Defining the Orientation of the ^{15}N Shielding Tensor Using ^{15}N NMR Relaxation Data for a Protein in Solution

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^{15}N relaxation studies provide information about protein backbone dynamics in solution.[1−3] These data are generally interpreted using the "model-free" formalism[4] which yields a generalized order parameter, S^2, and a time constant for internal motion, τ_e. Analysis of these values allows the distinction between backbone mobility on a picosecond to nanosecond time scale and static disorder in NMR-derived structures. Recently, it has been demonstrated that ^{15}N relaxation data are sensitive to rotational diffusion anisotropy;[3,5−9] variations in experimental T_1/T_2 ratios can be used to define the orientation and magnitude of the diffusion tensor. As a result, ^{15}N T_1/T_2 ratios can be used as constraints for NMR structure refinement[8] or to provide information about the conformation of domains in multidomain proteins.[5]

Two methods[5−9] have emerged for defining the orientation of the rotational diffusion tensor in the molecular frame using experimental ^{15}N relaxation data. In the first method,[5,9] a local diffusion coefficient, D_i, is determined for each residue i. In the second,[6−8] a direct fitting to the experimental ^{15}N T_1/T_2 ratios is performed. T_1/T_2 ratios are calculated for spherical, symmetric, and asymmetric diffusion models, and the goodness-of-fit to experimental data is assessed using the χ^2 statistic:

$$\chi^2 = \sum_{i=1}^{n}[(T_{1i}/T_{2i})^{\text{calcd}} - (T_{1i}/T_{2i})^{\text{expt}}]^2/\Delta^2 \qquad (1)$$

where $(T_{1i}/T_{2i})^{\text{calcd}}$ and $(T_{1i}/T_{2i})^{\text{expt}}$ are the calculated and experimental values, respectively, for residue i, Δ is the experimental uncertainty in T_1/T_2, and n is the number of residues. The selection of a spherical, symmetric, or asymmetric diffusion model for a protein, where each model has an increasing number of fitted parameters, is made by assessing the statistical significance of the additional parameters using the F-test.[6,9,10]

Solid-state NMR studies[11] and quantum mechanical calculations[12] of peptides and peptide analogues have shown that the ^{15}N shielding tensor is nearly axially symmetric with its principal axis located in the peptide plane. These studies indicate that the principal axis is not collinear with the $^{15}N-H$ bond vector but is inclined away from the $^{15}N-H$ bond toward the $^{15}N-C'$ bond by an angle α. A number of different values for α, typically ranging from 15 to 25°, have been reported from these studies. The value of α has also been investigated in a recent solution NMR study.[13] The small magnetic-field-dependent chemical-shift differences[13] observed from partially oriented anisotropic molecules depend on α; an analysis of these shift differences in a zinc-finger−DNA complex has found α to be in the range $13 \pm 5°$. ^{15}N chemical shift anisotropy (CSA) and $^{15}N-^{1}H$ dipolar cross-correlation is influenced by the value of α.[14] A detailed analysis of the CSA dipolar cross-correlation in ubiquitin has been presented;[14] the angle α was taken to be 20° for all ^{15}N nuclei.

The influence of α on the calculated ^{15}N T_1 and T_2 relaxation times, when an anisotropic rotational diffusion model is employed, has not previously been considered. Hitherto, calculations of these relaxation times, involving autocorrelation spectral densities,[15,16] have employed a single set of angles to define the orientation of *both* the $^{15}N-^{1}H$ internuclear vector and the principal component of the ^{15}N shielding tensor with respect to the principal axes of the diffusion tensor, implicitly assuming that α is zero. Here, we show that the orientation of the ^{15}N shielding tensor, as defined by α, has an influence upon calculated T_1/T_2 ratios and that this effect is detectable in experimental data.

The ratio $(T_{1i}/T_{2i})^{\text{calcd}}$ for the ith ^{15}N nucleus is calculated using two sets of spectral density functions defined by:

$$J_{ik}(\omega) = \sum_{j=1}^{n} A_{ijk}[\tau_j/(1 + \omega^2\tau_j^2)] \qquad (2)$$

where $n = 1$, 3, and 5 for the spherical, symmetric, and asymmetric top models, respectively. The spectral densities $J_{i1}(\omega)$ are used for $^{15}N-^{1}H$ dipolar contributions, and $J_{i2}(\omega)$ for ^{15}N CSA contributions to the T_1 and T_2 relaxation equations,[15,16] respectively. The time constants τ_j depend on the principle values of the diffusion tensor, as defined previously.[15,16] The coefficients A_{ij1}, for the dipolar terms, depend on the orientation of the $^{15}N_{(i)}-^{1}H_{(i)}$ internuclear vector with respect to the diffusion tensor whereas the coefficients A_{ij2}, for the CSA terms, depend on the orientation of the $^{15}N_{(i)}$ shielding tensor with respect to the diffusion tensor.[15,16] In the case of a symmetric top A_{ij1} depends on the angle θ between the $^{15}N_{(i)}-^{1}H_{(i)}$ vector and the principal axis of the diffusion tensor while A_{ij2} depends on the angle θ' between the principal axis of the ^{15}N shielding tensor and the principal

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Table 1. Comparison of χ^2 Values Obtained Using a Symmetric Diffusion Model with the ^{15}N Shielding Tensor Angle α Set to 0°(left) and with the Angle α Fitted (right) for Four Lysozyme Crystal Structures

structure[a]	χ^2 [b]	$D_\parallel/D_\perp$	α (deg)	χ^2 [c]	$D_\parallel/D_\perp$	α (deg)	*F*-value[d]
5LYM	140.2	1.29 ± 0.01	0.0	124.1	1.32 ± 0.02	28.5 ± 7.6	11.42
1UCO	142.8	1.29 ± 0.01	0.0	128.1	1.32 ± 0.02	27.2 ± 8.0	10.10
3LYM	145.2	1.28 ± 0.01	0.0	123.7	1.32 ± 0.02	32.2 ± 7.1	15.30
6LYT	146.6	1.29 ± 0.01	0.0	126.7	1.32 ± 0.02	33.9 ± 8.4	13.82

[a] X-ray coordinates for hen lysozyme[19] were obtained from the Brookhaven Protein Databank. Amide protons (H^N) were added to the X-ray structures using X-PLOR.[20] The structure was then energy minimized with the backbone heavy atoms constrained to remain at their initial positions. This energy minimization was necessary to ensure that H^N was located in the peptide plane. A vector representing the principal component of the ^{15}N shielding tensor for each residue $i + 1$ was generated using the coordinates of the atoms $H^N_{(i+1)}$, $N_{(i+1)}$, and $C'_{(i)}$; this vector lies in the plane defined by these atoms, inclined at an angle α to the ^{15}N−^{1}H bond. A ^{15}N−^{1}H internuclear distance of 0.102 nm was used in calculations of ^{15}N T_1 and T_2 relaxations times. [b] The angle α is fixed at 0° and the parameters $D_\parallel$ and $D_\perp$ and the orientation of the diffusion tensor are fitted to minimize χ^2. A total of 121 T_1/T_2 ratios have been measured experimentally at 50.6 MHz for lysozyme.[2] Residues with significant motions on the picosecond time scale, exchange broadening, or found in certain surface loops involved in crystal contacts have been removed from analysis;[6,18] 93 T_1/T_2 ratios are used in the present study. [c] $D_\parallel$, $D_\perp$, the orientation of the diffusion tensor, and α are fitted to minimize χ^2. The error in $D_\parallel/D_\perp$ and α has been estimated from 500 Monte Carlo simulations. The effective correlation time, $\tau_r = 1/(2D_\parallel + 4D_\perp)$, decreases by at most 0.002 ns when α is fitted. A value of 5.78 ns is found for all structures. [d] The *F*-test indicates that the symmetric diffusion model with the additional parameter α is a statistically significant improvement over the symmetric diffusion model with α = 0°.

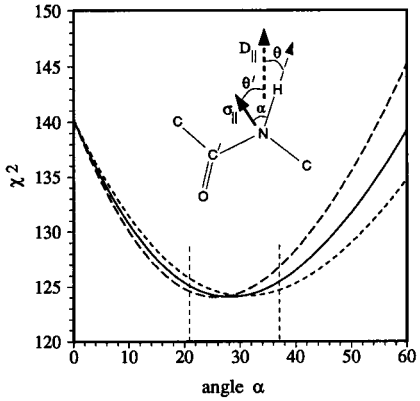

Figure 1. Plot of χ^2 as a function of the ^{15}N shielding tensor angle α. For each value of α, the parameters $D_\parallel$, $D_\perp$ and the orientation of the diffusion tensor were fitted. Calculations using uniform ^{15}N CSA values of either −160, −170, or −180 ppm are shown by short-dashed, solid, and long-dashed lines, respectively. The vertical dashed lines indicate the range of errors in the fitted value of α found using Monte Carlo simulations for a ^{15}N CSA value of −170 ppm. The inset shows the angles α, θ, and θ' which define the relative orientations of the principal component of the ^{15}N shielding tensor ($\sigma_\parallel$), the ^{15}N−^{1}H internuclear vector, and the principal component of the diffusion tensor ($D_\parallel$).

axis of the diffusion tensor. The value of θ' falls in the range of $\theta \pm \alpha$, the exact value depending on the orientation of the peptide plane with respect to the diffusion tensor (Figure 1). For the calculations performed here, we have assumed that the ^{15}N chemical shift asymmetry parameter is zero, that any contribution to^{15}N relaxation from the antisymmetric component of the shielding tensor[16,17] is small and can be neglected, and that the ^{15}N shielding tensor experiences identical dynamic behavior to the ^{15}N−^{1}H dipolar interaction.

The^{15}N T_1 and T_2 relaxation data were obtained at 50.6 MHz from a uniformly ^{15}N-labeled sample of hen lysozyme as described previously.[2] A recent analysis has shown that a symmetric rotational diffusion model best describes these data.[18] The change in the χ^2 statistic as the angle α is varied from 0 to 60° for the 5LYM lysozyme structure[19] is shown in Figure 1. The agreement between the experimental and calculated T_1/T_2 ratios improves as α is increased, with a minimum at 28.5°. Similar fits have been carried out using three other lysozyme X-ray structures,[19] the results are summarized in Table 1. For these structures, the optimal value of α is found to vary between 27.2 and 33.9°. The *F*-values listed in Table 1 correspond to a confidence level of greater than 99.8% indicating that the model which includes the angle α gives a significant improvement over the more widely used model which assumes that α = 0°. Monte Carlo simulations have been used to assess the error in α resulting from experimental uncertainty in the^{15}N T_1 and T_2 values; errors of ±7−8° are found for each of the four structures. This fairly large error is to be expected because the variation of χ^2 as a function of the angle α is found to exhibit a broad shallow minimum as shown in Figure 1. These values of α are obtained with a uniform ^{15}N CSA value of −170 ppm.[14] If this value is changed to −160 or −180 ppm, α increases or decreases by 2.4°, respectively, as shown in Figure 1. These values for α are slightly larger than those obtained previously.[11,13] The value of $D_\parallel/D_\perp$ is found to increase when the value of α is fitted. For example, $D_\parallel/D_\perp$ increases from 1.29 to 1.32, for the 5LYM structure, when α is increased from 0° to 28.5°. If $D_\parallel/D_\perp$ and the orientation of the diffusion tensor are fixed to the values found with α = 0° and only α is fitted, then somewhat lower values of α are found; α equals 21.9, 21.0, 23.9, and 24.3° for the 5LYM, 1UCO, 3LYM, and 6LYT structures, respectively. These values for α are now closer to the values reported from solid-state NMR[11] and from magnetic-field-dependent chemical-shift studies.[13]

Although the uncertainty in the value of α is quite large, the calculations for lysozyme show that improved agreement between the experimental and calculated T_1/T_2 ratios is obtained when using a symmetric rotational diffusion model in combination with the assumption that the ^{15}N shielding tensor is not collinear with the ^{15}N−^{1}H bond vector. The data analyzed here were collected at relatively low field for a protein of moderate anisotropy ($D_\parallel/D_\perp$ = 1.3). It is expected that the influence of α on T_1/T_2 ratios will be more pronounced at higher fields (18.8 T) and for proteins with a larger anisotropy. This has implications for the analysis of frequency-dependent ^{15}N relaxation data and for the use of T_1/T_2 ratios in the refinement of protein structure.

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Supporting Information Available: One table containing experimental and calculated T_1/T_2 ratios with α = 0° and α = 28.5° for the 5LYM lysozyme structure (4 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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