The conformation of a large RNA fragment from the E.coli ribosomal 16S-RNA. An X-ray and neutron small-angle scattering study

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ABSTRACT

A large 12S RNA fragment which constitutes the 5' two-thirds of 16S-RNA from the E. coli 30S subunit has been investigated by small-angle X-ray and neutron scattering.

The results indicate that in reconstitution buffer the 12S-RNA fragment has a molecular weight of 270,000 ± 20,000 and a radius of gyration of 7.1 nm. The scattering data are compatible with the RNA being folded into two major domains with the shapes of two adjacent, quite similar cylinders.

INTRODUCTION

Although, for more than a decade, there has been a general view that the proteins were the main functional components of the ribosome, there is now substantial evidence to suggest that the RNAs play an important functional role. This evidence derives mainly from studies on the ribosomal binding sites of tRNA, mRNA and protein factors (reviewed in 1). The recent determination of the nucleotide sequences of the large RNAs of E. coli (reviewed in 1) has provided a solid basis for determining the structure of RNAs and, eventually, for elucidating their structure-function relationships. Thus far, rapid progress has been made in ascertaining the probable secondary structure of the 16S-RNA, and for defining the long-range helical regions which provide large restraints on its folding (2,3). The results to date, for the 16S-RNA, support the conclusions drawn from several studies on protein-RNA complexes which indicate the presence of specific structural domains within the RNA to each of which one or a few proteins can independently associate (reviewed in 4,5,6).

There is accumulating biochemical evidence which suggests that the isolated 16S-RNA can be renatured into essentially the same secondary and tertiary structure that occurs in the ribosome (7-11). In this renatured state, the RNA can be treated with ribonuclease to yield specific fragments which appear to correspond to structural domains of the RNA structure; under
fairly extreme digestion conditions discrete subproducts of these domains can also be produced (e.g. 12-14). Thus, Zimmermann et al. (12,15) demonstrated that the 16S RNA can be cut by mild ribonuclease T1 treatment about two-thirds of the way along the molecule from the 5'-end. This produced two fragments, denoted 12S and 8S RNA. The 5' subproduct of 12S RNA, S4-RNA, binds the protein S4 but also the S16, S17, and S20 proteins. We have recently investigated S4-RNA and its complex with S4 using the small-angle X-ray scattering method (16,17).

In the present work we have continued our small-angle X-ray scattering studies on ribosomal RNA (16-18) by investigating the molecular parameters of 12S RNA in the solution. The results indicate that the data are consistent with RNA folded into two major domains with the shapes of two adjacent, quite similar cylinders.

**MATERIALS AND METHODS**

**Materials**

The 12S RNA was prepared from *Escherichia coli* ribosomes (strain A19), essentially as described by Zimmermann et al. (12). Thus, ribosomal 16S-RNA was incubated at 40°C for 5-10 min and then hydrolysed with T1 RNase (Sankyo, Japan) at an enzyme to substrate weight ratio of 1:1000 (which produced the best yield of 12S RNA) at 0°C for 30 min. The products were separated on 5-20% sucrose gradient in Tris/Mg/K buffer (0.03 M Tris-HCl, pH 7.6, 0.30 M KCl, 20 mM MgCl2) using a Beckman SW 27 rotor for 22 h at 25,000 rpm. Reseparation in the same sucrose gradient and buffer yielded an essentially symmetric peak. The RNA was precipitated with 1.7 volumes ethanol at -20°C overnight and it was dissolved in the TMK buffer and then dialysed against the same buffer. The product produced two closely migrating bands of variable relative intensity in 3% polyacrylamide tube gels containing 40 mM Tris acetate 5 mM Mg acetate, pH 8; no intact 16S RNA or smaller RNA fragments were detected. A gel electrophoresis assay (19) showed that the components of both bands bound the proteins S4, S8, and S15. These data, and the X-ray scattering data, indicate that the two bands have essentially the same composition; the difference in electrophoretic migration can most probably be ascribed to a minor extension or excision of sequence. Such effects are generally observed after ribonuclease digestion of ribosomal RNAs and their protein complexes (see e.g. 20). The sedimentation coefficient of the RNA fragment is probably an underestimate, since 12S was originally calculated from the sucrose gradient migration; as reference 16S-RNA was assumed to sediment
at 16S, whereas under reconstitution conditions, 16S-RNA sediments at 21S (21).

The concentrations of RNA were determined within ± 5 % by optical density measurements ($A_{260} = (20.1) \times 10^{-2}$ mg/cm$^2$) which were checked by nitrogen and carbon analyses (analytical error in the method ± 1 %), (22).

**X-ray measurements**

The X-ray small-angle scattering data were recorded with a camera developed by Kratky & Skala (23). The scattering angle was set by an on-line Hewlett-Packard computer 2100S, which also received and recorded the intensity data (Wingren, B.G., Sjöberg, B. & Österberg, R., unpublished data). Monochromatization was achieved with a nickel $\beta$-filter and a pulse height discriminator in conjunction with a proportional counter.

All measurements were made at 21°C on samples of 12S-RNA that had been dialysed at 4°C against the ribosome reconstitution Tris/Mg/K buffer (see above). Before each sample was subjected to the X-ray beam it was allowed to equilibrate at 21°C for 30 min. The small-angle X-ray scattering recorded for each sample was recalculated to absolute scattered intensities by using the scattering from a Lupolen sample (24). X-ray scattering from the same Tris/Mg/K buffer of the same kind as that used for the samples was used to correct the background scattering.

When 12S-RNA was subjected to prolonged X-ray exposure, it was found that the intensity decreased with time. The successively changing intensity eventually converged to an intensity curve which is very similar to that previously recorded for the smaller S4-RNA (16), suggesting some breakdown of 12S RNA to the size of S4-RNA. Therefore, the time exposure for each sample was carefully controlled, and for some concentrations, more than one sample was used for recording the complete scattering curve. Each sample used was checked for possible radiation damage by rerunning it under identical conditions; only those data are reported which were found to be consistent with the data obtained in the second run.

**Neutron scattering measurements**

The neutron small-angle scattering data were recorded with the Dll camera at the Institut Laue-Langevin, Grenoble (25). The sample-detector distance was set at 2.5 m, and neutron scattering was recorded for four solutions of different contrasts, 0, 20, 40, and 100 % $D_2O$. The wavelengths were 0.5 and 1.1 nm. The transmissions of the samples were recorded at a sample-detector
distance of 10.5 m. Samples of 12S-RNA were prepared in buffers of 0.05 Hepes pH 7.50, 20 mM MgCl₂ and 0.30 M KCl. Hepes was substituted for Tris, since its pK-value in H₂O is close to 7.55; thus, the pH or pD of the buffer could be obtained within ± 0.1 unit just by weighing solid Hepes and its salt, cf. (26). This eliminates the great uncertainty obtained by "pH measurements" of buffers containing D₂O in cells with liquid junctions.

ANALYSIS OF THE DATA

The X-ray scattering curve for 12S-RNA in the aqueous buffer is shown in Fig. 1. It is extrapolated from five slit-corrected curves measured at the

Fig. 1. Experimental and calculated X-ray scattering curves of 12S-RNA, a large fragment of the E. coli 16S-RNA. (——) Experimentally observed X-ray scattering curve corrected for the collimation effect and extrapolated to zero concentration; (o o o o o) experimentally observed contrast dependent neutron scattering, Ic, obtained via equation 2. Comparisons are made with the scattering from models consisting of two identical flat cylinders situated 1.0 nm apart and with their base areas in the same plane. (——, thin, full-drawn line) Cylinders with dimensions 11.0 x 11.0 x 5.0 nm; (-----) cylinders with dimensions 12.0 x 12.0 x 4.0 nm; (-----) cylinders with dimensions 10.0 x 10.0 x 6.0 nm; Q = (4π sin θ)/λ, where λ is the wavelength.
concentrations 27, 18, 8.6, 7, 3, and 2 mg/ml. From the extrapolated data the radius of gyration, \( R \), was found to be 7.2 ± 0.5 nm. The molecular weight of 12S-RNA was determined to be 278,000 ± 25,000 using the partial specific volume (16), \( \bar{\nu} = 0.53 \text{ cm}^3/\text{g} \) and the formula described by Kratky (27).

The X-ray data were further analysed by calculating the distance distribution function, \( p(r) \), using Glatter's computer program (28). The result, which is shown in Fig. 2, indicates that the largest distance within the 12S-RNA molecules is 23.0 ± 2.0 nm.

From the Guinier plots of Fig. 3 the radius of gyration, \( R \), and the extrapolated zero-angle intensities, \( I(0) \), were determined for the contrast of 20 \%, 40 \%, and 98 \% D_2O. The results of the \( I(0) \)-data are shown in Fig. 4 in the form of the plot \( \sqrt{I(0)} \) against \( \rho \), the scattering density of the solvent (29, cf. 30). The data can be fitted to a straight line yielding \( \rho_{\text{mean}} = 4.4 \times 10^{10} \text{ cm}^{-2} \) corresponding to 71.5 \% D_2O solvent (Fig. 4). (\( \rho_{\text{mean}} \) is the value of \( \rho \) at which \( I(0) \) vanishes). From the forward scattering in Fig. 4, the extrapolated value at zero D_2O concentration was determined, and this \( \rho_{\text{mean}} \) was used to estimate the molecular weight (31). The result was 250,000 ± 25,000 which agrees within ten per cent with that obtained via X-ray scattering.

From the value of \( \rho_{\text{mean}} \), the excess scattering density, \( \bar{\rho} \), was then calculated so that the variation of \( R \) with \( \bar{\rho} \) could be analysed; the quantity \( R^2 \) was plotted against \( (\bar{\rho})^{-1} \) according to the equation (32):

\[
R^2 = R_C^2 + \alpha(\bar{\rho})^{-1} - \beta(\bar{\rho})^{-2}
\]

(1)

\( R_C \) is the gyration radius at infinite contrasts, \( \alpha \), the second moment of the internal structure, \( \bar{\rho}(r) \), and \( \beta \), a constant related to the displacement of the centre of the scattering mass with contrast. Fig. 5 shows the result. As

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![Fig. 2. Distance distribution, \( p(r) \), of 12S-RNA as calculated from the X-ray scattering data.](image-url)
Fig. 3. Guinier diagrams of neutron scattering and X-ray scattering data obtained for 12S-RNA; the neutron scattering data were recorded for different H$_2$O/D$_2$O contrast. The slopes indicated yield the following radii of gyration: (a) X-ray scattering (corrected for the collimation effect and extrapolated to zero concentration), $R = 7.2$ nm; 20% D$_2$O, $R = 7.2$ nm; 40% D$_2$O, $R = 7.3$ nm; 100% D$_2$O, $R = 6.5$ nm; for H$_2$O, the slope indicated corresponds to the $R$-value obtained by X-ray scattering, $R = 7.2$ nm.

Fig. 4. Neutron zero-angle scattering from solutions of 12S-RNA plotted against the D$_2$O concentration. Zero-angle intensities were obtained by extrapolation of the Guinier diagram of Fig. 3.
Fig. 5. Contrast variation of radius of gyration of 12S-RNA.

indicated in this figure, the experimental data are subjected to relatively large errors and only a fairly crude estimate of $\alpha$ and $R_c$ is possible, yielding $\alpha = (7 \pm 4) \times 10^{-4}$ and $R_c = 7.1 \pm 0.2$ nm. Since the data can be fitted to a straight line we limit the number of parameters by tentatively assuming $\beta = 0$. The value of $\alpha$ is consistent with a particle where the regions of higher scattering density are located on average further from the centre of the particle than the regions of lower scattering density (cf. 30).

The small-angle scattering intensity observed depends on the contrast according to the following equation (cf. 30):

$$I(h) = \rho^2 I_c(h) + \beta I_{cs}(h) + I_s(h)$$

where $I_c$ is contrast-dependent, $I_s$ fluctuation-dependent and $I_{cs}$ the cross term. In this study the angular range, covered by the neutron scattering, is limited to $28 \leq 40$ mrad, and therefore, in the present quite small angular range, the contributions of $I_s$ and $I_{cs}$ are small. Therefore, in the comparison to models only the $I_c$ function was used.

When the data of the shape function $I_c$ were compared with theoretical curves, two-body models were used. This is due to the fact that the $p(r)$-curve consists of one maximum and one pronounced shoulder, which indicates the presence of at least two structural domains. The calculation of the theoretical scattering from the two-body models was done by using the procedure described by Pilz et al. (33). Of the various ellipsoid- and cylinder models tested, it was found that the scattering from two identical cylinders, situated 1.0 nm apart and with the base area of the cylinders in the same plane, best
explained the I_c scattering curve (Fig. 1). The best fit was obtained using cylinders of diameters 11.0 nm and height 5.0 nm. However, as indicated by the positive α-value, a compact model can only give a fairly crude description of the 12S-RNA molecule. In order to explain the whole sets of neutron- and X-ray data models more complex than those of uniform electron density are required.

DISCUSSION

The X-ray scattering results described in the previous section indicate that the 12S-RNA fragment of 16S-RNA has a molecular weight of 278,000 and a radius of gyration of 7.2 nm. These data agree with those obtained by neutron scattering, which yield a molecular weight of 255,000 and a radius of gyration at infinite contrast, R_C = 7.1 nm. The molecular weight obtained is also in agreement with the published analysis of the RNA indicating a molecular weight between 250,000 and 300,000 (12); as yet, however, the RNA sequence of 12S has not been determined exactly. The radius of gyration, on the other hand, is somewhat larger than that reported for the 30-S subunit, R_C = 6.85 nm, and for the RNA within the 30-S subunit, R^RNA = 6.1 nm (34). This might indicate that the present fragment of 16S-RNA has expanded somewhat compared to its native state and/or that the additional 16S-RNA is packed relatively close to the electron density centre of 12S RNA. The largest dimension of our model, 23 ± 2 nm, is close to that inferred for the 30-S subunit from electron microscopy, 24.0 - 26.0 nm (35) and X-ray scattering, 25.0 nm (36), and it is of the same order of magnitude as the largest dimension for RNA in the 30-S subunit observed by neutron scattering, 20.0 nm (34).

The present fragment of 16S-RNA is considerably larger than the fragment previously studied by X-ray scattering, denoted S4-RNA (16). The size of this latter fragment seems to be of the same order of magnitude as the fragment of 16S-RNA recently reported by Serdyuk et al. (37); however, they (37) report a larger molecular weight, 170,000 (S_20,w = 13.5), as compared to that of S4-RNA, 136,000 (16). Although the fragment of Serdyuk et al. (37) has not been analysed for composition, its size suggests that it may approximate to the 11S-RNA fragment of Zimmermann et al. (12), which is intermediate in size between S4-RNA and the 12S RNA described here. In their paper, Serdyuk et al. (37) claim that their 16S-RNA fragment is very compact in solution having an approximate spherical shape of radius 5.0 nm. However, given the partial specific volume of their fragment \( \bar{\nu} = 0.536 \text{ cm}^3/\text{g} \) (37), it can be calculated that it would then have a dry volume corresponding to a sphere of only 3.3 nm
radius. As a result, if the shape model of Serdyuk et al. (37) is correct, their RNA fragment would contain 71% water by volume and therefore be less compact.

As indicated in Fig. 1, the \( I_C \)-function of the neutron scattering data and the proximal part of the X-ray scattering curve can be explained by the scattering from a model consisting of two adjacent cylinders. These cylinders have quite similar dimensions as those of the flat superhelix recently reported by Finch et al. (38) from studies on the nucleosome core particles from chromatin. Attempts were therefore made to interpret the present data somewhat further using superhelical models where each base, sugar and phosphate were considered structural units. The results indicated that the data could not be explained by superhelices alone; however, distorted superhelices partly filled with minor "hairpin" loops yielded a scattering curve in fair agreement with the data (Österberg, R., unpublished results). However, it should be noted that such a detailed model involves too many parameters relative to the accuracy of the data and we cannot exclude the possibility that, within the proposed shape, the present X-ray data can be explained by many other detailed models.

Likewise, the previously reported small-angle X-ray scattering data of S4-RNA (16), a fragment from the 5' one-third of 16S RNA, has been reinterpreted. The shape of this latter fragment, which is about half the size of 12S-RNA, was previously estimated to correspond to an oblate ellipsoid model with the dimensions of 13.2 x 13.2 x 3.2 nm, assuming a compact structure. The reinterpretation of the X-ray data indicate that the S4-RNA data can be explained equally well or better by the scattering from a cylinder with the dimensions of 12 x 12 x 3 nm (Österberg, R., unpublished results). Since a compact cylinder of these dimensions is approximately equivalent to a less compact cylinder of the dimensions 11 x 11 x 5 nm, these data indicate that one of the 12S-RNA cylinders essentially corresponds to S4-RNA.

In some respects these data on 12S-RNA and S4-RNA would correlate with the biochemical data on 16S-RNA. For example, the limited ribonuclease digestion studies of Zimmermann et al. (13) suggest that the 3' half of 12S-RNA could yield a second domain which assembles with the group of proteins S8, S15, S6, and S18. The concept of two domains is also compatible with the more recent secondary structure model of Woese et al. (see 2) in which widely separated sequence regions make contact via base pairing.

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REFERENCES


