Structure Similarity of E. coli 5S rRNA in Solution and Within the Ribosome

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Abstract: The article presents translational and rotational diffusion coefficients of 5S rRNA determined experimentally by the method of dynamic light scattering (DLS) and its comparison with the values predicted for different models of this molecule. The tertiary structure of free 5S rRNA was proposed on the basis of the atomic structures of the 5S rRNA from E. coli and H. marismortui extracted from the ribosome. A comparison of the values of DT, τR, and Rg predicted for different models with experimental results for the free molecule in solution suggests that free 5S rRNA is less compact than that in the complex with ribosomal proteins. In general, the molecules of 5S rRNA consist of three domains: a short one and two longer ones. As follows from a comparison of the results of our simulations with experimental values, in the molecule in solution the two closest helical fragments of the longer domains remain collinear, whereas the short domain takes a position significantly deviated from them.


Keywords: 5S rRNA; structure; dynamic light scattering

INTRODUCTION

The ribosomal 5S rRNA was discovered in 1963 as a component of the E. coli ribosome. It was later identified in ribosomes of almost all organisms. The molecule of 5S rRNA is relatively small comprising about 120 nucleotides. In bacterial ribosomes it is a component of a large subunit (50S), in which it is tightly bound to three proteins (L5, L18, L25). In the eukaryotic ribosomes 5S rRNA is a component of a
large subunit (60S), but it is complexed to only one protein (L5). Although 5S rRNA has been studied for nearly 40 years, its biological function and spatial structure have not been fully determined yet. At present more than 200 sequences of different 5S rRNAs are known. A general secondary structure of ribosomal 5S RNA has been proposed. For the majority of known 5S rRNAs the secondary structure includes five helices (I–V), two hairpin loops (C, E), two internal loops (B, D), and a hinge region (A) (Figure 1). The main difference between the secondary structures of molecules from different organisms is the length of helix IV and a network of base pairing. The current prediction of a secondary structure was made with the help of different algorithms based on the sequence and a set of arbitrarily chosen physical data. The secondary structures predicted in this way for ribosomal 5S rRNA from *E. coli* and *X. laevis* differ from the general model in having three internal loops.

5S rRNA can assume different conformations depending on the interactions among particular fragments of its domains. The information on details of its spatial structure has been obtained by a wide spectrum of biochemical and biophysical methods. Combinations of results obtained by various experimental techniques have led to a few models of tertiary structures of 5S rRNA. Differences between them are probably a consequence of significant differences in the experimental conditions, for example, concentration of magnesium ions or some salts, different types of buffers, pH, and so forth.

Up to the year 2000 a detailed tertiary structure of the entire 5S rRNA had not been known. It could not be solved on the basis of the x-ray diffraction data because of insufficient quality of the crystals grown, or on the basis of NMR study because of its size (molecular mass of approximately 40 kDa). However, data for the spatial structure of fragments of 5S rRNA had been collected. Analysis of the experimental data in combination with the results of computer simulations has led to the first three-dimensional structural model of the ribosomal 5S rRNA from *X. laevis* published in PDB.

Crystallization of the whole 70S ribosome from *T. thermophilus* provided data allowing a reconstruction of the ribosomal RNA structure with a resolution of 7.8 and 5.5 Å. However, the most precise information about three-dimensional structure of 5S rRNA comes from the data collected with a 2.4 Å resolution from *Haloacuta marismortui* ribosome. It was possible to grow a crystal of 5S rRNA from *T. flavus* in the microgravity conditions and collect the data with a resolution not exceeding 7.5 Å. Since that time, the number of atomic structures of 5S rRNA or units containing this molecule, deposited at PDB, has increased to 6. The general shape of the molecules in ribosome is similar, although the molecules come from different organisms. It has been noted that some details of the structure of free 5S rRNA fragments differ from those in the complexes with ribosomal proteins. Similar conclusions can be drawn from a comparison of the general size and shape of a free 5S rRNA molecule with the results of a study on the 5S rRNA molecule bound with the ribosome. Reliable information on the effect of the interactions with the ribosomal proteins on the structure of the 5S rRNA molecule will be obtained from the x-ray diff-

**FIGURE 1** A general model of the secondary structure of procaryotic 5S rRNA based on crystal structure of *H. marismortui* 5S rRNA within ribosome.
fraction study of the free 5S rRNA and its complexes with proteins out of the ribosome. As long as it is impossible to grow the 5S rRNA crystals of sufficient quality, a possible alternative is the indirect method of general shape determination of dynamic light scattering (DLS). In this article we report the experimental studies of the translational and rotational diffusion coefficients of 5S rRNA measured by means of DLS: photon correlation spectroscopy and confocal Fabry-Perot interferometry. These studies were performed at a relatively low RNA and high salt concentrations, to minimize the effect of intermolecular interactions.

The postulated structure of the 5S rRNA molecule in solution was verified by comparing values of the translational and rotational diffusion coefficients calculated for different models of its atomic structure with the corresponding experimental values. The 5S rRNA structure was additionally verified by comparing the gyration radius and a maximum size of the 5S rRNA molecule calculated for different tertiary structure models with corresponding values obtained experimentally and reported in the literature.

MATERIALS AND METHODS

DLS

Hydrodynamic properties of molecules in solution can be investigated by means of various methods. An important argument to apply DLS is that both rotational and translational diffusion coefficients are measured for the same sample and by essentially the same method, the only difference being the way of analyzing the information provided by the scattered light intensity. The experimental error in DLS is typically less than 1%, and can be as low as 0.1% for monodisperse samples, which is also a good argument for using this technique. In a typical polarized DLS (photon correlation spectroscopy) experiment, a vertically polarized laser beam of a wavelength $\lambda$ enters the sample and the polarized component of light scattered at a certain angle $\theta$ in horizontal plane is measured with a photomultiplier. Fluctuations of the scattered light intensity, originating from Brownian motion of the molecules in solution, are analyzed in terms of their correlation function, whose analytical form in the limit of infinite dilution contains explicitly the translational diffusion coefficient $D_T$ of a single molecule:

$$g^{(2)}(\tau) = 1 + e^{-2q^2 D_T \tau}$$

where $q = 4\pi n \sin(\theta/2)/\lambda$, ($n$ being the refractive index of the solution) is the scattering vector magnitude.

In the depolarized DLS experiment the horizontal (depolarized) component of the scattered light is passed through a Fabry-Perot interferometer, and as a result, a depolarized spectrum is obtained, whose analytical form contains both translational ($D_T$) and rotational ($D_R$) diffusion coefficients:

$$I(\omega) = \frac{6D_R + q^2 D_T}{\omega^2 + (6D_T + q^2 D_R)^2}$$

For small and moderate size macromolecules the factor $q^2D_T$ is negligible when compared to $6D_R$, and only $D_R$ is evaluated from the depolarized spectrum. The rotational relaxation time is given by the relation

$$\tau_R = \frac{1}{6D_R}$$

Bead Model

The hydrodynamic parameters of 5S rRNA in solution have been calculated in terms of the so-called bead model, in which a molecule is represented by a set of rigidly bound spherical beads. There are many possible ways of transforming the molecule into the bead model, usually assuming the knowledge of the coordinates of its atoms. The choice of a particular one depends on the size of the molecule and the kind of information desired. A convenient model is bead per residue (BPR) model, which has been successfully applied for tRNA and DNA, and in which each nucleotide is replaced by a bead of the radius $\sigma$. In this article, we have assumed a more complex version of this model, namely a double bead per residue (DBPR) model, which has been tested for tRNA and short sections of DNA. In DBPR each nucleotide is represented by two overlapping beads of the same radius. The center of one of them is at the geometric center of the phosphate group and ribose, while the center of the other coincides with the geometric center of the amine base residue. The advantages this approach offers are a relatively exact representation of the shape of the molecule and a very easy way of controlling the thickness of the hydration shell by changing the radius of the beads. Correct calculation of the translational diffusion coefficient demands the use of a modified tensor of hydrodynamic interactions, while the introduction of the volume correction proposed by Antosiewicz and Pörshcke should lead to reliable results for the rotational diffusion time. A careful analysis of the theory and results of a number of tests have indicated that this approach can be used as long as the centers of the beads are outside the neighboring beads. For the overlapping beads of the same radii the Rotne-Prager tensor is used:

$$T_{ij} = (6\pi \eta_0 \sigma) \left( \frac{9}{32} \frac{R_{ij}}{\sigma^2} - \frac{1}{3} \frac{3}{32} \frac{R_{ij} R_{ij}}{\sigma^2} \right)$$

where $R_{ij}$ is the distance between the beads and $\sigma$ is their radius.

Samples

The sample of 5S rRNA from E. coli MRE 600 was purchased from Boehringer Mannheim (Mannheim, Germany).
It was dissolved in 10 mM Tris-HCl pH 7.5 buffer containing 100 mM NaCl and 1 mM MgCl₂. From 1 mL of the stock solution of 0.8 mg/mL, 200 µL was filtered (Millex-GV, 0.22 µm pore size; Millipore, Bedford, MA) directly into dust free scattering cell (Hellma, Müllheim, Germany) and centrifuged at 8000 × g for 1 hour to remove air bubbles. Dilution was performed by adding the proper volume of filtered buffer directly into the scattering cell.

Analysis of the structural changes of free 5S rRNA in solution was performed assuming the atomic model of 5S rRNA from *E. coli* (1CX2:C) obtained by cryo-EM with a resolution of 7.5 Å,²⁸ and for the sake of comparison, the model of this molecule from *H. marismortui* (1JJ2:9)¹³ obtained with a resolution of 2.4 Å by the x-ray diffraction method. The coordinates of the molecules have been extracted from the sets describing the structure of a large ribosome subunit.

**Light Scattering Setup**

For the polarized dynamic light scattering experiments (photon correlation spectroscopy) a setup consisting of Nd³⁺ YAG laser (ADLAS DPY 425 c, λ = 532 nm, 400 mW), a goniometer, and an ALV 5000 digital correlator (both from ALV GmbH) was used. All measurements were performed at 20 ± 0.05°C. The DISCRETE program was used to fit the experimental correlation functions.²⁹

For the depolarized DLS (Fabry-Perot Interferometry) a 150 MHz confocal Fabry-Perot interferometer with thermal housing (Burleigh CFT-500) with the same ADLAS laser was used. The measurement was also performed at 20°C. Special care was taken to ensure the best possible quality of optical components in the scattered light part of the setup. Achromatic lenses were used to collect and form the scattered light beam and a polarizing Glan-Thomson prism with the extinction coefficient of 10⁻⁷ was used as the analyzer. A very efficient (quantum efficiency 46%) photodetector SPCM-200-PQ (EG&G Optoelectronics, Canada) with very low dark counts allowed a remarkable reduction of accumulation times and noise in the spectra. An interference filter for 532 nm was mounted in front of the photodetector to avoid collecting fluorescence light whose intensity was comparable to or greater than that of the depolarized component of the scattered light. Although in a single, short sweep the finesse, a parameter characterizing the resolution of the instrument, amounted to 100, it was impossible to maintain finesse value above 30 in long runs without stabilization. Therefore, a special system of accumulation was applied in order to avoid the effect of the spectrum drift in the 50 cm long Fabry-Perot ethalon. The sweep duration was set to 0.5 s and the ramp amplitude allowed three orders to appear during this time. For the first 1/3 of the sweep the reference beam was passing through the instrument to indicate the exact position of the first peak (reference). A system of choppers then closed the reference beam and opened the path for scattered light, which was accumulated for the remaining 2/3 of the sweep. Data from a single sweep (1024 channels), collected with a multichannel scaler (PC card, SILENA, programming tools included) were analyzed in terms of the reference peak position and added to the accumulated spectrum after an appropriate shift. Thanks to some excess of the card memory, it was possible to use two memory banks for alternating sweeps, so that the whole analysis of a measured sweep could be made during accumulation of the next one, using just a regular, high level programming language. In this way the accumulation process was not disturbed by the data analysis and display. In order to account for the random fluctuations of the highest point position (taken as the peak position), the shift of a single channel was neglected. Two conditions had to be fulfilled to accept the incoming sweep: the intensity of the scattered light could not exceed a certain preset value and the peak shift could not be larger then 20 channels. The first condition allowed avoidance of rapid bursts of scattered light due to dust particles in solution (if present), while the second allowed a selection of the sweeps collected in identical conditions. A finesse of 60 (for accumulated spectrum) could be easily maintained even in 5 h long runs. Manual correction of the peak position (bias of the ramp) was required only occasionally (once per hour).

**Model Structures of the 5S rRNA Molecule**

At first, the molecular sites, which can work as hinges, facilitating relative rotations of molecular fragments, were identified. The distorted sites of possible bends were left as they were without optimization of the geometry, as the contribution of the local structural changes of individual nucleotides is negligible relative to that brought by rotation of whole domains. The supplest sites in the molecule structure are those without hydrogen bonds among the bases.

The structure of 5S rRNA from *E. coli* seems more extended or looser than those from other organisms (Figure 2). We have chosen a site in the β domain (helix II, loop B, helix III, loop C), about which the fragment of this domain could be rotated relative to the γ domain (helix V, loop E, helix IV, loop D) (Figures 2a and 3a). In 5S rRNA from *H. marismortui* we have chosen two sites of possible rotation in the β domain and a site permitting a rotation of the α domain (Figures 2b and 3b). In this way we have obtained groups of 5S rRNA models for *E. coli* and *H. marismortui*, differing in the patterns of domain distribution. The atomic models of the modified structures have then been converted into the bead models. In the DBPR model the radius of beads σ has been assumed as that for tRNA.²³ As we have chosen the model with overlapping beads, for the calculations of DT and Tg, we have had to take the tensor of hydrodynamic interactions, Eq.(4). The application of the model with overlapping beads is very convenient, although the tensor of hydrodynamic interactions used in this model still needs some improvements.²⁷ The calculations of DT and Tg were performed using the program HI4 courtesy of its author R. Pastor,³⁰ introducing a small modification to be able to carry out the calculations for modified forms of the interaction tensor T_{ij}. We also tested the program HYDROPRO, written and made accessible via the inter-
net by García de la Torre, at the address: http://leonardo.fc.u.m.es/macromol/programs.htm. For the molecule studied both programs gave essentially the same results. The time corresponding to rotation relaxation time measured in the DDLS experiment was taken to be that of the rotation about the axis perpendicular to the longest axis of the molecule. The gyration radii for the models were calculated directly from the atomic structure, taking into account all atoms except hydrogen atoms.

RESULTS

DLS

The PCS experiments were performed for four concentrations of 5S rRNA from the range 0.8 to 0.2 mg/mL. No dependence of $D_T$ on 5S rRNA concentration was observed. The final obtained value was $D_T = 6.00 \pm 0.06 \times 10^{-7}$ cm$^2$/s.

The analysis of the FPI spectrum was performed by convoluting the model function with the instrumental function obtained with use of a concentrated solution of high molecular weight DNA. Slow dynamics of this sample accompanied by high intensity of scattered light in VH geometry makes it a perfect choice for measuring the instrumental function while preserving the exact scattering geometry. A broadening of 1.49 MHz obtained from the fit corresponds to rotational relaxation time of 5S rRNA equal to $\tau_R = (107 \pm 7)$ ns.

Bead Model

The bead model calculations based on the atomic structure and calibrated on a series of nucleic acid molecules are expected to be more accurate compared to those based on the simple models like an ellipsoid of revolution or a rod. The assumption about a similar hydration level of tRNA and 5S rRNA allows a prediction of the diffusion properties of the latter on the basis of the postulated atomic structure. We expect the results of the bead model calculations to be precise enough to make certain statements concerning the 3D structure of the 5S rRNA molecule on the level of the relative orientation of its domains. The values of $D_T$ and $\tau_R$ calculated for the three available 5S rRNA atomic models are presented in Table I (upper part). Because none of the results fitted our experimental data, we decided to study the effect of the relative orientation of the molecule domains on the calculated

FIGURE 2  The tertiary structures of 5S rRNA drawn out of ribosome from (a) E. coli (1C2X:C); (b) H. marismortui (1JJ2:9) with the hinge regions marked by dashed line.
hydrodynamic properties. Simple manipulation performed on the three models involved only change of the angles between the domains. The bead model calculations performed on such modified structures allowed a determination of the most probable conformation of 5S rRNA molecule in solution. Also, the values of the longest dimension and radius of gyration were calculated for the modified structures and compared with literature data.

**Simple Hydrodynamic Models**

Because the hydrodynamic parameters of 5S rRNA are discussed in literature using simple hydrodynamic models, we have additionally used this approach here in order to allow for a direct comparison of identical quantities. We have calculated the size parameters for hydrodynamically equivalent ellipsoid of revolution (identical $D_f$ and $\tau_b$) to be $162 \times 35$ Å. Because all the diffraction experiments provide the value of the radius of gyration $R_g$, we have calculated $R_g$ for the ellipsoid, obtaining 37.9 Å. We shall use this $R_g$ value for comparison with those obtained more directly using other methods and the values of the model ellipsoid size parameters for comparison with the results of sedimentation experiments (Table I).

**DISCUSSION**

The bead model calculations of $D_f$ value based on the atomic structure of 5S rRNA extracted from the ribo-
some give results higher than the experimental value, whereas the value of \( \tau_R \) is too low (Table I). Moreover, the radius of gyration calculated for these structures varies from 32.7 to 34.0 Å. As follows from our considerations in the previous section, our experimental results \( (\text{DT}, \tau_R) \) analyzed using the bead model as well as simple hydrodynamic models suggest that the 5S rRNA molecule in solution has a radius of gyration in the range of 36–41 Å. In general, all these results consistently point out that its solution structure is less compact and more elongated (differences in \( D_T \) and \( \tau_R \)) compared to the structure maintained in ribosome. The other results reported in literature on the structure of free 5S rRNA seem to be ambiguous. A hypothetical model proposed by Westhof for 5S rRNA from *Xenopus laevis* (1RRN) has the value of \( R_g \) comparable to our results (38.5 Å). Also, the sedimentation experiment of Fox and Wong\(^3^2\) gave a hydrodynam-
ically equivalent ellipsoid 160 × 32 Å, corresponding to \( R_g = 37.2 \), which is close to our results. SAXS experiments on free 5S rRNA molecules revealed smaller estimates of \( R_g \). Österberg et al.\(^\text{33} \) reported 36.1 Å and Funari et al.\(^\text{8} \) \( R_g = 34.4 \) Å. While the former value might still correspond to an extended structure, the latter definitely falls in the region of values obtained for ribosome structures of 5S rRNA molecule. However, the buffer composition (low ionic strength) in the sample of Funari et al. suggests that their scattering profiles could be strongly affected by the solution structure factor, which has a peak at the values of the scattering vector close to the extrapolation region used for the estimation of \( R_g \). From the point of view of colloidal physics, such conditions (low salt buffer and strongly charged sample) are perfect for studying the electrostatic interactions, the effects of which dominate the shape of the SAXS spectrum.\(^\text{37–39} \) Österberg et al. used a buffer with much higher ionic strength, which might explain the differences in the values of \( R_g \) measured. As motioned above, we think the free 5S rRNA molecule is more extended and opened than that in ribosome. However, the structures suggested for this molecule so far do not deviate significantly from that in ribosome. The simplest way to achieve reasonable agreement with experimental data leads through manipulation of the domains’ orientation, that is, their folding and the angle they make with the other domains.

The first attempts to calculate the parameters showing the effect of changes in the mutual position of domains have been undertaken by us for the structure from \( H. \) marismortui (1JJ2:9). The deviation of the \( \alpha \) domain from the plane made by the other domains resulted in small changes. The orientation of the \( \alpha \) domain at the right angle to \( \beta \) and \( \gamma \) domains changed the value of \( D_T \) by \( \pm 3\% \), \( \tau_R \) by \( \pm 2.3\% \), and \( R_g \) by only 0.74\%. The maximum dimension of the molecule, that is the distance between extreme ends of the C and E loops, remains the same and equals 106 Å.

Much more pronounced is the effect of changes in the \( \gamma \) domain position on the values of \( D_T \), \( \tau_R \), and \( R_g \). The stretching out of this fragment of the molecule and its coaxial arrangement with the \( \beta \) domain in our calculations resulted in a significant change in all molecular parameters. The values of the hydrodynamic parameters \( (D_T \) and \( \tau_R \)) for the ribosomal 5S rRNA molecule stretched out in this way are very similar to those obtained experimentally. However the value of \( R_g \) and the maximum dimension of the model are much greater than those determined in x-ray scattering experiments. A similar elongated shape of the molecule has been assigned only in interpretation of sedimentation measurements.\(^\text{32} \) The molecule was hydrodynamically described as a prolate ellipsoid of the size 160 × 32 Å. A further deviation of the \( \alpha \) domain (helix I, loop A) leads to subsequent but less pronounced changes in the translation diffusion coefficient, rotational relaxation time, and radius of gyration.

We have performed analogous simulations for the atomic structure model of 5S rRNA extracted from the ribosome of \( E. \) coli (1C2X) (Figure 4a). On the basis of the experience from working on the 1JJ2 model, we have made an attempt to straighten up and stretch out the molecule, although it required procedures different from those used for 5S rRNA from the ribosome of \( H. \) marismortui. The full stretching out of the molecule, that is the coaxial arrangement of the \( \beta \) and \( \gamma \) domains, has brought about a decrease in \( D_T \), and a dramatic increase in \( \tau_R \) and \( R_g \). The coefficient
of translational diffusion is much less sensitive to changes in the maximum dimension of the model than $\tau_R$ and $R_g$. For the stretched out $\beta$ domain (helix II, loop B, helix III, loop C) arranged collinearly with the helix V, the maximum size of the 5S rRNA molecule is 155 Å and $\tau_R$ and $R_g$ are 113.7 ns and 38 Å, respectively (Figure 4c). A slight bent of the helix III with respect to the helix II, responsible for a decrease of the maximum size to 133 Å (Figure 4b), brings about a decrease in $\tau_R$ to 96.9 ns, whereas $R_g$ changes to 36.2 Å, and $D_T = 5.94 \times 10^{-7} \text{cm}^2/\text{s}$ shows practically no change. Thus, the structure presented in Figure 4(b) might be very similar to the real solution structure of the 5S rRNA molecule.

The DLS experiment reported by Müller et al. with the value of $D_T = (6.2 \pm 0.2) \times 10^{-7} \text{cm}^2/\text{s}$ was performed at low salt concentration and much higher concentrations of 5S rRNA. In these conditions as a result of electrostatic interactions the diffusion coefficient value extrapolated to the zero concentration is charged with a relatively great error (due to the strong dependence of $D_T$ on polymer concentration usually observed at low ionic strength), nevertheless the value obtained by Müller in the limit of error is comparable with that obtained by us (Table I).

**CONCLUSIONS**

We have measured the translational diffusion coefficient $D_T$ and the rotational relaxation time $\tau_R$ of 5S rRNA using DLS. We propose a solution structure of 5S rRNA that was obtained by modifying the structure of this molecule in the ribosome in such a way that the calculated values of $D_T$ and $\tau_R$ (using the bead model) matched the experimental results (Figure 4b). The effect of domain rotation leading to stretching of the molecule is illustrated in Figure 5. The proposed solution structure is less compact when compared to that in ribosome. As Funari suggested for *T. flavus*, 5S rRNA in solution assumes a Y-letter shape with two long arms (domains $\beta$ and $\gamma$) and one short arm ($\alpha$ domain). That model is similar to the hypothetical model of 5S rRNA from *X. laevis* in which $\alpha$ and $\beta$ domains are nearly collinear, while the $\gamma$ domain is inclined to them at a certain angle. Our analysis indicates that in solution the situation is probably different and the domains $\beta$ and $\gamma$ tend to assume a nearly collinear arrangement. The collinearity of the helices II and V has been postulated earlier by Burnel et al. and is preserved even in the compact structure of the 5S rRNA molecule extracted from the ribosome.

The hydrodynamic size of the ribosomal 5S RNA molecule determined on the basis of the measured translational diffusion coefficient and rotational relaxation time ($162 \times 35$ Å for a model of an ellipsoid) is in agreement with the results obtained from the sedimentation measurements. However, the maximum size of the structure proposed by us is only 133 Å, which is close to the results of SAXS measurements presented by Österberg. The size of the molecule and the values of $R_g$ obtained from the other SAXS studies imply a more compact solution structure of 5S rRNA, but the buffer conditions chosen in those experiments make the interpretation ambiguous.
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