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Hydrodynamic Properties of Rigid Macromolecules Composed of Ellipsoidal and Cylindrical Subunits

Abstract: A procedure is devised for the calculation of hydrodynamic properties of rigid macromolecules composed subunits that are modeled as ellipsoids of revolution and cylinders. Owing to the axial symmetry of these shapes, smooth shell models can be constructed for the subunit structure. The bead shell model so constructed is employed for the calculation of the properties. A computer program, HYDROSUB, has been written implementing both the model building and the hydrodynamic calculation. A detailed example of the use of this methodology is presented for the case of the solution properties of the human antibody molecule immunoglobulin G3 (IgG3). Finally, hints are given on other uses and applications of the procedure. © 2002 John Wiley & Sons, Inc. *Biopolymers* 63: 163–167, 2002; DOI 10.1002/bip.10013

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INTRODUCTION

In their pioneering work, Bloomfield et al.^{1–3} adapted the Kirkwood–Riseman hydrodynamic theory,^{4,5} initially conceived for rodlike or chainlike molecules modeled as string of beads, to the calculation of hydrodynamic properties of macromolecules of arbitrary shape. In works of one of the present authors, with Bloomfield^{6–8} and other co-workers,^{9–12} the methodology was improved in regard to theoretical aspects, model building, and computational procedures. In the early studies, distinction was made between two different classes of hydrodynamic models. In bead models in strict sense, the particle is represented by an array composed of a moderate number of beads of arbitrary sizes.¹ Alternatively, in shell models the contour of

the particle is represented by a shell composed of many identical “minibeads.”^{2,3} Shell models are hydrodynamically more appropriate, and have the further advantage that fine details of the macromolecular structure can be adequately modeled by decreasing the size of the minibeads.

We recently reviewed the alternative strategies for hydrodynamic modeling,¹² and hinted at general procedures to build shell models. For bodies of arbitrary shape, rough shell models can be constructed from a superimposed lattice, but the resulting models have local imperfections arising from the lattice discontinuities. We also suggested¹² that, for any axially symmetric body, a smooth shell model could be constructed by stacking rings of beads, a procedure that had already been proposed for building cylindrical models.^{13–17}

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There are a number of biologically relevant situations in which the rigid macromolecule comprises some number of subunits or domains. Examples that are typically mentioned are myosin and immunoglobulins. In a structural study based on hydrodynamic and other solution properties, the basic question is how those subunits are assembled. The finest details of the structure of each domain is less relevant than their mutual position and orientation. Thus, one could simplify the structural determination, assuming simple shapes (spherical, ellipsoidal, rodlike, etc.) for the subunits, in order to concentrate in the overall structure of their spatial arrangement. This approach is even more useful when the subunits can be isolated and their solution properties determined in the laboratory. Using available procedures for ellipsoidal^{18–21} or cylindrical^{8,15–17} particles, one could determine separately the shape and dimensions of the subunits. In another possibility, the three-dimensional shape of the subunits may be available from x-ray crystallography or NMR, and it is then possible to fit an equivalent ellipsoid to the atomic structure.²²

In the present work we describe a methodology in which the macromolecule is regarded as an assembly of subunits or domains whose shapes are simply assumed to be ellipsoids of revolution or cylinders. A shell model for the macromolecule is built from shell models of the individual subunits, which are easy to construct owing to their axial symmetry, and the solution properties are computed using methods developed for shell-model hydrodynamics. Illustrations of this procedure and hints for further applications are finally given.

METHODS

Our hydrodynamic model consists of a rigid assembly (for application to flexible systems, *vide infra*) of fixed shape, composed by subunits that can be (a) ellipsoids of revolution with semiaxes a , a (duplicate), b (distinct), and axial ratio $p = b/a$, including prolate ($p > 1$), oblate ($p < 1$), and spherical ($p = 1$) shapes; and/or (b) cylinders with length L , diameter d , and length-to-diameter ratio $p = L/d$, including rodlike ($p > 1$) and disklike ($p < 1$) shapes. A complete specification of the structure of the whole assembly, with reference to a laboratory-fixed system of coordinates, simply requires, for each subunit (a) its dimensions, a and b or L and d ; (b) the three Cartesian coordinates of its center of mass; and (c) the two sphericopolar angles, θ (polar) and ϕ (longitudinal), that determine the orientation of the main symmetry axis.

With this information, the model is easily built. A shell model for an ellipsoid or cylinder, with minibeads of radius σ , is constructed by stacking in the z' direction (z' being

the symmetry axis) rings placed in (x', y') -planes with variable circumference that correspond to the intersection of the particle envelope with perpendicular planes.¹² The coordinates of the minibeads are recalculated after the subunit is translated and rotated to its actual position within the structure. Doing this for every subunit, a shell model of the whole particle is calculated. For this model, the hydrodynamic properties are obtained using a HYDRO calculation.^{11,12} This procedure is repeated for a series of decreasing values of the minibead radius σ , and the results are extrapolated to the shell model limit of $\sigma = 0$. All the model building and computational procedures have been implemented in a computer program, HYDROSUB.

The radius of gyration R_g is measured from the scattering of radiation from the whole macromolecule (not just the surface, as in the case of hydrodynamic properties). Thus, this property can not be calculated from shell models, but instead requires models that fill the whole volume of the particle.¹² Fortunately, for a multisubunit structure, composed of n subunits (or arbitrary shape), whose individual radii of gyration are $R_{g,i}$, with subunits of arbitrary shape) can be calculated^{23,24} as $R_g^2 = \sum f_i (R_{g,i}^2 + s_i^2)$, $i = 1, \dots, n$, where $f_i = V_i / \sum V_i$ is the volume fraction of the i th subunit. For cylinders, the radius of gyration and volume are $V_i = \pi L d^2 / 4$ and $R_{g,i}^2 = L^2 / 12 + d_i^2 / 8$, and for ellipsoids we have $V_i = 4 \pi a^2 b / 3$ and $R_{g,i}^2 = (2a^2 + b^2) / 5$. These expressions have been implemented in HYDROSUB for the calculation of V and R_g .

APPLICATIONS

The purpose of this section is to provide a detailed example of the modelization of multisubunit structures for the calculation of their solution properties from HYDROSUB. We just emphasize the modeling and computational procedure; the experimental and biophysical aspects of the systems mentioned are described elsewhere. We also give some hints on the possible utilization of this methodology in other problems.

Human Antibody Immunoglobulin G3 (IgG3)

Antibody molecules are paradigmatic examples of multisubunit structures in which the fairly globular subunits, two Fab's and Fc, could be represented by ellipsoidal shapes, but the overall structure require a bead or shell model approach. Experimental data for the sedimentation coefficients and other solution properties of human antibodies and the separate fragments are available.^{25–27} With the properties of the subunits, and with the help of the crystallographically determined structure of the proteins, the shape and dimensions of the equivalent ellipsoids of revolution

FIGURE 1 Shell model for the human antibody molecule IgG3, showing the Fc subunit (left), the hinge (center), and the two Fab subunits (right). The size of the minibeads was the smallest used in the shell-model extrapolations, 2.2 Å. For this size, the number of minibeads is 1955.

can be determined. For Fab, the experimental data are $M = 47,500$ g/mol, $s_{20,w}^0 = 3.92$ S, and $\bar{v} = 0.73$ cm³/g, as reported by Carrasco et al., who also carried out an optimization of the axial ratio, finding a prolate shape with $p = 1.95$.²⁶ From this data, and using the standard formulas for the translational friction of ellipsoids of revolution, the semiaxes are found to be

$b_{ab} = 38$ Å and $a_{ab} = 43.7$ Å. Similarly, for Fc the experimental data are $M = 51,000$ g/mol, $s_{20,w}^0 = 3.85$ S, and $\bar{v} = 0.73$ cm³/g, with an optimum shape that is oblate with $1/p = 1.83$, longest semiaxis $a_c = 45.0$ Å, and shortest semiaxis $b_c = 24.6$ Å.²⁷

Among the various subclasses of antibody molecules, for the purpose of the present example we have chosen IgG3, with experimental properties: $M = 158,000$ g/mol, $s_{20,w}^0 = 5.90$ S, and $\bar{v} = 0.73$ cm³/g.^{25,27} IgG is particularly interesting for the present purpose because, in addition to the three subfragments, it has also a very long hinge, which can be represented by a rod of about $L_h = 90$ – 120 Å and $d_h = 15$ Å.^{25,27} With this information for the subunit dimensions, and the biochemical knowledge about the overall conformation of the antibody molecule, hydrodynamic modeling has been employed to investigate the details of the subunit assembly. Details are given by Carrasco et al.²⁷ A plausible structure for IgG3 is that depicted in Figure 1. The whole set of data used for the HYDROSUB calculation for this structure is listed in Figure 2B, which includes the file that specifies the geometry of the assembly. This was constructed with reference to a Cartesian system of axis, so that the hinge and the long axis of Fc are along the negative part of axis y . The centers of Fc and the hinge are at $(-a_c - L_h, 0, 0)$ and $(-L_h/2, 0, 0)$. Note that the symmetry axis of the oblate Fc is in the z direction ($\theta = 0^\circ$, $\phi = 0^\circ$), while that of the hinge is along y ($\theta = 90^\circ$, $\phi = 90^\circ$). The origin of the system is at the end of the hinge where the two Fab

```

41                                !Set HYDROSUB mode
igg100                           !Name of molecule
igg100                           !Main results file (OUTPUT)
igg100.txt                       !structure file (INPUT)
6,                               !NSIG
2.2,                             !Minimum radius of beads in the shell (SIGMIN)
4.7,                             !Maximum radius of beads in the shell (SIGMAX)
293.,                           !T (temperature, K)
158000.,                        !RM (Molecular weight)
0.01,                           !ETA (Viscosity of the solvent in poises)
0.730,                          !Partial specific volume, cm3/g
1.0,                            !Solvent density, g/cm3
-1                               End of file

```

```

4      (number of subunits)
3      0.  -145.0  0.0    0.0    0.0    45.0  24.6
2      0.  -50.0  0.0    90.0  270.0  50.0   7.5
1      0.   19.0  40.4   30.0   90.0   38.0  19.5
1      0.   19.0 -40.4  150.0   90.0   38.0  19.5

```

```

X+++++-----+++++-----+++++-----+++++
      Xc      yc      zc      theta      phi      semi      semi
                                     Long      short

```

FIGURE 2 Listing of the input files for HYDROSUB in the calculation for the IgG3 model. (A) The main data file and (B) the structure file specifying the geometry of the multisubunit assembly.

FIGURE 3 Plots of the shell-model extrapolations in the HYDROSUB calculations with the model. (a) Sedimentation coefficient and (b) intrinsic viscosity.

subunits are jointed; more specifically, it is at the center of the base of the cylinder. The orientation of the Fab fragments, and the orientation, i.e., their pair of angles (θ, ϕ) , was varied in the study of Carrasco et al.²⁷ The position of the centers of the two Fab depends on the orientational angle, being given in general by $(b_{ab} \sin \theta \cos \phi, b_{ab} \sin \theta \sin \phi, b_{ab} \cos \theta \pm d_h/2)$, where $\pm d_h/2$ is an allowance such that the tips of the ellipsoids just touch the edge of the cylinder. For the disposition shown in Figure 1, the angles of the two Fab fragments were $\theta = 30^\circ$ and 150° , and $\phi = 90^\circ$ for both. All this data are collected in Figure 2B.

With this input, HYDROSUB repeats the model building and computes the properties for various values of the minibead radius σ and carries out the shell-model-limit extrapolation. Although this an internal procedure, HYDROSUB reports the results in an ancillary file, and it is interesting to look at the intermediate results. In Figure 3 we show the extrapolation for two properties. The final output from HYDROSUB—namely, the main results file containing the final (extrapolated) values for all the proper-

ties—is presented in Figure 4. The sedimentation coefficient obtained from the program, $s_{20,w}^0 = 5.82$ S, compares very well with the experimental value, $s_{20,w}^0 = 5.90 \pm 0.10$ S.²⁷

Guidelines for Further Applications

In this paper we intended mainly to demonstrate the ability of the modeling and computational procedures implemented in SOLPRO to calculate hydrodynamic properties of rigid structures that can be modeled as assemblies of subunits of simple shapes. We have presented a detailed, step-by-step example of the use of HYDROSUB in the case of IgG3. Finally, we would like to mention that this methodology can be applied to a variety of problems.

One potential field of application would be the determination of the quaternary structure of oligomeric structures composed of globular subunits. This has been a common application of bead modeling procedures: oligomer-to-monomer ratios of solution properties have calculated^{10,28,29} for arrays of spherical subunits. However, the subunits may compact but be appreciably nonspherical. The possibility of modeling the oligomer with ellipsoidal monomers was suggested some time ago,³⁰ but using a rather primitive methods. Another related possibility is to make the subunits not tangent but slightly overlapping, in order to account for local details related to docking or hydration.

Another field of application is in the study of semiflexible structures. Usually, multisubunit struc-

SUMMARY OF DATA AND RESULTS	
This file:	igg100.res
Case:	igg100
Mode:	41
Structural file:	igg100.txt
Molecular weight:	158000. Da
Temperature:	293.0 K
Solvent viscosity:	0.010 poise
Partial specific volume:	0.730 cm ³ /g
Solvent density:	1.000 g/cm ³
Bouyancy factor:	0.270
Radius of PBM elements:	0.0 Å
Translational diffusion coefficient:	3.326E-07 cm ² /s
Stokes (translational) radius:	6.452E-07 cm
Radius of gyration:	7.315E+03 cm
Volume (from filling model):	3.474E+05 cm ³
Rotational diffusion coefficient:	5.029E+05 s ⁻¹
Relaxation time (1):	6.048E-07 s
Relaxation time (2):	4.338E-07 s
Relaxation time (3):	4.188E-07 s
Relaxation time (4):	2.260E-07 s
Relaxation time (5):	2.260E-07 s
Harm. mean relax.(correlation) time:	3.290E-07 s
Intrinsic viscosity:	1.258E+01 cm ³ /g
Sedimentation coefficient:	5.825E+00 svedberg

FIGURE 4 Main results file produced by HYDROSUB in the calculation for the IgG3 model, containing the final value of the solution properties.

tures are (using the term coined by Stryer et al.³¹) segmentally flexible; the subunits are essentially rigid, but the joints may be partially flexible, thus allowing a variety of conformations. A simple approach would consist of a HYDROSUB calculation for the most extended and the most compact of the possible conformations; the values of the properties so obtained could be considered as the upper and lower bounds of the actual values. In a more elaborated procedure, one would generate a number of allowed conformations, running HYDROSUB for each of them (the execution of the program permits successive cases, and CPU times for structures with up to 2000 beads are less than one hour in a typical personal computer). Then, according to the so-called rigid-body approximation,³² the properties would be calculated for each of such conformations, as if they were instantaneously rigid, and the final value of the properties would be calculated by averaging over the individual results.

COMPUTER PROGRAM

HYDROSUB will be freely available, along with other related programs, from our web site: <http://leonardo.fcu.um.es/macromol>.

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