# COMPACT GLOBULAR STRUCTURE OF PROTEIN S15 FROM *ESCHERICHIA COLI* RIBOSOMES

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## 1. Introduction

The ribosomal protein S15 is one of the 21 proteins of the small (30 S) subunit of the *Escherichia coli* ribosomes [1]. Its polypeptide chain consists of only 87 amino acid residues [2]. It became of special interest to study the conformation of protein S15 when it was claimed [3] that its antigenic determinants in the 30 S subunit were revealed by immunoelectron microscopy at the most distal sites of the particle (~200 Å apart), thus suggesting its extremely elongated or even fibrous conformation. Recently the concept of elongated, non-globular conformations of ribosomal proteins has become widely accepted (e.g., review [4]). A number of reports have appeared where measurements of physical parameters of isolated ribosomal proteins in solution were used as evidence for their expanded conformations. In particular, both hydrodynamic [5] and small-angle X-ray scattering [6] measurements of protein S15 in solution indicated that the axial ratio of its molecules is about 5:1 or 6:1 and that the molecular length is ~100 Å.

Recently in our group direct data were obtained showing that the ribosomal proteins S4, S7, S8 and S16, when prepared carefully, have compact globular conformations in solution [7], in contrast to a number of reports about their elongated shapes. This has forced us to undertake a special complex study of protein S15 which is usually assumed to be a typical elongated protein of the ribosome. Circular dichroism, proton magnetic resonance and neutron scattering measurements of protein S15 in solution have shown that it is a compact globular molecule with a high content of secondary structure, a well developed tertiary structure and an almost spherical shape. On the basis of a theoretical stereochemical analysis of its primary structure, a model of the tertiary structure of protein S15 is proposed and discussed.

## 2. Materials and methods

### 2.1. *Preparation of the ribosomal protein S15*

The 30 S subunits of *Escherichia coli* MRE-600 ribosomes were isolated by sucrose gradient centrifugation in the presence of 0.5 M $NH_4Cl$ and 1 mM $MgCl_2$ [8] using a zonal rotor [9]. The 30 S particles were treated with 3 M LiCl in the presence of 5 mM $MgCl_2$ in order to obtain the protein-deficient derivatives (core particles) retaining mainly the proteins S4, S7, S8, S15 and S16 [10]. Protein S15 was extracted from the 'core particles' by 4 M urea with 3 M LiCl and then purified by phosphocellulose column chromatography in the presence of 6 M urea [11].

The fraction of protein S15 eluted from the column was concentrated by Amicon ultracentrifugation using a UM-2 filter, and then re-chromatographed on Sephadex G-100 [11]. The protein solution was kept frozen at −80°C. Immediately before experiments the solution was thawed and dialyzed in the cold, first against 2 changes of 1 M KCl with 50 mM potassium phosphate (pH 5.6) then against several (5 or 6) changes of the standard solvent, 0.1 M NaCl–30 mM sodium phosphate (pH 5.6); if required, dialysis was continued against 3–6 changes of 0.1 M NaCl with 30 mM sodium phosphate prepared in 92% $D_2O$ (for neutron scattering), 99.8% $D_2O$ (for PMR

measurements) pD 5.3 (standard $D_2O$ solvents). The final solution of the renatured protein S15 was clarified by centrifugation at 16 000 rev./min for 30 min.

The identity, purity and homogeneity of the protein S15 preparations were checked by two-dimensional gel electrophoresis in urea [12], one-dimensional gel electrophoresis in SDS [13], N-terminal group determination [14], and amino acid analysis. The molecular weight of protein S15, determined by sedimentation equilibrium method [15] in the standard solvent, was 12 500 ± 1000.

### 2.2. *Absorption and CD spectra*

Measurements of absorption spectra were performed in an EPS-3T Hitachi instrument. In order to estimate the extinction coefficient, the micro-technique of nitrogen determination [16] was used, assuming a nitrogen content of 19.7% [2] and introducing the correction for solution turbidity [17].

CD spectra were measured in a J41A JASCO instrument. Calibration of the instrument was done according to [18]. The measurements were performed in cells 0.093, 0.186, 0.5 and 10.0 mm thick, in the far and near ultraviolet regions. For calculation of the ellipticity the mean molecular weight of an amino acid residue (MRW) was assumed to be 115.0 [2]. The estimation of the secondary structure content was done according to [19], using reported reference spectra [20].

### 2.3. *PMR spectra*

Proton NMR spectra were recorded on a Bruker WH 360 spectrometer operating in the Fourier transform mode, using a pulse-length of 6 μs with 1.8 s intervals; the number of accumulations was 2000. Chemical shifts were measured relative to sodium 2,2-dimethyl-2-silapentane sulphonate as an internal standard. Spectra were obtained in 5 mm tubes with 0.8–1.0 mg protein/ml in standard $D_2O$ solvent.

### 2.4. *Neutron scattering*

Neutron scattering experiments were done in the Institut Laue-Langevin, Grenoble, on the high-flux reactor using the D11 camera [21] as in [7]. The cell was 2 mm thick with 0.75 mg protein/ml in standard $D_2O$ solvent. The radius of gyration was calculated from the slope of the scattering curve in the Guinier coordinates ($\log I$ versus $\mu^2$). The molecular weight was calculated from the curves as in [7].

## 3. Results

### 3.1. *Extinction coefficient and secondary structure*

Figure 1 presents the absorption spectrum of protein S15 in the ultraviolet region recorded in standard solvent. The extinction coefficient at 277 nm ($A_{1\,cm}^{1\,mg/ml}$) is found to be 0.325 (±0.010). This extinction coefficient was used in all other experiments for determinations and checks of the protein concentration.

It has been shown in special experiments that the absorption spectrum and the extinction coefficient do not change in solvents with pH values from 5.6 to 7 and salt concentration from 30 mM to 0.4 M.

Figure 2 presents the CD spectrum of protein S15. It is seen that the spectrum corresponds to a highly α-helical protein. Estimation of the α-helical content in protein S15 from the CD spectrum [19,20] gives a value of 78%. The value of the helical content has been shown to remain unchanged, at least in the pH range 5.6 to 7 and salt concentration from 30 mM to 0.4 M.

### 3.2. *Tertiary structure*

Figure 3 shows the PMR spectra of protein S15 in standard solvent (A) and in the presence of 5 M urea

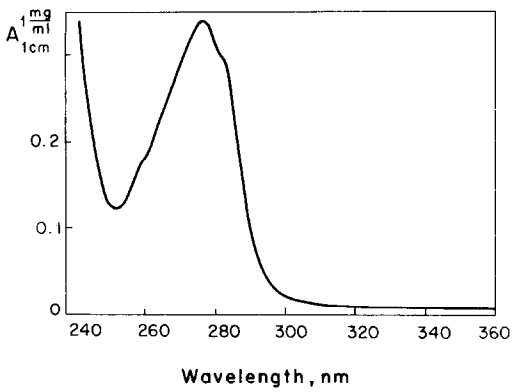


Fig.1. Absorption spectrum of protein S15 in the near ultraviolet region. Solvent: 0.1 M NaCl, 30 mM sodium phosphate (pH 5.6).

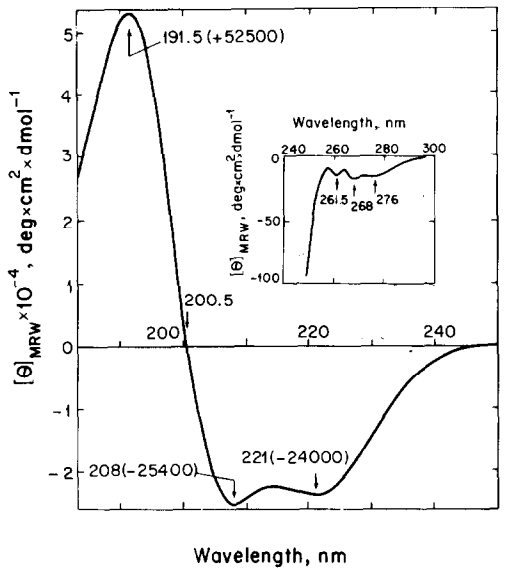


Fig.2. CD spectrum of protein S15 in the near (insertion) and the far ultraviolet regions. Solvent: 50 mM sodium phosphate (pH 5.6).

(B). Comparison of these spectra provides evidence that a well developed tertiary structure exists in protein S15 in standard solvent.

In the high-field region, between 0.9 and 3.5 ppm, the spectrum of the protein under non-denaturing conditions (fig.3A) contains wide overlapping resonance lines, mainly of apolar aliphatic residues and threonines. This indicates that the aliphatic side groups and the threonine residues are in different chemical surroundings within the protein, suggesting a specific folding of its chain.

In the extreme high-field region, between 0.8 and 0.5 ppm, the PMR spectrum of protein S15 has a number of resonance lines with chemical shifts which are absent in individual amino acid residues [22]. These resonances can be attributed to aliphatic amino acid residues located near to aromatic amino acids in a globular structure [22,23].

In the low-field region, between 6.5 and 8.5 ppm, the resonance lines of 4 histidine, 2 tyrosine and 2 phenylalanine residues are revealed. Four separate signals, 2 protons each (at 6.79, 6.85, 7.09 and 7.14 ppm), have been attributed to the 2 tyrosine residues since they underwent a shift into the high field at pH >9.5 (data not shown). Inasmuch as the resonance lines at 6.79 and 7.09 ppm of one of the tyrosine residues are broader and their resolution poorer than the resonances at 6.85 and 7.14 ppm of the other, it can be suggested that one of the residues rotates around the $C^{\beta}$–$C^{\gamma}$ bond more freely than does the other [24].

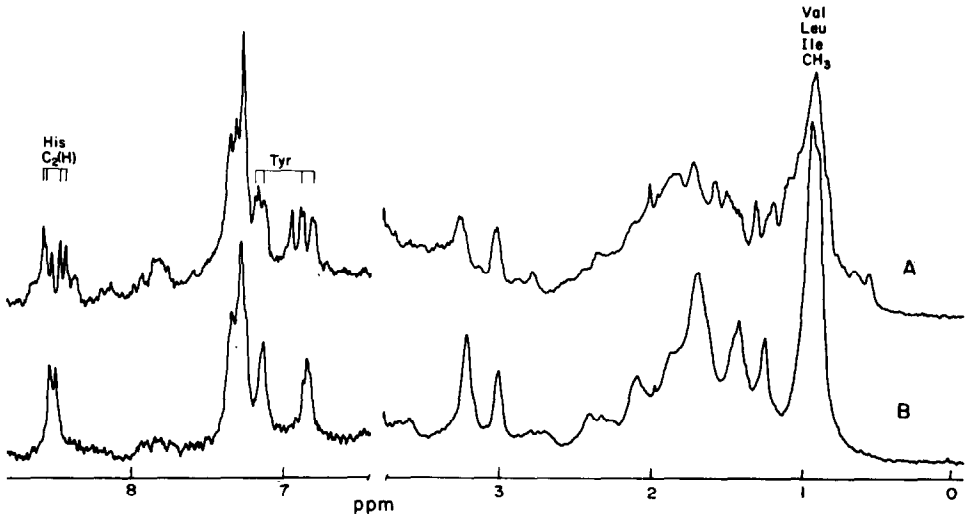


Fig.3. PMR spectra of protein S15. Solvent: 99.8% $D_2O$ with 0.1 M NaCl–30 mM sodium phosphate (pD 5.3) (A), and the same solvent in the presence of 5 M urea (B).

Protons at $C_2$ of the imidazole rings of the 4 histidine residues of protein S15 under non-denaturing conditions exhibit separate signals in the region from 8.4 to 8.6 ppm indicating their different chemical surroundings, i.e., specific chain folding. Since their width is <6 Hz it can be concluded that the histidine residues are mobile.

Thus, the existence of resonance lines in the extreme high field and the significant perturbations of the resonance lines of apolar aliphatic residues in the high field, as well as the perturbations of the resonances of the tyrosines and the histidines in the low field, provide definite evidence that protein S15 in solution is characterized by a specific folding of the polypeptide chain and, hence, by a well organized tertiary structure.

### 3.3. *Compactness*

Figure 4 shows the dependence of the neutron scattering intensity I on the scattering vector $\mu$ in the Guinier coordinates for protein S15 in standard $D_2O$

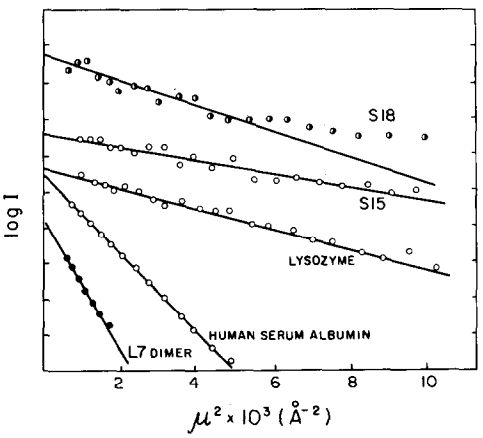


Fig.4. Dependence of the neutron scattering intensity I on the scattering vector $\mu$ ($\mu = \frac{4\pi}{\lambda} \sin\theta$, where $2\theta$ is a scattering angle), in Guinier coordinates, for protein S15. Solvent: 92% $D_2O$ with 0.1 M NaCl–30 mM sodium phosphate (pD 5.3). The data on protein S18 in 92% $D_2O$ with 10 mM NaCl–3 mM sodium phosphate (pD 6.8) and the dimer of the ribosomal protein L7 in 92% $D_2O$ with 0.1 M NaCl–10 mM Tris–HCl (pD 6.8) are presented for comparison. The data on lysozyme and human serum albumin [7] are plotted as controls. All the scattering curves were put on an absolute scale following normalization by the incoherent scattering from water; here they are presented on an arbitrary intensity scale for the sake of clarity.

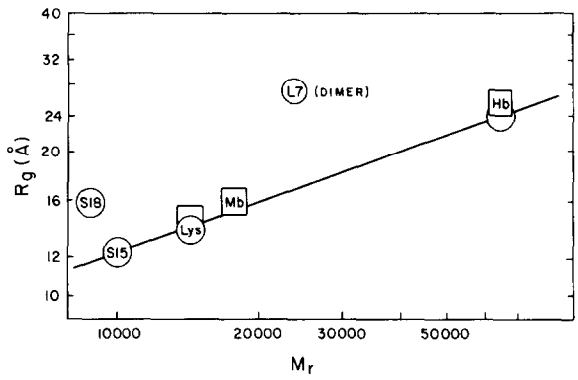


Fig.5. The ratio between the radius of gyration ($R_g$) and the molecular weight ($M_r$) for protein S15, in comparison with those of the non-compact protein S18, and the elongated protein dimer L7–L7. The ratios for lysozyme, myoglobin and hemoglobin [7] are presented as references of typical globular proteins. The straight line corresponds to the theoretical slope of 1/3 for compact globular proteins.

solvent. The radius of gyration calculated from the curve slope is found to be 12.5 (±1) Å. The molecular weight measured from the scattering is 10 000 which exactly coincides with the value calculated from the amino acid sequence [2].

Figure 5 presents a plot of the radii of gyration versus the molecular weights for compact globular proteins and the corresponding experimental values for protein S15. It is seen that the ratio between the radius of gyration and the molecular weight of protein S15 corresponds fully to a compact globular protein. For comparison, the results of neutron scattering for the dimer of protein L7 which seems to have a rod-like or dumbbell-like structure [25–27] and for protein S18 in the form of a partly disordered polypeptide chain ([28], Z.V.G., S.Yu.V., unpublished) are also plotted in fig.5.

## 4. Discussion

Thus, in our hands, isolated ribosomal protein S15 is found to be a typical globular protein characterized by a high content of secondary structure (78% α-helices), a well developed tertiary structure and a high compactness ($R_g$ = 12.5 Å).

A previous heat denaturation study of our prepara-

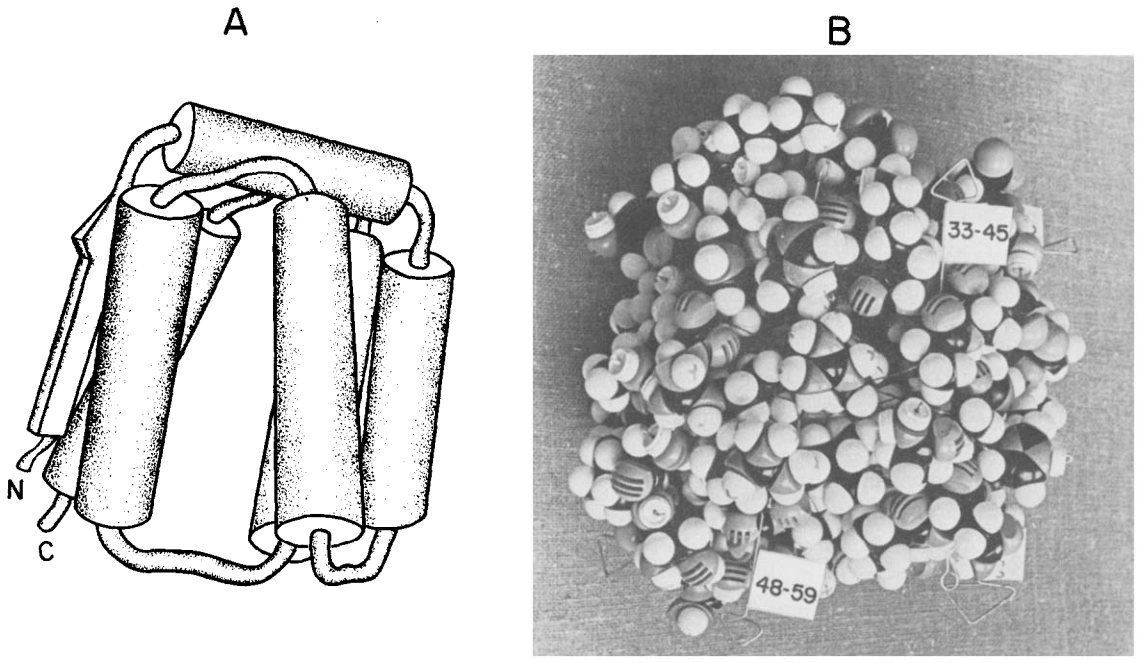


Fig.6. Predicted secondary and tertiary structure of the protein S15. (A) Scheme of the predicted folding pattern of the polypeptide chain. (B) Photograph of the atomic space-filled model built on the basis of the predicted folding.

tion of protein S15 has demonstrated the cooperativity and high thermal stability ($t^{\circ}_{m}$ = 72°C) of its tertiary structure [28], which is consistent with a compact globular state of the protein molecule in solution.

Earlier, a theoretical analysis of primary structures of a number of ribosomal proteins, including protein S15, led to the conclusion that the characteristic distribution of hydrophobic residues along their polypeptide chains will result in their folding into typical globular structures [29]. The use of a stereochemical algorithm for prediction of tertiary structures of globular proteins [30,31] has permitted one to predict a specific folding pattern of ribosomal protein S15 [29]. Here, following the folding pattern, a space-filled atomic model of protein S15 has been built, as shown in fig.6.

First of all, the folding pattern proposed earlier and based on completely a priori stereochemical rules has predicted the highly helical nature of protein S15 [29]. According to the model (fig.6), α-helical regions are located in the 7–18, 22–30, 33–45, 48–59, 61–69 and 75–84 positions of amino acid residues. The α-helical content of the model is 75% which is in good agreement with the experimental value (section 3.1.).

Further, the model predicts a well fitting tertiary structure. The third and the fourth helices form one hairpin and the fifth and the sixth helices are folded in the other hairpin; the sticking together of these 2 pairs of helices by their hydrophobic surfaces results in the formation of a hydrophobic core of the globular molecule (fig.6). The tyrosine residues 67 and 76 are found to be more or less exposed on the molecule surface, but Tyr 67 is more buried. The experiment supports this prediction: according to PMR spectra, one tyrosine ring is a freely rotating one whereas the other is somewhat inhibited, though still on the surface (section 3.2.). In the model, all the 4 histidine residues, in positions 37, 41, 48 and 49, are also more or less exposed, the His 41 being the least so. The PMR experiments show that all the 4 histidine rings are indeed exposed, but one significantly less than the others (section 3.2; fig.3).

The surface distribution of positively charged groups of arginine and lysine residues on the model is found to be very asymmetric, so that many of them

are concentrated on one side of the molecule and surround the two tyrosine residues. This is very consistent with the experimental data reported on tyrosine fluorescence of protein S15 in solution [32]: both tyrosines were shown to be rather exposed and their fluorescence was quenched by anions ($I^-$) but not cations ($Cs^+$).

Finally, the radius of gyration calculated from the model proposed (12.5 Å) proves to coincide fully with the experimental value (section 3.3.)

In connection with our results, the data published by others on an extended or expanded conformation of protein S15 in solution [5,6] seems surprising. We can explain this discrepancy only by the different quality of the preparations of protein S15 studied earlier [5,6]. This explanation is supported by a comparison of our (fig.3) and the earlier reported [33] PMR spectra: they are different. In any case, our results demonstrate at least the principal capability of the polypeptide chain of protein S15 to acquire and maintain a compact globular conformation with a stable, cooperative, and thus well fitting tertiary structure. We do not believe very much that such a conformation would undergo a complete transformation and unfolding into a 200 Å long thread [3,4] as a result of the interaction with the ribosomal RNA and the 30 S particle assembly.

Recently, an analogous complex study has been made in our group on ribosomal protein S4 (I.N.S. et al., in preparation). It was found that this protein preserves its compact globular conformation [7] after the interaction with the corresponding fragment of ribosomal 16 S RNA.

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