COMPACT STRUCTURE OF RIBOSOMAL PROTEIN S4 IN SOLUTION AS REVEALED BY SMALL-ANGLE X-RAY SCATTERING

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1. Introduction

In small-angle X-ray scattering studies of ribosomal proteins of the small subunit of *Escherichia coli* ribosomes in solution [1–7], X-ray diffractometers with high spatial resolution (≈1000 Å) and 5–15 mg ribosomal protein/ml were used. The values of the X-ray radii of gyration (5 g) were found to be 1.5–2-times as much as the r_g values of compact globular proteins with the same relative molecular masses (M_r). Thus, for protein S4 several r_g values, such as 34 Å [1], 42 Å [2] and 26 Å [5], have been reported. Relatively high r_g values were also obtained for other ribosomal proteins [2–7]. These data were interpreted as an evidence that ribosomal proteins in solution had strongly elongated or unfolded conformations [1–7].

In 1978 the small-angle neutron scattering technique was used to study conformation of ribosomal proteins in solution [8]. There are two advantages of neutron scattering in ^2H_2O over X-ray or neutron scattering in H_2O: lower absorption of the solvent and higher difference between the scattering power of the protein and the solvent (higher contrast). In the Laue-Langevin Institute (Grenoble) we have measured the neutron r_g of ribosomal proteins at 1.0–1.5 mg/ml. The r_g values of ribosomal proteins were found to be relatively small. Thus, the r_g of protein S4 was of 18.5 Å [8]. Small r_g values were also obtained for other ribosomal proteins [8,9]. They were consistent with the r_g values of compact globular proteins of the same M_r value. These results demonstrated the principal capability of some ribosomal proteins to acquire and preserve a compact globular conformation in solution [8].

At the same time, the discrepancy between the high X-ray r_g and the low neutron r_g of the same ribosomal proteins in solution requires elucidation and explanation.

Here we report the results of a small-angle X-ray scattering study of ribosomal protein preparations obtained by the neutron scattering method in [8,11]. The theoretical resolution of our diffractometer (Kratky camera, the entrance slit 80 μm, the receiving slit 190 μm, the sample-detector distance 20.4 cm) was the same as the resolution of X-ray diffractometers, on which high r_g values for ribosomal proteins were obtained. We used protein S4 adjusted to 20 mg/ml without any essential loss of solubility. The scattering indicatrix obtained in a wide range of angles has demonstrated that the X-ray r_g obtained here coincides with the earlier obtained neutron r_g and the outer part of the scattering curve is similar to that of slightly elongated compact bodies. We conclude that all discrepancies between our data on the study of ribosomal protein structure in solution [8–10] and the data in [1–7] are not connected with the characteristics of the instruments used but only with the quality of the protein preparations.

2. Materials and methods

2.1. *Preparation of ribosomal protein S4*

Preparation of ribosomal protein S4 was done as in [11]. The protein solution was kept frozen at −80°C. Immediately before experiment it was thawed and dialyzed in the cold against 2 changes of 1 M KCl, 0.05 M potassium phosphate, 0.01 M β-mercaptoethanol (pH 7.0) and then against 3 changes of 0.33 M KCl, 0.01 M Tris–HCl, 0.02 M $MgCl_2$, 0.001 M DTT (pH 7.0) for ⩾16 h. The CD and NMR spectra

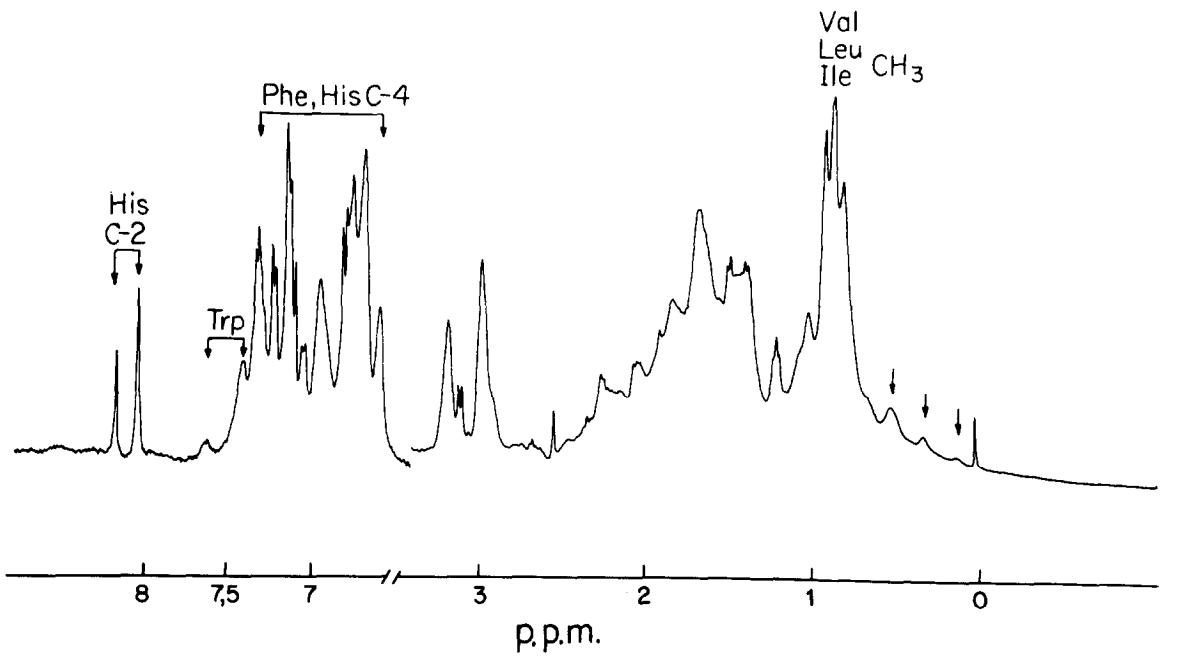

Fig.1. 360 MHz PMR spectra of protein S4. Solvent: 0.35 M KCl; 0.03 M potassium phosphate (pH 6.85) in 99.8% ^2H_2O; T = 25°C.

were examined to control the quality of the preparations. The CD spectrum quantitively coincided with that in [11]. A minor additional peak not seen in the spectrum of [11] was observed in the region of 3 ppm (fig.1) of the present NMR spectrum.

2.2. *X-ray scattering measurements*

X-ray scattering measurements were done at the Institute of Protein Research using the Kratky small-angle camera with an X-ray source in the form of an anode tube with CuK_α-radiation and the Geigerflex generator. Scattered radiation was registered by a scintillation counter combined with a nickel β-filter and an amplitude discriminator for isolation of a CuK_α-line (λ = 1.54 Å). All the instruments were in a thermostatted room (20 ± 0.3°C) and worked 24 h a day. The influence of the primary beam intensity drift and the lability of the electronic instruments were decreased by the technique of a many-fold automatic scanning with alternate measurements of the solution and solvent scattering in a bisectorial cell. Manyfold scanning was performed in a forward and opposite directions. The Multi-20 computer was on-line with the electronic instruments. After completion of measurements the information was processed by Zipper's algorithm [13]. The averaged curve was normalized for one of the scans accepted to be a reference scan. The first or last scan were chosen as a reference one in a continuous set of measurements (≈10–12 scans) because the primary beam intensity measurements were made by a standard Lupolen sample before or after a set of measurements. After completion of the measurements the solution and solvent in the cells were interchanged.

For ribosomal protein S4, X-ray scattering was measured in 3 ranges of angles corresponding to μ:

$$\mu = 4\pi/\lambda \cdot \sin\theta$$

where λ is the wavelength; 2θ is the scattering angle varying from: 0.008–0.09 $Å^{-1}$; 0.052–0.2 $Å^{-1}$; and 0.09–0.3 $Å^{-1}$.

Scattering curves from each μ range were used to plot a combined curve over 0.008–0.3 $Å^{-1}$. The collimation and receiving slit widths were 80 μ × 190 μ, 80 μ × 510 μ and 125 μ × 1000 μ, respectively, for the first, second and third ranges. The number of collected pulses provided for a 2%, 3% and 5% statistical accuracy for the first, second and third ranges, respectively. The measured protein concentrations were 3.17 mg/ml, 5.12 mg/ml, 7.22 mg/ml and 10.7 mg/ml for the first range, 10.7 mg/ml for the

second range and 12.1 mg/ml for the third range. Schedrin-Feigin's program was used in the collimation correction of the curves [14]. M_r was determined by normalization for a standard Lupolen sample. The partial specific volume ($\bar{v}$) of 0.739 cm^3/g was calculated from the amino acid sequence of protein S4 [16]. The protein concentration in solution was determined from the absorption spectrum [11].

3. Results and discussion

3.1. *Inner part of the scattering curve*

Fig.2 represents the dependence of the scattering intensity I *vs* scattering vector μ ($\mu = 4\pi/\lambda \cdot \sin\theta$, where λ is the wavelength, 2θ is the scattering angle) in Guinier coordinates for the 4 concentrations of ribosomal protein S4 in the buffer containing 0.33 M KCl, 0.01 M Tris–HCl, 0.02 M Mg^{2+}, 0.001 M DTT (pH 7.0). For 3.17 mg protein/ml and 5.12 mg protein/ml the scattering indicatrices in Guinier coordinates have a linear slope at μ varying from 0.01–0.08 $Å^{-1}$. The r_g obtained for these concentrations coincide in the experimental error limits and are 19.5 ± 1 Å. The intercept of the dependence of log I on μ^2 corresponds to the molecular mass of a monomer protein (M_r = 23 000).

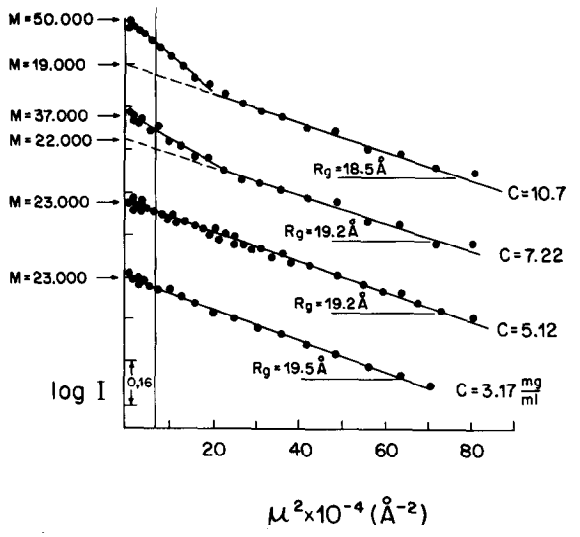

Fig.2. Guinier plot for ribosomal protein S4 at 10.7 mg/ml, 7.22 mg/ml, 5.12 mg/ml and 3.17 mg/ml in solution. The thin straight line parallel to the ordinate shows the least scattering vector μ obtained in our experiments on neutron scattering for ribosomal protein S4 [8].

At 7.22 mg protein/ml and 10.7 mg protein/ml, the scattering indicatrices in Guinier coordinates are distorted over (μ = 0.01–0.04 $Å^{-1}$). In this smallest range of angles the straight line has a slope corresponding to r_g = 26 Å and 33 Å for 7.22 mg protein/ml and 10.7 mg protein/ml, respectively. Such a rise of the scattering indicatrix in the range of the smallest angles is typical of the solution containing some amount of high M_r aggregates [15]. This interpretation is supported by overestimated M_r values obtained from the smallest scattering angles (37 000 for 7.22 mg protein/ml and 50 000 for 10.7 mg protein/ml) and by the coincidence of the scattering curves at $\mu > 0.06$ $Å^{-1}$ for all the 4 concentrations studied.

3.2. *Outer part of the scattering curve*

Fig.3 represents the experimental dependence of scattering intensity I *vs* scattering vector μ multiplied by r_g for ribosomal protein S4, plotted on a double logarithimic scale. The figure also shows theoretical scattering curves for different ellipsoids. As seen from fig.3, the experimental scattering indicatrix (○) is between theoretical scattering curves for prolate ellipsoids of revolution with the axial ratios of 2:1 and 3:1 (——) or for oblate ellipsoids of revolution with the axial ratios of 1:2 and 1:3 (solid lines). The best coincidence of the experimental and theoretical scattering curves is attained for a triaxial ellipsoid with an axial ratio of 3:1.5:1 (—·—).

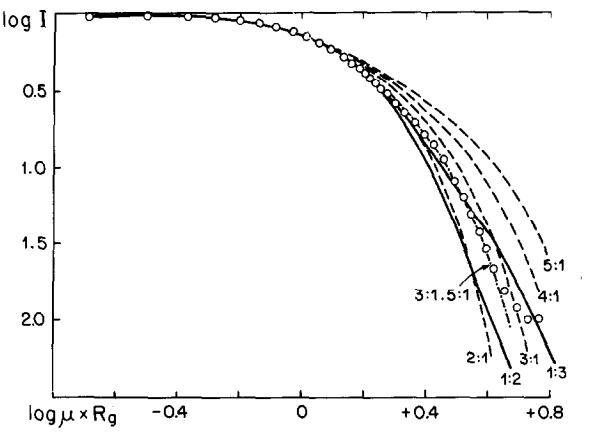

Fig.3. The experimental scattering curve for ribosomal protein S4 (○) in comparison with theoretical scattering curves for ellipsoids. Axial ratios are given in the figure: (– – –) prolate models; (——) oblate models; (—·—) triaxal ellipsoid with the ratio of 3:1.5:1.

4. Conclusion

The wide range scattering curve for ribosomal protein S4 shows that:

(i) The X-ray r_g (19.5 ± 1 Å) is consistent, within the limits of experimental error, with the published data on neutron scattering r_g (18.5 ± 1.5 Å) [8];

(ii) A triaxial ellipsoid with the axial ratio of 3:1.5:1 approximates well the outer part of the scattering curve.

Therefore, the discrepancy between our data on the compact globular conformation of ribosomal proteins [8,9,11] and the data in [1–7] on the elongated or unfolded conformation of ribosomal proteins in solution is not connected with the properties of the instruments used in physical methods. The only remaining reason is the quality of the ribosomal preparations.

The tendency of ribosomal protein S4 to aggregate at an increased concentration noted in [5] and clearly seen from our experiments, is a possible reason for the high r_g values observed in [1,2,5]. (The coincidence of the M_r estimated from X-ray data with the primary structure data could be occasional and might reflect an incorrect determination of the protein concentration in solution in the earlier experiments.) Another reason, such as partially denatured state of the protein in the earlier experiments, is not excluded either. A substantiation of the latter explanation is given in [17] which showed that the 'nativity' of ribosomal protein L11 according to the NMR evaluation depended on conditions of protein renaturation.

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