A preliminary investigation of the hydrodynamic properties of two novel monoclonal antibodies

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B72.3 is a murine monoclonal antibody which reacts with a range of ovarian, breast and colon carcinomas. Its immunoscintigraphic applications have been well reported in the literature and it has now been administered to over 300 patients [1]. A problem with mouse antibodies is the HAMA (human anti-mouse antibody) response which prevents further therapy. To negate the HAMA response, chimeric B72.3 (cB72.3) has been made and is in clinical trials [2]. The effectiveness of radioimmunoscintigraphy also depends on the tumour penetration properties of the administered antibody and the rate at which it is cleared from the body — factors favourably affected by the use of antibody fragments. The stability and tumour-binding efficiency of such fragments may be predictably linked to their solution conformations.

As a precursor to the investigation of novel antibody fragments, the solution conformations of B72.3 and cB72.3 are under investigation, with a view to developing a protocol for macromolecular modelling of this kind. X-ray crystallographic maps of appropriate hinge-deleted immunoglobulins provide a starting estimate for the overall dimensions of a similar antibody. To hydrodynamically model the protein, the TRV program developed by Garcia de la Torre and coworkers [3] is employed. In its most basic form the routine requires only the coordinates and radii of the bead assembly comprising the model. Among its output are parameters directly related to the diffusion and sedimentation coefficients, intrinsic viscosity and radius of gyration of the bead model. Consequently it is possible to iteratively refine the model to match the predicted values for these parameters with their experimentally determined counterparts.

Aside from the obvious problem of uniqueness of the model obtained (a problem which can be alleviated to some extent by increasing the number of experimentally determined parameters) there is the problem of hydration. Tanford [4] suggests an average protein hydration of 0.2 g water/g of protein. However, the work of Squire & Himmel [5] obliges one to consider higher values (of the order of 0.5 g of water/g of protein). Clearly solvation to this extent must play an important part in the hydrodynamic behaviour of protein macromolecules and in an attempt to estimate this effect for antibodies the Squire & Himmel [5] method has been employed by us.

The amino acid sequences of murine IgG1 and human IgG4 are very similar, even in the hinge region. Consequently, a conformational similarity between B72.3 (which is murine IgG1) and cB72.3 (which is human IgG4 with substituted B72.3 variable regions) would not be unexpected.

Sedimentation coefficients ($s^0_{20,w}$) for the two antibodies were determined via sedimentation velocity experiments performed on an MSE Centriscan analytical ultracentrifuge equipped with scanning absorption optics and a monochromator. Within the bounds of experimental error cB72.3 would appear to exhibit the same sedimentation behaviour as native human IgG4. B72.3 and cB72.3 have $s^0_{20,w}$ values of (6.51 ± 0.3) S and (6.89 ± 0.3) S, respectively, compared with a value of (6.73 ± 0.3) S determined for human IgG4 by K. G. Davis (personal communication). However, the positive slope of the $s_{20,w}$ versus concentration graph for B72.3 (see Fig. 1) suggests associative behaviour of the molecules in solution, a trend observed neither with cB72.3 nor with native human IgG4.

Preliminary intrinsic viscosity ($[\eta]$) determinations were performed using a 2.5 ml Ostwald capilliary microviscometer in conjunction with a Schott Gerate automated viscometry-measuring unit. Both proteins were studied in two buffers: I 0.1M, pH 6.9, potassium chloride with and without 3% (v/v) added glycerol. In non-glycerol buffer $[\eta]$ for B72.3 was (5.3 ± 0.6) ml/g and for cB72.3 was (4.4 ± 0.8) ml/g. In 3% (v/v) glycerol buffer $[\eta]$ for B72.3 was (5.5 ± 0.9) ml/g and for cB72.3 was (4.8 ± 0.6) ml/g. The relatively high errors associated with these values should hopefully be decreased as more data are acquired.

With further hydrodynamic analyses of both antibodies (and their fragments), the hydration of the proteins, their

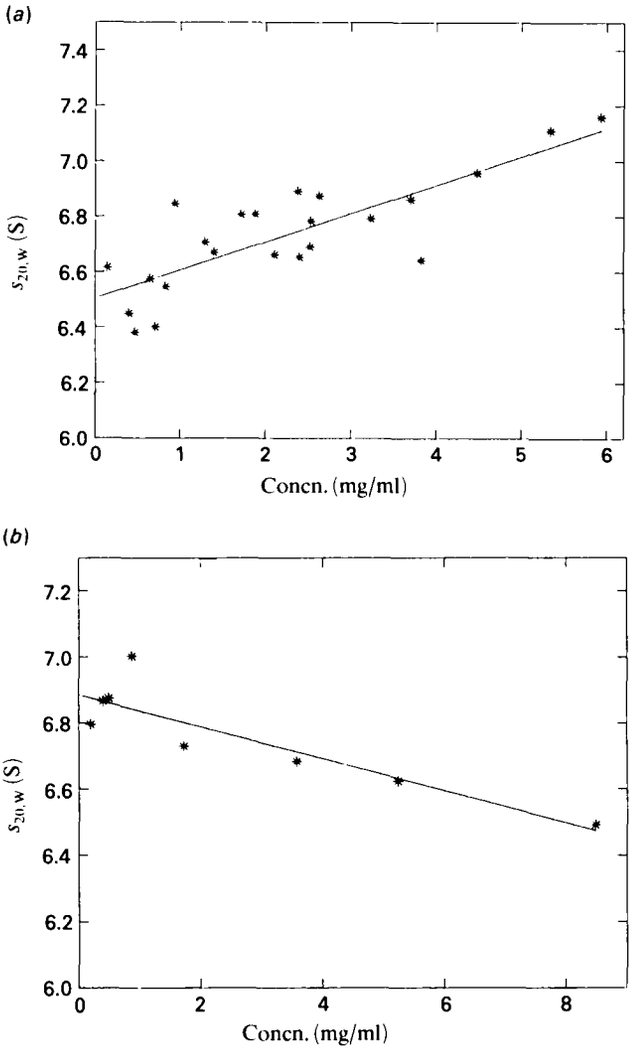

Fig. 1. *Plots of $s_{20,w}$ versus concentration for B72.3 and cB72.3*

Combined results from sedimentation velocity experiments for (*a*) B72.3: the positive gradient of the linear fit suggests associative behaviour of the molecules. (*b*) cB72.3: the negative gradient represents normal, non-associative behaviour. For both plots experiments were performed at approx. 40000 rev./min, 20°C.

Abbreviation used: HAMA, human anti-mouse antibody.

radii of gyration and their diffusion coefficients will be determined, allowing for the construction and subsequent refinement of hydrated bead models.

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Hydrodynamic characterization of *Chromobacter viscosum* lipase

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Lipases have growing commercial potential in the food and soap industries. For example, in detergent formulations it is considered that they may be effective, particularly at low washing temperatures, in removing fatty soil [1]. Microbial lipases exhibit molecular masses generally in the range 25000–50000 with some higher values reported [2, 3]. Very recently, the high resolution crystallographic X-ray structure of *Mucor miehei* phase (M_r ~ 29500) has appeared [4].

In this study, we consider the hydrodynamic properties of the lipase from *Chromobacter viscosum* in terms of (i) molecular mass in solution and (ii) sedimentation velocity behaviour. It has been the particular intention of this study to examine the possibility of self-association for *C. viscosum* lipase. Self-association has been suggested (possibly via a hydrophobic interaction) for *Aspergillus* sp. lipase [5] and, we believe, is also a possibility for *Geotrichum candidum* lipase [6].

Sedimentation velocity experiments were performed using an MSE Centriscan analytical ultracentrifuge equipped with scanning absorption and Schlieren optics, and the $s_{20,w}$ determined in the usual way [7]. Low-speed sedimentation equilibrium experiments were performed using a Beckmann Model E analytical ultracentrifuge equipped with a 5 mW He–Ne laser light source and Rayleigh interference optics. A low loading concentration of ~ 0.8 mg/ml was used throughout to minimize the possibility of thermodynamic non-ideality effects. 'Whole cell weight average' molecular masses, $M^0_{r,w}$, were determined according to the procedure of Creeth & Harding [8]. A value for $\bar{v}$ of 0.73 ml/g, calculated from the amino acid composition [9], was employed throughout.

The following values were obtained for $M^0_{r,w}$: 35000 ± 2000 in a phosphate chloride buffer, pH 6.8, I 0.1 M at 23.8°C; 35000 ± 2000 in a 40% (w/w) 1,4-dioxan/phosphate chloride buffer mixture, pH 6.8, I 0.1 M at 23.8°C and 40000 ± 2000 in phosphate chloride buffer at 10°C. These values are in agreement with molecular masses determined independently via SDS/PAGE (36000) and using the empirical equation of Squire & Himmel [10] from the infinite dilution sedimentation coefficient, $s^0_{20,w}$, of 3.17S (38700) (Fig. 1). The value for $s^0_{20,w}$ was obtained from a linear least-squares analysis of the data in Fig. 1. Taken in isolation these results appear to indicate that *C. viscosum* lipase has a molecular mass of 38000 ± 2000. However, close inspection of Fig. 1 may suggest, from the positive slope at low concentration, some self-association behaviour, although the absence of an observed decrease in $M^0_{r,w}$ for the 1,4-dioxane and low-temperature buffer systems would appear to suggest that the association, if present, is not hydrophobic in nature.

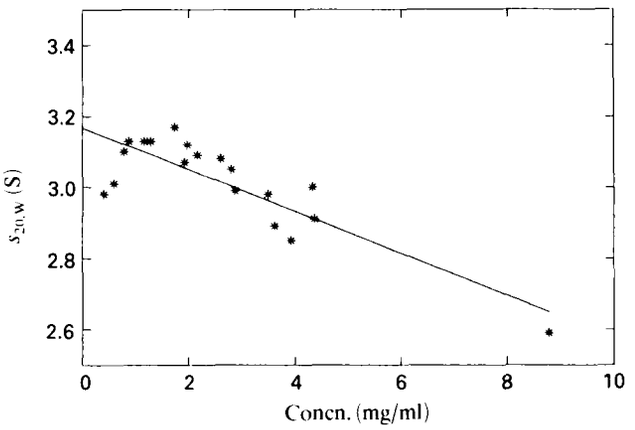

Fig. 1. *Sedimentation velocity profile for C. viscosum lipase*

Sedimentation velocity experiments were performed in a phosphate chloride buffer, pH 6.8, I 0.1 M, at 20.0°C, using a rotor speed of 40000 rev./min.

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