

A Neutron Scattering Study of the Distribution of Protein and RNA in the 30 S Ribosomal Subunit of *Escherichia coli*

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The radii of gyration of the RNA and protein distributions of the 30 S subunit from *Escherichia coli* have been measured by neutron scattering experiments on the intact subunit. In addition the radius of gyration of the whole structure has been found, permitting estimation of the distance between the centers of mass of the RNA and protein distributions in the subunit. The results indicate that the radius of gyration of the protein parts is 73.7 ± 1.2 Å, while that of the RNA is 67.2 ± 2.2 Å. The radius of gyration of the whole particle is 71.4 ± 0.6 Å giving a $17.1^{+9.0}_{-10.9}$ Å separation between the protein and RNA centers of mass. Corresponding figures for the 50 S subunit are 73.4 ± 2.0 Å, 72.5 ± 1.5 Å, 78.0 ± 0.95 Å, and 57.7 ± 1.0 Å (Moore *et al.*, 1974). Thus it appears that the two subunits differ substantially in overall organization.

1. Introduction

The spatial organization of the proteins and RNA molecules which combine to form the ribosome is in many respects a paradigm of the next level of structure beyond single protein molecules. The size of objects of this class and the absence of crystals in many cases put these objects beyond the reach of conventional structural techniques. Consequently, a number of novel approaches have been proposed recently for their study. At least four of these are currently being applied to ribosomal structure: electron microscopic localization of specific antibody binding sites (Wabl, 1973, 1974), energy transfer measurements (Cantor *et al.*, 1974), crosslinking (Bickle *et al.*, 1972; Chang & Flaks, 1972; Lutter *et al.*, 1972), and neutron scattering (Engelman & Moore, 1972; Moore *et al.*, 1974).

In this paper we describe experiments using small-angle neutron scattering measurements of the 30 S ribosomal subunit to obtain radii of gyration for the protein and RNA mass distributions within the particle, as well as the radius of gyration of the whole structure. Each radius of gyration, R , is the second moment of the scattering mass distribution in question referred to its own center of mass, or

$$R^2 = \frac{\sum m_i r_i^2}{\sum m_i}$$

where m_i is the scattering mass of the i th element, a distance r_i from the center of mass. The centers of mass of the RNA and protein distributions in a ribosomal subunit need not coincide. Knowing the total radius of gyration of the subunit, R_T , and the radii for the protein and RNA distributions, R_P and R_R , we can find the separation of their centers of mass, Δ , using the relation:

$$R_T^2 = f_P R_P^2 + f_R R_R^2 + f_P f_R \Delta^2, \quad (1)$$

where f_P and f_R are the fractions of the total scattering due to the protein and RNA ($f_P + f_R = 1$). Thus, in principle, our measurements allow us to characterize the relative distribution of material in the 30 S subunit as well as to obtain information about the distributions of protein and RNA, separately. In an earlier report (Moore *et al.*, 1974) we described a similar experiment done on the 50 S subunit.

The results obtained indicate that a substantial separation, Δ , exists between the RNA and protein centers of mass in the 50 S subunit (Moore *et al.*, 1974). The 30 S subunit, however, is far more symmetrical; Δ is small. The protein and RNA in the 50 S subunit have approximately equal radii of gyration. In the 30 S subunit, on the other hand, the radius of gyration of the protein is significantly larger than that of the RNA, suggesting that the protein is located more towards the outside of the 30 S particle than the 16 S RNA.

For the convenience of the reader the Results section of this paper is divided into two parts: (a) deals with the problem of determining the strength with which ribosomal subunits, both 50 S and 30 S, ribosomal protein, and ribosomal RNA scatter neutrons as a function of their deuterium content, information essential for estimating weighting factors used to solve equation (1); section (b) gives the results of the scattering measurements on 30 S subunits leading to estimates of R_P , R_R and Δ .

2. Materials and Methods

(a) Cells and ribosomes

Escherichia coli MRE600 was the source of ribosomes and ribosomal subunits in this work. All the media for cell growth contained the minimal salts mixture M9 (Anderson, 1946). The carbon source or sources used to support growth are specified in the text for each experiment, as is the D_2O/H_2O ratio in the medium solvent. It was observed that the yield of cells per weight of carbon source was substantially improved by doubling the concentration of the salts mixture when acetate was used as the carbon source. D_4 -acetic acid was obtained from Prochem (Lincoln Park, New Jersey). When necessary, exchangeable hydrogen in media components was converted to deuterium by dissolving the materials in 100% D_2O and then flash evaporating to dryness. Media were sterilized by filtration through Millipore HA filters. Cells were generally grown to late log phase, chilled to 5°C by passage through a heat exchanger and harvested using a JCF-Z continuous-flow rotor in a Beckman J21 centrifuge. About 15 min were required for the chilling and harvesting of a 10-1 culture.

To economize on D_2O , heavy water was recovered from spent medium by flash evaporation. The water recovered was assayed for D_2O content by measuring its density in bromobenzene/xylene gradients with suitable standards as markers (Low & Richards, 1952). When growth was done on D_4 -acetate, only fresh 100% D_2O was used as the solvent. For other growth purposes, 100% D_2O was operationally defined as material with a deuterium content greater than 98%.

Ribosomal subunits were prepared from cells as described before (Moore *et al.*, 1974). Subunits were purified by two cycles of sucrose gradient centrifugation. Contamination of subunit preparations by the other subunit was assayed for by analytical sucrose gradient

centrifugation and found to be undetectable (<5%). Subunits were prepared for scattering measurements by dialysis against several changes of buffer I (0.5 mM-MgCl₂, 50 mM-KCl, 10 mM-Tris·HCl (pH 7.5)) over the course of 36 h. The D₂O content of this buffer was adjusted to suit the experimental situation. Ribosome concentrations were determined from absorbance at 260 nm.

(b) *Estimation of the protein content of ribosomes and ribosomal subunits*

E. coli MRE600 was grown on M9 medium using uniformly labeled [¹⁴C]glucose (New England Nuclear, Boston, Mass.) as the sole carbon source. 70 S ribosomes and ribosomal subunits were prepared in the usual manner (Moore *et al.*, 1974). A small amount of each sample was set aside and the bulk subjected to phenol extraction in the presence of sodium dodecyl sulfate (Kurland, 1960). RNA samples were concentrated by ethanol precipitation and then dialyzed overnight against 1 mM-magnesium acetate, 100 mM-KCl, 10 mM-Tris·HCl (pH 7.5). Samples were removed from each of the RNA and ribosome samples for liquid scintillation counting to detect ¹⁴C radioactivity. Optical densities were read on each sample at 260 nm. All optical densities and radioactivities were measured in triplicate. Radioactivities per $A_{260\text{ nm}}$ were calculated for all samples assuming that the hyperchromicity of rRNA is the same as that of ribosomes (Schlessinger, 1960). The number of carbon atoms per weight of protein or RNA was calculated from base composition data for rRNA (Spahr & Tissieres, 1959) and from the amino acid composition of bulk ribosomal protein (Spahr, 1962). Specific radioactivities were converted into estimates of relative amounts of RNA and protein in each sample. RNAs isolated from 70 S, 50 S and 30 S ribosomes made from a given preparation of radioactive cells had identical specific radioactivities to within 1%, as expected.

(c) *Measurement of buoyant density*

Buoyant densities of ribosome and rRNA samples were measured by isopycnic equilibrium centrifugation in order to determine the extent of deuteration. In the case of ribosomes, samples were fixed with formaldehyde prior to centrifugation in CsCl. The conditions used and the method of data analysis were those described by Hamilton (1971). rRNA samples were centrifuged, unfixed, in Cs₂SO₄ solution. RNA data were analyzed following Ludlum & Warner (1965). Centrifugation was done on a model E analytical ultracentrifuge using a four-sample, An-F rotor. Centrifugation was carried out typically for 18 h at 33,450 revs/min at 4°C. The location of the bands was detected photographically using ultraviolet optics. In the case of ribosome samples, the photographs were traced with a Joyce-Loebl densitometer and band positions measured from their centers of gravity. The bands formed by RNA samples in Cs₂SO₄ were extremely sharp; their locations could be measured directly from the photographs. Every run included a standard sample, either all H† rRNA or all H subunits. All densities within a given run were calculated relative to the reference sample. At least two separate runs were done on every unknown sample. The density difference between the unknown and the reference was the average of all values. These density differences were converted to deuteration estimates by comparison with the density difference between authentic all H and all D specimens. The absolute density difference found between all H and all D rRNA was $99.6 \pm 4\%$ of that calculated from atomic composition data assuming all non-carbon-bonded hydrogen exchanges in rRNA. The corresponding figure for the density difference between all H and all D 30 S subunits was $102.6 \pm 7.5\%$ of that calculated from atomic compositions.

(d) *Data collection*

Scattering data were collected using the Söller slit spectrometer of the High Flux Beam Reactor at the Brookhaven National Laboratory, as previously described (Moore *et al.*, 1974). Data were collected in 5 periods of about 1 week each, over the course of a year. Small variations in the experimental arrangement resulted in a variation of λ in the range of $4.09 \pm 0.01 \text{ \AA}$ to $4.20 \pm 0.01 \text{ \AA}$ (peak wavelength) in different experimental runs. The full width at half maximum of the wavelength band used was 0.1 Å, and the flux was

† Abbreviations used: all H, fully hydrogenated; all D, fully deuterated.

4×10^6 neutron $\text{cm}^{-2} \text{min}^{-1}$ as neutrons counted at the ^3He detector. Samples (0.8 ml each) were examined at 5°C in a thermally controlled specimen holder for periods of 1 to 12 h. Data scans were taken over a range of 2θ from 0.3° to 1.2° for radius of gyration measurements, and over the range of 2θ from 0.3° to 2.1° for the extended scans used in slit correction.

(e) *Contrast experiments*

In order to establish values for the scattering density of hydrogenated and deuterated ribosomes, experiments were carried out in which the forward scatter of fully hydrogenated or fully deuterated ribosomal subunits was measured as a function of the D/H ratio in the surrounding medium. In each measurement a series of data points from $2\theta = 0.4^\circ$ to 1.2° was collected and extrapolated to $2\theta = 0$ following Guinier (1939) using a variance-weighted least-squares fitting program. The scatter in the forward direction (I_0) was obtained together with its statistical precision. I_0 values were corrected for absorption and scaled to constant counting time and sample concentration. The square roots of such values are proportional to the difference in scattering density between the specimen and the solvent. For each experiment, a preparation of a ribosomal subunits was split into two portions and dialyzed against 100% and 0% D_2O buffer. After measurement at these D_2O concentrations, the samples were mixed to give samples at 75% and 25% D_2O , equilibrated for several hours at 5°C , and measured. Finally, a 50% D_2O specimen was prepared by mixing equal portions of the 75% and 25% D_2O preparations, equilibrated and measured. Background curves were collected on a similar set of dilutions of the 100% and 0% D_2O dialysis buffers. In all cases the concentration of D_2O was monitored by neutron absorption of the direct beam and found to be correct within $\pm 0.8\%$.

(f) *Data analysis*

The data were processed essentially as previously described (Moore *et al.*, 1974). Background curves were subtracted without smoothing from the experimental curves after scaling (Schelten *et al.*, 1972). The data were then fitted with a straight line in a plot of $\log I$ versus $(2\theta)^2$ using the variance-weighted least-squares program of Bevington (1969) to propagate errors due to counting statistics in values for the slope and intercept of the line. The intercept is $\log I_0$, and the slope of the line, α , is related to the radius of gyration, R , by (Guinier, 1939):

$$R = 0.4183\lambda \sqrt{-\alpha}.$$

Measurements of R were made at a series of concentrations over the range of 10 to 40 mg/ml to test for concentration effects. Slit correction was done on extended scan data as described before (Moore *et al.*, 1974).

3. Results

(a) *Scattering densities*

(i) *General considerations*

In a neutron small-angle experiment, the physical quantities that correspond to the weighting factors in equation (1) are total coherent elastic scattering lengths. At small angles a macromolecule can be thought of as a volume of uniform scattering length per unit volume, or "scattering density". The absolute scattering length of the molecule is simply its volume, V , times its scattering density, ρ . For a molecule in a solvent, scattering amplitudes are measured relative to the solvent. Thus the appropriate expression at small angle for the effective scattering amplitude of a molecule will be $(\rho - \rho_{\text{solvent}}) \cdot V$. The weighting factor for a macromolecule which is part of a larger aggregate is simply the effective scattering amplitude of the macromolecule divided by that of the whole structure. Scattering densities can be calculated from atomic compositions:

$$\rho = \frac{\sum_i N_i b_i}{V}, \quad (2)$$

where N_i is the number of atoms of the i th species in the molecule, b_i is the atomic scattering length of the i th species and V is the molecular volume.

In order to obtain information about the structure of individual components of a two-component structure like the ribosome, using radius of gyration techniques, one must have a means of varying their relative contributions to the radius of gyration observed for the whole structure. From the discussion above it is clear that this requirement can be met by varying the scattering density of the components themselves and/or that of the solvent in which the structure is dissolved. The reason experiments of this type are easy to do by neutron scattering is that the required alterations in scattering density can be brought about by substituting deuterium for hydrogen in either the component in question or the solvent. These two isotopes of hydrogen have markedly different atomic scattering lengths. Thus by proper manipulation of H:D content, specimens can be prepared in which one or the other component dominates the small-angle scattering of the aggregate. Provided the weighting factors can be estimated, equation (1) can be applied to derive the radii of gyration of the two components and the separation of their centers of mass from radius of gyration data obtained on such specimens.

Because the contribution of hydrogen to the scattering densities of macromolecules is crucial, it is useful to reformulate equation (2) in such a way as to recognize that contribution directly. In so doing it should be remembered that two classes of hydrogen exist in macromolecules, those which exchange with solvent hydrogen and those which do not. Thus we have:

$$\rho = \frac{1}{V} \left[\sum_i N_i b_i + (1-s)N_{\text{F}}b_{\text{H}} + sN_{\text{F}}b_{\text{D}} + (1-x)N_{\text{E}}b_{\text{H}} + xN_{\text{E}}b_{\text{D}} \right]. \quad (3)$$

The summation in equation (3) now applies to all non-hydrogen atoms. N_{F} and N_{E} are the number of fixed and exchangeable hydrogens in the structure, and s and x are the fractions of the fixed and exchangeable hydrogens substituted with deuterium. b_{H} and b_{D} are the scattering lengths of H and D, respectively. Gathering constant terms together we get:

$$\rho = \frac{1}{V} \left[\left(\sum_i N_i b_i + N_{\text{F}}b_{\text{H}} + N_{\text{E}}b_{\text{H}} \right) + N_{\text{F}}(b_{\text{D}} - b_{\text{H}})s + N_{\text{E}}(b_{\text{D}} - b_{\text{H}})x \right]. \quad (4)$$

Thus we can express ρ for a macromolecule as:

$$\rho = A + Bs + Cx, \quad (5)$$

where

$$A = \frac{1}{V} \left(\sum_i N_i b_i + N_{\text{F}}b_{\text{H}} + N_{\text{E}}b_{\text{H}} \right),$$

$$B = \frac{N_{\text{F}}}{V} (b_{\text{D}} - b_{\text{H}}),$$

and

$$C = \frac{N_{\text{E}}}{V} (b_{\text{D}} - b_{\text{H}}).$$

Note that A is simply the scattering density of the molecule fully substituted with hydrogen. In making calculations based on equation (5) it is useful to remember that all hydrogens bonded to carbon are effectively non-exchangeable and most hydrogens not bonded to carbon will exchange. It is assumed in this work that x will always equal the fraction of deuterium in the aqueous buffer in which the macromolecule is suspended.

Atomic scattering lengths for thermal neutrons are available in the literature. Accordingly, coefficients A , B , and C can be calculated for a macromolecule provided one knows (a) its relative atomic composition; (b) its partial specific volume, and (c) the fraction of its hydrogens which exchange.

A calculation of this type was carried out to determine the dependence of scattering density on deuteration for the solvent, buffer I, used in these experiments. Since the atomic density of both H_2O and D_2O at the experimental temperature of $5^\circ C$ are known, an accurate estimate can be made *a priori*. Thus, since $s = 0$ for water and the buffer components,

$$\rho_0 = -0.553 + 6.914x \quad (6)$$

where the dimensions of ρ_0 are 10^{-14} cm/ \AA^3 . This equation is nearly the same as that for mixtures of pure H_2O and D_2O . In the analysis of experimental data, values for ρ_0 based on this equation are taken as the standard for comparison.

In principle, the scattering density for the 50 S or 30 S subunit from *E. coli* might also be calculated. The amino acid composition of the protein and the base composition of the RNA portions of the ribosome, and hence their atomic compositions, are known with reasonable accuracy. There are, however, three parameters required for the calculation which are less well established. The first of these is the weight fraction of both subunits which is protein. This parameter is difficult to measure, and, in addition, depends on the method of subunit preparation (Hill *et al.*, 1970). Second, \bar{v} , the partial specific volume of the ribosome, enters the calculation in a critical manner. Because one measures the square of the difference in ρ between object and solvent in a scattering experiment, an error in \bar{v} of 1% can lead to measurable discrepancies between calculated and observed intensities. Third, the fraction of the hydrogen in the ribosome which exchanges with solvent is unknown. In view of these obstacles to calculating scattering length equations for ribosomal subunits from first principles, we decided to approach the problem experimentally.

(ii) *Determination of the weight fraction of protein in ribosomal subunits*

When *E. coli* is grown in a minimal medium containing glucose as the only carbon source, and that glucose is uniformly labeled with ^{14}C , the molecules the cells make will also be uniformly carbon-labeled (Roberts *et al.*, 1957). Ribosomes and ribosomal subunits were prepared from cells labeled with ^{14}C in this manner using the technique described in Materials and Methods, and the amount of radioactivity per unit optical density at 260 nm was determined for 70 S, 50 S and 30 S ribosomes before and after phenol extraction. It is known that the hyperchromicity of free rRNA is the same as that of rRNA in the intact ribosome (Schlessinger, 1960), and that phenol extraction removes all protein from rRNA (Kurland, 1960). Hence a comparison of the specific radioactivity of the preparations before and after phenol extraction gives the relative amount of carbon in protein and RNA. This ratio is readily converted into a weight

TABLE 1

Composition of high-salt washed ribosomes

Species	% Protein (w/w)	M_r RNA	M_r protein	Total M_r
70 S	39.0 ± 0.85	1.65×10^6	$1,055,000 \pm 23,000$	2.70×10^6
50 S	30.2 ± 0.2	1.10×10^6	$476,000 \pm 3000$	1.58×10^6
30 S	36.4 ± 1.2	0.55×10^6	$315,000 \pm 10,000$	0.87×10^6

The weight fraction of protein in the three ribosomal species was measured as described in Materials and Methods. The values listed are averages of measurements on three independent preparations. The errors are standard errors of the mean. The molecular weight estimates for the protein contents and total molecular weights of the three ribosomal particles are calculated from weight percentages using values from the literature for the molecular weights of their RNA components (Kurland, 1960).

ratio using the atomic compositions of rRNA and bulk ribosomal protein. The weight fractions which result (Table 1) refer to the salt-free, anhydrous structure.

These data clearly indicate that the 30 S subunit is substantially richer in protein than the 50 S subunit. Assuming values from the literature for the molecular weights of rRNA (see Table 1), these compositions can be used to obtain estimates for the total molecular weight of the 70 S, 50 S, and 30 S ribosomes. The molecular weights found for the two subunits, 1.575×10^6 and 0.867×10^6 , are in excellent agreement with the values obtained in solution for high-salt washed subunits by Hill *et al.* (1970).

The 70 S particles obtained contained about 250,000 molecular weight of protein in addition to the contribution due to its subunits, leading to an estimate for the molecular weight of this structure of 2.7×10^6 , a value very close that obtained by Hill *et al.* (1970) of 2.65×10^6 . Thus it appears that the 70 S structure is appreciably larger than the sum of its two subunits, as normally isolated. This difference in weight is due to the loss of protein during subunit preparation (Hill *et al.*, 1969a; Moore, 1971).

(iii) *Calculation of the ratio (B + C)/A for the 30 S and 50 S subunits of Escherichia coli*

The base composition of rRNA (Spahr & Tissieres, 1959) and the amino acid composition of bulk protein from the two subunits (Spahr, 1962) are known. Therefore, the mass data in Table 1 lead directly to a calculation of the atomic composition of the two subunits (Table 2). These two compositions together with scattering length data for thermal neutrons (C. G. Shull, as quoted in Schoenborn & Nunes, 1972) allow one to calculate the ratio of (B + C) to A for the two subunits:

$$\frac{B + C}{A} = \frac{(N_F + N_E)(b_D - b_H)}{(\sum N_i b_i + N_F b_H + N_E b_H)}$$

(see equation (5)). The values found are 1.637 and 1.724 for the 50 S and 30 S subunits, respectively. These ratios are independent of assumptions about the molecular volume of the two subunits and hydrogen exchangeability. Accordingly, they provide us with a useful relation between the three coefficients needed to describe the scattering properties of each subunit.

TABLE 2

Atomic compositions of bulk ribosomal components

Atom	Ribosomal RNA ($1.1 \times 10^6 M_r$)	50 S protein (476,000 M_r)	30 S protein (315,000 M_r)
C	32,472	20,974	13,817
O	23,630	6127	4021
N	13,298	6225	4203
P	3394	—	—
S	—	132	84
H (C bonded)	26,084	26,551	17,352
H (non-C bonded)	10,518	9032	5524

Knowing the base composition of rRNA from *E. coli* (Spahr & Tissieres, 1959), the amino acid composition of bulk 50 S and 30 S protein (Spahr, 1962) and the molecular weight of protein and RNA in both subunits (Table 1), the atomic compositions of these materials are readily calculated.

(iv) *Determination of the absolute magnitude of A and C for the 50 S subunit*

50 S subunits were prepared from cells grown on minimal medium both in H₂O solvent with H glucose as the carbon source (H50) and in 100% D₂O solvent with D₄ acetate as the carbon source (D50). The 50 S subunits from the H₂O medium are fully hydrogen substituted (i.e. $s = 0$); the subunits from the D medium are fully D

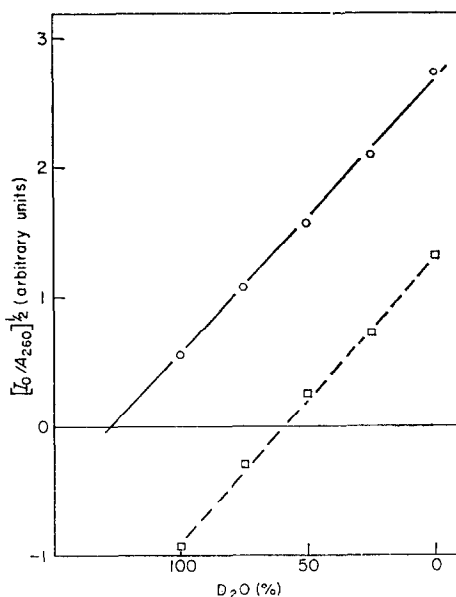


FIG. 1. Forward scatter of deuterated and hydrogenated 50 S subunits *versus* buffer deuteration. The scattering at zero angle, I_0 , was obtained by extrapolation of the small-angle scattering curves of 50 S subunits in solvents with D fractions of 0.0 to 1.0. The Figure is a plot of a_0 *versus* the D content of the solvent. $a_0 = (I_0/A_{260})^{1/2}$ where A_{260} is the absorbance of the sample at 260 nm and I_0 is the forward scatter per 1000 monitor counts corrected for absorption. —○—○—, a_0 for 50 S subunits which are all D in non-exchangeable positions; --□--□--, a comparable set of experiments with all H 50 S subunits.

substituted (i.e. $s = 1.0$) in non-exchangeable positions. Low-angle scattering profiles were collected on both specimens in solvents made from D_2O - H_2O mixtures ranging in composition from 0% to 100% (v/v). The forward scattering of each sample, I_0 , was obtained by extrapolation of the low-angle data to $2\theta = 0$ by the method of Guinier (1939). These observed I_0 values were then corrected for slit smearing effects, assuming that the extended scattering curve in each case could be approximated as a Gaussian having the observed radius of gyration. For corrected I_0 values it should be true that $(I_0/\text{weight})^{\frac{1}{2}}$ for any specimen is proportional to the difference in ρ between the specimen and the solvent. The error produced in $(I_0/\text{weight})^{\frac{1}{2}}$ by assuming a Gaussian shape for all scattering profiles is at most one or two per cent. Figure 1 is a plot of $(I_0/\text{weight})^{\frac{1}{2}}$ in arbitrary units for H50 and D50 subunits as a function of the D_2O content of the solvent. In both cases, as the concentration of D_2O in the solvent increases from 0% the scattering from the samples decreases, showing that ρ_{D50} and ρ_{H50} are both positive relative to ρ_{H_2O} . In the case of the H50 sample, $\rho_{H50} = \rho_0$ when the solvent is about 59% D_2O . At concentrations above this value, $\rho_{H50} < \rho_0$. In the case of the all D sample $\rho_{D50} > \rho_0$ for all solvent compositions.

Since forward scatter is proportional to $(\rho - \rho_0)^2$, equations (5) and (6) predict that plots of this kind should be straight lines, as observed (Schelten *et al.*, 1972). Furthermore, provided the partial volume of the structure is uninfluenced by substituting D for H in non-exchangeable sites, the slopes for the all D and all H samples should be identical. The slopes of the least-squares fit lines to the two data sets agree to within 8%. The statistics of the data are good enough for this difference to be real. The difference is sufficiently small, however, for us to assume constancy of the partial volume of the ribosome for the purposes of this work.

The data in Figure 1 provide us with the values for x that satisfy the condition that $\rho_{\text{particle}} = \rho_{\text{solvent}}$. Using equation (6) we can calculate ρ_{particle} for those conditions. Since we have these data for both the all H and the all D subunit, we can derive two independent relations between A , B , and C , from this information. The third relation necessary to solve for the three parameters is given by the ratio derived from atomic composition data (see above).

The outcome of the analysis is presented in Table 3. The values for A , B , and C obtained by *a priori* calculation taking $\bar{v} = 0.585 \text{ cm}^3/\text{g}$ (Hill *et al.*, 1969a) are in-

TABLE 3
Cross-section parameters for the 50 S subunit from E. coli

Parameter	Experimental	Calculated
A	2.969 ± 0.050	2.997
$B + C$	4.861 ± 0.080	4.907
B	3.861 ± 0.100	3.578 (min.)
C	1.000 ± 0.100	1.329 (max.)

The quantities A , B and C are defined in the text in equation (5). The calculated values given were derived from atomic scattering lengths (Schoenborn & Nunes, 1972) and the atomic composition data given in Table 2 assuming that $\bar{v}_{50} = 0.585 \text{ cm}^3/\text{g}$. The values calculated for B and C assume that all hydrogens not bonded to carbon will exchange, hence the former estimate, is a minimum value and the latter a maximum one. The experimental values given for A , B and C were derived from data given in the text. The dimensions of these parameters are $10^{-14} \text{ cm}^3/\text{A}^3$.

TABLE 4

Predicted scattering behaviour based on experimental values from Table 3 compared to observed scattering behaviour

Quantity	Prediction	Observation	Predicted/ observed
(1) Match to all D	1.248	1.268	0.985
(2) Match to all H	0.596	0.5887	1.012
(3) ρ_D/ρ_H in H_2O	2.096	2.13	0.984
(4) ρ_D/ρ_H in D_2O	-0.614	-0.608	1.010

A number of readily measured quantities may be calculated using the experimentally derived scattering parameters for the 50 S subunit (Table 3) and equation (6). These predicted values are compared to experimental data in this Table. The quantities shown are: (1) the fraction of D_2O in the solvent required so that $\rho_{50} = \rho_0$ for the all D 50 S subunit, (2) the fraction of D_2O in the solvent required to make $\rho_{50} = \rho_0$ for all H 50 S particles, (3) the ratio of the scattering lengths of all D and all H subunits in an H_2O solvent, and (4) the corresponding ratio on an all D_2O solvent.

cluded in Table 3 for comparison. Table 4 compares a number of measured quantities with the values predicted, using the experimentally determined parameters given in Table 3. All the predicted values agree with experimental findings to within 1.5%. This level of agreement is better than that obtained with the *a priori* calculation, regardless of what value is placed on C , as the reader may readily satisfy himself.

The value observed for A implies a partial volume for the 50 S subunit of 0.590 cm^3/g , well within the range found by Hill *et al.* (1969a) by direct measurement. The value found for C indicates that about 76% of the hydrogen not bonded to carbon exchanges in the 50 S subunit.

(v) *Scattering equations for 23 S ribosomal RNA and 50 S ribosomal protein*

The scattering equation for the ribosome should be the sum of the equations for rRNA and protein, weighted according to the volume fraction of the ribosome that each species occupies. In fact when the scattering length equation of the ribosome is calculated in this way, values for A , B and C are found that do not agree with observation. The reason the computation fails is that when standard values for \bar{v}_{RNA} and $\bar{v}_{protein}$ of 0.577 cm^3/g (Ortega & Hill, 1973) and 0.740 cm^3/g (Spahr, 1962), respectively, are used and $\bar{v}_{ribosome}$ is calculated from the weight composition of the particle, the value is found to be 0.625 cm^3/g . The measured value for 50 S subunits in solution is 0.590 cm^3/g . The 50 S subunit is more compact than the partial volumes of its components would suggest. In order to estimate scattering density equations for the two species *in situ*, the following procedure was used. Atomic compositions and standard values for \bar{v} were used to obtain coefficients for both species. All coefficients were then multiplied by a constant factor, the computed value for \bar{v} (0.625 cm^3/g) divided by the measured value for \bar{v} (0.590 cm^3/g). This procedure simply assigns the "volume deficit" equally between the two species. In addition, it was assumed that the percentage of the hydrogens which exchange with solvent in rRNA and protein separately is the same as the percentage which exchange in the whole particle. The scattering length equations that result are:

$$\rho_{50 \text{ S protein}} = 1.826 + 5.436s + 1.282x \quad (7)$$

and

$$\rho_{23 \text{ S RNA}} = 3.608 + 3.006s + 0.829x. \quad (8)$$

Because of the assumptions made in their derivation, these equations must be regarded as approximations, not definitive statements on the scattering properties of these materials *in situ*. (The impact of these assumptions on the final results obtained is discussed below.)

It might be noted in passing that equation (7) predicts that all H ribosomal protein should match a D_2O - H_2O solvent when the D_2O content of the solvent is about 42%. This value compares well with the value of 40% found for hemoglobin by Schelten *et al.* (1972), and with our own preliminary value of 44% for *E. coli* RNA polymerase.

(vi) *Scattering length equation for the 30 S subunit*

A scattering length equation can also be constructed for the 30 S subunit. The base compositions of 16 S RNA and 23 S RNA are virtually indistinguishable in *E. coli* (Spahr & Tissieres, 1959). Equation (8), therefore, should adequately describe the scattering properties of 16 S RNA when due allowance is made for differences in degree of exchangeability. The amino acid composition of 30 S protein differs somewhat from that of 50 S protein, requiring appropriate alterations in equation (7). By combining these computed parameters in the usual, volume-weighted ratio, estimates for A and $(B + C)$ can be obtained. The problem that remains is to measure C , the parameter for the scattering contribution of exchangeable hydrogens. This value can be calculated from an analysis of the dependence of scattering amplitude on solvent composition for fully H subunits. The results of such an experiment are shown in Figure 2. The all H 30 S subunit matches a solvent composed of $56.8 \pm 0.3\%$ D_2O . This value leads to the following equations for the 30 S subunit:

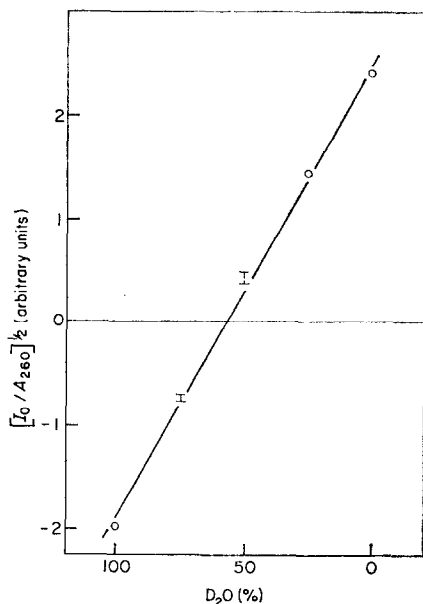


FIG. 2. Forward scatter of hydrogenated 30 S subunits *versus* buffer deuteration. The scattering at zero angle, I_0 , was obtained for 30 S subunits as a function of the D fraction in the buffer and the corrected amplitude obtained (see Fig. 1).

$$\rho_{30 \text{ S}} = 2.884 + 4.109s + 0.863x, \quad (9)$$

$$\rho_{16 \text{ S RNA}} = 3.608 + 3.0825s + 0.754x \quad (10)$$

and
$$\rho_{30 \text{ S protein}} = 1.905 + 5.466s + 1.067x. \quad (11)$$

These equations imply a partial volume for the 30 S subunit of $0.600 \text{ cm}^3/\text{g}$, in agreement with Hill *et al.* (1969a), and that $67.6 \pm 2.5\%$ of the hydrogens not bonded to carbon exchange.

The equations obtained for the 30 S and 50 S subunit can be checked for consistency by comparing the value they predict for the ratio

$$\frac{(I_0/O.D._{260})_{50 \text{ S}}}{(I_0/O.D._{260})_{30 \text{ S}}},$$

for fully hydrogen substituted particles in a 100% D_2O solvent, with the value found by observation. The predicted value for this ratio from the scattering equations is 1.276, allowing for slit correction; the observed ratio is 1.255. The difference between the observed ratio and predicted ratio is within experimental error. Efforts to verify the 30 S ribosome equations further, using fully deuterated particles, were frustrated by our inability to prepare fully deuterated preparations of adequate stability. The causes for this difficulty have not been determined.

(b) Scattering measurements

In this section we describe our results on the effects of growth conditions on the levels of deuteration of ribosomal protein and rRNA. Growth conditions were found which lead to marked differential uptake of deuterium into RNA and protein. Using the scattering length equations developed in section (a), solvent conditions were chosen so that the differentially labeled particles will scatter predominantly from RNA or protein. Radius of gyration measurements were done and values calculated for R_R , R_P and Δ .

(i) Determination of the extent of deuterium substitution in ribosomal protein and RNA

Throughout this work, the extent of deuteration of ribosome samples was estimated from I_0 . In order to compare I_0 values properly they first had to be corrected for slit smearing effects. This correction was done by the Gaussian approximation outlined above for data sets collected over a narrow angular range. Data collected over an extended range of angles were corrected by the standard technique (see Materials and Methods). In a typical experiment, following data correction, (I_0/A_{260}) of the unknown sample would be compared to (I_0/A_{260}) of the same subunit fully substituted with H in 100% D_2O , measured at the same time. Using the appropriate subunit scattering length equation and knowing the solvent composition, a value for s , the fraction of the fixed hydrogens which are substituted, was found.

Banding in CsCl was examined as an alternative to comparing I_0 values for obtaining s values for subunits (see Materials and Methods). A good correlation was observed between the results obtained by the two methods. Moreover, the absolute magnitude of the density difference between all H and all D subunits measured in CsCl agrees well with predictions based on atomic composition. However, the bands formed by formaldehyde-fixed ribosomes in CsCl are so wide that it is difficult to determine their midpoints accurately. The standard error of the mean for s as measured by the CsCl

method is about 8% for a typical sample measured in triplicate, which is an error of unacceptable magnitude. The error in the I_0 method, as estimated from repeated measurement on similarly prepared specimens, was about 2%.

To determine the deuterium content of a sample of RNA, its buoyant density was compared to that of all H RNA (see Materials and Methods). The density difference found between these two species relative to that between all H and all D RNA was taken as the degree of deuterium substitution for the sample in question. When these data were used for calculations on deuterium substitution of RNA in intact ribosomes, it was assumed that they were applicable to the non-exchangeable hydrogens not bonded to carbon as well as to carbon-bonded hydrogen. (These hydrogens are about 8% of the total.)

Using the atomic composition of the subunit and its degree of deuteration, s , the total number of deuterium molecules it contains can be estimated. This number less the number of D's in RNA is the number of D's in protein. Using the atomic composition of the protein, this figure is readily converted into an estimate of s for the protein.

(ii) *Dependence of ribosomal deuteration on cellular growth conditions*

Table 5 gives data on the deuteration of subunit preparations as a function of the medium on which the cells from which they were derived were grown. Levels of substitution were estimated as outlined above. It will be noted that the efficiencies of

TABLE 5

The dependence of RNA and protein deuteration on growth conditions

	Medium		RNA	% Deuteration Protein	Total
	Solvent (%D)	C source			
(1)	100	D ₃ acetate	100	100	100
(2)	100	Glycerol (H)	60	90	75
(3)	95	Glucose (H)	49	79	64
(4)	85	Glucose (H)	37	67	52
(5)	75	Glucose (H)	34	50	42
(6)	100	Glucose (H), nucleosides (H)	23.6	94.6	59.2
(6')	same as above,	for 30 S	23.6	101.9	67.8
(7)	65	Glucose (H), nucleosides (H)	11.4	48.3	29.9
(7')	same as above,	for 30 S	11.4	53.8	35.4
(8)	100	Pyruvate (H), amino acids (H)	68	20	40

The amounts of deuterium incorporated into ribosomes, ribosomal protein and ribosomal RNA are given as a function of the conditions under which cells are grown. In all cases the medium contains the salts mixture M9 (Anderson, 1946). The solvent composition is listed in each case as is the carbon source(s) used. When D₃ acetate was the carbon source, 10 g/l of medium were used and the salt mixture used was twice that for M9. For glycerol and glucose medium, 4 g of carbon source were used per liter. The pyruvate medium included 10 g pyruvate per liter. The media containing nucleosides included 0.1 g adenosine, uridine and cytidine per liter. Medium (8) included 50 mg of all 20 amino acids per liter. The extent of deuterium incorporation into ribosomes was estimated from their forward scattering by comparison with all H specimens using equation (6), and either equation (9) or Table 3, as appropriate. The deuteration of RNA was measured by equilibrium sedimentation in Cs₂SO₄ (see Materials and Methods). The deuteration of protein was taken as the difference between the overall deuteration and the deuteration of the RNA, due allowance being made for the extent of exchangeability of hydrogen in the two subunits.

transfer of hydrogen from carbon sources such as glucose or glycerol to RNA and protein in the presence of D_2O differ dramatically. Most of the non-exchangeable hydrogen in RNA is in its ribose moiety. Ribose is derived from glucose by a short pathway involving little hydrogen exchange with the medium. Amino acids, on the other hand, are derived from glucose by long pathways involving a great deal of exchange. Hence in samples (2) and (3), for example, we see very high levels of deuteration in protein and quite low levels in RNA. When pyruvate is used as the carbon source, on the other hand, ribose synthesis requires a long sequence of events. We expect a poor transfer of pyruvate hydrogen to RNA, as observed (sample (8)).

Entries (6') and (7') in Table 5 give composition values for 30 S subunits prepared under the same growth conditions as the 50 S particles described in entries (6) and (7), respectively. Taking the deuteration of RNA as the same in corresponding samples, we expect to find (nearly) identical values for the levels of deuterium in protein in the two subunits. The comparison of the protein deuterations for these sample pairs in fact provides a sensitive test of the internal consistency of the scattering density equations and the validity of the techniques used for estimating deuteration levels. The protein deuteration values agree to within 10% in both cases, a satisfactory outcome. It is impossible to have a substitution level of 101.9% in 30 S protein, of course. The appropriate value to take here is something like the mean of the 30 S and 50 S values.

(iii) *Sample preparation and solvent conditions for radius of gyration measurements*

Three different specimen-solvent pairs must be measured to estimate the radius of gyration of the protein and RNA parts of the ribosome and the separation between the two mass distributions. These specimens and solvents must be chosen so that the relative contribution of RNA and protein to the total scattering differs substantially among the three pairs.

TABLE 6
Calculation of weighting factors (f) for 30 S samples

Specimen	Degree of substitution	Deuteration of solvent		f
	s	x	$(\rho - \rho_0)$	
I. Fully substituted with H	Protein 0	1.0	-3.39	0.56
	RNA 0	1.0	-2.00	0.44
II. Protein weighted	Protein 0.95	0.7	+3.56	0.82
	RNA 0.24	0.7	+0.57	0.18
III. RNA weighted	Protein 0.54	1.0	-0.45	0.17
	RNA 0.11	1.0	-1.65	0.83

This Table shows the derivation of the weighting factors (f_s) for the RNA, and protein scattering from each of the three specimens measured in these experiments. The levels of deuteration in RNA and protein in each sample (see Table 5, entries (6') and (7')) are listed as well as the fraction of D_2O in the solvent (x) used. $(\rho - \rho_0)$ is the contrast between the solvent and the component in question (see equations (8), (10) and (11)). The weighting factors are the fractions of the total scattering length of each specimen due to RNA or protein.

30 S subunits were prepared from cells grown on minimal media containing H glucose and H nucleosides using 100% D₂O and 65% D₂O as the solvents. The degree of deuterium substitution into protein and RNA under the two sets of growth conditions are given in Table 5 entries (6) and (7). The third specimen used consisted of 30 S subunits grown on a 100% H₂O medium. The solvent chosen for the material derived from the cells grown on 100% D₂O was 70% D₂O; for the other two samples the solvent was 100% D₂O. Using the appropriate scattering length equations, the relative contribution of the RNA and protein portions to the neutron scattering of each specimen can be calculated. The result is given in Table 6. In the fully hydrogenated specimen, protein and RNA contribute roughly equally to the total scattering length of the particle. The particles grown on 100% D₂O and immersed in 70% D₂O scatter neutrons predominantly from protein, while the reverse is true of the particles grown on 65% D₂O in a 100% D₂O solvent.

(iv) Scattering measurements

Samples of the three varieties just described were prepared for scattering measurement by equilibration with buffer of the appropriate D₂O content. The scattering profiles of the samples and fractions of their final dialysis buffer were measured using the Soller slit diffractometer described previously (Moore *et al.*, 1974; see Materials and Methods).

The low-angle region of the scattering curve of a solution of identical particles is well approximated by a Gaussian. When such data are plotted as log of the scattered intensity *versus* the scattering angle squared, a linear plot should result. The slope of such a plot is proportional to the radius of gyration of the particles squared (Guinier, 1939). Figure 3 shows an example of a Guinier plot of low-angle data for each of the three types of specimen studied in this work. The data are arbitrarily scaled in this

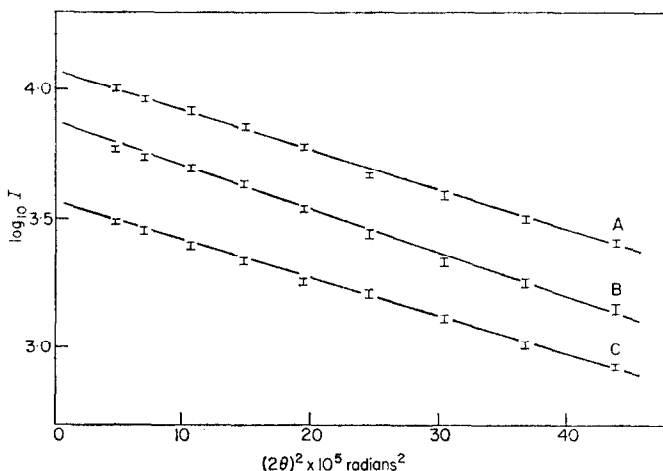


FIG. 3. Examples of Guinier plots for all H, protein-contrasting and RNA-contrasting 30 S subunits. Plots of $\log I$ versus $(2\theta)^2$ are shown for the small-angle scattering of different preparations of 30 S subunits. Each data set is fitted with a straight line by a variance-weighted least-squares procedure and the radius of gyration is obtained from the slope, a , as $R = 0.4183\lambda\sqrt{-a}$. The data are arbitrarily scaled with respect to $\log I$ for convenience in plotting them on the same graph. The uppermost curve (A) is the scatter of all H 30 S subunits, the middle curve (B) of protein-weighted 30 S subunits and the lower curve (C) of RNA-weighted subunits, and the radii obtained are $R_T = 69.34 \pm 0.47$, $R_P = 71.48 \pm 0.52$ and $R_R = 66.33 \pm 0.71$, respectively. Within error, the curves are linear to at least $I_0/4$.

case so they may be plotted conveniently on the same graph. The Guinier plots for all three specimens are linear at least to $I_0/4$, within the statistical accuracy of the data. Radii of gyration were calculated from such data sets by a variance-weighted least-squares determination of the slope of the corresponding Guinier plots. The radius of gyration of fully hydrogenated 30 S subunits was measured on 17 samples derived from four independent preparations at concentrations ranging from 100 to 700 A_{260}/ml . In the case of the protein and RNA weighted material 12 and 11 samples, respectively, were measured from four different preparations in the first case and three preparations in the second. The range of concentrations covered was the same as in the case of fully hydrogenated material.

In principle the radius of gyration observed for any specimen may have a concentration dependence. In order to determine the concentration dependence for 30 S subunits under these buffer conditions, a variance-weighted least-square procedure was used to extrapolate the radii of gyration obtained to zero concentration. The data considered were the all H data which was most abundant and of the best statistical quality due to the large scattering density of all H specimens relative to 100% D_2O . The data obtained from each preparation of all H subunits were extrapolated independently. No consistent indication of a non-zero slope for the extrapolation was found. The extrapolations for some data sets had small upward slopes, others had the opposite. Accordingly, the radius of gyration at zero concentration for all three types of specimen was calculated as the variance-weighted average of all values obtained. The averages are given in Table 7 as the "observed" radii of gyration. The error

TABLE 7
Observed and corrected radii

Sample	Description	Observed R_g (Å)	Corrected R_g (Å)
I	All H	70.4 ± 0.6	71.4 ± 0.6
II	Protein weighted	71.7 ± 0.3	72.9 ± 0.3
III	RNA weighted	67.7 ± 0.4	68.6 ± 0.4

The values found for the radius of gyration of each type of specimen are listed under Observed R_g . The error given for the all H specimen is the standard error derived from a variance-weighted least-squares extrapolation of values measured at a variety concentrations to zero concentration. The errors given for samples II and III are standard errors of the mean. The observed radii were corrected for slit smearing effects as given in Materials and Methods.

assigned to the all H data is the error which would be given them had they been extrapolated to zero concentration by variance-weighted least-squares fitting. The other two data sets are assigned errors which are standard errors of the mean. We assume, thereby, that the slope found for the all H data is appropriate for the other two data sets. The data are consistent with this assumption.

Low-angle data of the type considered here are subject to systematic error due to the size and shape of the beam used for irradiation of the specimen and the size and shape of the aperture of the detector used to measure scattered radiation. This aberration can lead to errors in estimation of the radius of gyration of a specimen. It is well known that the magnitude of error depends not only on the instrumental geometry,

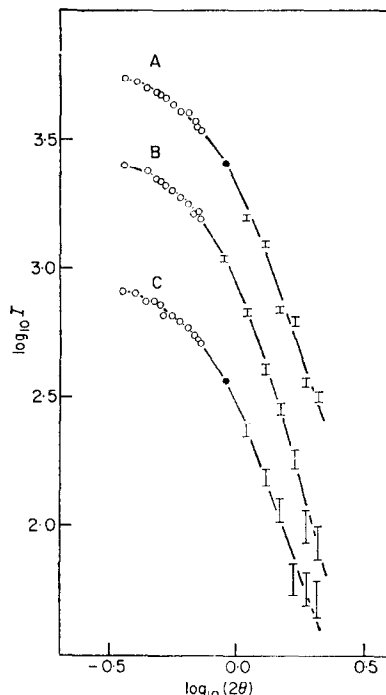


FIG. 4. Extended scattering curves of 30 S subunits with Hermite polynomial fits. Plots of $\log I$ versus $\log 2\theta$ are shown for all H (curve A), protein-weighted (curve B) and RNA-weighted (curve C) data sets for 30 S subunits. The vertical scale has been arbitrarily set for convenience in representation on a single graph. Each data point is represented together with its error, and the curves shown are the Hermite polynomial representation of the data used in the slit height correction algorithm of Hossfeld (1968).

but also on the shape of the scattering profile itself. A number of computational techniques are available for correcting data for this effect. The method used here was to correct first for slit height effects using the Hermite polynomial method of Hossfeld (1968) and then to correct for slit width aberrations by Fourier deconvolution (Guinier & Fournet, 1955). In order to carry out such procedures, the scattering profile must be measured to as high an angle as possible. Figure 4 shows extended data sets collected on all three specimen types at high ribosome concentration. The data are once again scaled arbitrarily to permit display on a single plot. All three scans were continued out to $2\theta = 2.1^\circ$ at which point the level of signal-to-noise had fallen to the point where the time available for data collection made it impractical to continue. The smooth curves through the data points are the optimal Hermite polynomial fits found by a variance-weighted, multiple regression method (Bevington, 1969). The smoothed curves were the input data for the remainder of the correction algorithm. The corrections found for these data sets were applied, additively, to the average radius of gyration for each type of specimen to yield the corrected radii of gyration given in Table 7.

(v) *Parameters for the 30 S subunit*

The corrected radii of gyration (Table 7) and the weighting factors for each specimen (Table 6) may be combined according to equation (1) to give three simultaneous

TABLE 8

Derived parameters for the 30 S and 50 S subunits

	30 S	50 S
R_R	$67.2 \pm 2.2 \text{ \AA}$	$72.5 \pm 1.5 \text{ \AA}$
R_P	$73.7 \pm 1.2 \text{ \AA}$	$73.4 \pm 2.0 \text{ \AA}$
Δ	$17.1 \pm_{19.0}^{9.0} \text{ \AA}$	$57.5 \pm 10 \text{ \AA}$

The values estimated for the radii of gyration of the RNA (R_R) and protein (R_P) mass distributions are given, as is the separation of their centers of mass (Δ). The values are computed using equation (1), the corrected radii given in Table 7 and the f_s values in Table 6. The 50 S data are taken from Moore *et al.* (1974).

equations in R_P , R_R , and Δ . When these equations are solved, values for R_P , R_R , and Δ are found for the 30 S subunit as given in Table 8. The corresponding values for the 50 S subunit are given for comparison (Moore *et al.*, 1974). The standard errors associated with each parameter were calculated by propagating the original counting errors in the data sets and combining them with the estimated error in the weighting factors (f), which is 2%. Accordingly, these errors reflect our best estimate of the precision of the determination. Systematic factors in the data collection and processing are not considered. It will be noted that the error in Δ is greater in the negative direction than in the positive, reflecting the fact that the parameter measured is Δ^2 not Δ .

4. Discussion

The basic result of these experiments can be deduced from the radius of gyration data in Table 7. Protein is distributed towards the periphery of the 30 S subunit and RNA localized around its center. The 30 S data are qualitatively different from the 50 S radius of gyration data we have obtained by the same means (Moore *et al.*, 1974). In the case of the 50 S subunit, both the protein and RNA weighted samples had radii less than that of the all hydrogenated 50 S subunit. This result indicates that the centers of mass of the RNA and protein distributions in the 50 S subunit must be separated by a substantial amount. Thus we can conclude that the two ribosomal subunits differ in overall organization.

These qualitative conclusions are independent of the assumptions we were required to make in deriving the scattering length equations. The partial volumes for ribosomal protein and RNA implied by the scattering length equations used above are $0.698 \text{ cm}^3/\text{g}$ and $0.544 \text{ cm}^3/\text{g}$, values less than normally assumed for these materials. These values were taken to obtain a simple additive relation between the volume of the ribosome and the volumes of the protein and RNA of which it is made. A reasonable question to ask is what would happen if different values were assumed for these partial volumes? For example, one could imagine that *in situ* in the ribosome the effective partial volume of the protein is $0.74 \text{ cm}^3/\text{g}$, the value found in the literature. If this is so, then \bar{v}_{RNA} would have to be $0.509 \text{ cm}^3/\text{g}$, in order to preserve additivity. When the consequences of this set of assumptions are worked out, one is led to values for R_R and R_P of 65.5 \AA and 76.2 \AA , respectively. Δ becomes small and imaginary. On

the basis of molecular packing considerations (Richards, 1974), the opposite extreme, i.e. the model in which all the "volume deficit" is applied to the protein moiety of the structure seems improbable. That model would imply that \bar{v} (protein) *in situ* is 0.613 cm³/g. Similarly we have considered a number of alternative methods for partitioning the exchangeable hydrogens between RNA and protein. In no case are the weighting factors changed enough to convert the protein weighted sample into an RNA weighted one, or *vice versa*.

While the qualitative picture that emerges from this work is secure, the quantitative values obtained for R_P , R_R and Δ do, of course, depend directly on the assumptions made in calculating the scattering length equations. To go beyond the present work and test these assumptions, it would be necessary to measure the partial volume of RNA and protein and their separate degrees of hydrogen exchangeability in the intact ribosome, a problem which goes well beyond anything contemplated here.

We find it interesting that the volume of the ribosome seems to be less than that of the sum of its parts taken separately. The sum is about 106% of the actual volume. It is possible that this discrepancy represents technical difficulties with the measurement of the partial volumes of rRNA or ribosomal protein or possibly counter ion effects. (The protein value is the product of calculation from the amino acid composition (Spahr, 1962) and has never been checked directly as far as we know.) A more interesting possibility is that this relatively small decrease in volume is the result of a rearrangement of the RNA and/or protein brought about by the forces which stabilize the particle.

The radius of gyration data we have obtained for the 30 S subunit agree well with the earlier work of Hill *et al.* (1969*b*). They found a value of 69 Å for the radius of gyration of the 30 S subunit by X-ray scattering. From our values of R_R , R_P and Δ , using appropriate weighting factors, we would predict a value of 69.6 Å for the X-ray radius of gyration. Smith's (1971) X-ray value for the radius of gyration of the 30 S subunit is 72 ± 1.6 Å.

The scattering length equations we give here for the 50 S subunit differ somewhat from those reported earlier (Moore *et al.*, 1974). The values given here are based on more extensive data and are, we believe, more accurate. In view of these revisions we have reprocessed our earlier 50 S scattering data. The outcome is that we find that our values for R_P , R_R , and Δ for the 50 S subunit have all changed slightly from our published values, mostly within the error limits given, and thus no significant improvement in our understanding of this structure has resulted (Moore *et al.*, 1974). The values we quote for R_P , R_R , and Δ for the 50 S subunit in this paper are the ones we published previously.

In the present work we chose to examine ribosomal subunits isotopically labeled to emphasize the differences in the contributions of protein and RNA to the total scattering from the subunit. Since protein and RNA differ in scattering density in all H particles, it should be possible to analyze the ribosome by varying solvent density without recourse to isotopic labeling. This method has been exploited in the special case of centrosymmetric particles (for a review see Schmatz *et al.*, 1974), and in principle would permit the determination of all relevant parameters for a non-centrosymmetric two-component structure as well. Seven independent measurements would give the scattering factors, radii of gyration and separation of centers for the two distributions as a solution of seven equations in seven unknowns. If the scattering factors were independently obtained, the problem would be reduced to

three unknowns, as in our present work. There are several difficulties with this approach, the most important one being that measurements necessarily must be made to high precision under low-contrast conditions. The signal-to-noise ratio in our experimental apparatus would be reduced to levels which would render such measurements impractical given available running time.

Our decision, then, was to devote a large effort to isotopic manipulation of the sample in order to reduce demands on instrument time. The availability of more advanced instruments may permit a more flexible and direct approach in the future.

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REFERENCES

- Anderson, E. H. (1946). *Proc. Nat. Acad. Sci., U.S.A.* **32**, 120-128.
- Bevington, P. R. (1969). *Data Reduction and Error Analysis for the Physical Sciences*, McGraw-Hill Book Co., New York.
- Bickle, T. A., Hershey, J. W. B. & Traut, R. R. (1972). *Proc. Nat. Acad. Sci., U.S.A.* **69**, 1327-1331.
- Cantor, C. R., Huang, K. H. & Fairclough, R. (1974). In *The Ribosomes*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., in the press.
- Chang, F. N. & Flaks, J. G. (1972). *J. Mol. Biol.* **68**, 177-180.
- Engelman, D. M. & Moore, P. B. (1972). *Proc. Nat. Acad. Sci., U.S.A.* **69**, 1997-1999.
- Guinier, A. (1939). *Ann. Phys. (Leipzig)*, **12**, 161-237.
- Guinier, A. & Fournet, G. (1955). *Small Angle Scattering of X-rays*, Wiley & Sons, New York.
- Hamilton, M. G. (1971). In *Methods in Enzymology* (Moldave, K. & Grossmann, L., eds), vol. 20, part C, pp. 512-521, Academic Press, New York.
- Hill, W. E., Rossetti, G. P. & Van Holde, K. E. (1969a). *J. Mol. Biol.* **44**, 263-278.
- Hill, W. E., Thompson, J. D. & Anderegg, J. W. (1969b). *J. Mol. Biol.* **44**, 89-102.
- Hill, W. E., Anderegg, J. W. & Van Holde, K. E. (1970). *J. Mol. Biol.* **53**, 107-122.
- Hossfeld, F. (1968). *Acta Crystallogr. sect. A*, **24**, 643-650.
- Kurland, C. G. (1960). *J. Mol. Biol.* **2**, 83-91.
- Low, B. W. & Richards, F. M. (1952). *J. Amer. Chem. Soc.* **74**, 1660-1666.
- Ludlum, D. B. & Warner, R. C. (1965). *J. Biol. Chem.* **240**, 2961-2965.
- Lutter, L. C., Zeichardt, H., Kurland, C. G. & Stöffler, G. (1972). *Mol. Gen. Genet.* **119**, 357-366.
- Moore, P. B. (1971). *J. Mol. Biol.* **60**, 169-184.
- Moore, P. B., Engelman, D. M. & Schoenborn, B. P. (1974). *Proc. Nat. Acad. Sci., U.S.A.* **71**, 172-176.
- Ortega, J. P. & Hill, W. E. (1973). *Biochemistry*, **12**, 3241-3243.
- Richards, F. M. (1974). *J. Mol. Biol.* **82**, 1-14.
- Roberts, R. B., Cowie, D. B., Abelson, P. H., Bolton, E. T. & Britten, R. J. (1957). *Studies of Biosynthesis in Escherichia coli*, Carnegie Institution of Washington, Washington, D.C.
- Schelten, J., Schlecht, P., Schmatz, W. & Meyer, A. (1972). *J. Biol. Chem.* **247**, 5436-5441.
- Schlessinger, D. (1960). *J. Mol. Biol.* **2**, 92-95.
- Schmatz, W., Springer, T., Schelten, J. & Ibel, K. (1974). *J. Appl. Crystallogr.* **7**, 96.
- Schoenborn, B. P. & Nunes, A. (1972). *Annu. Rev. Biophys. Bioeng.* **1**, 529-552.
- Smith, W. S. (1971). Ph.D. Thesis, University of Wisconsin.
- Spahr, P. F. (1962). *J. Mol. Biol.* **4**, 395-406.
- Spahr, P. F. & Tissieres, A. (1959). *J. Mol. Biol.* **1**, 237-239.
- Wabl, M. (1973). Ph.D. Thesis, Free University of Berlin.
- Wabl, M. (1974). *J. Mol. Biol.* **84**, 241-248.