THE SHAPE OF PROTEINS S15 AND S18 FROM THE SMALL SUBUNIT OF THE *ESCHERICHIA COLI* RIBOSOME

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1. Introduction

One of the steps toward an understanding of the structure and function of the ribosome, a large multicomponent biological entity, is the elucidation of the shape of its proteins. Several studies have been made in order to examine the shape of individual ribosomal proteins both in situ on the surface of the ribosome and in solution. The elucidation of a number of the specific antibody binding sites for individual proteins on the surface of the ribosome using immunoelectron microscopic techniques has given some evidence that many of the protein molecules have elongated shapes (reviewed [1]). Hydrodynamic studies have previously been performed on proteins S1, S3, S4, S5, S7, S8 and S20 [2–6] and proteins L7/L12, L6 and L11 [6–8]. Further information on the shape of the proteins has been obtained from low-angle X-ray scattering or neutron scattering studies on proteins S1, S4, S8, S15, S16 and S20 [2,5,9,10] L18 and L25 [10], L7/L12 and L10 [12,13] and S2, S5 and S8 [14], respectively.

In this study proteins S15 and S18 have been characterized by molecular weight determination, sedimentation and diffusion coefficients and viscosity measurements. Both proteins have been prepared using urea at low pH. The results of this study show that protein S18 has a more highly elongated shape than S15.

2. Materials and methods

2.1. *Purification of proteins*

Proteins S15 and S18 were extracted from ribosomal 30 S subunits with 66% acetic acid in the presence of 70 mM $MgCl_2$ [15] and further fractionated by CM-cellulose chromatography in 6 M urea followed by gel filtration on Sephadex G-100 in 15% acetic acid [16]. They were provided by Professor H. G. Wittmann.

The identity and purity of the proteins were established by two-dimensional gel electrophoresis [17] and by SDS gel electrophoresis.

Since the proteins were not soluble in high ionic strength buffer, the studies were made mainly in a buffer containing 10 mM KCl, 1 mM Tris–HCl, pH 7.0 and 3 mM 2-mercaptoethanol. The protein concentrations were determined by amino acid analysis and nitrogen assay as described [6].

2.2. *Sedimentation analysis*

Sedimentation equilibrium and sedimentation velocity experiments were performed as described [6]. The ultraviolet scanner and double-sector synthetic boundary cell were used for the latter experiments. Molecular weights were determined from the plots of $\ln C$ versus r^2.

2.3. *Diffusion coefficients*

The diffusion coefficients of the proteins were measured using a capillary-type double-sector synthetic boundary cell at low speed in a Beckman Model E analytical ultracentrifuge. The area of boundary was determined by numerical integration.

2.4. *Partial specific volume*

The apparent specific volumes (ϕ) were determined from density data of the proteins by Kupke's method [18]. The densities of protein solutions and their

dialysates were measured using a precision digital densitymeter (DMA 60 and DMA 601 M) at temp. 20 ± 0.01°C. Values of ϕ determined at several protein concentrations were extrapolated to infinite dilution to obtain a limiting value which would be equivalent to the partial specific volume.

2.5. *Viscosity*

The intrinsic viscosity values for the two proteins were determined from a plot of reduced viscosity versus concentration. The reduced viscosity data were obtained in an Ostwald type micro-capillary viscometer at temp. 20 ± 0.05°C. The data were collected using an automatic viscosity measuring system (AVS/G) fabricated by Schott Glass Co., Mainz.

Hydration and shape factors were determined from amino acid composition [19] and intrinsic viscosity values [20], respectively.

2.6. *Other physical parameters*

Stokes radii and radii of gyration were calculated as in [21]. Lengths of protein molecules were estimated as in [22].

3. Results

3.1. *Protein preparations*

Proteins S15 and S18 showed single bands on an SDS gel indicating a preparation of high purity. Very little protein degradation occurred during the experiments. Both proteins were best soluble in very low ionic strength buffer or water. In a buffer of higher ionic strength both proteins showed aggregation.

3.2. *Sedimentation (s) and diffusion (D) coefficient*

During the sedimentation velocity experiment the protein solutions showed a single, broad, typical protein boundary. The boundaries were mostly symmetrical in all the experiments (fig.1A,B). The app. s values were extrapolated to infinite dilution. $s^{\circ}_{20,w}$ values of 1.50 and 1.30 were obtained for proteins S15 and S18, respectively (fig.1C).

The app. D values corrected for viscosity and density of water, were also extrapolated to infinite dilution and a $D^{\circ}_{20,w}$ of 13.1 × 10^{-7} cm^2/s and 10.5 × 10^{-7} cm^2/s were obtained for proteins S15 and S18, respectively (fig.1D).

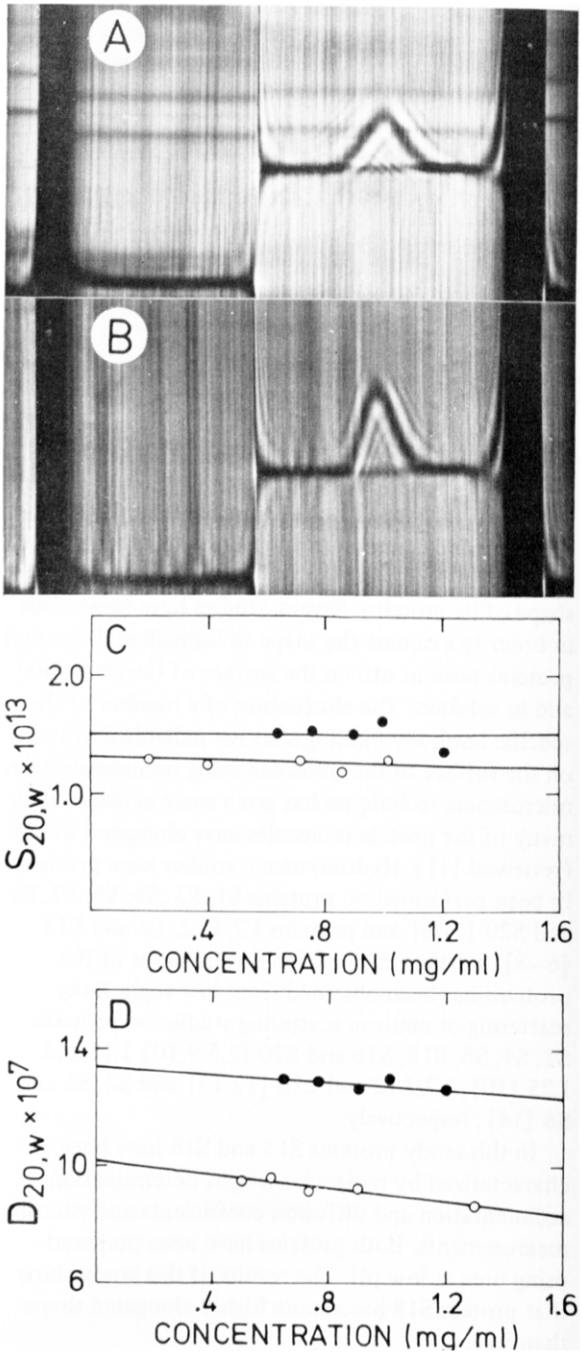

Fig.1. s and D values of proteins S15 and S18. (A, B) Schlieren pattern of proteins S15 (A) and S18 (B). A capillary-type double-sector centerpiece was used at a speed of 54 000 rev/min at 20°C. The pictures were taken 8 min after reaching the speed. (C) A graph of $s^{\circ}_{20,w}$ versus concentrations of proteins S15 (•) and S18 (○). (D) A graph of $D^{\circ}_{20,w}$ versus concentrations of proteins S15 (•) and S18 (○).

3.3. *Partial specific volume ($\bar{v}$)*

At concentrations below 1.7 mg/ml the plots of density versus protein concentration were linear and the app. $\bar{v}$ values did not significantly change with the concentration. A value of 0.72 ml/g and 0.70 ml/g were obtained for proteins S15 and S18, respectively. Protein S18 shows slightly smaller $\bar{v}$ than S15.

3.4. *Molecular weight*

Sedimentation equilibrium experiments were performed at several protein concentrations. A linear plot of $\ln C$ versus r^2 was obtained for both proteins at low protein concentration, e.g., 1.5 mg/ml (fig.2). These plots indicated that proteins were mostly monomeric. However, varying amounts of dimers were also observed at a comparatively higher protein concentration, and the $\ln C$ versus r^2 plots were not linear. This behavior was predominantly observed with protein S18.

The mol. wt values obtained for monomeric species were 10 200 and 9000 for proteins S15 and S18, respectively. These values are in excellent agreement with the mol. wt values determined from amino acid sequence data [23,24], namely 10 000 for S15 and 8951 for S18.

When the experimentally-determined $s^{\circ}_{20,w}$, $D^{\circ}_{20,w}$ and $\bar{v}$ data were combined in the Svedberg equation, a weight av. mol. wt for the two proteins was obtained which agrees well with the sedimentation equilibrium values (table 1).

3.5. *Intrinsic viscosity [η]*

When reduced viscosity values determined at several protein concentrations were extrapolated to

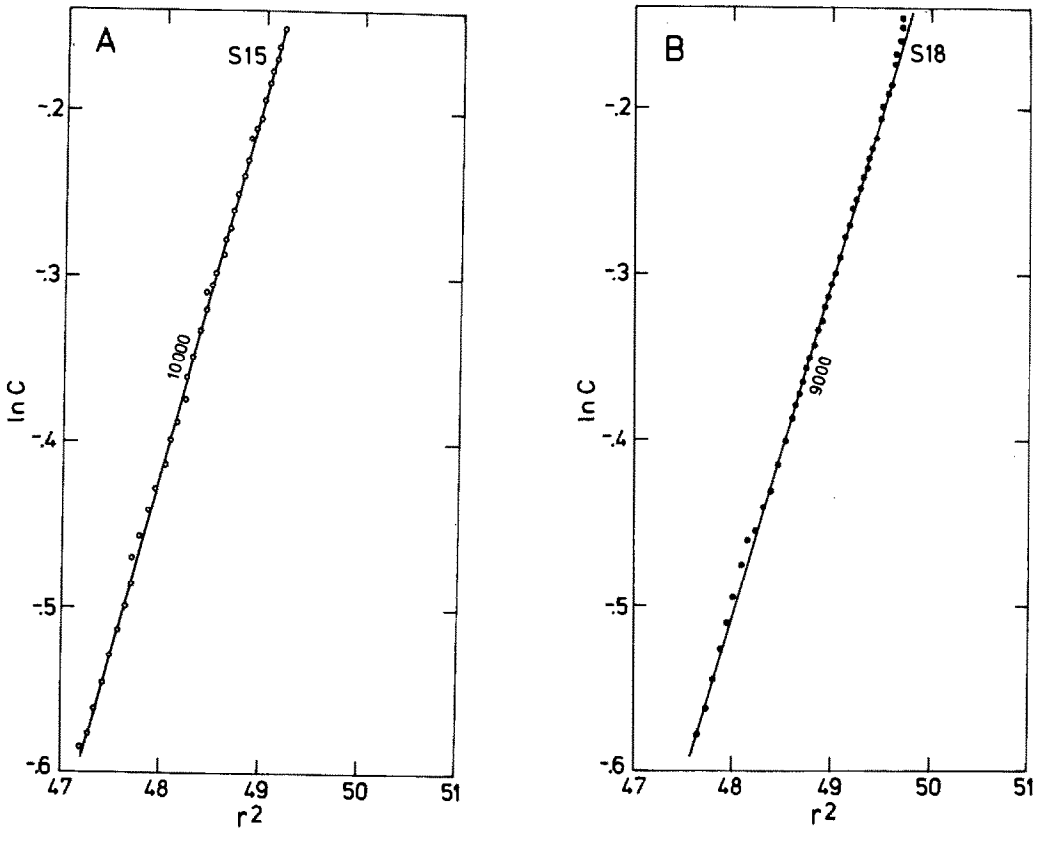

Fig.2. Sedimentation equilibrium experiments on proteins S15 (A) and S18 (B). Plots of $\ln C$ versus r^2 at a concentration of 1.5 mg/ml and a speed of 21 000 rev/min for 24 h.

Table 1
Physical properties of proteins S15 and S18

Parameter	S15	S18	unit
$s^{\circ}_{20,w}$	1.5	1.30	S
$D^{\circ}_{20,w}$	13.1 × 10^{-7}	10.5 × 10^{-7}	cm^2/s
$[\eta]$	6.0	10	ml/g
$\bar{v}$	0.72	0.70	ml/g
Mol. wt			
from sed. equilibrium	10 200	9000	–
from s and D	10 000	10 000	–
from s and $[\eta]$	12 000	11 600	–
Axial ratio			
prolate ellipsoid	5:1	8:1	–
oblate ellipsoid	6:1	12:1	–
Frictional ratio (f/f_o)	1.26	1.45	–
Radius of gyration (R_G)	20–25	28–33	A
Molecular length	90–100	125–140	A
Stokes radius (R_s)	16.5	18.3	A

infinite dilution, $[\eta]$ values of 6.0 ml/g and 10.0 ml/g were obtained for proteins S15 and S18, respectively. The concentration dependence was observed for both proteins, it was more pronounced with S18 (fig.3). The $[\eta]$ value for protein S18 is greater than that of most globular proteins.

Using the above $[\eta]$ values and a minimum hydration value of 0.30 g H_2O/g protein, shape factors of 5.9 and 9.9 are obtained for proteins S15 and S18, respectively. Assuming a prolate ellipsoidal shape for both proteins, these factors lead to an approx. axial ratio of 5:1 for S15 and 8:1 for S18. Assuming an oblate ellipsoidal shape an axial ratio of 6:1 and 12:1 for S15 and S18, respectively, can be approximated.

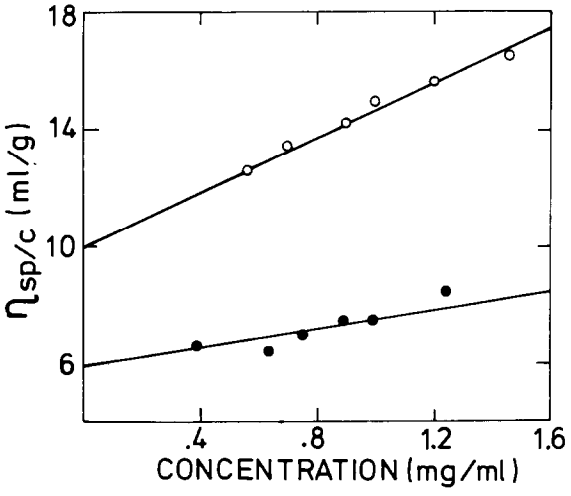

Fig.3. A graph of reduced viscosity versus proteins S15 (•) and S18 (○) concentrations.

Combining the experimental $[\eta]$ and $s^{\circ}_{20,w}$ values in the Scheraga-Mandelkern equation and using β-values of 2.23 × 10^6 and 2.35 × 10^6 [25] molecular weights of 12 000 and 11 600 for prolate ellipsoidal shapes were obtained. These values are somewhat higher than those obtained from sedimentation equilibrium and amino acid sequence.

Assuming again a prolate ellipsoidal shape, the total molecular lengths of proteins S15 and S18 can be estimated to be 90–100 Å and 120–140 Å, respectively, by combining mol. wt and $[\eta]$ data as in [22].

3.6. *Stokes radius (R_s), frictional ratio (f/f_o) and radius of gyration (R_G)*

The R_s values of the two proteins were calculated from the experimentally-determined values of $s^{\circ}_{20,w}$, mol. wt and $\bar{v}$. An R_s value of 16.5 Å for S15 and 18.3 Å for S18 was obtained. The f/f_o values for the two proteins, estimated from the viscosity data, are

1.25 for S15 and 1.45 for S18. From the above-calculated lengths, R_G values of 20–25 Å for S15 and 28–30 Å for S18 can be estimated.

4. Discussion

The main aim of this study was to characterize these proteins physically in order to obtain some information concerning their shape and behavior in solution. Globular proteins with molecular weights similar to that of S15 and S18 would be expected to have an $s^{\circ}_{20,w}$ of 1.65 and 1.55 S, respectively. As shown in table 1 the $s^{\circ}_{20,w}$ values for the two proteins are slightly smaller although the difference is not very large for S15. The $s^{\circ}_{20,w}$ value for S18 indicates an increase in frictional ratio. The axial ratios of 5:1 for S15 and 8:1 for S18 obtained from viscosity data indicate a slightly elongated shape for protein S15 and a highly elongated shape for S18. A similar axial ratio of 5:1 has been estimated from X-ray scattering study of protein S15 [10].

Protein S15 has been found to have two antibody binding sites on the ribosome surface. From the distance between these two antibody binding sites (160–200 Å) an elongated protein shape has been deduced [26]. Such a distance is, however, not in agreement with the molecular length of protein S15 estimated from this study.

Three antibody binding sites for protein S18 have been located on the 30 S subunit by immunoelectron microscopy [26]. This finding is compatible with the results of this study in that protein S18 possesses a highly elongated shape. However, the distance between the three antibody binding sites appears to be larger than the molecular length of S18 estimated from this study although one should keep in mind that the relatively large size of an antibody makes it difficult to measure the length of a protein by immunoelectron microscopy. Taking this into consideration the discrepancy between the two sets of data could be less pronounced. The maximum lengths of the proteins estimated from secondary structure predictions is 236 Å for S15 and 226 Å for S18 [27]. These lengths would correspond to the elongated shape proposed from immunoelectron microscopy, if the proteins are rather stretched in order to span the required distance.

Proteins prepared with urea are known to be partially denatured and some have different physical properties than those prepared under mild (non-denaturing) conditions [6,28]. However, proteins isolated in the presence of urea are biologically active since they can be subsequently reconstituted into active subunit [29,30].

It appears appropriate to consider the relationship between the shapes of proteins in solution and their conformations in the ribosome. It is difficult to predict how far any isolated protein would have the same physical structure as it has in the ribosome. However, the present study has provided us with some direct information on the physical properties of the two proteins. We conclude that in solution protein S15 is slightly and protein S18 is highly elongated.

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