SHAPE PROPERTIES OF PROTEINS L7 AND L12 FROM E. COLI RIBOSOMES[1]

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Summary: This paper reports physical-chemical properties of proteins L7 and L12 from E. coli 50 S subunits. Evidence is presented that these two proteins behave in their native state as a dimer of molecular weight 24000. From sedimentation velocity and intrinsic viscosity data the actual frictional ratio of the dimer has been obtained revealing an asymmetric particle which can be described as a rod with cell dimensions of L = 130 Å and a diameter of D = 17.0 Å. From small X-ray scattering the radius of gyration (Rg = 37.0 Å), the thickness factor, and the degree of hydration were determined. This indicates that the extended shape of the dimer is due to the asymmetry of the molecule and not to the hydration.

INTRODUCTION

Proteins L7 and L12 from E. coli 50 S subunits are involved in several ribosomal functions, e.g. elongation (for recent reviews see (1, 2)). The primary structure of these proteins is almost identical. The only difference is the acetylation of the N-terminal serine in the L7, but not the L12 protein (3, 4). Studies on the secondary structure of these proteins indicate an unusually high α-helix content in comparison to other ribosomal proteins (5, 6, 7). Theoretical predictions of the secondary structure (8) estimated the degree of helicity in the globular state to 50 % and in the unfolded state to 42 % with no indication for ß-structure. Measurements of the secondary

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structure by circular dichroism (5, 6) agree with these predictions, except of 20 % β-structure which has been found (6). Almost nothing is known about the tertiary structure as well as of the shape of these proteins. We report here the first experimental data of the shape properties of L7 and L12 in solution under physiological conditions.

MATERIALS AND METHODS

Proteins L7 and L12 from E. coli, strain MRE 600, was prepared according to (9) and kindly provided by Drs. G. STÖFFLER and B. KITTLER. The lyophilized material was dissolved in double destilled water and dialyzed against the appropriate buffers at 4°C for 12 hours. The buffers used throughout these investigations are:

A : 0.01 M TRIS-HCl, pH 7.0, 0.01 M KCl, 0.005 M $MgCl_2$
B : 0.01 M CH_3COONa, pH 5.5, 0.01 M NaCl, 0.005 M $MgCl_2$
C : 0.005 M potassium cacodylate, pH 7.5, 0.01 M KCl, 0.005 M $MgCl_2$
D : 0.001 M K_2HPO_4, pH 7.5, 0.01 M KCl, 0.005 M $MgCl_2$.

Protein concentrations ranged from 5.0-10 mg/ml for sedimentation velocity runs with Schlieren optics, for viscosity measurements, and for small angle X-ray scattering in solution. Sedimentation velocity experiments were performed in a Beckman ultracentrifuge (Model spinco E) using Schlieren optics in double sector cells with quarz windows. For very dilute protein solutions the electronic scanner at 274 nm was used in order to compare the concentration dependence of the sedimentation constant with protein concentration. Sedimentation coefficients (S) were obtained from log r vs. t diagrams and corrected for 20°C and water. Sedimentation equilibrium experiments were performed using the meniscus depletion method according to YPHANTIS (10); small angle X-ray scattering experiments in solution were performed in a Kratky camera, equipped with an electronically programmed step scanning device (MÜLLER-SEIFERT), using Cu K_α radiation. Scattering curves were recorded for different concentrations and temperatures as well as for the buffers. The apparent radius of gyration was determined by plotting the logarithm of the scattered intensities against $(2\theta)^2$ in the 0.9 I_o to 0.6 I_o region, where one obtains a

Table 1

Hydrodynamic properties of proteins L7 and L12 obtained by diffusion, sedimentation velocity, and equilibrium experiments.

MW*	24.000	Dimer
$S_{20,w}$	1.4×10^{-13} sec	sedimentation coefficient
$D_{20,w}$	5.59×10^{7} cm^2 · sec^{-1}	translational diffusion coefficient
f/fo	1.97 - 2.01	frictional ratio
$(n)_{c=o}$	27.7 ml g^{-1}	intrinsic viscosity
ß†	$2.64 - 2.70 \times 10^{-6}$	
$\bar{v}$§	0.735 ml g^{-1}	partial specific volume
$P = \frac{a}{b}$	19.2 - 20.1	axial ratio of the dimer
L = 2 a	125 - 130.0 Å	length of the dimer
D = 2 b	16.5 - 17.5 Å	diameter of the dimer
$\vartheta = 100 \frac{(h)}{v}$	38.2	viscosity increment, SIMHA parameter

* - depending om Mg^{2+} concentration and pH

† - according to MANDELKERN-SCHERAGA, using the equation

$$\beta = N \times \frac{S_{20,w} \cdot [h]^{1/3} \cdot h_s}{MW^{2/3} (1-\bar{v}\rho)}$$

§ - depending on buffer conditions.

straight line and the slope of this line is proportional to the apparent Rg^2. The values of the apparent Rg were plotted against concentration and extrapolated to infinite dilution. Viscosities were measured in special designed Ostwald viscosimeters at 4°C with flow times of 143 sec. for water.

RESULTS

From sedimentation and diffusion experiments the shape of the

dimer is estimated from the ratio of the frictional coefficients f/fo, from the SIMHA parameter ν (11), and from the radius of gyration obtained by small angle X-ray scattering. From Table 1 it is clear that the shape of the dimer in solution is not spherical. The ratio of the long to short axis of an ellipsoid of revolution, equivalent of the true dimer that would have the f/fo or $\nu = 100 \frac{(h)}{v}$ evaluated for proteins L7 and L12, is obtained from (12) by means of the PERRIN function (13). Applying ONCLEY's graphical representations of these two functions (14) an estimate of the influence of hydration on the shape can be made. Values for the degree of hydration were obtained from small angle X-ray scattering and found to be 0.26 g H_2O per 1 g protein, neglecting binding of cations. Under the assumption that v, the partial specific volume, represents the volume occupied by 1 g protein and that the hydrodynamic equivalent has the same volume as the protein, we obtain cell dimensions for the dimer of a length of L = 130 Å with a diameter of D = 17.0 Å, applying a molecular weight of 24000 for the dimer. Using the method of SCHERAGA and MANDELKERN (15) the above mentioned assumptions are not required for the Stokes' radius (Re) for a spherical particle, but their parameters define a hydrodynamically equivalent ellipsoid of revolution which does not necessarily have the same volume as the real molecule. Only a prolate ellipsoid of revolution is consistent with the value ß, whereas this parameter does not exceed 2.15×10^6 for oblate ellipsoids of any axial ratio.

Scattering curves were recorded in buffers A, B, and C and at different protein concentrations, and care was taken to measure the protein concentrations precisely and under identical experimental conditions. Table 2 summarizes the values obtained by small angle X-ray scattering in solution. In some cases, concentration dependence of the scattering curves were observed, e.g. by raising the magnesium concentration to 10 mM Mg^{2+}. No influence of monovalent cations on the radius of gyration has been detected, although there is an influence of the phosphate and cacodylate buffer in changing the radius of gyration about 10 %. Whether this effect is due to further aggregation and/or changing of the gross conformation of the dimer in solution is not yet clear. However, the feature of an extended shape

Table 2

Hydrodynamic properties obtained by small angle X-ray scattering.

Rg*	36.5 Å	radius of gyration
Rq	9.50 Å	cross section factor
Rd	6.68 Å	thickness factor
q	1.04	degree of swelling
v	0.740 ml g^{-1}	partial specific volume
MW	25.200	
V_h	3.05 x 10^4 $Å^3$	hydrodynamic volume
Degree of hydration	0.26 g H_2O per 1 g protein	
L	125.5 Å	
D	17.3 Å	

*- determined in buffer A, B, C.

with high axial ratio is still valid. The molecular weight of the dimer determined by small angle X-ray scattering is about 5 % higher than that measured by sedimentation equilibrium. The swelling factor q ≈ 1.04, equivalent of a degree of hydration of 0.26 g H_2O per 1 g protein, reveals unequivocally that the high radius of gyration and the actual frictional ratio are due to the asymmetry of the dimer and not to the hydration shell along the polypeptide chain.
From plots of the intrinsic viscosity of the dimer in the range of pH 5.0 to 8.0 an average value of 27.7 ml · g^{-1} was obtained. The SIMHA parameter $\nu = 100 \frac{(h)}{v} = 38.2$ is much larger than 2.5 for suspended spheres and indicates an extended rigid molecule (11).

DISCUSSION

From sedimentation, diffusion and viscosity measurements the

Table 3

Structural parameters of the dimer assuming different conformations.

Assuming random coil		Rg (Å)	Re (Å)
from $S^{o}_{20,w'}$	= 0.6650	22.60	19.81
$D^{o}_{20,w'}$	= 0.8875	17.20	15.05
$(h)_{c=o}$;		19.5	17.35
Assuming flexible rod			
from $S_{20,w}D_{20,w'}$ $(h)_{c=o}$		38.5	53.1
Experimental value (average from X-ray scattering, sedimentation, intrinsic viscosity, and diffusion)		37.0	50.9

proteins L7 and L12 were found to behave physically as a dimer of molecular weight 24000. The high axial ratio and the rather low degree of hydration in comparison to other ribosomal proteins (16) suggest that the high actual frictional coefficient f/fo is not due to hydration but more to its anisometric (nonspherical) shape. Table 3 shows conclusively that this dimer can be described as a rod with cell dimensions of L = 130 Å and D = 17.3 Å. The values given in Tables 1 and 2 are maximal values, corresponding to no degree of hydration. However, the proteins bind some water or even cations, regardless of configuration, so that the values of $\bar{v}$ have to be revised. This would lead to smaller values of a/b, but it would also give a greater value of V_h. The combined effect would leave the radius of gyration practically unchanged. Therefore, it is easy to discriminate between a random coil and a rod, as it is to be seen from Table 3. In conjunction with theoretical calculations (8) the degree of helicity of the proteins L7 and L12 is the same if the protein is changed from a compact spherical to an extended or even unfolded state. This seems to be very unusual in comparison with other ribosomal proteins from E. coli. Although the aggregation of L7 and/or

L12 occurs side by side rather than from tail to head, a certain fraction of overlap of each protein can be predicted (17), so that the rod length of L7 and/or L12 might be somewhat shorter than calculated in the present data. The high association constant of the dimer molecule as well as the peculiar solubility of the monomers and the dimers (Paradies, unpublished), suggest the existence of a highly cooperative protein in the translocation step of protein biosynthesis.

In the present report the main finding is that the shape of the L7 and L12 dimer in solution is not spherical. This is in agreement with crystallization studies (liquid crystals) and fiber X-ray diffraction studies on these proteins (H. H. Paradies, unpublished). It is clearly shown that the dimer is rather elongated and that its largest dimension L is about 130-140 Å, independent of the detailed shape of the hydrodynamic or scattering equivalent.

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