Measurement of the Radii of Gyration of Ribosomal Components
in situ by Neutron Scattering

BY DAVID H. HARRISON
Departments of Chemistry and of Molecular Biophysics and Biochemistry, Yale University,
New Haven, CT, USA

ROLAND P. MAY
Institut Laue-Langevin, Grenoble, France

AND PETER B. MOORE
Department of Chemistry, Yale University, New Haven, CT, USA

(Received 2 June 1992; accepted 24 September 1992)

Abstract

The radii of gyration of 16S rRNA, 30S proteins and S4 have been measured in situ in the 30S ribosomal subunit from Escherichia coli by the three-isotope method (TIM) [Pavlov & Serdyuk (1987). J. Appl. Cryst. 20, 105-110]. The values obtained were 70.0 (25), 66.7 (24) and 23.5 (10) Å, respectively. The data for ribosomal protein and RNA in this system are consistent with earlier findings, verifying that the TIM is reliable. The estimate obtained for S4 is one of the first high-quality estimates of the radius of gyration obtained for a ribosomal protein in situ.

Introduction

Most of the small-angle neutron scattering experiments performed on biological assemblies over the past 20 years have either exploited natural differences in the scattering-length densities of different types of biological materials or depended on scattering-length-density differences created by selective deuteration (see Zaccai & Jacrot, 1983). The primary goal of many of these studies has been the determination of the radii of gyration of components of macromolecular assemblies.

The 30S ribosomal subunit from the bacterium Escherichia coli, the macromolecular complex of interest here, consists of 21 different proteins and a single RNA molecule, 16S rRNA. Since proteins and nucleic acids differ significantly in scattering-length density, it has been possible to measure the radii of gyration of 16S rRNA and 30S proteins in the intact subunit by contrast-variation methods (see Koch & Stuhrmann, 1979). It is generally agreed that the radius of gyration of 16S rRNA is in the range 60-65 Å, but the radius of gyration of its protein parts is less certain.

Initial contrast-variation results suggested that the radius of gyration of the protein parts of the 30S subunit is about 80 Å (see Koch & Stuhrmann, 1979), but the protein map obtained by neutron scattering methods points to a much smaller value, 63.7 (27) Å (Capel, Kjeldgaard, Engelman & Moore, 1988). Ramakrishnan (1986) has found that this discrepancy is due to differences in protein composition. The native subunits used in the first contrast-variation studies contain a population of acidic proteins that contribute little or nothing to ribosome activity. When these proteins are removed by salt washing, the protein radius of gyration of the 30S subunit drops to about 67 Å.

Much less is known about the radii of gyration of individual ribosomal proteins in the 30S subunit, each one of which accounts for only a few percent of its total mass. Even if a single protein is deuterated and the rest of the particle left fully protonated, reliable estimates of the radius of gyration are difficult to obtain by contrast-variation methods for reasons that have been discussed elsewhere (Moore, 1981). The errors in such estimates can be reduced significantly by use of the 'glassy ribosome' approach and several radius-of-gyration estimates of this kind have appeared (Nowotny, May & Nierhaus, 1986). Most of the little that is known about the radii of gyration of individual proteins in the 30S ribosomal subunit has come from the neutron map, which yields radius-of-gyration estimates for components as well as information about their positions (Capel, Kjeldgaard et al., 1988). The errors associated with these estimates are, however, very large.

The most powerful method available for measuring the radii of gyration of components in macromolecular assemblies is the three-isotope method (TIM) of Pavlov & Serdyuk (1987). The TIM procedure
requires that the component of interest be produced in three forms; its fully deuterated form, its protonated form and a form that is roughly 50% deuterated. Three samples of the assembly must be prepared that differ only in the level of deuteration of the component of interest. Assemblies containing the deuterated and protonated versions of the component are then mixed so that the average deuteration of that component is the same in the mixed sample as it is in the assemblies containing the partially deuterated component. The TIM signal is the scattering given by the mixed sample minus the scattering given by the partially deuterated sample, measured in the same buffer and, of course, suitably normalized for differences in concentration.

The special virtue of the TIM is that the results it gives should be completely independent of the distribution of scattering-length densities in the rest of the assembly. In this paper, we report the results of three TIM measurements done on the 30S ribosomal subunit from *Escherichia coli*. The radius of gyration of 16S rRNA has been measured by the TIM to confirm that it gives results that correspond to earlier experience, where it seems reliable. The protein radius of gyration has been measured by the TIM as well, to verify the results of Ramakrishnan (1986). Finally, the protein composition has been measured by the TIM as well, to verify the results of Ramakrishnan (1986). Finally, the protein radius of gyration of a single 30S protein, $4$, has been determined. The implications of these results for our understanding of ribosome structure are discussed, as are the prospects for further TIM studies of the ribosome.

**Materials and methods**

**Ribosomes**

30S ribosomal subunits were prepared from *Escherichia coli* MRE600 by zonal centrifugation (Moore, 1979). Most of the cells used were grown on a medium that contained per liter: 4 g succinic acid, 24 g KH$_2$PO$_4$, 3.7 g NaOH, 1.68 g (NH$_4$)$_2$SO$_4$, 0.3 g MgSO$_4$.7H$_2$O and 3 mg FeSO$_4$. The pH value was 6.5. Protonated succinate and H$_2$O were used when protonated cells were required, 50% deuterated succinate and 50% D$_2$O were used when 50% deuterated components were needed and the medium was perdeuterated when maximally deuterated components were required. Deuterated succinate was prepared from succinic anhydride (Stella, 1973; LeMaster & Richards, 1982). Some ribosomal materials were prepared from cells grown on a D$_2$O minimal medium using protonated glucose, with protonated nucleosides as carbon sources (Capel, Kjeldgaard *et al.*, 1988).

**Ribosomal protein S4**

Bulk 30S protein (see below) was fractionated initially by ion-exchange chromatography on carboxymethyl cellulose and the S4-containing fractions were purified further by chromatography on Sephadex G100 (Moore, 1979). The last step was HPLC on a Synchropak RP-P column (Capel, Datta, Nierras & Craven, 1988).

**Determination of deuteration of protein S4**

0.2 µg each of protonated, fractionally deuterated and perdeuterated S4 protein were injected into an electrospray ionization mass spectrometer designed by Analytica (Branford, CT, USA) (Fenn, Mann, Meng, Wong & Whitehouse, 1989). The mass spectra of multiply charged protein peaks were deconvoluted to determine the masses of the three protein species (Whitehouse & Labowsky, 1992). The relative fractional deuteration of the partially deuterated S4 protein was calculated as the ratio of the excess mass of the partially deuterated molecule to the excess mass of the perdeuterated molecule.

**Reconstitutions**

Reconstitutions were performed as described previously (Moore, 1979) except that the buffers used contained NH$_4$Cl in place of KCl. The 16S rRNA concentrations were 7.5 to 10.0 $A_{260}$ nm cm$^{-3}$ in the reconstitution mixtures and the molar ratio of protein to RNA was usually 4. Once incubation was complete, precipitated protein was removed by low-speed centrifugation. The mixtures were then concentrated by ultrafiltration and frozen at 203 K until just before scattering measurements were to begin. Aliquots of each reconstitution mixture were assayed for activity in poly U-directed, polyphenylalanine synthesis (Moore, 1979). They were also cosedimented with trace quantities of $^3$H-labeled 70S ribosomes on sucrose gradients in 0.050 M KCl, 0.0005 M magnesium acetate and 0.010 M tris–HCl, at pH 7.5, to verify that 30S particles had formed. The protein compositions of all samples were examined after data collection was completed by two-dimensional gel electrophoresis; all samples were normal in this respect.

**Final preparation of samples**

30S subunits were transported to the reactor site as frozen reconstitution mixtures. Once there, they were thawed and the 30S subunits they contained were purified on sucrose gradients as previously described (Moore, 1979). Fractions containing monomeric 30S subunits were pooled and were concentrated using Amicon Centriprep ultrafiltration units. The concentrated samples were divided in two and one half dialyzed against D$_2$O scattering buffer, while the other half was dialyzed against H$_2$O scattering buffer. The scattering buffer contained 0.001 M tris, 0.006 M 2-mercaptoethanol, 0.050 M KCl and 0.0005 M
magnesium acetate, at pH 7.5. Samples of intermediate
\(\text{D}_2\text{O}\) concentration were obtained by mixing the \(\text{D}_2\text{O}\) and \(\text{H}_2\text{O}\) samples together and allowing them to
dialyze overnight against the appropriate buffer. The
samples were not frozen from the time their final
purification began until after data collection because
freezing promotes aggregation.

Data acquisition

Neutron data were collected using the D11
low-angle spectrometer at the Institut Laue–Langevin,
Grenoble, France (Ibel, 1976; Lindner, May &
Timmins, 1992). All measurements were made at 278 K
with a water-jacketed sample holder, which was
maintained in a dry-air atmosphere to avoid
condensation. Round quartz Helma cells with a 1.9 cm
interior diameter and a path length of 0.100 cm were
used. The aperture of the sample holder allowed a
beam of 1.6 cm diameter to pass through the sample.
1.0 nm neutrons were used to collect data at two
sample-to-detector distances so that a wide range of
Q would be sampled. Data-collection times were
adjusted so that the statistical uncertainties con-
tributed by each data set to the final data would be
roughly equal.

Data reduction

The scattering curves obtained for each sample at
both distances were merged using the program
\textit{ADAPT} (May, 1992). It multiplies the data obtained
at short distance by a scalar chosen to minimize the
least-squares differences between the two spectra in
the overlap region. When merged scattering curves
had been obtained, buffer contributions were sub-
tracted from them and they were normalized for
differences in concentration, \(C\), transmission, \(T\), and
cell thickness, \(t\), as follows:

\[
I(Q) = \left(\frac{1}{CtT_{\text{30S}}}\right) \times \left(\frac{\{J(Q) - \text{Cd}(Q) - T_{\text{30S}}[E(Q) - \text{Cd}(Q)]\}}{\{\text{H}_2\text{O}(Q) - \text{Cd}(Q) - T_{\text{H}_2\text{O}}[E(Q) - \text{Cd}(Q)]\}^{-1}}\right) \\
- \left[\gamma(1 - T_{\text{buf}})[\text{Buf}(Q) - \text{Cd}(Q) - T_{\text{buf}}[E(Q) - \text{Cd}(Q)]]\right) \\
\times \left(\frac{\{J(Q) - \text{Cd}(Q) - T_{\text{H}_2\text{O}}[E(Q) - \text{Cd}(Q)]\}}{\{\text{H}_2\text{O}(Q) - \text{Cd}(Q) - T_{\text{H}_2\text{O}}[E(Q) - \text{Cd}(Q)]\}^{-1}}\right),
\]

where \(Q = \frac{4\pi}{\lambda}\) sin \(\theta\), \(\lambda\) is the neutron wavelength,
\(2\theta\) is the scattering angle and \(J(Q), \text{Cd}(Q), E(Q), \text{H}_2\text{O}(Q)\)
and Buf(Q) are the scattering profiles measured for the
sample, a cadmium-blocked beam, an empty cell, a
water sample and a buffer sample, respectively. \(\gamma\) is a
parameter whose value lies between 1 and 2, depending
on the amount of \(^1\text{H}\) in the sample (May, Ibel & Haas,
1982). The reader will note that the \(I(Q)\) function that
results is the scatter given by the solute of interest
alone scaled to the scatter given by water.

We also processed the data following the method
described by Ramakrishnan & Moore (1981). The
results were indistinguishable.

Forward-scattering values, \(I(0)\), can be computed
for samples of known structure as follows:

\[
I(0) = 4\pi N(V\Delta\rho)^2/(1 - T_{\text{H}_2\text{O}}),
\]

where \(N\) is the number of particles per mg, \(V\) is the
volume occupied by a single particle and \(\Delta\rho\) is the
difference in average scattering-length density between
the particle and the solvent (Zacchai & Jacrot, 1983).

Results

Experimental design

As Pavlov and co-workers have demonstrated, a
scattering curve for a component of a macromolecular
assembly can be obtained by subtracting the scattering
curve of a sample consisting of assemblies in which
the component of interest is approximately 50%
deuterated from the scattering curve of a mixture of
assemblies in which the component is either
perdeuterated or protonated, but whose average
deuteration level is also approximately 50%. Provided
the isotopic composition of the rest of the assembly
is constant in all samples and the buffer deuteration
is varied, the difference curve that results is the
scattering curve that would be given by the array of
nonexchangeable H atoms in the component of interest
in isolation (Pavlov & Serdyuk, 1987; Pavlov et al.,

The most difficult part of a TIM experiment is
determining the level of deuteration of the partially
deuterated component. To the extent that determina-
tion is in error, the isotopic composition of the mixed
sample will also be in error and the difference curve
that results will not be the one desired. Since
neutron scattering measurements are often the best
way to obtain the estimates required, there can be
practical constraints to getting this part of the
experiment done properly. The measurement time
available is often so short that analysis of the
deuteration data cannot be fully completed before
samples must be mixed and the final data taken. For
these reasons, it is important to be aware of the impact
of ‘mixing errors’ on the outcome of TIM experi-
ments.

The scattering of an assembly composed of a
component of interest, \(p\), and a set of other
components, \(o\), can be written:

\[
I(Q) \propto \langle[A_p(Q)(\rho_p - \rho_{\text{sol}}) + A_o(Q) + A_p(Q)(\rho_p - \rho_{\text{sol}})]^2\rangle,
\]

where \(A_p(Q)\) is the Fourier transform of a particle
having unit scattering-length density and the same shape and position as the component of interest, \( A_o(Q) \) is the Fourier transform of an object having unit scattering-length density and the same shape and position as the other parts of the assembly. \( A_i(Q) \) is the Fourier transform of the fluctuations in scattering-length density within the region occupied by the other parts of the assembly. \( \rho_p \) is the average scattering-length density of the particle of interest, \( \rho_o \) is the average scattering-length density of the other parts of the assembly and \( \rho_{sol} \) is the scattering-length density of the solvent. The \( \langle \cdots \rangle \) notation indicates averaging over all possible configurations in solution.

The difference scattering function that is arrived at in a TIM experiment, \( I_\delta(Q) \), has the form

\[
I_\delta(Q) = \delta I_p(Q) + (1 - \delta) I_i(Q) - I_{1BD}(Q), \tag{3}
\]

where \( \delta \) is the fraction of the mixed-sample particles that carry the component of interest in the deuterated form and \( I_p \) is the scattering given by those particles. \( I_i \) is the scatter given by particles that carry the component in the protonated form and \( I_{1BD} \) is the scatter given by particles carrying the component of interest in partially deuterated form.

Suppose that the level of deuteration of the component of interest in the \( I_{1BD} \) particles is \( f \), not \( \delta \). If the scattering-length density of the component of interest in the protonated form is designated \( \rho_H \) and that of the same component in the deuterated form is designated \( (\rho_H + A\rho) \), (2) and (3) can be combined to give

\[
I_\delta(Q) = \delta \langle [A_o(Q)(\rho_o - \rho_{sol}) + A_i(Q) + A_p(Q)(\rho_H + A\rho - \rho_{sol})]^2 \rangle
+ (1 - \delta) \langle [A_o(Q)(\rho_o - \rho_{sol}) + A_i(Q) + A_p(Q)(\rho_H - \rho_{sol})]^2 \rangle
- \langle [A_o(Q)(\rho_o - \rho_{sol}) + A_p(Q)(\rho_H + fA\rho - \rho_{sol})]^2 \rangle. \tag{4}
\]

When (4) is expanded out, the following expression results:

\[
I_\delta(Q) = I_p(Q)[2 A\rho(\delta - f)(\rho_H - \rho_{sol}) + (\delta - f^2)A\rho^2]
+ I_{1BD}(Q)[(\delta - f)(\rho_H - \rho_{sol})A\rho]
+ I_i(Q)[(\delta - f^2)A\rho^2]. \tag{5}
\]

where \( I_p(Q) = \langle A_o(Q) \rangle \), \( I_{1BD}(Q) \) is the interference scatter between the particle of interest and the average scatter of the rest of the particle and \( I_i(Q) \) is the interference scatter between the particle of interest and the inhomogeneities in the rest of the aggregate. In the event that \( \delta = f \), \( I_\delta(Q) = \delta(1 - \delta)A\rho^2 I_p(Q) \), as expected (Pavlov & Serdyuk, 1987), and the TIM difference curve becomes independent of the scattering-length densities of the solvent and of the rest of the assembly.

Let us call the part of a particle which remains constant in a TIM experiment the ‘reference particle’. (The reference particle for the S4 experiment, for example, would be the entire particle except for S4.) From the point of view of minimizing coherent background scattering, the best choice of buffer will be one whose scattering-length density matches that of the reference particle. Since the coherent scattering of an inhomogeneous reference particle is \( Q \) dependent at the match point and unknown \textit{a priori}, the argument is less straightforward. Nevertheless, since at low \( Q \) the scattering increases strongly with any deviation from the match point, the best contrast will never be far from it.

Both \( \rho_H \) and \( \rho_o \) can also be manipulated to minimize the sensitivity of \( I_\delta(Q) \) to errors in mixing. In addition, the signal-to-noise ratio of the experiment can be improved by manipulating the isotopic composition of the assembly. For example, the signal-to-noise ratio of the experiment will be improved if D\textsubscript{2}O-based buffers are used throughout so that incoherent scatter is minimized. Note also that whenever \( \rho_{sol} = \rho_o \), the scattering-length density of the rest of the assembly, the \( I_{1BD}(Q) \) contribution to \( I_\delta(Q) \) vanishes. Thus, there are significant advantages to be gained by arranging the experiment so that \( \rho_{sol} = \rho_o = \rho_{D2O} \). Ideal experimental conditions are achieved when the scattering-length density of the partially deuterated form of the particle of interest matches the scattering-length density of the other components in the assembly, which in turn matches the scattering-length density of a D\textsubscript{2}O-based solvent. Under these conditions, the difference scattering curve will be essentially that of the mixed sample alone and mixing errors will also disappear. The problem that remains is the practical one of producing samples which meet these characteristics.

**Samples**

Because S4 represents only 3% of the mass of the 30S subunit, it was clear from the outset that the signal-to-noise ratio would be critical when measuring its radius of gyration. For that reason, the non-S4 portions of all S4 samples were made from ribosomal materials extracted from cells grown on 100% D\textsubscript{2}O, protonated glucose, protonated nucleosides medium (see Materials and methods). Reconstituted subunits made this way contrast match in 100% D\textsubscript{2}O (Capel, Kjeldgaard et al., 1988), which makes 100% D\textsubscript{2}O buffers ideal. In addition, 50% deuterated protein is close to contrast matched in D\textsubscript{2}O. Thus, the samples and buffer conditions used for the S4 experiments described below were close to ideal.

Since 16S rRNA accounts for more than half the mass of a 30S subunit, and its protein portion, as a whole, is also very large, signal-to-noise considerations were much less important in the design of the samples required for the measurement of their radii of gyration.
Protonated protein or protonated RNA was used as the ‘other’ material in these samples and 70% D$_2$O was the buffer chosen in both cases. Subunit samples that contain 50% deuterated RNA or protein and have everything else protonated are roughly contrast matched in 70% D$_2$O, which usefully reduces the scatter given by such a sample. Because 70% D$_2$O is also close to the match point of protonated RNA, mixing errors were noticeably suppressed in the protein series of samples but not for their RNA counterparts.

**Determination of levels of deuteration**

Because substantial changes in average scattering-length density can be achieved by deuterating either the RNA or protein parts of the 30S ribosomal subunit, it was appropriate to determine the deuteration levels of the samples in the protein and RNA series by neutron scattering. Each series had three samples: a fully protonated sample, a sample carrying the component of interest in partially deuterated form and a sample with the component fully deuterated. Scattering curves were measured for all samples in both series in buffers differing in D$_2$O concentration. Following data reduction and radius-of-gyration determination, the square root of $I_o$ was plotted versus the fraction of D$_2$O in the sample's buffer, as estimated from transmission data, with the result shown in Fig. 1.

Linear regressions were calculated for each of the six samples by least-squares methods. Since the particles in these samples all have the same chemical structure, the forward-scattering data they give should all be fit by regression lines having identical slopes and that condition was forced during fitting. Once the regression line was determined for each sample, the fractional deuteration of the partially deuterated sample in each series could be found by comparing the partially deuterated intercept with the protonated and deuterated intercepts. This analysis indicated that the relative deuteration for the partially deuterated RNA sample was 53%, while the relative deuteration of the partially deuterated protein sample was 58.5%. Two independent sets of samples were generated in the course of these experiments; the forward-scattering data they gave were identical within error.

Because S4 is such a small fraction of the 30S subunit, no effort was made to determine the levels of S4 deuteration by measurements of forward scattering. Instead, the deuterium contents of purified samples of S4 were measured by ion-spray mass spectrometry (Gulicsek, Harrison & Shen, 1992). The value obtained for the partially deuterated sample was 52%. We note that this estimate was obtained at the expense of less than 1 µg of protein and is accurate to 1% or so. There is little doubt that mass spectrometry is the best method for such measurements.

Both times these experiments were run, only a preliminary analysis of the forward-scattering data was possible before samples had to be mixed for TIM data collection and that analysis indicated that the fractional deuteration was about 55% in both the RNA and protein samples. Consequently, the mixed samples for the RNA, protein and S4 series were formulated on that basis, and in retrospect it is clear that a small mixing error was made.

**The radius of gyration of 16S rRNA**

Fig. 2 shows the scattering curves for the mixture of protonated and deuterated RNA particles and for the partially deuterated particles and the difference between these curves. It is clear that the subunits containing partially deuterated RNA are almost perfectly contrast matched in the chosen buffer. The forward scattering that one predicts for this sample using the regression data in Fig. 1 is 0.07. The value observed is 0.08 (1).

It is equally obvious that the mixed sample was not contrast matched under these conditions. The forward scatter given by the mixed sample is 0.71 (2). Using the regressions in Fig. 1, one predicts a value of 0.53, which would be the value actually observed if the particles in the sample scattered independently. The radius of gyration of the mixed-particle samples has no physical significance per se, since there are two kinds of particles present in the sample.

The difference curve should correspond to the scattering curve of the RNA in vacuo, except for
exchanged protons. The forward scattering expected for that curve may be calculated in two ways: (i) using the contrast-variation regression curves, which again is equivalent to assuming that all the particles in each sample scatter independently and (ii) using (1) and the fact that 16S rRNA has 11,913 nonexchangeable protons in it. The forward scatter predicted the first way is 0.51 while that calculated the second way is 0.65. The forward scatter observed is 0.64.

The radius of gyration of the difference curve is 70.0 (25) Å, which is larger than the estimate obtained by contrast variation on native subunits (Koch & Stuhrmann, 1979) but similar to the value obtained by Ramakrishnan with both S1-stripped native subunits and reconstituted particles (Ramakrishnan, 1986). The points used for determining this radius of gyration start at a $Q_{\text{min}}R_g$ of 0.77 and finish at a $Q_{\text{max}}R_g$ of 2.1. It appears that points at $Q < Q_{\text{min}}$ are influenced by aggregation but the addition of a point or two inside $Q_{\text{min}}$ to the data analyzed would make little difference to the value obtained for the radius of gyration.

The radius of gyration of 30S proteins

Fig. 3 shows the scattering curves obtained for the mixture of protonated and deuterated protein particles and for the partially deuterated protein particle and the difference between these two curves. It is clear that the scattering-length density of 70% D$_2$O is appreciably different from that of the partially deuterated particles. The forward scatter predicted for the partially deuterated sample is 0.16, while the value actually observed is 0.27. The forward scatter predicted for the mixed-sample scattering profile using contrast-variation data is 0.88.

The difference in scattering between the mixed-protein particles and the HD protein particles is also shown in Fig. 3. Before a prediction of the forward scattering expected from this profile can be made using (1), the number of nonexchangeable protein H atoms each particle contains must be determined. The easiest way to do this is to compare the (normalized) forward scattering values for the fully deuterated RNA and protein samples with the fully protonated sample, all measured in H$_2$O. This comparison indicates that the number of nonexchangeable protein H atoms in the subunit is about 14,800. Note that if every particle contained one copy of every protein except S1, the number would be 15,848 (Wittmann-Liebold, 1986). The forward scattering predicted on this basis is 1.01. The forward scatter of the difference curve predicted using the regression method is 0.80. The forward scatter observed is 0.46, which is significantly less than either estimate (see below). The radius of gyration of the 30S proteins in vacuo is 66.7 (24) Å.

Stuhrmann plots

The data presented above indicate that the protein and RNA portions of the 30S ribosomal subunit have about the same radius of gyration as the 30S subunit as a whole. A simple way to test this conclusion further is to display the contrast-variation data collected for determination of deuteration levels as a Stuhrmann plot, i.e. as a plot of radius of gyration squared versus $(\Delta \rho)^{-1}$. Fig. 4 shows such a plot of the data obtained on the fully protonated samples examined in this study.

The data in Fig. 4 fit a straight line and the radius of gyration of the particle is about 67 Å at $(\Delta \rho)^{-1} = 0$. The radius of gyration at $(\Delta \rho)^{-1} = 0$ is that of an object having the shape of the 30S subunit, but no interior variation in scattering-length density. Furthermore, the slope of the line defined by the data,
which is a measure of the distribution and magnitude of the interior scattering-length density inhomogeneities, is quite small, \( x = -0.8 \times 10^{-4} \). Stuhrmann plots of the other particle types used in these experiments gave similar results (data not shown).

Fig. 4 resembles the Stuhrmann plot published by Ramakrishnan (1986) for salt-washed particles and is very different from what has been found for native 30S subunits (Koch & Stuhrmann, 1979). Stuhrmann plots of native 30S data have similar intercepts at \((\Delta \rho)^{-1} = 0\), but large negative slopes \((x \approx -1 \times 10^{-3})\). For our particles, as for those of Ramakrishnan (1986), there is not much difference between the radii of gyration of protein and RNA.

**Protein S4**

Before beginning TIM experiments, the radii of gyration of all of the samples to be used in the S4 experiments were measured in H\(_2\)O. They were all normal; their radii of gyration were in the range 66.5 to 66.8 Å. This indicates that S4 was present in all of them, since S4 adds to 16S rRNA early in reconstitution and is essential if 30S-like particles are to be recovered (Held, Mizushima & Nomura, 1973). The presence of S4 in these samples was verified electrophoretically afterwards (see Materials and methods).

Fig. 5 shows the scatter given by the mixed S4 sample and the partially deuterated S4 sample. The reader will note that both scattering profiles are very weak, as one would expect knowing that S4 is a tiny fraction of the whole assembly being examined and that the buffer used, D\(_2\)O, is nearly a perfect contrast match. The intensity of the partially deuterated sample scatter is higher than the intensity of the scatter given by the mixed sample at high angles, which is not consistent with the expectation that, at large scattering angles, the difference between the two profiles should go to zero.

It appeared from transmission differences that there was a small difference in the D\(_2\)O content of these samples, which would produce a difference in incoherent scatter that could account for the difference. The HD S4 scattering curve was corrected by subtracting a constant amount from each point so that the difference between it and the mixed sample was reduced to zero at high angle. The amount that had to be subtracted was of the order indicated by the transmission difference.

The difference curve that results from the adjustment just described is shown in Fig. 6. Its Guinier region ranges from a \(Q_{\text{min}}R_g\) of 0.88 to a \(Q_{\text{max}}R_g\) of 2.0. The forward scatter, 0.011, agrees well with the calculated forward scatter of 0.009 that is predicted by (1), when one takes 1278 to be the number of nonexchangeable

---

![Fig. 4. Stuhrmann plot of data collected on protonated 30S subunits. Radius-of-gyration data obtained for several reconstituted protonated 30S subunit samples are plotted as \(R_g^2\) versus \((1/\Delta \rho)\), where \(\Delta \rho\) is the contrast of the sample. Relative contrasts were determined by comparison of forward-scattering intensities. Absolute values were estimated from the known compositions of the particles and the buffers used.](image)

![Fig. 5. S4 data. The data collected on the sample containing both deuterated and protonated S4 (†) and on the sample containing \(50\%\) deuterated S4 (○) are displayed in the form \(I(Q)\) versus \(Q\). Data were obtained as described in Materials and methods. The buffer used was 100% D\(_2\)O. Error bars are shown for the sample containing both deuterated and protonated S4.](image)

![Fig. 6. TIM analysis of corrected S4 data. The S4 data shown in Fig. 5 were corrected for their differences in transmission prior to subtraction and are presented here in the same format as the protein and RNA data in Figs. 2 and 3. All data were collected using a 100% D\(_2\)O buffer. ○ represents the sample containing particles that have either protonated S4 or deuterated S4, † the sample with particles containing \(50\%\) deuterated S4 and □ the TIM difference of the first two profiles. The line drawn through the difference curve shows the region used for radius-of-gyration estimation.](image)
protons in S4. The radius of gyration of S4 deduced from this curve is 23.5 (10) Å.

We also examined what happened if the negative excursion of the raw difference curve was ascribed to errors in the determination of concentrations. In this instance, the correction required was unreasonably large, 0.6, and the forward scatter of the corrected profile is much greater than 0.011. The radius of gyration is not changed by treating the data this way; it remains 23.4 Å. The Guinier region of the curve is defined by \( Q_\text{min} R_g = 0.94 \) to \( Q_\text{max} R_g = 2.0 \), this is a slightly more restricted Guinier region than was used above. However, the forward scatter obtained using this method is greater than the forward scatter using the transmission-corrected data, which is an additional indication that a correction of this kind would be inappropriate.

**Discussion**

The data reported above are self-consistent in all respects except for the strength of the TIM difference obtained in the protein experiment, which is weaker than predicted by a factor of about two. We know that the mixing ratio used in this experiment, 55%, was not the right one, 58.5% is the ratio the data call for. We have examined the impact of this mixing error using (4), which predicts a reduction in the forward scatter of only 10%, and dominance of \( I_p \) in the scattering measured. The reason \( I_p \) dominates is that the impact of the \( I_q \) term is strongly diminished because protonated RNA is nearly contrast matched in 70% D_2O. The agreement between predicted and observed values of \( I_o \) for the other profiles is so good that it is unlikely that the mixing errors made in those samples are serious.

Nevertheless, the radius of gyration the protein TIM experiment gives is consistent with the other data obtained in this study. The Stuhrmann plots of the contrast-variation data indicate that the radius of gyration of the whole subunit is not much different from the radii of gyration of its RNA and protein portions, which is what the TIM data are also reporting. Thus, this study provides strong support for Ramakrishnan's conclusion that contaminating acidic proteins are responsible for the high protein radius of gyration seen in native subunits (Ramakrishnan, 1986).

It should be noted that the radius of gyration of the 30S protein assembly determined by neutron mapping, which was cited earlier, includes S1. S1 is located close to the center of gravity of that assembly and, since its own radius of gyration is less than that of the assembly as a whole, the radius of gyration of the assembly actually increases when S1 is left out. It becomes 65.4 Å, which is close to the value obtained in this study, 66.7 Å, and the value obtained by Ramakrishnan (1986), 68–70 Å.

It is also noteworthy that the radius of gyration of the RNA portions of native subunits appears to be smaller than the radius of gyration of 16S rRNA when it is part of a salt-washed particle or a reconstituted particle (Ramakrishnan, 1986; Koch & Stuhrmann, 1979). The difference is of the order of 5 Å. The RNA in native subunits is reported to have a radius of gyration in the low 60’s while that of the RNA in reconstituted subunits is closer to 70. The source of this discrepancy is hard to understand at this point. Could it reflect some systematic difficulty in interpreting contrast-variation data when \( \alpha \) is large?

While the TIM worked satisfactorily for S4, there are clearly limits as to the size of the minimal part of an assembly that can be analyzed through its use. In our estimation, S4, which is about 3% of the 30S subunit, is close to that limit. Clearly, the signal-to-noise ratio was a problem in this instance, even though experimental conditions were close to ideal. These experiments were done at 10 mg ml^{-1}, which is not high, and it would be possible to improve the signal-to-noise ratio by increasing concentrations. One might hesitate to do so, however, since aggregation is a major problem in ribosome samples and the inner parts of the scattering curves obtained in this study suggest that its effects may not have been fully controlled.

Because the geometry at D11 allows the use of very large cells, the total amount of material exposed to the beam was about the same as in the neutron mapping experiments that we have reported in the past (e.g. Capel, Kjeldgaard et al., 1988). The reason those experiments worked with proteins much smaller than S4 is that the difference signal in a mapping experiment is four times as strong as the signal in a TIM experiment, all else being equal.

The radius of gyration for the S4 protein that we report here, 23.4 (9) Å, is smaller than the one we reported earlier, 30.2 (46) Å (Capel, Kjeldgaard et al., 1988). This is not surprising. The radius-of-gyration estimates for individual proteins that resulted from mapping were known to be crude; many of them had standard errors of 10 Å. There is little doubt that TIM measurements can give more accurate radius-of-gyration estimates than those that are the by-products of mapping measurements, provided an adequate signal-to-noise ratio can be achieved.

We thank Mrs Betty Freeborn for the assistance she provided in making and analyzing the samples used in these studies. We also thank Dr Erol Gulcicek for doing the mass spectroscopy on protein S5. We are grateful to Dr Reuben Leberman for providing us with the facilities we needed to prepare samples immediately before our runs at ILL. This work was supported by a grant from the NIH to PBM (AI-09167).
References
