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### On the Sedimentation Behavior and Molecular Weight of 16S Ribosomal RNA from Escherichia coli

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On the sedimentation behavior and molecular weight of 16S ribosomal RNA from *Escherichia coli*

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### INTRODUCTION

Although there have been several studies made on the physical characteristics of rRNA in the past [1,2,3], there is still continuing discussion on the molecular weight and sedimentation behavior of 16S rRNA. A recent study by Pearce *et al.* [4] reported on anomalous concentration dependence of the sedimentation coefficient of the Na<sup>+</sup> salt of 16S rRNA. They also reported the molecular weight for this particle to be  $5.2 \times 10^5$  which is considerably less than  $6.4 \times 10^5$  that was previously reported from this laboratory [3] for the K<sup>+</sup> salt of the 16S rRNA. In this paper we provide additional data with respect to the dependence of the sedimentation behavior on concentration for the K<sup>+</sup> salt of 16S rRNA and confirm the previous  $6.4 \times 10^5$  value for the molecular weight. This latter value was obtained using combined sedimentation and diffusion data.

### MATERIALS AND METHODS

Ribosomal RNA from *E. coli* MRE 600 was isolated using the phenol extraction procedure described by Ortega and Hill (1973). The purified 16S rRNA was dialyzed to equilibrium and then diluted to the concentrations to be used in the sedimentation velocity and diffusion studies (0.05 to 1 mg/ml).

Sedimentation velocity experiments were carried out in a Beckman Model E analytical ultracentrifuge using the ANE rotor at 48,000 rpm and 4°C. Pictures of the schlieren patterns were taken at 8 minute intervals and measured on a Nikon 6C microcomparator equipped with IKL digital micro-meters. Sedimentation coefficients were calculated using a program developed in this laboratory.

Diffusion coefficients were determined using intensity fluctuation

spectroscopy [5,6,7] from the correlation function

$$g(\tau) = e^{-DK^2\tau}$$

where

$g(\tau)$  is the normalized first-order electric field correlation function

$\tau$  is the delay time

$K$  is the magnitude of the scattering vector

$D$  is the translational diffusion coefficient

The correlation function was obtained using a Malvern 4300 spectrometer system containing a digital autocorrelator. Samples were monitored and analyzed by methods reported by Koppel [7] as well as those described by Pusey *et al.* [5].

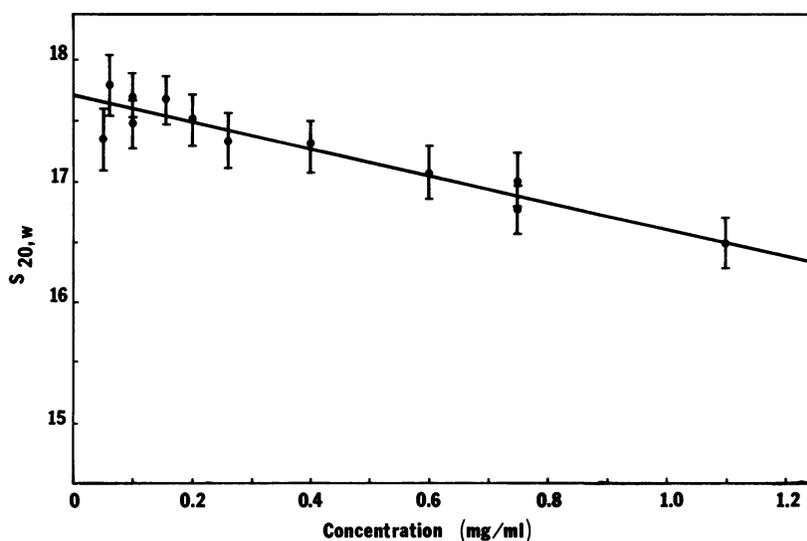
The diffusion and sedimentation coefficients, along with the previously determined density increment ( $\frac{\partial \rho}{\partial c}$ ) [3] were used in the Svedberg equation

$$M = \frac{sRT}{D\left(\frac{\partial \rho}{\partial c}\right)}$$

to give the molecular weight of the sample.

## RESULTS

The results of sedimentation velocity studies made on various concentrations of two separately prepared samples of 16S rRNA are shown in Fig.1.



**Fig. 1.** A plot of sedimentation coefficient ( $S_{20,w}$ ) versus concentration for the  $K^+$  salt of 16S rRNA. The experiments were made by spinning an ANE rotor in a Beckman Model E analytical ultracentrifuge at 48,000 rpm. The resulting sedimentation pattern was monitored using schlieren optics.

The error bars indicate the possible error of each value due to temperature variations, plate-reading errors, speed variations and concentration determinations. Duplicate experiments were made on selected samples to check the reproducibility of the data. One duplicate experiment was run at 60,000 rpm to check for any pressure-induced discrepancy. No such discrepancy was found.

Schlieren optics were used for all concentrations with phase-plate angles varying from 75° for the 0.4 mg/ml and higher concentrations to 55° for the 0.05 to 0.1 mg/ml region. The large error noted for the 0.05 mg/ml point was due to some imprecision caused by the reading of that particular plate as well as low initial temperature of the experiment. The line was obtained from a linear least-squares fit of the data and  $S_{20,w}^{\circ}$  was found to be 17.7 ( $\pm$  0.2) S.

The diffusion coefficient was determined by averaging the results of 32 measurements on three different sample preparations having concentrations of 0.5 to 2 mg/ml. No concentration dependency was observed in this range. The  $D_{20,w}^{\circ}$  value so obtained was found to be  $1.63 (\pm 0.04) \times 10^{-7}$  cm<sup>2</sup>/sec.

Calculation of the molecular weight was carried out using the above  $S_{20,w}^{\circ}$  and  $D_{20,w}^{\circ}$  values coupled with the density increment ( $\frac{\partial \rho}{\partial c}$ ) of 0.418 ( $\pm$  0.004) determined previously [3]. The molecular weight was determined to be  $6.3 (\pm 0.3) \times 10^5$ . The total error using this technique is about 5% as compared with the better than 3% precision expected from sedimentation equilibrium experiments.

## DISCUSSION

The results of these studies on the K<sup>+</sup> salt of 16S RNA confirm our previously reported values for the molecular weight of 16S ribosomal RNA [3]. Sedimentation velocity results show a rather typical linear dependence of the sedimentation coefficient on concentration, even in the region below 0.2 mg/ml. This is in marked contrast to the results reported by Pearce *et al.* [4] on the Na<sup>+</sup> salt of 16S RNA in which the sedimentation coefficient reached a maximum value at about 0.4 mg/ml and then decreased with decreasing concentration to a lower value at infinite dilution. In an effort to reproduce their results, we also studied the 16S rRNA in Na<sup>+</sup> salt and found quite anomalous results from sedimentation velocity experiments. We were unable to obtain reproducible results from many experiments on several different sample preparations. The cause of this anomalous behavior with Na<sup>+</sup> salt rRNA is yet unknown.

Molecular weights for the  $K^+$  salt 16S rRNA were obtained by coupling the sedimentation coefficient, diffusion coefficient and density increment in the Svedberg equation. The value which we obtained of  $6.3 (\pm 0.3) \times 10^5$  confirms our previously reported values of  $6.4 (\pm 0.2) \times 10^5$  which was determined from sedimentation equilibrium experiments.

Both values are considerably higher than the value of  $5.2 \times 10^5$  reported by Pearce *et al.* [4] for the  $Na^+$  salt of the 16S RNA and somewhat higher than 5.6-5.8  $\times 10^5$  reported by others [1,2]. It should be noted that difference in molecular weight between  $K^+$  and  $Na^+$  ions may account for approximately  $0.3 \times 10^5$ . Therefore, using the value of  $6.4 \times 10^5$  Daltons for the  $K^+$  salt form of 16S RNA as a standard, the  $Na^+$  salt form should have a molecular weight of approximately  $6.1 \times 10^5$  and the value obtained for the rRNA as calculated from either end-group analysis or from sequencing data should be approximately  $5.7 \times 10^5$ . Our results suggest that 16S rRNA contains approximately 1700-1750 nucleotides which is still somewhat higher than the 1580-1600 nucleotides accounted for from sequencing results as reported by Ehresmann *et al.* [8].

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