Model-Independent Analysis of ^{15}N Chemical Shift Anisotropy from NMR Relaxation Data. Ubiquitin as a Test Example

David Fushman and David Cowburn*

The Rockefeller University, 1230 York Avenue New York, New York 10021

Received February 19, 1998

This paper describes a method of obtaining ^{15}N chemical shift anisotropy information in solution using a direct, model-independent method. The complete chemical shift tensor, $\underline{\sigma}$, contains significant information about the local electronic environment of a NMR-detectable nucleus. In solution, only the isotropic chemical shift, $\sigma^{iso} = tr(\underline{\sigma})/3$, is available, and the useful elements are averaged out. The isotropic chemical shifts in proteins have been widely identified as useful structural indicators,[1−3] and the anisotropic elements would likely be even more valuable.

An elegant method of assessment of the chemical shift anisotropy (CSA) by measuring cross correlation between ^{15}N−^{1}H dipolar interaction and ^{15}N CSA was recently suggested.[4,5] This CSA data analysis[4] relies on the "model-free" approach[6] and therefore has limited applicability in the cases of (1) nonspherical proteins when structural information is not available, (2) when other processes (domain motion in multidomain proteins; protein dimerization, protein−ligand interaction, etc.) restrict the validity of the model-free approach, and (3) where the residue-specific variability of the CSA renders estimates of S^2 imprecise.

The characterization of ^{15}N CSA, without use of the model-free assumption is presented here. This approach is based on analysis of R_2 and the cross correlation between CSA and dipolar interaction, and does not require knowledge of the additional relaxation parameters needed for the model-free analysis.

For amide ^{15}N nuclear spin relaxation, the observed transverse relaxation rate, R_2, is given in terms of the spectral density function, $J(\omega)$,[7] as $R_2 = {}^1/_2(d^2 + c^2)[4J(0)+3J(\omega_N)] + P_{HF} + R_{ex}$, where $P_{HF} = {}^1/_2 d^2[J(\omega_H - \omega_N) + 6J(\omega_H) + 6J(\omega_H + \omega_N)]$ denotes contribution to R_2 from high-frequency motions and R_{ex} corresponds to conformational exchange contribution, if any. Here ω_N and ω_H are Larmor frequencies of the nitrogen and hydrogen nuclear magnetic moments respectively, $d = (\mu_o/(4\pi))\gamma_H\gamma_N h/(4\pi r_{HN}^3)$, $c = -\omega_N(\sigma_{\|} - \sigma_{\perp})/3$, where r_{HN} is the internuclear ^{1}H−^{15}N bond length, $\sigma_{\|} - \sigma_{\perp}$ is anisotropy of the ^{15}N chemical shift tensor, γ_H and γ_N are gyromagnetic ratios of the nuclei, and h is Planck's constant. The term P_{HF} can be obtained directly from the experimental data, without any assumption about ^{15}N CSA, using the reduced spectral density approach:[8,9] $P_{HF} = 13(\gamma_N/\gamma_H)(1 - \text{NOE})R_1/10$. For the majority of the amide NH groups, $J(\omega_H)$ is much smaller than $J(\omega_N)$ and $J(0)$,[8,9] which then allows us to safely neglect P_{HF} to the first order, $R_2 \approx {}^1/_2(d^2 + c^2)[4J(0) + 3J(\omega_N)]$, assuming R_{ex} is negligible.

The effect of cross correlation between ^{15}N−H dipolar interaction and ^{15}N CSA was shown[10] to result in different transverse relaxation rates for the doublet components of an isolated ^{15}N−^{1}H spin pair. The cross correlation term can be written in the following form,[4] $\eta = dc[4J(0) + 3J(\omega_N)]P_2(\cos\theta)$, where θ is the angle between the unique axes of the CSA and dipolar tensors and $P_2(x)$ is the second-rank Legendre polynomial. This expression, valid for small values of θ, provides a good approximation for peptide ^{15}N nuclei, where the observed θ angles are less than about 24°.[11−16]

Our approach is based on the observation that η and the dominant term in R_2 contain exactly the same combination of the spectral density functions. The ratio of the two quantities then gives $\eta/R_2 \lesssim \eta/(R_2 - P_{HF}) = [2dc/(d^2 + c^2)]P_2(\cos\theta)$. Since the η/R_2 ratio does not contain any direct dependence on protein dynamics, this equation provides a basis for a direct, model-independent determination of the ^{15}N CSA from experimentally measured parameters, without explicit knowledge of the microdynamic parameters and without any assumption about the model of overall motion. Indeed, given R_2, η and the structure-related angle θ, the CSA can be obtained:

$$\sigma_{\|} - \sigma_{\perp} = -3\frac{d}{\omega_N}\frac{R_2P_2(\cos\theta)}{\eta}\left[1 - \sqrt{1 - \left(\frac{\eta}{R_2P_2(\cos\theta)}\right)^2}\right]$$

(for a complete derivation see the Supporting Information). On the other hand, if the ^{15}N CSA is known, then the η/R_2 ratio allows estimation of the θ angle:

$$\theta = \cos^{-1}(\{[1 + (\eta/R_2)(d^2 + c^2)/(dc)]/3\}^{1/2})$$

The approach suggested here assumes absence of conformational exchange contribution to R_2. Those residues with significant R_{ex} are to be excluded from the analysis. The high sensitivity of η/R_2 to R_{ex} contributions (Supporting Information, Figure 2c) suggests that the η/R_2 ratio can be used as a tool for identification of those amide groups subjected to conformational exchange.

The expected errors associated with the neglect of the high-frequency components of the spectral density in R_2 are small (Supporting Information, Figure 2a,b), suggesting that in most cases measurements of η and R_2 are likely to be sufficient for this approach and that R_1 and NOE measurements are not necessarily required.

To illustrate this approach, we analyzed published ^{15}N relaxation data for human ubiquitin.[4,17] To test observed ratio of η/R_2, the values of the cross-correlation term η were plotted (Figure 1a) against the corresponding values of R_2, modified to subtract the high-frequency contribution, P_{HF}. On the basis of the spectral density functions derived from published relaxation data, R_1, R_2, and NOE for ubiquitin,[17] contributions to R_2 from the high-frequency components are small (Supporting Information, Figure 3), less than 2.3% (1.3% on average) for all but the four C-terminal residues, Leu^{73}-Gly^{76}. The two rightmost points in

(1) Wüthrich, K. *NMR of proteins and nucleic acids*; John Wiley and Sons: New York, 1986.
(2) Wishart, D. S.; Sykes, B. D. *Methods Enzymol.* **1994**, *239*, 363−92.
(3) Oldfield, E. *J. Biomol. NMR* **1995**, *5*, 217−25.
(4) Tjandra, N.; Szabo, A.; Bax, A. *J. Am. Chem. Soc.* **1996**, *118*, 6986−91.
(5) Tessari, M.; Mulder, F. A. A.; Boelens, R.; Vuister, G. W. *J. Magn. Reson.* **1997**, *127*, 128−3.
(6) Lipari, G.; Szabo, A. *J. Am. Chem. Soc.* **1982**, *104*, 4559−70.
(7) Abragam, A. *The Principles of Nuclear Magnetism*; Clarendon Press: Oxford, 1961.
(8) Ishima, R.; Nagayama, K. *Biochemistry* **1995**, *34*, 3162−71.
(9) Farrow, N. A.; Zhang, O.; Szabo, A.; Torchia, D. A.; Kay, L. E. *J. Biomol. NMR* **1995**, *6*, 153−62.
(10) Goldman, M. *J. Magn. Reson.* **1984**, *60*, 437−52.
(11) Harbison, G. S.; Jelinski, L. W.; Stark, R. E.; Torchia, D. A.; Herzfeld, J.; Griffin, R. G. *J. Magn. Reson.* **1984**, *60*, 79−82.
(12) Oas, T. G.; Hartzell, C. J.; Dahlquist, F. W.; Drobny, G. P. *J. Am. Chem. Soc.* **1987**, *109*, 5962−6.
(13) Hartzell, C. J.; Whitfield, M.; Oas, T. G.; Drobny, G. P. *J. Am. Chem. Soc.* **1987**, *109*, 5966−9.
(14) Hiyama, Y.; Niu, C.; Silverton, J.; Bavoso, A.; Torchia, D. *J. Am. Chem. Soc.* **1988**, *110*, 2378−83.
(15) Shoji, A.; Ozaki, T.; Fujito, T.; Deguchi, K.; Ando, S.; Ando, I. *Macromolecules* **1989**, *22*, 2860−3.
(16) Mai, W.; Hu, W.; Wang, C.; Cross, T. A. *Protein Sci.* **1993**, *2*, 532−42.
(17) Tjandra, N.; Feller, S. E.; Pastor, R. W.; Bax, A. *J. Am. Chem. Soc.* **1995**, *117*, 12562−6.

S0002-7863(98)00565-4 CCC: $15.00 © 1998 American Chemical Society
Published on Web 07/03/1998

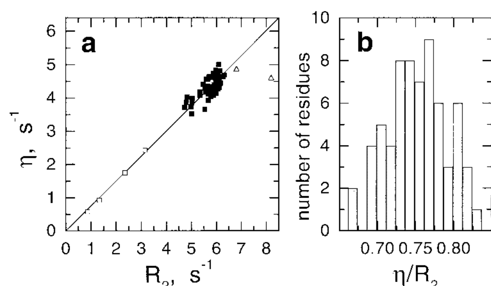

Figure 1. Correlation between the cross-correlation term η and the rate of ^{15}N transverse relaxation, R_2, (a) and histogram of η/R_2 values (b) for ubiquitin. Solid squares represent data having no significant conformational exchange contribution; residues Asn[25] and Ile[36] indicated by open triangles have significant R_{ex}, and the four C-terminal residues, Leu[73]-Gly[76], having significant high-frequency component of the spectral density function are indicated by open squares. To provide an accurate fit for all residues exhibiting no R_{ex} contribution, those which have a significant contribution from high-frequency motions (73-76) and those which have not, all R_2 values were modified to subtract the P_{HF} term, determined using R_1 and NOE data as described in the text. The straight line corresponds to $\eta = (0.753 \pm 0.005)R_2$, as derived from linear regression using all residues not subject to conformational exchange: the correlation coefficient between η and R_2 is 0.96. A two-parameter fit was not statistically significantly better. A very similar proportionality coefficient, 0.743 ± 0.005, was obtained for raw R_2 data, without correction for the high-frequency contribution (Supporting Information, Figure 4).

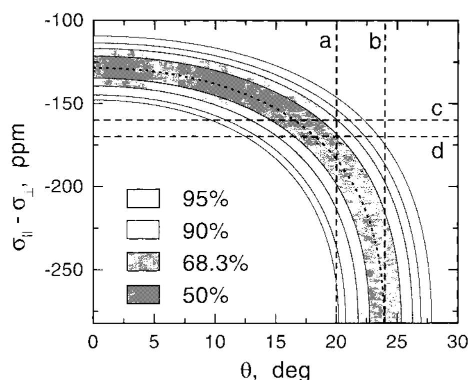

Figure 2. Contour map of possible sets of CSA and θ values, representing solutions of equation $\eta/R_2 = [2dc/(d^2 + c^2)]P_2(\cos\theta)$ for ubiquitin. The solid lines correspond to η/R_2 values corresponding to 95, 90, 68.3, and 50% of distribution of the experimental η/R_2 values, Figure 1b. The dotted line in the middle of the 50% area corresponds to the average value of $\eta/R_2 = 0.753$ (Figure 1a). Dashed lines corresponding to $\theta = 20°$ (a), $\theta = 24°$ (b), CSA = −160 ppm (c), and CSA = −170 ppm (d) indicate a range of values of the parameters expected from either solid-state NMR studies of peptides[11−15] or from NMR solution studies of ubiquitin.[4]

Figure 1a belong to Leu[23] and Asn[25], exhibiting a substantial conformational exchange contribution.[17] These two residues are excluded from further analysis. The spread in the η/R_2 values (Figure 1b) can be attributed to deviations in the individual values of θ, to variations in the CSA values, and/or to experimental errors. Analysis in terms of CSA relies on the assumption that r_{NH} is constant. The variations in η/R_2 arising from variations in the ^{15}N−^{1}H dipolar coupling with r_{NH} are small, ±3% for the range 1.02 ± 0.01 Å for r_{NH}.

In Figure 2, the surface of appropriate CSA = θ values is shown for given values of η/R_2. This makes determination of CSA solely from NMR relaxation data at one frequency ambiguous. Additional information regarding the θ angle, as e.g. from protein crystal NMR studies, from *ab initio* calculations, or methods sensitive to the relative orientation of the N−H vector and the CSA tensor (e.g. like ref 18) should resolve this issue.

(18) Yang, D.; Konrat, R.; Kay, L. E. *J. Am. Chem. Soc.* **1997**, *119*, 11938−40.

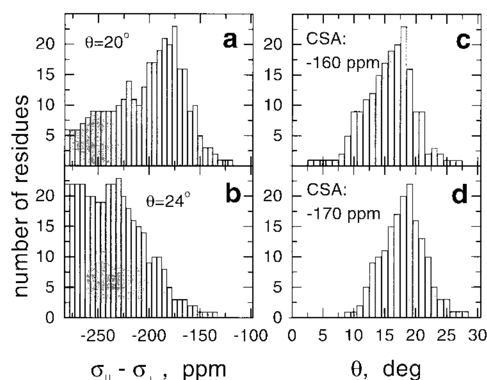

Figure 3. Examples of a statistical distribution of CSA (a, b) and θ (c, d) values corresponding to a section of the CSA−θ distribution surface (Figure 2) along the corresponding dashed lines, as indicated in Figure 2. The height of each vertical bar equals the number of residues for which a given set of $\sigma_{\|} - \sigma_{\perp}$ and θ values satisfies the observed values of η/R_2 within experimental error.

The experimental data for ubiquitin[4,17] provide a basis for statistical analysis of possible values of these parameters in proteins. Figures 2 and 3 depict the range of statistically significant values of θ and ^{15}N CSA. These values are somewhat different from what is expected from the solid-state NMR studies on peptides ($\theta \approx 20−24°$, CSA = −160 ppm, dashed lines *a*, *b*, and *c*, Figure 2). The value of CSA = −170 ppm reported in ref 4 (dashed line *d* in Figure 2) is in a somewhat better agreement with Figure 2, although this statistical analysis of ubiquitin data suggests larger absolute values of ^{15}N CSA in proteins, or smaller values of θ angle, or both. A trend toward smaller values of θ is consistent with data for gramicidin A in lipid bilayers[16] and for a protein−DNA complex.[19] Additional studies on other proteins are in progress (Supporting Information, Figure 5).

A constant value of CSA (−160 ppm) is currently assumed for relaxation data analysis, both in the model-free analysis[6] and in the spectral density function approach.[20] A broad distribution of possible CSA values at a given θ (e.g., half-width at half-height of ~30 ppm at $\theta = 20°$, Figures 2 and 3) suggests significant variations in CSA. A variation, δCSA, will cause a relative variation of $-2dc/(d^2 + c^2)(\delta\text{CSA/CSA})$ in the spectral density functions $J(0)$ and $J(\omega_N)$ derived from relaxation data, R_1, R_2, and NOE. Similar relative variations are expected for S^2 in the model-free approach. At 600 MHz and CSA = −160 ppm, the factor $2dc/(d^2 + c^2) = 0.5$, so that e.g. a 20% variation in CSA (Figures 2 and 3) might result in 10% error in apparent S^2, which exceeds the commonly reported precision of the order parameter. An incorporation of CSA information into relaxation data analysis appears to be necessary to improve the accuracy of obtained order parameters or spectral density values. This may be particularly important for the correct interpretations of changes in order parameters observed as a result of protein−ligand interactions or conformational transitions, where the effect of perturbation of CSA tensor has to be deconvolved from the real changes in protein dynamics.

Acknowledgment. This work was supported by NIH GM-47021. We are grateful to Dr. A. Bax for discussion.

Supporting Information Available: Derivation and analysis of the equations; two figures illustrating the η/R_2 approach and expected errors; two figures showing analysis of ubiquitin data; one figure with η vs R_2 for dynamin PH domain (9 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

JA980565J

(19) Ottiger, M.; Tjandra, N.; Bax, A. *J. Am. Chem. Soc.* **1997**, *119*, 9825−30.

(20) Peng, J.; Wagner, G. *J. Magn. Reson.* **1992**, *94*, 82−100.