COMMUNICATIONS

A combined transient electric birefringence and excluded volume approach to macromolecular shape determination in solution

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The appropriateness of a general triaxial ellipsoid for modelling the gross conformation of biological macromolecules in solution has been described in detail (Harding & Rowe, 1983). To perform such modelling however, it is necessary to combine information from two or more techniques: one such technique is transient electric birefringence. The decay of birefringence of a monodisperse solution of asymmetric scatters after removal of the orienting electric field is characterized by two decay constants. Resolution of such decays of more than one exponential is notoriously difficult (Jost & O'Konski, 1978; O'Connor *et al.*, 1979). A further problem is that satisfactory allowance for macromolecular hydration has to be made; this has been overcome (Harding, 1983; Harding & Rowe, 1983) by combining reduced decay constants with intrinsic viscosity information to give hydration independent functions δ_+ and δ_-. These are each line-solution functions of the two axial ratios a/b, b/c which characterize a triaxial ellipsoid (semi-axes $a \geqslant b \geqslant c$).

The decay of birefringence Γ (as represented by the optical retardation) with time t is characterized (see, e.g., Harding & Rowe, 1983) by the following relation:

$$\Gamma = A_+ e^{6\theta_+ t} + A_- e^{6\theta_- t}$$

where the $A_\pm$ are 'pre-exponential' factors and $\theta_\pm$ are the decay constants. Once $\theta_\pm$ are known the shape functions $\delta_\pm$ can be found from:

$$\delta_\pm (a/b, b/c) = (6\eta_0[\eta] M_r / N_A kT)\theta_\pm$$

where η_0 is the solvent viscosity, $[\eta]$ the intrinsic viscosity and M_r the relative molecular mass. A given value of δ_+ or δ_- has a line solution of possible values for $(a/b, b/c)$; a unique solution can be found from their intersection (Fig. 1*a*). Unfortunately, the difficulty in resolving the decay constants requires use of another line-solution hydrodynamic function as a constraint in the fitting procedures.

In an earlier study the usefulness of the R-function line solution (which can be obtained, after certain assumptions, from sedimentation concentration dependence and intrinsic viscosity) as a constraint was examined. The rigorous hydration independent function Π is now available as an alternative (Rallison & Harding, 1985), which depends on the second thermodynamic virial coefficient B (excluded volume part) and intrinsic viscosity:

$$\Pi (a/b, b/c) = \frac{2BM}{[\eta]} - f(Z, I)$$

where $f(Z, I)$ is a charge correction term (Rallison & Harding, 1985). The Π line solution for a typical protein of $(a/b, b/c) = (3.0, 3.0)$ is also given in Fig. 1(*a*).

There are several curve-fitting procedures for multi-exponential decay analyses (see, e.g., Jost & O'Konski, 1978; O'Connor *et al.*, 1979; Isenberg & Small, 1982). We found a satisfactory procedure for resolving two (closely spaced) component decays with expected experimental noise was a constrained non-linear least squares procedure using

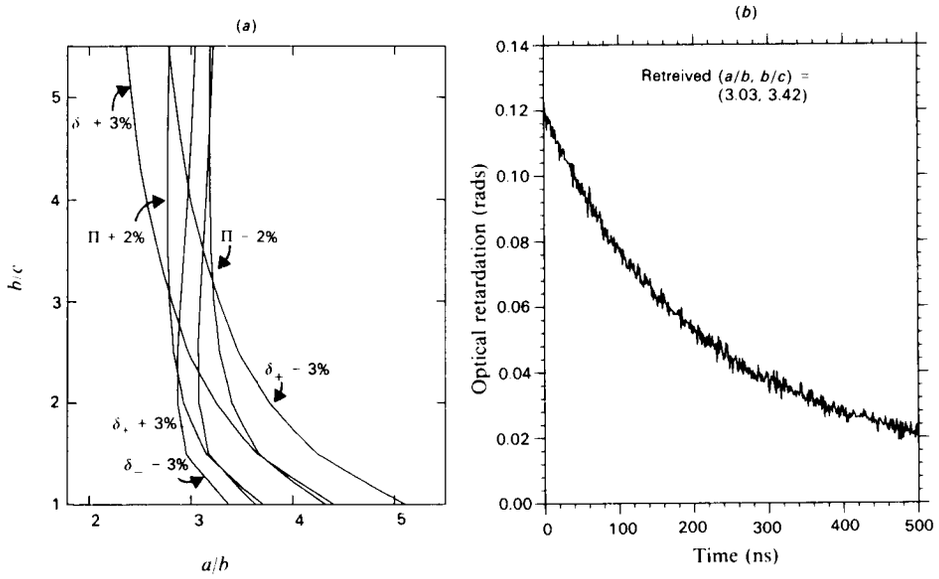

Fig. 1. *Contour plots and decay curve*

(*a*) Plots of contours of constant value of the shape functions $\delta_\pm$ and Π in the $(a/b, b/c)$ plane for a macromolecule with true $(a/b, b/c) = (3.0, 3.0)$, allowing for experimental error. (b) Simulated decay curve for a protein of true $(a/b, b/c) = (3.0, 3.0)$, M_r 240 000, partial specific volume 0.730 ml/g and (volume) swelling ratio 1.3. True $A_+ = 0.07$; $A_- = 0.05$. Cut off time, 500 ns; 500 data points with 0.1 deg random S.E. A similar decay curve (100 ns cut off, 100 data points) for a protein of M_r 170 000 and true $(a/b, b/c) = (2.0, 2.0)$ gave a retrieved $(a/b, b/c) = (2.02, 1.92)$.

NAG statistical packages (Harding & Rowe, 1983; Harding, 1983). We use now a modified analysis, the modification being the Π line solution as the constraint, namely the estimates for (a/b, b/c) from the $\delta_{\pm}$ line solutions must also lie on the Π line. The problem is reduced from one of four variables to three (b/c, A_+, A); since b/c contrains a particular value for a/b [because of the nature of the dependence of Π on (a/b, b/c) it is more convenient to use b/c as the independent variable as opposed to a/b].

For typical decay curves with expected noise (e.g. Fig. 1*b*), satisfactory recovery of the axial ratios are obtained for several streams of simulated data for two hypothetical proteins of (a/b, b/c) = (2.0, 2.0) and (3.0, 3.0). These fits were obtained assuming no error inΠ: the accuracy of recovery appears to be limited by how accurately Π can be obtained. Since Π is insensitive below (2.0, 2.0) and δ_- above (10.0, 10.0) the method also appears to be restricted to particles within this range of axial ratios. The other restrictions with regard to experimental applicability are as before (Harding & Rowe, 1983), namely the requirement for monodisperse solutions, the need to extrapolate 'apparent' b/c values to zero concentration, the need for rapid instrumental response times, choice of optimum cut-off time and ionic strength restrictions. Recent progress in the experimental methodology however has been considerable and has been described by, for example, Bernengo (1981).

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Localization of proteins in bovine central nervous system myelin with surface-specific and photoactivatable hydrophobic reagents

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In the central nervous system (CNS) of vertebrates myelin is produced as an extension of the plasma membrane of oligodendrocytes. The mature membrane forms a multilamellar sheath of insulation around nerve fibres which allows rapid conduction of nerve impulses by saltatory conduction. CNS myelin has a relatively simple protein composition: the proteolid protein, myelin basic protein and 2,3′-cyclic nucleotide-3-phosphohydrolases (2,3-CNPases) account for approximately 40, 30 and 4% by weight of total protein respectively.

The unique ability of myelin to form a highly compact multilayered structure must be determined by its distinctive composition and by the manner in which the proteins are organized in the bilayer. Here we report the use of chemical probes to assess the manner in which the major proteins of myelin interact with the myelin membrane.

Diazotized [^{125}I]iodosulphanilic acid was used as a surface-specific reagent to determine the degree to which the major proteins of myelin were exposed at the hydrophilic/hydrophobic bilayer interface. Incorporation of radioactivity into the proteolipid protein and myelin basic protein was measured after separation by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis in the system of Laemmli (1970). Protein bands were visualized by staining with Coomassie Blue, cut out and their radioactivity determined. The ratio of radioactive label in the proteolipid protein compared with the myelin basic protein was 2:1, which suggests that extensive portions of the proteolipid protein project out of the bilayer. Recent models of the organization of the proteolipid protein support this view (Laursen *et al.*, 1984; Stoffel *et al.*, 1984).

Hydrophobic photoactivatable reagents are excellent probes for the labelling of those segments of membrane proteins that penetrate the lipid bilayer. A chemically inert precursor such as an azide or diazirine is first introduced into the membrane where it is subsequently converted to a highly reactive species by u.v. radiation. Photoactivation of azides and diazirines yields highly reactive nitrenes and carbenes respectively. Therefore these are attractive reagents for the labelling of the rather unreactive side-chains of non-polar amino acids buried in the membrane. Carbenes are more reactive than nitrenes; however, both have been used to map the disposition of membrane proteins in lipid bilayers (Bayley & Knowles, 1978*a*,*b*; Wells & Findlay, 1979; Brunner & Semenza, 1981). The hydrophobic reagents that we used were 1-[^{3}H]spiro(adamantane-4,4′-diazirine) (Bayley & Knowles, 1978*b*), 1-[^{125}I]iodo-5-azidonaphthalene (Bercovici & Gitler, 1978) and 3-(trifluoromethyl-3-(*m*-[^{125}I]iodophenyl)diazirine (Brunner & Semenza, 1981).

Each of the hydrophobic reagents labelled the proteolipid protein much more heavily than myelin basic protein. Glutathione, which scavenges those free radicals that migrate to the bilayer/water interface, did not affect either the extent or the pattern of labelling. Both diazirines labelled the proteolipid protein to a similar extent and the ratio of proteolipid protein to myelin basic protein labelling was 8:1. Incorporation of label into the proteolipid protein was 17-fold greater than myelin basic protein with the azide reagent of Bercovici & Gitler (1978). The degree of penetration of myelin basic protein into the lipid bilayer is a matter of some debate (Boggs *et al.*, 1981). From the labelling results presented here it is clear that if the myelin basic protein does penetrate the bilayer, it does not do so to any great extent or depth. Like the myelin basic protein the 2,3-CNPases were found to be susceptible to labelling by the surface-specific reagent but did not label with the hydrophobic reagents. Therefore it appears that the 2,3-CNPases are peripheral-type proteins like the myelin basic protein.

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Abbreviations used: CNS, central nervous system; 2,3-CNPase, 2,3′-cyclic nucleotide-3-phosphohydrolase.

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