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A Molecular Weight Determination of the 16S Ribosomal Ribonucleic Acid from *Escherichia coli*†

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ABSTRACT: Sedimentation equilibrium experiments coupled with an accurate determination of the partial specific volume ($\bar{v}$) were used to obtain a mol wt of 6.4×10^5 for 16S ribosomal RNA from *Escherichia coli*. This value is significantly greater than those previously reported, which were obtained through a combination of sedimentation velocity, viscosity, and light scattering experiments or by chemical methods. This new value for the molecular weight of 16S rRNA implies that there are 260,000 daltons of protein present on the 30S ribosomal subunit and about 1900 nucleotides present in the 16S rRNA strand. It also gives clear evidence that the 30S ribosomal subunit is heterogeneous with respect to its protein complement.

The molecular weight of the 16S ribosomal RNA (rRNA) was first determined by Kurland (1960) using combined sedimentation velocity, viscosity, and light scattering measurements. Later Stanley and Bock (1965) obtained a similar molecular weight using sedimentation velocity and viscosity measurements. Midgley (1965) also obtained the molecular weight of this macromolecule by calculating a value based on the stoichiometry of periodate-oxidized RNA with [*carbonyl*-^{14}C]isonicotinic acid hydrazide. As a result of these studies, the molecular weight of 16S rRNA has been given as 5.5–5.6 $\times 10^5$, with a precision of about $\pm 15\%$.

However, due to the recent studies by Kurland *et al.* (1969), Voynow and Kurland (1971), and Traut *et al.* (1969), showing the possible heterogeneity of the 30S ribosomal protein complement, as well as the meaningful work of Fellner *et al.* (1972) in sequencing the 16S rRNA, it has become apparent that there is a critical need for a more refined determination of the molecular weight of the 16S rRNA. This study was made for this purpose.

Materials and Methods

Escherichia coli strain MRE 600 (RNase I$^-$) supplied by Dr. James Young, University of Wisconsin, was grown in glucose and minimal salts media, harvested in the middle of the logarithmic growth phase, and stored at $-76°$ until needed.

Preparation of 30S Ribosomal Subunits. The 30S subunits were isolated using the method of Hill *et al.* (1969b) except

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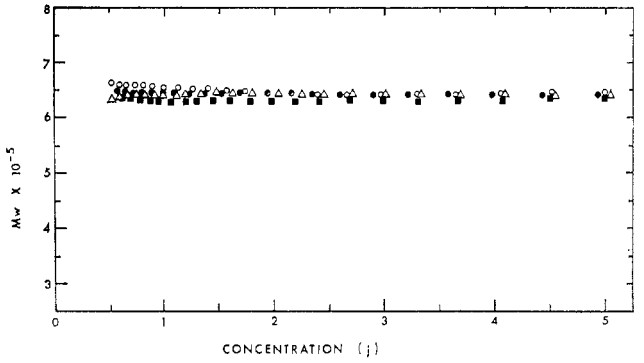

FIGURE 1: Weight-average molecular weights *vs.* concentration in fringe numbers (*j*) for four different preparations of fresh 16S rRNA. The RNA concentration in each case was approximately 0.5 mg/ml, spun at 6800 rpm at 4° for 18 hr or more.

that in the final steps the separation of 50S and 30S subunits was achieved with a 10–30% exponential sucrose gradient formed in a Ti-15 Beckman zonal rotor spun at 121,750*g* for 9 hr (Eikenberry *et al.*, 1970). The 30S subunits were recovered from the sucrose solution by increasing the Mg^{2+} concentration tenfold and precipitating with 1.5 vol of 95% ethanol. The precipitate was pelleted by centrifugation at 20,000*g* for 30 min, dissolved in buffer (0.01 M Tris-HCl–0.08 M KCl–0.0015 M $MgCl_2$, pH 7.4), checked for purity by means of sedimentation velocity in the Beckman Model E ultracentrifuge, and used immediately for the extraction of the RNA or stored at −76° for future use.

16S rRNA Preparation. The 16S ribosomal RNA was extracted from the 30S subparticles by the method of Stanley and Bock (1965) with slight modification as follows: to the aqueous phase of the last phenol extraction (~3 ml) was added 2 vol of 95% ethanol, mixed well, after which the mixture was centrifuged at 20,000*g* for 30 min. The supernatant fluid then was discarded and the precipitate dissolved slowly in 3 ml of buffer (0.05 M Tris-HCl–0.1 M KCl, pH 7.0) after which 6 ml of 95% ethanol was added and mixed, and the mixture was centrifuged at 20,000*g* for 30 min to pellet the RNA. The supernatant fluid was again discarded and the precipitate dissolved slowly in 3 ml of the above buffer. This solution was dialyzed for 12 hr against 1000 ml of buffer, with a change of buffer every 3 hr. The concentration of the sample was then determined by measuring the absorption at 260 nm using 22.3 as the extinction for a 1-mg/ml solution of the potassium salt of RNA in 0.05 M Tris-HCl–0.1 M KCl, pH 7.0 (Stanley and Bock, 1965). A dilution was made to give an RNA concentration of about 0.5 mg/ml, after which this solution was dialyzed for about 4 hr before being used in the sedimentation equilibrium experiments. The rest of the RNA solution together with the original dialysate was used as such for density and dry weight determinations. In all steps, maximum precautions were taken to avoid ribonuclease contamination of glassware and equipment.

Sedimentation Equilibrium. The high-speed or meniscus-depletion technique of Yphantis (1964) was used for the sedimentation equilibrium experiments in a Beckman Model E analytical ultracentrifuge equipped with Rayleigh interference optics. One sector of a double sector cell was filled with 0.11 ml of the dialysate and the other sector with 0.10 ml of the dialyzed ribosomal RNA giving column heights of about 3 mm. The cell was spun in an AN-J rotor at 6800 rpm at 4° for 18–24 hr. The interference patterns were photographed on Kodak II-G spectroscopic plates. Displacements were measured for at least five fringes at spacings of 50–100 μ in a Nikon Model 6C profile projector with a 50× objective.

Partial Specific Volume. To determine the partial specific volume of the 16S RNA in the buffer employed in our study, the density of the dialysate and that of 16S ribosomal RNA solutions were measured with a Digital Density Meter DMA 02C manufactured by Anton Paar (Graz, Austria) in accord with the design of Kratky *et al.* (1969). Each set of measurements was preceded and followed by a verification of the calibration constant of the instrument with three different freshly prepared salt solutions of density previously determined pycnometrically at the same temperature. The rRNA samples were then equilibrated to 4° and then carefully inserted into the hollow glass oscillator tube of the instrument to avoid the generation of air bubbles. After equilibration in the density meter for 30 min, readings were taken from which the density was calculated. The precision of the measurements is $\pm 1.5 \times 10^{-6}$ when the temperature is controlled to ±0.01°. The temperature we used was 4 ± 0.005°.

The concentration of both the dialysate and the RNA solution was obtained by weighing 3–4 ml of the solutions into stoppered flasks, lyophilizing them, and then drying them in a vacuum oven at 98–100° to constant weight (Hill *et al.*, 1969a). From the densities and concentrations, the apparent specific volume for the RNA was obtained by use of the relation

$$\Phi = (1/\rho_0)[1 - (\rho - \rho_0)/c)]$$

where Φ is the apparent specific volume, ρ_0 is the density of the dialysate, ρ is the density of the RNA solution, and c is the concentration of the RNA in grams per milliliter. Since no variation of Φ with concentration was apparent we concluded that this value is equal, within experimental error, to the partial specific volume ($\bar{v}$) of RNA.

This value was then used in conjunction with data from the sedimentation equilibrium experiments which were smoothed and then used to obtain weight-average molecular weights across the solution column.

Results

Uniform preparations of 16S rRNA were routinely obtained by the methods given in the previous section. The sedimentation velocity pattern always showed a single peak with no apparent contaminants.

However, in sedimentation equilibrium experiments, it was found that in all cases where the sample had been frozen and stored at −76°, some degradation occurred, giving a much lower molecular weight in the meniscus region. These results were therefore not used and all molecular weights were obtained using fresh preparations.

The results of the measured RNA solution densities at various concentrations are given in Table I. The resultant partial specific volume is 0.577 ± 0.004 ml/g. When this value was used in conjunction with the weight-average molecular weight across the solution column, the values shown in Figure 1 were obtained. The reason for the slight decrease in molecular weight across the cell in the one experiment is probably due to fringe distortion, but it was felt that the results at the higher concentrations were valid. It should be noted that both nonideality and aggregation are virtually ruled out when one analyzes the results given in Figure 1. The point-average molecular weights across the cell are a sensitive indication of any nonideality or aggregation taking place. There is a finite possibility that the two effects may cancel each other, but this is

TABLE I: Concentrations and Corresponding Densities of 16S rRNA Solutions.[a]

Concn (mg/ml)		Density (g/ml)	
1	2	1	2
0.00	0.00	1.00750	1.00757
2.37	2.35	1.00849	1.00855
3.36	3.15	1.00891	1.00889
4.54	4.47	1.00940	1.00944

[a] Each measurement was made using individual preparations of 16S rRNA. 1 and 2 correspond to different buffer preparations used.

very unlikely in four different preparations. We therefore give $6.4 \pm 0.2 \times 10^5$ as the molecular weight of 16S rRNA.

Discussion

The mol wt of 6.4×10^5 for the 16S ribosomal RNA found by us is significantly greater than the $5.3–5.6 \times 10^5$ daltons reported previously by Kurland (1960), Stanley and Bock (1965), and Midgley (1965). The disparity can best be analyzed by detailing the techniques used and the data gathered in each study.

Kurland (1960) used in his calculations a partial specific volume ($\bar{v}$) for the rRNA of 0.57 ml/g which he measured pycnometrically. He combined sedimentation coefficients from sedimentation velocity experiments and weight-average molecular weights from light scattering experiments to obtain the empirical equation $s_w = 0.98 \times 10^{-2} M_w^{0.56}$ from which a mol wt of 5.6×10^5 resulted. He found the intrinsic viscosity of the RNA to be close to 0.3 dl/g which when substituted together with the previously determined molecular weight into the Scheraga–Mandekern equation (Scheraga and Mandelkern, 1953) gave a value for the shape–volume coefficient, β, of 2.25×10^6.

On the other hand, Stanley and Bock (1965) in their sedimentation velocity and viscosity studies assumed a partial specific volume for the rRNA of 0.53 ml/g and a shape–volume coefficient (β) of 2.16×10^6. They found the intrinsic viscosity to be 0.25 dl/g. These three values are considerably lower than those obtained in the experiments above, but used in the Scheraga–Mandelkern equation they produce a mol wt of 5.5×10^5 in accord with that of Kurland (1960). Therefore, although there is agreement between the two studies with respect to the molecular weight of the 16S rRNA, it is clear that there are rather major differences in their parameters, which can be attributed to the lack of accurate data and/or contaminated samples. When we used the values of Stanley and Bock for the viscosity and β along with our measured $\bar{v}$ of 0.577 in the Scheraga–Mandelkern equation, the resulting mol wt was 6.25×10^5, which is in excellent agreement with the value determined in this study by sedimentation equilibrium.

While it is possible to ascertain the cause of the rather low molecular weights obtained in these previous studies, we are unable at the moment to find the specific reason(s) for the still lower mol wt of 5.3×10^5 reported by Midgley (1965) based on the stoichiometry of the reaction of periodate-oxidized RNA with [*carbonyl*-^{14}C]isonicotinic acid hydrazide. The results of all four studies are presented in Table II for comparison.

TABLE II: Molecular Weight of 16S rRNA from *Escherichia coli* Determined by Four Different Methods.[a]

Method	$M_w \times 10^{-5}$ Daltons (%)	$\bar{v}$ (g/ml)
Sedimentation–viscosity[b]	5.6 ± 15	0.57 ± 0.02
Sedimentation–light scattering[b]	5.6 ± 12	0.57 ± 0.02
Sedimentation–viscosity[c]	5.5	0.53
Periodate–isonicotinic acid hydrazide[d]	5.3 ± 3	
Sedimentation equilibrium[e]	6.4 ± 3	0.577 ± 0.004

[a] The partial specific volume ($\bar{v}$) used in the calculations is also tabulated. [b] Kurland, 1960. [c] Stanley and Bock, 1965. [d] Midgley, 1965. [e] Present study.

It should be emphasized that using sodium or other salts, the molecular weight may vary by as much as 5% from our value. It should also be noted that it is possible that the tRNA from this RNase I$^-$ strain differs from that obtained from other strains. However, this difference should be negligible.

The value of 6.4×10^5 which we obtained for the molecular weight of the potassium salt of 16S rRNA implies that there are about 1900 nucleotides present on the 16S rRNA strand and that the protein complement is about 260,000 daltons for the 30S subunit (see Hill, 1969a).

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