ological actions of HRG in vivo.

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Physical Studies on the Ribosomal Protein S2 from the *Escherichia coli* 30S Subunit†

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ABSTRACT: The protein S2 has been isolated from the 30S subunit of *Escherichia coli* A19 ribosomes [Littlechild, J., & Malcolm, A. L. (1978) *Biochemistry 17*, 3363–3369]. This salt-extracted protein is soluble and does not aggregate at salt concentrations of 0.3–0.4 M as used under reconstitution conditions. This differs from the S2 protein extracted by the acetic acid and urea method. The molecular weight from sedimentation equilibrium was found to be 29 200, and the protein was found to have a $s^0_{20,w}$ value of 2.36 S. The apparent specific volume at 20 °C was 0.726 mL·g^{-1}, and the $D^0_{20,w}$ was 7.37×10^{-7} cm^2 s^{-1}. The value for intrinsic viscosity was found to be 6.42 mL·g^{-1}. An axial ratio of (5–6):1 for a prolate ellipsoid of revolution was estimated by using these parameters. The circular dichroism and proton magnetic resonance studies show that protein S2 has both substantial amounts of α helix and β-pleated sheet in solution and appears as a "folded" protein and not a random coil structure.

To understand the structural significance of the many components that make up the bacterial ribosome, it is essential to obtain information about the structure of individual ribosomal proteins. Such information is now available for many of the 53 different protein molecules as regards their secondary and tertiary structure and shape in solution [reviewed by Wittmann et al. (1979)].

The ribosomal protein S2 is the second largest protein in the 30S subunit of the *Escherichia coli* ribosome. Its molecular weight has been estimated from NaDodSO$_4$[1]–polyacrylamide gel electrophoresis to be 28 300 (Dzionara et al., 1970) and 29 000 (Littlechild & Malcolm, 1978), respectively. Values obtained from sedimentation studies vary from 24 000 (Dzionara et al., 1970) to 30 000 (Craven et al., 1969).

Neutron scattering experiments (Engelman et al., 1975) and immunological studies (Tischendorf et al., 1975) have both suggested that protein S2 has an elongated shape.

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[1] Abbreviations used: PhCH$_2$SO$_2$F, phenylmethanesulfonyl fluoride; BAM, benzamidine hydrochloride; NaDodSO$_4$, sodium dodecyl sulfate; DTT, dithiothreitol; EDTA, (ethylenedinitrilo)tetraacetic acid; CM, carboxymethyl; TP30, total protein extracted from the 30S ribosomal subunit with acetic acid according to Hardy et al. (1969); CD, circular dichroism.

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Several mutants have been produced from this protein (Okuyama et al., 1974; Yoshikawa et al., 1975; Nashimoto & Uchida, 1975; Isono et al., 1976; Dabbs, 1978). A resistance to the antibiotic kasugamycin is in some cases linked to an altered S2 protein (Okuyama et al., 1974; Yoshikawa et al., 1975). This mutation is located near the *purE* locus at 12 min in the *E. coli* chromosomal map at a site distinct from the locations of all other genes for ribosomal proteins known. Chemical cross-linking studies (Traut et al., 1974; Bollen et al., 1975) have identified S2 to be near the binding site for the initiation factor IF-2. The protein S2 when isolated under mild conditions was found to bind weakly to 16S rRNA (Littlechild et al., 1977). It showed more secondary and tertiary structure and completely different solubility characteristics (Morrison et al., 1977) to S2 isolated with urea and acetic acid (Wittmann et al., 1979).

Materials and Methods

Preparation of Protein. The ribosomal protein S2 was prepared by a new method that avoids the use of urea, acetic acid, and lyophilization (Littlechild & Malcolm, 1978). The proteins were removed from the ribosome subunits by washing with 1 M LiCl and 0.001 M EDTA, pH 7.0. Further fractionation was as described above. This enabled us to perform the physical measurements with concentrations ranging from 0.5 to 3.0 mg/mL, where previously this protein had been shown to dimerize (Rohde & Aune, 1975).

Before concentration and storage (−80 °C), the protein was dialyzed against a buffer containing 0.05 M potassium phosphate, pH 7.8, 0.3 M KCl, 1×10^{-5} M $PhCH_2SO_2F$, 2×10^{-5} M BAM, and 1×10^{-4} M DTT (buffer B). All physical measurements were performed in this buffer unless otherwise stated. The protein was concentrated either by dialysis against dry Sephadex G-150 (using Spectrapor 3 dialysis tubing obtained from Spectrum Medical Industries Inc., Los Angeles; molecular weight cutoff 3500) or by vacuum dialysis with collodium bags (Sartorius).

The protein was identified and checked for purity by two-dimensional gel electrophoresis (Kaltschmidt & Wittman, 1970) and by slab gel electrophoresis in the presence of $NaDodSO_4$ (Laemmli & Favre, 1973).

For determination of Stokes radius (Ackers, 1970), 0.5 mL of protein sample (1 mg·mL^{-1}) was applied on a Sephadex G75 superfine column (145 × 1 cm) equilibrated with buffer A. The absorbance of the eluant was monitored at 235 nm by using a Gilson Spectrochrom UV monitor, and the fractions were checked for protein content by slab gel electrophoresis in the presence of $NaDodSO_4$ (Laemmli & Favre, 1973). The column was calibrated with markers of known Stokes radii: aldolase (46 Å), serum albumin (35 Å), chymotrypsinogen (22 Å), cytochrome *c* (16 Å).

For the hydrodynamic measurements, the protein solution was dialyzed to equilibrium. The dialysate was filtered through Nucleopore filters (0.4-μm pore size) and clarified by centrifugation before use.

Circular Dichroism (CD). Circular dichroism spectra were measured with 300 μL of protein solution by using a cell of 0.101-cm path length. The concentration was ~0.1 mg·mL^{-1}, and the sample was dialyzed against 0.3 M potassium fluoride and 0.001 M potassium phosphate, pH 7.0 (since KCl would absorb at the low wavelengths employed). The instrument used was a Roussel Jouan CD 3 dichrograph, and spectra were recorded between 260 and 180 nm at room temperature.

Proton Magnetic Resonance. Protein S2 was concentrated to 1–2 mg·mL^{-1} and was dialyzed against 0.05 M potassium phosphate, pH 7.0, 0.35 M KCl, and 5×10^{-4} M DTT in ^2H_2O. Spectra were recorded over a period of 4 h at 20 °C and 270 MHz on a Bruker WH 270 magnetic resonance spectrometer operating in a Fourier-transform mode, using a pulse length of 12 μs and data collection over 0.5 s for each pulse. The free induction decay pattern was multiplied by an exponential function equivalent to line broadening of approximately 2 Hz. After the ^1H NMR spectra had been recorded, the protein was checked for proteolytic degradation by $NaDodSO_4$ slab gel electrophoresis.

Sedimentation and Diffusion Experiments. Sedimentation velocity experiments were performed by using the AnD rotor at 52 000 rpm at 20 °C. A capillary type double-sector centerpiece was used to generate a synthetic boundary. Schlieren optics were used between concentrations of 2.9 and 1.0 mg·mL^{-1}, and the ultraviolet scanner at 280 nm was employed for lower concentrations.

Sedimentation equilibrium experiments were performed by using the AnD and AnG rotors at a wide range of speeds at 20 °C. The UV scanner at 280 nm was employed, and several scans were made from 16 to 24 h for each concentration. Molecular weights were determined from the plots of ln *c* vs. r^2.

Diffusion coefficients of the protein were measured by using the AnH rotor at 5600 rpm at 20 °C. A capillary type double-sector centerpiece was used to generate a synthetic boundary. Schlieren optics were used, and the areas of the boundaries were determined by the height–area method (Kawahara, 1969; Wei & Deal, 1976).

Stokes radius and the approximate radius of gyration were calculated as previously described (Tanford, 1961; Giri & Dijk, 1979). The frictional ratio was estimated from $s^0_{20,w}$, $\bar{v}$, and viscosity data. The maximum hydration was assumed to be 0.3 g of H_2O/g of protein.

Intrinsic Viscosity and Partial Specific Volume. Reduced viscosity data were obtained in an Ostwald-type microcapillary viscometer. All data were collected at 20 °C, using a automatic viscosity measuring system (AVS/G, Schott Glass Co., Mainz). The densities of protein aliquots and their dialysates, used in viscosity measurements, were determined with a precision digital density meter (DMA 60 and DMA 601 M, Paar KG, Graz, Austria). The shape factor was determined from intrinsic viscosity, hydration, and $\bar{v}$ (Van Holde, 1971).

Protein concentrations were determined by amino acid analysis and by a nitrogen assay, using ammonium sulfate as a standard (Jaenicke, 1974). For amino acid analysis, the hydrolysis was performed in 6 N hydrochloric acid containing 0.2% 2-mercaptoethanol under a nitrogen atmosphere at 110 °C for 23 h. The amino acid analysis was performed on a Durrum D-500 analyzer. Norleucine was used as an internal standard.

Results

Protein S2 showed a single spot on the two-dimensional gel electrophoresis system and a single band in $NaDodSO_4$ slab gel electrophoresis, indicating a pure preparation (Figure 1).

The circular dichroism study (Figure 2) showed that the protein has a high α-helix content [53% estimated from measurement at 222 nm and by using the value of $[\theta] \times 10^{-3}$ of 30 to represent 100% α helix (Chen et al., 1974)] and some β structure. When these data were curve fitted by the method of Chen et al. (1974), the values of 58% α helix and 22% β structure were found (J. Littlechild, A. M. Freund, and J. Pouyet, unpublished experiments).

The proton magnetic resonance study (Figure 3) showed that protein S2 must have substantial tertiary structure in solution. This can be seen by the width of the resonances, and

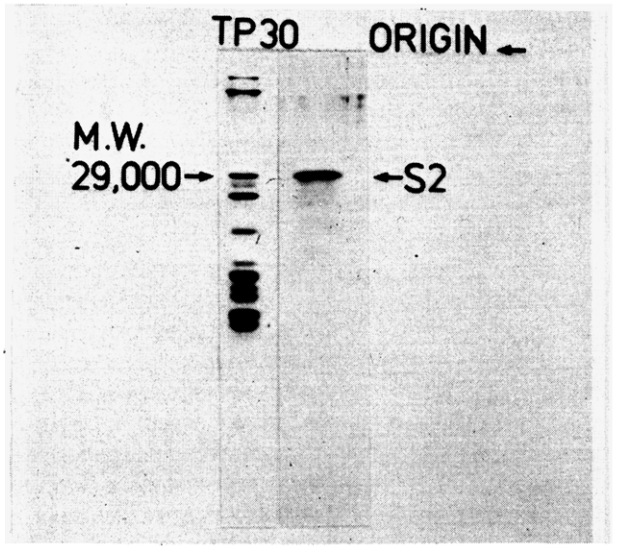

FIGURE 1: Gel electrophoresis of protein S2 in $NaDodSO_4$-containing slab gels. TP30 represents total proteins extracted from the 30S subunit according to Hardy et al. (1969). The molecular weight found for protein S2, by comparison with marker proteins of known molecular weight, is shown on the left.

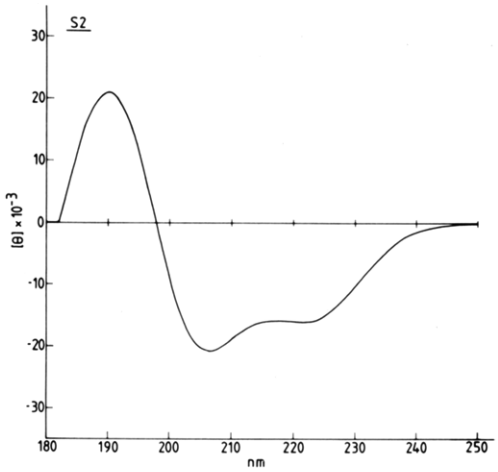

FIGURE 2: Circular dichroism spectrum of protein S2. The molar ellipticity is plotted against wavelength. Measurements were from 260 to 180 nm.

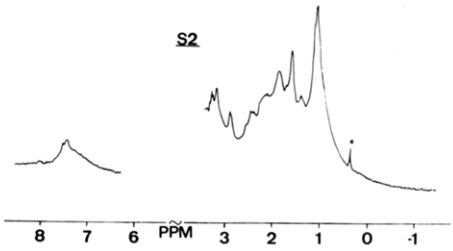

FIGURE 3: Spectrum (270 MHz) of protein S2, showing the aromatic and high-field regions.

the indication of perturbed resonances in the aromatic region and apolar region of the spectrum, which are due to folding within the protein chain. Hence, one can exclude the possibility of a random coil structure for this protein. The spectra obtained are similar to what would be found for a compact protein of a similar molecular weight.

Plots of apparent sedimentation coefficients against c were linear, indicating the presence of one component and little concentration dependence. The extrapolation of apparent diffusion coefficients, reduced viscosities, and apparent specific volumes showed a similar behavior. The limiting values of these parameters, after extrapolation to infinite dilution, are listed in Table I.

Table I: Physical Properties of Protein S2

parameter	protein S2
$s^0_{20,w}$ (S)	2.36 ± 0.07
$D_{20,w} \times 10^{-7}$ ($cm^2 \cdot s^{-1}$)	7.4 ± 0.20
$[\eta]$ ($mL \cdot g^{-1}$)	6.4 ± 0.10
$\bar{v}$ ($mL \cdot g^{-1}$)	0.726 ± 0.002
molecular weight	
from SE[a]	29 200 ± 800
	25 200[d]
from s and D	28 500
from s and $[\eta]$	30 400
from $NaDodSO_4$ gel electrophoresis	29 000
frictional ratio (f/f_{min})	1.45
from $s^0_{20,w}$, $\bar{v}$, and mol wt	
corrected frictional ratio f/f_0, hydrated	1.29
axial ratio	
from $s^0_{20,w}$, $\bar{v}$, and mol wt	PE[b] 5.6, OE[c] 6.5
from viscosity	PE 5.4, OE 7.5
Stokes radius (A)	
from $s^0_{20,w}$, $\bar{v}$, and mol wt	28
from gel filtration	26
average radius of gyration (A)[e]	26–29

[a] SE, sedimentation equilibrium. [b] PE, prolate ellipsoid. [c] OE, oblate ellipsoid. [d] Rohde & Aune (1975). [e] Calculated for an ellipsoid of revolution with semiaxes a, b, $c = b$ according to Pilz (1973).

In the sedimentation equilibrium experiments, the plots of ln c vs. r^2 were found to be linear at concentrations below 1.5 $mg \cdot mL^{-1}$. Varying amounts of dimers were present at higher concentrations. Extrapolation of M_{app}^{-1} vs. c gave a monomeric molecular weight of 29 200 ± 800 in the concentration range 0.3–1.5 $mg \cdot mL^{-1}$.

The R_s value of protein S2 was calculated from the experimentally determined values of $s^0_{20,w}$, molecular weight, and $\bar{v}$, and molecular weight data by using the appropriate equation (Tanford, 1961).

Discussion

From these results, it is clear that when S2 is prepared in a gentle manner (Littlechild & Malcolm, 1978) it does have a unique structure. The protein although slightly elongated contains a substantial secondary and tertiary structure as revealed from the circular dichroism and proton magnetic resonance studies.

The $s^0_{20,w}$ value for S2 was found to be 2.36 S. We have assumed a maximum hydration of 0.3 g of H_2O/g of protein. A globular protein with the molecular weight of S2 would be expected to have a sedimentation coefficient of ~3 S.

The intrinsic viscosity value of 6.42 $mL \cdot g^{-1}$ is higher than expected for a globular protein. Nevertheless, with these data and the assumption of 30% hydration, an oblate ellipsoid would correspond to an axial ratio of 7.4:1 and a prolate ellipsoid to an axial ratio of 5.3:1. We will consider for this purpose that an axial ratio of (4–5):1 or a frictional ratio of 1.3 is the limit for a globular protein (Tanford, 1961; Yang, 1961). When the frictional ratio of S2, 1.45, is corrected for hydration, a value of 1.29 is obtained. This would correspond to an axial ratio of 5.5:1 for a prolate ellipsoid and 6.5:1 for an oblate ellipsoid.

The determination of the β parameter calculated from Perrin and Simha equations can provide us with the most reliable axial ratios; however, 1–2% error in β leads to widely different values. This makes the precision required often beyond the precision realistically obtainable. A value of 2.22

$\times 10^6$ was found for β, reflecting a 5:1 axial ratio for a prolate ellipsoid in this study. This is in good agreement with independently calculated values of axial ratios for prolate ellipsoids from molecular weight $s_{20,w}$, and viscosity measurements (Table I).

The immune electron microscopy studies indicate (Tischendorf et al., 1975) an elongated shape for protein S2 due to the two distinct antibody recognition sites on the 30S subunit. Our results do not contradict this idea; however, a direct comparison in shape is not possible due to the limitation of the techniques involved.

From these results, we can conclude that protein S2 prepared in a gentle manner is a slightly elongated protein with an axial ratio of (5–6):1 with a discrete secondary and tertiary structure. It is, however, important to keep in mind that the dimensions of shape described in this study are only approximations, assuming equivalent ellipsoidal models of revolution.

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