GLOBULAR CONFORMATION OF SOME RIBOSOMAL PROTEINS IN SOLUTION

I. N. SERDYUK, G. ZACCAI[+] and A. S. SPIRIN

Institute of Protein Research, USSR Academy of Sciences, 142292 Poustchino, Moscow Region, USSR and
[+]Institut Laue-Langevin, 156X Centre de Tri, 38042-Grenoble Cédex, France

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1. Introduction

During recent years the idea that ribosomal proteins are not ordinary globular proteins but are asymmetric, 'elongated' structures has become widespread. The first grounds for this were immunoelectron microscopy data indicating that some proteins in the ribosomal particles display their antigenic determinants removed from each other by > 50 Å (reviewed [1]). In particular, this was shown for such proteins in the *Escherichia coli* ribosomal 30 S subparticle as S4, S7, S15, S18. The data on neutron scattering of the 30 S particles in which specific proteins were inserted in deuterated form supported the above observations concerning proteins S4 and S7 [2]. Measurements of small-angle X-ray diffuse scattering of isolated ribosomal proteins in solution also demonstrated that they are not compact globular molecules; the data on such proteins as S4, S7, S8, S15, S16 and others were interpreted as evidence of their elongated conformation [3–6]. Hydrodynamic measurements also indicated that they are not as compact as globular proteins [7,8].

At the same time, when known primary structures of ribosomal proteins (reviewed [1]) were analyzed according to the stereochemical algorithm developed [9–11], it was found that many proteins including S6, S8, S15, S16, S17 of the 30 S subparticle have all the structural prerequisites for the formation of a compact hydrophobic core and, consequently, for acquiring a globular conformation in solution [12].

We have explored experimentally the possibility that such RNA-binding proteins of the 30 S subparticle as S4, S7, S8 and S16 exist in the form of compact globules in solution. To this end we have studied these proteins in D_2O solution by neutron scattering to measure their radii of gyration. This type of radiation using D_2O as a solvent provides the maximum 'contrast', that is the maximum difference between the scattering of the protein and the solvent. It allowed measurements to be made using protein at $\leqslant 1.5$ mg/ml. The radii of gyration for the ribosomal proteins S4, S7, S8 and S16 were found to be relatively small corresponding to the radii of gyration of compact globular proteins of the same molecular weights.

2. Materials and methods

2.1. *Preparation of ribosomal proteins*

Ribosomal 30 S subparticles of *E. coli* MRE 600 were isolated by sucrose gradient centrifugation in the presence of 0.5 M NH_4Cl and 1 mM $MgCl_2$ [13], using a zonal rotor [14]. The ribosomal proteins were prepared by extraction with 4 M urea–3 M LiCl followed by chromatography on a phosphocellulose column [15] in a 50 mM phosphate buffer, pH 5.8, containing 6 M urea, 15 mM methylamine and 10 mM β-mercaptoethanol. If necessary, the fractions were rechromatographed on a Sephadex G-75 column in the same buffer with 6 M urea. Purity of the individual proteins was checked by two-dimensional polyacrylamide gel electrophoresis [16] and amino acid analysis. The solutions of individual proteins were concentrated by ultrafiltration on UM-2 membranes (Amicon) and frozen in liquid nitrogen. The proteins were stored at $-70°C$ and transported to the Institut Laue-Langevin in dry ice. The proteins were thawed just before the neutron scattering experiment

and thoroughly dialyzed; proteins S7, S8 and S16 were dialyzed against an H_2O buffer containing 10 mM Tris–HCl, pH 7.0, 100 mM NaCl, 1 mM β-mercaptoethanol (3 changes) and then against a D_2O buffer of the same composition (again 3 changes); protein S4 was dialyzed against an H_2O buffer containing 0.01 M Tris–HCl, pH 7.0, 1 M KCl, 20 mM $MgCl_2$ (3 changes), then against either H_2O or D_2O buffer containing 0.01 M Tris–HCl, pH 7.0, 330 mM KCl, 20 mM $MgCl_2$ (3 changes).

2.2. *Neutron scattering*

Neutron scattering was done at the Institut Laue-Langevin on the high-flux reactor using the D11 camera [17]. It has a 64 × 64 cm^2 two-dimensional detector with resolution elements of 1 cm^2. The sample–detector distance was 2.55 m, the wavelength $\lambda = 7.0$ Å and $\Delta\lambda/\lambda = 8\%$. The measured interval of reciprocal vectors μ was from 0.025–0.12 $Å^{-1}$ ($\mu = 4\pi/\lambda \sin\theta$; 2θ is scattering angle). The cell was 2 mm thick for the D_2O buffer and 1 mm thick for the H_2O buffer. At the concentrations of ribosomal proteins studied the difference between the scattering intensities of the solution and the solvent was < 10–25%. This compelled us to focus attention on the reproducibility of the values of neutron radii of gyration obtained. With this in view 3 independent measurements of the solution and the solvent were made for each ribosomal protein. The calculation of the radius of gyration from any solution–solvent combination showed that the error was $< 10\%$. To check the values of absolute neutron radii of gyration obtained, measurements of the radii of gyration of human serum albumin (c = 5 mg/ml) and hen egg-white lysozyme (c = 1.3 mg/ml) were made as controls (see table 1).

The molecular weights of the ribosomal proteins were calculated from the neutron scattering data following the procedure in [18,25]. The scattering curves were put on an absolute scale by normalising to the incoherent scattering from water. Since the normal procedure requires the intensity scattered in H_2O buffer, it was calculated from the D_2O data using a linear extrapolation of $\sqrt{I}$ with a match-point of 42% D_2O for all the proteins. Protein concentrations were determined by measurement of nitrogen content. The uncertainty in the molecular weights thus derived is estimated to be ± 20%, arising from uncertainties in the concentration and in the assumption about the match-point, as well as the accuracy of the measured $I(O)$.

3. Results and discussion

Figure 1 presents the dependence of scattering intensity I on the scattering vector μ in the Guinier coordinates, $\log I$ versus μ^2, both in H_2O and in 92% D_2O buffers (0.01 M Tris–HCl, pH 7.0, 0.33 M KCl, 0.02 M $MgCl_2$) for protein S4 and in 92% D_2O buffer (0.01 M Tris–HCl, pH 7.0, 0.1 M NaCl, 0.001 M β-mercaptoethanol) for proteins S7, S8 and S16. Hen egg-white lysozyme and human serum albumin

Table 1
Comparison of values of radii of gyration obtained for ribosomal proteins from neutron and X-ray scattering data

Protein	M_r from primary structure	M_r from neutron scattering (our data)	R_g, Å (our data)			R_g, Å (literature data)
			neutrons H_2O	neutrons D_2O	X-rays	X-rays
S4	22 550 [19]	24 000	18.5 ± 1.5	17.5 ± 1	–	34 [3], 42 [4], 26 [5]
S7	20 000 [20]	16 000	–	14.5 ± 1	–	27 [5]
S8	12 200 [21]	12 000	–	13.0 ± 1	–	23 [6]
S16	9200 [22]	11 000	–	12.2 ± 1	–	21 [6]
Lysozyme	14 300	14 100	–	13.8 ± 1	14.3 ± 0.5	14.3 [23]
Human serum albumin	68 000	72 000	–	27.0 ± 1	27.5 ± 0.5	–

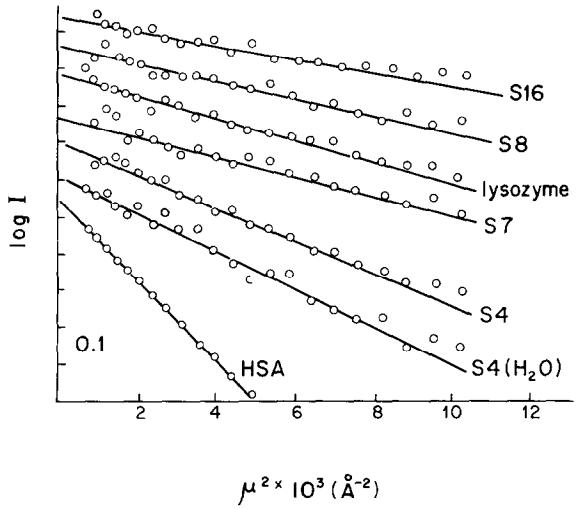

Fig.1. Dependence of the logarithm of scattering intensity on the square of the reciprocal vector (μ^2) for the ribosomal proteins studied. Concentrations used: S4 in H_2O buffer, 1.1 mg/ml; S4 in D_2O buffer, 1.2 mg/ml; S7 in D_2O buffer, 1.0 mg/ml; S8 in D_2O buffer, 0.9 mg/ml; S16 in D_2O buffer, 1.2 mg/ml. Hen egg-white lysozyme in D_2O buffer (c = 1.3 mg/ml) and human serum albumin in D_2O buffer (c = 5 mg/ml) are also presented as controls. Though all scattering curves have been put on an absolute scale following normalisation by the incoherent scattering from water, they are presented here on an arbitrary intensity scale for the sake of clarity. They are in order of increasing molecular weight starting at the top.

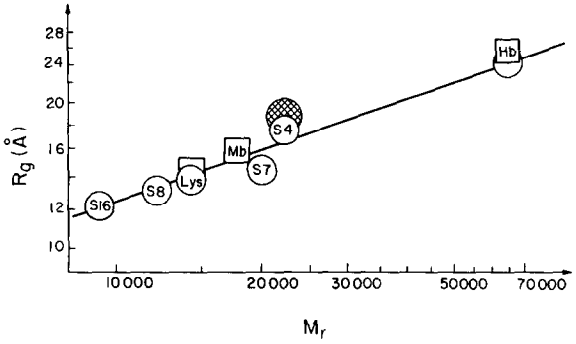

Fig.2. Dependence of the radii of gyration (R_g) on the molecular weights (M_r) of the proteins studied, in logarithmic coordinates. Open circles correspond to neutron measurements in D_2O buffer; filled circles, to neutron measurements in H_2O buffer; data for hemoglobin are taken from [24]; dimensions of the circles are proportional to the measurement error. Squares correspond to X-ray measurements for lysozyme (our data), myoglobin (our data) and hemoglobin [24]; dimensions of the squares are 2-times larger than the measurement error. The straight line corresponds to the theoretical slope of 1/3 for compact globular proteins.

in 92% D_2O are also presented here as controls. The radii of gyration calculated from the slope of these curves are given in table 1, together with the molecular weights of the proteins studied. It is seen from table 1 that the radii of gyration of the ribosomal proteins measured by us are relatively small. They are typical of compact particles, neither elongated, nor unfolded. They correspond to the radii of gyration of ordinary globular proteins with the same molecular weights. The molecular weights measured correspond to the molecular weights calculated from the known amino acid sequences, within limits of experimental error (± 20%).

Figure 2 shows that the ratio between the radius of gyration and the molecular weight characteristic for compact globular proteins (hemoglobin, myoglobin, lysozyme) is completely obeyed by the ribosomal proteins studied. Thus, these data unambiguously provide evidence that the ribosomal proteins S4, S7, S8 and S16 can assume a compact globular conformation in solution.

At the same time the data on radii of gyration of the same proteins [3–6] give values about 2-times higher than ours (see table 1). Those values cannot fit a compact conformation typical of ordinary globular proteins.

At present we do not know how to explain the discrepancy between the results given above and the data in [3–8] on expanded conformations of ribosomal proteins in solution. We believe that this discrepancy can be connected mainly with the difference in the quality of the protein preparations and in that special attention was paid to their renaturation and maintenance of the non-denatured state in our experiments. In any case, our results demonstrate the capability, in principle, of some ribosomal proteins to acquire and preserve a compact globular conformation in solution. The question of how this conformation is transformed as a result of the ribosomal particle assembly (if it is transformed indeed) remains open.

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