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Bead modeling using HYDRO and SOLPRO of the conformation of multisubunit proteins: sunflower and rape-seed 11S globulins

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Abstract

Oil seed globulins from sunflower and rape seed are multi-subunit, oligomeric proteins whose native 11S form is a hexamer. In this work we try to determine the spatial structure in which the six subunits of 11S globulin are arranged. Experimental values of solution properties, including radius of gyration, sedimentation and diffusion coefficients and intrinsic viscosity, are compared with theoretical predictions for hexamers of various geometries. Bead model calculations of solution properties are carried out using the HYDRO and SOLPRO computer programs. A most compact shape, the regular octahedron, is the hexameric structure that fits best the experimental values.

1. Introduction

The seed globulins represent an interesting class of multi-subunit proteins: their main purpose in nature is as a nitrogen reserve for the plant [1,2]. Commercially they have been of considerable interest for food use, particularly the soy-bean globulins. Seed globulins from a variety of species appear to have a number of common properties, particularly concerning their structure: they each exhibit a multiplicity of quaternary structures as a result of polymorphism and the range of subunit compositions is reflected in a series of sedimentation coefficients.

The 11S sunflower and rapeseed globulins (known as Helianthin and Brassin, respectively) like other 11S seed globulins dissociate into subunits according to the following scheme: [3–5]

$$11S \rightarrow 2 \times 7S \rightarrow 6 \times 3S \xrightarrow{\text{6M GuHCl or 8M urea}} 12 \times 2S \quad (1)$$

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Changing the pH and ionic strength can be used to cause native intact 11S globulin to dissociate into the six 3S subunits [1]. To get this reduced further to the 2S subunits requires much stronger dissociating agents: the addition of 8 M urea or 6 M GuHCl, and so to a reasonable extent the 11S sunflower and rape seed globulins can be regarded as being made up of 6 subunits [5]. However, if the pH and ionic strength are not substantially altered, the native intact 11S form does not itself significantly dissociate, at least at concentrations above 0.3 mg/ml [1].

It should also be pointed out that despite the fact that some of the so-called 11S seed globulins have sedimentation coefficients $\sim$12S at 20.0°C, they are still referred to as 11S, and the sunflower and rapeseed globulins are no exception [1].

The solution properties of both proteins have been studied in detail by K.D. Schwenke and co-workers [4,6] and V. Prakash and co-workers [1,2] although the most detailed study of the conformation appears to have been by Plietz et al. [5] who used small angle X-ray scattering to show the most likely arrangement of the subunits as a trigonal biprism.

In this study we extend their work by incorporating sedimentation and viscosity data and taking advantage of the recently developed HYDRO and SOLPRO (SOLution PROperties) algorithms for solution conformation determination.

Oligomeric proteins, composed by a 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 were among those which 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 been over the years the subject of bead modeling work [9,10,12–14].

Solution properties of rigid macromolecules and macromolecular complexes depend on both the size and shape (including rugosity, as a local scale of the shape). The hydrodynamic properties will also depend on hydration. 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 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 modeling by interfacing HYDRO with a new computer program, SOLPRO, which calculates a variety of complex (derived) SOLution PROperties, including the shape functions. In the present work, 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.

2. Recall of experimental data

For the present work, we have used literature data for the solution properties of globulins. These data (Table 1) include the sedimentation coefficient, the molecular weight, the partial specific volume and the diffusion coefficient from two articles, one of them about globulin from sunflower seed and the another one about rape seed globulin, both from Schwenke et al. [4,6]. In their study of sunflower globulin, those authors reported a value of 4.2 ml/g for the intrinsic viscosity of this protein. On the other hand, Prakash [2] in an overview about a family of oilseed proteins, gives a range of 3.5 ± 0.7 ml/g for the intrinsic viscosity of these proteins. We can obtain the values for the sunflower and rape seed proteins from Fig. 3 of the Prakash article. For the sunflower globulin the intrinsic viscosity data is slightly different from the Schwenke et al. value (see Table 1), although it will be shown here that, for our purposes both data lead to the same conclusion.

Table 1
Values of the different properties obtained from the literature (when pertinent, the values are referred to water 20°C)

Protein	Property	Value	Reference number	Reference
Sunflower seed				
	$\bar{v}$	0.73 ml/g	[4]	Schwenke et al., 1979
	M	305 000 Da	[4]	Schwenke et al., 1979
	$D \times 10^7$	3.76 cm^2/s	[4]	Schwenke et al., 1979
	s	12.8 S	[4]	Schwenke et al., 1979
	$[\eta]$	4.2 ml/g	[4]	Schwenke et al., 1979
	$[\eta]$	3.6 ml/g	[2]	Prakash, 1994
	R_g	3.96 nm	[5]	Plietz et al., 1983
Rape seed				
	$\bar{v}$	0.73 ml/g	[6]	Schwenke et al., 1980
	M	300 000 Da	[6]	Schwenke et al., 1980
			[27]	Harding et al., 1987
	$D \times 10^7$	3.8 cm^2/s	[6]	Schwenke et al., 1980
	s	12.7 S	[6]	Schwenke et al., 1980
	$[\eta]$	3.8 ml/g	[2]	Prakash, 1994
	R_g	4.08 nm	[5]	Plietz et al., 1983

3. Size-independent (universal) shape functions

The solution properties of macromolecules reflect essentially two aspects of the macromolecular solute: its size and its shape. The size of the hydrodynamic particle, in turn, includes the volume occupied by the macromolecule itself and the volume and the distribution of the hydration water. The dependence of properties on hydrated volume can be eliminated by formulating combined quantities which 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 volume.

$$P = \frac{f}{6\pi\eta_0(3V_h/(4\pi))^{1/3}} \tag{2}$$

where V_h is the hydrodynamic volume, including hydration, which can be expressed in terms of the anhydrous volume, $V_{anh} = \bar{v}M/N_A$

$$V_h = h^3\left(\frac{\bar{v}M}{N_A}\right) \tag{3}$$

where $h^3 = 1 + \{\delta/(\bar{v}\rho)\}$, M is the molecular weight, 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]. In terms of the hydration expansion factor, h, we can write

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

The friction coefficient f is derived from the measured sedimentation coefficient, s

$$f = \frac{M(1-\bar{v}\rho)}{N_A s} \tag{5}$$

or from the translational diffusion coefficient,

$$f = \frac{kT}{D} \tag{6}$$

where k is the Boltzmann constant and T the absolute temperature.

Similarly we formulate a combination of viscosity and hydrated volume:

$$\nu = \frac{[\eta]M}{N_A V_h} = \frac{[\eta]}{\bar{v}h^3} \tag{7}$$

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 the regard), then, the radius of gyration measured in these techniques can be combined with the anhydrous volume. An adequate combination is the 'Mittelbach function'

$$G = \frac{R_g^2}{[3V_{anh}/(4\pi)]^{2/3}} = \frac{R_g^2}{[3\bar{v}M/(4\pi N_A)]^{2/3}} \quad \text{(Case A)} \tag{8}$$

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

$$\Phi_o = \frac{[\eta]M}{6^{3/2}R_g^3 h^3} \quad \text{(Case A)} \tag{9}$$

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

$$P_o = \frac{f}{6^{1/2}R_g\eta_0 h} \quad \text{(Case A)} \tag{10}$$

In the above analysis we have made the assumption that the R_g value reflects the anhydrous size of the particle. (This is hereafter referred to as the case A). However, it has been proposed by some authors [20] that, in the scattering of *X-rays* at least part of the hydration water contributing to hydrodynamic properties contributes similarly in scattering (case B) then the R_g would be expanded by a factor of h. This would affect the compound quantities involving R_g: the h or h^3 factor Eq. (9) and Eq. (10) should be removed, while the G function should be multiplied by a factor of h^2:

$$G = \frac{R_g^2}{[3\bar{v}M/(4\pi N_A)]^{2/3}h^2} \quad \text{(Case B)} \tag{11}$$

$$\Phi_o = \frac{[\eta]M}{6^{3/2}R_g^3} \quad \text{(Case B)} \tag{12}$$

$$P_o = \frac{f}{6^{1/2}R_g\eta_0} \quad \text{(Case B)} \tag{13}$$

From the experimental data in Table 1 we can calculate these size-independent, universal functions. The degree of hydration is unknown, and therefore we analyze the results for three 'typical' values of $\delta = 0.20$ g/g (low), 0.35 g/g (medium) and 0.50 g/g (high) [15,21]. Using these alternatives for δ, we obtain the experimental values of the shape functions P, ν, G, P_o and Φ_o listed in Tables 2 and 3. Table 2 corresponds to case A.

Table 2
Experimental values for the Universal Shape Functions (accurate to $\sim \pm 5\%$) as a function of hydration, δ (case A; see text)

Hydration, δ	Low (0.20 g/g)	Medium (0.35 g/g)	High (0.50 g/g)
Sunflower seed			
P	1.18(p)	1.12(o)	1.07(o)
G	0.79(o)	0.79(o)	0.79(o)
ν^a	4.51(p)	3.89(o)	3.41(o)
ν^b	3.87(p)	3.33(o)	2.92(o)
P_o	10.2(o)	9.7(o)	9.3(p)
$10^{-23} \times \Phi_o{}^a$	11.0(o)	9.5(o)	8.3(o)
$10^{-23} \times \Phi_o{}^b$	9.4(o)	8.1(o)	7.1(p)
Rape seed			
P	1.18(p)	1.12(o)	1.07(o)
G	0.84(p)	0.84(p)	0.84(p)
ν	4.08(p)	3.52(o)	3.09(o)
P_o	9.9(o)	9.4(p)	9.0(p)
$10^{-23} \times \Phi_o$	9.1(o)	7.8(p)	6.9(p)

Abbreviations. Values are best fitted by an octahedron (o) or a trigonal prism (p).
[a] $[\eta] = 4.2$ ml/g.
[b] $[\eta] = 3.6$ ml/g.

Table 3
Experimental values for the Universal Shape Functions (accurate to $\sim \pm 5\%$) as a function of hydration, δ (case B; see text)

Hydration, δ	Low (0.20 g/g)	Medium (0.35 g/g)	High (0.50 g/g)
Sunflower seed			
G	0.67(o)	0.61(o)	0.56(o)
P_o	11.0(o)	11.0(o)	11.0(o)
$10^{-23} \times \Phi_o$ [a]	14.0(o)	14.0(o)	14.0(o)
$10^{-23} \times \Phi_o$ [b]	12.0(o)	12.0(o)	12.0(o)
Rape seed			
G	0.72(o)	0.65(o)	0.60(o)
P_o	10.6(o)	10.6(o)	10.6(o)
$10^{-23} \times \Phi_o$	11.4(o)	11.4(o)	11.4(o)

Abbreviations. Values are best fitted by an octahedron (o) or a trigonal prism (p).
[a] $[\eta] = 4.2$ ml/g.
[b] $[\eta] = 3.6$ ml/g.

The functions that are changed by the assumption made in case B are listed separately in Table 3.

4. Theory and models

Oligomeric structures, and particularly multisubunit proteins are usually found exhibiting rather regular, symmetric structures. If the individual subunits are compact and roughly globular, the oligomer can be modeled as a regular, polygonal or polyhedral array of spheres, to which the bead modeling approach can be readily applied for the prediction of its hydrodynamic properties. From the earliest stages of bead-model theory, this rationale has been followed and various compilations of hydrodynamic properties of rigid, regular arrays of spheres have been published [10,12–14]. When rotational properties or the intrinsic viscosity is involved in the analysis, the hydrodynamic calculations must include the so-called volume correction [10,22,23]. While this correction works well in a majority of the cases, it has been recently found that it can give abnormally high results for the intrinsic viscosity of very compact arrays [23], such as the regular structures appropriate to the seed globulins. The problems that underpin the volume correction have been tackled at the modeling level (rather than by modifications in theory) using the cubic substitution strategy [24], and this procedure has been applied to oligomeric structures by García Bernal and García de la Torre (GB-GT) [14].

At the present time, we consider that the GB-GT results can be safely applied to the solution properties of oligomeric structures, and we employ them in this paper for the structural analysis of seed globulins.

In addition to the hydrodynamic properties of the bead models, other properties like V and R_g are calculated from the primary bead models, i.e. without cubic substitution.

Table 4
Predicted values for the different size-independent functions obtained from HYDRO and SOLPRO using cubic substitution

Structure	P	G	ν	P_o	$\Phi_o \times 10^{-23}$
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

5. Results and discussion

Using the procedures described in the previous section, we now calculate the various shape functions for several hexameric models: 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 since it is actually a regular polyhedron). The results are listed in Table 4, where we have included the reference values for a spherical particle.

The comparison of experimental values (Tables 2 and 3) and calculated results (Table 3) is complicated not only by the existence of various alternative models, but also by the uncertain degree of hydration. We proceeded as follows. Each of the values in Tables 2 and 3 was compared with the results in Table 4 for the corresponding property, observing which of the hexameric structures produced the best fit. In this comparison we detect

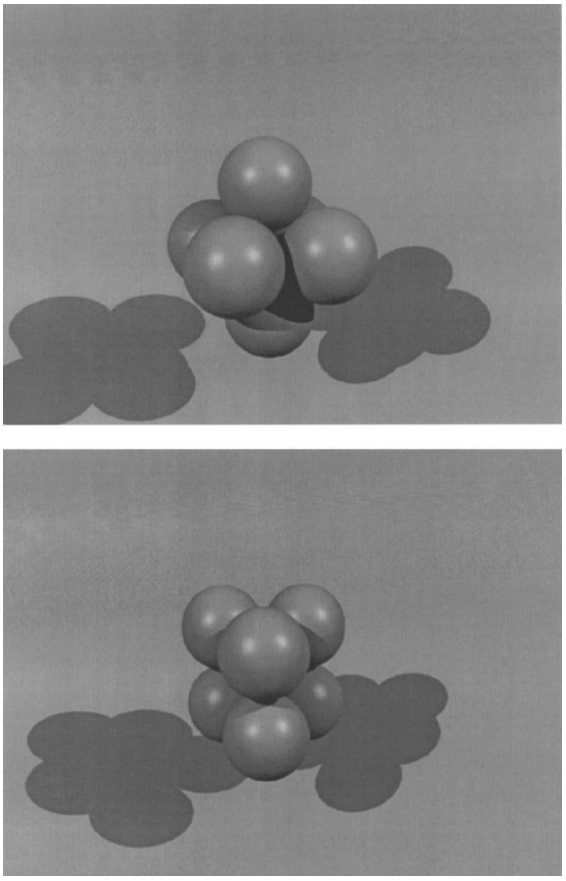

Fig. 1. Bead models for the arrangement of subunits in the sunflower and rape seed 11S globulins. Top panel: Octahedron (or trigonal antiprism); Bottom panel: Trigonal prism.

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 Tables 2 and 3 were best fitted either by the octahedron or by the trigonal prism [which is indicated by (o) or (p)], respectively, whose three-dimensional representations are displayed in Fig. 1.

Distinguishing between these two 'compact' models of octahedron and trigonal prism is as expected less clear-cut: this is hardly surprising since hydrodynamic parameters are usually dependent on the maximum asymmetry or aspect ratio of a macromolecule (rather than local detail of surface topology) and this appears to be similar for both models. For the sunflower seed 11S globulin the octahedron model does appear, however, on the basis of the data of Tables 2 and 3 to give the most satisfactory result, particularly for so-called 'moderate' hydration values [25]. With the rape-seed 11S 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 modeling 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.

From all the evidence manifested in our bead modeling analysis of hydrodynamic and other solution properties of 11S globulins from rape seed and sunflower, we can conclude that the arrangement of subunits possesses a geometry best represented by a regular octahedron, which is the most compact shape for an hexameric structure.

In our work we have collected a number of experimental data of various properties from various sources, for two types of globulins. Our conclusion confirms the earlier results concerning the prismatic or, more probably, octahedral shape of globulins, as it had been proposed by other authors [5,26]. Our study also serves as an example of bead-modeling methodology (including the hydration effects). This procedure can be indeed applied to rigid particles of arbitrary shape [9–11,18] and in the present paper we have shown its usefulness for compact oligomeric proteins.

6. Computer Programs

HYDRO and SOLPRO are public-domain programs. The latest versions of these programs, along with samples of input data and user information, can be downloaded from the Web site of the Group of Macromolecules, University of Murcia, http://leonardo.fcu.um.es/macromol

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