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Models for the multisubunit conformation of oil-seed globulins

B. Carrasco*, J. García de la Torre*[1] and S. E. Harding†[1]

*Departamento de Química Física, Universidad de Murcia, 30071 Murcia, Spain, and †Department of Applied Biochemistry and Food Science, University of Nottingham, Sutton Bonington LE12 5RD, U.K.

Introduction

Seed globulins provide a nitrogen reserve for the plant [1,2], and have a considerable commercial interest for food use. From a structural point of view, these globulins are an interesting example of multi-subunit proteins for which the determination of the spatial arrangement of the subunits can be attempted from measurements and modelling of solution properties.

The native structure of sunflower and rapeseed globulin is the so-called 11 S form, despite the fact that the precise value of their sedimentation coefficients is approx. 12 S at 20.0 °C. The 11 S globulins dissociate into subunits according to the following scheme [3–5]:

$$11\,S \rightarrow 2 \times 7\,S \rightarrow 6 \times 3\,S\,(\rightarrow 12 \times 2\,S) \qquad (1)$$

where the last step requires much stronger dissociating agents (6 M guanidinium chloride or 8 M urea) and so to a reasonable extent the 11 S sunflower and rapeseed globulins can be regarded as being made up of six subunits [5].

A detailed study of the conformation of these proteins has been undertaken by Plietz [5], who used small-angle X-ray scattering to show the most likely arrangement of the subunits as a trigonal biprism. It would be most interesting to carry out the structural determination from other solution properties, which have been studied in detail by K. D. Schwenke and co-workers [4,6] and V. Prakash and co-workers [1,2]. A summary of published values is presented in Table 1.

Oligomeric proteins, composed of moderate number of subunits arranged in a rather specific manner, with a polygonal or polyhedral geometry, are good examples of biological macromolecules whose solution properties cannot be described by simple ellipsoidal models. Instead, this important class of structures was among those that motivated the development of bead models for solution properties [7,8], for which the existing theory [9,10] has been implemented in the HYDRO computer program [11]. Indeed, oligomeric structures have, over the years, been the subject of bead modelling work [9,10,12–14].

From the early studies based on ellipsoidal shapes, it is known that it is possible to model the shape of the particle directly without ambiguities induced by having to include particle size as well [15]. This is done by combining two or

[1]To whom correspondence should be addressed.

Table 1

Values for the different properties obtained from the literature

When pertinent, the values are referred to water at 20 °C.

Protein	Property	Value	References
Sunflower seed	$\bar{v}$	0.73 ml/g	[4]
	M	305 kDa	[4]
	$10^7 D$	3.76 cm^2/s	[4]
	s	12.8 S	[4]
	$[\eta]$	4.2 ml/g	[4]
	$[\eta]$	3.6 ml/g	[2]
	R_g	3.96 nm	[5]
Rapeseed	$\bar{v}$	0.73 ml/g	[6]
	M	300 kDa	[6,26]
	$10^7 D$	3.8 cm^2/s	[6]
	s	12.7 S	[6]
	$[\eta]$	3.8 ml/g	[2]
	R_g	4.08 nm	[5]

more solution properties in a single quantity that is universal, i.e. size-independent, and is a function of shape only. The theory for hydrodynamic properties of ellipsoids has been employed to formulate such shape functions [16], and they have been implemented in the ELLIPS suite of algorithms [17]. Recently we [18] have extended the use of size-independent analysis in bead modelling by interfacing HYDRO with a new computer program, SOLPRO, which calculates a variety of complex (derived) SOLution PROperties, including the shape functions. Here we illustrate the usefulness of bead models, treated by HYDRO and SOLPRO, to model the conformation of seed globulins. From well-characterized values of hydrodynamic and scattering properties, we are able to elucidate the subunit arrangement in these proteins.

Theory and models

The solution properties of macromolecules depend essentially on two aspects of the macromolecular solute: its size and its shape; the size, in turn, includes the volume occupied by the macromolecule itself and the hydration water. The dependence of properties on hydrated volume can be eliminated by formulating combined quantities that will be size-independent. (For a summary of such size-independent shape functions see [16] and [18].) One such function is the 'Perrin frictional' P function, which combines the translational friction coefficient, f, and the particle's hydrated volume, V_h. The latter can be written in terms of the anhydrous volume, $V_{anh} = \bar{v}M/N_A$ in the form $V_h = h^3(\bar{v}M/N_A)$, where $h^3 = 1 + \{\delta/(\bar{v}\rho)\}$, M is the molecular mass, N_A is the Avogadro number, $\bar{v}$ is the partial specific volume of the macromolecule, η_0 and ρ are respectively the viscosity and the density of the solvent, and δ is the degree of hydration, expressed as grams of water associated with the macromolecule per gram of macromolecule [15]. Thus the Perrin function reads:

$$P = \frac{f}{6\pi\eta_0(3\bar{v}M/(4\pi N_A))^{1/3}h} \qquad (2)$$

The friction coefficient f is derived from the measured sedimentation coefficient, s, as $f = M(1-\bar{v}\rho)/(N_A s)$ or from the translational diffusion coefficient, $f = kT/D$, where k is the Boltzman constant and T the absolute temperature.

Similarly we formulate a combination, ν, of viscosity and hydrated volume:

$$\nu = \frac{[\eta]M}{N_A V_h} = \frac{[\eta]}{\bar{v}h^3}. \qquad (3)$$

This is usually named the Einstein coefficient or the Simha function.

If it can be reasonably assumed that the radiation scattering techniques 'see' only the macromolecular material (i.e. hydration water is transparent in this regard), then the radius of gyration, R_g, measured in these techniques can be combined with the anhydrous volume. An adequate combination is the Mittlebach function:

$$G = \frac{R_g^2}{(3V_{anh}/(4\pi))^{2/3}} = \frac{R_g^2}{(3\bar{v}M/(4\pi N_A))^{2/3}} \qquad (4)$$

Another possibility consists of combining $[\eta]$ and R_g in the Flory Φ_0 value [19]. However, hydration must be considered here, since $[\eta]$ corresponds to the hydrated particle. The proper definition is

$$\Phi_0 = \frac{[\eta]M}{6^{3/2}R_g^3 h^3} \qquad (5)$$

For completeness, we mention another Flory function, P_0, relating R_g and f:

$$P_0 = \frac{f}{6^{1/2}R_g\eta_0 h} \qquad (6)$$

From the experimental data in Table 1 we can calculate these size-independent universal functions. The degree of hydration is unknown, and therefore we analyse the results for three 'typical' values of $\delta = 0.20$ g/g (low), 0.35 g/g (medium) and 0.50 g/g (high) [15,20]. Using these alternatives for δ, we obtain the experimental values of the shape functions P, v, G, P_0 and Φ_0 listed in Table 2.

Multisubunit proteins usually exhibit rather regular, symmetrical structures. If the individual subunits are compact and roughly globular, the oligomer can be modelled as a regular, polygonal or polyhedral array of spheres, for which the hydrodynamic properties can be readily obtained by bead modelling. Indeed, several compilations of hydrodynamic properties of rigid, regular arrays of spheres have been published [10,12–14]. A theoretical aspect that remains unclear refers to the so-called volume correction, which in principle should be applied to rotational properties or the intrinsic viscosity [10,21,22]. This correction works well in most cases but, for very compact arrays [22], such as the regular structures appropriate to the seed globulins, it has been recently found that it can give abnormally high results for intrinsic viscosity [22]. The problems that underpin the volume

Table 2

Experimental values for the universal shape functions (accurate to $\sim \pm 5\%$ as a function of hydration, δ

The letters in parentheses indicate that the value is best fitted by an octahedron (o) or a trigonal prism (p).

		Hydration (δ) (g/g)		
Protein source	Function	Low (0.20)	Medium (0.35)	High (0.50)
Sunflower seed	P	1.18(p)	1.12(o)	1.07(o)
	G	0.79(o)	0.79(o)	0.79(o)
	$[\eta]^{(a)}$	4.51(p)	3.89(o)	3.41(o)
	$[\eta]^{(b)}$	3.87(p)	3.33(o)	2.92(o)
	P_0	10.2(o)	9.7(o)	9.3(o)
	$10^{-23}\ \Phi_0{}^{(a)}$	11.0(o)	9.5(o)	8.3(o)
	$10^{-23}\ \Phi_0{}^{(b)}$	9.4(o)	8.1(o)	7.1(p)
Rapeseed	P	1.18(p)	1.12(o)	1.07(o)
	G	0.84(p)	0.84(p)	0.84(p)
	$[\eta]$	4.08(p)	3.52(o)	3.09(o)
	P_0	9.9(o)	9.4(p)	9.0(p)
	$10^{-23}\ \Phi_0$	9.1(o)	7.8(p)	6.9(p)

[a] $[\eta] = 4.2$ ml/g.
[b] $[\eta] = 3.6$ ml/g.

Table 3

Predicted values for the different size-independent functions obtained from HYDRO and SOLPRO by using cubic substitution

Structure	P	G	v	P_0	$10^{-23}\ \Phi_0$
Sphere	1.010	0.600	2.405	10.03	8.882
Sixfold planar ring	1.248	1.393	4.859	8.137	5.073
Trigonal prism	1.153	0.888	3.688	9.418	7.562
Octahedron	1.126	0.787	3.470	9.761	8.525
Linear	1.418	3.715	9.209	5.660	2.208

correction have been tackled at the modelling level (rather than by modifications in theory) using the cubic substitution strategy [23], and this procedure has been applied to oligomeric structures by García Bernal and García de la Torre [14]. At the present time, we consider that the results in [14] can be safely applied to the solution properties of oligomeric structures, and we employ them in this paper for the calculations of hydrodynamic properties. Other properties such as V and R_g are calculated from the primary bead models, i.e. without cubic substitution. Finally, with the full set of solution properties, the 'universal', size-dependent functions are finally evaluated from eqns. (2–6).

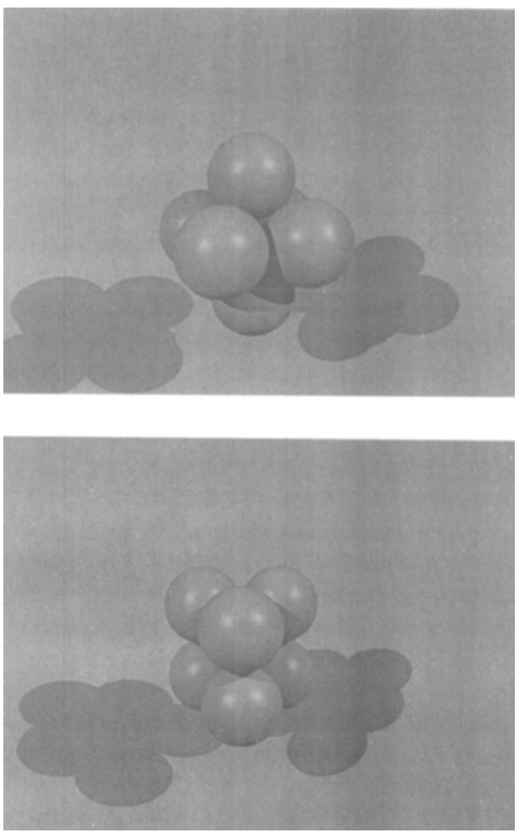
Results and discussion

As described in the Introduction section, seed globulins are regarded as hexameric structures. Then, using the procedures described in the previous section, we calculate the various shape functions for several polygonal or polyhedral structures: a linear string of six beads, a sixfold planar ring, a trigonal prism and an octahedron (the octahedron is sometimes called a trigonal antiprism, but we prefer the former name because it is actually a regular polyhedron). The results are listed in Table 3, where we have included the reference values for a spherical particle.

Figure 1

Bead models for the arrangement of subunits in the sunflower and rapeseed 11 S globulins

Upper panel, octahedron (or trigonal antiprism); lower panel, trigonal prism.



The experimental values for P, G, ν, P_0 and Φ_0 listed in Table 2 can be compared with the theoretical predictions for various geometries in Table 3. The comparison is complicated not only by the existence of various alternative models but also by the uncertain degree of hydration. Each of the values in Table 2 was compared with the results in Table 3 for the corresponding property, observing which of the hexameric structures produced the best fit. In this comparison we detect that, from the lowest to the highest degree of hydration, the best-fitting structure is never the linear one or the ring. It is absolutely clear that the present analysis discriminates against these two, less compact structures. All the values in Table 2 were fitted best either by the octahedron or by the trigonal prism [indicated by (o) or (p)] respectively, whose three-dimensional representations are shown in Figure 1.

The differentiation between these two, most compact models of octahedron and trigonal prism is, as expected, less clear-cut: this is hardly surprising because hydrodynamic parameters are usually dependent on the maximum asymmetry or aspect ratio of a macromolecule (rather than local detail of surface topology) and this seems to be similar for both models. For the sunflower seed 11 S globulin the octahedron model does seem, however, on the basis of the data of Table 2 to give the most satisfactory results, particularly for so-called 'moderate' hydration values [24]. With the rapeseed 11 S globulin it is impossible to distinguish between the two, although the data are at least consistent with the conclusions of Plietz et al. [5], who, on the basis of modelling of the subsidiary maxima in the small-angle X-ray scattering diagrams, found that the octahedron (trigonal antiprism) model is the most appropriate for both proteins.

As the final conclusion of our bead modelling analysis of hydrodynamic and other solution properties of 11 S globulins from rapeseed and sunflower, we can affirm that the arrangement of

subunits possesses a geometry best represented by a regular octahedron, which is the most compact shape for a hexameric structure. Our conclusion confirms the earlier results concerning the prismatic or, more probably, octahedral shape, as had been proposed by the other authors [5,25].

In our studies we have collected a number of experimental data of various properties from various sources, for two types of globulins, and confirm the octahedral arrangement of the six subunits in seed globulins.

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