Determination of Protein Backbone ^{13}CO Chemical Shift Anisotropy Tensors in Solution

Yuxi Pang[†] and Erik R. P. Zuiderweg*[,†,‡]

Biophysics Research Division
Departments of Biological Chemistry and Chemistry
The University of Michigan, 930 N University Avenue
Ann Arbor, Michigan 48109-1055

Received November 30, 1999
Revised Manuscript Received February 29, 2000

We obtain complete parametrization of peptide backbone ^{13}CO chemical shift anisotropy (CSA) tensors for a protein in solution, by measuring the interference (cross correlation) between the ^{13}CO CSA transverse relaxation and three different dipole−dipole relaxations, ^{13}CO−^{13}Cα, ^{13}CO−^{15}N, and ^{13}CO−^{1}HN for all sites.

The transverse cross-correlated relaxation rate $\Gamma_{I,IS}^{CSA/DD}$ for a rhombic CSA tensor of spin I (^{13}CO) with the dipolar interaction between two spins I (^{13}CO) and S (^{15}N, ^{13}Cα or ^{1}HN) is[1−3]

$$\Gamma_{I,IS}^{CSA/DD} = \frac{1}{6}\left(\frac{\mu_0}{4\pi}\right)\frac{\hbar\omega_I\gamma_I\gamma_S}{r_{IS}^3}\{(\sigma_{11}-\sigma_{33})\{4J^{11,IS}(0)+3J^{11,IS}(\omega_I)\}+(\sigma_{22}-\sigma_{33})\{4J^{22,IS}(0)+3J^{22,IS}(\omega_I)\}\} \quad (1)$$

where the cross correlation spectral density functions are[2]

$$J^{ii,IS}(\omega) = \frac{2}{5}\left\{\frac{S_{ii,IS}\tau_c}{1+(\omega\tau_c)^2}+\frac{(P_2(\cos\theta_{ii,IS})-S_{ii,IS})\tau}{1+(\omega\tau)^2}\right\}$$

ω_I is the angular resonance frequency of spin I, γ_S and γ_I the gyromagnetic ratios for spin S and I, and r_{IS} the distance between the two nuclei. τ_c is the rotational correlation time; $\tau^{-1} = \tau_c^{-1} + \tau_e^{-1}$, where τ_e is the local correlation time. The principal values of the CSA tensor of spin I are indicated by σ_{ii} , where i =1, 2, 3. The angle between the principal axis ii of the CSA tensor of I and the IS dipolar interaction vector is $\theta_{ii,IS}$ and $P_2(\cos\theta_{ii,IS}) = (3\cos^2\theta_{ii,IS} - 1)/2$. The quantity $S_{ii,IS}$ is the "cross-correlation order parameter"[2] ($|P_2(\cos\theta_{ii,IS})| \geq |S_{ii,IS}| \geq 0$). The other symbols are natural constants.

Under the assumption (temporary, see below) of absence of local motion, the order parameters reduce to $S_{ii,IS} = P_2(\cos\theta_{ii,IS})$. By also assuming that σ_{11} and σ_{22} lie in the peptide plane, eq 1 contains only four unknown variables: σ_{11}, σ_{22} and σ_{33} and the two angles $\theta_{ii,IS}$ and $\theta_{22,IS}$ that are related by 90°. We measured by NMR the transverse interferences ^{13}CO (CSA)/^{13}CO−^{15}N (DD), ^{13}CO (CSA)/^{13}CO−^{13}Cα (DD), and ^{13}CO (CSA)/^{13}CO−^{1}HN (DD) that depend on the same three CO tensor elements and for which the six angles are related by known transformations. The isotropic ^{13}CO chemical shift provides a fourth observable; ^{15}N relaxation yields τ_c. Together, these measurements allow the calculation of all variables in eq 1 for every ^{13}CO nucleus in a protein in solution.

We carried out the measurements for the protein Binase, a 12.3 kDa ribonuclease; the data are available as Supporting Information. For 71 sites, the values for the three ^{13}CO CSA tensor principal axes and its orientation were obtained from fitting to eq 1 assuming no internal motion, $S_{ii,IS} = P_2(\cos\theta_{ii,IS})$. Figure 2a shows that the parameters for the ^{13}CO tensor show large statistically significant variations (see Figure 2 legend). These cannot be caused by the effect of overall anisotropic diffusion on the primary cross correlation data[4] and are also much larger than the dispersion in the values reported from solid-state NMR[5] (see Table 1). Our data lead to the conclusion that the different peptide planes of proteins in solution have very different properties. The differences are for the most part not correlated with the protein secondary structure (see Table 1). While such large static variations in ^{13}CO CSA tensors may exist for proteins in solution not unlike recent reports for the ^{15}N tensor,[6] an extended analysis indicates that anisotropic local motion could account for a large part of the variations, and that motionally averaged, apparent tensors have been measured.

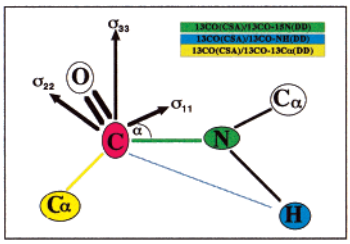

Figure 1. Definitions of the ^{13}CO tensor components and the three dipolar interactions that give rise to structurally independent CSA/DD cross correlations. The most shielded component of the ^{13}CO tensor is defined as σ_{33}.

Table 1 shows that the average value of σ_{22} is about 9 ppm more shielded and that σ_{33} is about 11 ppm less shielded in solution than in the solid. These differences are significant at confidence levels at 94%, using the *Z*-score test.[7] The average value of σ_{11} in solution does not differ significantly from the value in solid. While there is not a general scaling effect of the tensors in solution, the selective discrepancy can be well accounted for by effects of rotational fluctuations around an axis nearly parallel to the Cα−Cα axis. Starting with the average literature values for the tensors, we compute the effects of three orthogonal motions on the three cross correlation rates through the order parameter formalism (see legend to Figure 2), and subsequently reinterpreted the results in terms of apparent static tensors using eq 1. Figure 2b shows that a Gaussian fluctuation along σ_{11} of ±15° reduces the apparent value of σ_{22} by 10 ppm, increases the apparent value of σ_{33} by 10 ppm, and leaves the apparent value of σ_{11} unaffected, in agreement with the experiment. Such so-called crank-shaft motions of similar amplitude were inferred from molecular dynamics simulations,[8] and in cross correlation measurements.[9] Motions around axes perpendicular to the crank-shaft axis could potentially account for much of the observed spread of apparent tensor values.

* To whom correspondence should be addressed at zuiderwe@umich.edu.
† Biophysics Research Division.
‡ Departments of Biological Chemistry and Chemistry.

(1) Goldman, M. *J. Magn. Reson.* **1984**, *60*, 437−52.
(2) Daragan, V. A.; Mayo, K. H.*J. Magn. Reson. B* **1995**, *107*, 274−8. Daragan, V. A.; Mayo, K. H. *Prog. Nucl. Magn. Reson. Spectrosc.* **1997**, *31*, 63−105.
(3) Fischer, M. W. F.; Majumdar, A.; Zuiderweg, E. R. P. *Prog. Nucl. Magn. Reson. Spectrosc.* **1998**, *33*, 207−72.
(4) Pang, Y.; Wang, L.; Pellecchia, M.; Kurochkin, A. V.; Zuiderweg, E. R. P. *J. Biomol. NMR* **1999**, *14*, 297−306.
(5) Oas, T. G.; Hartzell, C. J.; McMahon, T. J.; Drobny, G. P.; Dahlquist, F. W. *J. Am. Chem. Soc.* **1987**, *109*, 5956−62. Teng, Q. Determination of tensor orientations and the applications in the study of gramicidin A by solid-state NMR. Ph.D. Thesis, Florida State University, 1990. Teng, Q.; Iqbal, M.; Cross, T. A. *J. Am. Chem. Soc.* **1992**, *114*, 5312−21.
(6) Fushman, D.; Cowburn, D. *J. Am. Chem. Soc.* **1998**, *120*, 7109−10. Fushman, D.; Tjandra, N.; Cowburn, D. *J. Am. Chem. Soc.* **1998**, *120*, 10947−52. Fushman, D.; Tjandra, N.; Cowburn, D. *J. Am. Chem. Soc.* **1999**, *121*, 8577−82.
(7) Devore, J. L. *Probability and Statistics for Engineering and the Sciences*; Duxbury Press: Belmont, 1995; p 349.
(8) Chandrasekhar, I.; Clore, G. M.; Szabo, A.; Gronenborn, A. M.; Brooks, B. R. *J. Mol. Biol.* **1992**, *226*, 239−50. Fadel, A. R.; Jin, D. Q.; Montelione, G. T.; Levy, R. M. *J. Biomol. NMR* **1995**, *6*, 221−6.
(9) Lienin, S. F.; Bremi, T.; Brutscher, B.; Brüschweiler, R.; Ernst, R. R. *J. Am. Chem. Soc.* **1998**, *120*, 9870−9.

10.1021/ja994184x CCC: $19.00
Published on Web 04/29/2000

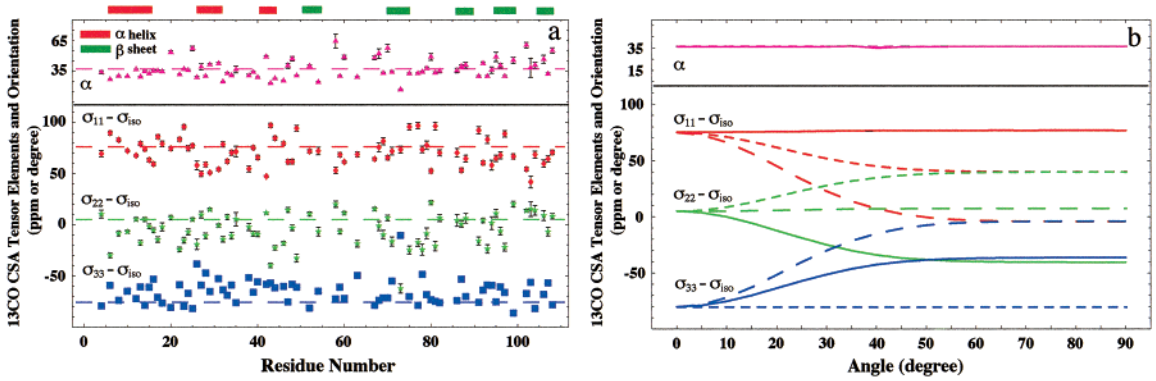

Figure 2. (Panel a, experimental) Values of the ^{13}CO CSA tensor principal components for the individual amino acid residues of the protein Binase in solution. The tensor components are expressed as differences from the isotropic chemical shifts, and the angle α is defined in Figure 1. A rotational correlation time of 6.0 ns, determined from standard ^{15}N relaxation measurements, was used. The cross correlation data were obtained at 303 K, using a Bruker AMX-500 spectrometer, with uniformly ^{15}N/^{13}C labeled Binase (12.3 kDa) at ~1.5 mM (90:10 H_2O:D_2O, pH 5.2). The transverse cross correlation rates ^{13}CO (CSA)/^{13}CO−^{13}Cα (DD) were obtained from the ratios of the ($^1J_{CO-C\alpha}$ = 55 Hz) doublet intensities in a coupled constant-time 3D HNCO experiment.[11] The cross correlation rates were independent of the length of the constant time period used. The transverse cross correlation rates ^{13}CO (CSA)/^{13}CO−^{15}N (DD) were obtained from the ratios of the ($^1J_{CO-N}$ = 17 Hz) doublet intensities in a coupled constant-time 3D HNCO experiment.[12] As the two-bond scalar coupling $^2J_{CO-HN}$ is small, the ^{13}CO (CSA)/^{13}CO−^{1}HN (DD) transverse cross correlation rates were obtained using a E.COSY strategy.[4] The error bars in the figure represent ±70% confidence intervals, and were obtained as follows. Experimental uncertainties were derived from the signal-to-noise ratios (taken to correspond to one standard deviation) in the original NMR spectra and were expressed as lower and upper limits. Eight combinations of lower and upper limits for the three cross correlations were subsequently used for every residue to compute a range of apparent tensor values according to eq 1. These ranges are indicated by the bars in this figure, and thus correspond to two standard deviations. The dashed horizontal lines give the averages of the corresponding tensor elements as determined from solid-state NMR measurements reported in the literature.[5] Chemical shift assignments for Binase were extended from ref 13; the secondary structure from ref 14. (Panel b, theoretical) Computations of the effect of local anisotropic motions on the apparent values of the tensor elements. The abscissa indicates the extend of Gaussian axial fluctuations[11] along three different axes in ±deg. The solid traces give the effect of fluctuations around an axis parallel to σ_{11} (crank shaft motion), the traces with large dashes around an axis parallel to σ_{22}, and the traces with small dashes around an axis parallel to σ_{33}. The apparent tensor values were obtained using the following two-step procedure. First, theoretical values for the three different cross correlation rates ^{13}CO (CSA)/^{13}CO −^{15}N (DD), ^{13}CO (CSA)/^{13}CO−^{13}Cα (DD), and ^{13}CO (CSA)/^{13}CO−^{1}HN (DD) were computed according to eq 1, taking local motion modeled as Gaussian axial fluctuations around the three different directions into account. Average solid-state NMR parameters were used for the ^{13}CO tensor, and the effects of the Gaussian fluctuations on the order parameters were calculated by numerically solving the expressions[12,13] $S_{ii,IS} = (4\pi/5)\sum_{m=-2}^{2}\langle Y_{2m}(\theta_{ii}, \varphi_{ii})\rangle\langle Y^*_{2m}(\theta_{IS}, \varphi_{IS})\rangle$, where, e.g., $\langle Y_{2m}(\theta_{ii}, \varphi_{ii})\rangle = \int_0^{2\pi}\int_0^{\pi} Y_m(\theta_{ii}, \varphi_{ii})\, P_{ii}(\theta_{ii}, \varphi_{ii})\, \sin\theta\, d\theta\, d\varphi$. Here, the terms Y_{2m} are spherical harmonics while $P_{ii}(\theta_{ii}, \varphi_{ii})$ gives a probability distribution of the angles θ_{ii} and φ_{ii} in the molecular frame, modeling the local motion. Second, these theoretical local motion-affected cross correlation data were used as synthetic input to re-compute the quantities σ_{11}, σ_{22}, and σ_{33} as well as the angles θ according to eq 1, now assuming that no local motion occurs (and assuming an ^{13}CO isotropic chemical shift of 170 ppm). In effect, with this procedure we calculate apparent, motionally averaged, tensor values from the theoretical data.

Table 1. Average ^{13}CO Chemical Shift Tensors[a]

	solid (9)	α helix (17)	β sheet (18)	loop (36)	all (71)
$\sigma_{11}-\sigma_{iso}$	73.6 ± 6.0	68.5 ± 15.1	69.1 ± 9.9	72.2 ± 13.5	70.5 ± 13.1 (1.2)
$\sigma_{22}-\sigma_{iso}$	4.0 ± 6.3	−7.9 ± 15.1	−3.2 ± 19.2	−4.1 ± 14.3	−4.8 ± 15.7 (3.1)
$\sigma_{33}-\sigma_{iso}$	−77.6 ± 3.8	−60.5 ± 9.7	−65.9 ± 16.8	−68.2 ± 9.5	−65.8 ± 12.0 (6.2)
σ_{iso}	170 ± 2.0	178.1 ± 1.4	174.9 ± 1.4	175.4 ± 1.8	175.9 ± 2.1
α	35.7 ± 1.5	35.8 ± 6.9	41.4 ± 9.9	41.2 ± 9.8	40.0 ± 9.4 (3.5)

[a] The average ^{13}CO chemical shift tensor parameters for residues in α-helical, β-sheet, loop, and all areas of Binase, as derived from this work. Also given are average ^{13}CO chemical shift tensor parameters in the solid state.[5] The number of measurements used to calculate averages is included in parentheses. Tensor components and orientations are given in ppm and deg, respectively. Standard deviations are given. *Z* test values[7] evaluating the significance of the differences of the averages in the solid and all column are given in parentheses in the last column.

Anisotropic rotational fluctuations cannot account for σ_{11} solution tensor values that are larger than the solid-state values; such effects may be caused by variations in strength of hydrogen bonding to the carbonyl groups.[10] Anisotropic dynamics cannot explain the (apparent) variations of the angle α of the principal axis system of the ^{13}CO tensor. These effects may be due to static variation.

Acknowledgment. We thank Drs. Anil Kumar and Mark W.F. Fischer for helpful discussions. Drs. Alexander V. Kurochkin and Lincong Wang are gratefully acknowledged for the preparation of double-labeled binase. This work was supported by grant MCB 9814431 from the National Science Foundation.

Supporting Information Available: Tables with the three measured transverse cross correlation rates and the calculated tensor values for the protein Binase (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA994184X

(10) De Dios, A. C.; Oldfield, E. *J. Am. Chem. Soc.* **1994**, *116*, 11485−8; Sitkoff, D.; Case, D. A. *Prog. Nucl. Magn. Reson. Spectrosc.* **1998**, *32*, 165−90.

(11) Fischer, M. W. F.; Zeng, L.; Pang, Y.; Hu, W.; Majumdar, A.; Zuiderweg, E. R. P. *J. Am. Chem. Soc.* **1997**, *119*, 12629−42.

(12) Ghose, R.; Huang, K.; Prestegard, H. *J. Magn. Reson.* **1998**, *135*, 487−99.

(13) Kurochkin, A. V.; Kirpichinov, M. P.; Rüterjans, H. *Dokl. Biochem.* **1991**, *321*, 282−6.

(14) Pavlovsky, A. G.; Vagin, A. A.; Vainstein, B. K.; Chepurnova, M. K.; Karpeisky, M. Y. *FEBS Lett.* **1983**, *162*, 167−70.