Validation of Protein Structure from Anisotropic Carbonyl Chemical Shifts in a Dilute Liquid Crystalline Phase

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Received April 14, 1998

The change in chemical shift ($\Delta\delta$) observed for a given nucleus, when shifting from an isotropic medium to a strongly oriented, liquid crystalline phase, contains valuable information on the orientation of its chemical shift anisotropy (CSA) tensor relative to the molecular alignment tensor.[1] Analogously, we have demonstrated[2] a dramatic improvement in the agreement between observed and predicted magnetic field dependence of ^{15}N chemical shifts in a very weakly, magnetically aligned protein−DNA complex upon inclusion of dipolar coupling constraints in the structure calculation.[3] In that case, the minute changes in ^{15}N shift and the very small one-bond ^{15}N−^{1}H and $^{13}C^{\alpha}$−$^{1}H^{\alpha}$ dipolar couplings resulted from the field-dependent degree of molecular alignment,[3] induced by the magnetic susceptibility anisotropy of the complex.[4] Use of a dilute liquid crystalline medium consisting of phospholipid bicelles[1,5] makes it possible to obtain much higher degrees of molecular alignment while nevertheless retaining the spectral simplicity of the regular isotropic phase.[6] As we show here, it is straightforward to measure the difference in chemical shift frequency when switching from the liquid crystalline to the isotropic phase. The change is largest and most easily measured for backbone carbonyl atoms. The $\Delta\delta^{13}C'$ values can either be used as constraints in the structure calculation or to evaluate the quality of the structure. This latter application is illustrated here, using the protein ubiquitin as a test case.

The change in chemical shift for a given atom upon switching from the liquid crystalline to the isotropic phase is given by

$$\Delta\delta = \sum_{i=x,y,z} \sum_{j=x,y,z} A_{jj} \cos^2 \theta_{ij}\, \delta_{ii}^{\,t} \qquad (1)$$

where θ_{ij} is the angle between the δ_{ii}^{t} principal axis of the traceless CSA tensor and the A_{jj} principal axis of the diagonalized traceless molecular alignment tensor. The alignment tensor **A** and its orientation relative to the molecular structure are obtained from a least-squares fit between observed one-bond N−H and/or C−H dipolar couplings and those calculated on the basis of the orientations of these bond vectors in the protein's structure.[7] The dipolar coupling between two nuclei, A and B, in a solute macromolecule of fixed shape is related to the traceless alignment tensor according to:

$$D^{AB} = \sum_{i=x,y,z} -(\mu_o h/8\pi^3)\gamma_A\gamma_B\langle r_{AB}^{\,-3}\rangle \cos^2 \phi_i\, A_{ii} \qquad (2)$$

where ϕ_i is the angle between the A−B bond vector and the A_{ii} principal axis of the alignment tensor, γ_A and γ_B are the gyromagnetic ratios of the two nuclei, and $\langle r_{AB}^{\,-3}\rangle$ is the vibrationally averaged inverse cube of the distance between the two nuclei.

As chemical shifts are extremely sensitive to sample conditions, it is preferred to measure $\Delta\delta$ from a single sample and to switch from the liquid crystalline to the isotropic phase by lowering the temperature. The effect of the temperature on the change in chemical shift can be eliminated either by measuring its magnitude for a sample without bicelles, or by measuring the shifts at two temperatures in the isotropic phase, and assuming that the chemical shift has a linear dependence on temperature over this range. This latter approach was used for ubiquitin.

$^{13}C'$ NMR chemical shifts were measured at three temperatures, 292, 298 and 304 K, for a sample containing 0.7 mM uniformly ^{13}C/^{15}N-enriched ubiquitin in a 220 μL Shigemi microcell, and 5% w/v bicelles consisting of a 3:1 molar ratio of dimyristoyl-phosphatidylcholine (DMPC) and dihexanoyl-phosphatidylcholine (DHPC),[5] 93% H_2O, 7% D_2O, 10 mM phosphate buffer, pH 6.6. Experiments were carried out at 600 MHz ^{1}H frequency, using a two-dimensional version of the highly sensitive HNCO experiment.[8] Spectra and measurement parameters are included as Supporting Information. The dependence of $^{13}C'$ chemical shift on temperature, measured for a sample without bicelles, was found to be highly linear over the 292−304 K range, and the CSA contribution to the $^{13}C'$ shift was calculated from $\Delta\delta = \delta^{304} - 2\delta^{298} + \delta^{292}$, where δ^{k} is the $^{13}C'$ shift measured in the bicelle containing sample at temperature k. ^{1}H−^{15}N HSQC spectra, without ^{1}H decoupling in the ^{15}N dimension, were recorded under conditions identical to those of the HNCO experiment, and the change in ^{15}N−{^{1}H} splitting between the aligned (304 K) and isotropic (298 K) measurements was used for deriving the dipolar couplings for the backbone ^{15}N−^{1}H pairs (Supporting Information).

Assuming a 1.02-Å distance for $\langle r_{NH}^{\,-3}\rangle^{-1/3}$, and using ubiquitin's X-ray crystal structure[9] with hydrogens added with the

Table 1. Alignment Tensor Orientation and Magnitude of Ubiquitin in the Liquid Crystalline Phase[a]

structure	α	β	γ	$10^4 A_{zz}$	$10^4 A_{yy}$	$10^4 A_{xx}$	Q
1.8-Å X-ray[b]	42°	35°	42°	5.6	−3.6	−2.0	0.227
2.3-Å X-ray[c]	48°	37°	33°	5.4	−3.3	−2.0	0.266
NMR1[d]	44°	37°	44°	5.4	−3.3	−2.1	0.247
NMR2[e,f]	44°	37°	41°	5.6	−3.6	−2.0	0.163

[a] Using 5% w/v bicelles in 93% H_2O, 7% D_2O, with a 3:1 ratio of DMPC:DHPC, at 304 K. The Euler angles α, β, and γ define the alignment tensor relative to the coordinate frame of the 1.8-Å X-ray structure. The NMR structures and 2.3-Å X-ray structures were oriented to yield a best fit to the C^{α} atoms of the 1.8-Å structure prior to calculating the alignment tensor. [b] From ref 9. [c] From ref 11. [d] Calculated using 2727 NOEs and 98 dihedral constraints. [e] Calculated using 2727 NOEs, 98 dihedral, and 372 dipolar constraints. [f] Pairwise rmsd for the backbone atoms of residues 1−70: NMR1 vs NMR2 = 0.33 Å; 1.8 vs 2.3-Å X-ray = 0.62 Å; NMR1 vs 1.8-Å X-ray = 0.56 Å; NMR2 vs 1.8-Å X-ray = 0.45 Å.

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S0002-7863(98)01261-X CCC: $15.00
Published on Web 06/23/1998

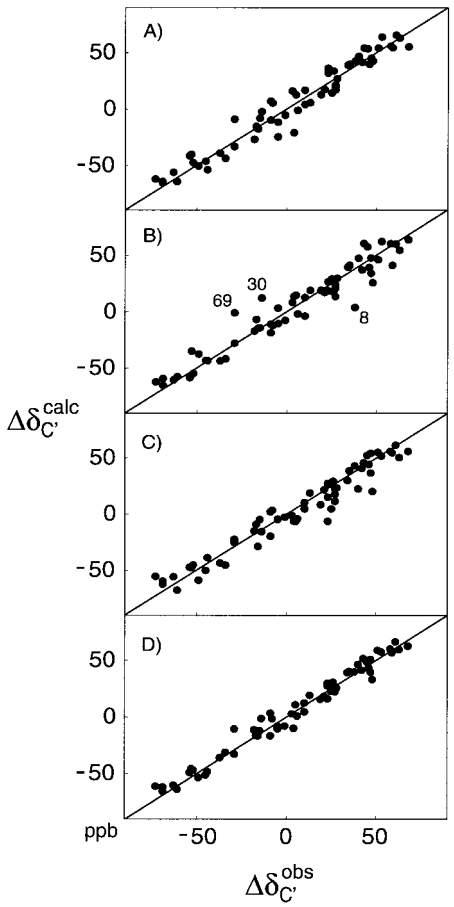

Figure 1. Plot of the predicted versus the experimentally observed change in chemical shift when changing from an isotropic orientation to that defined by the parameters listed in Table 1, for four different structures: (A) 1.8-Å crystal structure;[9] (B) 2.3-Å crystal structure;[13] (C) NMR structure based on 2727 NOE restraints and 63 ϕ and 35 χ_1 restraints derived from *J* couplings; (D) As (C), but including also 59 $^{15}N-^{1}H$, 56 $^{15}N-^{13}C'$, 44 $^{13}C'-^{13}C^{\alpha}$, 68 $^{13}C^{\alpha}-^{1}H^{\alpha}$, 55 $^{13}C'-^{1}H^{N}$, 41 $^{13}C^{\alpha}-^{13}C^{\beta}$, and 49 side chain $^{13}C-^{1}H$ dipolar couplings. Except for residues 71−76 (which are highly disordered), the plots include all residues for which measurements of $\Delta\delta C'$ could be made. The correlation coefficients, R^2, are (A) 0.949; (B) 0.930; (C) 0.943; and (D) 0.974. If all scatter in D were attributed solely to uncorrelated random fluctuations in δ_{11}, δ_{22}, and δ_{33}, $R^2 = 0.974$ corresponds to a rms variation of 11 ppm in δ_{nn} from its average value; 11 ppm is therefore an upper limit for the rms variation in δ_{nn}.

program X-PLOR, best-fitting[7] yields $A_{zz} = 5.6 \times 10^{-4}$, $A_{yy} = -3.6 \times 10^{-4}$, and $A_{xx} = -2.0 \times 10^{-4}$ for the principal components of the alignment tensor. Using NMR structures, slightly smaller or larger values for the alignment tensor are obtained (Table 1), depending on the degree of refinement, but the orientation of the alignment tensor does not shift significantly.

Knowing the magnitude and orientation of the molecular alignment tensor, and assuming a uniform $^{13}C'$ CSA tensor, $\Delta\delta(^{13}C')$ can be calculated straightforwardly from eq 1. In Figure 1, correlations between $\Delta\delta(^{13}C')$ calculated in this manner (using $^{13}C'$ CSA values of Teng et al.[10]) and experimentally obtained values are shown for several different structures. The 1.8-Å crystal structure (Figure 1A) shows considerably better agreement than the 2.3-Å structure (Figure 1B), taken from one-half of di-ubiquitin.[11] Outliers marked in Figure 1B correspond to peptide bonds which adopt slightly different orientations in the two crystal structures. Figure 1C is with respect to the average of 10 NMR structures, calculated on the basis of 2727 NOEs and 98 dihedral angle restraints derived from homo- and heteronuclear *J* couplings. Figure 1D shows the correlation for the average of NMR structures when in addition to the above NOEs and dihedral constraints, 372 dipolar coupling restraints are added to the structure calculation.

We propose the use of a quality or *Q* factor to evaluate the agreement between the structure and the observed $\Delta\delta$ values:

$$Q = \mathrm{rms}(\Delta\delta^{meas} - \Delta\delta^{pred})/\mathrm{rms}(\Delta\delta^{meas}) \qquad (3)$$

Q factors calculated in this manner range from 25% for the NMR structure calculated without dipolar couplings, 27% for the 2.3-Å structure, 23% for the 1.8-Å crystal structure, to 16% for the NMR structure calculated with the dipolar couplings included.

The remarkable agreement between measured $\Delta\delta(^{13}C')$ values and those predicted by the NMR structure testifies to the high quality of the backbone geometry of this structure. It also indicates that the $^{13}C'$ CSA tensor must be rather uniform (see legend to Figure 1). This provides experimental validation for recent studies which interpret $^{13}C'$ relaxation rates in terms of backbone dynamics.[12]

In contrast to X-ray crystallography, where *R* factors are commonly used to describe the agreement between structure and experimental data, the many factors which influence the relation between internuclear distance and NOE have impeded the general use of an analogous *R* factor for NMR.[13] The *Q* factor, defined above, offers a straightforward criterion for evaluating the quality of NMR structures, provided they can be studied in the liquid crystalline state. Analogous *Q* factors, of comparable magnitude, can also be defined using one-bond dipolar couplings, provided these couplings were not included in the structure calculation process (Marquardt, J., unpublished results).

Acknowledgment. Work by G.C. is in partial fulfillment for the Ph.D. degree at the University of Maryland, College Park, MD. We thank F. Delaglio and D. Garrett for assistance and use of their software and M. Clore, A. Gronenborn, R. Tycko, and D. Torchia for many useful discussions. This work was supported by the AIDS Targeted Anti-Viral Program of the Office of the Director of the National Institutes of Health.

Supporting Information Available: One table containing the ubiquitin $^{13}C'$ shifts, recorded at 292, 298, and 304 K; one table containing the $^{1}H-^{15}N$ dipolar couplings, obtained from the difference in $^{1}J_{NH}$ splitting in the aligned and isotropic phase; and small regions of the overlaid 2D H(N)CO spectra, recorded at 298 and 304 K (4 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

JA9812610

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