SMALL-ANGLE X-RAY AND QUASI-ELASTIC LIGHT SCATTERING STUDIES ON 11 S GLOBULIN FROM SUNFLOWER SEED

P. PLIETZ, Hilde DAMASCHUN, D. ZIRWER, K. GAST, K. D. SCHWENKE+, Wilma PAEHTZ+ and G. DAMASCHUN

Zentralinstitut für Molekularbiologie der AdW der DDR, DDR-1115 Berlin-Buch, Lindenberger Weg 70 and *+Zentralinstitut für Ernährung Potsdam-Rehbrücke der AdW der DDR, DDR-1505 Bergholz-Rehbrücke, Arthur-Scheunert-Allee 114–116, GDR*

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1. Introduction

The globulins from plant seeds represent mainly storage proteins of oligomeric structure. The molecular weight of sunflower 11 S globulin was determined to be about 3.05×10^{-5} g/mol [1]. The shape of the globulin molecule was approximated by ellipsoids of revolution assuming 30% hydration (oblate, $a/b = 0.25$; prolate, $a/b = 3.6$) or 82%, respectively, ($a/b = 1.0$) using hydrodynamical data [2]. From the small-angle X-ray scattering data we derive as a shape model for the sunflower globulin an oblate ellipsoid of revolution ($a/b = 0.6$). Data from both methods used are compared and discussed.

2. Materials and methods

The 11 S globulin from sunflower seed was prepared according to [3,4]. As solvents 0.05 M Tris–HCl buffer, pH 8.0, containing different NaCl quantities were used (prep. C1, ionic strength $\mu = 0.39$; prep. C2, $\mu = 0.44$). The globulin concentrations varied from 6–25 g/l.

Small-angle X-ray scattering measurements were made at constant room temperature and humidity with a highly stabilized X-ray generator (VEB Freiberger Präzisionsmechanik, DDR) using a copper tube. A Kratky-diffractometer and an automated 4-slit diffractometer were used. Collimation effects were eliminated using a computer program described in [5,6]. Measurements of the absolute intensity were performed by using a calibrated Lupolen sample [7].

Quasi-elastic light scattering measurements [8,9] were made by means of an apparatus consisting mainly of a helium–neon laser (HNA 188, VEB Carl Zeiss Jena, ~ 50 μW), a goniometer with a temperature-controlled index matching bath (20°C) for the cell, a photomultiplier EMI 9558 BQ, and a power spectrum analyzer (laboratory-built). The measurements were done using homodyne detection. The half-width (Γ) of the photomultiplier current power spectrum and the translational diffusion coefficient D_T of the scattering particles are connected by [7]:

$$\Gamma = 2 D_T q^2, q = \frac{4\Pi n}{\lambda_o} = \sin\Phi/2$$

n = refractive index of the solution, λ_o = wavelength of the laser in vacuo, Φ = scattering angle. D_T is determined by measuring Γ at different scattering angles and plotting Γ in the dependence on $\sin^2\Phi/2$. The slope of the resulting curve gives D_T.

For the quasi-elastic light scattering measurements the freshly prepared globulin solution (prep. C1, 25 g protein/l) was filtered through 3 layers of membrane filters (Synpor 1.5 μm, CSSR) directly into the scattering cell in order to eliminate dust particles.

3. Results

In fig.1 the inner portions of the slit smeared X-ray scattering curves of prep. C1 for various concentrations are shown as Guinier plots ($s = 4\pi\lambda^{-1}\sin\Theta$, 2Θ-scattering angle). From the desmeared scattering curve, after extrapolation to zero concentration, several molecular parameters which do not depend on the special structure model were calculated (table 1). The experimental scattering data of prep. C1 are consistent with the scattering curve of an oblate ellipsoid of revolution with the axial ratio $a/b = 0.6$ and the axes (11.8 × 11.8 × 7.1) nm up to a scattering vector of $s = 0.95\ \mathrm{nm}^{-1}$ (fig.2, table 1). An arrangement of 6 spherical subunits into a trigonal antiprism

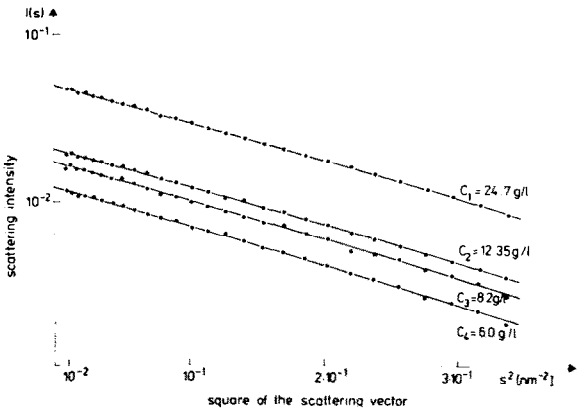

Fig.1. Guinier plot of the smeared scattering curve $J(s)$ of sunflower globulin (prep. C1) for various concentrations.

Table 1

Experimentally determined parameters, parameters derived from the shape model (oblate ellipsoid of revolution: $2a = 11.8$ nm, $2b = 7.1$ nm) of the globulin molecule of prep. C1 and radii of spheres derived from the experimental parameters

	Experimental parameters	Parameters of the shape model	Radius of an equivalent sphere
Radius of gyration R_G (nm)	4.05	4.05	5.23
Solvation m (g solvent/g protein)	0.22		
Largest diameter L (nm)	11.8	11.8	
Intersection length d_C (nm)	5.36	5.27	4.02
Correlation length l_C (nm)	6.97	11.7	4.65
Correlation area f_C (nm²)	57.9	56.9	4.8
Volume V (nm³)	480	518	4.85
Surface S (nm²)	368	109	5.4
Translational diffusion coefficient $D_{20,W}$ ($\mathrm{cm^2 s^{-1}}$)	$(3.77 \pm 0.08) \times 10^{-7}$		
Stoke's radius[a] R_S (nm)			5.65 ± 0.12
Molecular weight[b] M (g/mol)	$(3 \pm 0.1) \times 10^5$		
Ratio of frictional coefficients f/f_0	1.28 ± 0.05	1.02	

a $R_S = \dfrac{kT}{6\pi\eta_0 D_T}$; $\eta_0 = 1.026\ cP$, D_{20}, buffer $= 3.7 \cdot 10^{-7}\mathrm{cm^2 s^{-1}}$

b $M = \dfrac{RTs}{D(1-\bar{\nu}\rho_0)}$, s_{20}, buffer $= 11.8$ S, $\rho_0 = 1.019$ g/ml, $\bar{\nu} = 0.73$ ml/g [1]

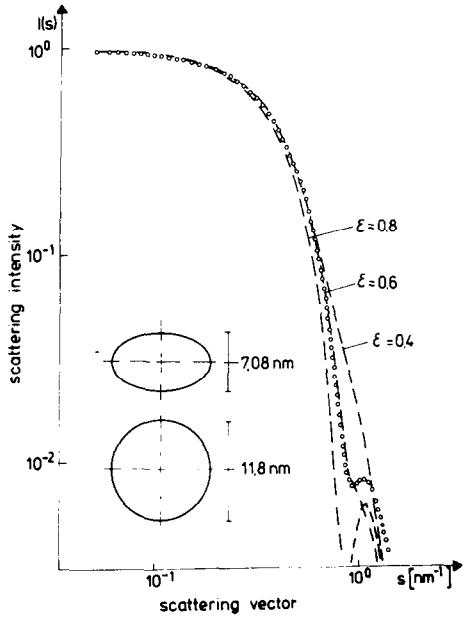

Fig.2. Desmeared sunflower globulin scattering curve $I(s)$ (prep. C1) and comparison with the scattering curves of ellipsoids of revolution with the axial ratio ϵ.

with the point group symmetry 32 leads to a further improvement of the approximation to the experimental data. The decrease of scattered intensity of prep. C1 and prep. C2 up to $s = 0.9$ nm^{-1} is approximately identical but the position of the first submaximum of prep. C1 at $s = 1.06$ nm^{-1} shifts to $s = 1.18$ nm^{-1} for prep. C2.

The comparison of the scattering curves of edestin $R_G = 4.96$ nm (data from [10]) and sunflower globulin $R_G = 4.05$ nm in the interval $s < 0.9$ nm^{-1} shows that the shapes of both molecules are similar.

The results of the quasi-elastic light scattering measurements give a linear dependence of the half-width of the photomultiplier current power spectrum on $\sin^2\Theta/2$ up to $\Theta = 120°$. One gets a straight line through zero. The spectrum is accurately Lorentzian. This shows that Γ depends only on the translational diffusion of the protein molecules, and that rotational diffusion or other phenomena have no measurable effect upon Γ [9]. The results of the quasi-elastic light scattering measurements are summarized in table 1.

4. Conclusions

The oblate ellipsoid of revolution with $a/b = 0.6$ is a shape model of the sunflower globulin. There exists a shape equivalence between edestin and sunflower globulin and small differences in the molecule dimensions. The largest diameters of sunflower globulin and of edestin are $L = 11.8$ nm and $L = 16.5$ nm [11], respectively. The deviation between the scattering curves of prep. C1, prep. C2 and edestin may be explained by small alterations in the subunit arrangement.

The large difference between f/f_0 calculated from the diffusion coefficient and from the shape model may be caused by solvation effects. If one assumes a solvation of the sunflower globulin of $m = 0.22$ g solvent/g protein and solvation shell of a thickness of 0.5 nm the ratio of frictional coefficients derived from D_T is $f/f_0 = 1.06$ which is in much better correspondence to the shape model.

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