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Usefulness of the Bead Model Algorithm SOLPRO for Modeling the Conformation of Seed Globulins

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SOLPRO is a new program for the calculation of SOLution PROperties of rigid macromolecules and bioparticles (Garcia de la Torre *et al.*, 1997). These properties have traditionally been valuable sources of information on the size and shape of biological macromolecules, and their interest has been increasing over the years.

Recently, two sets of computer algorithms have been presented which permit direct modeling of the shape of macromolecules and macromolecular assemblies without ambiguities induced by the need to include particle size as well. These are the ELLIPS suite of algorithms (ELLIPS1-4) for the representation of shape in terms of either simple ellipsoids of revolution with 2 equal axes or general triaxial ellipsoids with three distinct unequal axes (Harding *et al.*, 1997). For the representation of macromolecules with complex shapes, and particularly for representing the conformation of proteins with several (~globular) subunits, hydrodynamic bead modeling is more appropriate with the algorithm SOLPRO. Both SOLPRO and ELLIPS1-4 are *completely compatible*, in that they use "universal shape functions" which are functions only of the shape of the bioparticle. {SOLPRO also has other useful features, including the prediction of rotational diffusional decay profiles from electric birefringence or fluorescence anisotropy depolarization and the form factor in radiation scattering, which gives the angular dependence of light or X-rays scattered by the macromolecule in solution.} These universal shape functions include the "Perrin frictional" P function (which can be calculated from the sedimentation coefficient together with molecular weight and an estimate for protein hydration) and the "Mittelbach" G function (from the radius of gyration). For example, for a sphere, P and G have the same values (1.0 and 0.6) independent of the size of the sphere.

For a user interested only in the simpler case of the shape of the macromolecule, the advantage of universal shape functions is that only an arbitrarily sized or scaled bead model with the desired shape is required; the use of absolute coordinates and radii is not necessary.

SOLPRO can improve our understanding of the subunit arrangement of seed globulins, a significant protein class. For this purpose, hydrodynamic data (sedimentation and diffusion coefficients, molecular weight and radius of gyration data) already published for the 11S sunflower seed and oilseed rape globulins were used to confirm the compact globular representation of the solution structure of both these molecules.

Application to 11S globulins from sunflower seed and rapeseed

Plietz *et al.* (1983) described the use of solution X-ray scattering on these proteins to compare the measured angular scattering intensity envelope with different six-bead models. They proposed a trigonal antiprism model with the dihedral point group symmetry 32 as the most plausible structure. Published hydrodynamic data for these proteins (Schwenke *et al.*, 1979; Plietz *et al.*, 1983) can now be used in support of the choice of this model: $s^{\circ}_{20,w}$ = 12.8 S, M = 305,000 Da, R_g = 3.96 nm, $\bar{v}$ = 0.73 ml/g for sunflower, and $s^{\circ}_{20,w}$ = 12.7 S, M = 300,000 Da, R_g = 4.06nm and the same $\bar{v}$ for rapeseed. Since the precise dimensions of the molecule are not clear, we decided to study the shape of the assembly using SOLPRO and hence avoid ambiguities concerning the size of the assembly. We considered 4 different models arraying six beads: lineal, sixfold ring, trigonal prism and trigonal antiprism, together with the case of a pure spherical model. SOLPRO provided the values of P and G for each model from the relative coordinates of the beads (Tab. 1).

Tab. 1. Predicted values for universal shape functions P and G.

	P	G
Sphere	1.000	0.600
Trigonal antiprism	1.019	0.787
Trigonal prism	1.059	0.888
Sixfold planar ring	1.172	1.393
Lineal (linear array)	1.387	3.715

For its measurement from experimental parameters, the P function requires an estimate for the hydration of the molecule, δ (g H_2O/g protein). If it is assumed that the density of bound water is not significantly different from that of free solvent, then G will be ~ independent of δ.

Table 2 considers the predicted values of P, G from the experimental data and for 3 "typical" values of δ (Tanford 1961; Squire and Himmel, 1979).

Tab. 2. Experimental values for the universal shape functions P and G (accurate to ~ ±5%) as a function of hydration, δ.

Hydration, δ	Low (0.2 g/g)	Medium (0.35 g/g)	High (0.5 g/g)
Sunflower			
P	1.18	1.12	1.07
G	0.79	0.79	0.79
Rapeseed			
P	1.18	1.12	1.07
G	0.84	0.84	0.84

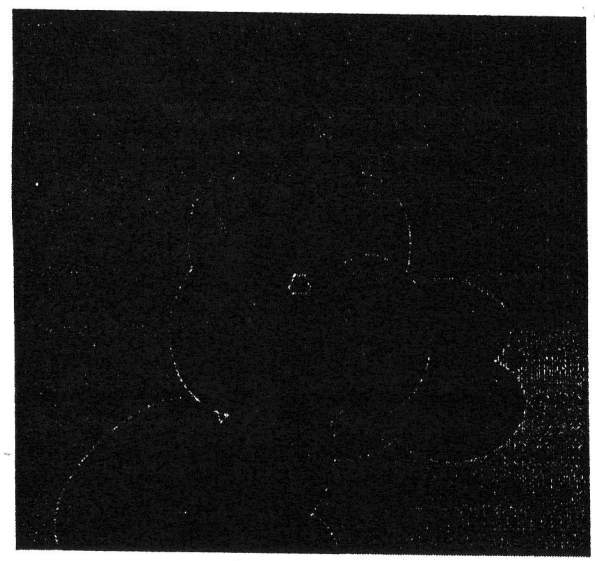

Fig. 1. Trigonal antiprism hydrodynamic bead model for the arrangement of subunits in sunflower and rapeseed 11S globulins.

The experimental data for both proteins rule out the two most asymmetric models of Table 1 (lineal and planar ring) and are at least consistent with the trigonal antiprism model (or "octahedron," Fig. 1) proposed earlier for both proteins based around the model fitting of subsidiary maxima in X-ray solution scattering envelopes (Plietz *et al.*, 1983). Although we cannot be more precise just on the basis of sedimentation (or diffusion) and the radius of gyration measurements alone, this approach does provide support for the solution angular X-ray scattering data. Of course, there are other universal shape functions based on other hydrodynamic measurements (notably intrinsic viscosity and rotational diffusion parameters) which can be used, a possibility which we are now actively exploring.

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