A GENERAL METHOD FOR MODELING MACROMOLECULAR SHAPE IN SOLUTION

A Graphical (II–G) Intersection Procedure for Triaxial Ellipsoids

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ABSTRACT A general method for modeling macromolecular shape in solution is described involving measurements of viscosity, radius of gyration, and the second thermodynamic virial coefficient. The method, which should be relatively straightforward to apply, does not suffer from uniqueness problems, involves shape functions that are independent of hydration, and models the gross conformation of the macromolecule in solution as a general triaxial ellipsoid. The method is illustrated by application to myosin, and the relevance and applicability of ellipsoid modeling to biological structures is discussed.

INTRODUCTION

Hydrodynamic measurements have for several decades provided a rapid and useful way of obtaining information concerning the gross conformation of biological macromolecules in solution by modeling them in terms of rather simple models: spheres, rods, and oblate and prolate ellipsoids of revolution (i.e., ellipsoids with two equal axes; see, for example, Nichol and Winzor, 1985). A relatively recent and significant development has been the ability to model macromolecules in terms of complicated structures by representing their structure as a series of spheres that interact in a way described by the Burgers-Oseen (or modifications thereof) tensor (see Garcia de la Torre and Bloomfield, 1978). The idea is to take a model and successively "iterate" it until the predicted hydrodynamic property (usually the sedimentation coefficient) agrees with the experimentally measured value. This type of modeling has found many applications ranging from virus assembly (Wilson and Bloomfield, 1979) to the structure of complement (Perkins, 1985). There are, however, considerable limitations to this type of modeling: (*a*) Uniqueness; depending on the complexity of the model there can be an enormous number of other models, equally as complex as the one chosen, which can give the same calculated value for the hydrodynamic coefficient. (*b*) The frictional ratio and, hence, the sedimentation coefficient is notoriously insensitive to shape. (*c*) Assumptions have to be made concerning macromolecular solvation (including physically trapped or entrained solvent). For complex modeling, there is a strong likelihood of significantly different hydrations for the chosen complex models (see Perkins, 1985). Hydration (which includes physically entrained as well as chemically bound solvent) is a notoriously difficult parameter to measure. As a result, complex modeling of this type can only be satisfactory when (*a*) a close starting estimate is available from, for example, electron microscopy (which itself may give a quite different representation from the true solution structure) or x-ray crystallography, and (*b*) the hydration is accurately known, and the relative hydration of the different models is also accurately known.

The most general shape now available, which can be applied unambiguously and using hydration-independent shape functions, is the general triaxial ellipsoid (i.e., an ellipsoid with three unequal axes). Earlier work (Harding and Rowe, 1982*a*, *b*, 1983; Harding, 1980, 1986) was concerned with developing much of the necessary methodology. A whole range of analytic expressions are now available enabling us to predict both hydration-dependent and hydration-independent hydrodynamic parameters for any given value of the two axial ratios a/b, b/c, which characterize a general triaxial ellipsoid of semi-axes $a \geq b \geq c$ (Harding, 1982). All these triaxial shape functions share the common property of having a line solution of possible values for the axial ratios (a/b, b/c) for any given value of the hydrodynamic function. A unique solution for the two axial ratios may be found from the intersection of two or more of these "line solutions." The problem centers around finding two suitable (preferably hydration-independent) parameters that (*a*) are experimentally determinable to a reasonable precision, (*b*) are sensitive to shape (and insensitive to experimental error), and (*c*) give a reasonable intersection (i.e., as orthogonal as possible). These criteria are quite restrictive and the methodology described earlier has involved the use of shape functions

(such as those from transient electric birefringence measurements) that are difficult to measure or involve functions (such as from fluorescence depolarization) that are affected by internal and segmental flexibility of the macromolecule.[1] The procedure described here, however, appears to satisfy these stringent criteria.

In this study, we examine the usefulness of the hydration-independent Π function (from intrinsic viscosity and excluded volume measurements), which is now available following the recent evaluation of the excluded volume for triaxial ellipsoids (Rallison and Harding, 1985). In particular, we examine the feasibility of combining this function with a shape function for triaxial ellipsoids, G, which can be obtained from the radius of gyration of a macromolecule (as measured by, for example, light scattering or low-angle x-ray scattering).

Triaxial Shape Functions

The hydration-independent shape function Π is a compound function involving both excluded volume and viscosity parameters and is defined by (Harding, 1981)

$$\Pi = \frac{u_{red}}{\nu} = \frac{U}{[\eta]M}, \tag{1}$$

where u_{red} is the "reduced" excluded volume (ml/g), ν is the viscosity increment, U is the molar covolume (ml/mol), M is the molecular weight (g/mol), and $[\eta]$ is the intrinsic viscosity (ml/g). Precise relationships relating both u_{red} and ν with the axial ratios $(a/b, b/c)$ for a general triaxial ellipsoid are now available;

$$u_{red} = 2 + \left(\frac{3}{2\pi abc}\right) R \cdot S,$$

where R and S are the double integrals

$$S = \frac{8}{3}\int_0^{\pi/2}\int_0^{\pi/2} \cos u \, du \, dv \left\{\left(\frac{bc}{a} + \frac{ca}{b} + \frac{ab}{c}\right)\Delta - \sin^2 v \cos^2 v \cos^2 u \, \Delta^3 c \left(\frac{b}{a} - \frac{a}{b}\right)\left(\frac{1}{a^2} - \frac{1}{b^2}\right) - \sin^2 u \cos^2 u \, \Delta^3 \left(\frac{\cos^2 v}{a^2} + \frac{\sin^2 v}{b^2} - \frac{1}{c^2}\right) \cdot \left[c\left(\frac{b\cos^2 v}{a} + \frac{a \sin^2 v}{b}\right) - \frac{ab}{c}\right]\right\} \tag{2}$$

and

$$R = \frac{2}{3\pi}\int_0^{\pi/2}\int_0^{\pi/2} \cos u \, du \, dv \left\{\left(\frac{a}{bc} + \frac{b}{ac} + \frac{c}{ab}\right)\Delta^2 - \sin^2 v \cos^2 v \cos^2 u \, \Delta^4 \left(\frac{1}{a^2} - \frac{1}{b^2}\right)\frac{1}{c}\left(\frac{b}{a} - \frac{a}{b}\right) - \sin^2 u \cos^2 u \, \Delta^4 \left(\frac{\cos^2 v}{a^2} + \frac{\sin^2 v}{b^2} - \frac{1}{c^2}\right) \cdot \left[\frac{1}{c}\left(\frac{b\cos^2 v}{a} + \frac{a \sin^2 v}{b}\right) + \frac{c}{ab} - \frac{b}{ac} - \frac{a}{bc}\right]\right\}, \tag{3}$$

where

$$\Delta^{-2} = \frac{\cos^2 u \cos^2 v}{a^2} + \frac{\cos^2 u \sin^2 v}{b^2} + \frac{\sin^2 u}{c^2},$$

u and v being the ellipsoid surface parameters (essentially dummy variables in Eqs. 2 and 3). The integrations may be readily solved without convergence problems by using standard numerical packages (Rallison and Harding, 1985).

A numerical method (involving matrix inversion procedures) for evaluating ν for triaxial ellipsoids was given by Rallison (1978). An analytic solution was given by Harding et al. (1979, 1981);

$$\nu = \frac{1}{abc}\left\{\frac{4(\alpha_0'' + \beta_0'' + \gamma_0'')}{15(\beta_0''\gamma_0'' + \gamma_0''\alpha_0'' + \alpha_0''\beta_0'')} + \frac{1}{5}\left[\frac{\beta_0 + \gamma_0}{\alpha_0'(b^2\beta_0 + c^2\gamma_0)} + \frac{\gamma_0 + \alpha_0}{\beta_0'(c^2\gamma_0 + a^2\alpha_0)} + \frac{\alpha_0 + \beta_0}{\gamma_0'(a^2\alpha_0 + b^2\beta_0)}\right]\right\} + \delta, \tag{4}$$

where the α_0, β_0, γ_0, etcetera are elliptic integrals and can be solved using standard numerical routines (Harding, 1982), again without convergence problems.

The term $\delta = 0$ for axisymmetric particles (viz., "ellipsoids of revolution"), is negligible for general ellipsoids of $(a/b, b/c) \lesssim (2.0, 2.0)$ and $<1\%$ for higher axial ratios, and represents the small deviation of asymmetric particles from rotating with the same angular velocity as the local unperturbed solvent. Its value was worked out by J. M. Rallison (personal communication) and Haber and Brenner (1984);

$$\delta = -\frac{1}{5abc}\left[\frac{\left(\frac{a^2 - b^2}{a^2\alpha_0 + b^2\beta_0} + \frac{b^2 - c^2}{b^2\beta_0 + c^2\gamma_0} + \frac{c^2 - a^2}{c^2\gamma_0 + a^2\alpha_0}\right)^2}{\left(\frac{a^2 + b^2}{a^2\alpha_0 + b^2\beta_0} + \frac{b^2 + c^2}{b^2\beta_0 + c^2\gamma_0} + \frac{c^2 + a^2}{c^2\gamma_0 + a^2\alpha_0}\right)}\right].$$

Table I gives values of Π as function of a:b and b:c and Fig. 1A gives the corresponding contour plot; both illustrate the line solution properties of Π as a function of a/b, b/c.

The molecular covolume is related to the second virial coefficient, B, from sedimentation equilibrium, osmotic pressure, or light scattering measurements by (Tanford

[1] Functions involving fluorescence depolarization have however been applied with some success to a study of neurophysin monomers and dimers (Harding and Rowe, 1982*b*) and also to a study of myosin light chains by Stafford and Szent-Gyorgi (1978), the latter using adapted Perrin rotational frictional relations (Perrin, 1934; Small and Isenberg, 1977) and a procedure for coping with the further difficulty of resolving decay data of more than one exponential term.

TABLE I
VALUES OF Π AS A FUNCTION OF ($a/b/c$) FOR A GENERAL TRIAXIAL ELLIPSOID ($a \geq b \geq c$)

a/b	b/c									
	1‡	2	3	4	5	6	7	8	9	10
1*	3.200	3.180	3.179	3.192	3.208	3.225	3.241	3.255	3.268	3.280
2	3.122	3.043	3.029	3.035	3.046	3.058	3.070	3.080	3.090	3.098
3	2.960	2.814	2.776	2.768	2.770	2.775	2.781	2.786	2.792	2.797
4	2.778	2.584	2.530	2.513	2.509	2.510	2.512	2.515	2.518	2.521
5	2.601	2.378	2.315	2.293	2.285	2.283	2.283	2.284	2.286	2.288
6	2.438	2.199	2.131	2.106	2.096	2.092	2.091	2.091	2.092	2.093
7	2.291	2.043	1.973	1.947	1.936	1.931	1.929	1.928	1.929	1.929
8	2.159	1.908	1.838	1.811	1.799	1.794	1.791	1.790	1.790	1.791
9	2.041	1.791	1.721	1.694	1.682	1.676	1.673	1.672	1.672	1.672
10	1.935	1.688	1.619	1.592	1.580	1.574	1.571	1.570	1.569	1.569

*This row corresponds to an oblate ellipsoid.
‡This column corresponds to a prolate ellipsoid.

[1961] and Ogston and Winzor [1975]);

$$U = 2BM^2 - Z^2/2I, \tag{5}$$

where Z is the charge on the macromolecule and I is the ionic strength, viz., the concentration of uni-univalent supporting electrolyte. An improvement to the second term on the right side of Eq. 5 has recently been given by Wills et al. (1980). If charge effects are negligible (either by working, for example, at the isoelectric point or using solutions of high enough ionic strength), then Eq. 1 simplifies to $\Pi = 2BM/[\eta]$. If not, the full form of Eq. 5 must be used since the charge or "Donnan" term can be significantly larger than the excluded volume term. This point is discussed further below.

Since BM is normally more readily measurable from nonideality measurements (for a dilute monodisperse, noninteracting solution) as opposed to B itself, a precise knowledge of the molecular weight may, therefore, not be necessary; the product BM is normally directly obtainable from, for example, plots of apparent molecular weight versus concentration (sedimentation equilibrium) or "Zimm" plots from light scattering (see, Harding and Johnson, 1985).

A relationship between the root mean square radius about the center of Mass, R_g,[2] and the axial dimensions of a triaxial ellispoid has been derived by Mittelbach (1964);

$$R_g^2 = 1/5\,(a^2 + b^2 + c^2). \tag{6}$$

[2] R_g is almost ubiquitously referred to in the literature as an operational "radius of gyration," although this differs from its usage in a classical mechanical sense, where it is defined with respect to a fixed axis of rotation. However, providing its usage is consistent, no errors are introduced.

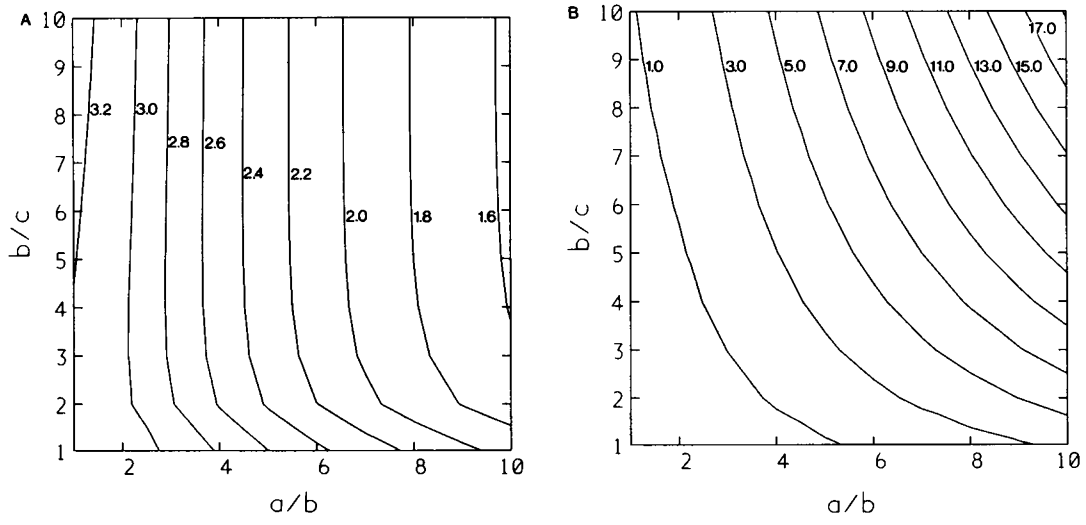

FIGURE 1 Plots of contours of constant value for the shape functions Π (A) and G (B) for a general triaxial ellipsoid of semi-axes $a \geq b \geq c$, in the (a/b, b/c) plane. A 10 × 10 matrix of grid values for the plot was used; intermediate values were calculated by interpolation using the Cambridge CAMPLOT routine GRCT2D.

TABLE II
VALUES OF G AS A FUNCTION OF $(a/b/c)$ FOR A GENERAL TRIAXIAL ELLIPSOID $(a \geq b \geq c)$

a/b	b/c									
	1	2	3	4	5	6	7	8	9	10
1	0.600	0.714	0.878	1.039	1.193	1.339	1.479	1.612	1.741	1.866
2	0.756	1.050	1.339	1.607	1.857	2.092	2.315	2.528	2.732	2.930
3	1.058	1.564	2.022	2.438	2.823	3.184	3.526	3.852	4.165	4.467
4	1.429	2.173	2.825	3.412	3.955	4.463	4.943	5.402	5.842	6.267
5	1.847	2.850	3.715	4.492	5.208	5.878	6.513	7.118	7.698	8.258
6	2.302	3.582	4.676	5.657	6.560	7.406	8.205	8.968	9.700	10.405
7	2.787	4.360	5.697	6.895	7.997	9.028	10.004	10.934	11.827	12.687
8	3.300	5.179	6.772	8.197	9.509	10.736	11.896	13.003	14.065	15.087
9	3.837	6.035	7.895	9.558	11.089	12.520	13.874	15.164	16.402	17.596
10	4.395	6.925	9.062	10.973	12.730	14.374	15.928	17.411	18.832	20.202

To use it as a triaxial ellipsoid shape parameter we reduce it to the dimensionless quantity "G";

$$G = \left(\frac{4\pi}{3V}\right)^{2/3} R_g^2 = \frac{1}{5}\left[\frac{a^2 + b^2 + c^2}{(abc)^{2/3}}\right], \quad (7)$$

where, for a sphere of uniform density, G is simply 0.6. Values of G for other various ellipsoidal axial ratios are given in Table II, with the corresponding contour plot as Fig 1 *B*. Assuming there are no internal cavities and that the bound solvent does not differ in density from free solvent, V can reasonably be taken as the dry volume of a macromolecule (see Jarcot, 1976; Martin, 1964), which is related to the partial volume $\bar{v}$ and the molecular weight by $V = \bar{v}M/N_A$ where N_A is Avogadro's number. $\bar{v}$ is readily available for many macromolecules; thus, measurement of G normally poses no real difficulties.

Graphical Combination of Line Solutions

We now examine the feasibility of employing the intersection method described above for obtaining the axial ratios of a macromolecule modeled by a triaxial ellipsoid. The criteria for judging the suitability are as before, viz., sensitivity to axial ratio, insensitivity to experimental error, hydration independence and experimental measurability. Fig. 2 illustrates the type of intersection for two macromolecules with typical axial ratios, allowing for expected experimental error in the functions; a globular macromolecule of true axial ratio (a/b, b/c) = (2.0, 2.0) and a more extended molecule of (a/b, b/c) = (5.0 , 5.0). Since a precise measurement of molecular weight may not be required as described above and intrinsic viscosity can be measured to a precision of 1% (see Harding and Rowe, 1983) we have allowed an error of ±3% for Π. This would

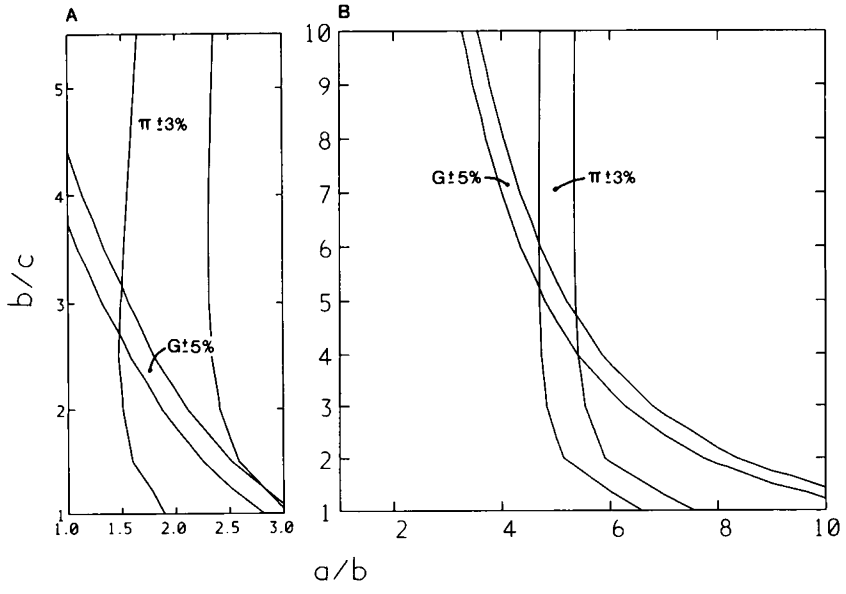

FIGURE 2 Details same as in Fig. 1, but contours for Π and G are shown in both plots: (*A*) True $(a/b, b/c)$ = (2.0, 2.0); (*B*) (a/b, b/c) = (5.0, 5.0). For a macromolecule with a given value for $(a/b, b/c)$ [(2.0, 2.0) and (5.0, 5.0) in this example], Π is calculated to be a certain number, n. The two lines on Fig. 1 give the a/b and b/c values that correspond with n + 3% and n − 3%, respectively. Similarly, for the G lines where the error is now ±5%.

not appear to be unreasonable when compared with some reported accuracies for B in the literature (Gellert and Englander, 1963; Tomimatsu, 1964; Emes and Rowe, 1978; Jeffrey et al., 1977); the effect of error in B is discussed in more detail below. For the G function, we have allowed an error of ±5%. In practice, the radius of gyration would provide the largest source of error to G, although this will depend on the size of the particle and the method employed, as discussed below.

It is apparent from Fig. 2 that our procedure is more sensitive for more asymmetric particles than for "globular," although it is still useful even for near-spherical particles. The method of measurement, however, is relatively rapid, does not suffer from the need to crystallize the sample or to work on "low" molecular weight macromolecules, and refers directly to the solution structure without the uncertainties of electron microscopy. An important consideration, on the other hand, when applying this procedure, is that the axial ratios (a/b, b/c) from the G function refer to the dry particle surface, whereas Π refers to the hydrated particle; the axial ratios of both these particles may not, therefore, be quite "identical," depending on the extent of the surface hydration layer relative to the gross particle dimensions.

For a protein with typical surface hydration levels, the difference is not likely to be significant compared with the errors in measurement.

Application to Myosin

The procedure can usefully be illustrated by applying it to data available in the literature for myosin (monomers). Myosin, as visualized by electron microscopy, is a fairly rigid rod-shaped molecule with a potential hinge point at the interface between the heavy meromyosin (HMM) and light meromyosin (LMM) polypeptide chains and with two head regions (S1 and S2) at one end (see, for example, Squire, 1981). Myosin has been well characterized in solution and has been the subject of an exhaustive hydrodynamic study involving Burgers-Oseen interaction theory (Garcia de la Torre and Bloomfield, 1980). We seek now to model the myosin molecule in terms of a general ellipsoid, without prior assumptions about molecular hydration or using prior information from electron microscopy. That is, given the wide range of shapes that a triaxial ellipsoid will allow (ranging from tapes, discs, rods, and intermediate shapes), what does the method predict without prior knowledge about what the shape may be? The results thus found will reveal how the method is prone to error through local variations in shape (in the case of myosin, primarily through the S1 heads) and rigidity (viz., effects of possible variable orientation of the S1 heads and flexibility of the HMM/LMM interface).

Values for the virial coefficient from a range of measurements are in very good agreement and have been reviewed by Emes and Rowe (1978). For this example, we take BM to be 52.6 ml/g (Emes and Rowe, 1978), $[\eta]$ = 217 ml/g, giving a value for Π of 0.47. We also take R_g as 468×10^{-8} cm (Holtzer and Lowey, 1959), and $\bar{\nu}$ = 0.728 ml/g and M = 474000 (Emes and Rowe, 1978), giving a value for G of 82.0. The corresponding line solutions for each of these functions, after allowance for the same experimental error as in Fig. 2, are shown in Fig 3. It is seen that, even allowing for the extra degree of freedom the general ellipsoid gives, the myosin molecule appears as a prolate ellipsoid of (a/b, b/c) ≈ (80, 1).

Of course we could have arrived at the same results from either Π or G independently by assuming a prolate ellipsoid in the first place; or we could have used the Scheraga-Mandelkern (1953) β function for prolate ellipsoids. The superiority of the present approach stems from the fact that (*a*) a prolate ellipsoid does not have to be assumed a priori, and (*b*) the shape functions are far more sensitive than β. The difficulties of applying the β function have been well described (see, Harding and Rowe, 1982*a*);

Because of the higher concentration of mass towards one end due to the presence of the heads, the axial ratio of 80 for the rod shape is likely to be an overestimate from the radius of gyration information but by no more than 15% (M. Dampier, personal communication). Nonetheless, nonwithstanding the difficulties of modeling an ellipsoid to a particle that has a "lop-sided end", this result is in good agreement with predicted results from electron microscopy, and would appear to suggest that the S1 heads are in a "closed" rather than an "open" form in solution in the native state, and that the majority of the myosin molecules in solution are linear and not significantly bent at the HMM/LMM interface. A much more thorough determination of the extent of flexibility in the myosin molecule has been given by Garcia de la Torre and Bloomfield (1980).

DISCUSSION

Effects of Experimental Error and some Potential Pitfalls

The most likely source of experimental error involved in this approach concerns the measurement of the molar covolume, U, via the second virial coefficient, B. Nichol, Winzor, and coworkers (Nichol and Winzor, 1985; Jeffrey et al., 1977; Nichol, 1981) have considered the experimental determination of B in some detail. Because of the greater problems of sample polydispersity, clarification, etc. association with light scattering, it is my opinion that low speed sedimentation equilibrium procedures, such as those described by Jeffrey et al. (1977), provide the more accurate method for obtaining B (or BM). Since light scattering may also have to be used as well (unless low angle x-ray scattering (LAXS) or neutron scattering is employed) to obtain R_g, the "Zimm" plot method would still be useful for providing a check on the value obtained for B. Whatever method employed, it is essential that the contribution of the Donnan term be taken into account,

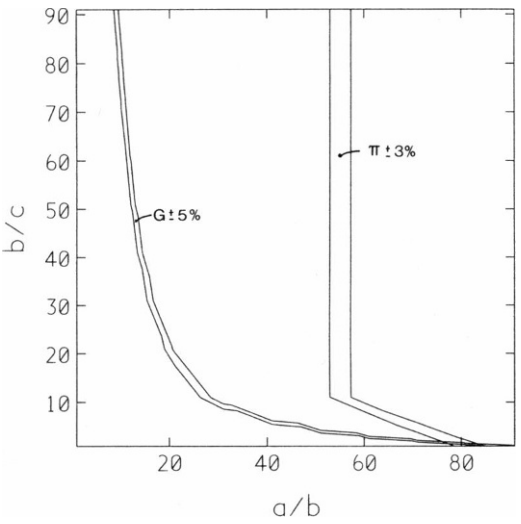

FIGURE 3 Plots of constant values for Π and G in the $(a/b, b/c)$ plane for myosin. Other details are the same as in Fig. 2.

along the lines described above and illustrated for ovalbumin by Jeffrey et al. (1977). These authors also describe how any appreciable flexibility in the macromolecule will also be a source of error (it will tend to lower the covolume); the treatment would, therefore, be unsuitable for randomly coiled polymers.

The major contribution to experimental error in the G function would appear to be from measurement of the radius of gyration, since the partial specific volume or molecular weight can normally be measured to a much higher precision, particularly if, for a protein, the amino acid composition or (better) the sequence is known. R_g is commonly obtained from the "Zimm plot" procedure (Zimm, 1948; Tanford, 1961). The accuracy to which R_g can be obtained depends largely on the wavelength of the radiation employed and the size of the particle. For example, using light scattering, for vaccinnia virus ($M = 2.7 \times 10^9$). Fiel et al. (1970) report an acurracy of ~1.4%, whereas for many proteins the accuracy can be considerably lower (Muller and Burchard, 1981; Holtzer and Lowey, 1958). However, for these macromolecules, LAXS (or neutron scattering) would be the method of choice (wavelength of the radiation employed $\lesssim$ particle diameter). For example, using LAXS, Witz et al. (1964) obtain a value for R_g of lactoglobulin of 3.44 ± .04 nm. It should be stressed, however, that because of the high sensitivity of G to change in axial ratio (Figs. 2–4), it is the accuracy in Π (or equivalently, B) that will be the principal limiting factor, particularly in the low axial ratio range (both a/b and b/c $\lesssim$ 3).

Another potential source of systematic error is in sample heterogeneity. It is essential that solvent conditions should be such that the sample is monodisperse and that self-association (or dissociation in the case of assemblies) phenomena are minimized. This requirement is, of course, common to other modeling procedures using scattering or hydrodynamic methods.

Ellipsoids of Revolution Versus Triaxial Ellipsoids

For many macromolecules, the "ellipsoid of revolution" model (i.e., an ellipsoid with two equal axes) can give a very reasonable representation of the gross conformation of macromolecules in solution; Squire and Himmel (1979) have shown that, for several globular proteins, the predicted dimensions from such modeling (albeit based on "assumed" hydration levels) agrees well with that from x-ray crystallography. However, for many macromolecular systems, the restriction to two equal axes is inadequate (Harding, 1980). In addition, either an "oblate" two equal major axes) or "prolate" (two equal minor axes) has to be assumed a priori, together with (more often than not) a "typical" hydration level: values of between 0.2–0.35 g water/g protein are commonly chosen (see, Tanford, 1961). The significance of the presently defined approach is that (a) neither ellipsoid has to be assumed a priori (as illustrated with the myosin example), and (b) a value for the hydration need not be assumed. Indeed, if the hydration could be measured independently, and accurately, we could have used hydration-dependent functions for our intersection procedure (including, for example, ν) without having to use the virial coefficient at all. Lack of adequate hydration information is, of course, one of the limitations of the Burgers-Oseen multiple sphere approach.

Some of the macromolecular systems that would appear to benefit from the general ellipsoid approach include C-protein (which is thought to be "asymmetric" from electron microscopy studies) from muscle filaments, α-actinin, another muscle protein whose function is closely related to its conformation in situ, and fibrinogen, for which there still appears considerable discrepancy in the recent literature as to its solution conformation.

The Relevance of Ellipsoid Modelling

There is no biological macromolecule whose surface is exactly described by a triaxial ellipsoid. How misleading are the dimensions for the gross conformation obtained by using a general triaxial ellipsoids as a hydrodynamic model? Indeed, for the somewhat related case of rotational diffusion, Wegener et al. (1979) have demonstrated that some values for the rotational diffusion coefficients do not correspond to any ellipsoid at all, for example, for the fork-shaped immunoglobulins. Although rotational diffusion parameters are not used here, it is possible that there exist similar particles that have π and G functions not corresponding to ellipsoids. It is unlikely, however, that merely surface roughness alone would invalidate the model, since calculations performed by Bloomfield et al. (1967) for the analagous case of frictional coefficients have

shown that holes and crevices have little effect; the same has also been demonstrated for the cases of intrinsic viscosity and rotational diffusion (see Wilson and Bloomfield, 1979). (This, of course, is the whole basis for the multiple sphere approximation of macromolecular structure using the Burgers-Oseen tensor as mentioned above). Broersma (1960) has confirmed this experimentally (see also Squire and Himmel, 1979, who find the ellipsoid of revolution approximation quite adequate). In any case, the effects of surface hydration would be to "smooth over" the surface topology, further strengthening the ellipsoid approximation. Although with the relative simplicity of the approach compared to x-ray crystallography, or Burgers-Oseen modeling, it is not possible to examine the richness in detail and the possible local flexibility of the molecular in question, we have demonstrated that, even for myosin, the overall gross conformation is still very adequately represented.

It is my view that the Burgers-Oseen multiple sphere approach of Bloomfield and coworkers and the ellipsoid approach described here should prove complementary; the first, when close starting estimates for the structure are available from other sources, and where the hydration is known accurately, thereby facilitating a complex model; the second, when no prior shape or hydration information is available, and only the gross solution dimensions of the macromolecule in solution are required.

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