Determining the Magnitude of the Fully Asymmetric Diffusion Tensor from Heteronuclear Relaxation Data in the Absence of Structural Information

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Heteronuclear relaxation data have been used to study internal dynamics and characterize the rotational anisotropy of macromolecules.[1] Recently, it has been shown that the dependence of heteronuclear relaxation times on rotational diffusion anisotropy can provide structural restraints for simulated annealing structure refinement that characterize long-range order *a priori*.[2] While a detailed analysis of internal dynamics requires that both the magnitude and orientation of the diffusion tensor be known,[3,4] only the magnitude of the tensor is required to derive information on the shape of the molecule and for structure refinement.[2] (In the latter case knowledge of the orientation of the diffusion tensor is not required since it is allowed to float during the calculations). If the structure is already known to high accuracy (≤1.8 Å resolution crystal structure), the six parameters that describe the magnitude and orientation of the diffusion tensor can be obtained by least squares optimization on the basis of the measured relaxation times and the orientations of the internuclear vectors.[3,4] In this paper, we describe a simple and robust procedure, based on an examination of histograms of heteronuclear T_1/T_2 ratios, to obtain the magnitude of the fully asymmetric diffusion tensor.

For the general case of rigid body anisotropic reorientation, the spectral density function, $J(\omega)$, in the limit of very fast internal motions, is given to a good approximation[5] by $J(\omega) = \boldsymbol{S}^2 C_k[\tau_k/(1 + \omega^2\tau_k^2)]$ where ω is the angular frequency; $\boldsymbol{S}$ is the generalized order parameter; τ_k are time constants that are functions of the elements D_{zz}, D_{yy}, and D_{xx} of the second rank diagonal diffusion tensor **D**, with D_{zz} defined as the unique component of **D**; and the coefficients $C_k = f_k(D_{xx}, D_{yy}, D_{zz}, \cos^2\theta, \sin^2\phi)$, where θ is the angle between the interactomic vector and the z axis of the diffusion tensor, and ϕ the angle between the projection of the interatomic vector on the $x-y$ plane and the x axis.[6] The effective correlation time $\tau_{c,eff}$ is given by $(2D_{zz} + 2D_{yy} + 2D_{xx})^{-1}$, the diffusion anisotropy A by $2D_{zz}/(D_{xx} + D_{yy})$, and the rhombicity η by $3/2(D_{yy} - D_{xx})/[D_{zz} - 0.5(D_{yy} + D_{xx})]$. The heteronuclear T_2 is a function of frequency-dependent and -independent terms, while the heteronuclear T_1 is only a function of the frequency dependent terms.[7] Thus the heteronuclear T_1/T_2 ratio is given by $f(\omega, \tau_{c,eff}, A, \eta, \cos^2\theta, \sin^2\phi)$. For an oblate ellipsoid, (i.e., $A < 1$ and $D_{xx} \geq D_{yy} > D_{zz}$), the minimum and maximum heteronuclear T_1/T_2 ratios always correspond to vectors lying along the z ($\theta = 0°$) and x ($\theta = 90°$, $\phi = 0°$) axes of the diffusion tensor, respectively. For a prolate ellipsoid (i.e., $A > 1$ and $D_{zz} > D_{yy} \geq D_{xx}$), the maximum heteronuclear T_1/T_2 ratio always corresponds to vectors lying along the z ($\theta = 0°$) axis of the diffusion tensor, but the value of θ corresponding to the minimum heteronuclear T_1/T_2 ratio depends on ω, $\tau_{c,eff}$, and A and very weakly on η. The ω and $\tau_{c,eff}$ dependence of the cutoff value of A below which the minimum ^{15}N T_1/T_2 ratio corresponds to vectors lying along the x axis is depicted in Figure 1. Thus, for $\tau_{c,eff} = 4$ ns, the cutoff value of A is 3.6, 4.1, and 4.7 at ^{1}H frequencies of 500, 600, and 750 MHz, respectively; for $\tau_{c,eff} = 10$ ns, the cutoff value of A is 5.9, 6.2, and 6.5 at ^{1}H frequencies of 500, 600, and 750 MHz. As A increases above these values, the minimum ^{15}N T_1/T_2 value shifts from vectors lying along $\theta = 90°$, $\phi = 0°$ to vectors lying at $\theta = 54.7°$, $\phi = 0°$ (i.e., θ is at the magic angle). Thus, for globular proteins (which invariably have anisotropies below these cutoff values) the minimum ^{15}N T_1/T_2 ratio will always correspond to vectors lying along the x axis.

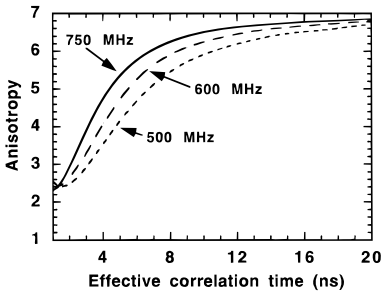

Figure 1. $\tau_{c,eff}$ and ω dependence of the cutoff value of the diffusion anisotropy A for a prolate ellipsoid ($A > 1$) below which the minimum and most probable value of ^{15}N T_1/T_2 coincide with the x and y axes of the diffusion tensor, respectively. Curves are shown for ^{1}H frequencies of 750 (—), 600 (– – –), and 500 (- - -) MHz and are calculated for the axially symmetric case ($\eta = 0$). The introduction of rhombicity results in a very small increase in the cutoff values of A. Thus, as $\tau_{c,eff} \rightarrow \infty$, the cutoff value for A is 6.99 for $\eta = 0$ and 7.12 for $\eta = 1$.

For this class of proteins, how can one find the heteronuclear T_1/T_2 ratio that corresponds to vectors lying along the third (i.e., y) principal axis of the diffusion tensor? By analogy with solid-state NMR line shapes, if the distribution of vectors is isotropic, one would expect this T_1/T_2 ratio to correspond to the maximum of the T_1/T_2 distribution function. This has been confirmed by numerical simulations. The distribution of heteronuclear T_1/T_2 values obtained by generating a million random, isotropically distributed vectors, does have a powder pattern-like appearance, and the most probable T_1/T_2 value does indeed coincide with the T_1/T_2 value at $\theta = 90°$, $\phi = 90°$. This is illustrated in Figure 2.

The values of $(T_1/T_2)_{zz}$ and $(T_1/T_2)_{xx}$ are obtained from the extreme values of the experimentally observed heteronuclear T_1/T_2 ratios. Thus, for a prolate ellipsoid ($A > 1$) the value of $(T_1/T_2)_{zz}$ is determined by taking the average of the cluster of high T_1/T_2 values within one experimental standard deviation of the maximum observed T_1/T_2 value. The same procedure is used to estimate $(T_1/T_2)_{xx}$ from the cluster of observed low T_1/T_2 values. The value of $(T_1/T_2)_{yy}$ is obtained from the maximum of the

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(1) (a) Torchia, D. A.; Nicholson, L. K., Cole, H. B. R.; Kay, L. E. In *NMR of Proteins*; Clore, G. M., Gronenborn, A. M., Eds.; MacMillan Press: London, 1993; pp 190−219. (b) Wagner, G.; Hyberts, S.; Peng, J. W. In *NMR of Proteins*; Clore, G. M., Gronenborn, A. M., Eds.; MacMillan Press: London, 1993; pp 220−257. (c) Palmer, A. G. III *Curr. Opin. Biotechnol.* **1993**, 4, 385−391.

(2) Tjandra, N.; Garrett, D. S.; Gronenborn, A. M.; Bax, A.; Clore, G. M. *Nature Struct. Biol.* **1997**, 4, 443−449.

(3) (a) Tjandra, N.; Feller, S. E.; Pastor, R. W.; Bax, A. *J. Am. Chem. Soc.* **1995**, *117*, 12562−12566. (b) Brüschweiler, R.; Liao, X.; Wright, P. E. *Science* **1995**, 268, 886−889. (c) Lee, L. K.; Rance, M., Chazin, W. J.; Palmer, A. G. III. *J. Biomol. NMR* **1997**, 9 287−298. (d) Phan, I. Q. H.; Boyd, J.; Campbell, I. D. *J. Biomol. NMR* **1996**, 8, 369−378.

(4) Tjandra, N.; Wingfield, P. T., Stahl, S. J.; Bax, A. *J. Biomol. NMR* **1996**, 8, 273−284.

(5) Lippari, G.; Szabo, A. *J. Am. Chem. Soc.* **1982**, *104*, 4546−4559.

(6) Woessner, D. E. *J. Chem. Phys.* **1962**, 36, 647−654.

(7) Abragam, A. *The Principles of Nuclear Magnetism*; Clarendon Press: Oxford, 1961.

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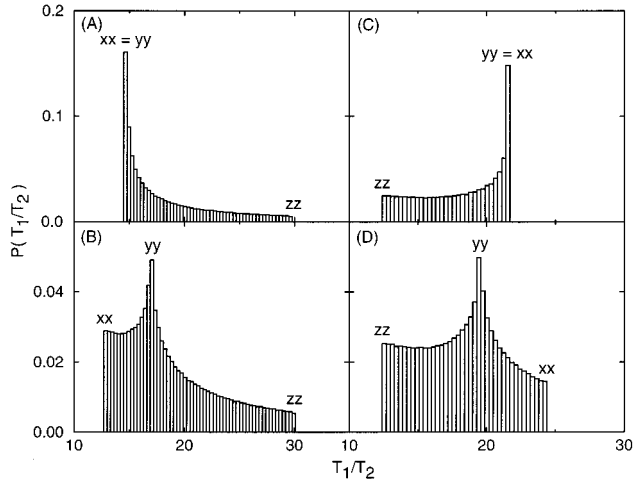

Figure 2. Theoretical distribution of ^{15}N T_1/T_2 values simulated for a million random, isotropically oriented vectors for prolate (A and B) and oblate (C and D) ellipsoids. $\omega_H = 2\pi \times 600$ MHz; $\tau_{c,eff} = 13$ ns; the anisotropy $A = 2$ in (A) and (B) and 0.5 in (C) and (D). The axially symmetric cases are shown in (A) and (C) and examples with a rhombicity η of 0.6 in (B) and (D).

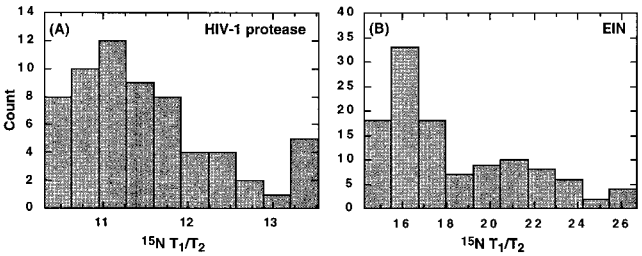

Figure 3. Experimental distribution of ^{15}N T_1/T_2 values for (A) HIV-1 protease complexed to DMP323 and (B) the N-terminal domain of enzyme I (EIN) measured at 600 MHz. The approximate rms error in the experimental T_1/T_2 ratios is ± 0.5 for HIV-1 protease and ± 1 for EIN. The values of $(T_1/T_2)_{xx}$, $(T_1/T_2)_{yy}$, and $(T_1/T_2)_{zz}$ are 10.6, 11.1, and 13.3, respectively, for HIV-1 protease, and 14.8, 16.3, and 26.5, respectively, for EIN. The experimental ^{15}N T_1/T_2 ratios, comprising 64 values for the HIV-1 protease complex[4] and 116 values for EIN[2], were obtained on perdeuterated ^{15}N-labeled protein. The data only comprises residues for which there is no significant internal motion.[2,4]

histogram of the observed heteronuclear T_1/T_2 ratios. With three unknowns ($\tau_{c,eff}$, A, and η) and three observables, $(T_1/T_2)_{zz}$, $(T_1/T_2)_{yy}$, and $(T_1/T_2)_{xx}$, the values of $\tau_{c,eff}$, A, and η (and hence D_{xx}, D_{yy}, and D_{zz}), are readily obtained by nonlinear least-squares optimization.

In practice, the number of heteronuclear T_1/T_2 ratios is limited, and the assumption of a uniform isotropic distribution of orientations need not necessarily apply. To illustrate how this approach fairs in practice, we have examined ^{15}N T_1/T_2 data from two proteins, HIV-1 protease, a dimer of 99 residues per subunit, complexed to the inhibitor DMP323,[4] and the N-terminal domain of enzyme I (EIN), a 259 residue monomer.[2] Figure 3 illustrates histograms of ^{15}N T_1/T_2 ratios obtained for the two proteins. A comparison of the results obtained using the present method with those of a six parameter nonlinear least-squares fit on the basis of the vector orientations derived from a known structure are summarized in Table 1 and demonstrates good agreement between the two methods.[9]

Table 1. Comparison of Parameters Characterizing the Magnitude of the Fully Asymmetric Diffusion Tensor Derived from ^{15}N Heteronuclear Relaxation Data by Analysis of a Histogram of ^{15}N T_1/T_2 Values and by a Best-Fitting Procedure Based on the N−H Vector Orientations in a Known Structure[a]

	HIV-1 protease/DMP323		N-terminal domain of enzyme I	
	histogram	structure-based[b]	histogram	structure-based[c]
$\tau_{c,eff}$ (ns)	10.4	10.6	13.2	13.1
A	1.25	1.35	1.70	1.46
η	0.33	0.46	0.27	0.10
D_{xx} (μs^{-1})	14.3	13.4	9.6	10.9
D_{yy} (μs^{-1})	15.1	14.8	10.9	11.2
D_{zz} (μs^{-1})	18.4	19.0	17.4	16.1

[a] The experimental ^{15}N T_1/T_2 data, displayed in Figure 3, are taken from refs 2 and 4. [b] Taken from ref 4 and calculated on the basis of the N−H vector orientations in the 1.8 Å resolution crystal structure of the HIV-1 protease/DMP323 complex.[8a] [c] Calculated on the basis of the N−H vector orientations in the 2.5 Å resolution crystal structure of EIN,[8b] using the procedure described in ref 4. As the EIN crystal structure is not of the same high accuracy as that of the HIV-1 protease/DMP323 complex, the degree of anisotropy is underestimated and it becomes difficult to assess whether there is a significant rhombic component.

In conclusion, we have presented a simple method for obtaining the magnitude of the diffusion tensor from the distribution of heteronuclear T_1/T_2 ratios without the need for any prior structural information.[10] This not only permits one to derive information regarding the shape and hydrodynamic properties of the macromolecule under consideration (without the need for any resonance assignments) but also permits the heteronuclear T_1/T_2 ratios to be used for structure refinement, with the proviso that the difference between the maximum and minimum T_1/T_2 ratios exceeds the measurement error by a factor of at least 10.

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(8) (a) Lam, P. Y. S.; Jadhav, P. K.; Eyerman, C. J.; Hodge, C. N.; Ru, Y.; Bacheler, L. T.; Meek, J. L.; Otto, M. J.; Rayner, M. M.; Wong, Y. N.; Chang, C.-H.; Weber, P. C.; Jackson, D. A.; Sharpe, T. R.; Erickson-Viitanen, S. *Science* **1994**, 263, 380−384. (b) Liao, D.-I.; Silverton, E.; Seok, Y. -J.; Lee, B. R., Peterkofsky, A.; Davies, D. R. *Structure* **1997**, 4, 861−872.

(9) Good agreement is also obtained for the 76 residue protein ubiquitin with values of $\tau_{c,eff}$, A, and D_{yy}/D_{xx} of 4.1 ns, 1.16, and ≤ 1.03, respectively, calculated on the basis of the known structure,[3a] compared to 4.1 ns, 1.19, and 1.05 calculated on the basis of the distribution of T_1/T_2 values.

(10) Care should be taken to exclude (a) residues with significant internal motion characterized by ^{15}N{-^{1}H} NOE ≤ 0.65[2−4] and (b) residues undergoing conformational exchange characterized by $[(\langle T_2\rangle - T_{2,n})/\langle T_2\rangle) - (\langle T_1\rangle - T_{1,n})/\langle T_1\rangle)] \rangle 1.5 \times$ SD, where the average is taken only over residues that have not been excluded because of a low NOE, $T_{1,n}$ and $T_{2,n}$ are the T_1 and T_2 values of residue n, and SD is the standard deviation calculated for these residues using the left-hand side of the equation.[4] This criterion is based on the fact that slow conformational exchange shortens T_2 but not T_1, whereas rotational diffusion anisotropy results in equal but opposite fractional changes in T_1 and T_2.[4]