SMALL-ANGLE X-RAY SCATTERING STUDY OF THE PROTEINS S1, S8, S15, S16, S20 FROM *ESCHERICHIA COLI* RIBOSOMES

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

and

Jenny LITTLECHILD
Max-Planck-Institut für Molekulare Genetik (Abt. Wittmann), Berlin, Germany

Received 15 April 1978
Revised version received 19 June 1978

1. Introduction

A series of biochemical data indicates that the ribosomal proteins S4, S8, S15 and S20 are specifically bound to 16S RNA, for a review see ref. [1]. However, very little is known regarding how these proteins interact with the nucleic acid. One approach to a better understanding of this interaction may be to first analyse the conformation of the individual components and then to analyse the specific rRNA–protein complexes by using the small-angle X-ray scattering method. As the first step of this general study, we have characterized both S4 and its binding area on 16S RNA from their small-angle X-ray scattering curves [2,3]. Here, we report the results from similar studies on the proteins S1, S8, S15, S16 and S20. The results indicate that these proteins are elongated: the proteins S8, S15 and S16 appear to have a largest dimension of about 100 Å and they are similar to the L18 and L25 proteins [4]; the protein S1 is more elongated and appears to have a maximum dimension of about 260 Å. It should be noted that all the proteins were prepared by a new, gentle method [5] that appears to preserve their native conformations, cf. [6].

2. Materials and methods

The proteins were prepared by a salt extraction procedure, where a stepwise LiCl extraction of the protein was used followed by chromatography on CM-Sephadex C-25, using LiCl gradients [5]. Further purification was obtained by gel filtration on Sephadex G-100 at high ionic strength. With the present method of preparation, denaturing conditions such as the use of urea, low pH, and lyophilization were avoided [5]. The identity and purity of the proteins were established by two-dimensional gel electrophoresis [7] and by one-dimensional slab gel electrophoresis in the presence of sodium dodecylsulphate [8]. Also, as indicated by an electrophoretic method [9], the preparations of the proteins S8, S15 and S20 bound specifically to 16S RNA.

When the protein samples were studied with the small-angle X-ray scattering method, the protein S1 was run in a 50 mM K-phosphate buffer of pH 7.80, containing 0.3 M KCl, 0.01 mM phenylmethylsulfonylfluoride (PMSF), 0.02 mM benzamidine (BAM), and 0.2 mM dithioerythritol (DTE); the protein S8 was run in a 0.05 M NaAc buffer of pH 5.6, containing 0.6 M LiCl, and the same concentrations of PMSF, BAM, and DTE as the previous buffer; the other proteins, S15, S16, and S20, were run in a 0.05 M NaAc buffer of pH 5.6, containing 0.4 M LiCl and PMSF, BAM, and DTE of the concentrations indicated above. The final protein concentration of the solutions subjected to X-ray measure-

ments was determined by nitrogen and carbon analyses [10].

The X-ray small-angle scattering data were recorded with a camera developed by Kratky and Skala [11]. 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 data). Monochromatization 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. The absolute scattered intensities were obtained using a standard Lupolen sample [12]; the Lupolen sample had been previously calibrated at the Graz Institut für Physikalische Chemie.

3. Results

The small-angle X-ray scattering data were recorded for the following concentrations: 2–6.2 mg/ml (S1); 1.5–3.5 mg/ml (S8); 1–3 mg/ml (S15); 2–8.8 mg/ml (S16); and 2–4 mg/ml (S20). When the normalized intensity ($\tilde{I}/c$) was plotted against the scattering angle, no significant concentration dependence was observed (c is the concentration in mg/ml). The data were analysed by first calculating the $p(r)$-function (a Patterson analogue function), the radius of gyration, and the slit-corrected $I(0)$-values, using a computer program developed by Glatter [13]; the slit-corrected data in the form of Guinier diagrams are shown in fig.1 and the radii of gyration are listed in table 1. On the basis of the $p(r)$-function, various triaxial bodies, described by two parameters, were assumed and smeared scattering curves were then calculated. Using a curve-fitting procedure, the calculated curves were superimposed on the primary data and, from this comparison, the best fitting two-parameter model was chosen. As a control of a certain model, the molecular weight is determined via the $I(0)$-value, obtained as the difference in readings on the vertical axes of the experimental and calculated graphs [4] (cf. figs 2 and 3). As an example, fig.2 shows the experimental data of S16 in the best fit relative to the scattering curve of a two-parameter model.

For the refinement of the two-parameter models, a weighted least-squares computer program was used [14]. The results, as far as proteins S8 and S15 are

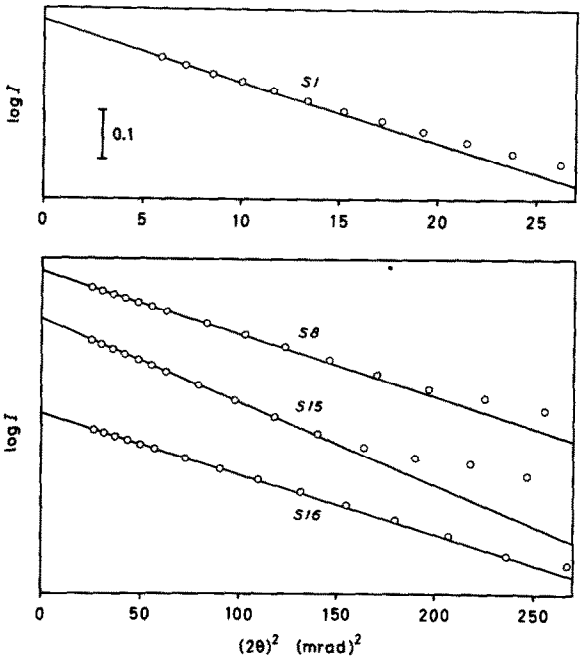

Fig.1. Guinier plots, $\log I$ versus $(2\theta)^2$, of slit-corrected scattering data. The straight lines are calculated assuming the radii of gyration listed in table 1.

concerned, are listed in table 1. It should be noted that apart from the prolate ellipsoid, the results obtained indicated that the S8-data give equally good agreement with scattering data calculated from an oblate ellipsoid with the semiaxes $A = B = 30.5$ Å and $C = 7.5$ Å. However, such an ellipsoid corresponds to

Table 1
Molecular parameters of ribosomal proteins from *Escherichia coli*

Protein	Ellipsoid model	Axes	R
S 8	Prolate	$A = 48$ Å $B = C = 14$ Å	23 Å
S15	Prolate	$A = 58$ Å $B = C = 10.5$ Å	26.5 Å
S16	Prolate	$A = 43$ Å $B = C = 12$ Å	21 Å
S 1	See fig.2 and the text	$D_{max} = 260$ Å[a]	72 Å

[a] Maximal distance within the particle obtained from the $p(r)$ curve, cf. [13]

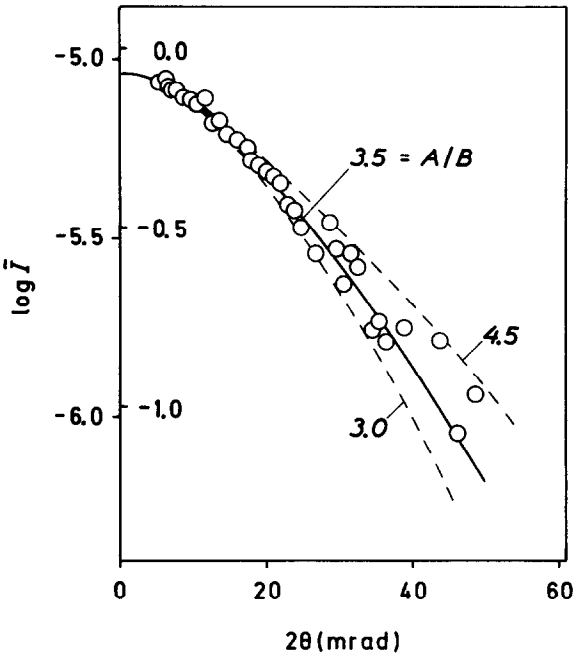

Fig.2. Experimental X-ray scattering data for the S16 protein compared with the scattering curves calculated for three prolate ellipsoids with a gyration radius of 21 Å and the axial ratio A/B of 4.5, 3.5 and 3.0; the B and C semiaxes are equal and the A semiaxes are 42, 43 and 44 Å, respectively. Please note that the theoretical curves, $\log \tilde{I}$ versus 2θ, are smeared; they are normalized so that their intensity (not smeared) at zero angle, $I(0)$, is equal to 1.0. The upper dashed curve is shifted downwards (0.015 units) and the lower dashed curve is shifted upwards (0.02 units).

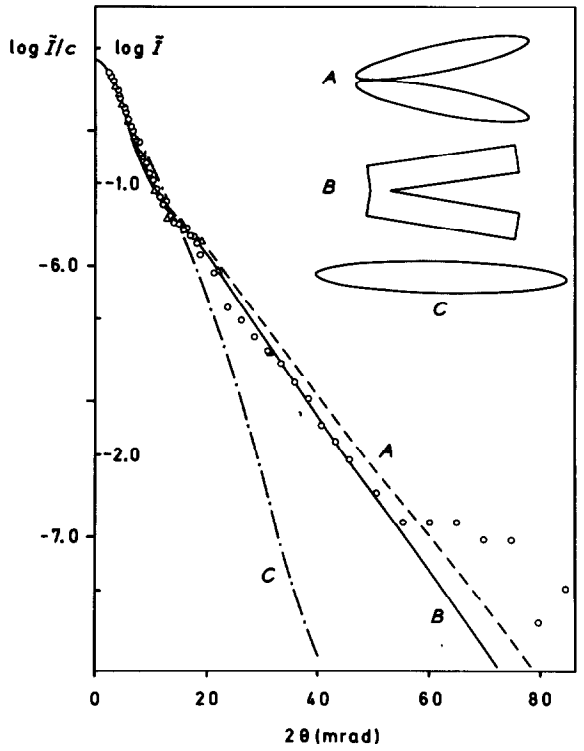

Fig.3. Experimental X-ray data for the S1 protein compared with scattering curves calculated for three models (○, 6.2 mg/ml; △, 3.2 mg/ml): (A) two identical ellipsoids (A = 130, B = 20, C = 8 Å) with A and B semiaxes in the same plane and with an angle of 23° between the A axes; R = 68 Å; (B) two identical elliptic cylinders (A = 20, B = 8, H = 220 Å) with the A and H axes in the same plane and with an angle of 20° between the H axes; R = 72 Å; (C) prolate ellipsoid with the semiaxes A = 186 Å, and $B = C$ = 23 Å; R = 85 Å. Please note that the theoretical curves, $\log \tilde{I}$ versus 2θ, are smeared; they are normalized so that their intensity (not smeared) at zero angle, $I(0)$, is equal to 1.0. The upper dashed curve (A) is shifted downwards (0.02 units) and the lower dashed-dotted curve (C) is shifted upwards (0.01 unit).

a radius of gyration, R = 19.7 Å, which appears to be too low; therefore, as a first approximation, the shape of S8 may be described by a prolate ellipsoid (table 1). The data of protein S20 did not yield satisfactory numerical stability at the calculation of the $p(r)$-function, cf. [13]. Also, the $I(0)$-value as well as the form of the scattering curve indicated that the S20 protein, to a certain extent, is aggregated in the solution. Therefore, for S20, no comparison with models was made.

The $p(r)$-curve of protein S1 indicated a structure that is more complicated than that produced by a simple two-parameter ellipsoid. Figure 3 shows the comparison between the data of S1 and those calculated for three different models; the scattering curve for a two-parameter ellipsoid is included for comparison. As follows from fig.3, the best agreement with the experimental data is obtained by the scattering from a V-shaped model, formed by two identical, flat elliptic cylinders (A = 20, B = 8, H = 220 Å) with an angle of 20° between their main axes. Also, in fair agreement with the experimental data, there is the V-shaped model of two flat ellipsoids (fig.3). The prolate ellipsoid model, the scattering curve of which only fits in the most proximal angular range (fig.3), has a quite unlikely maximum dimension

of 392 Å. As indicated by a least-squares data treatment [14], a better fit in the more distal angular range can be obtained, but then an even more unlikely ellipsoid results, one with a maximum dimension of 800 Å.

The partial specific volumes, $\overline{\nu}$, of the proteins were either analysed by a digital densitometer (S1 and S20), [15], or calculated from the primary structures (S8, S15, S16); the results were, $\overline{\nu}_1 = 0.74_1$ (S1), $\overline{\nu}_1 = 0.75_0$ (S8), $\overline{\nu}_1 = 0.73_6$ (S15), $\overline{\nu}_1 = 0.73_8$ (S16), and $\overline{\nu}_1 = 0.75_0$ cm^3/g (S20). On the basis of these $\overline{\nu}_1$-values and the $I(0)$-values obtained [4], the molecular weights were calculated. The results were 75 000 (S1), 10 600 (S8), 9500 (S15) and 10 000 (S16). These values are in essential agreement with the molecular weights of S8 (12 200), S15 (10 000) and S16 (9200) reported from analyses of the primary structures [16–18].

4. Discussion

The results described in the previous section are in agreement with our previous studies [2,4,19,20] on ribosomal proteins, indicating that these proteins generally are highly elongated with axial ratios from 1:3 to 1:10. The S8, S15, and S16 proteins seem to belong to a class of proteins having a maximum dimension of about 100 Å. Of these, the S8 and S16 proteins seem to be somewhat more compact than S15; they have axial ratios of 3.4 and 3.6, respectively, as compared with 5.5 for S15. Such a relatively compact structure is further indicated for S8 from electron microscopy using antibody markers, since only one single antibody site was detected on the 30S subunit [1,21]. Regarding the protein S15, on the other hand, two antibody sites were detected in the 30S unit which were far apart, thus indicating, in agreement with our data, that S15 has quite an elongated structure [1,21].

The elongated nature of protein S1 has been indicated from previous studies involving both electron microscopy [1] and X-ray scattering [22]. Our molecular weight, 75 000, and radius of gyration, 72 Å, are higher than those previously reported [22], 66 500 and 58 Å. In this study both $I(0)$ and the R-value are obtained by the integration of the $p(r)$-function [13] rather than from a Guinier plot. Also, the curve-fitting procedure using smeared theoretical curves yields a satisfactory fit for R = 72 Å and a molecular weight of 75 000 (cf. p. 116 and Model B in fig.3). However, the uncertainty is considerable as indicated in fig.3 where the smeared scattering curve corresponding to an R-value of 68 Å (curve A) yields an even better fit in the proximal angular range. In excellent agreement with our results, the previous X-ray scattering study indicated a maximal distance, D_{max}, within the molecule of 260 Å, and a high water content [22]. In addition, our $p(r)$-data indicate that more than one triaxial body is required in order to describe the data. As shown by fig.3, a model consisting of two flat, elliptic cylinders arranged as the letter V, yields a scattering curve that essentially describes our data. It is then interesting to note that the functions of S1 support the idea of at least two structural domains, cf. [23–25]. However, it must be kept in mind that many models might explain the X-ray scattering data and at this stage of the investigation where only models having a limited number of parameters can be considered, the V-shaped cylinder model of fig.3 should only be considered as the very first, plausible model.

Acknowledgement

We thank Ms Ailsen Malcolm and Mr Knut Mørk-Olsen for expert technical assistance. This study was supported by a grant from the Swedish Natural Science Research Council (to R. Österberg and B. Sjöberg).

References

[1] Stöffler, G. and Wittmann, H. G. (1977) in: Molecular Mechanisms of Protein Biosynthesis (Weissbach, H. and Pestka, S. eds) Academic Press, London.
[2] Österberg, R., Sjöberg, B., Garrett, R. A. and Littlechild, J. (1977) FEBS Lett. 73, 25–28.
[3] Österberg, R., Sjöberg, B., Garrett, R. A. and Ungewickell, E. (1977) FEBS Lett. 80, 169–172.
[4] Österberg, R., Sjöberg, B. and Garrett, R. A. (1976) FEBS Lett. 65, 73–76.
[5] Littlechild, J. and Malcolm, A. (1978) Biochemistry, in press.
[6] Morrison, C. A., Bradbury, E. M., Littlechild, J. and Dijk, J. (1977) FEBS Lett. 83, 348–352.

[7] Kaltschmidt, E. and Wittmann, H. G. (1970) Anal. Biochem. 36, 401–412.
[8] Laemmli, U. K. and Favre, M. (1973) J. Mol. Biol. 80, 575–599.
[9] 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.
[10] Kirsten, W. J. (1971) Microchem. J. 16, 610–625.
[11] Kratky, O. and Skala, Z. (1958) Z. Elektrochem. 62, 73–77.
[12] Pilz, I., Kratky, O., Cramer, F., Von der Haar, F. and Schlimme, E. (1970) Eur. J. Biochem. 15, 401–409.
[13] Glatter, O. (1977) J. Appl. Cryst. 10, 415–421.
[14] Sjöberg, B. (1978) J. Appl. Cryst. 11, 73–79.
[15] Kratky, O., Leopold, H. and Stabinger, H. (1969) Z. Angew. Physik 27, 273–277.
[16] Stadler, H. and Wittmann-Liebold, B. (1976) Eur. J. Biochem. 66, 49–56.
[17] Morinaga, T., Funatsu, G., Funatsu, M. and Wittmann, H. G. (1976) FEBS Lett. 64, 307–309.
[18] Vandekerckhove, J., Rombauts, W. and Wittmann-Liebold, B. (1977) FEBS Lett. 73, 18–21.
[19] Österberg, R., Sjöberg, B., Liljas, A. and Pettersson, I. (1976) FEBS Lett. 66, 48–51.
[20] Österberg, R., Sjöberg, B., Pettersson, I., Liljas, A. and Kurland, C. (1977) FEBS Lett. 73, 22–24.
[21] Lake, J. A. personal communication.
[22] Laughrea, M. and Moore, P. B. (1977) J. Mol. Biol. 112, 399–421.
[23] Szer, W., Hermoso, J. M. and Boublik, M. (1976) Biochem. Biophys. Res. Comm. 70, 957–964.
[24] Bear, D. G., Ray, N. G., Van Derveer, D., Johnson, N. P., Thomas, G., Schleich, T. and Noller, H. F. (1976) Proc. Natl. Acad. Sci. USA 73, 1824–1828.
[25] Draper, D. E., Pratt, C. W. and Von Hippel, P. H. (1977) Proc. Natl. Acad. Sci. USA 74, 4786–4790.