Direct Measurement of ^{15}N Chemical Shift Anisotropy in Solution

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Abstract: The magnitude and orientation of the ^{15}N chemical shift anisotropy (CSA) tensors are determined for human ubiquitin in solution from ^{15}N relaxation data at 600, 500, and 360 MHz. The analysis uses the model-independent approach [Fushman, D.; Cowburn, D. *J. Am. Chem. Soc.* **1998**, *120*, 7109−10] based on a ratio, η/R_2, of the cross correlation (η) between ^{15}N CSA and ^{15}N−^{1}H dipolar interaction and of the rate (R_2) of ^{15}N transverse relaxation. Since the η/R_2 ratio does not contain any direct dependence on protein dynamics, the present approach is free from assumptions about overall and local motions. The ^{15}N CSA values fall in the range −125 to −216 ppm, with the average value of −157 ± 19 ppm; the average angle between the NH bond and the unique principal axis of the ^{15}N CSA tensor was 15.7 ± 5.0° (range 6−26°). The results indicate the importance of residue-specific ^{15}N CSA for accurate analysis of dynamics from relaxation data, and provide access to the CSA in solution, which may be structurally useful.

The chemical shift tensor contains information about the local environment of a nucleus and therefore is a valuable source of information on local structure and conformation of molecules. Information on the individual components of the chemical shift tensor is not generally available from signal positions in solution NMR spectra because it is averaged by fast reorientational motion in solution, so that only the isotropic chemical shift, $\sigma^{iso} = tr(\underline{\sigma})/3$, is available from high-resolution NMR studies. Anisotropy of the chemical shift tensor (CSA), on the other hand, contributes to nuclear spin relaxation caused by reorientational motion, and therefore, in principle, can be determined from NMR relaxation studies.

A model-independent approach to analysis of ^{15}N CSA was suggested recently,[1] based on the similarity of the dependence on spectral density function $J(\omega)$ of the cross correlation rate, η, between ^{15}N CSA and ^{15}N−^{1}H dipolar interaction:

$$\eta = dc[4J(0) + 3J(\omega_N)]P_2(\cos\theta) \quad (1)$$

and of the rate, R_2, of ^{15}N transverse relaxation,

$$R_2 = {}^1/_2(d^2 + c^2)[4J(0) + 3J(\omega_N)] + P_{HF} + R_{ex} \quad (2)$$

where $P_{HF} \equiv {}^1/_2 d^2[J(\omega_H - \omega_N) + 6J(\omega_H) + 6J(\omega_H + \omega_N)]$ denotes the contribution to R_2 from *h*igh-*f*requency 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; θ is the angle between the ^{15}N−H vector and the unique principal axis of the ^{15}N chemical shift tensor; $d = -(\mu_o/(4\pi))\gamma_H\gamma_N h/(4\pi r_{HN}^3)$, $c = \gamma_N B_o(\sigma_{||} - \sigma_\perp)/3$ (ref 2); r_{HN} is the internuclear ^{15}N−H bond length; $\sigma_{||} - \sigma_\perp$ is the anisotropy of the ^{15}N chemical shift tensor, assumed axially symmetric; $P_2(x) = (3x^2 - 1)/2$ is the second-rank Legendre polynomial; 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;[3,4] $P_{HF} = 13(\gamma_N/\gamma_H)(1 - NOE)R_1/10$. For the majority of the amide NH groups $J(\omega_H)$ is much smaller than $J(\omega_N)$ and $J(0)$,[3,4] which then allows P_{HF} to be safely neglected to first order:

$$R_2 \approx {}^1/_2(d^2 + c^2)[4J(0) + 3J(\omega_N)] \quad (3)$$

assuming R_{ex} is negligible (see ref 1 for P_{HF} analysis in ubiquitin). The ratio

$$\frac{\eta}{R_2} = \frac{2dc}{d^2 + c^2}P_2(\cos\theta) \quad (4)$$

is then independent of protein dynamics,[1] and therefore contains only "structural" information, in the form of the ^{15}N CSA and the angle θ between the ^{15}N−H vector and the unique principal axis of the ^{15}N chemical shift tensor.[5]

The η/R_2 ratio, eq 4, contains the product $(\sigma_{||} - \sigma_\perp)P_2(\cos\theta)$, and therefore does not directly permit independent determination of the magnitude and orientation of the ^{15}N CSA tensor from single-frequency ^{15}N relaxation data.[1] Here we demonstrate a method of separating the magnitude and orientation of the CSA tensor using relaxation data collected at different magnetic field strengths. This method is used to determine both the magnitude and orientation of the ^{15}N chemical shift anisotropy tensor in human ubiquitin. It is worth noting that since the η/R_2 ratio does not contain any direct dependence on protein dynamics, the present approach does not require assumptions about overall and/or local motion (like, e.g., the

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(5) An astute reviewer points out that eq 4 can be cast into a form suitable for linear least squares, $2\omega_N R_2/\eta = 3d/\{\Delta\sigma P_2(\cos\theta)\} + \Delta\sigma/\{3dP_2(\cos\theta)\}\omega_N^2$. The linear fit of the left-hand side vs ω_N^2 producing a slope m, and an intercept, b, which then permits direct evaluation as $P_2(\cos\theta) = 1/\sqrt{mb}$ and $\Delta\sigma = 3d\sqrt{m/b}$.

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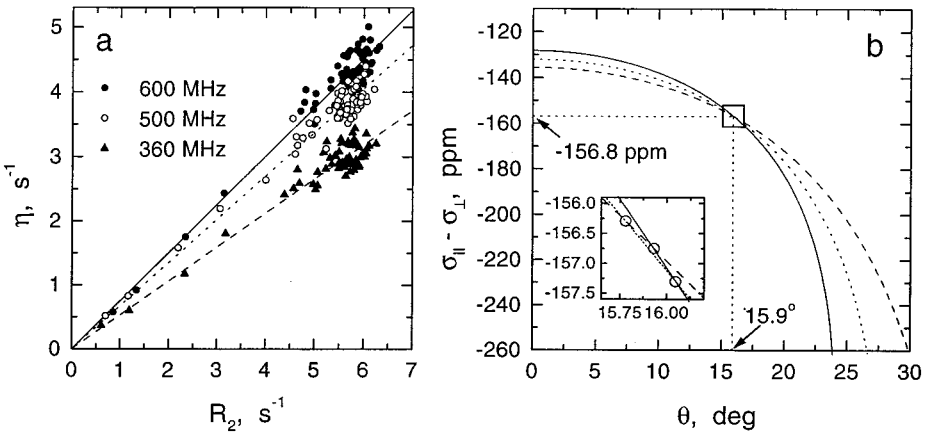

Figure 1. (a) Correlation between η and R_2 in ubiquitin, from the 600 (solid circles), 500 (open circles), and 360 MHz (solid triangles) data; and (b) graphical illustration of the present method of derivation of CSA and θ from η/R_2 data at multiple fields. The straight lines in (a) correspond to a linear fit, $\eta = (0.752 \pm 0.005)R_2$ (solid), $\eta = (0.675 \pm 0.004)R_2$ (dotted), and $\eta = (0.531 \pm 0.004)R_2$ (dashed), of the data for 600, 500, and 360 MHz, respectively, using all residues not subjected to conformational exchange. The correlation coefficient between η and R_2 was 0.96 (600 MHz), 0.96 (500 MHz), and 0.95 (360 MHz). The curves in (b) represent loci of CSA and θ value solutions for the average values of η/R_2 (from the linear fits in part a at 600 (solid), 500 (dotted), and 360 MHz (dashed) separately; the intersection of any two curves provides a unique solution for a given pair of η/R_2 values at two frequencies, as indicated in the inset. The values of R_2 were modified to subtract the contribution, $P_{HF} = (13/2)d^2J(\omega_H)$, from high-frequency components of the spectral density function, assuming the reduced spectral density function approach.[3,4] This contribution is small for most residues in ubiquitin.[1] $J(\omega_H)$ at 600 MHz was derived directly from the experimental data: $J(\omega_H) = (\gamma_N/\gamma_H)d^{-2}R_1(1\text{-NOE})/5$, whereas $J(\omega_H)$ values at 500 and 360 MHz were estimated as $J(\omega_H)$ values at 600 MHz scaled by $(600/500)^2$ and $(600/360)^2$, respectively. Residues Ile[23] and Asn[25], exhibiting a conformational exchange contribution, are not shown. These two residues were excluded from further analysis. The error bars in (a) are of the size or smaller than the symbols. The errors in modified R_2 were computed as $[(\delta R_2)^2 + (13d^2/2)^2(\delta J(\omega_H))^2]^{1/2}$, where $\delta J(\omega_H) = J(\omega_H)[(\delta R_1/R_1)^2 + (\delta\text{NOE}/(1\text{-NOE}))^2]^{1/2}$, assuming normal distribution of measurement errors. Experimental errors, δR_1, δR_2, δNOE, and $\delta\eta$, in the corresponding relaxation parameters were estimated based on data reproducibility in separate measurements, as described in ref 6.

correlation time and the degree of anisotropy of the overall rotation, order parameters, correlation times of local motion, etc). Therefore, the results of this study are model-independent, free from any systematic errors associated with the analysis of relaxation data with use of terms for either overall or micro-dynamic characterization.

As pointed out in ref 1 and illustrated in Figure 1b, for any given value of η/R_2 eq 4 has multiple solutions in terms of CSA and θ. Since both CSA and θ are independent of the magnitude B_o of the external magnetic field, eq 4 can be used to derive these parameters from analysis of η/R_2 values at multiple fields. For a given pair of η/R_2 values at two fields, the intersection of the corresponding curves provides a unique solution in terms of CSA and θ (this pairwise procedure of deriving CSA and θ by direct solution of eq 4 for η/R_2 data at two fields is described in detail in the Supporting Information). The presence of data at more than two fields makes the system overdetermined. For example, in the case of the three fields considered here, up to three pairs of η/R_2 values per residue are available. Due to experimental uncertainties, the curves corresponding to various pairs of η/R_2 data might intersect at somewhat different points in the CSA$-\theta$ plane, as illustrated in the inset of Figure 1b. To satisfy various pairs of η/R_2 simultaneously, the least-squares-fit method was used here. The values of ^{15}N CSA and θ for each residue were derived by fitting the experimental data to eq 4, based on minimization of the following target function:

$$\chi^2 = \sum_{B_o}\left[\frac{(\eta/R_2)^{exp}_{B_o} - (\eta/R_2)^{calc}_{B_o}}{\sigma_{B_o}}\right]^2 \qquad (5)$$

Here σ_{B_o} is the experimental error in η/R_2 at field B_o, and superscripts exp and calc indicate measured and calculated values, respectively. Representative examples of the quality of fit are shown in Figure 2.

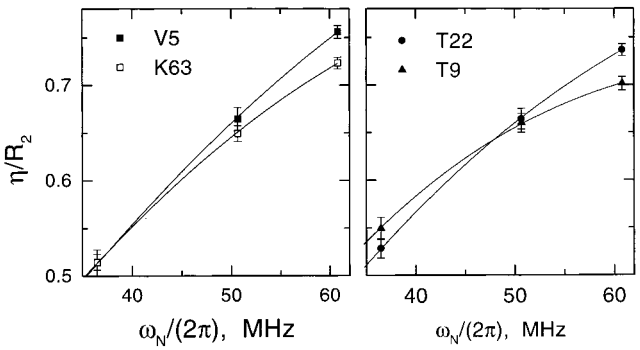

Figure 2. Representative examples of fitting the experimental η/R_2 data at three fields to eq 4. Lines represent the best fit obtained with use of the least-squares fit procedure described in the text.

Materials and Methods

In this paper, we use ^{15}N relaxation data for human ubiquitin, collected at three fields corresponding to 600, 500, and 360 MHz, at 27 °C. The 600 MHz measurements of ^{15}N R_1, R_2, and NOE and η measurements at both 600 and 360 MHz were reported previously,[6,7] with the average data precision of 0.5%, 0.7%, 2%, 0.7%, and 1.7%, respectively. The R_1, R_2, and η values at 500 MHz and R_1 and R_2 data at 360 MHz were obtained on the same sample by using the same methods as in ref 6, and the level of experimental error was 1.1%, 1.3%, and 1.1% (500 MHz) and 1.5% and 1.3% (360 MHz data). The η measurements at 500 MHz used a straight 180° proton pulse instead of a composite 180° pulse for conversion of the in-phase component of the $^{15}N_Z$ magnetization in experiment A.[7] To compensate for the effect of this pulse that is absent in reference experiment B, the ratio I_A/I_B of cross-peak intensities from the two experiments was scaled by 5.8%, based on the η consistency test, eq 6 (Figure 3a). At the fields used here, $c < d$, $c/d = 0.34$ at 360 MHz, 0.47 at 500 MHz, and 0.57

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at 600 MHz (for CSA=-160 ppm), so high precision relaxation measurements are required in order to determine *c* from this comparison.

Testing Consistency of Multiple-Field Data. The approach suggested here relies on experimental data acquired at different fields, hence on different spectrometers. Matching multiple-field relaxation data is not a trivial problem.[8] Care has to be taken to avoid any systematic errors/deviations caused by differences in the instrument-associated experimental setup, like, e.g., temperature calibration and control, pulse widths, etc. Since the η/R_2 ratio does not directly depend on protein dynamics, the present approach can tolerate small deviations in temperature or other sample conditions between different instruments used for data collection. However, to check consistency of the present multiple-field data, the following internal calibration tests were introduced here.

***η*-test.** On the basis of eq 1, η scales with magnetic field as $\eta \propto B_o[4J(0) + 3J(\omega_N)]$. Assuming the "model-free" form of $J(\omega)$,[9] the ratio

$$F_\eta = \frac{\eta}{B_o\{4J(0) + 3J(\omega_N)\}} \propto \frac{\eta}{B_o\{4 + 3[1 + (\omega_N\tau_c)^2]^{-1}\}} \quad (6)$$

is expected to be independent of the magnetic field strength, at least for those residues with restricted local mobility. Note that the only "theoretical" parameter required for performing this internal calibration test is the overall rotational correlation time τ_c. Figure 3a,b indicates an excellent agreement between the values of the ratio measured at different fields.

***R*$_2$-test.** A similar field-independent ratio for R_2,

$$F_{R_2} = \frac{R_2 - P_{HF}}{[4J(0) + 3J(\omega_N)](d^2 + c^2)} \propto \frac{R_2 - P_{HF}}{\{4 + 3/[1 + (\omega_N\tau_c)^2]\}[d^2 + (CSA\omega_N/3)^2]} \quad (7)$$

follows from eqs 2 and 3. Consistency of the transverse relaxation data at three fields is illustrated in Figure 3c,d.

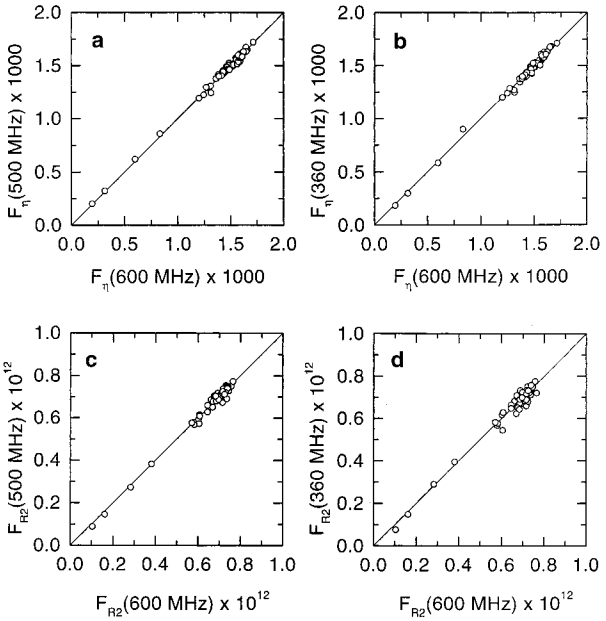

Figure 3. Illustrations of the data consistency tests for the experimental values of η (a, b) and R_2 (c, d), according to eqs 6 and 7. Solid lines correspond to theoretically expected correlation. The correlation coefficient between the η values was 0.996 (600 vs 360 MHz data), 0.997 (600 vs 500), and 0.994 (500 vs 360). The corresponding values of the correlation coefficient between R_2 values at the same frequencies were 0.994, 0.983, and 0.986. An overall rotational correlation time of 4.1 ns was assumed.[6] In addition to τ_c, the R_2-test requires a value of CSA. An average value of −157 ppm was assumed. Some spread in the data in parts c and d is caused by variations in individual CSA values, as well as experimental errors.

Results and Discussion

The correlation between the experimental values of η and R_2 is illustrated in Figure 1a, consistent with the previous prediction[1] that both these quantities contain the same combination of spectral density function components.

Figure 4a shows the distribution and the level of precision of η/R_2 values derived from ^{15}N relaxation data for human ubiquitin, collected at 600, 500, and 360 MHz. This distribution of η/R_2 values is due to variation in CSA−θ values and not to local protein dynamics. The magnitude of the ^{15}N CSA tensor and its orientation (angle θ) derived as described above are shown in parts b and c of Figure 4, respectively. The angle θ could not be determined for Thr[12], Leu[15], Val[26], Ile[44], and Glu[51], as the derived values turned out to be smaller that the estimated errors, and these values are not included in further analysis. These results then allow detailed mapping of the CSA−θ values in human ubiquitin, as depicted in Figure 5.

Until recently, solid-state NMR remained the only source of experimental information on individual components of chemical shift tensors in biomacromolecules. On the basis of numerous solid-state NMR studies,[10−16] mostly focused on small (di- or tri-) peptides with a limited range of residue types (Gly, Ala, Tyr, and Leu) being ^{15}N labeled, the ^{15}N CSA values fall into a range of −144 to −165 ppm, while the θ angle is found to be from 12 to 24°. Studies on oriented samples of a 15-residue peptide gramicidin A,[17] which provide data for a wider range of residues (Ala, Gly, Val, Trp, D-Val, and D-Leu) and sites, represent only a certain (α-helical) conformation of the polypeptide chain. The question yet remains of how adequately the existing solid-state NMR data on peptides represent the situation with ^{15}N CSA in a real protein molecule, taking into account both its structural and sequence diversity. The approach suggested here provides a means to directly measure chemical shift anisotropy in uniformly labeled proteins.

Present results (Figure 4b,c) indicate a distribution of ^{15}N CSA in ubiquitin in a range from −125 to −216 ppm, with the average value of −157 ± 19 ppm. The average value of angle θ, 15.7 ± 5.0°, range 6° to 26°, is consistent with a recent report.[18] The observed spread of CSA magnitude and θ values is somewhat larger than anticipated, based on solid-state NMR studies of peptides,[10−17] but the limited range of structural and residue variability in the solid-state studies does not permit a conclusion that the solid state and solution measurements are inconsistent. Interestingly, the range of CSA and θ values

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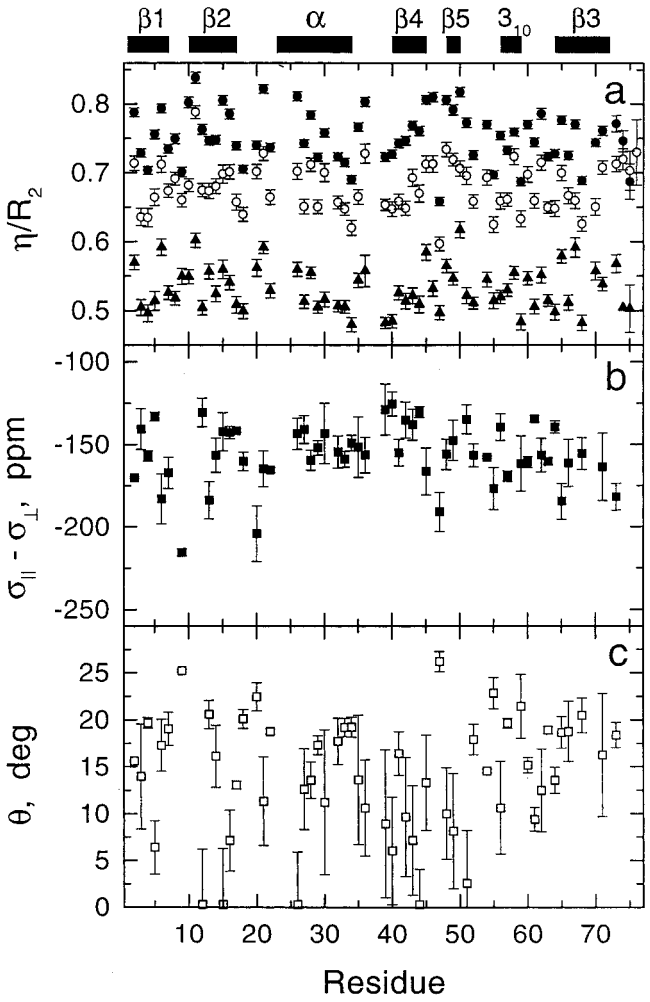

Figure 4. Values of the (a) η/R_2 ratio (solid circles, 600 MHz, open circles, 500 MHz, triangles, 360 MHz), (b) ^{15}N CSA, and (c) the θ angle versus residue number in human ubiquitin. Horizontal bars (top) indicate elements of secondary structure. CSA and θ were calculated by the least-squares fit method as described in the text (eq 5). The fitting was performed with Matlab programs based on the simplex algorithm.[32] For 12 out of 65 amide groups analyzed here, the minimized χ^2 value exceeds the 95% confidence limit (3.84) for the goodness-of-fit test.[33] These residues are not presented in parts b and c. The errors in η/R_2 were calculated as $(\eta/R_2)[(\delta\eta/\eta)^2 + (\delta R_2/R_2)^2]^{1/2}$. The standard errors in the derived parameters (CSA, θ) were estimated by Monte Carlo simulation of 5000 synthetic data sets and subsequent analysis of these "experimental" data using the same fitting routine.[33] Estimated errors in angle θ in five residues, Thr[12], Leu[15], Val[26], Ile[44], and Glu[51], exceeded the derived values of θ. The average relative errors are 5.6% for CSA values and 25% for θ, and in the latter case, the above-mentioned five residues are not included. No significant difference in average values of CSA or θ was observed between β-sheet (-151.8 ± 17.5 ppm, $13.9 \pm 5.0°$), α-helix (-151.2 ± 7.2 ppm, $16.6 \pm 2.8°$), and 3_{10}-helix (-156.8 ± 15.5 ppm, $17.2 \pm 5.8°$). Residues with the largest θ and absolute CSA values are mostly located in the turns, although average values of CSA and θ (-170.4 ± 22.8 ppm, $18.7 \pm 5.2°$) here are not significantly different from those of the rest of the protein.

observed in the α-helix (-141 to -160 ppm, $11-19°$) is much smaller than that in the rest of the protein, and is similar to the one (-144 to -162 ppm, $12-20°$) reported for the helical conformation of gramicidin A.[17] This might suggest a rather uniform characteristics of the ^{15}N chemical shift tensor in the α-helical conformation.

What effects on the apparent dynamics would these values of ^{15}N CSA have? To avoid hydrodynamic-dependent features

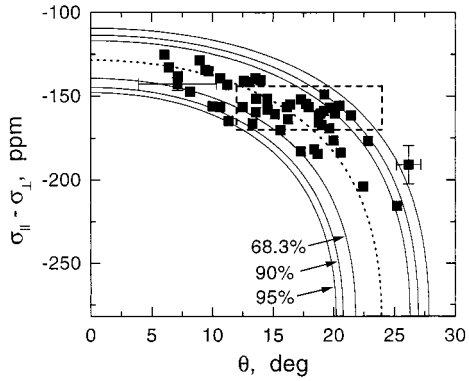

Figure 5. Map of loci of CSA and θ value solutions for NMR data of human ubiquitin. Symbols represent the results of this paper. The solid line loci correspond to 95%, 90%, and 68.3% probabilities of distribution of the experimental η/R_2 values from 600 MHz data for ubiquitin.[1] The dotted line in the middle of the 68.3% area corresponds to the locus for the average value of $\eta/R_2 = 0.752$ for ubiquitin.[1] The dashed rectangle indicates a range of values of the parameters, $\theta = 12-24°$, CSA from -144 to -170 ppm, expected from solid-state NMR studies of peptides,[10-16] and from previous analysis of NMR solution studies of ubiquitin.[7] The level of precision in the CSA and θ values is indicated for two residues, Glu[16] and Gly[47].

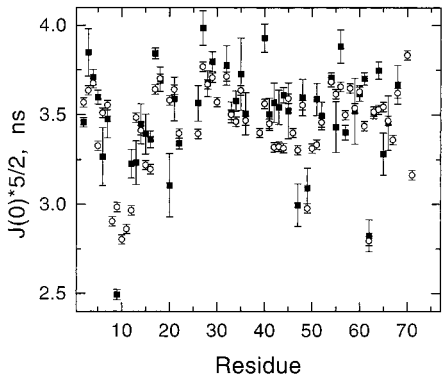

Figure 6. Comparison of the zero-frequency components, $J(0)$, of the spectral density in ubiquitin derived from the relaxation data (R_1, R_2, and NOE) at 600 MHz, using ^{15}N CSA values obtained here (solid squares) and assuming CSA $= -160$ MHz (open circles). Note that in the model-free aproach[9] the squared order parameter is linearly related to $J(0)$, e.g., $S^2 = (5/2)J(0)/\tau_c$ for local motions faster than the overall tumbling ($\tau_{loc} \ll \tau_c$); therefore differences in $J(0)$ suggest differences in the apparent S^2 values.

of the model-free approach,[9] the most effective comparative analysis is to calculate the spectral density at zero frequency, $J(0)$, from the values of R_1, R_2, and NOE, using either the residue-specific values of CSA derived here or the standard value of CSA of -160 ppm.[13] Figure 6 indicates that the $J(0)$ values from these two approaches do differ significantly, for about 50% of the residues have the two values differing by more than the sum of their standard errors of the mean.

How are these results related to protein structure? The nature of the amide ^{15}N chemical shift in the protein backbone is complex (reviewed in refs 19 and 20). The isotropic chemical shift of ^{15}N is sensitive to backbone conformation.[21,22] However, a direct relation of the ^{15}N chemical shift to a single structural parameter is not straightforward, due to contributions

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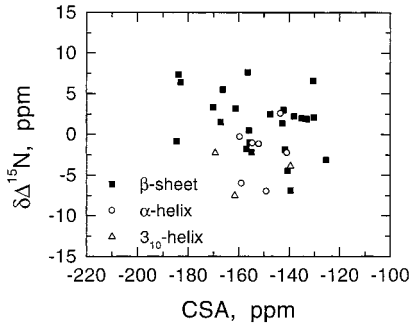

Figure 7. Correlation between ^{15}N CSA and deviation, $\delta\Delta^{15}N$, of the isotropic ^{15}N chemical shift[24] from the random coil value[24] for backbone amides in human ubiquitin. Only those residues belonging to well-defined elements of secondary structure are shown, indicated as follows: β-sheet (squares), α-helix (open circles), and 3_{10}-helix (open triangles).

to shielding from various sources, including torsion angles of both current and preceding residues (ϕ_i, ψ_i, χ_i, ϕ_{i-1}, ψ_{i-1}), hydrogen bonding, solvent accessibility, local sequence effects, longer range electrostatics, etc.[19] Whether the anisotropic component of the tensor has similar sensitivity remains to be elucidated with a much larger set of data. Figure 7 indicates a weak correlation of observed ^{15}N CSA values with deviation of ^{15}N isotropic chemical shift from its random coil value.[23] In particular, the amide ^{15}N nuclei in the α-helix display a trend toward smaller absolute values of CSA as compared to the average level of the chemical shift anisotropy in the β-sheet. A larger database is necessary to get an insight into ^{15}N CSA correlation with protein structure. ^{15}N CSA studies on other proteins are currently in progress.

The values of ^{15}N CSA obtained here, in conjunction with the isotropic ^{15}N chemical shift data,[24] then allow determination of the individual (parallel and orthogonal) components of the ^{15}N chemical shift tensor:

$$\sigma_{||} = (3\sigma_{iso} + 2\mathrm{CSA})/3; \qquad \sigma_{\perp} = (3\sigma_{iso} - \mathrm{CSA})/3 \quad (8)$$

These results are presented in Table 1.

Analysis of Possible Errors. Besides experimental errors in the relaxation parameters, three assumptions made here might influence the derived CSA and θ values.

First, conformational exchange contributions, R_{ex}, are assumed negligible. Two residues, Ile[23] and Asn[25], exhibiting a significant conformational exchange contribution,[1,6] were excluded from the analysis. Absence of systematic (uniform) R_{ex} contribution follows from the fact that the η vs R_2 dependence at each field can be nicely fit to a linear dependence going through the origin (Figure 1a). Possible contributions to apparent CSA due to the presence of small R_{ex} can be approximated as follows:

$$\mathrm{CSA}_{app} = \mathrm{CSA}(1 + 0.39R_{ex}) \quad (9)$$

where the subscript app indicates the apparent values corresponding to $R_{ex} > 0$. For R_{ex} values below 0.25 s^{-1} (larger values of R_{ex} would be relatively easily detected by other methods[25]) this suggests possible overestimation of the absolute value of CSA by less than 10%.

Table 1. Parallel and Orthogonal Components of Amide ^{15}N Chemical Shift Tensor in Human Ubiquitin, Restored from Isotropic Chemical Shifts[22] and CSA Values (present work) according to Eq 8[a]

residue	$\sigma_{||}$, ppm	$\sigma_{\perp}$, ppm	residue	$\sigma_{||}$, ppm	$\sigma_{\perp}$, ppm
Q2	9.7(0.4)	180.0(0.2)	Q40	33.2(4.8)	158.6(2.4)
I3	21.6(7.6)	162.2(3.8)	Q41	14.4(5.8)	169.4(2.9)
F4	13.5(2.1)	170.4(1.1)	R42	32.6(7.5)	167.7(3.8)
V5	32.4(1.8)	165.3(0.9)	L43	32.0(6.3)	170.1(3.1)
K6	5.5(9.5)	188.5(4.7)	I44	35.1(2.3)	165.3(1.1)
T7	3.9(6.3)	171.1(3.2)	F45	14.5(9.6)	180.8(4.8)
T9	−38.1(1.4)	177.4(0.7)	G47	−24.9(7.7)	166.0(3.8)
T12	33.4(5.7)	164.0(2.9)	K48	17.6(6.3)	173.6(3.2)
I13	4.5(7.2)	188.4(3.6)	Q49	24.1(7.5)	171.6(3.8)
T14	17.2(7.2)	173.7(3.6)	E51	33.0(5.6)	167.9(2.8)
L15	30.0(6.9)	172.2(3.5)	D52	15.6(4.8)	172.2(2.4)
E16	27.0(2.5)	169.8(1.2)	R54	13.8(0.6)	171.7(0.3)
V17	22.8(0.8)	164.4(0.4)	T55	−9.4(8.2)	167.3(4.1)
E18	12.1(3.8)	172.4(1.9)	L56	24.9(5.4)	164.4(2.7)
S20	−32.7(10.6)	171.5(5.3)	S57	0.5(2.2)	169.8(1.1)
D21	13.7(7.6)	178.4(3.8)	Y59	7.8(11.2)	169.4(5.6)
T22	−1.5(1.0)	164.0(0.5)	N60	8.7(2.4)	169.5(1.2)
V26	26.1(6.5)	169.5(3.3)	I61	28.9(1.5)	163.4(0.7)
K27	24.9(5.7)	165.8(2.8)	Q62	20.4(6.8)	176.9(3.4)
A28	17.1(4.2)	176.6(2.1)	K63	13.3(1.3)	173.8(0.7)
K29	18.6(2.8)	170.6(1.4)	E64	20.9(2.5)	160.5(1.2)
D32	16.3(6.2)	170.9(3.1)	S65	−8.3(7.5)	176.3(3.7)
K33	9.2(3.4)	168.1(1.7)	T66	9.6(9.8)	170.9(4.9)
E34	14.5(3.1)	163.6(1.5)	H68	14.6(6.5)	170.3(3.2)
G35	7.6(12.2)	159.2(6.1)	L73	3.0(5.4)	184.8(2.7)
I36	15.6(7.0)	172.0(3.5)			

[a] Numbers in parentheses indicate standard errors in the corresponding parameters. An error of 0.1 ppm was assumed for the isotropic chemical shifts.

Second, the values of $J(\omega_H)$ at 500 and 360 MHz were estimated from $J(\omega_H)$ at 600 MHz (see Figure 1 caption). Calculations show that for typical values of order parameter ($S^2 \sim 0.87$) and correlation time of local motion (τ_{loc} from 0 up to 50 ps) expected errors introduced by this approximation are less than 0.7% in η/R_2 and 2.4% in CSA, which are much smaller than estimated uncertainties in these parameters caused by experimental errors.

Last, variations in $^{15}N-^{1}H$ dipolar coupling may arise from variations in the internuclear distance. All calculations in this paper assume $r_{NH} = 1.02$ Å. Variations in r_{NH} in the range from 1.01 to 1.03 Å will cause ±3% variation in the value of d, which in turn will result in ±(1.5, 1.9, and 2.4)% variation in η/R_2 at 600, 500, and 360 MHz, respectively, of the order of experimental errors.

In conclusion, we demonstrated for the first time that CSA's may be obtained by analysis of η/R_2 at multiple frequencies in solution. The variability of the ^{15}N CSA tensor is unexpected. First, the range of magnitude of the CSA suggests that precise estimation of the order parameters from relaxation data for, e.g., estimation of segmental entropy,[26−28] requires CSA measurement, and that the variability of apparent S^2 from variations of CSA could be of the order of 10%. Second, the tensor properties, in conjunction with the isotropic chemical shift, are

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likely to be sensitive indications of the local electron environment about the $^{15}N-^{1}H$ bond,[19,20,29−31] permitting analysis of the relationship of chemical shift to protein structure.

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Supporting Information Available: Detailed analysis and illustration of the η/R_2 method in the case of two fields; one table containing the values of η/R_2,^{15}N CSA, and θ for human ubiquitin (6 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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