Macromolecular Preprints (F.G.W. Butwell ed.)
Macro Group UK [Royal Society of Chemistry UK/
Society of Chemistry in Industry UK], 1986

GENERAL ELLIPSOID MODELLING OF MACROMOLECULAR SHAPE USING LIGHT SCATTERING & VISCOMETRY

Stephen E. Harding

Department of Applied Biochemistry & Food Science
University of Nottingham, NG7 2RD, U.K.

INTRODUCTION

For several decades now it has been possible to model the gross conformation of macromolecules in solution in terms of simple models, such as spheres, rods, or more commonly "ellipsoids of revolution" (viz. ellipsoids with two equal axes). Hydrodynamicists have for a long time recognised the advantages of being able to apply the general triaxial ellipsoid (three unequal axes) to uniquely describe the triaxial dimensions of a particle from solution measurements. Until recently either the necessary theoretical relationships or experimentation were not available to permit this.

The general ellipsoid (of semi-axes a>b>c) can now be applied unambiguously and using hydration - independent shape functions. A general method is described here which should be relatively straightforward to apply, and involves a function, Π (from intrinsic viscosity & excluded volume measurements) now available for triaxial ellipsoids and a new function G, from radius of gyration measurements.

THE SHAPE FUNCTIONS

The Π function is defined by (1)

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

where u_{red} is the 'reduced' excluded volume (ml/g) ν the viscosity increment, U the molar covolume (ml/mol), M the molecular weight (g/mol) and $[\eta]$ the intrinsic viscosity (ml/g). Precise relationships relating both u_{red} and ν with the axial ratios (a/b, b/c) of a general triaxial ellipsoid are now available (2), and hence Π can be given for triaxial ellipsoids. Like other triaxial ellipsoid shape functions, a given value of Π has a line solution of possible values of (a/b, b/c). The experimental measurement of Π involves the determination of $[\eta]$ and U: the latter can be determined, after allowance for charge effects, from the second virial coefficient, B from light scattering (from 'Zimm plots'), sedimentation equilibrium or osmotic pressure measurements (3,2).

To get a unique solution for (a/b, b/c), it is necessary to combine Π graphically with another triaxial ellipsoid shape function, whose line solution is as orthogonal as possible to that of Π. The other important criterion for the shape function, besides hydration independence is sensitivity to shape (and insensitivity to experimental error). One such function can be obtained from measurements of the radius of gyration of the macromolecule.

The relationship between the radius of gyration, R_G and the ellipsoid semi-axes is (4):

$$R_G^2 = \{a^2 + b^2 + c^2\} / 5$$

To use it as a shape parameter we reduce it to the new dimensionless quantity "G"

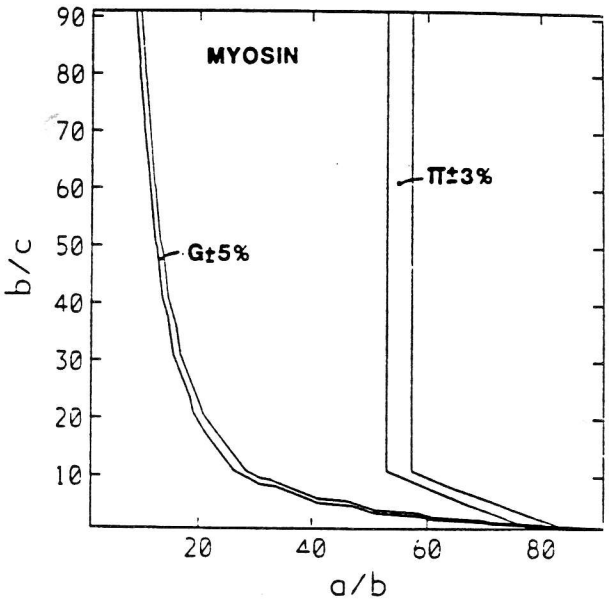
$$G = \left(\frac{4\pi}{3V}\right)^{2/3} R_G^2 = \frac{1}{5}\left[\frac{a^2 + b^2 + c^2}{(abc)^{2/3}}\right]$$

Assuming no internal cavities and that bound solvent does not differ in density from free solvent, V can be reasonably taken as the dry volume of a macromolecule, related to the partial specific volume, $\bar{v}$ and the molecular weight M by $V = \bar{v} M/N$, where N is Avogadro's number. G posseses the extremely useful property of being a highly sensitive function of axial ratio (a/b, b/c), for both globular and extended forms.

To obtain a unique solution for (a/b, b/c) we combine the line solutions for Π and G graphically. We have shown that the procedure is more sensitive for asymmetric particles than for 'globular' (both axial ratios <3.0). The limiting factor in its use for globular particles is the sensitivity of Π: accuracies in Π greater than 2% will be required. In addition the presence of surface hydration may alter the effective surface dimensions of the particle represented by Π compared with G: however, unless this is substantial (i.e. considerably more than a monolayer), the errors introduced are not likely to be significant when compared to experimental errors.

APPLICATION TO MYOSIN

The procedure can usefully be illustrated by application to data available for myosin. Taking BM to be 52.6 ml/g (5), $[\eta]$ = 217 ml/g (6), R_G = 468x10^{-8} cm (6), $\bar{v}$ = .728 ml/g and M = 474000 (5) we obtain a value of 0.47 for Π and 82.0 for G. The corresponding line solutions, allowing for experimental error are:



(This contour plot was produced on the Cambridge IBM 3081/B using a CAMPLOT routine with a 10x10 array of grid values, intermediate values being evaluated by interpolation).

Even allowing for the extra degree of freedom which the triaxial ellipsoid gives, the myosin molecule appears to behave as a prolate ellipsoid of axial ratio (a/b, b/c) ≈(80,1). Therefore, without any prior assumption about the shape (other than the wide range of shapes which a general ellipsoid allows) the result for the overall or gross conformation is consistent with data from electron microscopy: the 'fine' structural detail of the myosin molecule (protruding heads and potential flexibility at the HMM/LMM interface) does not therefore appear to affect the result for the overall conformation, providing encouragement for the application of our method to other macromolecular systems, whose surface features may not be as severe as myosin. Finally, it should be pointed out that BM, R_G and M can all be measured using light scattering 'Zimm plots' measurements (as well as by other methods). It would therefore appear possible to perform such modelling using light scattering alone (in conjunction with some simple viscometry measurements)

The support of the Nuffield Foundation is gratefully acknowledged

REFERENCES

1. Harding (1981) Int. J. Biol. Macromol. 3, 340
2. Rallison & Harding (1985) J. Coll. Int. Sci. 103, 284
3. Tanford (1961) 'Physical Chemistry of Macromolecules' Wiley, NY.
4. Mittelbach (1964) Acta Phys. Austriaca 19, 53
5. Emes & Rowe (1978) Biochim. Biophys. Acta. 537, 110
6. Holtzer & Lowey (1959) J. Am. Chem. Soc. 81, 1370