.e., under optimal magnesium ion concentration, ionic strength, and temperature required for reconstitution (27). The hydrodynamic shape properties obtained by intrinsic viscosity and sedimentation velocity measurements are significantly different from those previously obtained in other buffers. These results confirm the observation that Mg$^{2+}$ and ionic strength play a significant role in maintaining the structure of the RNA. Furthermore, a comparison of the results of 16 S RNA with those of the 30 S subunit under identical conditions indicates that the effective hydrodynamic radii of 16 S RNA and 30 S subunit are essentially the same albeit the degree of asymmetry is significantly less for the 30 S subunit. Circular dichroism (CD) was used to characterize the conformation of 16 S RNA and it was found that the conformation of 16 S RNA free in solution and bound in the 30 S subunit is similar with only subtle differences. In addition, a comparative UV melting study presented here indicates that the binding of proteins stabilizes the structure of the RNA. We have also done a comparative study on the physical chemical properties of the 16 S RNA prepared by phenol extraction and that by acetic acid/urea. The hydrodynamic shape, conformation, and thermal stability for the two 16 S RNA preparations are essentially the same.

MATERIALS AND METHODS

Ribosomes and 30 S subunits were isolated from E. coli strain MRB 900 cells as described previously. The 16 S RNA was extracted from the 30 S subunit by the phenol/sodium dodecyl sulfate procedure (28) and by the acetic acid/urea method (29). All physical chemical studies were conducted under the optimal conditions for reconstitution, i.e., in the TMK$_{600}$ buffer (20 mM Tris, 20 mM MgCl$_2$, 360 mM KCl, pH 7.6) at 37°C, unless otherwise noted. These physical chemical data should yield important information about the shape and conformation of the 16 S RNA, and the structural role of RNA in the assembly of the 30 S subunit. CD spectra were obtained using a Jasco J-20 spectropolarimeter equipped with a temperature-regulated cell holder with the temperature maintained by a Lauda K2/R circulating water bath and thermal isolation compartments. A calibration curve correcting for the temperature difference between the solution in the spectrophotometric cuvette and circulating water bath was obtained before the studies were begun. A drop of silicone oil was layered on top of the samples to avoid evaporation. The observed absorbance was not corrected for volume expansion as a function of temperature. The reference cell holder was maintained at 55°C, while the sample cell holder was heated in increments of approximately 5°C. Samples were allowed to preincubate 25 min before the absorbance was recorded. The thermal denaturation profile of the 30 S subunit and phenol/sodium dodecyl sulfate-extracted RNA were conducted in TM buffer (10 mM Tris, 0.1 mM MgCl$_2$, pH 7.6). These conditions of low salt and magnesium ion concentration are similar to those in physical chemical studies cited previously and circumvent a problem of precipitation of the subunit which results when ribonucleoproteins are heated to high temperature in the presence of Mg$^{2+}$.

The concentrations of 30 S subunit and 16 S RNA were 0.075 and 0.046 mg/ml, respectively, as determined spectrophotometrically. An A$_{260}$ value of 14.5 (17) was assumed for concentration determinations of the 30 S subunit. Molar absorption, $e$, was determined at each temperature using Beer-Lambert's law, A = e.l.c., where A is the observed absorbance at 260 nm, $e$ is the concentration in moles per liter, and l is the optical path length. The difference in melting profiles for the phenol/sodium dodecyl sulfate-extracted 16 S RNA and the acetic acid/urea-extracted 16 S RNA was obtained by melting both preparations simultaneously. 1.0 A$_{260}$ of acetic acid/urea-extracted RNA was placed in the reference cuvette, and 1.0 A$_{260}$ of phenol/sodium

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dodecyl sulfate-extracted RNA was placed in the sample cuvette. The RNAs had been dialyzed against TMK$_{360}$ buffer after their extraction and then against TK$_{360}$ buffer (20 mM Tris, 360 mM KCl, pH 7.6). The difference in absorbance as a function of temperature was obtained and expressed in terms of absolute absorbance for both preparations.

All pH measurements were made with a Radiometer model 26 pH meter or a model PHM 64 research pH meter with a combined glass electrode (GR2302C). The pH meters were routinely calibrated with pH 7.0 and pH 4.01 standard buffers. Sequential grade sodium dodecyl sulfate was purchased from Pierce Chemical Co. Phenol was redistilled. All other chemicals used were analytical or reagent grade. The water used in all experiments was redistilled with a glass still, deionized, and passed through a column of organic remover.

RESULTS

Sedimentation Velocity—The sedimentation velocity pattern for phenol-extracted 16S RNA in TMK$_{360}$ buffer at 37°C is shown in Fig. 1. A single symmetric peak was observed through its whole passage through the centrifuge cell. The RNA concentration dependence of the observed sedimentation coefficients is shown in Fig. 2 and is extrapolated to 29.2 S at zero concentration. The corresponding $s_{0,w}$ was calculated to be 21.1 S. At first glance, this sedimentation coefficient may seem anomalous when compared to published values; however, after a careful analysis of the conditions used in previous studies (see Table I), it is apparent that 21.1 S may more truly reflect the hydrodynamic behavior of the native RNA isolated from the 30 S subunit of E. coli ribosome. The "16 S" sedimentation coefficient reported was determined in a low ionic strength environment with no Mg$^{2+}$ ion present (16, 29, 32), a condition in which the RNA is likely to be unfolded. The sedimentation coefficient of this RNA has also been shown to increase with increasing Mg$^{2+}$ concentration (16, 24). Therefore, the higher value of 21.1 S that we obtained under the optimal reconstitution conditions (i.e. ionic conditions: 360 mM KCl, 20 mM MgCl$_2$, and at 37°C) is not unexpected. The effective hydrodynamic radius calculated from sedimentation measurements is 114 Å. The frictional coefficient ratio, $f/f_{o,s}$ was calculated to be 2.15. This indicates that the "native" 16 S RNA is probably not spherical and suggests a rather asymmetric shape or a higher degree of hydration, or both.

Viscosity—The viscosity of 16 S RNA under the conditions of reconstitution was determined and the concentration dependence of reduced viscosity is shown in Fig. 3. An intrinsic viscosity of 14.3 ml/g is obtained by extrapolation to zero RNA concentration. This value suggests again a rather asymmetric shape for 16 S RNA and is significantly smaller than the value of 24.8 ml/g reported by Stanley and Bock (16) and 30.5 ml/g reported by Kurland (32). The hydrodynamic radius calculated from the intrinsic viscosity is 113 Å. Once again, the differences in intrinsic viscosity between the present result and those reported earlier can be explained by the observation that the hydrodynamic shape of the 16 S RNA is sensitive to changes in ionic strength and the absence or presence of Mg$^{2+}$ ions. The small angle x-ray scattering, sedimentation velocity, and intrinsic viscosity studies of other workers could reflect hydrodynamic properties of unfolded or partially unfolded particles, if done at low ionic strength and in the absence or low concentration of Mg$^{2+}$ ions.

Circular Dichroism—The circular dichroism spectra of phenol-extracted 16S RNA are shown in Fig. 4. The general shape of the spectrum in the near UV region (320 to 240 nm) is similar to that of the 30 S subunit. The CD spectrum for the 16 S RNA is characterized by a small negative trough at 296 nm which is almost one-half the magnitude of the trough for the 30 S subunit and a large peak at 265 nm which is approximately 4% larger than that of the 30 S subunit. The 30 S subunit shows crossovers at 292.5 and 247 nm, while the free 16 S RNA shows crossovers at 292.5 and 246 nm. The far UV CD spectrum of 16 S RNA differs from the 30 S subunit in that it is characterized by two troughs at 237 and 208 nm. There is zero net ellipticity at 222 nm for the 16 S RNA, and the trough at 208 nm is approximately one-half the magnitude of the trough for the 30 S subunit.

UV Hyperchromic Melting Studies—In an effort to understand the forces which maintain the three-dimensional structure of the 16 S RNA and that of the 30 S subunit, we have compared the melting profile of the 16 S RNA with that of...
TABLE I
Hydrodynamic properties of ribosomal 16 S RNA

<table>
<thead>
<tr>
<th>m × 10^(-6)</th>
<th>η</th>
<th>η, [η']</th>
<th>Effective hydrodynamic radius, R_e</th>
<th>Radius of gyration, R_g</th>
<th>Salt conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.56, S⁺</td>
<td>0.570</td>
<td>16.3</td>
<td>30.5</td>
<td>0.01 M Acetate, 0.1 M NaCl, pH 4.6</td>
<td>(32)</td>
<td></td>
</tr>
<tr>
<td>0.55, S⁻</td>
<td>0.560</td>
<td>17.4</td>
<td>24.8</td>
<td>0.05 M Tris, 0.1 M KCl, pH 7.0</td>
<td>(16)</td>
<td></td>
</tr>
<tr>
<td>0.64, SE⁺</td>
<td>0.577</td>
<td>189; S</td>
<td>161; [η']</td>
<td>0.05 M Tris, 0.1 M KCl, pH 7.0</td>
<td>(29)</td>
<td></td>
</tr>
<tr>
<td>0.53, SE⁻</td>
<td>15.9</td>
<td>176, x-ray</td>
<td>37.5 mm Tris, pH 7.4</td>
<td>10 mm Tris, 1 mm EDTA, 200 mm NaCl, pH 7.4</td>
<td>(13)</td>
<td></td>
</tr>
</tbody>
</table>

η, intrinsic viscosity. S⁺, sedimentation velocity. SE⁺, sedimentation equilibrium. 

0.64, SE⁺ 0.577 176, x-ray

The 30 S subunit as shown in Fig. 5. The change in molar absorption (Δε) at 260 nm was calculated using the following relationship: Δε = (ε_f - ε_0)/ε_0, where ε_f is the molar absorption at T°C, and ε_0 is the molar absorption at 25°C. The melting profile for the 16 S RNA was moved upward in Fig. 5 so that its absorbance at 95°C coincides with the corresponding absorbance of the 30 S subunit. This is done by assuming that the final denatured states at 95°C are the same for the 30 S subunit and 16 S RNA. Thus, the final absorbance was used as a reference to compare the denaturation profiles. The absolute change in molar absorption for the 30 S subunit and 16 S RNA was 1.46 × 10⁷ to 1.89 × 10⁷ M⁻¹ cm⁻¹, respectively. One difficulty that arises in these melting studies is that 30 S subunits have a tendency to aggregate at higher temperatures when Mg⁺⁺ ion is present at concentrations greater than 1 mM. Therefore, these melting studies were conducted in a 10 mM Tris buffer, pH 7.4, with 0.1 mM MgCl₂. Under these conditions, the free 16 S RNA shows a less cooperative melting curve, and apparently it has fewer base pairs than the intact subunit. The melting temperatures, T_m, observed by absorbance at 260 nm are 56°C and 64°C for the free 16 S RNA and 30 S subunit, respectively. However, because it was necessary to conduct this study in a low ionic strength medium with low concentration of Mg⁺⁺, the initial conformation of the 16 S RNA was probably partially unfolded, and the estimation of the relative number of base pairs under this condition should be used with caution. Our estimations of 27.7% for the 30 S and 25% for the 16 S RNA are not much different from the respective values of 28% and 27% in Miall and Walker's work (7) which was done in 1 mM MgCl₂ instead of the absence of Mg⁺⁺ used in our work. With respect to the melting temperature, T_m, Miall and Walker reported 64°C and 63°C for the 30 S and the 16 S RNA, respectively. These values were obtained at 1 mM MgCl₂. When the Mg⁺⁺ was removed by addition of EDTA, drastic changes occurred. The melting curves for both the 16 S RNA and the 30 S subunit were shifted to lower temperatures by about 20°C. Furthermore, the melting profile for the 16 S RNA did not reach zero slope. Our results are similar to Miall and Walker's and the conditions we used are intermediate between the two conditions studied by Miall and Walker. The important conclusion from comparing the melting profiles of 30 S and the 16 S RNA is that binding of proteins stabilizes the secondary structure of 16 S RNA because there is no change in the absorption of the 30 S subunit at 260 nm until after 45°C.

**Studies on Acetic Acid/Urea-extracted 16 S RNA**—The 16 S RNA obtained by the acetic acid/urea extraction procedure of Craven and co-workers (26), capable of binding six to seven
more proteins than the 16 S RNA isolated by phenol extraction, has also been studied. Craven (26) has suggested that the acetic acid/urea-extracted RNA may have a more expanded or open structure, which may reflect a more "native" conformation. The fact that attempts to reconstitute the 50 S subunit from phenol-extracted ribosomal RNA have failed, supports this contention. However, reconstitution using an alternative RNA extraction procedure of 4 M LiCl with 0.5 M magnesium acetate has been successful (34). The question is raised whether the traditional phenol extraction procedure denatures the 16 S RNA. The primary role of sodium dodecyl sulfate used in conjunction with phenol presumably is to dissociate the proteins from the RNA, while phenol denatures proteins. The phenol is also saturated with sodium dodecyl sulfate-extracted RNA (1.0 A250/ml) and acetic acid/urea-extracted RNA (1.0 A250/ml) were placed in the sample and reference cell holders, respectively. The heat source was connected to both cell holders. After adjusting each temperature increment, the samples were allowed to equilibrate for 25 min. No differences were observed in the CD spectra, and only minor differences were detected from the melting studies. The melting curves for the two RNAs as a function of temperature are shown in Fig. 6. Since the RNAs may undergo some denaturation during their respective extraction procedure, each sample of the 16 S RNA was renatured by dialyzing against TMK500 buffer before dialysis against TK300 buffer, which was used for the melting studies. Fig. 6 shows that the phenol-extracted 16 S RNA exhibits about 0.5% more hyperchromicity. Thus, we have been unable to detect any significant hydrodynamic or conformational differences between the RNAs extracted by the two methods. 

**DISCUSSION**

The availability of accurate structural information of 16 S RNA in "reconstitution" and "native" buffer is essential to the elucidation of the molecular mechanism of 30 S assembly. Since most of the available physical chemical data on 16 S RNA reported in the literature were conducted in buffers which were likely to render the RNA unfolded, we have characterized hydrodynamic shape and conformation of 16 S RNA under the conditions of reconstitution and compared them with the corresponding properties of the 30 S subunit. Intrinsic viscosity and sedimentation velocity studies yield information about the hydrodynamic shape of biomolecules, and changes in these physical chemical properties reflect differences in shape or amount of hydration (or both). The $s_{20w}$ of 21.1 S found for the 16 S RNA in reconstitution buffer...
may have some relevance to the use of empirical methods in determination of S values of other RNA or ribonucleoprotein particles by density gradient centrifugation, since 16 S RNA is commonly used as a 16 S marker. The 16 S RNA does not appear to behave hydrodynamically as a sphere. Not only is the intrinsic viscosity value much greater than the 3.3 to 4.0 mI/g range which is usually indicative of compact globular protein molecules, but the frictional coefficient ratio is also greater than the 1.1 to 1.3 range normally found for globular proteins (31). It is also evident from the results that the hydrodynamic properties of 16 S RNA are stabilized by the presence of Mg2+ and salt. The effective hydrodynamic radius of 16 S RNA under reconstitution conditions reported here is significantly smaller than those determined without Mg2+ (16). The Mg2+ ion and salt may be required to shield the negative charges of phosphate groups, hence permitting the structure of the 16 S RNA to assume a more folded structure in reconstitution buffer than has been described in the absence of Mg2+ ion and salt. Electron microscopic studies of ribosomal RNA as a function of ionic strength indicate that the shape of the RNA is sensitive to the ionic environment (25). The electron micrographs of RNA in the medium of no Mg2+ and low ionic strength indicate that the shape of the RNA is more extended than that in a medium containing 10 mM Mg2+ ion with an ionic strength of 0.10.

The recent finding by Craven and co-workers (26) that 16 S RNA extracted by acetic acid/urea treatment was able to bind six to seven more 30 S subunit proteins than the phenol-extracted 16 S RNA suggested that these two RNA preparations might have different structures. In fact, these workers postulated that the acetic acid/urea-extracted 16 S RNA might have a more "open" structure. The results of our investigation indicate that the overall conformation and shape of the two preparations of 16 S RNA are the same. However, the possibility still exists that the alleged difference in binding capacity of the 16 S RNA might be due to subtle conformational differences which are not detectable by CD, thermal denaturation, and hydrodynamic studies. In this regard, it should be noted that further studies by Hochkeppel and Craven (35) have demonstrated the existence of electrophoretic mobility differences between the 16 S RNA extracted by the two different procedures.

A comparison of the hydrodynamic shape and conformation properties of the 16 S RNA and 30 S subunit both under conditions of reconstitution should yield important information about the role of RNA in the assembly of the 30 S subunit. Since the near UV CD spectra of ribosome and its subunits arise primarily from the RNA, conformational changes of the 16 S RNA should be manifested in this spectral region. There are only subtle differences between the CD spectra of RNA and the 30 S subunit in reconstitution buffer. The magnitudes of the 265 nm peak and 296 nm trough for the intact ribosomal 30 S subunit are only slightly lower than those for the 16 S RNA. A decrease of the 265 nm peak has been attributed to base unstacking (36, 37); thus, the binding of proteins to the 16 S RNA may cause a subtle change of the conformation of the RNA. However, a recent report by Cox et al. (38) suggests that an increase in the number of G-C pairs will cause a decrease in the magnitude of the CD bands at 265 and 296 nm. Thus, if the ratios of G-C to A-U pairs were to increase upon protein binding, this qualitative change could be due to electrostatic interaction of the basic ribosomal proteins with the anionic RNA. A model CD study in which the conformational changes induced in polyadenylic acid by oligopeptides containing lysyl and aromatic residues (39) supports this interpretation. However, these authors suggest that it is the stacking of the aromatic amino acids with the adenine bases that contributes to most of the decrease in ellipticity observed at 265 nm.

The binding of ribosomal proteins to the 16 S RNA to form the 30 S subunit appears to stabilize the conformation of the 16 S RNA as shown by our melting studies presented here. In addition, studies in our laboratory indicate that the 30 S subunit is more stable toward denaturation by ethylene glycol than the 16 S RNA (40). Recent binding studies of protein S4 with its specific RNA-binding fragments suggest that protein S4 stabilizes the RNA structure (41). These observations are consistent with the notion that RNA-protein interactions play an important role in maintaining the three-dimensional structure of the ribosome. It appears that the optimal conditions required for reconstitution, i.e., 10 to 20 mM Mg2+, ionic strength equal to 0.37, 37°C, and pH 7.6 (37) indirectly influence these RNA-protein interactions (22), thus allowing a conformational state of RNA that can be recognized by its binding proteins. This is further supported by the fact that hydrodynamic properties of free 16 S RNA in reconstitution buffer are significantly different from those reported in a medium of low ionic strength and low Mg2+ concentration (16).

The hydrodynamic behavior and conformational properties of the 30 S subunit have also recently been characterized in TMK100 buffer at 37°C and a comparison with the results of 16 S RNA is summarized in Table II. At first glance, it appears that there are significant differences between the hydrodynamic shape of the 30 S subunit and its isolated 16 S RNA free in solution. The frictional coefficient ratio of the RNA is 40% greater than that for the 30 S subunit, and likewise the intrinsic viscosity of the RNA is 30% greater than the value for the 30 S subunit. Comparison of the data in this manner could result in the interpretation that the hydrodynamic shape of the 16 S RNA is larger than the 30 S subunit. This is reported in the hydrodynamic studies of Miall and Walker (7) and implied from comparison with other studies of the 16 S RNA (13) and the 30 S subunits (14, 15). As a consequence, it has been suggested that the ribosomal proteins "organize" the tertiary structure of the RNA and that the extended 16 S RNA molecule collapses as the proteins are added during assembly (21). However, a direct comparison of these hydrodynamic values for the ribonucleoprotein particle and the RNA is misleading because these parameters are also dependent upon the mass of the biomolecules or biomolecular complexes. Perhaps a better way is to compare the effective hydrodynamic radius of the 16 S RNA with that of the 30 S subunit.

The effective hydrodynamic radius of the 16 S RNA is nearly identical with that of the 30 S subunit as determined from both sedimentation velocity and intrinsic viscosity measurements as indicated in Table II. The difference between our conclusion and those of other workers (7) can be analyzed by describing the experimental conditions of each of these studies. Miall and Walker reported that the hydrodynamic volume

| Table II: Comparison of hydrodynamic properties of 16 S RNA and 30 S subunit under conditions of reconstitution |
|--------------------------------------------------|--|--------------------------------------------------|
| Hydrodynamic parameter | 16 S RNA | 30 S Subunit* |
| s0.5 | 21.1 | 32.6 |
| f/fo | 2.15 | 1.54 |
| Effective hydrodynamic radius determined from s0.5, Rsed | 114 Å | 108 Å |
| [η], mI/g | 14.3 | 10.9 |
| Effective hydrodynamic radius determined from [η], Rη | 113 Å | 120 Å |

* S. H. Allen and K.-P. Wong, manuscript submitted for publication.
of 16 S RNA ($V_{RNA}$) is larger than that of the 30 S particle ($V_{30 S}$) in 1 mM Mg$^{2+}$; the $V_{30 S}/V_{RNA}$ ratio is 0.48. But under this low concentration of Mg$^{2+}$, the 16 S RNA is partially unfolded (13). Furthermore, no reconstitution is achieved at this Mg$^{2+}$ concentration from the studies of Tsukub and Nomura (27). Since, in the 30 S ribosomal subunit the binding of the proteins to the 16 S RNA stabilized the 16 S RNA against the unfolding induced by decreasing the concentration and removal of Mg$^{2+}$, this low ratio of $V_{30 S}/V_{RNA}$ (0.48) merely reflects that the low concentration (1 mM Mg$^{2+}$) used in Miall and Walker’s work (7) results in unfolding of the RNA but to a much less extent with respect to the 30 S particle. The critical requirements of 10 to 20 mM Mg$^{2+}$ were not obvious at the time of this work.

The addition of EDTA to remove the remaining Mg$^{2+}$ ion as reported by Miall and Walker, increases the $V_{30 S}/V_{RNA}$ to 0.7 (at 5 mM EDTA). This simply suggests that the further removal of Mg$^{2+}$ now also unfolds the 30 S particle so that $V_{30 S}$ also increases and the $V_{30 S}/V_{RNA}$ ratio increases. Upon the addition of 10 mM EDTA, both the 16 S RNA and the ribonucleoprotein particle are extensively unfolded. However, the 16 S RNA alone probably exists as a flexible coil, while the unfolded ribonucleoprotein chain is stiffened by the bound protein and exists in a more extended form. Hence, an even higher value of the $V_{30 S}/V_{RNA}$ ratio was observed. Thus, at low Mg$^{2+}$ concentration, identical values of effective hydrodynamic volumes of the 16 S RNA and the 30 S particle may indicate that both are unfolded to the extent that their hydrodynamic volumes are similar.

Since at this low Mg$^{2+}$ concentration no reconstitution of active ribosomal particle can be accomplished (27), this coincidence is artificial. But, under the conditions of reconstitution as used in studies described here (i.e at 20 mM Mg$^{2+}$), the fact that ribosomal proteins can bind to the 16 S RNA and form an active ribosomal 30 S particle with the same effective hydrodynamic radius suggests that the compact 16 S RNA with a hydrodynamic radius similar to that of the 30 S particle under the proper environmental condition (i.e. Nomura’s reconstitution buffer (27): high salt, 10 to 20 mM Mg$^{2+}$) assumes a unique three-dimensional structural matrix. This structural matrix presumably contains the primary binding sites for the initial binding proteins to bind through molecular recognition to form the active particle via a number of steps involving more subtle conformational changes of part(s) of the RNA matrix, and the proteins. This latter subtle type of conformational change has been studied in our laboratory and by Hochkeppel and Craven using electrophoretic mobilities studies (35). In the study of Folkhard et al. (35), the radius of gyration ($R_g$) measurements of the 16 S RNA were conducted in 37.5 mM Tris buffer with no Mg$^{2+}$, a condition which probably renders the RNA partially unfolded. The corresponding studies of the ribosomal subunit (17) were conducted at low Mg$^{2+}$ ion concentrations (1 mM). However, because ribosomal proteins appear to stabilize the structure of ribosomal RNA and thus may protect the RNA from unfolding under this low Mg$^{2+}$ concentration, it is not surprising that the radius of gyration for the 16 S RNA determined in the conditions as outlined would be larger than that for the 30 S subunit. Clearly, a comparison of the $R_g$ values of the 30 S subunit and free 16 S RNA in reconstitution buffer would provide more relevant information about the structures of the RNA and the end product of assembly, the 30 S subunit.

Thus, the primary interpretation dictating the conformation of the RNA appears to lie within its polynucleotide sequence. Albeit the conformation of 16 S RNA is sensitive to environmental conditions, the binding of ribosomal proteins to the

\[ V_{16 S RNA} / V_{30 S Subunit} \]
The hydrodynamic and spectroscopic properties of 16 S RNA from Escherichia coli ribosome in reconstitution buffer.

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