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

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1. Introduction

It is indicated from recent small-angle X-ray scattering studies [1–3] that the L18, L25 and L7/L12 ribosomal proteins are highly elongated. Similar conclusions have recently been drawn for many other ribosomal proteins using other methods. For instance, electron microscope studies using antibody markers [4,5] show that proteins S4, S5, S11, S12, and perhaps also S2, S7, S15 and S18 are elongated; neutron scattering data indicate protein S2 to be elongated [6]. However, very little is known regarding how these highly elongated proteins are packed within the ribosomal subunits; previous ribosome models are based almost entirely on spherical protein models [7]. An idea of how the various ribosomal components might be packed together seems to be emerging from small-angle X-ray scattering studies of protein–protein complexes and protein–RNA complexes [8,9]; for instance, the L7/L12–L10 complex [8] appears to have a conformation similar to an elongated, flattened disc, and the S4 binding site on 16 S RNA [10] appears to be a flattened, oblate ellipsoid.

This report deals with a separate study of the protein S4 from the 30 S subunit. The main results indicate that protein S4, by itself, has a conformation very similar to a flat, elongated ellipsoid with the semiaxes $a = 90$, $b = 25$, and $c = 4$ Å.

The protein S4 was prepared via two different methods, one involving denaturing conditions and the other non-denaturing conditions.

2. Materials and methods

2.1. *Preparation of the protein S4*

The first method of preparation involved the standard procedure of Hindennach et al. [11]: the proteins were fractionated on CM-cellulose in the presence of 6 M urea. However, the procedure was modified in the following way: prior to gel filtration, the sample was dissolved in 15% acetic acid and then run on the Sephadex G-100 column in the same solvent (instead of 6 M urea). After lyophilizing, the protein was dissolved in a small amount of distilled water and then stored at −80°C.

The second method of preparation, using non-denaturing conditions, involved a procedure where the protein was not subjected to urea, acetic acid or lyophilization [12]. Protein S4 was identified and checked for purity by two-dimensional gel electrophoresis. No contaminating proteins were detected.

Both protein samples were studied with the small-angle X-ray scattering method using an acetate buffer of pH 5.6 containing 0.05 M sodium acetate, 0.4 M LiCl and 0.006 M 2-mercaptoethanol. Apart from this buffer, the sample prepared by the second method [12] was also studied in the TMK-buffer

(0.03 M Tris–HCl, pH 7.6, 0.30 M KCl, 20 mM $MgCl_2$, 6 mM mercaptoethanol). The final protein concentration of the solutions subjected to X-ray measurements was determined by nitrogen and carbon analyses [13].

As a criterion for the retention of the native S4 structure, the capacity of each protein preparation to bind specifically to 16 S RNA was established by an electrophoretic method described elsewhere [14]. There were no marked differences between the protein–RNA complexes prepared in the TMK-buffer or those prepared in the acetate buffer, cf. [15].

2.2. *X-ray measurements*

The X-ray small-angle scattering data were recorded with a camera developed by Kratky and Skala [16]. 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 [17]; 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 concentrations (c) from 2–7 mg/ml. When the normalized intensity ($\tilde{I}/c$) was plotted against the scattering angle, no significant concentration dependence was observed. As indicated in fig.1, the two different preparations of S4 yield essentially the same X-ray scattering curves. However, in the distal angular range there is a tendency to higher intensity for the samples prepared by the gentle technique [12] indicating some slight degradation (fig.1); in the proximal angular range there is a tendency to higher intensity for the sample prepared via the urea method indicating some aggregation. The absolute intensity scattering curves, corrected for background, were essentially the same whether

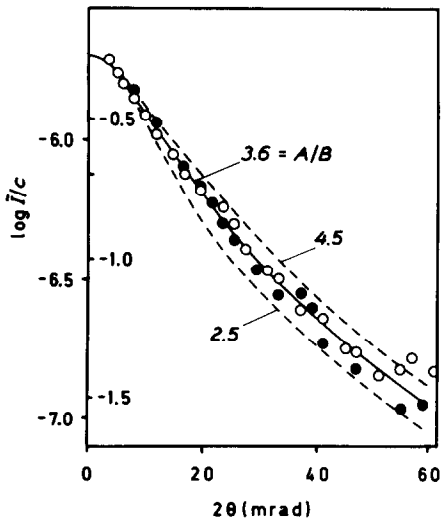

Fig.1. Normalized experimental X-ray scattering data ($\tilde{I}/c$) recorded for two samples of the S4-protein prepared by a gentle technique (7.0 mg/ml) [12] (○) and by a urea method (5.6 mg/ml) (●) are compared with theoretical scattering curves calculated for ellipsoids with a gyration radius of 42 Å, the semiaxis, c = 4 Å and the a/b axial ratio of 4.5, 3.6 and 2.5. The best fit corresponds to an ellipsoid with a = 90, b = 25 and c = 4 Å.

S4 was run in the acetate buffer or the TMK-buffer. After slit correction (de-smearing) of the data [18], the radius of gyration was determined to be 42 ± 2 Å. When the X-ray scattering data were compared with theoretical curves, calculated for different triaxial bodies, the experimental data rather than their de-smeared counterparts were used; the set of theoretical curves was smeared using a computer program [18]. Fig.1 illustrates the comparison between the experimental data and three different theoretical curves: the best fit obtained indicates that it is an ellipsoid with the semiaxis a = 90, b = 25, and c = 4 Å. The volume of this ellipsoid is 37 700 $Å^3$. It should be noted that the theoretical curves calculated for two parameter oblate ellipsoid models do not fit the experimental data as well as that of the three parameter ellipsoid described above; the scattering curves calculated for prolate ellipsoids do not in any appropriate way fit the experimental data. On the other hand, as indicated from a least-squares computer program [19], an elliptic cylinder with a = 111, b = 20, and H = 5 Å (H = the height)

yields an equally good fit to the experimental data. However, this latter model corresponds to an I_0/P_0-value (cf. [2]) that yields too large a mol. wt, 32 000; also its radius of gyration is too large, 56 Å, compared to that observed, 42 Å.

The partial specific volume, $\bar{v}$, of the S4 protein was calculated from the primary structure [20]; the result was $\bar{v} = 0.74$ cm^3/g. Using this value and the formula described by Kratky [21], the molecular weight of S4 was calculated to be 23 800. This is in agreement with the molecular weight of S4 as calculated from the primary structure, 22 550 [20].

4. Discussion

The results described in the previous section indicate that the 30 S ribosomal protein S4 has the shape of a flattened, elongated disc with a thickness of about 8 Å. The samples of protein S4, prepared by the two different methods described above, yield very similar X-ray scattering curves (fig.1); this indicates that the overall conformation is essentially conserved in both protein preparations.

Our data agree with those of a recent paper by Paradies and Franz [22] indicating that the protein S4 is considerably elongated. However, our data do not support the particular models suggested by these authors [22], i.e., it is neither an ellipsoid with the semiaxes $a = 62.5$, $b = 5.25$, and $c = 2.5$ Å nor a rod with a length of 140 Å and a diameter of 10 Å. These two S4 models of Paradies and Franz [22] seem most unlikely because of their small volumes. They yield volumes of only 3400 and 11 000 Å^3 which is much less than the dry volume, 27 800 Å^3, calculated from the mol. wt, 22 550, and the partial specific volume, $\bar{v} = 0.74$, of the S4 protein. Moreover these volumes do not compare at all with the volume that Paradies and Franz calculated from the experimental data using Porod's invariant [22] which gave 121 000 Å^3 [22]. Also, the radii of gyration for the models of Paradies and Franz, 28 and 41 Å, are not consistent with what they report based on experiments, 33.6 Å [22].

The conformation of S4 is quite different from the 50 S subunit proteins L18 and L25, which were found to be highly elongated prolate ellipsoids [2]; however, the L7/L12 protein appears to be a similar flattened, elongated ellipsoid with a thickness of 11 Å [3]. The S4 protein is known to interact with a large region of RNA (about 430 nucleotides) situated close to the 5′-end of the 16 S RNA [23,24]. The conformation of this RNA binding site has recently been found to be similar to that of an oblate ellipsoid with a thickness like that of a double-helix (30 Å) [10]. A possible idea for the interaction of S4 with its RNA binding site might be that the flattened, elongated S4-molecule interacts with a cleft in the RNA molecule, perhaps formed by a series of double helical grooves [9]. This flat, elongated disc-like conformation of S4, with the dimensions of 180 × 50 × 8 Å, indicates that protein S4 must extend throughout the 30 S subunit, which, according to electron microscopy [5], has the dimensions 180 × 100 × 80 Å. Support for this idea is strongly indicated from electron microscopy studies with antibody markers, where S4 has been found to be accessible at multiple sites on the 30 S subunit surface [4,5].

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