Construction of Hydrodynamic Bead Models from High-Resolution X-Ray Crystallographic or Nuclear Magnetic Resonance Data

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ABSTRACT Computer software such as *HYDRO*, based upon a comprehensive body of theoretical work, permits the hydrodynamic modeling of macromolecules in solution, which are represented to the computer interface as an assembly of spheres. The uniqueness of any satisfactory resultant model is optimized by incorporating into the modeling procedure the maximal possible number of criteria to which the bead model must conform. An algorithm (*AtoB*, for atoms to beads) that permits the direct construction of bead models from high resolution x-ray crystallographic or nuclear magnetic resonance data has now been formulated and tested. Models so generated then act as informed starting estimates for the subsequent iterative modeling procedure, thereby hastening the convergence to reasonable representations of solution conformation. Successful application of this algorithm to several proteins shows that predictions of hydrodynamic parameters, including those concerning solvation, can be confirmed.

INTRODUCTION

One central goal of structural biology is the definition of atomic coordinates for macromolecular structures. The number of coordinate sets submitted to databases such as the Brookhaven Protein Data Bank (PDB) (Abola et al., 1987; Bernstein et al., 1977) increases constantly. Yet questions remain unanswered even by these sophisticated data. Is the crystal lattice structure the same as that adopted by the macromolecule in solution? If the molecule crystallizes as a homodimer, is the orientation of interaction representative of that observed in vitro, if such self-association is detected at all? In addition, are the structures that can remain unseen by diffraction—macromolecules that contain flexibly linked domains—large, noncovalently associated systems. The modeling of the solution conformation of macromolecules can provide answers to these questions, but the models constructed for this purpose should take, as their starting point, the highest possible resolution data available. Such an approach will be described in this paper.

The modeling of macromolecular solution conformation has been limited by progress in the development of rigorous theory. At present, the solution behavior of only two general geometrical structures has been defined: the sphere and the general triaxial ellipsoid (Harding et al., 1981; Harding and Rowe, 1982). The exact mathematical description for spheres has been extended to multi-sphere assemblies (Bloomfield et al., 1967; Kirkwood, 1954; Kirkwood, 1949) and can be used to represent the more complex conformations of macromolecules for solution modeling. This approach has been effective in the study of several biological macromolecular systems, for example, the solution conformation of immunoglobulins (Byron, 1992; Davis et al., 1990; Gregory et al., 1987), myosin (Garcia de la Torre and Bloomfield, 1980), components of the human complement system (Perkins et al., 1993; Perkins, 1985), complexes between Fc fragment and its receptor in the allergic response (Keown et al., 1995), and $\alpha_{IIb}\beta_3$ integrin (Rocco et al., 1993).

Currently, progress is being made in the development of algorithms incorporating segmental flexibility (Diaz et al., 1990; Garcia de la Torre, 1994), the phenomenon that has precluded the generation of atomic coordinates for intact flexible molecules such as the immunoglobulins and myosin. Therein lies the need for hydrodynamic modeling: to generate what, by virtue of the current knowledge gap in terms of bending potentials, must be viewed as a time-averaged conformational model of a macromolecule in dilute solution. Swanson et al. (1978) appreciated the need for a precise structural basis on which to build hydrodynamic bead models. This approach is summarized by Teller et al. (1979) who found that by representing the protein with surface coordinates alone the frictional coefficient calculated was too low and that by encasing the structure in a shell of test spheres of radius 1.4 Å (representing the radius of a water molecule, the smallest unit to interact with the protein surface) the frictional coefficient was too high, but by placing test spheres onto the surface at the sites of charged residues good agreement with experimental data was obtained. The authors believed that this protocol optimally represented the surface rugosity of the macromolecule together with a realistic hydration (see below). The progress of this approach was, at that time, hindered mainly by limitations in computational power, taking approximately 120 hours to perform an approximate calculation for hemoglobin.

For current purposes, such a level of fine tuning is probably a poor use of computer resources. The current need is

Received for publication 26 June 1996 and in final form 16 September 1996.

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0006-3495/97/01/408/08 $2.00

for an easily accessible tool for testing putative conformations of newly engineered protein molecules, oligomeric structures, and heterogeneous complexes. Here, an algorithm (*AtoB*, for atoms to beads) for the construction of initial bead models from atomic coordinates is described that, first, allows for the testing of conformation as defined by a system observed in the crystal lattice or at very high concentrations and, second, hastens the convergence to a satisfactory dilute solution model, concurrently decreasing the often ad hoc nature of alternative model construction. This algorithm is then applied to two globular protein systems.

HYDRODYNAMIC BEAD MODELING

The first useful representation of macromolecules by spherical bead assemblies is attributable to Kirkwood (1954, 1949) who developed a hydrodynamic interaction tensor incorporating the Burgers (1938) and Oseen (1927) perturbation term to account for the effect of other assembly elements on the solvent flow pattern experienced by any given bead. This theory has subsequently been upgraded in at least three important respects so that the finite size of the beads is recognized and the elements are no longer treated as point masses (Rotne and Prager, 1969; Yamakawa, 1970); nonoverlapping beads of unequal size can be used (Garcia de la Torre and Bloomfield, 1977), and overlapping beads of equal size can be modeled. To date, there is no satisfactory treatment of the interaction tensor for overlapping beads of differing size. The hydrodynamic modeling program *HYDRO* (Garcia de la Torre et al., 1994) reverts to an unmodified Burgers-Oseen tensor in the event of an overlapping bead pair with the resultant incurrence of a small but relatively insignificant error.

From the Cartesian coordinates and Stokes radii of the composite beads together with the solvent viscosity, solute molar mass and buoyancy factor, *HYDRO* (via the original core program *TRV* (Garcia de la Torre, 1989)), generates a radius of gyration (R_g), translational diffusion coefficient (D_T), sedimentation coefficient (s), Scheraga-Madelkern parameter (β), intrinsic viscosity ($[\eta]$), and five rotational relaxation times, which can then be compared with experimental data. Clearly, the sedimentation and diffusion coefficients so generated pertain to a solvated molecule, therefore the bead radii (i.e., volumes) ideally reflect a reasonable degree of molecular hydration (δ (g solvent/g solute)); this factor, however, is simply another variable in the modeling procedure. Although the density of water is clearly significantly lower than that of many biological macromolecules, there is presently no mathematical facility for the hydrodynamic modeling of bead models composed either of spheres of differing density or of regions of density gradient within individual spheres (e.g., those on the surface of a model, where hydration is most prevalent).

So far as the size of beads selected is concerned, the computational time required for the matrix manipulations central to the implementation of the modified Burgers-Oseen tensor increases proportionally with N^3 for a model composed of N beads. The supermatrix of interaction tensors requires $9N^2$ memory positions for storage (Garcia de la Torre and Bloomfield, 1981). Both factors place a limit on the practicable number of beads incorporated in a model.

Bead models have historically been constructed on the basis of several criteria. Gregory et al. (1987) designed representations of the subclasses of human immunoglobulin (Ig)G by ensuring the total volume of the cubes enclosing the spheres equalled the volume of the particular molecule (with the inclusion of a degree of hydration). In common with a later study on rat IgE (Davis et al., 1990), the actual arrangement of beads in the constituent molecular domains was intended to mimic the low-resolution structure evident in the x-ray crystallographic data for the hingeless mutant antibody Mcg (Rajan et al., 1983). Perkins and co-workers (Perkins, 1985; Perkins and Sim, 1986; Perkins et al., 1993) have modeled the components of the human complement system via the combined use of small-angle x-ray and neutron scattering curve emulation and hydrodynamic modeling. The resultant models were also compared with electron microscopy data. Fibronectin has been modeled extensively by Rocco and co-workers (Rocco et al., 1987, 1984) who have used a similar approach to model the solution structure of an integrin (Rocco et al., 1993). Here a set of models is randomly generated. Those that satisfy certain pre-set steric criteria are then evaluated in terms of their frictional coefficients and other solution data.

The algorithm outlined below presents the hydrodynamic modeler with an additional alternative for bead model construction.

Transformation of atomic coordinates to spherical assemblies

Atomic coordinate databanks such as the PDB are easily accessible stores of structural and conformational data. At the time of writing, the PDB contains in excess of 4700 entries, of which 4300 are files of atomic coordinates for proteins, enzymes, and viruses. Although any significant degree of segmental flexibility within a macromolecule frequently prevents either its successful crystallization or results in a region of preclusively low electron density (thereby rendering the coordinates of that flexibly linked segment insoluble), the database contains the atomic coordinates of many individual segments, crystallized independently of their parent molecules. Increasingly, the problem of flexibility is being addressed by the generation of atomic coordinates for mutant molecules, engineered or chemically modified to have restricted degrees of freedom.

AtoB permits the construction either of an intact bead model directly from the atomic coordinates of a molecule or of bead models of molecular domains that can then be connected by more hypothetical linker regions. The interdomain spatial relationships can then be iteratively refined

(simultaneously with, for example, molecular hydration) in an attempt to reproduce experimental hydrodynamic data.

To implement *AtoB*, the coordinates for a selected molecule (or molecular segment) are transferred from the Brookhaven PDB (or a similar database) and edited to remove the header text. *AtoB* is designed to cope with missing atoms and residues and will alert the user in such an instance. Models can also be constructed from α-carbon backbone structures; the mass density of each residue is simply centered on the coordinates of the main-chain α-carbon atom. Once in the appropriate format, the atomic resolution coordinates are submitted to the FORTRAN program *AtoB*, which generates the multisphere model required as input for the program *HYDRO* (Garcia de la Torre et al., 1994). Both programs are run locally on the Silicon Graphics Instruments Challenge XL mainframe computer at the University of Leicester.

Modeling with *AtoB* proceeds as outlined in the flow chart in Fig. 1. By searching through the entire data file, the coordinates of the spatial extremes of the molecule are determined. The structure is subsequently enclosed in a cuboid of appropriate extreme dimensions. This cuboid is then subdivided into equally sized cubes, the dimensions of which are determined by the user's choice of resolution (e.g., 5, 10, or 20 Å or whatever is appropriate to the overall dimensions and structure of the molecule). If the length of any given side of the cuboid is not equal to an integral multiple of the resolution, its length is rounded up accordingly. The centers of gravity of the constituent amino acid residues are then determined and are correspondingly assigned to the appropriate cube. The center of gravity of each cube is calculated and at this location is positioned a sphere the radius of which is calculated (by *AtoB*) from the molecular mass and partial specific volume, which are either calculated from the PDB data file or, when the coordinates are incomplete, are supplied by the user in the input file.

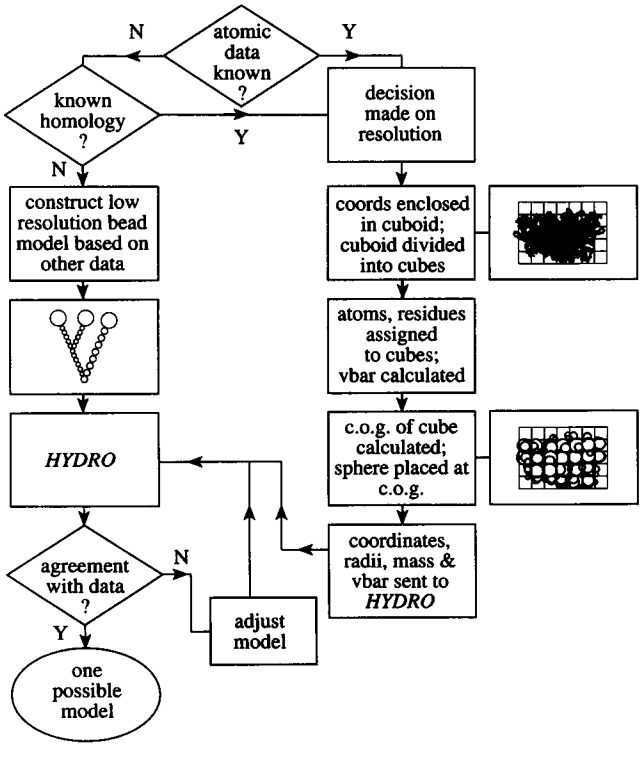

FIGURE 1 Flow chart illustrating the general procedure of hydrodynamic bead modeling and the particular reduction of data by *AtoB*.

Resolution

The term resolution is used in a nominal sense. If a cuboid is subdivided into cubes that are subsequently occupied by spheres of diameter equal to that of the cube dimension, the space left unfilled by the spheres is equal to 0.476 times the volume of the original cuboid. This illustrates the need to use overlapping spheres to simultaneously satisfy the gross dimensions of the particle, its mass, and its partial specific volume. Normally, 1 sphere can only reasonably be expected to overlap with a maximum of 26 other spheres. Thus, inaccuracies in the calculation of hydrodynamic parameters within *HYDRO* can be minimized by increasing the resolution and minimizing the proportion of the entire model with which a particular sphere overlaps. The diameters of the composite spheres will generally therefore exceed the resolution.

AtoB includes an option for creating a model with equally sized beads when the user wishes to model the hydrodynamic behavior of the macromolecule with a precise modified Burgers-Oseen tensor. The user chooses a nominal resolution to set the grid size within the cuboid. The actual bead size is then calculated by the program.

Hydration

The nature of hydration has been considered at length by Kuntz and Kauzmann (1974) who identified at least four different types of hydration. One of the four is termed hydrodynamic hydration (the remaining three being preferential, structural, and low-temperature hydration), which defines a mass of solvent that migrates with a hydrodynamic particle. If the amino acid content is known for a protein, for example, it is possible to estimate a value for this hydration based on the data presented in their Table XXII, determined from nuclear magnetic resonance studies. Otherwise, the level of hydration can, with care, be experimentally determined as described by Eisenberg (1994).

Hydrodynamic hydration itself can be subdivided into two categories that for reasons of clarity shall be referred to here as experimental hydrodynamic hydration (EHH) and modeled hydrodynamic hydration (MHH). EHH (see Squire and Himmel (1979) who calculated a mean hydration of 0.53 ± 0.26 g H_2O/g protein for a series of 21 proteins) includes surface-associated solvent and solvent contained in cavities within the macromolecule. MHH, on the other hand, is the amount of hydration seen by *HYDRO* and includes surface solvent only. It is unsurprising that Teller et

al. (1979), who opted for the lower hydration treatment of Kuntz and Kauzmann (1974) but placed test spheres (see above) only onto spheres representing surface-charged groups, calculated frictional coefficients in good agreement with experimental data. This approach appears to be more appropriate for hydrodynamic bead modeling but is clearly only of use when the surface amino acid distribution is known. In the modeling of highly elongated structures, the use of hydrations estimated from experimentally determined hydrodynamic parameters appears to be justified (i.e., where there is a minimum of buried residues and MHH approaches EHH) and, usefully, these hydrations can be estimated without knowing the surface residues or their distribution. Unfortunately, globular proteins present more of a problem. But, for globular structures lacking in significant internal crevices (unlike apoferritin below), the need for a modeled hydration of less than 0.25 g water/g protein or higher than 0.60 g water/g protein is indicative of a poor model.

In the absence of a totally satisfactory alternative, the hydration of bead models generated by *AtoB* is achieved by isomorphous expansion, i.e., the swelling of beads concerned to give uniform expansion (Garcia de la Torre and Bloomfield, 1980). This approach is also taken by Perkins and co-workers (see, for example, Perkins et al., 1993) who assign a standard hydration of 0.39 g H_2O/g protein to all protein models. It is important to note that for the hydration of elongated models the expansion should be only radial; otherwise, the axial ratio is artificially elevated. The radial expansion factor (e) is the cube root of the ratio of solvated to anhydrous volume ($e = \{(\bar{v} + \delta_1 v_1)/\bar{v}\}^{1/3}$ where v_1 is the specific volume of the solvent). The successful computational implementation of this hydration strategy results from the rapid regeneration of the interbead vectors **Rij** ($\mathbf{i} = 1, N; \mathbf{j} = 1, N; \mathbf{i} \neq \mathbf{j}$), the calculation of which would otherwise be tedious, especially for high-resolution bead models originating from atomic data.

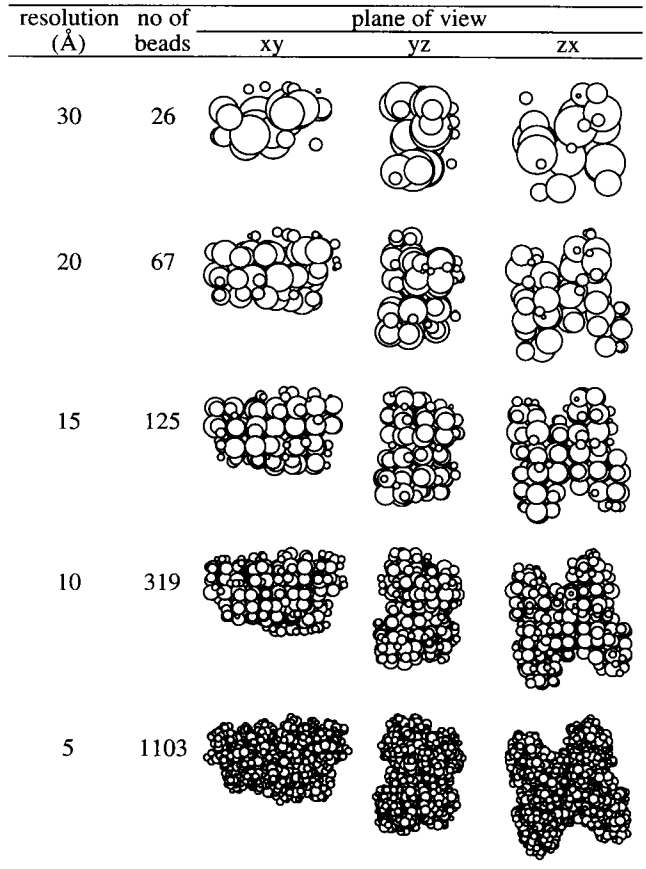

FIGURE 2 Bead models of aldolase generated by the program *AtoB* using the atomic coordinates obtained by x-ray crystallography to 1.9 Å by Hester et al. (1991). The resolution of the bead models is nominal (see text) and refers merely to the grid size used to subdivide the molecule into spheres.

Effect of bead size upon simulated parameters

The chosen resolution of models should reflect the limit in sensitivity of the hydrodynamic parameters being modeled. Although the use of very small spheres does impose a considerable demand on computer time, this continues to be less of a concern as computers become more powerful. Certainly small beads are desirable to minimize the proportion of beads that overlap in the model, as discussed above. The use of spheres that are too large also produces a model with an overall shape that may be a poor representation of the actual molecule. This is illustrated with a model for the globular enzyme, aldolase, for which bead models were generated from the atomic coordinate data (Hester et al., 1991) over a range of different resolutions (Fig. 2) between 5 and 30 Å. It is evident that resolutions of 10 and 20 Å are sufficient to create a model that retains a reasonable similarity to the original crystal structure (Fig. 6). At lower resolutions, important shape and mass distribution information is lost. This resolution dependence is naturally a function of molecular size and of the complexity of surface topography. A more globular molecule could easily be well represented by beads of larger size. The convergence of modeled data as a function of bead size is illustrated in Fig. 3, confirming the adequate resolution of the 10- and 20-Å models but nonetheless indicating the desirability to adopt the highest available resolution.

Testing the validity of using only the α-carbon backbone

To extend the use of this algorithm to low resolution atomic coordinate data, a subroutine has been incorporated to enable the construction of bead models from α-carbon backbone data. This was used in the generation of bead models for myosin (see below). The validity of this approach was tested using the atomic coordinates and edited α-carbon coordinates for aldolase. By definition, the α-carbon backbone model will be more compact, so the 1.5% elevation in sedimentation coefficient observed is unsurprising. This

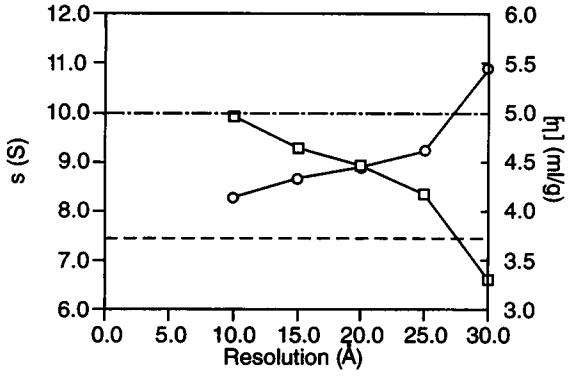

FIGURE 3 The dependence of sedimentation coefficient (○) and intrinsic viscosity (□) upon bead size for aldolase. Experimental data are indicated by horizontal dashed lines (- - -, sedimentation coefficient; –·–·–, intrinsic viscosity). Intersection with modeled data is not expected as hydration has not been included in the models.

approach therefore does produce a model with different hydrodynamic characteristics, but the difference is not significant when compared with experimental error on the original data and the uncertainties in hydration inherent in most hydrodynamic modeling procedures.

Effect of an absolute frame of reference

It is important to assess the dependence of *HYDRO*-generated parameters upon the absolute frame of reference used for the construction of bead models by *AtoB*. This is vital if different models are to be comparable with each other. It is also of importance when designing models of interacting species. If the interaction is modeled with a standard molecular graphics program and the coordinates are then transferred to the algorithm, the individual molecules will be modeled in different ways depending on where the initial all-encompassing cuboid lies. This is once again illustrated with aldolase in Fig. 4. Here the coordinates of the molecule were shifted in the y direction by 2-Å increments away from a fixed arbitrary point, and 10-Å resolution bead models were accordingly generated. There is a slight visible difference between the models, as expected. But the variation in hydrodynamic parameters for the models (e.g., 8.41 S $\leq$ s $\leq$ 8.53 S for the five anhydrous models tested) is insignificant within the accuracy of this modeling technique.

Application to two well characterized proteins

Globular proteins: aldolase

Aldolase is a well characterized globular enzyme, consisting of four polypeptide chains, easily studied with hydrodynamic techniques. The molecular mass of the rabbit muscle aldolase used in this study was determined to be 156 kDa by sedimentation equilibrium studies (data not shown), in agreement with the known amino acid sequence. This had a sedimentation coefficient of 7.40 (± 0.2) S, a partial

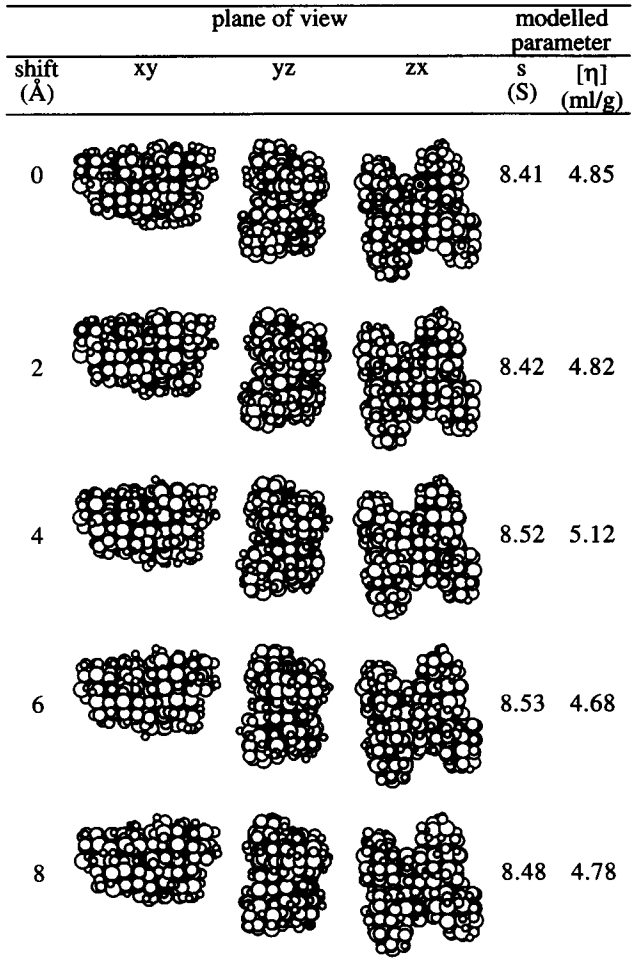

FIGURE 4 The 10-Å nominal resolution bead models of aldolase generated by *AtoB* from atomic resolution coordinates have been shifted repeatedly by 2 Å in the y direction. Neither the models nor the parameters subsequently calculated by *HYDRO* depend greatly on the positioning of the 10-Å grid.

specific volume of 0.742 ml/g and an intrinsic viscosity of 5.0 (± 1.0) ml/g (data not shown). First, even though aldolase is a globular protein, its representation by a single sphere is highly unsatisfactory. A hydration of approximately 0.9 g H_2O/g protein is required to reproduce the experimental data with the single-sphere model. By representing aldolase as a tetramer of four equally sized spheres, this large hydration can be reduced, with the less compact geometry of the model contributing to the hydrodynamic parameters. The curves plotted for the three proposed low-resolution models (Fig. 5) demonstrate the relative insensitivity to shape of the modeled parameters. It is clear, however, that the linear model cannot to be representative of the solution conformation as the modeled and experimental values for intrinsic viscosity fail to intersect. Instead, the subunits of aldolase must adopt a conformation somewhere between that of the tetrahedral and square planar arrangements. This conclusion is consistent with the atomic resolution structure for aldolase in which the square planar

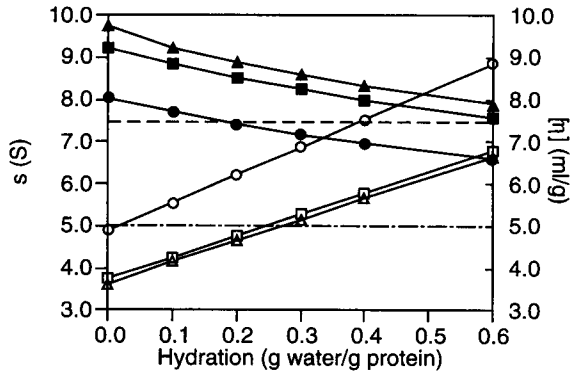

FIGURE 5 The dependence of sedimentation coefficient (▲, ■, ●) and intrinsic viscosity (△, □, ○) upon hydration modeled by isomorphous expansion of three different four-bead structures for aldolase: tetrahedral (▲, △); square planar (■, □); linear (●, ○). Experimental data are indicated by horizontal dashed lines (- - -, sedimentation coefficient; —·—·—, intrinsic viscosity).

arrangement can be observed in one orientation, whereas the skewed nature of this configuration is apparent upon rotation through 90° (Fig. 6). A 10-Å resolution bead model was generated for aldolase from the atomic coordinate data (Fig. 2). This model does reproduce the experimentally determined data, although at a rather lower than average hydrodynamic hydration, in agreement with the earlier postulated difference between experimentally determined (EHH) and modeled (MHH) hydration for globular particles (see above).

Spherical hollow proteins: apoferritin

The frictional properties of macromolecules are determined by the topography and hydration of the solvent-exposed surface. Therefore, the hollow, spherical protein apoferritin represents a good test of hydrodynamic bead modeling. The hydration required to reproduce measured hydrodynamic parameters should simply be that of the surface residues and should not include any solvent entrapped in the sizeable central cavity. For apoferritin, the difference between these two hydrations (EHH and MHH, above) will be easily distinguishable. Hydrodynamic data (M^0_w = 502 kDa; $s^0_{20,w}$ = 18.3 S; $\bar{v}$ = 0.728 ml/g; $[\eta]$ = 5.16 ml/g) were acquired for commercially available horse spleen apoferritin via standard analytical ultracentrifugation, viscosimetric, and densimetric methods (data not shown). Additionally, the radius of gyration has been determined by Zipper et al. (1993) to be 53.3 Å. Bead models were generated at resolutions of 10 Å (1088 beads) and 20 Å (375 beads; Fig. 7), although limitations on computational time restricted actual calculations of hydrodynamic parameters (via the use of *HYDRO*) to the 20 Å model. The resultant dependence upon hydration of the intrinsic viscosity and sedimentation coefficient is plotted in Fig. 8. Unquestionably, the intercept between experimental and modeled parameters occurs only at very low hydrations (up to 0.2 g H_2O/g protein) confirming the exclusive contribution of the surface-associated solvent to the frictional behavior of the model and supporting the proposed difference between EHH and MHH (above).

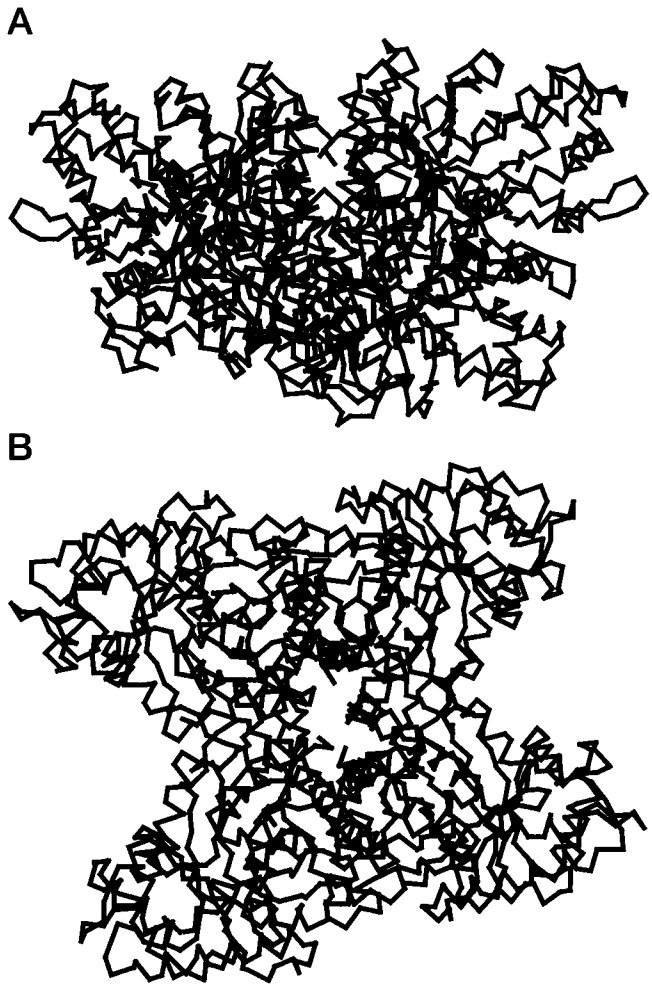

FIGURE 6 The α-carbon backbone of fruitfly aldolase (Hester et al., 1991) shown in two orientations. In *b*, the molecule appears to be a square planar arrangement of the constituent domains whereas rotation through 90° in *a* reveals the skewed nature of its conformation.

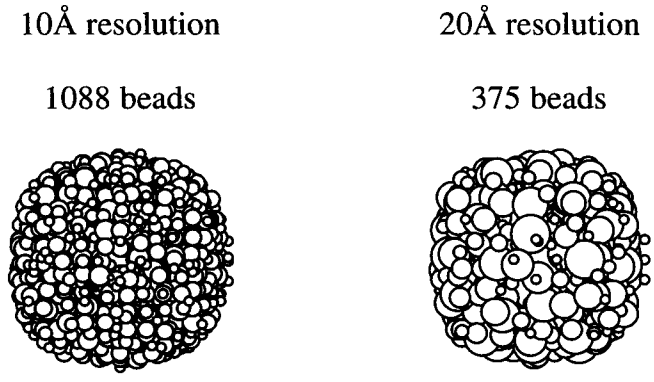

FIGURE 7 Apoferritin models at 10 and 20 Å nominal resolution constructed from atomic resolution data.

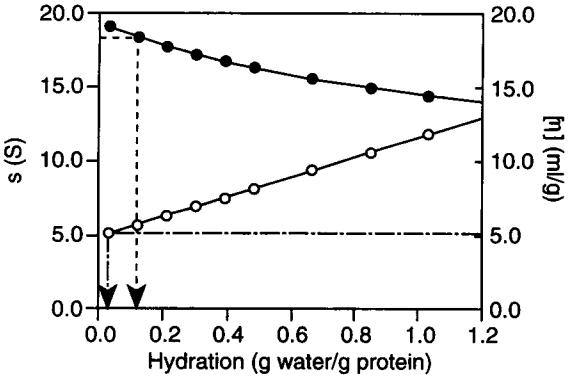

FIGURE 8 Sedimentation coefficient (●) and intrinsic viscosity (○) as a function of model hydration for the 20-Å resolution model of apoferritin. The intersection of modeled and experimental data confirms the surface-only nature of hydration as seen by *HYDRO*.

DISCUSSION AND SUMMARY

An algorithm facilitating the straightforward construction of bead models from atomic resolution data has been designed and implemented via a FORTRAN program (*AtoB*). In the first instance, this provides a means of testing how well an atomic resolution model represents the macromolecular solution conformation. Clearly, if the hydrodynamic data generated in response to an initial bead model (constructed solely on the basis of high-resolution data) are in poor agreement with their experimentally determined counterparts, the original atomic coordinates are likely to be those of a molecule perturbed in some way, perhaps by the constraints of a crystal lattice or by interparticle effects in a high concentration environment. In the second instance, this algorithm provides a reduction in the ad hoc nature of solution macromolecular modeling. Even without the atomic coordinates of the actual molecule of interest, a model can be constructed by mapping the residues of the molecule of interest onto the known structure of one with which it shares a significant degree of sequence homology (R. Sowdhamini, T. J. Mitchell, P. W. Andrew, and P. J. Morgan, in preparation). The solution conformation of a newly expressed or modified protein can be probed by representation of the molecule with a bead model constructed from the atomic coordinates of the wild type. Hydrodynamic data can be obtained from relatively small amounts of material compared with the requirements of x-ray crystallographic and nuclear magnetic resonance techniques. Furthermore, these high-resolution studies take longer to perform and are limited to crystallizable and lower molecular weight systems, respectively. Finally, molecular graphics software can be used to model covalently bound groups such as carbohydrate residues or polymers. The final bead model can then be based on these modeled coordinates.

AtoB is a fast running program that easily links to other related software. It can construct models from atomic or α-carbon backbone data and has been successfully applied not only to the two proteins discussed here but also to other systems such as the glycosylated extracellular domains of cell adhesion molecules CD2, CD48, and CD4 (Silkowski et al., in preparation) and pneumolysin (P. J. Morgan and O. Byron, unpublished). Furthermore, modeling reported here has confirmed the theoretical view that hydration, as modeled by *HYDRO* has a particular physical meaning distinct from experimentally observed hydrodynamic hydration.

An alternative procedure for the generation of bead models from atomic coordinates is simply to represent each residue by a bead of 4 Å in diameter. This approach is unlikely to yield models that differ from those generated at a 4 Å resolution with this algorithm, certainly within the inescapable uncertainties of hydration, for example. But when a large structure is being modeled and computer time is at a premium, this algorithm is able to provide the user with the coordinates of a lower resolution model, containing fewer elements.

AtoB has the potential for further development. Certainly it would be interesting to observe the effect on modeled parameters of assigning each bead a partial specific volume corresponding to that of the residues contained therein. It will also be vital to develop a more satisfactory method for estimating and modeling the surface-bound hydration. The current isomorphous expansion strategy is unsatisfactory because the spatial arrangement of all residues is altered upon hydration. This is clearly a poor representation of surface-bound water. In the case for which high-resolution data are available, the possibility exists for a knowledge-based approach to this problem. This might be based on data concerning the residence times of hydrating water molecules. Brunne et al. (1993), in a comparison of the experimental and theoretical residence times of water molecules solvating bovine pancreatic trypsin inhibitor, found that the duration of residence is dependent on the type of side-chain atom (i.e., the time of residence near polar atoms exceeds that for nonpolar atoms, which in turn is greater than that for charged atoms). The same study also revealed that there is no correlation with these residence times (for solvent-accessible atoms) and the type of secondary structure in which they are presented to the solvent.

Consideration in this present discussion has been limited to protein molecules. The program can generate models for glycoproteins but has yet to be expanded to handle nucleic acids or synthetic polymers. Increasing interest in the interactions between protein and nucleic acids makes this an essential but simple next step. *AtoB* is available upon request from the author in the form of a FORTRAN file, which can then be compiled and linked locally. It will also be posted on the World-Wide Web (WWW) pages of the Biophysical Society (http://biosci.cbs.umn.edu/biophys/biophys.html) where *HYDRO* is also available. The output from *AtoB* can be easily fed into *HYDRO*. WWW pages are currently being compiled locally (http://www.ccc.nottingham.ac.uk/~ sczles/ncmhp), and eventually *AtoB* will be available there.

I thank Dr. Steve Harding and Dr. Arthur Rowe for their enthusiastic support and initial direction. Thanks also go to Prof. Jose Garcia de la Torre for his continuing advice and encouragement. Ms. Nima Mistry performed analytical ultracentrifugation runs for this work for which she is thanked, as is Mr. Anil Pancholi who performed viscosimetric and densimetric analyses.

This work was supported in part by the SERC/BBSRC/EPSRC.

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