A small angle X-ray scattering study of a fragment derived from *E. coli* 5S RNA

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ABSTRACT

The structure of a 62 base nuclease resistant fragment of *E. coli* 5S RNA (bases 1-11, 69-87, 89-120) has been examined by small angle x-ray scattering. The results obtained are indistinguishable from those expected if this oligonucleotide complex were a perfect RNA double helix of about 30 base pairs. These results indicate that this portion of 5S RNA is in a configuration which is approximately double helical, even though proper base pairing is possible over only half its length.

INTRODUCTION

Many bacterial 5S ribosomal RNAs are efficiently reduced to a nuclease resistant core when lightly digested with RNase A. In the case of *E. coli*, the resistant fragment consists of bases 1-11, 69-87, and 89-120 of the parent molecule (see Fig. 1). It includes the sequences which comprise the terminal stem helix and the procaryotic loop helix in conventional models of 5S RNA secondary structure. Fragment retains the protein L25 binding activity characteristic of intact 5S RNA (1).

Recent NMR results have demonstrated the existence of the terminal stem helix and the procaryotic loop helix in both 5S RNA (2) and fragment (3). The spectra of the two molecules are remarkably similar suggesting that fragment is a domain of 5S RNA. The similarity of fragment and 5S RNA is underlined by the fact that L25 binding causes alterations in the fragment's NMR spectrum which are identical to those seen in the case of 5S RNA (2). Interest in this system has been further stimulated by the finding that both fragment and its complex with L25 can be crystallized (4).

In order to get some information about the overall conformation of the fragment, a small-angle x-ray scattering (SAXS) study was carried out, the results of which are reported here. (A preliminary report on this work has appeared elsewhere (5).) Data were collected at the same

Figure 1: 5S RNA secondary structure. *E. coli* 5S RNA is depicted in the three-stem model proposed by Fox and Woese (20). This figure is taken, with minor changes, from Figure 1 of reference (24).

time on 5S RNA as a control. In brief, the fragment scatters x-rays as though it were a cylindrical rod about 85Å long and 20Å in diameter, exactly the dimensions it would have if it were a normal RNA double helix about 30 base pairs long. Apparently the bases which lie between the two helical stems visible by NMR (69-78, 98-109), even though their sequences do not permit normal helix formation, nevertheless have a structure which gives the overall molecule about the same properties it would have if these bases were in perfect double helix.

MATERIALS AND METHODS

Samples

All 5S RNA and fragment samples were prepared from *E. coli* rrnB 5S RNA obtained from *E. coli* strain HB101/pKK5-1 under overproducing conditions, as previously described (3). The purity of all RNA preparations was checked by polyacrylamide gel electrophoresis.

Sample concentrations were determined spectrophotometrically using the approximate extinction coefficient 20 OD₂₆₀ /cm (mg/ml). The relative hypochromicity of 5S and fragment was checked by alkaline hydrolysis (0.3M KOH, 18 hours at 37°). No difference was found within an error of 3%. Thus relative concentrations of 5S RNA and fragment should be proportional to relative optical density.

Samples were dialyzed against 0.1M KCl, 3mM MgAcetate, 10mM cac-

dylic acid, pH 7.0, and concentrated by ultrafiltration through a YM5 membrane (Amicon).

DATA COLLECTION

SAXS data were collected on two instruments both of which are based on focussing mirror optics and linear position-sensitive detectors as described (6). One instrument makes use of a Phillips 1.2 kW fine-focus copper x-ray tube and the other an Elliot GX21 rotating anode. Copper $K\alpha$ x-rays ($\lambda = 1.54\text{\AA}$) were used. Samples were held in thin walled quartz sample tubes 1mm in diameter.

SAXS data were typically collected over the range $s = 0.0024$ to $0.038\text{\AA}^{-1}$ ($s = 2\sin\theta/\lambda$, where θ is half the scattering angle) with an interchannel spacing of $0.000340\text{\AA}^{-1}$. The beam width at half height was less than 1 channel.

All data sets were collected at room temperature (293 to 298 K). Two independent data sets were collected for each molecule. Each set included 7 to 8 concentrations equally spaced between 5 and 40 mg/ml. Background scattering data were collected in the same sample tubes on the respective dialysates.

DATA ANALYSIS

After subtraction of background scattering, the data were folded about the beam center, scaled by concentration and collection time and extrapolated channel by channel to zero concentration by a variance weighted, linear least squares routine (7). Each of the extrapolated data sets so generated was analyzed by indirect transformation (8) to obtain the length distribution function, $P(r)$, of the solute molecule, as well as its zero-angle scattering, I_0 , and its radius of gyration, R_g . (The length distribution is the distribution of lengths of all interatomic vectors within the molecule giving rise to the scattering.) The parameters I_0 and R_g were also calculated by indirect transformation of the experimental data sets obtained followed by extrapolation of the values found at each concentration to zero concentration. The data for fragment were analyzed for cross-sectional radius of gyration, R_x , by plotting $\ln(sI(s))$ against s^2 (9). The slope of such a plot in the linear region, if it exists, corresponds to $-2(\pi R_x)^2$ (10).

The maximum dimension, $D_{\max}$, of each molecule was obtained by noting the value of r at which $P(r)$ reaches zero.

Table I. Scattering Parameters of 5S RNA and Fragment.

Experimental Series 1:		
	<u>Fragment</u>	<u>5S RNA</u>
R_g	$26.1 \pm 0.4 \text{ \AA}$	$32.9 \pm 0.4 \text{ \AA}$
I_0	3595 ± 50	8030 ± 100
$D_{\max}$	$86 \pm 4 \text{ \AA}$	$105 \pm 5 \text{ \AA}$
Experimental Series 2:		
	<u>Fragment</u>	<u>5S RNA</u>
R_g	$26.9 \pm 0.5 \text{ \AA}$	$33.7 \pm 0.3 \text{ \AA}$
$D_{\max}$	$86 \pm 4 \text{ \AA}$	$105 \pm 5 \text{ \AA}$

RESULTS

Values for forward scatter (I_0), radius of gyration (R_g) and maximum linear dimension ($D_{\max}$) for both experimental series are summarized in Table I. All values given are extrapolated to zero concentration and were obtained by analysis of the scattering curves extrapolated to zero concentration. Extrapolation of the R_g 's and I_0 's from individual data sets to zero concentration gave effectively identical results. The radius of gyration found for 5S RNA $33.3 \pm 0.6 \text{ \AA}$, is in reasonable agreement with previous work. Connors and Beeman (11) reported a value of $34.5 \pm 1.5 \text{ \AA}$ for *E. coli* 5S RNA and Oesterberg *et al* (12) observed it to be $36.1 \pm 1.0 \text{ \AA}$.

The distribution of lengths of vectors between 5S RNA atoms found by Fourier inversion of the entire 5S RNA scattering curve is shown in Fig. 2. It is unusual in being bimodal, but is in fact quite similar to the length distribution published recently by Müller *et al* (13) for rat liver ribosomal 5S RNA. The maximum vector length we obtain for *E. coli* 5S RNA is in the neighborhood of $105 \text{ \AA}$, a value again in agreement with the results of Müller *et al* (13). Thus, there is every reason to believe the data collection and processing techniques being used here are adequate.

The radius of gyration of fragment is $26.5 \pm 0.5 \text{ \AA}$, substantially less than that of its parent molecule. Its length distribution (Fig. 3) also

Figure 2: Length distribution function of 5S RNA. $P(r)$ was calculated by Fourier inversion of 5S scattering data, extrapolated to zero concentration, covering the angular range $s = .0024 \text{ \AA}^{-1}$ to $.0381 \text{ \AA}^{-1}$.

differs from 5S in being monomodal, not bimodal. It rises sharply with increasing r to a peak at about $18\text{\AA}$ and then decreases in a more or less linear manner until it reaches a maximum distance of $85\text{\AA}$. (The ripples apparent in the descending part of length distribution are series termination effects, not real features of the data (see below).) This type of length distribution is characteristic of long objects of constant cross-section such as prisms and cylinders (14).

Figure 3: Length distribution function of 5S RNA fragment. $P(r)$ was calculated from scattering data, extrapolated to zero concentration, covering the angular range $s = .0024 \text{ \AA}^{-1}$ to $.0373 \text{ \AA}^{-1}$.

Figure 4: Cross-sectional scattering curves of fragment. Data from two independent experimental series, extrapolated to zero concentration, and smoothed by indirect transformation, are shown plotted as $\ln(sI(s))$ vs s^2 .

Information about the cross-sectional profile of such scatterers can be obtained by plotting the scattering data they produce in the form $\ln(sI(s))$ versus s^2 (9). Such a presentation of fragment scattering data is shown in Fig. 4. Two zero concentration data sets are shown derived from independent experimental series. The long linear regions they contain confirm the interpretation given to the length distribution, namely that fragment is a long molecule of constant cross-sectional profile. The slope of the linear regions yields an estimate of the radius of gyration of the cross-section of the molecule, $8.0 \pm 0.7 \text{ \AA}$. Similar values have been obtained in the past for the radius of gyration of the cross-section of RNA double helices (see (15) and references therein).

The forward scattering values shown are in arbitrary units and can be used only for comparing the molecular weights of molecules studied in the same experimental series. The relative I_0 's for 5S RNA and fragment should be proportional to their molecular weights, assuming similar partial specific volumes. RrnB 5S RNA produced in the presence of chloramphenicol, as this material was, is 4 to 6 bases larger than normal 5S RNA (Normanly & Noller personal communication) due to a failure of the material to mature fully (16). The fragment's sequence includes 62 bases (Moore, unpublished data). Thus the I_0 's should be in the ratio of 2:1, which they nearly are in the first series of experiments, in which data

for the fragment and whole 5S were collected simultaneously on the same instrument.

SIMULATIONS

The data summarized above strongly suggest that fragment is a cylinder whose diameter is that of a double-helical nucleic acid. Computations were done to test the degree to which such a model is consistent with the data. The first simulation was done by modeling the fragment as a solid cylinder of a length and diameter which would accommodate all the fragment's bases as A type double helix.

The length distribution function, $P(r)$, of such a cylinder (radius $10\text{\AA}$ and length $84.3\text{\AA}$) was calculated by a Monte Carlo method. Pairs of points lying in the cylinder were generated randomly and binned according to the distance between them. The bin width was typically $0.5\text{\AA}$ and up to 10^6 pairs of points were generated. The resulting $P(r)$ function showed the characteristic sharp initial rise and linearly decreasing tail expected for rodlike objects (data not shown) (14). Because $I(s)$, the scattering profile, and $P(r)$, the length distribution, are related by Fourier transformation, the effect of truncation of the scattering function, $I(s)$, on $P(r)$ could be investigated by calculating $I(s)$ from $P(r)$, truncating $I(s)$ at various values of s , and recalculating $P(r)$. Strong ripples were evident in the linear region of $P(r)$ calculated from $I(s)$ truncated at $s = 0.040\text{\AA}^{-1}$, about the value where the experimental data ended. These disappeared when $I(s)$ was extended to $s = 0.100\text{\AA}^{-1}$ or further. (The indirect transformation algorithm employed to calculate $P(r)$ from measured data used Porod's $(1/s^4)$ Law (10) to continue the scattering data beyond the point of observation in order to minimize truncation effects in the resulting length distribution. This continuation of the data is a good one for globular molecules, but for more elongated shapes, like the one encountered here, is much less effective.) R_g was calculated by indirect transformation and found to be $25.6\text{\AA}$, in agreement with the value $25.3\text{\AA}$ calculated using the formula:

$$\text{Eq. 1.} \quad R_g^2 = \frac{R^2}{2} + \frac{H^2}{12}$$

where R is the radius and H is the height of the cylinder.

The scattering from fragment was also simulated assuming it is a perfect 30 base pair A-RNA double helix. Atomic coordinates obtained for an A-U basepair by fiber diffraction (17) were used to generate the helix by incrementing the polar angle by 32.7° and the axial variable by $2.81\text{\AA}$

Figure 5: Length distribution function calculated for 30 AU base-pair A-RNA double-helix. $P(r)$ obtained by indirect transformation of scattering data computed from Debye formula (Eq. 2) for the angles $s = .001 \text{ \AA}^{-1}$ to $.040 \text{ \AA}^{-1}$.

for each successive base pair. The scattering was calculated for this hypothetical molecule using the Debye scattering formula (18):

$$\text{Eq. 2.} \quad I(s) = \sum_{ij} f_i f_j \frac{\sin(2\pi s r_{ij})}{(2\pi s r_{ij})}$$

where f_i and f_j are the atomic structure factors of atoms i and j and r_{ij} is the distance between them. For most molecules of the fragment's molecular weight the number of terms which must be included in the Debye sum is prohibitive. However, the symmetry of this model permitted a substantial reduction in the number of distinct terms that had to be calculated, making this approach feasible. The atomic form factors were assumed to be independent of angle for the small scattering angles in question. Displacement of solvent was accounted for simply by subtracting the average electron density of the solvent from the electron density of each atom, as determined using volumes calculated for each atom in the structure by Richard's Voronoi volume program (19). This program takes into account the accessibility to solvent of pockets and crevices in the molecule.

The scattering was calculated out to $s = 0.040 \text{ \AA}^{-1}$ in increments of $.001 \text{ \AA}^{-1}$. Inversion of this calculated scattering curve resulted in the $P(r)$ shown in Fig. 5. The calculated radius of gyration is $25.3 \text{ \AA}$, in good agreement with observation. The value of R_x calculated from the slope of the cross-sectional scattering curve is $7.8 \text{ \AA}$.

Calculations were also carried out to determine the degree to which

the fragment could be bent and still give rise to the data observed. $P(r)$ functions were calculated using the Monte Carlo method for two circular cylinders each 42Å in length and 20Å in diameter, positioned end to end with their axes making angles of 165°, 150°, 135° and 120° to each other. A significant effect on R_g was evident for angles of 150° and smaller ($R_g = 24.9$ Å for 150° compared to 25.6 Å for 180°). The effect of bending on $P(r)$ was less obvious at angles above 150°, but change was noticeable by an angle of 135° as compared to the straight cylinder.

DISCUSSION

It is clear that the data obtained for 5S RNA fragment match quite well what one would anticipate if the molecule were a perfect A-type RNA helix, in a word, a cylindrical molecule 20Å in diameter, about 85Å long. The data do not rule out a modest bend (less than 30°) in the molecule over its contour length, however.

This result is a surprising one. Secondary structure models for 5S RNA based on comparative studies of the sequences of 5S RNA from different species generally propose two helical segments within the region included in fragment (see Fig. 1)(20). The NMR data available show that both these helical stems exist, but that there are only 13 base pairs of well developed double helix in the two of them, a stretch of 7 involving bases 2-8 and a stretch of 6 including bases 79-84. The sequence permits a number of additional base pairs to be hypothesized between the two stable helices, but nothing extensive or continuous. Moreover, the fragment includes an interruption in its covalent structure between bases 10 and 69 of the parent molecule. Yet apparently it is able to maintain itself in a structure which roughly approximates normal, straight, A-type double helix over its entire length. In this connection it is interesting to note that the consensus structure for eucaryotic 5S RNA includes a substantial helix in the middle of the region which is poorly structured in the *E. coli* fragment (21 and 22). Moreover, there exists at least one procaryotic 5S RNA, that from *Sulfolobus acidocaldarius* (23) whose sequence permits a near perfect helix for the entire region homologous to the *E. coli* fragment. Clearly one of the interesting aspects of future studies of this molecule will be the determination of the details of how this structure is achieved. This result also suggests that one should be alert to the possibility that in other rRNAs there may exist a lot of quasi-helical structure in addition to the more perfect helices already

postulated using standard base pairing rules.

Having seen what the length distribution of 5S fragment looks like, namely like that of an RNA double helix, one returns to the length distribution of the intact parent molecule with renewed interest. Its steep initial rise to a maximum at 20Å is characteristic of a double helix. Were one to suggest that 5S RNA consists of two straight helical structures, one being the fragment portion, and the other including bases 11-68, the sharp rise observed would be anticipated but the linear descent to zero would be obscured by the presence of interhelix vectors at longer distances, resulting in a bimodal distribution as observed. Previous workers have proposed three stem models for 5S RNA having Y shapes, based on scattering data (12). Minor modifications of these models would easily accommodate the data presented here on fragment.

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