SHAPE OF PROTEIN S5 FROM THE 30 S SUBUNIT OF *ESCHERICHIA COLI* RIBOSOME DETERMINED IN TWO DIFFERENT IONIC ENVIRONMENTS

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1. Introduction

In order to understand the structure and function of bacterial ribosomes, the study of ribosomal components has been undertaken using different techniques. Immuno-electron microscopy studies (reviewed in [1]) have shown that some proteins have multiple antibody binding sites and suggest that these proteins span a large distance inside or on the surface of the ribosome. Corroborative information concerning the physical structure of ribosomal proteins is available from hydrodynamic and other physical studies of these proteins in solution (reviewed in [2]).

These physical studies are rendered difficult by low solubility of ribosomal proteins and their tendency to aggregate in aqueous solution. In particular, ribosomal proteins isolated by a conventional method using acetic acid and urea have been found to be less soluble in high ionic strength buffer than those isolated by a non-denaturing method [3]. Due to this limited solubility behavior the hydrodynamic studies on some of the ribosomal proteins have been carried out in low salt buffer [4].

In this study protein S5 was found to be moderately soluble in buffers of low or high ionic strength. Therefore, this protein was characterized in two different buffers by molecular weight determination, sedimentation and diffusion coefficients and viscosity measurements. The goal of this study was to elucidate the shape of protein and to obtain information about the influence of low salt buffer on the shape.

2. Materials and methods

Protein S5 was extracted from ribosomal 30 S subunits with 66% acetic acid in the presence of 70 mM $MgCl_2$ [5] and further fractionated by CM-cellulose chromatography in 6 M urea followed by gel filtration on Sephadex G-100 in 15% acetic acid [6]. The protein used in this study was provided by Dr H. G. Wittmann. The identity and purity of the protein were established by two-dimensional gel electrophoresis [7] and by SDS–gel electrophoresis.

All the measurements were made in two different buffers containing:

(i) 0.35 M KCl, 2 mM $MgCl_2$, 30 mM Tris–HCl, pH 7.4 (TMK);

(ii) 0.04 M KCl, 1 mM Tris–HCl, pH 7.0 (low salt buffer).

The protein solutions were dialysed to osmotic equilibrium and filtred through 0.45 μm Millipore before the measurements were performed. Protein concentrations were determined by amino acid analysis as in [3].

Sedimentation velocity and sedimentation equilibrium experiments were carried out in a Beckman Model E ultracentrifuge using AN-D or AN-G rotors. Schlieren optics were used in sedimentation velocity studies. A capillary type double sector centerpiece was used to generate a synthetic boundary. The ultraviolet-scanner and double sector or the 6 channel centerpiece were used in conventional sedimentation equilibrium experiments. The apparent $s_{20,w}$ and molecular weight

were determined at several protein concentrations. The latter was determined from a least squares fit of ln C versus radius squared.

The diffusion coefficients of the protein were measured in a capillary type synthetic boundary double sector centerpiece in AN-H rotor of the Beckman Model E ultracentrifuge, at low speed. The area of the boundary was determined by numerical integration.

The intrinsic viscosity, $[\eta]$, was determined from a plot of reduced viscosity versus protein concentrations. The reduced viscosity data were obtained in an Ostwald type microcapillary viscometer with a flow rate of 192 s for water at 20 ± 0.01°C. All the data were collected using an automatic viscosity measuring system (AVS/G, Schott Glass Co., Mainz). No correction for shear gradient was made. Densities of protein solutions and their dialysates were measured using a precision density meter (DMA 60 and DMA 601 Graz, Austria) at 20 ± 0.01°C.

To determine the molecular weight from sedimentation equilibrium experiments, the partial specific volume $\bar{\nu}$, was calculated from the amino acid composition of S5. Shape factors and axial ratios were determined from intrinsic viscosity. Stokes radii, frictional ratios (f/f_o), radius of gyration and maximum dimensions were calculated from experimentally determined $s^0_{20,w}$, molecular weight and calculated $\bar{\nu}$ [8,9].

3. Results

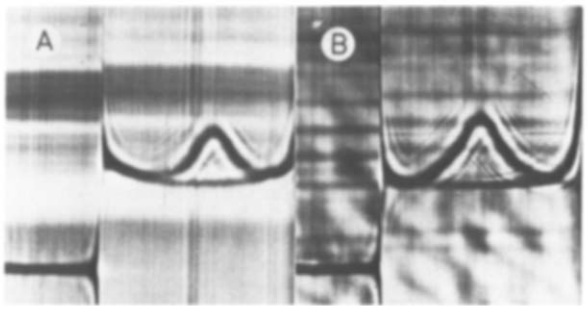
Protein S5 showed a single band on an SDS–gel and two-dimensional gel electrophoresis indicating a preparation of high purity. After the hydrodynamic measurements the protein remained intact in both buffers. The protein was soluble in both TMK and low salt buffer and no precipitate was visible up to 1.5–2 mg/ml.

During the sedimentation velocity experiments protein S5 showed a single broad peak in both buffers (fig.1). The apparent $s_{20,w}$ values were extrapolated to zero protein concentration and an $s^\circ_{20,w}$ value of 1.4 S and 1.36 S were found in TMK and low salt buffer, respectively. In low salt buffer, a large concentration dependence of $s_{20,w}$ was observed. The apparent $s_{20,w}$ value increased with the increase in protein concentration (fig.1).

Upon extrapolation of the apparent diffusion coefficients to zero protein concentration a limiting $D^\circ_{20,w}$ of 8×10^{-7} and 7.2×10^{-7} cm^2/s were obtained in TMK and low salt buffer, respectively (fig.2).

In sedimentation equilibrium experiments a linear plot of ln$C$ versus r^2 was obtained in both buffers even at 1.5 mg/ml. This indicates that protein was monomeric up to this concentration (fig.3). However, varying amounts of dimers were observed at higher protein concentration. This was more pronounced in low than in high salt buffers. Molecular weights of 17 000–17 200 were obtained for the monomeric proteins in both buffers.

Molecular weights obtained from the Svedberg equation gave 18 000 in TMK and 17 600 in low salt buffer. These values are in good agreement with those obtained from sedimentation equilibrium experiment.



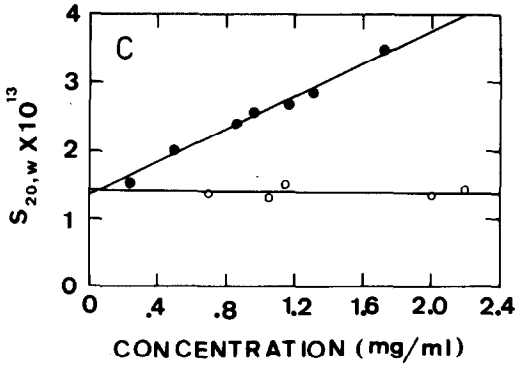


Fig.1. Sedimentation velocity of protein S5. Schlieren patterns of S5 in TMK (A) and low salt buffer (B). A capillary type double sector centerpiece was used at 52 000 rev./min at 20°C. The pictures were taken 20 min after reaching the speed. Protein was 1.2–1.35 mg/ml (C). The apparent $s_{20,w}$ was plotted against protein concentration in TMK (○) and low salt (●) buffers.

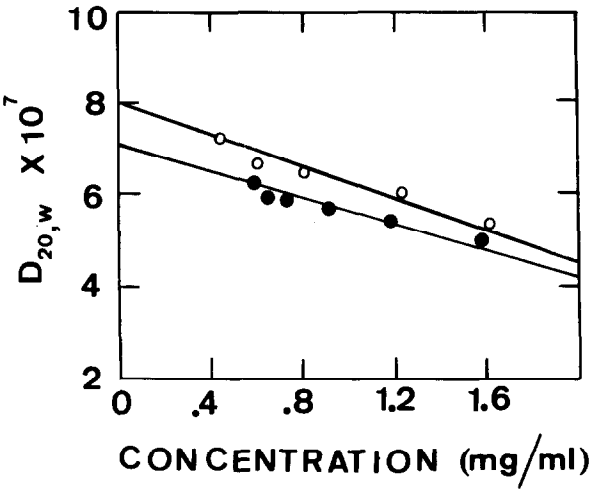


Fig.2. Diffusion coefficients. The apparent $D_{20,w}$ was plotted against protein concentration in TMK (○) and low salt (●) buffers.

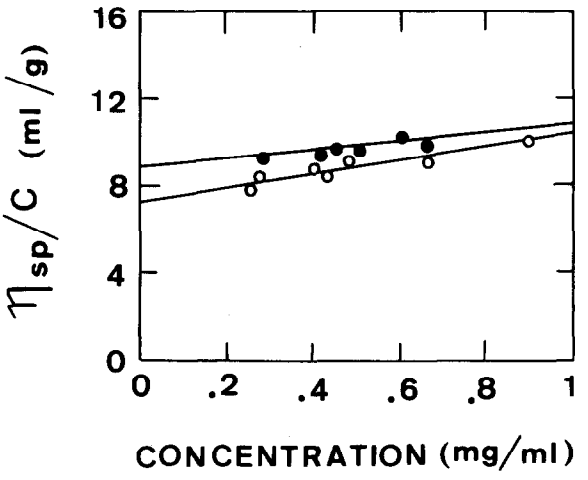


Fig.4. Viscosity. The reduced viscosity was plotted against protein concentration in TMK (○) and low salt (●) buffers.

The sedimentation coefficient and molecular weight obtained for S5 in this study are in good agreement with those in [10,11] and also with that obtained from the amino acid sequence data, namely, 17 515 [12].

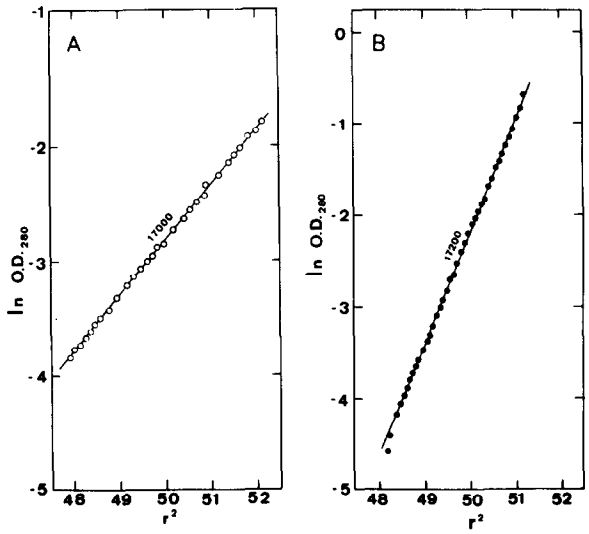


Fig.3. Sedimentation equilibrium. The extinction at 280 nm was plotted against radius squared in TMK (A) and low salt (B) buffers. The speed was 22 000 rev./min (A) and 36 000 rev./min (B) for 24 h at 20°C. An ultraviolet scanner was used. Protein was 0.5 mg/ml and 1.0 mg/ml, respectively.

The viscosity experiment gave intrinsic viscosity values of 7.3 and 9.0 ml/g in TMK and low salt buffer, respectively. The concentration dependence of the reduced viscosity was very similar in both buffers (fig.4). A summary of all the physical parameters is given in table 1.

Table 1
Comparison of physical properties of S5 in TMK and low salt buffer

Parameter	TMK buffer	Low salt buffer
$s^0_{20,w}$ (S)	1.44	1.36
$D^0_{20,w}$ X 10^{-7} (cm^2/s)	8.0	7.2
$[\eta]$ (ml/g)	7.3	9
Mol. wt:		
from SE[a]	17 000	17 200
from S + D	17 600	18 200
from S and $[\eta]$	18 000	17 600
Axial ratio from $[\eta]$:		
Prolate ellipsoid	6:1	8:1
Oblate ellipsoid	8:1	11:1
Frictional ratio:		
f/f_{min}	1.47	1.62
$f/f_{o,\,asym}$	1.32	1.44
Stokes radius (Å)	25.3	28.0
Radius of gyration (Å)	28–30	30–32
Maximum dimension (Å)	120–130	125–135

[a] Sedimentation equilibrium

4. Discussion

A globular protein of molecular weight and hydration value similar to protein S5 would be expected to have a sedimentation constant of ~2 S. As shown in table 1 the $s^0_{20,w}$ values found for S5 are smaller than the expected value for globular protein. An increase in the frictional coefficient (f) would be the most plausible explanation for this decrease in the $s^0_{20,w}$.

The sedimentation coefficient at zero protein concentration is expected to be somewhat lower in buffers of low than of high ionic strength due to the primary and/or secondary charge effects and the electrostatic interaction. In the present study the difference of the $s^0_{20,w}$ values in the two buffers is very small and it is within the range of the experimental error. This indicates that low salt buffer does not affect the $s^\circ_{20,w}$ values of protein S5. The increasing $s_{20,w}$ values with the increase in protein concentration observed in low salt buffer (fig.1) can be attributed to a pressure dependent aggregation because there was no significant protein aggregation observed at these concentrations in sedimentation equilibrium and viscosity experiments. The intrinsic viscosity of S5 determined in low salt buffer is somewhat higher than that obtained in TMK buffer. This could be due to molecular expansion in an electrostatic field.

Comparison of the sedimentation coefficients of S5 in TMK and low salt buffers with its molecular weight (17 000) gives maximal frictional coefficient ratios (f/f_{min}) of 1.47 and 1.62, respectively. If a minimal hydration of 30% is taken [4] the axial ratios, a/b for prolate ellipsoids of revolution are then 6.0:1 and 7.0:1, respectively. A similar axial ratio approximation was obtained independently from viscosity data.

The results presented in table 1 are in good agreement with those in [10] and allow the general conclusion that protein S5 is an elongated or asymmetric protein in solution. An elongated shape for S5 protein has also been deduced by immuno-electron microscopy [1]. The low salt buffer does have some influence on the limiting hydrodynamic parameters. However, the deviation is very small to have any significant impact on the overall shape of the protein S5. Further studies have to be done in this direction, before this conclusion can be extrapolated to other ribosomal proteins.

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