HYDRODYNAMIC PROPERTIES OF PROTEIN S1 FROM *ESCHERICHIA COLI* RIBOSOME

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1. Introduction

Several studies on the shape of individual ribosomal proteins both in situ on the ribosomal surface and in an isolated state have been reported. They include hydrodynamic studies [1–4], low angle X-ray scattering [3,5–7], neutron scattering [8] and immune-electron microscopy [9,10]. The results from these experiments have shown that some of the ribosomal proteins, e.g., L7/L12, S4 and S7 have highly elongated shapes whereas others, e.g., S8 and L6, are nearly spherical.

In the present paper we describe hydrodynamic studies on protein S1 which is the largest and a functionally important protein of the *E. coli* ribosome. The results of our study show that protein S1 has a highly elongated shape in solution.

2. Materials and methods

2.1. *Purification of S1*

Protein S1 was isolated from *E. coli* (strains MRE600 and A19) ribosomes by extraction with 2 M LiCl + 4 M urea followed by chromatography in the presence of 6 M urea at 4°C. The extracted protein mixture was passed through a column of Sephadex G-150 and the high molecular weight peak which contains S1 and the A-protein [11] was then fractionated on DEAE-cellulose essentially as described elsewhere [11]. Protein S1 emerges from this column after the A-protein. It was concentrated by ultrafiltration and freed of urea by dialysis against 10 mM Tris–HCl (pH 7.6), 7 mM 2-mercaptoethanol. The S1 solution was finally passed through a Sephadex G-150 column to remove traces of aggregates and other impurities. The purity of S1 was checked by SDS disc gel [12,13] and two-dimensional gel [14,15] electrophoresis. Preparations used in this study were at least 95% pure. Identity of S1 was established by two-dimensional gel electrophoresis [14,15] and N-terminal sequence analysis in a sequenator (unpublished). S1 prepared in this manner was fully active in a functional assay which measures its stimulation of *B. stearothermophilus* ribosomes in the translation of f_2-RNA coat protein cistron [16].

Protein concentrations were determined by the method of Lowry et al. [17] using bovine serum albumin as a standard, by amino acid analysis and by nitrogen determination as described earlier [4]. Good agreement was found between all three methods. Values from Lowry procedure and amino acid analysis were used in all calculations.

All measurements were made in 10 mM Tris–HCl (pH 7.6), 100 mM KCl, 5 mM MgAc and 7 mM 2-mercaptoethanol. Protein solutions were dialyzed for 12–20 h with three changes of 300–400 ml buffer, before each measurement.

2.2. *Sedimentation analysis*

Sedimentation equilibrium and sedimentation velocity experiments were performed as described in an earlier work [4] using both UV-scanner and synthetic boundary cell for the latter experiments.

2.3. *Diffusion coefficient by laser light scattering*

The diffusion coefficient was determined by digital autocorrelation of scattered photons from a laser beam. By this method accurate determination of the diffusion coefficient has been described by previous workers [18]. The photo-correlation measurements were made with a Malvern System 4300 photon

correlation spectrometer with a temperature controlled specimen cell and a Malvern autocorrelator. The light source was a Spectra-Physics He-Ne laser (λ = 632 nm). Measurements were made at 20°C and θ = 60°, 90° and 120°. The experimentally determined correlation function was fitted by least-squares single exponential. The diffusion coefficient was determined from the plot of $g^{(2)}(\tau)-1$ versus delay time and corrected for solvent difference.

2.4. *Partial specific volume*

The apparent specific volumes ($\emptyset$) were obtained from density data according to the method of Kupke [19]. The densities of protein solutions and their dialysates were determined using an Anton Paar digital densitymeter at 20 ± 0.005°C. Values of $\emptyset$ determined at several protein concentrations were extrapolated to infinite dilution to obtain the partial specific volume ($\bar{v}$).

2.5. *Viscosity*

The intrinsic viscosity was determined at 20 ± 0.005°C using an Ubbelohde type microcapillary viscometer. Dilutions of the protein solutions were made in the viscometer and an aliquot removed prior to each dilution for density and concentration determination. Solution and solvent densities were measured as described above. No correction for shear gradient was made. Hydration and shape factor (Simha factor) were determined from amino acid composition and intrinsic viscosity, respectively [20–22].

2.6. *Other physical parameters*

Stokes radius and frictional ratios were calculated according to Tanford [23]. Length and radius of gyration (R_G) were calculated according to Yang [24] and Tanford [23].

3. Results

3.1. *SDS gel electrophoresis and gel filtration*

Figure 1 shows the position of S1 *vis à vis* phosphorylase a (mol. wt 94 000) and bovine serum albumin (mol. wt 68 000) in two procedures used for estimating molecular weights. S1 eluted slightly ahead of phosphorylase *a* in gel filtration while it migrated between the two standard proteins in SDS gels under a variety of conditions (6–12% acrylamide concentrations, phosphate or Tris buffer, sample heated to 65°C or 95°C etc.). The apparent molecular weight of S1 by SDS gel electrophoresis was 76 000, and slightly above 94 000 by gel filtration.

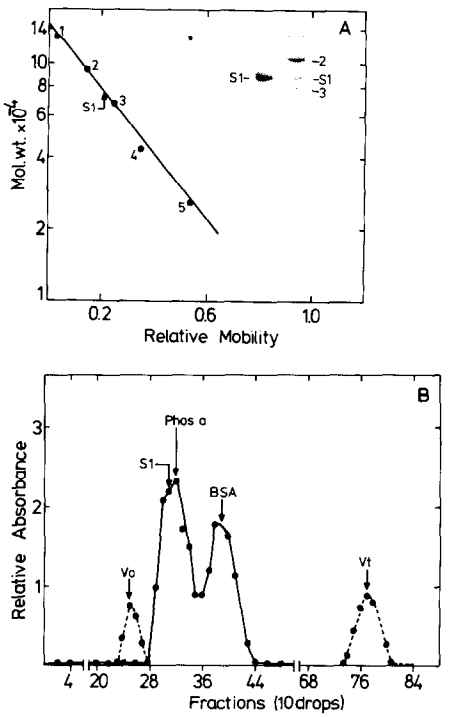

Fig.1. SDS electrophoretic behavior and Sephadex elution profile of protein S1. (A) SDS electrophoresis according to Weber and Osborn [12] using standard proteins (numbered 1 to 5), *E. coli* β-galactosidase, rabbit muscle phosphorylase a, bovine serum albumin, ovalbumin and bovine chymotrypsinogen. Insert shows S1 electrophoresed alone and in the presence of two standard proteins. (B) Elution of S1 on 65 × 1.0 cm Sephadex G-150 column at 3 ml/h. Fractions were electrophoresed in SDS gel to determine positions of each protein. V_0 and V_t were determined with Blue dextran and NaN_3, respectively.

3.2. *Sedimentation and diffusion coefficients*

Values for the apparent sedimentation coefficient obtained by two methods (UV-scanner and synthetic boundary cell) were consistent. These values, extrapolated to infinite dilution (fig.2A) gave $s^{\circ}_{20,w}$ of 3.2 S. The plot showed only a slight concentration dependency.

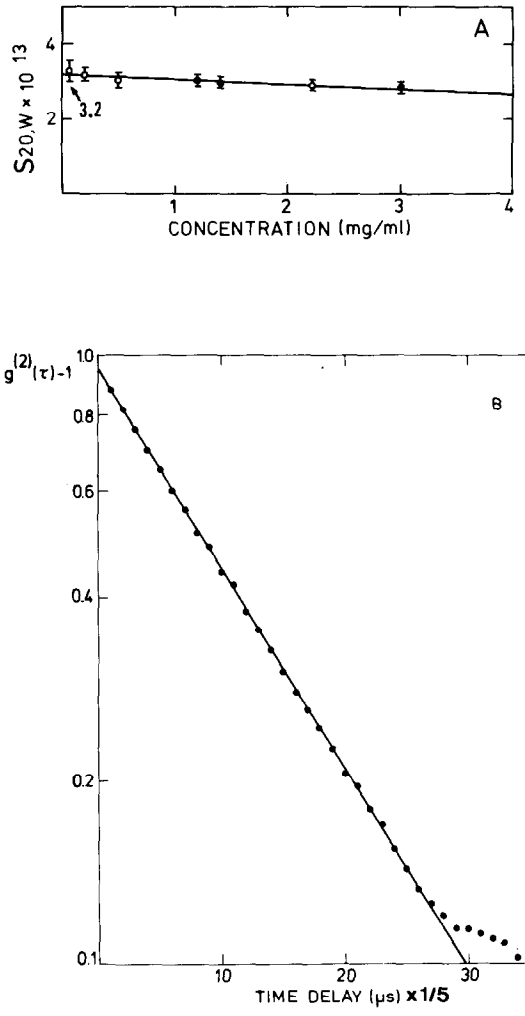

Fig.2. Sedimentation and diffusion coefficients of protein S1. (A) Apparent $s_{20,W}$ vs. protein concentration. The centrifugation runs were made at 52 000 rev./min at 20°C. Open and filled circles represent UV-scanner and Schlieren experiments, respectively. (B) A plot of autocorrelation function for monodisperse preparation of protein S1 at 1.0 mg/ml. A $D_{20,W}$ of 4.5×10^{-7} cm²/sec was calculated.

Figure 2B shows the plot of $g^{(2)}(\tau)-1$ on a logarithmic scale versus time delay at $\theta = 90°$. The plot gives virtually a straight line indicating a monodisperse system. From the slope of this line a corrected $D_{20,W}$ value of 4.5×10^{-7} cm²/sec was obtained. The values obtained at $\theta = 60°$ and 120° in a similar manner were within 1–2% of the 90° value.

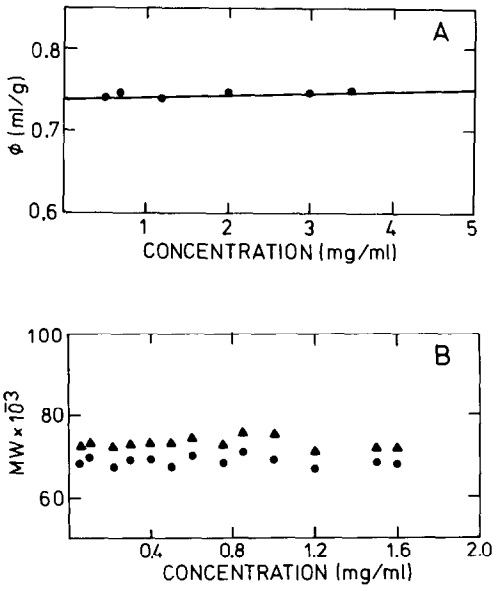

Fig.3. Partial specific volume and molecular weight of protein S1. (A) A plot of apparent-specific volume vs. protein concentration. (B) A plot of apparent M_w and M_z vs. protein concentration. The sedimentation equilibrium experiments were made at 15 000–20 000 rev./min for 24 h at 20°C. (•) weight average and (▲) z-average molecular weight.

3.3. *Molecular weight and partial specific volume*

Molecular weight determined by sedimentation equilibrium is critically dependent on the accuracy of the partial specific volume ($\bar{\nu}$). The ϕ values of S 1 varied from 0.740 – 0.750 ml/g over the concentration range used in the present study. A limiting $\bar{\nu}$ value of 0.740 ml/g was obtained upon extrapolation (fig.3A).

Sedimentation equilibrium experiments were performed at several protein concentrations. A linear plot of lnC vs. r^2 was obtained indicating a single molecular species. An extrapolation procedure was used to ascertain the weight average (M_w) molecular weight of S1 (fig.3B), and a value of 68 000 was obtained. The z-average molecular weight (M_z) was also determined to estimate possible heterogeneity. Up to a protein concentration of 1.6 mg/ml the ratio of M_z to M_w was not significantly different from unity.

When the experimentally determined $s^0_{20,W}$, $D_{20,W}$ and $\bar{\nu}$ data were combined in the Svedberg equation a

Table 1
Physical properties of protein S1

Parameter	Value
$s^{o}_{20,w}$	3.2 S
$D_{20,w}$	4.5×10^{-7} cm²/sec
$[\eta]$	9.8 ml/g
$\bar{v}$	0.74 ml/g
Molecular weight:	
M_w from SE	68 000
M_w from s^o and D^o	67 700
M_w from s^o and $[\eta]$	65 000
Frictional ratio:	
f/f_{min} observed	1.7
$f/f_{min,asym}$	1.55
Axial ratio from s^o and M_w	
for a PE	10 : 1
for an OE	11 : 1
Axial ratio from viscosity:	
for a PE	7.5 : 1
for an OE	11 : 1
Stokes radius (R_S)	40–45 Å
Length	220–230 Å
Radius of gyration (R_G)	65–70 Å

SE = Sedimentation equilibrium; PE = prolate ellipsoid; OE = oblate ellipsoid.

weight average molecular weight was obtained which agrees with the sedimentation equilibrium value (table 1).

3.4. *Intrinsic viscosity*

The reduced viscosity was determined at several concentrations of S1 and the intrinsic viscosity was obtained by extrapolation to infinite dilution (fig.4). The value 9.8 for the intrinsic viscosity of S1 is higher than that for most globular proteins of similar molecular weight. Interestingly the plot of reduced viscosity versus protein concentration showed only a low degree of concentration dependency.

Using the above $[\eta]$ value and a hydration of 0.35–0.40 g H_2O/g protein estimated from the amino acid composition of S1, a shape factor (Simha factor)

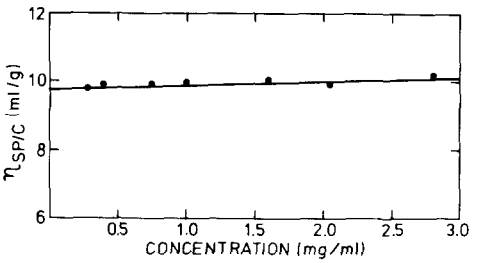

Fig.4. Intrinsic viscosity of protein S1. The reduced viscosity values were plotted against protein concentration.

of 9.2 is obtained. From this value for the shape factor an approximate axial ratio of 7.5 is obtained assuming a prolate ellipsoid.

A calculation of molecular weight using the Scheraga-Mandelkern equation [25] gave a molecular weight of 65 000 for a prolate ellipsoid (if the shape were assumed to be an oblate ellipsoid the calculation yields 67 000). Considering the uncertainty in β values used in this calculation these molecular weights are in good agreement with that obtained from sedimentation equilibrium.

3.5. *Stokes radius and frictional ratio*

The stokes radius of protein S1 was obtained from the experimentally determined $s^{o}_{20,w}$, M_w and $\bar{v}$ as well as from diffusion coefficient data. Both methods gave a value of 40–45 Å.

The frictional ratio (f/f_{min}) of protein S1 calculated from $s^{o}_{20,w}$ and $D_{20,w}$ are 1.73 and 1.76, respectively. If we correct these values for the estimated hydration, we obtain $f/f_{min,asym}$ of 1.50–1.55. Assuming a prolate ellipsoid f/f_{min} value of 1.5 would correspond to an axial ratio of approximately 10.

4. Discussion

The main object of the present study was to physically characterize protein S1 in order to gain some insight about its shape in solution. Table 1 summarizes the results from this study.

A globular protein of molecular weight 68 000 would be expected to have sedimentation and diffusion coefficients in the neighborhood of 4.8 S and 6.7×10^{-7} cm²/sec, respectively (e.g. the values for

these parameters for bovine serum albumin are 4.4 S and 6.0×10^{-7} cm^2/sec). The sedimentation and diffusion coefficents of S1 are considerably smaller; these values therefore yield a high frictional ratio. The f/f_{min} is a measure of molecular asymmetry and hydration. Typical values for f/f_{min} for globular proteins are in the range of 1.1–1.3 [23]. Thus protein S1 appears to have an extended hydrodynamic shape. The high intrinsic viscosity of S1 similarly indicates a highly elongated shape. Such a shape is also reflected in the apparent high molecular weights of S1 by gel filtration and gel electrophoresis.

Assuming a prolate ellipsoid and using the experimental intrinsic viscosity and molecular weight values the length of S1 is calculated to be about 220–230 Å. A radius of gyration of 60–70 Å is obtained from the above data for the length, sedimentation and molecular weight. It is important to keep in mind that these dimensions are only approximations assuming an ellipsoidal model. Further studies which are in progress, e.g., by low angle X-ray scattering, are expected to yield more accurate dimensions.

Although the accurate dimensions of protein S1 must await further experiments, the present studies show that it is highly elongated like proteins S4 and L7/L12. Indeed the approximate linear dimension of S1 derived from this study is as large as the small ribosomal subunit itself. Clearly any theory on the function of protein S1 must explain this unique size relationship between this protein and the ribosome.

Note: When this manuscript was ready for publication, a paper by M. Langhrea and P. B. Moore reporting several physical properties of S1 appeared (J. Mol. Biol. (1977) 112, 399–421). The results from the two studies, as far as they can be compared, are in good agreement with each other.

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References

[1] Wong, K. P. and Paradies, H. H. (1974) Biochem. Biophys. Res. Commun. 61, 178–184.
[2] Rohde, M. F., O'Brian, S., Cooper, S. and Aune, K. C. (1975) Biochemistry 14, 1079–1087.
[3] Paradies, H. H. and Franz, A. (1976) Eur. J. Biochem. 67, 23–29.
[4] Giri, L., Littlechild, J. and Dijk, J. (1977) FEBS Lett. 79, 238–244.
[5] Österberg, R., Sjöberg, B. and Garrett, R. A. (1976) FEBS Lett. 65, 73–76.
[6] Österberg, R., Sjöberg, B., Pettersson, I., Liljas, A. and Kurland, C. G. (1977) FEBS Lett. 73, 22–24.
[7] Österberg, R., Sjöberg, B., Garrett, R. A. and Littlechild, J. (1977) FEBS Lett. 73, 25–28.
[8] Engelmann, D. M., Moore, P. B. and Schoenborn, B. P. (1975) Proc. Natl. Acad. Sci. USA 72, 3888–3892.
[9] Tischendorf, G. W., Zeichhardt, H. and Stöffler, G. (1975) Proc. Natl. Acad. Sci. USA 72, 4820–4824.
[10] Lake, J. A. and Kahan, L. (1975) J. Mol. Biol. 99, 631–644.
[11] Subramanian, A. R., Haase, C. and Giesen, M. (1976) Eur. J. Biochem. 67, 591–601.
[12] Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412.
[13] Laemmli, U. K. (1970) Nature 227, 680–685.
[14] Kaltschmidt, E. and Wittmann, H. G. (1970) Anal. Biochem. 36, 401–412.
[15] Subramanian, A. R. (1974) Eur. J. Biochem. 45, 541–546.
[16] Isono, S. and Isono, K. (1975) Eur. J. Biochem. 56, 15–22.
[17] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[18] Foord, R., Jakeman, E., Oliver, C. J., Pike, E. R., Blagrove, R. J., Wood, E. and Peacoke, A. R. (1970) Nature 227, 242–245.
[19] Kupke, D. E. (1973) in: Physical Principles and Techniques of Protein Chemistry, Part C (Leach, S. J. ed) pp. 1–75, Academic Press, New York.
[20] Kuntz, I. D. (1971) J. Am. Chem. Soc. 93, 514–516.
[21] Van Holde, K. E. (1971) in: Physical Biochemistry, p. 147, Prentice Hall, Englewood Cliffs, N. J.
[22] Giri, L., Tam, M. F. and Hill, W. E. (1976) Biochemistry 15, 5188–5192.
[23] Tanford, C. (1961) Physical Chemistry of Macromolecules. John Wiley, New York.
[24] Yang, J. T. (1961) Advan. Protein Chem. 16, 323–400.
[25] Scheraga, H. A. and Mandelkern, L. (1953) J. Am. Chem. Soc. 75, 179–184.