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COMMUNICATIONS

HYDRONMR: Prediction of NMR Relaxation of Globular Proteins from Atomic-Level Structures and Hydrodynamic Calculations

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The heteronuclear NMR relaxation of globular proteins depends on the anisotropic rotational diffusion tensor. Using our previous developments for prediction of hydrodynamic properties of arbitrarily shaped particles, by means of bead models, we have constructed a computational procedure to calculate the rotational diffusion tensor and other properties of proteins from their detailed, atomic-level structure. From the atomic coordinates file used to build the bead model, the orientation of the pertinent dipoles can be extracted and combined with the hydrodynamic information to predict, for each residue in the protein, the relaxation times. All of these developments have been implemented in a computer program, HYDRONMR, which will be of public domain. © 2000 Academic Press

Key Words: **quasirigid atomic structure; NMR relaxation; hydrodynamics; rotational diffusion; correlation and relaxation times.**

1. INTRODUCTION

In addition to the well-known capabilities of NMR spectroscopy as a powerful structure-determination tool, the measurement of heteronuclear relaxation can be particularly helpful for solving the complex, tridimensional structure of biological molecules (*1, 2*). This is, essentially, a consequence of two concomitant aspects. One of them is the dependence of the relaxation rates on the dynamics of the macromolecule in solution. In the particular case of quasirigid protein molecules, the dynamics can be regarded as a superposition of global reorientational dynamics of the whole particle and internal dynamics at a more local level. In such cases, the two classes of dynamics take place in separate time scales and, as learned from the model-independent treatment (*3, 4*), they can be readily separated out (*5*). The overall rotational dynamics of the quasirigid structure can be expressed in terms of a single quantity, the correlation time, which is derivable from the ratio of longitudinal and transversal relaxation times (*6, 7*). For nonspherical proteins, rotational dynamics is governed by a tensorial quantity, the rotational diffusion tensor, $\mathbf{D}_{rr}$, and methods have been recently proposed for the determination of its components. From our knowledge of rigid-body hydrodynamics (*8–10*), it is accepted that, among the various hydrodynamic properties of rigid particles, those related to rotational diffusion are most sensitive to size and shape. These aspects of overall macromolecular structure are traditionally investigated using other, standard hydrodynamic techniques such as sedimentation velocity, translational diffusion measurements, solution viscometry, and rotational decay of birefringence of fluorescence anisotropy.

Since the pioneering studies of Bloomfield and co-workers (*11, 12*), the hydrodynamic behavior of rigid particles can be modeled and computed using bead models, composed by spherical frictional elements. The theoretical and computational aspects of this modeling procedure have evolved over the years (*8, 13–15*) and have gained further popularity since the publication of our public-domain software packages HYDRO (*16*) and SOLPRO (*17, 18*) (the latest version of SOLPRO (*18*) already includes a primary calculation of NMR relaxation). On the other hand, over these years, the amount of three-dimensional, atomic-level structural information on macromolecules has grown enormously. Thus, the prediction of hydrodynamic properties from bead models based on those structures is a promising possibility. However, special care has to be taken when applying continuous hydrodynamics at the atomic level. Also, some problematic aspects of bead-modeling calculations, such as bead overlapping and volume corrections (*10, 19, 20*), may influence the calculations, particularly those of rotational properties.

In a recent paper (*10*) we discussed the various procedures for bead modeling, pointing out that the particular strategy known as shell modeling, as originally proposed by Bloomfield and co-workers (*11, 21, 22*), is the most appropriate one for the description of fine details of macromolecular structure. In a subsequent work (*23*) we have shown how this strategy can be applied to predict hydrodynamic properties of quasirigid, globular proteins from their atomic structure. Our methodology avoids the above-mentioned problematic aspects [which other authors pursuing the same goal may have incurred somehow (*23*)] and is able to predict simple hydrodynamic coefficients, such as the translational diffusion and sedimentation coeffi-

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cients and intrinsic viscosity, with remarkable precision. This is the methodology that we propose in this paper for the calculation of the full rotational diffusion tensor of the quasi-rigid structures of globular proteins.

The components of the rotational diffusion tensor contain detailed information on the *external* shape of the macromolecular particle. The second, concomitant structural aspect, mentioned at the begin of this Introduction, refers to structure at an even more local, *internal* level. Recently, heteronuclear relaxation NMR studies of proteins permit the characterization of the longitudinal and transverse relaxation times T_1 and T_2 for each individual amino acid residue within the protein [for recent examples, see (*24, 25*)]. The T_2/T_1 ratios, in which the effects due to an eventual presence of internal motion at the residue are eliminated, depend not only on the overall, anisotropic rotational diffusion tensor but also on the orientation of the amide N–H or C–H bond vector with respect to the eigenvectors of rotational diffusion. Thus, a list of the T_2/T_1 ratios for each residue has great information content about the structure of the protein.

We have developed a computer program, HYDRONMR, whose main input (in addition to simple constants about the protein and the NMR experiment) is the atomic coordinates contained in a protein data bank (PDB) file or similar format. The program builds an appropriate hydrodynamic model of the protein and computes the fully anisotropic rotational diffusion tensor. A primary NMR quantity, the correlation time, is obtained from this tensor. Then, HYDRONMR proceeds, again reading the PBD file to extract the vectors or the bonds involved in the dipolar relaxation, and for each residue it calculates T_1, T_2, the nuclear Overhauser effect (NOE), and the T_2/T_1 ratio. We also suggest a procedure for using these ratios in a way that is practically independent of choice of some hydrodynamic parameters.

2. THEORY, MODELS, AND METHODS

2.1. Rigid-Body Rotational Diffusion

In this section we give a brief summary of rigid-body rotational diffusion. For more details we refer the reader to our extended description in Ref. (*18*). The rotational diffusion of a rigid body is governed by the rotational diffusion tensor, $\mathbf{D}_{\rm rr}$. This can be obtained from exact formulas in a few cases (ellipsoidal particles), and for an arbitrarily shaped particle it can be calculated using bead modeling methods (see below). $\mathbf{D}_{\rm rr}$ can be expressed in terms of its three eigenvalues D_1, D_2, and D_3, and the corresponding eigenvectors, which give the three principal directions of the tensor. In many instances, like in dynamic NMR, rotational diffusion is observed from the time decay of a correlation function $\langle P_2(t)\rangle \equiv \langle 3(\mathbf{u}(t)\cdot\mathbf{u}(0))^2 - 1\rangle/2$ of some vector $\mathbf{u}$ within the particle. It can be shown (*26, 27*) that

$$\langle P_2(t)\rangle = \sum_{l=1}^{5} a_l \exp(-t/\tau_l), \qquad [1]$$

where the five rotational relaxation times are $\tau_1 = (6D_{\rm r} - 2\Delta)^{-1}$, $\tau_2 = (3(D_{\rm r} + D_1))^{-1}$, $\tau_3 = (3(D_{\rm r} + D_2))^{-1}$, $\tau_4 = (3(D_{\rm r} + D_3))^{-1}$, and $\tau_5 = (6D_{\rm r} + 2\Delta)^{-1}$, determined by the mean of the three eigenvalues, which is the (scalar) rotational diffusion coefficient,

$$D_{\rm r} = (1/3)\mathrm{Tr}\,\mathbf{D}_{\rm rr} = (1/3)(D_1 + D_2 + D_3), \qquad [2]$$

and their anisotropy, $\Delta = (D_1^2 + D_2^2 + D_3^2 - D_1D_2 - D_1D_3 - D_2D_3)^{1/2}$. The amplitudes, a_l, depend on the components of the unitary vector along the dipole that is being monitored; their expressions are not listed here for the sake of brevity; they can be found elsewhere (*18, 25, 27, 28*).

In NMR, dynamics is observed in the frequency domain, and the pertinent function is the spectral density, $J_0(\omega)$, that can be directly obtained as the Fourier transform of $\langle P_2(t)\rangle$ as

$$J_0(\omega) = \int_0^\infty \langle P_2(t)\rangle \cos(\omega t)\,dt \qquad [3]$$

(this definition differs by a factor of 2/5 from the one used by other authors). For the rigid, anisometric particle, $J_0(\omega)$ is a sum of up to five Lorentzians:

$$J_0(\omega) = \sum_{k=1}^{5} \frac{a_k\tau_k}{1 + \tau_k^2\omega^2}. \qquad [4]$$

If the particle is spherical (or more generally, an isometric body), or if it is considered to be spherical just as an approximation, then the three eigenvalues are identical, $D_1 = D_2 = D_3 = D_{\rm r}$, and there is a simple relaxation time $\tau_{\rm sph} = 1/(6D_{\rm r})$, so that

$$J_0(\omega) = \frac{\tau_{\rm sph}}{1 + \omega^2\tau_{\rm sph}^2} \qquad [5]$$

and $\tau_{\rm sph} = \tau_{\rm mean}$, whatever the orientation of the dipole is.

Even when the particle is anisometric, there exists the possibility of expressing the rotational dynamics in terms of a single Lorentzian like in Eq. [5], with a single, effective relaxation time, $\tau_{\rm c}$:

$$J_0(\omega) = \frac{\tau_{\rm c}}{1 + \tau_{\rm c}^2\omega^2}. \qquad [6]$$

For a particle of arbitrary shape, $\tau_{\rm c}$ is given by the set of the five τ_k's:

$$\frac{1}{\tau_c} = \frac{1}{5}\sum_k \frac{1}{\tau_k} = 6D_r. \quad [7]$$

τ_c is denoted in other instances as τ_h because, as seen in Eq. [7], it is the harmonic mean of the five τ_k's. We employ here the notation τ_c because it is what in NMR terminology is termed the correlation time.

2.2. NMR Relaxation Times

The primary quantities determined in NMR relaxation are the spin–lattice and spin–spin relaxation times, T_1 and T_2, and the NOE. These quantities are related to values of the spectral density function, $J(\omega)$, for specific values of the frequency ω, that are combinations of the Larmor frequencies of the nuclei involved in the dipolar interaction, X (= ^{13}C or ^{15}N) and ^{1}H. Thus, T_1 and T_2 and the NOE are given by

$$\frac{1}{T_1} = d^2[J(\omega_X - \omega_H) + 3J(\omega_X) + 6J(\omega_X + \omega_H)] + c^2 J(\omega_X) \quad [8]$$

$$\frac{1}{T_2} = (1/2)d^2[4J(0) + J(\omega_X - \omega_H) + 3J(\omega_X) + 6J(\omega_H) + 6J(\omega_X + \omega_H)] + (1/6)c^2[4J(0) + 3J(\omega_X)] \quad [9]$$

$$\mathrm{NOE} = 1 + (\gamma_H/\gamma_X)d^2[6J(\omega_X + \omega_H) - J(\omega_X - \omega_H)]T_1, \quad [10]$$

where

$$d^2 = (1/10)(\mu_0/4\pi)\hbar^2\gamma_H^2\gamma_X^2 r_{XH}^{-6} \quad [11]$$

$$c^2 = (2/15)\omega_X^2(\Delta\sigma)^2. \quad [12]$$

In Eqs. [11] and [12], $\hbar = h/2\pi = 1.054510^{-27}$ erg · s, where h is Planck's constant; γ_X and γ_H are the gyromagnetic ratios of ^{1}H and X, respectively (2.6753×10^4 and -2.71×10^3 rad · s^{-1} · G^{-1}, respectively); ω_H and ω_N are the Larmor frequencies ($2\pi \times 600.13 \times 10^6$ and $2\pi \times 60.80 \times 10^6$ for ^{1}H and ^{15}N at a spectrometer frequency of 600 MHz); r_{HX} is the length of the X–H bond ($r_{HN} = 1.02 \times 10^{-8}$ cm for the N–H bond); $\sigma_\parallel - \sigma_\perp$ is the anisotropy of the chemical shift tensor of the X spin ($\sigma_\parallel - \sigma_\perp = -160$ ppm $= -160 \times 10^{-6}$ for ^{15}N). [The values given in parentheses correspond to the amide ^{15}N spin relaxed by dipolar coupling to the proton to which it is bound, at an operating frequency of 600 MHz, taken from Ref. (*28*).]

If the X–H bond belongs to an absolutely rigid structure, $J(\omega)$ is given by Eq. [4]. However, in most practical cases, the X–H bonds experience some amount of internal motion that is superimposed to the overall (anisotropic) rotational diffusion. For nearly rigid structures such as globular proteins or oligonucleotides, it is usually assumed that the time of scale of internal motions is faster, well separated from rotational diffusion. Thus, the effective relaxation time, τ_e, is appreciably smaller than the relaxation times of the rigid structure, $\tau_e \gg \tau_k$, and, according to the model-free approach (*3, 24*),

$$J(\omega) = S^2 J_0(\omega) + (1 - S^2)J_{int}(\omega), \quad [13]$$

where S is the order parameter in the model-free treatment ($0 < S < 1$, with S close to 1 if the amplitude of internal motion is small); $J_0(\omega)$ is the rigid-body spectral density function, given by Eq. [4], and $J_{int}(\omega) = \tau/(1 + \omega^2\tau^2)$, where τ is a combination of τ_e and the harmonic mean relaxation time of the rigid particle, τ_c (also denoted as τ_{iso}) (Eq. [7]), given by

$$\tau^{-1} = \tau_c^{-1} + \tau_e^{-1}. \quad [14]$$

More explicitly, we can write

$$J(\omega) = S^2 \sum_{k=1}^{5} \frac{a_k\tau_k}{1 + \omega^2\tau_k^2} + (1 - S^2)\frac{\tau}{1 + \omega^2\tau^2}. \quad [15]$$

In order to gain information on the overall structural of the macromolecule, the effect of internal motion can be eliminated: the ratio T_1/T_2 is given by (*1, 2*)

$$T_1/T_2 = \{4J_0(0) + J_0(\omega_X - \omega_H) + 3J_0(\omega_X) + 6J_0(\omega_H) + 6J_0(\omega_X + \omega_H) + (c^2/d^2)[4J_0(0) + 3J_0(\omega_X)]\}/\{2J_0(\omega_X - \omega_H) + 6J_0(\omega_X) + 12J_0(\omega_X + \omega_H) + 2(c^2/d^2)J_0(\omega_X)\} \quad [16]$$

and depends only on the relaxation times for overall anisotropic rotational diffusion.

Hydrodynamic calculations, such as the bead modeling procedure (*8, 16, 10*), provide the eigenvalues and eigenvectors of the anisotropic rotational diffusion tensor. From the eigenvalues, the five τ_k's are calculated. From the eigenvectors, knowing the orientation of the X–H bond, the amplitudes are also calculated, so that $J_0(\omega)$ can be obtained for any value of ω. This, along with the other NMR constants, allows the calculation of the T_1/T_2 ratio for any X–H bond within the macromolecule.

As introduced above, a simplified treatment of NMR relaxation of proteins is possible in terms of an overall relaxation time, τ_c (Eq. [7]). The fact that the global shape of globular proteins is approximately spherical gives some support to this approach. Then, it can be shown that the general expression for T_2/T_1 (Eq. [16]) reduces to a polynomial of fifth order in τ_c (*6*). Thus, an experimental τ_c value can be extracted from T_2/T_1

data. An extensive, useful list of τ_c values for 75 proteins has been recently published (*7*).

2.3. Hydrodynamic Models for Proteins

The procedure for modeling globular proteins from a list of atomic coordinates (PDB file) has been described in detail in our previous, recent publication (*23*), as an adaptation to this problem of the general procedures that we have developed for building shell models of arbitrary structures (*10*).

We give here just a brief summary of the essential aspects. A primary hydrodynamic model (PRH) model is constructed first, replacing each nonhydrogen atom by a spherical element, having a radius, a, which we call the atomic element radius (AER). As we have discussed elsewhere, a lower bound for this radius should be the (typical) van der Waals radii of the atoms (1.8–2 Å), which determine the volume occupied by the molecule *in vacuo*. However, as a consequence of hydration, one should expect larger values of the AER; the difference between the AER and the van der Waals radius would amount to the thickness of the hydration shell. The AER is in principle considered an adjustable parameter; in the calculations, it is varied typically in the range $a = 2$–5 Å. Figure 1A displays the PRH for lysozyme.

Although some authors (*29*) have reported calculations with our HYDRO software (*16*) for PRH models, we think that this practice is nonadvisable or even risky, for several reasons. As the distance between spherical elements for neighbor (bonded) atoms is about 1.5 Å (the typical bond length), the spherical radius for the larger a's is much larger, which gives rise to an exaggerated amount of overlap. As discussed elsewhere (*20*) the hydrodynamic theory and our HYDRO program contain some provisions for moderate, occasional overlapping, but the performance of the procedure when overlapping is so extensive it is not guaranteed. This is due to difficulties in the description of hydrodynamic interactions and in the determination of the volume correction. Another drawback of PRH models is that the number of elements, N, is equal to the number of nonhydrogen atoms. As the HYDRO CPU time is proportional to N^3, this procedure may be applicable for small proteins, but it is not feasible for proteins with molecular weights over, say, 50 kDa.

We propose that these difficulties can be avoided if the PRH model is in turn replaced by a shell model. In shell models (*11, 21, 22*), the surface of an arbitrarily shaped particle is represented by a shell of tangent, small beads of radius σ. The resulting model, in the example of lysozyme, is shown in Fig. 1B. The hydrodynamic calculations are carried out for various values of σ and extrapolated to $\sigma = 0$. In our previous paper (*10*) we have described the procedures and computer programs for constructing the shell models and for the extrapolations. These procedures are readily applied to the present case in which the particle, i.e., the PRH, is the union (in the mathematical sense) of a set of overlapping spheres. HYDRONMR includes our own subroutines for shell modeling and extrapolation.

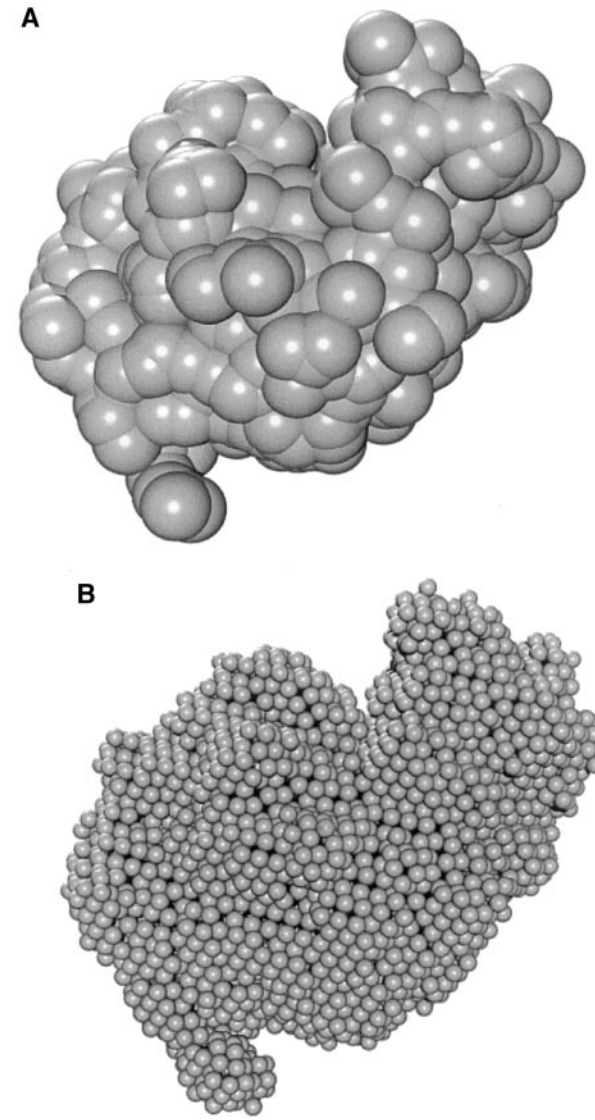

FIG. 1. (A) Primary hydrodynamic (PRH) model of lysozyme, with $a = 3$ Å. (B) Shell model, with $\sigma = 0.8$ obtained from the PRH model.

2.4. Hydrodynamic Calculations

The procedure for the calculation of hydrodynamic properties is based on the well-known bead-model methodology originally proposed by Bloomfield and co-workers (*11, 12*), further developed by Garcia de la Torre and co-workers (*8–10, 13, 14*) and implemented in the computer program HYDRO (*16*). We have recently presented a compilation of the underlying theory (*10*).

At the core of HYDRONMR there is a simplified version of HYDRO (*16, 30*) in which some features or options of the general version are removed or preset. Thus, we have suppressed the calculation of the sedimentation coefficient and the intrinsic viscosity, which requires data for molecular weight and the partial specific volume of the macromolecule. These properties are less important for the present purpose; the sedimentation coefficient is somehow equivalent to the transla-

tional coefficient and, for globular (protein) structures, the intrinsic viscosity is noticeably insensitive to size and shape. Still, HYDRONMR gives the full 6×6 diffusion tensor $\mathbf{D}$ (*8, 10*), which contains the 3×3 tensors that describe translational diffusion, $\mathbf{D}_{tt}$, translational-rotation coupling, $\mathbf{D}_{tr}$, and rotational diffusion, $\mathbf{D}_{rr}$. The diffusion center (*10, 31*) is also provided. These properties have a variety of applications, including some in NMR techniques. As mentioned above, the rotational diffusion coefficients and relaxation times are derived from $\mathbf{D}_{rr}$. It is noteworthy that translational diffusion coefficients can be determined by NMR (*32–34*). Thus, HYDRONMR also evaluates the D_t as

$$D_t = (1/3)\mathrm{Tr}(\mathbf{D}_{tt}). \qquad [17]$$

It is pertinent to recall here that rotational diffusion, albeit being so structure-sensitive (and essential for dynamic NMR), presents difficulties for bead-model theory development and computations that have been reported (*35, 36*), approximately corrected in terms of the so-called volume correction (*19*) and, finally, fully understood and avoided although at the cost of some extra computing time (*37*). It has been recently shown (*10*) that for the shell-modeling strategy employed in this work, the volume correction for rotational diffusion is misleading (or at least unnecessary). Therefore, in HYDRONMR the volume correction is simply ignored.

3. RESULTS AND DISCUSSION

3.1. Rotational Correlation Times

As mentioned above, the rotational correlation time τ_c is an useful indicator of the overall, rotational diffusivity of the macromolecule. Experimentally, it can be determined from T_1/T_2 ratios using the polynomial relationships between this ratio and τ_c (*6*). This procedure has been applied recently to an extensive set of proteins by Krishnan and Cosman (*7*). We shall use some experimental values from the compilation by these authors. We standardize values at different temperatures by referring them to 20°C, using the transformation

$$\tau_c(20°\mathrm{C}) = \frac{\eta_{20}}{\eta_t}\,\frac{(273 + t)}{293}\,\tau_c(t°\mathrm{C}), \qquad [18]$$

where η_t is the viscosity of water at the Celsius temperature t, which can be obtained within 0.5% of its true value (*38*) using the formula

$$\eta_t = 1.7753 - 0.0565t + 1.0751 \times 10^{-3}t^2 - 9.2222 \times 10^{-6}t^3. \qquad [19]$$

From hydrodynamic calculations, τ_c is extracted from the rotational diffusion tensors as the harmonic mean relaxation time, according to Eq. [7]. HYDRONMR gives the calculated value of τ_c, after shell modeling and extrapolation, from the PDB file for a given value of the AERs, a, in the PRH. The fitting of this parameter is done as described in our previous paper (*23*). First, calculations are carried out for $a = 2$, 3, 4, and 5 Å. The variation of τ_c with a is slight and nearly linear. Then, by interpolation in a graph of τ_c vs a, we obtain the value of a that fits the experimental value of τ_c. We have applied this procedure to a subset of 15 proteins of the large set of Krishnan and Cosman (*7*) that cover a broad range of molecular weight, from 2.93 to 26.7 kDa. The resulting values for the fitted AER are listed in Table 1. Most of the cases are fitted with values between $a = 2$ and 4 Å, with an average of $a = 3.3$ Å. The dispersion resulting value of the AER is small but, as we discuss next, it may be biophysically significant.

In their study on τ_c using a PRH model (*29*) fixed AER ($a = 5$ Å), Krishnan and Cosman (*7*) observed this variability in a related aspect: the calculated values are sometimes larger and sometimes smaller than the experimental ones. As discussed by these authors, an immediate origin (apart from other effects, like segmental mobility) of the variability is the assumed equivalence of the experimental τ_c and the theoretical τ_h, which may fail for highly anisotropic rotors. This observation is correct, but there can be an additional, significant reason. In a separate work (*23*) we have applied the same shell-modeling methodology employed here for globular proteins covering a much wider range of molecular weight (up to about 200 kDa). We calculated various hydrodynamic properties including translational diffusion and sedimentation coefficients, rotational diffusion, and intrinsic viscosity $[\eta]$. In our work, for a given protein, the fitted values of a for different properties were quite similar; however, from one protein to another we found a small but noticeable variability of a, around an average value of 3.3 Å. This average coincides very precisely with the one obtained here from τ_c. We have ascribed the variability to the different degree of hydration of different proteins, a fact that is classically admitted (*39–41*). The average AER is about 1.5 Å larger than the average van der Waals radius; this can be ascribed, on the average, to a monolayer of water molecules. Differences in the thickness of the hydration shell may be responsible in part for the fluctuations that we observe in a. If we take a common AER, $a = 3.3$ Å for all the proteins, we can calculate τ_c (directly with our program or simply, by interpolation in the set of results for 3, 4, and 5 Å) and compare the result with the experimental value. The average of the absolute percentage deviations, listed in Table 1, is 9%. With the τ_c calculations of Krishnan and Cosman (*7*) for this set of proteins, the deviation is slightly larger, about 14%.

Anyhow, differences between predicted and experimental τ_c for small proteins, like those studied by NMR, should be judged generously: a difference in global size of only 0.5 Å in an approximately spherical protein of radius 15 Å would introduce an uncertainty in the calculated τ of 10%.

The uncertainty or ambiguity introduced in the bead-model analysis of rotational diffusion by the choice of the AER parameter, a, can be removed somehow if another solution

TABLE 1
Experimental and Calculated Correlation Times

Protein	PDB file	M(KD)	τ_c(20°C) exp. (ns)	a (Å)	This work: τ_c(20°C) calc. (ns)	This work: % Diff.	Krishnan and Cosman: τ_c(20°C) calc., K-C	Krishnan and Cosman: % Diff.
Savinase	1svn	26.70	12.4	2.6	13.67	10.3	13.28	7.1
HIV-1 protease	1bvg	21.58	13.2	3.5	13.73	4.0	11.33	−14.2
Leukemia inh. factor	1lki	19.10	14.9	4.3	12.73	−14.5	11.31	−24.1
Interleukin-1β	6i1b	17.40	12.4	3.6	11.88	−4.2	10.31	−16.9
Lysozyme	1hwa	14.32	8.3	3.1	8.60	3.6	8.45	1.8
Trp-repressor	1wrt	11.89	23.1	2.3	26.29	13.6	24.36	5.3
Barstar C40/82A	1bta	10.14	7.4	4.2	6.20	−16.3	4.81	−35.0
Cytochrome b_5	1wdb	9.61	6.1	2.8	6.60	8.2	5.35	−12.3
Ubiquitin	1ubq	8.54	5.4	3.0	5.87	8.8	5.24	−3.0
Calbindin-D9k apo	1clb	8.43	4.9	2.8	5.44	11.0	4.77	−2.7
Calbindin-D9k apo	2bca	8.43	5.1	3.2	5.16	1.1	4.5	−11.8
Eglin c	1egl	8.15	6.2	3.2	6.28	1.3	5.45	−12.1
BPTI	1pit	6.16	4.4	3.0	4.73	7.6	4.48	1.8
Xfin-zinc finger DBD	1znf	2.93	2.4	3.8	2.15	−11.4	1.73	−28.8
Mean value				3.2		8.4		13.0

property is included in the analysis. An appropriate way to estimate the a value to be used for the calculation of relaxation times can be based on the translational diffusion coefficient, D_t, which, as commented above, is another outcome of dynamic NMR measurements. The variation of the calculated D_t with a is practically linear, and this estimation can be precisely made by linear intrapolation in a list of D_t vs a values (Table 2), where a would take, for instance the values $a = 2$, 3, and 4 Å. Choosing lysozyme as well-documented case, from the experimental $D_t = 10.9 \times 10^{-7}$ cm^2 s^{-1} (*42–45*), we obtain $a = 3.0$ Å, for which we interpolate $D_r = 2.04 \times 10^7$, i.e., $\tau_c = 8.16$ ns, in very good agreement with the experimental value, $\tau_c = 8.33$ (*46*).

A change in the value of a modifies the size of the model uniformly, while its shape remains unchanged. Two hydrodynamic properties can be combined into compound quantities which are size-independent, i.e., that remain constant when the particle is uniformly expanded. With D_t and D_r we can simply formulate the combination $D_r^{1/3}/D_t$. As noticed in Table 2, the calculated values of this quantity are practically independent of a and agree well with the value from experimental data.

TABLE 2
Rotational and Translational Diffusion Constants for Lysozyme

	Calculated			
	$a = 2$ Å	$a = 3$ Å	$a = 4$ Å	Experimental
$D_t \times 10^7$ cm^2 s^{-1}	11.5	10.9	10.2	10.9
$D_r \times 10^{-7}$ s^{-1}	2.38	2.04	1.67	2.0
$D_r^{1/3}/D_t \times 10^{-8}$ cm^{-2} s$^{2/3}$	2.50	2.51	2.51	2.49
$D_x \times 10^{-7}$ s^{-1}	2.37	1.94	1.64	
$D_y \times 10^{-7}$ s^{-1}	2.30	1.88	1.59	
$D_z \times 10^{-7}$ s^{-1}	3.41	2.66	2.21	
$2D_z/(D_x + D_y)$	1.460	1.397	1.367	
D_x/D_y	1.032	1.031	1.029	
Rhombicity factor	0.102	0.117	0.119	

Note. D_x, D_y, and D_z are the three eigenvalues, $D_r^{(1)}$, $D_r^{(2)}$. $D_r^{(3)}$, assigned so that (a) D_x and D_y are the pair of eigenvalues closest to each other, with $D_x > D_y$. The rhombicity factor is defined as $\frac{3}{2}(D_x - D_y)/(D_z - \frac{1}{2}(D_x + D_y))$.

Recently, the characterization of the fully anisotropic rotational diffusion, i.e., particularly the determination of the main components of the rotational diffusion tensor, is being attempted (*24, 25, 47*). Two or more such rotational quantities can be combined into some dimensionless form which, as illustrated in Table 2, is very insensitive to the value of a and depends essentially on the shape of the protein. This is illustrated with calculated values and experimental results (*24*) for HIV-1 protease in Table 3. It is clear that the values of ratios such as $2D_x/(D_y + D_z)$ or D_x/D_y depend very slightly on the a parameter. With the experimental τ_c we obtain for this protein $a \approx 3.5$ Å. The numerical values of the ratios that corresponding to this are in acceptable agreement with the experimental data (regarding the limitations of the rigid body assumption; see below). All of these quantities are evaluated by HYDRONMR.

3.2. T_1 and T_2 Relaxation Times and the $T_1/T2$ Ratio

As indicated above, the second stage in the HYDRONMR calculations begins with the extraction of the unitary X–H, vectors (X being the amide N or C-α) from the same PDB file (alternatively, a user-supplied file may be given). Then, the program calculates straightforwardly the values of T_1, T_2, NOE, and the T_1/T_2 ratio. This calculation only require as additional data the various NMR constants that determine the constants c and d in Eqs. [8]–[12]. A list of the values of T_1, T_2, NOE, and T_1/T_2 for each vector (for each amino acid

TABLE 3
Anisotropic Rotational Diffusion of HIV-1 Protease

	Calculated			
	a = 2 Å	a = 3 Å	a = 4 Å	Experimental (a)
τ_c (ns)	10.21	12.21	14.1	10.8
$D_x \times 10^{-7}$ s^{-1} [a,b]	1.412	1.188	1.036	
$D_y \times 10^{-7}$ s^{-1} [a,b]	1.355	1.149	1.001	
$D_z \times 10^{-7}$ s^{-1} [a,b]	2.130	1.785	1.507	
$2D_z/(D_x + D_y)$	1.540	1.528	1.480	1.37
D_x/D_y	1.042	1.034	1.035	1.08
cos θ [c]	0.98	0.98	0.98	0.99

[a] Referred to 20°C.
[b] D_x, D_y, and D_z, as indicated in Table 2.
[c] θ is the angle between the third eigenvector and the z axis of the PDB coordinates.

residue) is produced by the computer program. In Fig. 2 we present the results of such a calculation for lysozyme (which is chosen for this illustrative purpose because it has a quite large rotational diffusion anisotropy). As in the case of τ_c, calculations of these quantities are done for some values of the AER, a, on which all these quantities show a remarkable dependence, as illustrated in Fig. 2. This was to be expected; thus, the T_1/T_2 values depend primarily (apart from their fluctuation) on τ_c, which in turn depends on AER.

We have already indicated that the AER should be somewhere in the range a = 2–4 Å, with a consensus value of a = 3.3 Å. If a previous analysis of the value of τ_c has been made (eventually including D_t) and a has been fitted, it would be used for the calculation of T_1/T_2. Anyhow, the ambiguity in the choice of this parameter again poses some doubt in the final results. Fortunately, from our numerical results for various values of a we have discovered a trend that may be extremely useful for the joint analysis of computed and experimental results. If, rather than the ratios for each of the $(T_1/T_2)_i$ with $i = 1, \ldots N_{res}$, we employ their relative deviations, ∇_i,

$$\nabla_i = ((T_1/T_2)_i - \langle T_1/T_2 \rangle)/\langle T_1/T_2 \rangle \qquad [20]$$

with respect to the average over the N_{res} residues in the protein,

$$\langle T_1/T_2 \rangle = (1/N_{res}) \sum_i (T_1/T_2)_i, \qquad [21]$$

then the values of ∇_i are remarkably insensitive to the value of a. The series of ∇_i values for the successive residues of the protein contains a great deal of information regarding not only the detailed three-dimensional shape of the macromolecule, but also the disposition of the residues within it.

In order to compare HYDRONMR computed values of T_1/T_2 and ∇_i with experimental results, the user should bear in mind the validity and limitations of the underlying methodology. If the protein molecule were absolutely rigid, the results for τ_c and those for the individual components of the rotational tensor (i.e., the coefficients characterizing rotational diffusion anisotropy) would be most reliable, as it has been proved for other hydrodynamic coefficients. As described in the NMR relaxation literature, the effect of fast, small-amplitude motions is eliminated in the T_1/T_2. Discarding the residues for which this is not valid requires decisions based on a previous examination of the protein structure and the relaxation data. More problematic may be the existence of flexibility at a larger scale (hinge-bending motions, flexible tails, or protruding parts (*48*), etc.). Then not only would the T_1/T_2 and ∇_i values be affected, but also the apparent rotational diffusion anisotropy can be influenced (*48*). In such a case, other techniques (perhaps molecular dynamics simulation) will be needed to complement HYDRONMR. Another aspect to be recalled in this context is that the hydration-independent ∇_i values are close to zero because globular proteins are not too elongated (obviously, $\nabla_i = 0$) for an isotropic rotor. The experimental errors in T_1 and T_2 accumulate in T_1/T_2 and they are further propagated and enlarged in the values of ∇. So, the user should judge whether the experimentally observed variations of ∇ along the protein sequence will be significantly over those errors. On the basis that all of these limitations are properly considered, the

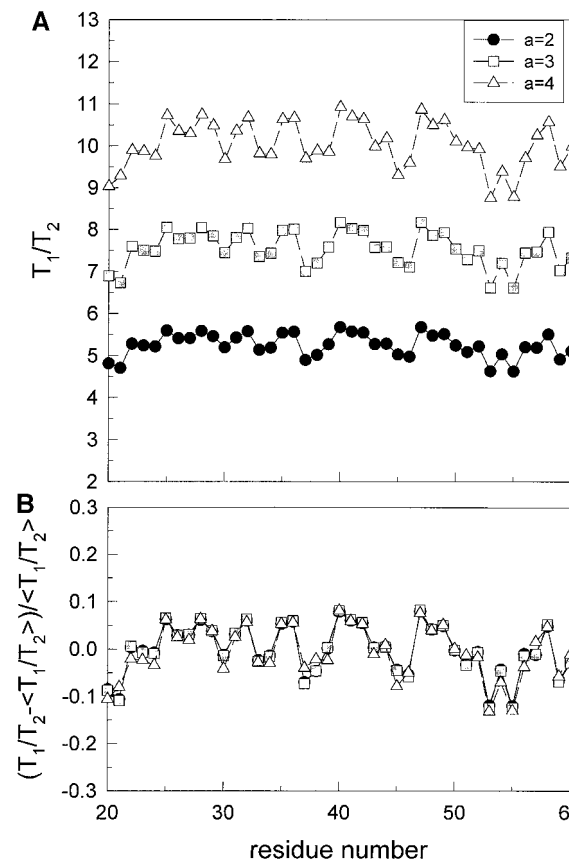

FIG. 2. Typical results from a HYDRORMN calculation of residue-specific T_1/T_2 ratios. (A) Calculated ratios of T_1/T_2 for residues 30–70 of lysozyme in ^{15}N NMR relaxation, at 600 MHz, with different values of the a parameter. (B) Results calculated for the quantities ∇_i (Eqs. [20] and [21]).

possibility of analyzing T_1/T_2 ratios in this way, using the computer program HYDRONMR, makes it a promising tool for structural characterization of proteins and other macromolecules from NMR relaxation.

4. CONCLUDING REMARKS AND COMPUTER PROGRAMS

We have presented HYDRONMR, a new computational tool for the prediction of translational and fully anisotropic, rotational diffusion coefficients and NMR relaxation times of quasirigid macromolecules. The calculation is made directly from the atomic coordinates, just taken from a PDB file. In this paper we describe the theory and, mostly, the modeling procedures that are implemented in HYDRONMR. The prediction of the simplest relaxation quantity, the correlation time, is tested for a number of proteins, and the results are discussed in terms of the choice of a hydrodynamic parameter; a standard value of about 3.3 Å may yield satisfactory values. On the basis that the protein shape is appreciably rigid and anisotropic, HYDRONMR enables the calculation of the residue-specific quantities T_1/T_2 and ∇. HYDRONMR allows for further calculation of the translational diffusion coefficient, which is of further help for model building and parameterization. Obviously, further work will be needed to assess the performance of this tool and to apply it to cases of special interest. In order to make HYDRONMR widely available, it will be of public domain, downloadable in the form of source code from our Web site, http://leonardo.fcu.um/macromol, where other computational tools for prediction of solution properties, including HYDRO, SOLPRO, and HYDROPROT, are also available.

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