Characterization of ^{15}N Chemical Shift Anisotropy from Orientation-Dependent Changes to ^{15}N Chemical Shifts in Dilute Bicelle Solutions

Jonathan Boyd and Christina Redfield*

Department of Biochemistry and Oxford Centre for Molecular Sciences Oxford University, South Parks Road Oxford, OX1 3QT, U.K.

Received April 9, 1999
Revised Manuscript Received June 24, 1999

^{15}N relaxation studies provide information about protein backbone dynamics in solution.[1] Analysis of ^{15}N relaxation data using the "model-free" formalism,[2] to yield generalized order parameters S^2, requires information about the ^{15}N chemical shift anisotropy (CSA).[3] The magnitude of the CSA, $\Delta\sigma$, determines the relative importance of dipolar and CSA contributions to relaxation, and, in particular, influences the frequency dependence of T_1 and T_2 values. A value of −160 ppm was used in the first ^{15}N relaxation study of a protein[1a] and has subsequently been widely adopted in other studies. Recently a value of −170 ppm was determined for ubiquitin from field dependent ^{15}N T_1 and T_2 data, and also from ^{15}N dipolar−CSA relaxation interference measurements.[4,5] A decrease of −10 ppm to the^{15}N CSA decreases the order parameters, S^2, obtained from a relaxation analysis at 17.6 T, by about 4% which is greater than typical experimental uncertainties. The orientation of the principle component of the ^{15}N CSA with respect to the NH bond vector, as defined by the angle α, has been shown to have a significant influence on the analysis of ^{15}N relaxation data when anisotropic rotational diffusion models are used.[6a,b] Recently, $\Delta\sigma$ and α have been reported for individual residues of ubiquitin from analysis of cross- and autocorrelated ^{15}N relaxation rates, significant variations to both $\Delta\sigma$ and α were found.[6c]

Changes to ^{15}N chemical shifts occur when a protein is partially oriented in the magnetic field.[7−9] Very small field-dependent ^{15}N chemical shift changes for a protein−DNA complex oriented by magnetic alignment have been used to estimate values of −168 ± 20 ppm for $\Delta\sigma$ and 13 ± 5° for α.[8] Larger shift changes are observed when a protein is dissolved in a dilute liquid crystalline phase such as bicelles.[9] Recently it has been shown that carbonyl ^{13}C chemical shift changes, measured in bicelles, can be used to evaluate the quality of X-ray and NMR structures.[9] In this paper we use changes to the ^{15}N chemical shifts, measured at 17.6 T, for hen lysozyme resulting from orientation of the protein in bicelle solutions to determine the magnitude and orientation of the ^{15}N chemical shift tensor.

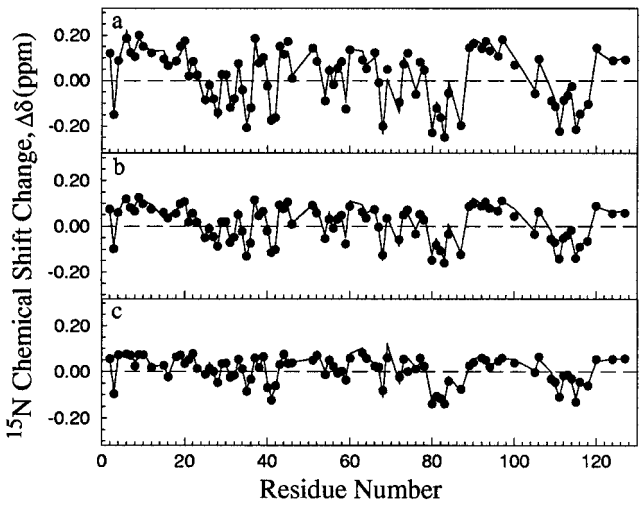

Figure 1. Experimental and calculated orientation-dependent ^{15}N chemical shift changes for lysozyme are plotted as a function of residue number for three phospholipid compositions. Experimental shift changes are indicated by filled circles, calculated shift changes by a solid line. Only those residues for which shift changes can be measured in all three samples and for which significant motions on a picosecond time scale have not been observed[1b] are included in the analysis (88 residues). The calculated shifts are based on the alignment tensors calculated with S = 1 and r_{NH} = 0.104 nm and a fully asymmetric ^{15}N tensor. ^{15}N chemical shift changes were measured for three solutions of 0.5 mM lysozyme in bicelles: (A) 7.5% w/v dimyristoyl−phosphatidlycholine (DMPC) and dihexanoyl−phosphatidylcholine (DHPC), q = 2.9, 10 mM phosphate buffer, pH 6.5, 93/7% H_2O/D_2O with a small amount of dioxane for ^{1}H chemical shift referencing (7.5% M:H); (B) as in (A) but 5% w/v DMPC:DHPC (5% M:H); (C) as in (A) but with cetyltrimethylammonium bromide (CTAB) added, DMPC:DHPC:CTAB = 2.9:1.0:0.1 (7.5% M:H:C). The sample preparation protocol followed that described by Ottiger and Bax.[13]

^{15}N chemical shift changes and ^{15}N−^{1}H residual dipolar couplings[10,11] have been measured for three hen lysozyme samples with distinct phospholipid compositions[12−14] (see legend to Figure 1). The magnitude and orientation of the alignment tensor, A_{ii}, were obtained from a fit of the experimental residual dipolar couplings to those calculated from the 1.3 Å X-ray structure[15] of hen lysozyme (193L) using published procedures;[9,11] the values are summarized in Table 1. Two sets of calculations were performed. The first used an order parameter (S_{NH}) of 1 with r_{NH} = 0.104 nm, the effective NH bond length determined by Ottiger and Bax,[11] the second used the experimentally determined[1b] values of S with r_{NH} = 0.102 nm. The orientation of the alignment tensors is nearly identical in the 5 and 7.5% DMPC:DHPC solutions with the magnitude of A_{ii} increased by 50% in the latter. The addition of CTAB to the 7.5% bicelle solution leads to a 10° change in the orientation of the principle component and to a decrease in the rhombic component of the alignment tensor.

(1) (a) Kay, L. E.; Torchia, D. A.; Bax, A. *Biochemistry* **1989**, *28*, 4000−4009. (b) Buck, M.; Boyd, J.; Redfield, C.; MacKenzie, D. A.; Jeenes, D. J.; Archer, D. B.; Dobson, C. M. *Biochemistry* **1995**, *34*, 4041−4055. (c) Palmer, A. G.; Williams, J.; McDermott, A. *J. Phys. Chem*. **1996**, *100*, 13293−13310.
(2) Lipari, G.; Szabo, A. *J. Am. Chem. Soc*. **1982**, *104*, 4546−4558, 4559−4570.
(3) Duncan, T. M. *A Compilation of Chemical Shift Anisotropies*; Farragut-Press: Madison, WI, 1990.
(4) Tjandra, N.; Wingfield, P.; Stahl, S.; Bax, A. *J. Biomol. NMR* **1996**, *8*, 273−284.
(5) Tjandra, J.; Szabo, A.; Bax, A. *J. Am. Chem. Soc*. **1996**, *118*, 6986−6991.
(6) (a) Boyd, J.; Redfield, C. *J. Am. Chem. Soc*. **1998**, *120*, 9692−9693. (b) Fushman, D.; Cowburn, D. *J. Biomol. NMR* **1999**, *13*, 139−147. (c) Fushman, D.; Tjandra, N.; Cowburn, D. *J. Am. Chem. Soc.* **1998**, *120*, 10947−10952.
(7) (a) Buckingham, A. D.; McLauchlan, K. A. *Progr. Nucl. Magn. Reson. Spectrosc.* **1967**, 63−110. (b) Bothner-By, A. A. In *Encyclopedia of Nuclear Magnetic Resonance*; Grant, D. M., Harris, R. K., Eds.; 1996; pp 2932−2938.
(8) Ottiger, M.; Tjandra, N.; Bax, A. *J. Am. Chem. Soc.* **1997**, *119*, 9825−9830.
(9) Cornilescu, G.; Marquardt, J. L.; Ottiger, M.; Bax, A. *J. Am. Chem. Soc.* **1998**, *120*, 6836−6837.
(10) (a) Gayathri, C.; Bothner-By, A.A.; Van Zijl, P. C. M.; Maclean, C. *Chem. Phys. Lett*. **1982**, *87*, 192−196. (b) Tjandra, N.; Bax, A. *Science* **1997**, *278*, 1111−1114.
(11) Ottiger, M.; Bax, A. *J. Am. Chem. Soc*. **1998**, *120*, 12334−12341.
(12) (a) Sanders, C. R.; Hare, B. J.; Howard, K. P.; Prestegard, J. H. *Prog. Nucl. Magn. Reson. Spectrosc.* **1994**, *26*, 421−444. (b) Sanders, C. R.; Landis, G. C. *Biochemistry* **1995**, *34*, 4030−4040.
(13) Ottiger, M.; Bax, A. *J. Biomol. NMR* **1998**, *12*, 361−372.
(14) Ramirez, B. E.; Bax, A. *J. Am. Chem. Soc*. **1998**, *120*, 9106−9107.
(15) Vaney, M. C.; Maignan, S.; Ries-Kautt, M. and Ducruix, A. *Acta Crystallogr.* **1996**, *D52*, 505−517.

10.1021/ja9911216 CCC: $18.00 © 1999 American Chemical Society
Published on Web 07/29/1999

Table 1. Alignment Tensors for Hen Lysozyme

bicelle composition [a]	$A_{xx}/10^{-4}$ [b]	$A_{yy}/10^{-4}$	$A_{zz}/10^{-4}$	ϕ	θ	ψ	Q[c]	R^2 [d]
7.5% M:H (1)[e]	5.7	17.3	−23.0	155	126	93	0.191	0.964
(2)	5.8	17.5	−23.3	155	126	92	0.190	0.964
5% M:H (1)	3.9	10.8	−14.6	155	127	91	0.179	0.968
(2)	3.9	10.9	−14.8	155	127	90	0.178	0.968
7.5% M:H:C (1)	4.8	7.2	−12.1	157	137	53	0.182	0.967
(2)	4.8	7.3	−12.2	157	137	54	0.182	0.966

[a] Details of the bicelle solutions are given in Figure 1. [b] The principle components (A_{xx}, A_{yy}, and A_{zz}) and orientation (Euler angles ϕ, θ, and ψ) of the molecular alignment tensor in the X-ray structure were fitted to minimize the χ^2 between experimental and calculated residual dipolar couplings. [c] Q is the quality factor[8] that describes the agreement between calculated and observed residual dipolar couplings. [d] R^2 is the square of the sample correlation coefficient. [e] Calculations (1) and (2) used $r_{NH} = 0.104$ nm and 0.102 nm, respectively.

Changes in ^{15}N chemical shifts due to orientation of the protein were measured from ^{15}N−^{1}H HSQC spectra recorded in the presence and absence of phospholipid at 34.7 °C. To compensate for the effects of small differences in the samples upon the ^{15}N chemical shifts spectra were also recorded at 21.5 °C for both samples. The orientation-dependent shift changes, for each bicelle composition, were then calculated from the following relationship:

$$\Delta\delta = (\delta_{21.5C}{}^{bic} - \delta_{34.7C}{}^{bic}) - (\delta_{21.5C}{}^{iso} - d_{34.7C}{}^{iso}) \quad (1)$$

where the subscripts indicate the sample temperature and the superscripts indicate a sample with or without phospholipid. A positive value of $\Delta\delta$ represents a change to lower ^{15}N frequency. The change to the ^{15}N chemical shift is determined by the relative orientation of the principal axes of the traceless chemical shift tensor and the diagonalized traceless molecular alignment tensor, determined from the residual dipolar couplings. Two models were used for the chemical shift tensor. First, the tensor was assumed to be axially symmetric with the principal component (σ_{11}) located in the peptide plane inclined at an angle α to the NH bond. In the second model a fully asymmetric tensor was used with σ_{11} and σ_{33} in the peptide plane. Global values for $\Delta\sigma$ and α, for the two models, and the chemical shift asymmetry parameter η, for the fully asymmetric model, were obtained from a fit of experimental and calculated shift changes. The results are summarized in Table 2, and the experimental and calculated shift changes are compared in Figure 1. The agreement between the experimental and calculated shift changes is excellent, even assuming uniform values for the CSA. The use of a fully asymmetric chemical shift tensor gives a statistically significant improvement in the χ^2 value compared to the axially symmetric tensor as assessed by the F-test (p-value $= 2.8 \times 10^{-10}$).[16] These calculations have been repeated for 31 other lysozyme X-ray structures. The 10 structures which give Q values below 0.22, for the fully asymmetric model, give $\Delta\sigma$, α, and η values of -173.6 ± 2.1 ppm, $18.2 \pm 0.8°$ and 0.16 ± 0.01, respectively, when the alignment tensors are calculated with $S = 1$ and $r_{NH} = 0.104$ nm, and values of -171.5 ± 2.2 ppm, $18.3 \pm 0.8°$ and 0.16 ± 0.01, respectively, when the alignment tensors are calculated with experimental S values and $r_{NH} = 0.102$ nm. Thus, the values of α and η are independent of the NH bond length and S values used. The value of $\Delta\sigma$ shows some dependence on these parameters. The rigid molecule value of -173.6 ppm probably represents a lower limit for $\Delta\sigma$ since it is calculated using an effective NH bond length of 0.104 nm which assumes a value of $S = 1$ for the N−Ca bond;[11] a lower value for S_{N-Ca} will increase the value obtained for $\Delta\sigma$.

Table 2. Orientation and Magnitude of the ^{15}N Chemical Shift Tensor

	axially symmetric tensor[a]				fully asymmetric tensor[b]				
model[c]	$\Delta\sigma$	α	Q	R^2	$\Delta\sigma$	η	α	Q	R^2
(1)	−174.2	18.4	0.223	0.951	−174.4	0.144	18.6	0.207	0.958
(2)	−172.1	18.5	0.223	0.951	−172.4	0.145	18.7	0.206	0.958

[a] For the axially symmetric model $\Delta\sigma$ ($\Delta\sigma = \sigma_{||} - \sigma_{\perp}$, $\sigma_{||} = \sigma_{11}$, $\sigma_{\perp} = (\sigma_{22} + \sigma_{33})/2$) and α are fitted to minimize χ^2. [b] For the fully asymmetric model $\Delta\sigma$ ($\sigma_{11} - (\sigma_{22} + \sigma_{33})/2$), η ($(\sigma_{22} - \sigma_{33})/\sigma_{11}$), and α are fitted assuming the isotropic shift ($(\sigma_{11} + \sigma_{22} + \sigma_{33})/3$) is zero. [c] Fits were carried out using the two sets of alignment tensors (1) and (2) described in Table 1.

Solid-state NMR studies of ^{15}N shift tensors have usually constrained both the σ_{11} and σ_{33} components to the peptide plane on the basis of the planar symmetry of the peptide bond.[17] We have explored a third model which employs a fully asymmetric ^{15}N tensor with only the σ_{11} component constrained to the peptide plane. This model gives a statistically significant improvement to the fit compared to the model with both σ_{11} and σ_{33} located in the peptide plane (p-value $= 1.8 \times 10^{-4}$). The σ_{33} component is rotated out of the peptide plane on average by only $4.5 \pm 0.6°$ in the 10 X-ray structures analyzed; this is somewhat smaller than that found with solid-state NMR.[18] In this model $\Delta\sigma$ changes by less than 0.1 ppm, and the asymmetry parameter increases by 0.01. The rigid molecule value for $\Delta\sigma$ obtained here is close to the value of -170 ppm determined by Bax and co-workers.[4,5] These values are somewhat lower than values reported from solid-state NMR. The decreased value for the ^{15}N CSA has been justified[4,5,19] by noting that the influence of rapid internal motions on the powder pattern width had typically been neglected and that these motions would reduce this width. A value for α of $28 \pm 8°$ has been estimated previously from ^{15}N T_1/T_2 ratios.[6a] The value of α reported in this study is close to the lower end of this range.

The analysis described here has assumed a uniform value for $\Delta\sigma$ and α. In a protein with a variety of backbone conformations and hydrogen bond strengths some variation in $\Delta\sigma$ and α is possible; residue specific CSA values have been reported for ubiquitin.[6c] In principle, the methods used here can be used to define CSA values for individual residues. This could be achieved by significantly decreasing the experimental error in the shift measurements, possibly by using perdeuterated protein, and by measuring shifts for a variety of oriented samples with a bigger range of alignment tensor orientations[14,20] than found here.

Acknowledgment. This is a contribution from the Oxford Centre for Molecular Sciences which is supported by BBSRC, EPSRC, and MRC. C.R. thanks the BBSRC for an Advanced Research Fellowship.

Supporting Information Available: One page containing details of the measurement of residual dipolar couplings and orientation-dependent ^{15}N chemical shift changes (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA9911216

(16) Bevington, P. R.; Robinson, D. K. *Data Reduction and Error Analysis for the Physical Sciences*, 2nd ed.; McGraw-Hill: New York, 1992.
(17) Harbison, G. S.; Jelinski, L. W.; Stark, R. E.; Torchia, D. A.; Herzfeld, J.; Griffin, R. G. *J. Magn. Reson.* **1984**, *60*, 79−82.
(18) Lee, D. K.; Wittebort, R. J.; Ramamoorthy, A. *J. Am. Chem. Soc.* **1998**, *120*, 8868−8874 and references therein.
(19) Lienin, S. F.; Bremi, T.; Brutscher, B.; Bruschweiler, R.; Ernst, R. R. *J. Am. Chem. Soc.* **1998**, *120*, 9870−9879.
(20) Prosser, R. S.; Hunt, S. A.; DiNatale, J. A.; Vold, R. R. *J. Am. Chem. Soc.* **1996**, *118*, 269−270.