

Hydration from hydrodynamics. General considerations and applications of bead modelling to globular proteins

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Abstract

The effect of hydration on hydrodynamic properties of globular proteins can be expressed in terms of two quantities: the δ (g/g) parameter and the thickness of the hydration layer. The two paradigms on hydration that originate these alternative measures are described and compared. For the numerical calculation of hydrodynamic properties, from which estimates of hydration can be made, we employ the bead modelling with atomic resolution implemented in programs HYDROPRO and HYDRONMR. As typical, average values, we find 0.3 g/g and a thickness of only approximately 1.2 Å. However, noticeable differences in this parameter are found from one protein to another. We have made a numerical analysis, which leaves apart marginal influences of modelling imperfections by simulating properties of a spherical protein. This analysis confirms that the errors that one can attribute to the experimental quantities suffice to explain the observed fluctuations in the hydration parameters. However, for the main purpose of predicting protein solution properties, the above mentioned typical values may be safely used. Particularly for atomic bead modelling, a hydrodynamic radius of approximately 3.2 Å yields predictions in very good agreement with experiments. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Protein hydrodynamics; Solution properties; Hydration layer

1. Introduction

One of the major manifestations of macromolecular hydration or solvation is its influence

on the hydrodynamic behaviour of the macromolecule in solution. The macromolecular material is surrounded by a more or less strongly adsorbed layer of solvent (water in aqueous solutions of biological macromolecules). When the macromolecule moves under the influence of an external effect, or simply as a consequence of Brownian motion, that layer moves rigidly at-

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tached to the macromolecular material. Thus, the effective hydrodynamic particle includes the hydration water, and the hydrodynamic size should be larger than what would correspond to the anhydrous macromolecule.

When the macromolecule is fairly compact and of a moderate size (so that the size increase is relatively important), as it is the case with globular proteins, the influence of hydration on hydrodynamic behaviour is quite notorious. This was realised from the earliest days of macromolecular hydrodynamic research [1], when it was also commonly accepted that the shape of globular proteins could be approximately represented by an ellipsoid of revolution. Thus, in addition to the overall size, the other key factors determining the solution properties would be the axial ratio of the ellipsoid p , and the amount of hydration, which was usually represented as, δ , the grams of attached water per gram of protein. It is well known [1] that the evaluations of these two parameters require data of at least two solution properties.

The compilation of a considerable amount of results obtained from this type of analysis [2–4] indicated a noticeable variability in the δ hydration parameter. A typical value could be $\delta = 0.3$ g/g, but results fluctuate widely in a range of approximately 0.15 to 0.50 g/g. This circumstance concerned hydrodynamics researchers, because this uncertainty would influence the determination of the macromolecular size and shape. From another point of view, it was unclear whether such variability in hydration from protein to protein is molecularly true, or an artefact of the data analysis.

In more recent years, with the advent of bead-modelling methodologies [5,6], it is possible to model the detailed irregular shapes of rigid macromolecules. Particularly significant for the purpose of the present work is the possibility of modelling the finest details of globular proteins at a nearly atomic level [7,8]. At this level, hydration effects can be treated with an alternative point of view that allows the determination of the thickness of the hydration layer.

The gram-per-gram and the hydration-layer descriptions of macromolecular hydration may be

compatible, but their relationship should be clearly understood. Therefore, in the first part of this paper we describe and compare the general aspects of the two approaches. Then, we proceed making a specific application of the atomic-level bead modelling procedure, which allows the estimation of both hydration measurements. The variability in hydration that this calculation evidence is discussed, pointing out that one of its main origins may be the experimental errors in the data of solution properties.

2. General considerations

The hydrated macromolecule is regarded as a core of macromolecular material and an envelope of bound water. The frontier between bound and bulk water defines the contour of the *hydrodynamic particle*, whose size and shape will determine the hydrodynamic properties in solution. As indicated in Fig. 1, hydration the obvious effect of increasing the size or volume of the particle. The hydrated volume, V_{hyd} , is larger than the anhydrous volume, V_{anh} which we would obtain from the molecular weight, M , and the specific volume, \bar{v} , of the protein:

$$V_{\text{anh}} = M\bar{v}/N_A \quad (1)$$

where N_A is Avogadro's number. As illustrated in Fig. 1, hydration also influences the overall shape of the protein, mainly in the sense of smoothing out some structural details. Thus, while in certain regions hydration will just coat the protein surface (3 in Fig. 1), in others the surface details will be blurred Fig. 1, and it may even happen that pockets or cavities in the protein will be entirely filled by hydration water Fig. 2.

Apart from the above, qualitative description of the hydration effect, we face the problem of how hydration can be quantified in a manner that could have a general validity for globular proteins and other rigid macromolecules. The two paradigms can be applied for such purposes are described next.

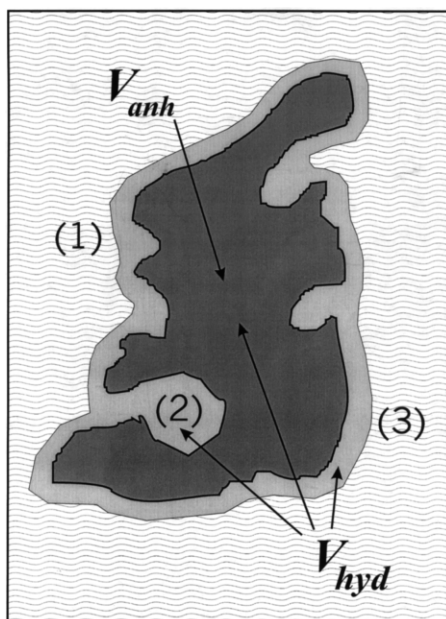


Fig. 1. Schematic view of a rigid, hydrated macromolecule. The various effects of hydration on size and shape.

2.1. The δ (g/g) paradigm

The classical scheme for expressing protein hydration has been the one based on the δ (g/g) parameter, which expresses the ratio of the mass of the bound water to that of the protein:

$$\delta = \frac{\text{grams (water)}}{\text{grams (protein)}} \quad (2)$$

If ρ is the density of the aqueous dilute solution, which will be close to that of either bound or bulk water (approx. 1 g/cm³), then we have:

$$\delta = \frac{(V_{\text{hyd}} - V_{\text{anh}})\rho}{V_{\text{anh}}/\bar{v}} = \left(\frac{V_{\text{hyd}}}{V_{\text{anh}}} - 1 \right) \bar{v}\rho \quad (3)$$

A common version of the δ paradigm is the uniform expansion hypothesis. It originates in the classical, primitive representation of globular proteins as ellipsoidal particles [1]. The axial ratio b'/a' of the ellipsoid that represents the hydrated particle is assumed to be the same as that of the shape of the anhydrous core b/a (b or b' ,

is the duplicate semiaxis of the ellipsoid of revolution, and a or a' is the single one). For a particle of arbitrary shape (Fig. 2), the uniform expansion assumes that any linear dimension, u of the particle is expanded by a constant factor, h ,

$$h = \frac{u_{\text{hyd}}}{u_{\text{anh}}} \quad (4)$$

such that

$$h^3 = \frac{V_{\text{hyd}}}{V_{\text{anh}}} \quad (5)$$

and it easily follows that in this representation h is related to the δ parameter by

$$\delta = (h^3 - 1)\bar{v}\rho \quad (6)$$

or

$$h = \left(1 + \frac{\delta}{\bar{v}\rho} \right)^{1/3} \quad (7)$$

As an example with typical values for globular proteins, if $\rho = 1$ cm³/g, $\bar{v} = 0.72$ g/cm³, and $\delta = 0.35$ g/g, then $h = 1.14$, which amounts to a 14% expansion in linear size, and a 48% in volume.

The uniform expansion is applicable not only to ellipsoidal shapes, but also to compact, not too elongated structures of any shape [9,10], and this is the case with the family of globular proteins that we specifically consider in this paper. However, some words of caution are pertinent to warn that the uniform expansion is not valid for elongated particles, such as fibrous proteins, filamentous or rodlike particles, etc. Suppose that the anhydrous dimensions of a long (approximately rodlike) protein are 18 Å in length and 1440 Å in diameter (the data are for myosin rod). Due to hydration, the diameter is reasonably increased by, say, $h = 1.14$ or 14%, to approximately 20 Å. But if the same expansion would apply to the protein length, this would increase approximately 1640 Å, i.e. 100 Å at each end. Obviously, this is molecularly erroneous, and would bring about a very abnormal change in solution properties (ap-

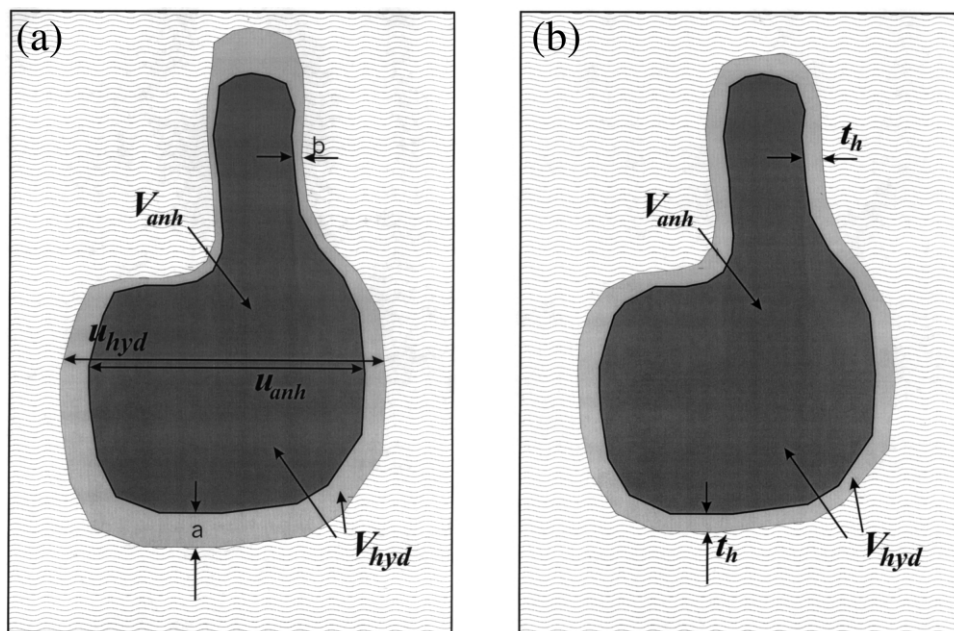


Fig. 2. Comparison of effects of the two hypothesis for hydration. (a) Uniform expansion (b) Uniform hydration layer.

prox. 50% increase in intrinsic viscosity and relaxation time). For such elongated structures, it would be more reasonable to assume that the thickness of the hydration layer is the same at the tips as at the centre.

2.2. The hydration layer paradigm

The above considerations introduce an alternative view of hydration. In the hydration layer paradigm, one accepts that the anhydrous core is coated by a layer of bound water which has a constant thickness, t_h , measured in the direction normal to the protein surface. As illustrated in Fig. 2, this is in contrast with the effect of the uniform expansion, which gives a thicker hydration layer in directions where the particle is longer. The uniform thickness contradicts the uniform expansion, but is compatible with the general δ (g/g) paradigm. Indeed, for the hydrodynamic particle, defined by the contour of the hydration layer, the hydrated volume V_{hyd} may be calculated, and one can formulate again the δ parameter as in Eq. (6).

One important advantage of the hydration layer paradigm is that it appears as the proper, physically significant way to account for hydration in hydrodynamic models with atomic resolution, as described next.

3. Bead modelling of globular proteins with atomic resolution

Due to the simultaneous progresses, along the last two or three decades, in the structurally detailed knowledge of macromolecules and in hydrodynamic theory and methodology, it is now possible to predict hydrodynamic and other solution properties of macromolecules from their atomic-level structure (i.e. from the atomic coordinates) [7,8]. In the hydrodynamic model, the friction elements are the atoms themselves, and their effective hydrodynamic size must include obviously a contribution from hydration. This methodology is described in earlier work [7]. Here, we will just mention the aspects that are more

related to hydration, presenting a list of results that expands that in our previous publication.

3.1. Procedure

In the bead modelling of globular proteins with atomic resolution, a *primary hydrodynamic model* is built representing each non-hydrogen atom by a sphere of radius a . The values of a must have a lowest bound corresponding to the typical van der Waals radius of the protein atoms, approximately 2 Å. This is required for the model to reproduce, at least, the anhydrous volume of the protein given by Eq. (1). However, as one could expect, calculation with such a value of a predicts erroneous results for the hydrodynamic properties; actually, larger values of a are required due to the size-increasing effect of hydration.

The *primary hydrodynamic model* is the superposition of a set of spheres, whose radius, including hydration, may be typically $a_{\text{hyd}} \approx 3$ Å (vide infra), which is more than twice the typical distances (bond lengths) between contiguous spheres.

Thus, the resulting bead model presents an impressive extent of overlapping, as illustrated in Fig. 3a, and it is well known that this may influence badly the prediction of some properties. That is why in the modelling procedure, implemented in HYDROPRO [7], there is a further step (before hydrodynamic calculations), in which the model of overlapping beads is next modelled by a shell of minibeads. For the present purpose of the present paper, the shell model phase of the model building can be considered as an internal of HYDROPRO; the presentation of results and their discussion is essentially based on the *primary hydrodynamic model* (Fig. 3b)

For the model of overlapping spheres, its volume can be numerically obtained as a function of the sphere radius, a . An example of the results is displayed in Fig. 4b. As the anhydrous volume of the protein is known from Eq. (1), its value can be interpolated to find a anhydrous atomic radius, a_{anh} , as illustrated in Fig. 4b. We anticipate that, as expected, this value is always very close to the typical van der Waals radius of protein atoms, approximately 2 Å.

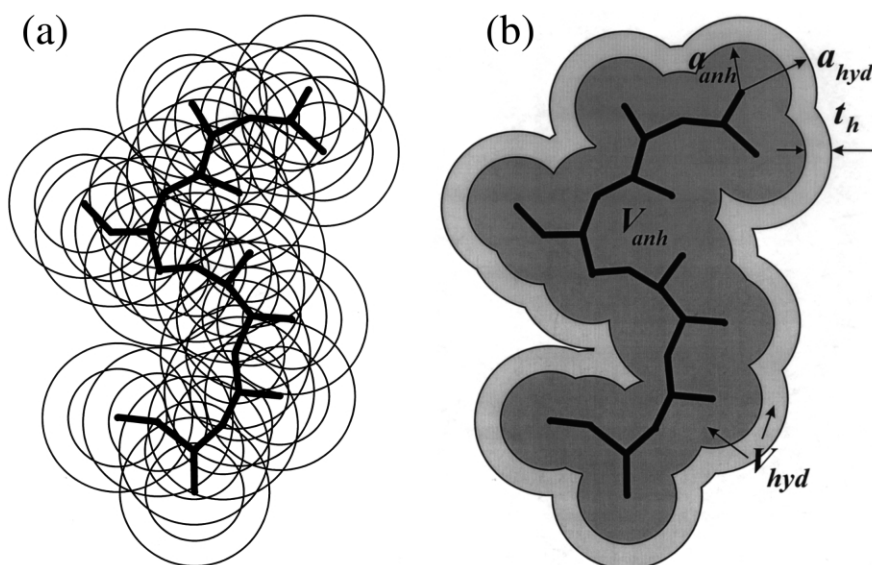


Fig. 3. Schematic construction of the atomic bead model for a hypothetical (small, planar) molecule. In (a), the contours of the van der Waals and hydrodynamic spheres are superimposed to illustrate the great extent of compactness. In (b) we note more clearly the envelopes of the protein core and the hydrodynamic particle.

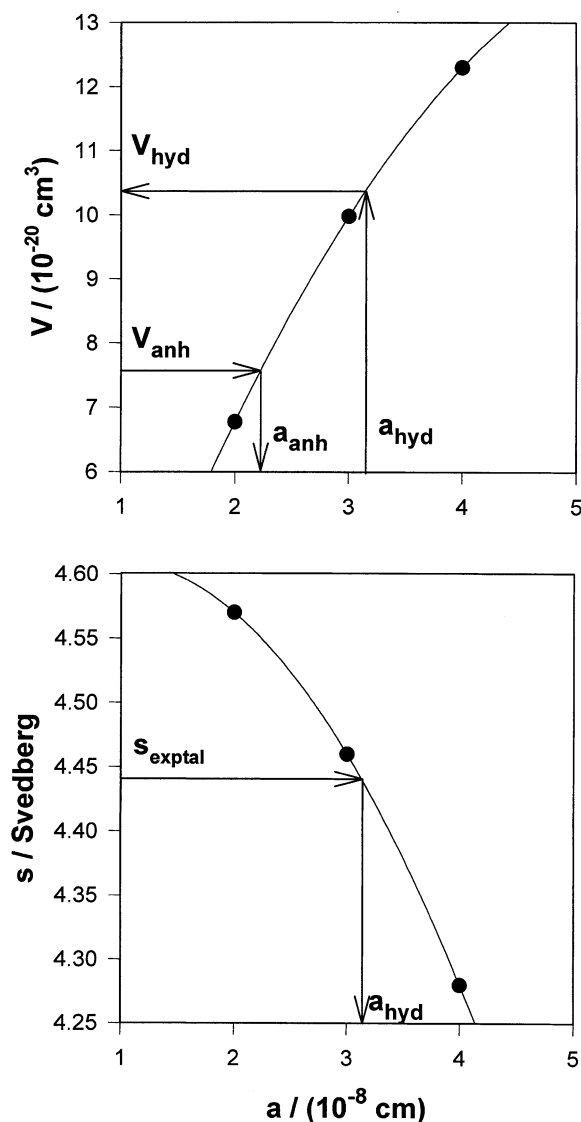


Fig. 4. Results for lysozyme. Data points are calculated for some values of a and the curves are intra/extrapolating polynomials. (a) Fitting a from the experimental value of the sedimentation coefficient, s , or predicting s from a given value of a . (b) Determination of a_{anh} from V_{anh}

3.2. Results

The estimation of hydration requires the comparison of computed properties with experimental values for a large set of proteins. In this work we have further expanded the collection used in our

previous work: we now include 19 proteins, listed in Table 1. The experimental values are taken from a variety of sources, like the compilation of sedimentation coefficients by Smith [11] and that of intrinsic viscosity's by Harding [12], and other papers in which properties of various proteins were jointly analysed [13–17].

In principle (i.e. in the absence of other preliminary information), the hydrodynamic radius of the atoms, a_{hyd} (we shall distinguish several versions of it) can be treated as an adjustable parameter, whose value is assigned as to fit some experimental hydrodynamic property. Calculations with HYDROPRO are done for a few values of a , and the value that fits the experimental value of some available property is obtained by inter/extrapolation, as indicated in Fig. 4a. The results for the 19 proteins are reported in Table 1.

The results from either sedimentation or translational diffusion coefficients are denoted as a_t . When experimental values for the intrinsic viscosity and the rotational diffusion coefficients were available, they were used also to fit the hydrodynamic radius, with results denoted as a_η and a_r , respectively. The available values of a , for hydrodynamic properties are represented by their average, a_h . When available, we have also included in our analysis a non-hydrodynamic property, the radius of gyration, which comes usually from X-ray scattering measurements. The atomic radius that fits the radius of gyration is denoted as a_g . For each protein, the final value of the effective atomic radius, a , which represents the whole set of solution properties is either a_h or the mean of a_h and a_g . For the different classes of a , Table 1 gives the mean over the whole set of 19 proteins.

One of the first conclusions that emerged from Table 1 is that (apart from noticeable fluctuations, that we shall discuss below) the typical values of a_g and a_h , represented by their means over the whole set of proteins, are quite similar 3.1 and 3.3 Å, respectively. Thus, the effective size of the protein, as seen by X-ray scattering and detected by hydrodynamics is practically the same. In other words, the size-increasing effect of hydration is the same for scattering and from

Table 1

Results from atomic-level bead-modelling calculations for a set of 19 proteins.

Protein	a_t	a_r	a_η	a_h	a_g	a	% Dif. in			
							D_t	D_r	$[\eta]$	R_g
BPTI	3.6	2.6		3.1		3.1	3.1	−11.8		
Ribonuclease A	3.1		2.5	2.8	2.3	2.6	2.8		2.4	2.0
Lysozyme	3.0	3.1	1.9	2.7	2.5	2.6	2.3	8.8	4.1	1.4
Myoglobin	1.9	2.8	2.6	2.4	3.3	2.7	−4.9	0.9	2.2	−4.1
α -Lactalbumin	2.5			2.5		2.5	2.1			
Trypsin	3.4			3.4		3.4	1.7			
Chymotrypsinogen	1.7		1.3	1.5	2.9	2.0	−1.9		21.4	−7.7
β -Lactoglobulin	2.7	2.7		2.7	2.5	2.6	0.7	1.5		1.0
Pepsin	2.7			2.7		2.7	−2.0			
Subtilisin	2.8			2.8		2.8	−1.5			
Ovalbumin	1.4		3.3	2.3		2.3	−4.5		−9.1	
Hemoglobin	2.7	3.2	3.8	3.2	2.8	3.1	0.5	−0.3	2.0	1.5
Serum albumin	2.2	2.0	3.0	2.4	4.8	3.6	−1.5	10.0	4.1	15.0
Citrate synthase	1.7		4.1	2.9	3.0	2.9	−3.0		−9.9	−0.4
GPD	1.9		2.0	2.0	2.2	2.0	−0.4		0.3	−0.7
Lactate dehydrogenase	2.4		3.8	3.1	5.1	3.8	−2.9		−0.2	−3.8
Aldolase	3.3		3.8	3.6		3.6	−0.3		−1.3	
Nitrogenase MoFe	5.3			5.3		5.3	0.0			
Catalase	5.6		5.6	5.6	5.5	5.6	−0.1		−0.2	0.6
Mean value				3.1	3.3	3.2	2.1	5.8	5.1	2.2

Results for the AER's, a , of each protein, and percent difference between the properties calculated for $a_{\text{hyd}} = 3.3 \text{ \AA}$ and the experimental values.

hydrodynamics. This is in agreement with recent, independent findings by Perkins [18]. Thus it is valid to employ a single value for the effective radius a . Looking in Table 1 for the a values of the various proteins, we find an average of $a = 3.2 \text{ \AA}$, which will be considered as a representative or *standard* hydrodynamic radius of the atoms to be used in the HYDROPRO predictions of hydrodynamic properties. When the various properties of all the proteins are recalculated with this standard value, the results deviate from experimental values typically about 2% for translation (sedimentation and diffusion), 6% for rotation and 5% for intrinsic viscosities. These discrepancies between predictions and experiments are quite small, in relation to the order of the uncertainties of the experiments.

Recently, we have conducted a similar study of another hydrodynamic property, the NMR correlation time, τ_c , (other times denoted as harmonic-mean relaxation time, τ_h) of small globular proteins, for which a collection of experi-

mental data is available [19]. This rotational quantity can be evaluated from HYDRO in a way analogous to that of the other properties [10]. Indeed, we have published a variant of the HYDROPRO program, named HYDRONMR [8], which evaluates not only the correlation time, but also other quantities relevant in dynamic NMR, such as residue-specific spin–spin and spin–lattice relaxation times. Proceeding with, τ_c as with the other solution properties we obtain the results reported in Table 2, which shows the very same trend already noticed in Table 1 for the other properties. The fitted values of a , differ somehow from protein to protein, but have an average, standard value practically coincident with the previous one, and when this is used for the calculation, the expected errors are around 10%, not worse than typical experimental errors.

3.3. Estimation of hydration parameters

Both the hydration ratio δ (g/g) and the thick-

Table 2
NMR correlation time, τ_c , for a set of 15 proteins

Protein	PDB file	M(KD)	τ_c (20°C)	a	τ_c (20°C)	%dif.
Savinase	1svn	26.70	12.4	2.6	13.67	10.3
HIV-1 protease	1byg	21.58	13.2	3.1	13.73	4.0
Leukemia inh. factor	1lki	19.10	14.9	4.3	12.73	−14.5
Interleukin-1 β	6i1b	17.40	12.4	3.6	11.88	−4.2
Staphylococcal Nucl.	1stn	15.51	13.3	4.7	10.30	−22.6
Lysozyme	1hwa	14.32	8.3	3.1	8.60	3.6
Trp-repressor	1wrt	11.89	23.1	2.3	26.29	13.6
Barstar C40/82A	1bta	10.14	7.4	4.2	6.20	−16.3
Cytochrome b_5	1wdb	9.61	6.1	2.8	6.60	8.2
Ubiquitin	1ubq	8.54	5.4	3.0	5.87	8.8
Calbindin-D9k apo	1clb	8.43	4.9	2.8	5.44	11.0
Calbindin-D9k apo	2bca	8.43	5.1	3.2	5.16	1.1
Eglin c	1egl	8.15	6.2	3.2	6.28	1.3
BPTI	1pit	6.16	4.4	3.0	4.73	7.6
Xfin-Zinc Finger DBD	1znf	2.93	2.4	3.8	2.15	−11.4
Mean value				3.3		9.2

Experimental values and calculations for atomic-level bead-models. Fitted values of a and errors in calculated results for $a = 3.3\text{\AA}$.

ness of the hydration layer, τ_h , can be obtained from the bead models with atomic resolution. From the V (volume) vs. a plots, we can determine a_{anh} for V_{anh} , as indicated above (Fig. 4b). Also, from the hydrated radius, a_{hyd} (a in

Tables 1 and 2), we can also evaluate V_{hyd} (Fig. 4b). Then, combining the hydrated and anhydrous volume as in Eq. (3), the δ parameter is evaluated.

On the other hand, for each protein we have obtained the effective anhydrous radius, with results very close to the typical van der Waals radius of the protein atoms. For the 19 proteins in Table 1 the results can be condensed as $a_{\text{anh}} = 2.1 \pm 0.1 \text{\AA}$, where we note that the standard deviation is very small. Subtracting, for each protein, the two radii, we obtain the thickness of the hydration layer (Fig. 3.b) as:

$$t_h = a_{\text{hyd}} - a_{\text{anh}} \quad (8)$$

For the proteins in Table 1, results for δ and t_h are given in Table 3, indicating their mean and the standard deviation. Each of these two statistical parameters has its own special significance.

The mean value of δ is approximately 0.3 g/g. This estimate is in perfect agreement with those from the classical treatment of protein hydrodynamics in terms of ellipsoidal models, of which a number of examples are available. A specific result from the atomic bead model is the hydration

Table 3
Hydration parameters from atomic-level bead-modelling calculations.

Protein	t_h (\AA)	δ (g/g)
BPTI	1.5	0.66
Ribonuclease A	0.9	0.36
Lysozyme	0.9	0.33
Myoglobin	0.4	0.14
Trypsin	1.3	0.36
β -Lactoglobulin	0.3	0.10
Pepsin	0.6	0.07
Subtilisin	0.8	0.19
Hemoglobin	1.0	0.27
Serum Albumin	0.3	0.17
Lactate dehydrogenase	0.6	0.13
Nitrogenase MoFe	3.4	0.56
Aldolase	1.4	0.31
Catalase	3.8	0.54
Mean value	1.2	0.30
S.D.	1.0	0.18

thickness, for which the mean is approximately 1.2 Å. This scarcely amounts to a monolayer of water molecules surrounding the protein, which is in agreement with recent estimates from bead-model and finite-element calculations [20–23]. Thus, the predictive capability of our procedure is complemented by such a consistent description of hydration.

4. Discussion and conclusions

4.1. Variability in the calculated hydration parameters

A remarkable aspect of the results in Table 3 remains to be discussed: the variability in the values δ and t_h from protein to protein, clearly perceived in the list of values, and manifested in the large value of their standard deviation (some fluctuations were noticed in the values of hydrodynamic sizes in Tables 1 and 2). As the evaluation of δ and t_h are both made by difference between two values (hydrated and anhydrous) that are not too far apart, fluctuations or uncertainties are amplified in the subtraction.

One could speculate about possible differences in the extent of hydration from protein to protein. In order to reach a firm conclusion about this hypothesis, other influences in the hydration parameters should be considered. Clearly, one of the aspects could be the theoretical and computational procedures themselves; in spite of the detailed modelling and the advanced hydrodynamics that have been employed, their residual imperfections could produce some influence in the final values of the hydration parameter. However, we think that there is a much more primary and influential source that comes from the errors of the experimental data used to fit the parameters.

4.2. Influence of experimental errors on the hydration parameters

In order to show that experimental errors can be by themselves responsible for the fluctuations in δ and t_h , we leave now the bead-model description of complex shapes, and restrict the

numerical analysis to a spherical particle, for which the hydrodynamic theory is exact. For the sphere, the anhydrous radius r_{anh} is obtained from V_{anh} given in Eq. (1). From expressions for each of the considered solution properties [R_g , D_t , s , (η) , τ_c , etc.], expressions can be derived for the hydrated radius r_{hyd} , and from it V_{hyd} can be obtained. Thus, final expressions for δ and t_h can be formulated in terms of each solution property. As examples, we give those for the sedimentation coefficient:

$$\delta = \bar{v}\rho \left[\frac{4\pi N_A}{3M\bar{v}} \left(M \frac{(1 - \bar{v}\rho)}{6\pi\eta_0 N_A s} \right)^3 - 1 \right] \quad (9)$$

$$t_h = \frac{M(1 - \bar{v}\rho)}{6\pi\eta_0 N_A s} - \left(\frac{3\bar{v}M}{4\pi N_A} \right)^{1/3} \quad (10)$$

and from the rotational correlation time

$$\delta = \bar{v}\rho \left(\frac{kT\tau N_A}{\eta_0 M\bar{v}} - 1 \right) \quad (11)$$

$$t_h = \left(\frac{3kT\tau}{4\pi\eta_0} \right)^{1/3} - \left(\frac{3\bar{v}M}{4\pi N_A} \right)^{1/3} \quad (12)$$

The equations (9)–(12) illustrate that the hydration parameters depend on a number of solvent and solute properties, and it is clear that errors in these properties can accumulate in the final values of the parameter. The error in the results due to errors in the data can be estimated using numerical techniques for error propagation. Suppose, for instance that we wish to determine the influence of experimental errors on the determination of t_h from the sedimentation coefficient. According to Eq. (10), t_h is a function of various quantities: $t_h \equiv t_h(M, \bar{v}, \rho, T, s)$. Then the error Δt_h in t_h could be estimated from the errors in the intervening quantities as

$$\Delta t_h = \left| \frac{\partial t_h}{\partial M} \right| \Delta M + \left| \frac{\partial t_h}{\partial \bar{v}} \right| \Delta \bar{v} + \left| \frac{\partial t_h}{\partial \rho} \right| \Delta \rho + \left| \frac{\partial t_h}{\partial T} \right| \Delta T + \left| \frac{\partial t_h}{\partial s} \right| \Delta s \quad (13)$$

However, due to the complexity of equations like (9)–(12), the algebraic calculus is cumbersome, and we have adopted an alternative numerical simulation method. The steps are as follows. (1) Fix a ‘true’ values M , \bar{v} , ρ , and T . Fix ‘true’ value of hydration parameter, for instance t_h . Calculate true hydrodynamic property, for instance s . (2) Assign ranges of experimental errors: ΔM , $\Delta \bar{v}$, $\Delta \rho$, ΔT , and Δs . (3) Do the following many times: (3a) Assign to M a random value with Gaussian distribution of mean M and std. deviation ΔM . Do the same for \bar{v} , ρ , M , s and T (the solvent viscosity η_0 is determined by T). (3b) Calculate individual value of t_h . (4) Make a simple statistics of the values so generated; the final result is their mean, and their standard deviation is taken as Δt_h . This can be done for the two hydration parameters, δ and t_h , and for the various properties: s , D , τ , $[\eta]$.

Suppose that there were a perfectly spherical protein (let us call it *spherin*) having a moderate molecular weight, $M = 40$ kDa and common values of the hydration parameters $t_h = 1.5$ Å or $\delta = 0.3$ g/g. We take $M \pm \Delta M = 40\,000 \pm 400$ (1%), $\bar{v} \pm \Delta \bar{v} = 0.730 \pm 0.005$ cm³/g (<1%), $T \pm \Delta T = 293.16 \pm 0.50$ K, and $\rho = 1.00$ g/cm³ (exact). Taking for the solution properties two choices of the errors as indicated in Tables 4 and 5, we obtained the results indicated there. The correct values of t_h and δ of *spherin* are recovered, as expected, as the mean values of the simulated results. can predict as arising from the reasonably assumed errors in the experimental data. These uncertainties depend obviously on the choice of experimental errors, and are more sensitive for some properties. As indicative val-

ues, we may take $\Delta t_h \approx \pm 1$ Å and $\Delta \delta \approx \pm 0.15$ (g/g), so that the values found for the hydration parameters would be in the ranges $t_h = 3 \pm 1 = 2\text{--}4$ Å and $\delta = 0.30 \pm 0.15 = 0.15\text{--}0.45$ g/g. As seen in Table 3 for our atomic-level bead-model calculation, and noted by other authors for the δ values [2–4], most of the values found for globular proteins fall within this range. In conclusion, the differences in the values of the hydration parameter from protein to protein may be just a consequence of the numerical errors of the experimental data.

5. Concluding remarks

The estimation of hydration from hydrodynamic properties is sensitive to several types of errors because the extent of hydration is determined as the difference between two quantities that are not much different, namely the hydrated and anhydrous sizes. We have shown that the main source of uncertainty in the estimation of hydration from hydrodynamic parameters may be experimental errors in the data of the hydrodynamic and other solution properties. Actually, many of the tabulated data for common proteins are up to 50 years old, and it is evident that, for a quantitative, more accurate evaluation of hydration, newer and more precise data are required.

From another point of view, hydration is an effect that one has to consider in the prediction of solution properties from the macromolecular size and shape. The most commonly used value of δ , 0.3 g/g, can be safely used for calculations based on ellipsoidal models (or, more generally,

Table 4
Estimation of the errors in t_h and δ for the indicated estimates of the experimental errors

Property	Relative error In property	Recovered t_h	Recovered δ
From D	$\Delta D/D = \pm 3\%$	1.52 ± 0.78	0.31 ± 0.10
From s	$\Delta s/s = \pm 3\%$	1.53 ± 0.93	0.31 ± 0.12
From $[\eta]$	$\Delta[\eta]/[\eta] = \pm 5\%$	1.51 ± 0.43	0.30 ± 0.06
From τ	$\Delta\tau/\tau = \pm 5\%$	1.51 ± 0.42	0.30 ± 0.05

Table 5

Estimation of the errors in t_h and δ for the other estimates of the experimental errors

Property	Relative error in property	Recovered t_h	Recovered δ
From D	$\Delta D/D \pm 6\%$	1.59 ± 1.46	0.32 ± 0.19
From s	$\Delta s/s \pm 6\%$	1.61 ± 1.57	0.32 ± 0.21
From $[\eta]$	$\Delta[\eta]/[\eta] \pm 10\%$	1.47 ± 0.80	0.30 ± 0.11
From τ	$\Delta\tau/\tau = \pm 10\%$	1.48 ± 0.82	0.30 ± 0.12

in uniform expansion approaches. The recent advances in bead-model hydrodynamics has made it now possible to predict the hydrodynamic properties of proteins from atomic-level structures, using the computer programs HYDROPRO [7] and HYDRONMR [8]. In these calculations, one has to take a value of the effective hydrodynamic radius, a , of the protein atoms, that must be larger than the van der Waals values ($\approx 2 \text{ \AA}$) due to hydration. The present study indicates that with $a \approx 3 \text{ \AA}$ the predictions of our procedure are in very good agreement with the experimental data, well within the experimental errors.

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