ϕ Angle Restraints in Protein Backbones from Dipole−Dipole Cross-Correlation between $^1H^N-^{15}N$ and $^1H^N-^1H^\alpha$ Vectors

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Measurement of the dihedral angle ϕ between NH and $C^\alpha H$ bonds provides important restraints for protein structure determination by nuclear magnetic resonance (NMR) spectroscopy. ϕ angle restraints can be obtained from scalar couplings between amide and α protons, $^3J_{HN,H\alpha}$, with the help of the Karplus relation (Figure 1)[1−3] or from dipole−dipole cross-correlation effects, for example, between the $^{15}N-^1H$ and $^{13}C^\alpha-^1H^\alpha$ vectors in a ^{15}N/^{13}C-double-labeled protein.[4] It has further been noted that the appearance of an amide proton resonance from a ^{15}N-labeled protein is influenced by cross-correlation effects, including dipole−dipole and dipole-CSA cross-correlations between $^1H^N$, $^1H^\alpha$, and ^{15}N spins in a ϕ angle-dependent manner.[5] Here we show that the dipole−dipole cross-correlation effect of $^1H^\alpha$ and ^{15}N spins on the $^1H^N$ spin yields a simple criterion for identification of amino acid residues with positive ϕ angles as well as providing ϕ angle restraints for glycine residues.

In the absence of ^{15}N decoupling, the $^1H^N$ resonance is a multiplet of four lines arising from scalar $^1J_{HN,N}$ and $^3J_{HN,H\alpha}$ couplings. The ϕ angle dependent dipolar cross-correlation effects are most strikingly manifested in the relative line widths of the pairs of inner and outer multiplet components, originating from the correlation of the dipolar fields of $^1H^\alpha$ and ^{15}N spins at the site of the $^1H^N$ spin.[5] In the slow tumbling limit, this cross-correlation results in a cross-correlated relaxation rate $\Gamma_{HN,N\,HN,H\alpha}$[4−6]

$$\Gamma_{HN,N\,HN,H\alpha} = \left(\frac{\hbar\mu_0}{4\pi}\right)^2 \frac{\gamma_H\gamma_N}{(r_{HN,N})^3}\frac{\gamma_H\gamma_H}{(r_{HN,H\alpha})^3}\frac{3\cos^2\theta - 1}{2}\frac{2S^2\tau_c}{5} \tag{1}$$

where $\hbar$ denotes Planck's constant divided by 2π, μ_0 the induction constant, γ_H and γ_N the gyromagnetic ratios of the ^1H and ^{15}N spins respectively, $r_{HN,N}$ the distance between the $^1H^N$ and ^{15}N spins, $r_{HN,H\alpha}$ the distance between H^N and H^α, θ the angle between the H^N-N and H^N-H^α internuclear vectors, S the generalized Lipari−Szabo order parameter,[7,8] and τ_c the global rotational correlation time. The cross-correlated relaxation rate $\Gamma_{HN,N\,HN,H\alpha}$ can be measured by comparing the transverse relaxation rates of

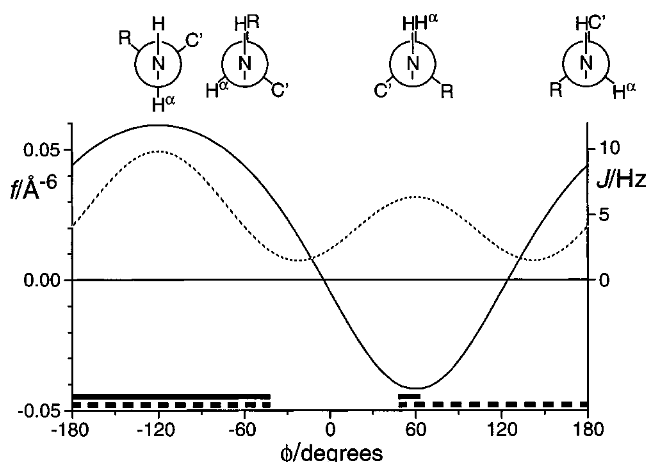

Figure 1. Dipole−dipole cross correlation factor $f = (3\cos^2\theta - 1)/(r_{HN,N}r_{HN,H\alpha})^3$ (solid line) and $^3J_{HN,H\alpha}$ coupling constant (dashed line) plotted as a function of the dihedral angle ϕ. The factor was calculated using ECEPP/2 standard geometry with $r_{HN,N} = 1.01$ Å.[15,16] Newman projections along the N−C^α bond are shown for $\phi = -120°$, $-60°$, 60°, and 180°, where R denotes the amino acid side chain. Bars identify the sterically allowed range of ϕ angles for amino acids with side chains (solid bar) and glycine (dashed bar).[17] Over the entire ϕ angle range, $r_{HN,H\alpha}$ varies between 2.26 and 2.97 Å and θ between about 66° and 21°.

the outer lines ($\Gamma_{\alpha\beta}$ and $\Gamma_{\beta\alpha}$) and the inner lines ($\Gamma_{\alpha\alpha}$ and $\Gamma_{\beta\beta}$) of the $^1H^N$ resonance:[6]

$$\Gamma_{HN,N\,HN,H\alpha} = 0.25\,(\Gamma_{\alpha\alpha} - \Gamma_{\alpha\beta} - \Gamma_{\beta\alpha} + \Gamma_{\beta\beta}) \tag{2}$$

All four multiplet components must be evaluated to cancel cross-correlation effects between dipolar and CSA relaxation mechanisms.[6] A plot of the angle and distance dependent part of $\Gamma_{HN,N\,HN,H\alpha}$, $f = (3\cos^2\theta - 1)/(r_{HN,N}r_{HN,H\alpha})^3$ (eq 1), versus the dihedral angle ϕ shows that it is clearly negative only for amino acid residues with positive ϕ angles (Figure 1). Steric hindrance confines nonglycine residues with positive ϕ angles to a narrow region around $\phi = 60°$. Such residues can readily be identified by qualitative inspection of their $^1H^N$ multiplets measured without ^{15}N decoupling, as the cross-correlation effect has a maximum at this ϕ angle.

Experimental data were recorded using ^{15}N-labeled plastocyanin from the cyanobacterium *Phormidium laminosum*. This protein contains 105 amino acid residues and has a rotational correlation time of 5.1 ns under the conditions used. A crystal structure of *P. laminosum* plastocyanin at 2.8 Å resolution is available.[9] The $^1H^N$ multiplets were observed in a ^{15}N HSQC spectrum recorded without ^{15}N-decoupling during acquisition, using an α/β-half-filter to direct the low-field and high-field components of the multiplet into different subspectra.[10] The crystal structure reports two non-glycine residues with positive ϕ angles, Leu 36 ($\phi = 48°$) and Ala 51 ($\phi = 56°$). Figure 2 shows the $^1H^N$ multiplets of these residues together with those of Leu 59 and Ala 95 which have similar $^3J_{HN,H\alpha}$ coupling constants but negative ϕ angles. As predicted, both Leu 36 and Ala 51 give rise to a multiplet fine structure in which the peak heights of the inner two multiplet components were on average lower than the peak heights of the outer two multiplet components. For all other non-glycine residues the multiplets were tilted as illustrated for Leu 59 and Ala 95

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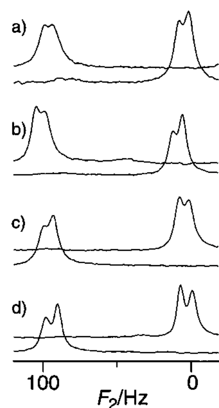

Figure 2. Cross sections along the F_2 dimension through selected $^1H^N$ multiplets observed in a ^{15}N HSQC spectrum with α/β-half-filter before the acquisition time t_2 (see Figure 2B in ref 10), using a 1.5 mM solution of ^{15}N-labeled *P. laminosum* plastocyanin at 27 °C, pH 6. The spectrum was recorded on a Bruker DRX 500 NMR spectrometer with an acquisition time of 439 ms and a total recording time of 3.5 h. The subspectra contain, respectively, the low-field and high-field multiplet components which are separated by $^1J_{HN}$. They were processed with multiplication by a cosine window function before Fourier transformation. Cross sections containing the $^1H^N$ multiplets of (a) Leu 36, (b) Ala 51, (c) Leu 59, and (d) Ala 95 are shown from the sum and difference spectra.

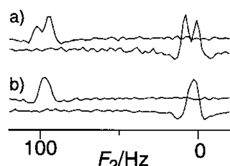

Figure 3. Cross sections along the F_2 dimension through the $^1H^N$ multiplet of Gly 12 in *P. laminosum* plastocyanin recorded as the spectra of Figure 2 but with selective irradiation (100 Hz) of (a) $^1H^{\alpha 1}$ or (b) $^1H^{\alpha 2}$ of Gly 12 during the acquisition time t_2. Each pair of subspectra was recorded with an acquisition time of 293 ms and a total recording time of 4 h. The subspectra were processed with resolution enhancement by multiplication with a sine bell window function before Fourier transformation.

(Figure 2, c and d), or otherwise the $^3J_{HN,H\alpha}$ coupling constant was too small to be resolved.

The same analysis can be applied to glycine residues, if the $^1H^N$ multiplet is simplified by selective decoupling of one of the $^1H^\alpha$ resonances during data acquisition. Figure 3 shows the $^1H^N$ multiplet of Gly 12 recorded with irradiation of the resonances of either $^1H^{\alpha 1}$ or $^1H^{\alpha 2}$ during acquisition. The three coordinate sets presented in the crystal structure report ϕ angles of 83° and 92° for Gly 12.[9] As shown in Figure 3, $^3J_{HN,H\alpha 1}$ is significantly smaller than $^3J_{HN,H\alpha 2}$, suggesting that 92° is the preferred ϕ angle in solution (Figure 1). Although the $^3J_{HN,H\alpha 1}$ coupling is barely resolved even after resolution enhancement, the outer lines of the $^1H^N$ multiplet appear more intense than the inner lines when the $^1H^{\alpha 2}$ resonance is irradiated (Figure 3b), while the opposite holds when the $^1H^{\alpha 1}$ resonance is irradiated (Figure 3a), as one would predict for $\phi = 92°$ (Figure 1). The $^1H^N$ multiplets of a further six glycine residues were investigated in the same way (Supporting Information). Those split by large $^3J_{HN,H\alpha}$ coupling constants were found to behave as Gly 12 (Figure 3a), in agreement with the left half of Figure 1. On the other hand, $^1H^N$ multiplets with small $^3J_{HN,H\alpha}$ coupling constants were often unresolved. In the case of Gly 50 the cross-correlation effects disagreed with the ϕ angle reported in the crystal structure, suggesting conformational averaging of this highly solvent-exposed residue.[9]

In conclusion, observation of dipole−dipole cross-correlation effects on the $^1H^N$ resonance in ^{15}N-labeled proteins provides a sensitive criterion for the identification of residues with positive ϕ angles. Conventionally, these residues are identified by their $^1J_{C\alpha,H\alpha}$ coupling constant[11,12] or by evaluation of intraresidual nuclear Overhauser effects (NOE) between α and amide protons.[13] The technique described here is analytically appealing in that it does not require ^{13}C-labeling and avoids the evaluation of intraresidual NOEs which are beset with zero-quantum effects[14] and prone to spectral overlap. The cross-correlation effects described above not only eliminate ambiguities between positive and negative ϕ angles in protein structure determinations, which cannot be resolved with the use of $^3J_{HN,H\alpha}$ couplings alone (Figure 1), but also provide additional conformational restraints for glycine residues which frequently show only few NOEs. As positive ϕ angles for non-glycine residues are scarcely observed in protein structures,[13] such data provide most valuable starting points for sequential resonance assignments of proteins for which structures are available from X-ray crystallography or molecular modeling.

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Supporting Information Available: A table of cross sections from ^{15}N HSQC spectra with α/β-half-filter from glycine residues recorded with selective $^1H^\alpha$ irradiation (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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