Accurate Orientation of the Functional Groups of Asparagine and Glutamine Side Chains Using One- and Two-Bond Dipolar Couplings

Mengli Cai, Ying Huang, and G. Marius Clore*

Laboratories of Chemical Physics and Molecular Biology
National Institute of Diabetes and Digestive and Kidney
Diseases, National Institutes of Health
Bethesda, Maryland 20892

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Residual dipolar couplings measured in a dilute liquid crystalline phase provide unique long-range orientational information that has been shown to lead to substantial improvements in backbone accuracy for NMR structures of proteins and protein complexes. Much of the focus has been on backbone dipolar couplings. Recently, it has been shown that $\chi_1$ rotamers can be identified from analysis of $^1$D$_{CC\alpha}$, $^1$D$_{CON}$, $^1$D$_{CON}$, and $^1$D$_{CON}$, couplings. In this paper, we present a simple approach based on dipolar couplings for obtaining accurate orientations of the carboxamide functional group of Asn ($\chi_2$) and Gln($\chi_2$) side chains. These are of considerable interest since the functional groups of Asn and Gln are often involved in specific hydrogen bonding interactions.

There are five easily measured dipolar couplings that can be used to determine the orientation of the carboxamide group of Asn and Gln in an NMR-based protein structure refinement: namely, $^1$D$_{N\beta-O\beta}$, $^1$D$_{N\beta-O\beta}$, $^1$D$_{O\beta-C\beta}$, $^1$D$_{O\beta-C\beta}$, and $^1$D$_{O\beta-C\beta}$ for Asn, and $^1$D$_{N\beta-O\beta}$, $^1$D$_{N\beta-O\beta}$, $^1$D$_{N\beta-O\beta}$, $^1$D$_{O\beta-C\beta}$, and $^1$D$_{O\beta-C\beta}$ for Gln. To make use of these couplings, it is first necessary to stereospecifically assign the side chain NH$_2$ protons of Asn and Gln. Methods based on three-bond scalar couplings between the NH$_2$ protons and C$\beta$ atom for Asn and C$\gamma$ atom for Gln have been described. These experiments, however, are relatively time-consuming. We demonstrate a different approach using two-bond heteronuclear scalar ($^2$J$_{HN\gamma}$) couplings between the NH$_2$ protons and side chain carbonyl carbon atoms (C$\gamma$ atom for Asn and C$\delta$ for Gln). The H$\delta$21 (Asn) and H$\epsilon$21 (Gln) protons are trans to the side chain oxygen (O$\delta_1$ and O$\epsilon_1$, respectively) of the carboxyamid group, analogous to the relationship between the backbone amide proton and carbonyl oxygen in a trans peptide bond, and have a positive $^2$J$_{HN\gamma}$ coupling of 2.5–5.0 Hz; the H$\delta$22 (Asn) and H$\epsilon$22 (Gln), on the other hand, are cis to the side chain oxygen, and have a negative $^2$J$_{HN\gamma}$ coupling of ~2.5 to ~5.0 Hz. These couplings are readily measured from a carbonyl-coupled n = 2 multiplicity-edited 2D $^1$H–$^15$N heteronuclear quantum coherence (HSQC) correlation spectrum in which only cross-peaks corresponding to the side chain NH$_2$ groups are observed.

Figure 1. Stereospecific assignment and measurement of residual dipolar couplings for the side chain amide groups of Asn and Gln. (a) The basic fully decoupled, gradient-based, n = 2 multiplicity-edited $^1$H–$^15$N HSQC experiment designed to selectively observe NH$_2$ correlations. Narrow and wide pulses correspond to flip angles of 90° and 180°, respectively. The length ($\tau_{900}$) of the 180° $^1$C$\beta$/ $^1$C$\beta$/ $^1$C$\beta$ resonances is chosen such that they have a null at the positions of the $^1$C$\beta$ and $^1$C$\beta$/ $^1$C$\beta$/ $^1$C$\beta$/ $^1$C$\beta$ resonances, respectively ($\tau_{900}$ = $\sqrt{2A}$, where A is the frequency difference between the $^1$C$\beta$/ $^1$C$\beta$/ $^1$C$\beta$/ $^1$C$\beta$ and $^1$C$\beta$/ $^1$C$\beta$/ $^1$C$\beta$/ $^1$C$\beta$ resonances). The delay $\tau$ is set to $\tau_{900}$ = 2.6 ms. The delay $\delta$ has a value of 400 μs. All pulse phases are x, unless otherwise specified. Phase cycling: $\varphi_1 = 2(y(2\pi-y]], \varphi_2 = x_0 − x_1; \varphi_3 = 4(x(4(y)), 4(−x), 4(−y)); and receiver phase = x_2(x_2(x_2), x_0 − x_1).$ Rance–Kay $\tau_1$ quadrature detection is used, alternating the phase of $\varphi_4$ between x and −x in concert with the polarity of the pulsed field gradient (PFG) $g_5$. All PFGs are sine–bell shaped (30 G/cm except for which is 21 G/cm). The durations of g1, g2, g3, g4, and g5 are 1.5, 2.0, 0.3, 0.3, and 0.203 ms, respectively. For stereospecific assignments of the H$\delta$21 and H$\epsilon$22 hydrogens of Asn and the H$\epsilon$21 and H$\epsilon$22 hydrogens of Gln, and for the measurement of side chain $^1$D$_{NC}$ and $^1$D$_{H\delta21}$ dipolar couplings, the 180° carbonyl pulse during the evolution period $\tau_t$ is omitted; for measurement of $^1$D$_{NC}$ side chain dipolar couplings, no broad-band decoupling is employed during acquisition. (b) 2D $^1$F$_\gamma$–carbonyl multiplicity-edited $^1$H–$^15$N HSQC spectrum of LAP2 collected in isotropic medium (water) using the pulse sequence shown in (a) omitting the carbonyl 180° pulse during $\tau_t$.

for the side chain NH$_2$ groups are observed (Figure 1a). The basic pulse sequence (fully decoupled version) employed is given in Figure 1a, and the F$_\gamma$–carbonyl coupled spectrum for the 168 residue protein LAP2, whose structure has recently been solved, (1)
is shown in Figure 1b. The $^3J_{\text{HN}}$ couplings are measured from the displacement of the upper component of the multiplet relative to the lower one in the $^1H$ ($F_2$) dimension of the spectrum with positive and negative slopes indicative of positive and negative $^3J_{\text{HN}}$ couplings, respectively (Figure 1b). In this particular instance all the H$^\alpha$21 and H$^\beta$21 protons of Asn and Gln are downfield from their respective H$^\alpha$22 and H$^\beta$22 partners (Figure 1b).

The five dipolar couplings involving the side chain amides of Asn and Gln in LAP2 were measured in a liquid crystalline medium of phage pf10 (15 mg/mL). The side-chain $^1D_{\text{HN}}$ and $^2D_{\text{HN}}$ couplings are determined from the splittings in the $F_1$ and $F_2$ dimensions, respectively, of the $F_1$-carbonyl coupled version of the experiment shown in Figure 1a. The side-chain $^1D_{\text{NH}}$ couplings are measured in the $t_2$ acquisition dimension from the $F_2^1$-$^15N$ coupled $^13C$-decoupled version of the experiment.10

The presence of significant motion about $\chi_2$ for Asn and $\chi_3$ for Gln was assessed by recording a steady-state $^15N$–$^1H$ NOE experiment:11 the side-chain amide groups of Asn23, Gln49, Gln52, and Gln132 have $^15N$–$^1H$ NOE values of 0.87, 0.65, 0.50, and 0.30, respectively; all other side-chain amide groups had $^15N$–$^1H$ NOE values that were either close to zero or negative. Thus, the side-chain amide of Asn23 is essentially as rigid as the backbone, while the side-chain amide groups of the other Asn and Gln display varying degrees of internal motion.

We therefore chose to use the data on Asn23 to assess the usefulness and impact of side-chain dipolar couplings on the refinement of the positions of the carboxamide groups of Asn and Gln. Structures were calculated in torsion angle space by simulated annealing structures for the main chain atoms of residues 18–25 and the side-chain amide atoms of Asn23 of 20 simulated annealing structures calculated (a) without and (b) with $^1D_{\text{HN}}$, $^1D_{\text{NH}}$, $^2D_{\text{HN}}$, $^1D_{\text{NH}}$, and $^2D_{\text{NH}}$ dipolar couplings for Asn23. The main chain atoms are shown in green and the backbone carbonyl (C–O) of Glu19 in red; the side chain amide of Asn23 is in blue and the remainder of the Asn23 side chain in yellow. The distances in Å from the N02, H021, and H022 atoms of Asn23 to the backbone carbonyl oxygen atom of Glu19 are indicated.

$\pm$ 17% for the second cluster. (In both cases, the $\chi_3$ angle is very close to $-60^\circ$.) In the first cluster, the NH$_2$ group of Asn23 could form a potential hydrogen bond with the main chain carbonyl oxygen atom of Glu19 (Figure 2a). While the N02(23)–O(19) distance of 2.64 Å is reasonable for such a hydrogen bond, the H021 and H022 atoms of Asn23 are almost equidistant from the carbonyl oxygen of Glu19 (2.74 and 3.07 Å, respectively) and the N02(23)–H021(23)–O(19) angle has a value of $84 \pm 2^\circ$. Thus, the hydrogen bond geometry for the first cluster is very poor. For the second cluster, the amide group of Asn23 is too far away and in an inappropriate orientation to form a hydrogen bond with the backbone of Glu19 (Figure 2a). When the side chain dipolar couplings of Asn23, however, are included in the calculation (Figure 2b), $R_{\text{exp}}$ is reduced to 22 ± 1% and all simulated annealing structures display the same side chain orientation of Asn23 with a $\chi_3$ angle of $-70 \pm 3^\circ$.

The distances from the N02 and H021 atoms of Asn23 to the backbone carbonyl oxygen of Glu19 are 2.82 ± 0.10 and 2.05 ± 0.09 Å, respectively, and the N02(23)–H021(23)–O(19) angle is $135 \pm 4^\circ$, fully consistent with good stereochemistry for a hydrogen bond between the H021 atom of Asn23 and the backbone carbonyl oxygen of Glu19.

We have shown that side-chain dipolar couplings involving the carboxamide group of Asn and Gln are readily measured and can have a significant impact on the accuracy with which the orientation of the functional group of Asn and Gln can be determined by NMR, thereby shedding light on and improving the geometry of specific hydrogen bonding interactions.

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