SMALL-ANGLE X-RAY SCATTERING STUDY OF S4-RNA, THE 16 S RNA BINDING SITE FOR THE PROTEIN S4 FROM *ESCHERICHIA COLI* RIBOSOMES

Ragnar ÖSTERBERG and Bo SJÖBERG
Department of Medical Biochemistry, University of Göteborg, Fack, S-400 33 Göteborg 33, Sweden

and

Roger A. GARRETT and Ernst UNGEWICKELL
Max-Planck-Institut für Molekulare Genetik (Abt. Wittmann), Berlin, Germany

Received 26 April 1977
Revised version received 9 June 1977

1. Introduction

The ribonucleic acid (16 S RNA) of the 30 S *Escherichia coli* ribosomal subunit binds many of the proteins in this subunit, such as, for instance, S4, S7, S8, S15 and S20 [1,2]. The 16 S RNA binding site for the S4 protein, S4-RNA, can be isolated in pure form [3–9]; and it is indicated that this binding site consists mainly of two regions of approximately 150 and 160 nucleotides, originating from the 5′ terminal third of the 16 S RNA molecule [7]. These two regions, which are separated by about 120 nucleotides of the 16 S RNA sequence, seem to be stabilized by a specific RNA–RNA interaction [7]. Although the sequence of the S4-RNA region, prepared with both T_1 and pancreatic ribonucleases, has been partially analysed [7,9], the correct molecular weight and the size and shape of S4-RNA are not known.

We have analysed S4-RNA by using the small-angle X-ray scattering method; the results yield a molecular weight of 136 000 and a radius of gyration of 43.5 Å. The X-ray scattering curve can be explained, in its proximal angular range, by the scattering from a two-parameter, uniform electron density model with a shape of an oblate ellipsoid and the dimensions of 132 × 132 × 32 Å.

2. Materials and methods

2.1. *Preparation of S4 RNA from 16 S RNA*

16 S RNA was prepared from *E. coli* ribosomes (strain K12) in the presence of 0.5 mM $MgCl_2$ using the phenol-dodecylsulphate method [10]. In order to renature the 16 S RNA, it was first dissolved in the TMK buffer (0.03 M Tris–HCl of pH 7.6, 0.30 M KCl, 20 mM $MgCl_2$), then incubated for 1 h at 42°C, and finally cooled to 0°C. This renatured RNA was digested with carrier-bound RNAase A (Boehringer) for 5 h at 21°C, using 0.0018 Kunitz units per mg RNA. The solution was cooled to 0°C, centrifuged at 8000 × *g* for 10 min, to remove the enzyme, and then fractionated by agarose gel filtration in the TMK buffer at 4°C. The S4-RNA fraction eluted first from the agarose column (Biorad), well resolved from the other RNA fragments. The S4-RNA was precipitated with two volumes of cold ethanol. It migrated as one homogeneous band in 5% polyacrylamide gel electrophoresis, at pH 7.6, using a 0.02 M Tris buffer containing 5 mM Mg^{2+} ions. The capacity of S4-RNA to bind the S4 protein specifically was checked by an electrophoretic binding assay [11].

The concentration of S4-RNA was obtained from the optical density; the unit used (A_{260}) was defined

as the amount of material which, when dissolved in 1.0 ml, gave an absorbance of 1.0 at 260 nm with a light path of 1.0 cm. The UV absorbance of S4-RNA was determined by nitrogen and carbon analyses [12]; $A_{260} = (20.1)^{-1}$mg/cm^2

2.2. *X-ray measurements*

The X-ray small-angle scattering data were recorded with a camera developed by Kratky and Skala [13]. 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. and Österberg, R., unpublished results). Monochromatization of the copper radiation was achieved with a nickel β-filter and a pulse height discriminator in conjunction with a proportional counter.

All measurements were made at 21°C on samples of S4-RNA that had been dialysed against either the TM buffer (0.02 M Tris–HCl pH 7.4, 5 mM $MgCl_2$) or the TMK buffer mentioned above. The absolute scattered intensities were obtained using a standard Lupolen sample [14]; the Lupolen sample had been previously calibrated at the Graz Institut für Physikalische Chemie.

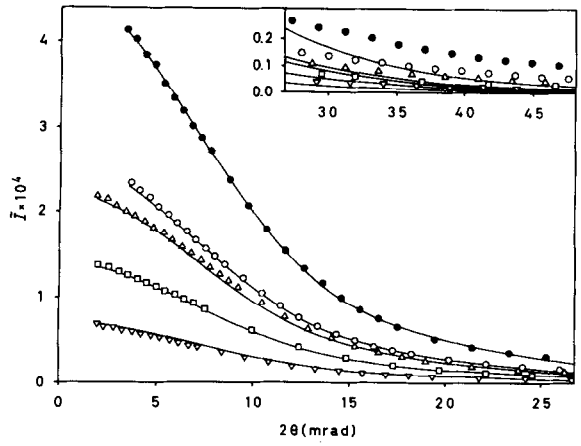

Fig.1. Small-angle X-ray scattering of the S4-binding site on 16 S RNA. The symbols are the experimental points for the concentrations 3.1 (▽), 6.3 (□), and 9.4 (△) mg/ml, run in the TM buffer, and the concentrations 10.7 (○) and 19.1 (●) mg/ml, run in the TMK buffer (every fourth point is plotted). The two sets of data have been recorded with S4-RNA from different preparations, and with different settings of the small-angle X-ray camera. The curves have been calculated from an oblate ellipsoid with the semiaxes $A = B = 66$, and $C = 16$ Å, and for a molecular weight of 136 000, cf. the text. Please note that the theoretical curves, $\tilde{I}$ versus 2θ, are 'smeared'.

3. Results

The small-angle X-ray scattering data were recorded for concentrations (c) equal to 3.1, 6.3, 9.4, 10.7 and 19.1 mg/ml, of the S4-RNA up to a scattering angle of 150 mradians. When the normalized intensity ($\tilde{I}/c$), obtained from the same setting of the camera, was plotted against the scattering angle, no significant concentration dependence was observed (fig.1). Furthermore, as indicated in fig.1, the scattering curves were the same whether the TM buffer or the TMK buffer was used. After slit correction (desmearing) of the data [15], the radius of gyration was determined to be 43.5 ± 2.0 Å. In order to calculate the molecular weight of S4-RNA, the partial specific volume must be determined with high accuracy. For this purpose the densities of three solutions (6.3, 10.7 and 19.1 mg/ml) and the density of the dialysis buffer were measured by a precision densitometer [16] at 21°C. From the results obtained the specific volume was calculated to be 0.528 cm^3/g. Using this value, and the formula described by Kratky [17], the molecular weight of S4-RNA was then determined; the result was 136 000.

When the X-ray scattering data were compared with theoretical curves, calculated for different triaxial bodies, the experimental data rather than their desmeared counterparts were used; therefore, the theoretical curves were 'smeared'. For this purpose and for the comparison, a computer program was developed (Sjöberg, B., manuscript in preparation), where, *via* a least-squares procedure, the model is searched for, which gives the best-fitting scattering curve relative to the primary experimental data. Figure 1 illustrates the result where the set of theoretical curves which give the best fit are compared with the present experimental data; the theoretical curves correspond to a two-parameter, oblate ellipsoid model with the semiaxes A = B = 66 Å and C = 16 Å. Theoretical curves calculated for other triaxial bodies did not give as good agreement with the experimental data as that obtained for the oblate ellipsoid. For instance, the oblate ellipsoid mentioned gave the error

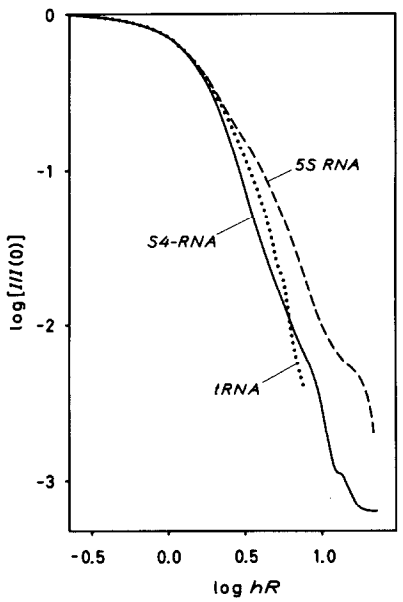

Fig.2. Comparison of experimental scattering curves obtained for different ribonucleic acids. Solid line, S4-binding site of 16 S RNA, S4-RNA; long-dash line, *E. coli* 5 S RNA [18]; dotted line, yeast phenylalanine tRNA (data from the study of Pilz et al. [14]).

square sum, U, = 1.2×10^{-3} as compared to $U = 7.2 \times 10^{-3}$ for the best prolate ellipsoid. Also, the prolate ellipsoid gave a radius of gyration that was much too high, 61 Å. In agreement with the oblate ellipsoid a triaxial ellipsoid gave the semiaxes A = 66 Å, B = 67 Å and C = 16 Å and a U-value of 1.2×10^{-3}. Thus, as far as uniform electron density models are concerned, the scattering from an oblate ellipsoid yields the best agreement with experimental data (cf. fig.1). As indicated in figs 1 and 2, however, the internal structure of S4-RNA contributes to the scattering data in the distal angular range, and the present oblate ellipsoid model should therefore only be considered as the very first approximation. A detailed model (most probably involving double-helical structures) that may explain the complete scattering curve, would require a better knowledge of the primary structure.

A comparison of the scattering curves recorded for S4-RNA, 5 S RNA and tRNA illustrates that S4-RNA is more symmetrical than tRNA or 5 S RNA. The comparison is shown in fig.2, where log $[I(h)/I(0)]$ is plotted against log (hR) for S4-RNA, *E. coli* 5 S RNA [18] and yeast $tRNA^{Phe}$ [14]; here, I denotes the desmeared [15] intensity; R, the radius of gyration and $h = (4\pi/\lambda) \sin\theta$, where θ is half the scattering angle. At very small angles, in the Guinier region, the scattering curves of all particles are the same function of hR. At somewhat larger angles the curves depend on the shape of the particle but not on the size.

It follows from fig.2 that, in the proximal angular range, the scattered intensities from 5 S RNA fall off more slowly with the angle than those of tRNA and the scattered intensities from tRNA fall off more slowly than those from S4-RNA. This implies that S4-RNA is less elongated than tRNA and that tRNA is less elongated than 5 S RNA.

4. Discussion

The results presented in the previous section on the S4-binding site of 16 S RNA have furnished a two-parameter model of this site consisting of an oblate ellipsoid with the dimensions of 132 X 132 X 32 Å. The mol. wt, 136 000, indicates that S4-RNA contains about 420 nucleotides. This large number of nucleotides requires a quite closely packed model, perhaps mainly consisting of double-helices. The existence of a compact structure is supported by the fact that this RNA region is inaccessible to T_1 ribonuclease digestion [19] and kethoxal modification [20] within the 30 S subunit.

The fact that the X-ray intensity of S4-RNA drops faster in the proximal angular range than those of 5 S RNA or tRNA (fig.2), indicates that S4-RNA is less elongated than 5 S RNA or tRNA, thus indirectly supporting the idea of a fairly symmetrical model for S4-RNA, such as an oblate ellipsoid.

As indicated from a recent electron microscope study [21], the 30 S ribosomal subunit has an elongated shape of the approximate dimensions 200 X 100 X 80 Å. Since the largest dimension of S4-RNA is 132 Å, these data [21] indicate that the S4-binding region of 16 S RNA may extend through the entire ribosomal subunit. In addition, the protein S4 which specifically binds to S4-RNA seems to have the shape of a flattened, elongated ellipsoid with the largest dimension equal to 180 Å [22]. As a result, the large dimensions of the S4-binding region of 16 S RNA and the corresponding

S4 protein indicate that they may extend through the 30 S subunit; their complex may constitute an important part of the central core in the 30 S ribosome subunit. Here, it is assumed that S4 and S4-RNA do not undergo any major conformational change during the assembling process. Support for this idea is provided via two independent electron microscope studies, using antibody markers, both indicating that S4 exists at multiple sites on the 30 S surface, situated fairly far apart [23,24].

Acknowledgements

We thank Mrs Christa Hennig and Mr Kunt Mørk-Olsen for expert technical assistance. This work was supported by grants from the Deutsche Forschungsgemeinschaft (to R. Garrett) and the Swedish Natural Science Research Council (to R. Österberg and B. Sjöberg).

References

[1] Zimmermann, R. A. (1974) in: Ribosomes (Nomura, M., Tissières, A. and Lengyel, P., eds) pp. 225–269, Cold Spring Harbor Laboratory, New York.
[2] Hochkeppel, H.-K., Spicer, E. and Craven, G. R. (1976) J. Mol. Biol. 101, 155–170.
[3] Schaup, H. W., Sogin, M., Woese, C. and Kurland, C. G. (1971) Mol. Gen. Genet. 114, 1–12.
[4] Zimmermann, R. A., Muto, A., Fellner, P., Ehresmann, C. and Branlant, C. (1972) Proc. Natl. Acad. Sci., USA 69, 1282–1286.
[5] Muto, A., Ehresmann, C., Fellner, P. and Zimmerman, R. A. (1974) J. Mol. Biol. 86, 411–432.
[6] Ungewickell, E., Garrett, R., Ehresmann, C., Stiegler, P. and Fellner, P. (1975) Eur. J. Biochem. 51, 165–180.
[7] Ungewickell, E., Ehresmann, C., Stiegler, P. and Garrett, R. (1975) Nucl. Acids Res. 2, 1867–1888.
[8] Zimmermann, R. A., Mackie, G. A., Muto, A., Garrett, R. A., Ungewickell, E., Ehresmann, C., Stiegler, P., Ebel, J.-P. and Fellner, P. (1975) Nucl. Acids Res. 2, 279–302.
[9] Mackie, G. A. and Zimmermann, R. A. (1975) J. Biol. Chem. 250, 4100–4112.
[10] Folkhard, W., Pilz, I., Kratky, O., Garrett, R. and Stöffler, G. (1975) Eur. J. Biochem. 59, 63–71.
[11] Garrett, R. A., Rak, K. H., Daya, I. and Stöffler, G. (1971) Mol. Gen. Genet. 114, 112–124.
[12] Kirsten, W. J. (1971) Microchem. J. 16, 610–625.
[13] Kratky, O. and Skala, Z. (1958) Z. Elektrochem. 62, 73–77.
[14] Pilz, I., Kratky, O., Cramer, F., von der Haar, F. and Schlimme, E. (1970) Eur. J. Biochem. 15, 401–409.
[15] Glatter, O. (1974) J. Appl. Cryst. 7, 147–153.
[16] Kratky, O., Leopold, H. and Stabinger, H. (1969) Z. Angew. Physik, 27, 273–277.
[17] Kratky, O. (1963) Progr. Biophys. 13, 105–172.
[18] Österberg, R., Sjöberg, B. and Garrett, R. A. (1976) Eur. J. Biochem. 68, 481–487.
[19] Fellner, P., Ehresmann, C., Ebel, J.-P. and Blasi, O. (1970) Eur. J. Biochem. 13, 583–588.
[20] Noller, H. F. (1974) Biochemistry 13, 4694–4703.
[21] Lake, J. A. (1976) J. Mol. Biol. 105, 131–159.
[22] Österberg, R., Sjöberg, B., Garrett, R. A. and Littlechild, J. (1977) FEBS Lett. 73, 25–28.
[23] Lake, J. A., Pendergast, M., Kahan, L. and Nomura, M. (1974) Proc. Natl. Acad. Sci. USA 71, 4688–4692.
[24] Tischendorf, G., Zeichhardt, H. and Stöffler, G. (1975) Proc. Natl. Acad. Sci. USA 72, 4820–4824.