SMALL-ANGLE X-RAY SCATTERING STUDY OF THE 5S RNA BINDING PROTEINS L18 AND L25 FROM *ESCHERICHIA COLI* RIBOSOMES

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1. Introduction

The elucidation of the structure and function of ribosomes at the molecular level is a major objective in molecular biology. The specific complex formation between 5S RNA and a few ribosomal proteins is a part of this general problem and consequently has received much attention [1–6]. Nevertheless, we are very far from a complete understanding of the mechanisms involved in the ribosomal protein–nucleic acid interaction, and this will eventually require a thorough knowledge of the structures of 5S RNA, the binding proteins, and their complexes. Since, as yet, none of these ribosomal components have been crystallized, and consequently the prospects of X-ray crystallographic analyses are curtailed, we have initiated a small-angle X-ray scattering study of these components. In a previous report, a tertiary model for 5S RNA was described [7]. This report describes the shapes of the 5S RNA-binding proteins L18 and L25 [8].

Proteins L18 and L25, like many other ribosomal proteins, exhibit low solubility in aqueous solution and tend to aggregate; as a result, it was necessary to study the monomeric forms of the proteins at rather low concentrations. This limited the amount of information that could be gained about the protein structures.

2. Materials and methods

2.1. *Preparation of the proteins L18 and L25*

Proteins were prepared from 50S subunits of *Escherichia coli* A19. Either 2 M LiCl–urea dissociated proteins [1] or total 50S subunit proteins were fractionated by CM-cellulose chromatography and Sephadex gel filtration [8]. Proteins fractionated from total 50S subunit proteins were generously provided by Dr H. G. Wittmann. Proteins were identified and checked for purity by two-dimensional gel electrophoresis, using an apparatus one half of the size of that described earlier [9]. Contaminating proteins were estimated at less than 3% of the total amount of protein detected.

Lyophilized proteins were dissolved in water and dialysed against TMK reconstitution buffer (30 mM Tris-HCl, pH 7.4, 20 mM $MgCl_2$, 0.30 M KCl and 6 mM 2-mercaptoethanol) for 8 h and centrifuged to remove insoluble proteins. The final protein concentration of the supernatant was determined from analyses of both nitrogen and carbon [10] after correction for the Tris buffer. Three batches of each protein were examined.

As a criterion for the retention of their native structures, the capacity of each protein to bind specifically to 5S RNA was established by an electrophoretic method described elsewhere [6].

2.2. *X-ray measurements*

The X-ray small-angle scattering data were recorded with a camera developed by Kratky and Skala [11]. 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 X-ray scattering data were recorded for concentrations (c) from 1 to 5 mg/ml. This concentration range is limited due to the low solubilities of the L18 and L25 proteins in the TMK buffer. As indicated by comparison to extrapolated data, for which the normalized intensity, $\tilde{I}/c$, was extrapolated to zero concentration, the protein solutions were monomeric for $c \leqslant 2$ mg/ml; for the L25 proteins, some batches were monomeric up to $c = 3$ mg/ml. Fig.1 shows the extrapolated small-angle X-ray scattering data obtained for the L18 protein and fig.2 shows those recorded for the L25 protein at $c = 2.6$ mg/ml.

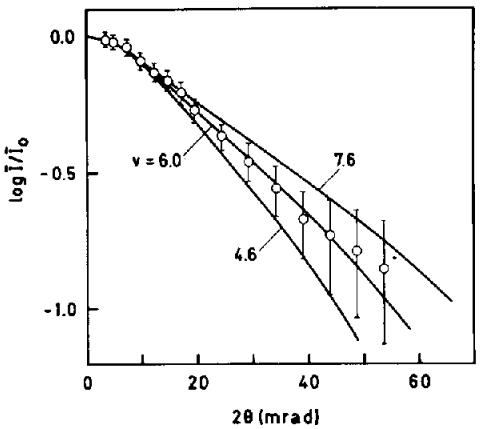

Fig.1. Experimental X-ray scattering data recorded for the L18 protein, extrapolated to zero concentration (cf. the text), compared with the theoretical scattering curves calculated for prolate ellipsoids with a gyration radius of 26 Å and an axial ratio of $\nu = 4.6$, 6.0 and 7.6; 2θ = scattering angle.

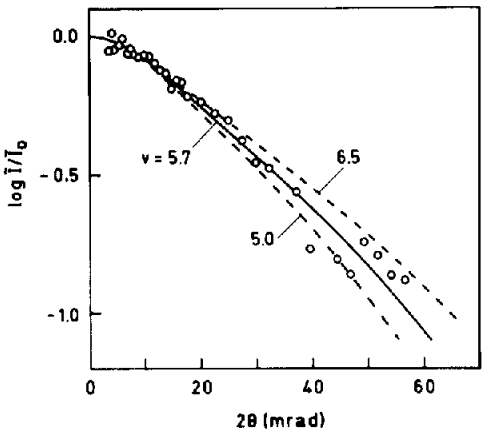

Fig.2. Experimental X-ray scattering data recorded for the L25 protein at $c = 2.6$ mg/ml compared with the theoretical scattering curves calculated for prolate ellipsoids with a gyration radius of 24 Å and an axial ratio of $\nu = 5.0$, 5.7 and 6.0.

When the scattering curves of L18 and L25 were compared with the theoretical curves calculated for different models, the experimental intensities ($\tilde{I}$) were used rather than the desmeared experimental curves; therefore, the theoretical curves were smeared using a computer program [13]. As a first approximation, two parameter ellipsoid models were used: i.e., oblate and prolate ellipsoids.

As illustrated in fig.1, the data recorded for L18 can be explained by the scattering from a prolate ellipsoid having a length of 113 Å and an axial ratio of 6 to 1. The data obtained for L25 can be explained by the scattering from a similar ellipsoid having a length of 104 Å and an axial ratio of 5.7 to 1 (fig.2). When oblate ellipsoid models were assumed, only those theoretical curves fitted the present data which corresponded to very small volumes, $< 10\,000$ Å^3. Since the minimum volumes of the present protein molecules are about 15 700 and 13 300 Å^3, respectively, the scattering from oblate ellipsoids cannot explain the experimental data. It should be noted that the present data which, within the experimental errors, can be explained by a minimum of parameters assuming prolate ellipsoids, are equally well (or even 'better') explained by three-parameter ellipsoids. For instance, the data of the L18 protein are compatible with the scattering from an ellipsoid having a length

of 91 Å and an axial ratio of 9:3.6:1 and those of L25 with the scattering from an ellipsoid having a length of 93 Å and an axial ratio of 7:2:1.

The gyration radii of L18 and L25 were determined to be 26 ± 5 Å and 24 ± 5 Å, respectively. The molecular weights were calculated using the formula described by Kratky [14]. For this purpose the desmeared absolute intensity at zero angle, $I(0)$, must be calculated. In this case, $\log I(0)$ was obtained from the comparison between the experimental data and the theoretical curves. In the position of the best fit, $\log I(0)$ is obtained as the difference between the reading on the experimental scale and that of the calculated scale according to the equation:

$$\log I(0) = \log \tilde{I}_E(0) - \log \tilde{I}_C(0) \quad (1)$$

where $\tilde{I}_E(0)$ is the experimental absolute intensity at zero angle and $\tilde{I}_C(0)$ is the zero angle smeared intensity of the theoretical curve; $I_C(0)$ is normalized so that $\log I_C(0) = 0$. The molecular weights were found to be 13 400 ± 2000 (L18) and 14 500 ± 4000 (L25). Within the experimental error these values are in agreement with those reported from the primary structure analyses of the L18 and L25 proteins, 12 770 [15] and 10 700 [16,17], respectively.

The volumes of the protein molecules were calculated via Porod's invariant (cf. [14]); the results were 21 700 Å^3 (L18) and 17 400 Å^3 (L25). Although these volumes only should be considered as the very first approximation, it is interesting to note that the prolate ellipsoids of the indicated shapes (figs.1 and 2) yield very similar volumes: 21 000 Å^3 (L18) and 18 100 Å^3 (L25); the triaxial ellipsoids mentioned above yield 17 500 Å^3 and 17 200 Å^3, respectively, which agree within the experimental errors.

4. Discussion

The results described in the previous section indicate that the ribosomal proteins L18 and L25 have highly elongated shapes. Similar conclusions have recently been drawn for some other ribosomal proteins. Electron microscope studies using antibody markers [18,19] show proteins S4, S5, S11, S12 as well as S2, S7, S15, and S18 to be elongated, and neutron scattering studies indicate protein S2 to be elongated [20]. However, in the same neutron scattering study the proteins S5 and S8 were found to have more compact sphere-like shapes [20]. It seems likely, therefore, that the sizes and shapes of the different ribosomal proteins do vary considerably; these collective results suggest that many of the ribosome models with spherical protein models will require revision (reviewed in [21]).

As far as the secondary structures of the ribosomal proteins are concerned, very little detailed information is available. So far, infrared and circular dichroism spectra have provided the idea that a certain amount of both α-helices and β-structures are present [22–24]; for instance, in a recent CD-study on some 30S proteins (S4, S6, S7, and S8) 25–30% α-helices and about 20% β-structures were reported [24]. Such a mixture of different secondary structures also seem to exist in the present proteins [15, 17], and our X-ray data do not contradict these findings. They eliminate the possibility of a single extended α-helix, since the prolate ellipsoid models of L18 and L25 are too short. The lengths of these models were 113 and 104 Å, respectively; and they must be considered maximum lengths; extended α-helices would have the lengths of 180 and 140 Å. Ellipsoid models having three different semiaxes yielded even shorter lengths, 91 and 93 Å, respectively.

The proteins L18 and L25 individually form specific and stable complexes with 5S RNA [1–3]. The low angle X-ray data, to be reported for 5S RNA [7], together with those presented here for L18 and L25, have enabled us to undertake a study of the L18-5S RNA and L25-5S RNA complexes and to draw conclusions about the location of the proteins on the 5S RNA [6,25].

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References

[1] Gray, P. N., Garrett, R. A., Stöffler, G. and Monier, R. (1972) Eur. J. Biochem. 28, 412–421.
[2] Gray, P. N., Bellemare, G., Monier, R., Garrett, R. A. and Stöffler, G. (1973) J. Mol. Biol. 77, 133–152.
[3] Horne, J. R. and Erdmann, V. A. (1972) Mol. Gen. Genet. 119, 337–344.
[4] Horne, J. R. and Erdmann, V. A. (1973) Proc. Natl. Acad. Sci. US 70, 2870–2873.
[5] Feunteun, J., Monier, R., Garrett, R. A., Le Bret, M. and Le Pecq, J. B. (1975) J. Mol. Biol. 93, 535–541.
[6] Österberg, R., Sjöberg, B., Garrett, R. A. and C. Schulte (1975) Abstracts Tenth International Congress of Crystallography, Acta Cryst. A31, S59.
[7] Österberg, R., Sjöberg, B. and Garrett, R. A., manuscript in preparation.
[8] Hindennach, I., Kaltschmidt, E. and Wittmann, H. G. (1971) Eur. J. Biochem. 23, 12–16.
[9] Kaltschmidt, E. and Wittmann, H. G. (1970) Anal. Biochem. 36, 401–412.
[10] Kirsten, W. J. (1971) Microchem. J. 16, 610–625.
[11] Kratky, O. and Skala, Z. (1958) Z. Electrochem. 62, 73–77.
[12] Kratky, O., Pilz, I. R., Schmitz, P. J. (1966) J. Colloid Sci. 21, 24–34.
[13] Glatter, O. (1974) J. Appl. Cryst. 7, 147–153.
[14] Kratky, O. (1963) Progr. Biophys. 13, 105–172.
[15] Brosius, J., Schiltz, E. and Chen, R. (1975) FEBS Lett. 56, 359–361.
[16] Dovgas, N. V., Markova, L. F., Mednikova, T. A., Vinokurov, L. M., Alakov, Yu. B. and Ovchinnikov, Yu. A. (1975) FEBS Lett. 53, 351–354 and erratum (1975) FEBS Lett. 57, 305.
[17] Bitar, K. G. and Wittmann-Liebold, B. (1975) Hoppe Seyler's Z. Physiol. Chem. 356, 1343–1352.
[18] Lake, J. A., Pendergast, M., Kahan, L. and Nomura, M. (1974) Proc. Natl. Acad. Sci. US 71, 4688–4692.
[19] Tischendorf, G., Zeichhardt, H. and Stöffler, G. (1975) Proc. Natl. Acad. Sci. US 72, 4820–4824.
[20] Engelmann, D., Moore, P. B. and Schoenborn, B. (1975) Proc. Natl. Acad. Sci. US 72, 3888–3892.
[21] Traut, R. R. (1974) in: The Ribosomes (M. Nomura, P. Lengyel and A. Tissières, eds.) p. 271–308. Cold Spring Harbor Press.
[22] Cotter, R. and Gratzer, W. B. (1969) Eur. J. Biochem. 8, 352–356.
[23] Dzionara, M. (1970) FEBS Lett. 8, 197–200.
[24] Lemieux, G., Lefevre, J.-F. and Daune, M. (1974) Eur. J. Biochem. 49, 185–194.
[25] Österberg, R., Sjöberg, B. and Garrett, R. A., manuscript in preparation.