Two-Dimensional Nuclear Magnetic Resonance Method for Identifying the H^{α}/C^{α} Signals of Amino Acid Residues Preceding Proline

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Heteronuclear multidimensional NMR methods[1-3] and isotope-labeling[4] have extended the range of protein structures that can be determined by NMR.[5,6] An important first step in a structure determination is the assignment of the NMR signals, a process which has been greatly facilitated by recently developed triple-resonance experiments in which adjacent amino acid spin systems are identified via through-bond heteronuclear scalar couplings.[7,8] Typically, the amide ^1H and ^{15}N resonances of each amino acid are correlated to the C^{α}, H^{α}, or C′ signals of the i and $(i-1)$ residues, allowing the neighboring amino acids to be linked together. The sequential assignments are then obtained by identifying the spin systems by amino acid type and matching the strings of neighboring residues with the amino acid sequence. One of the major problems in this assignment strategy is the identification of the amino acid spin systems preceding the prolines which lack an amide proton. Although this may be accomplished using NOEs between the H^{δ} or H^{α} protons of proline and the H^{α} of the preceding residue,[9] experimental evidence based on through-bond scalar couplings versus NOE data would be preferable.

In this paper, we describe a simple two-dimensional NMR method for identifying the H^{α} and C^{α} resonances of amino acids preceding proline residues. The method involves a 2D HACA-(CO)(N) experiment[7] in which the H^{α}/C^{α} signals of the residues preceding the prolines are selectively observed by eliminating the magnetization corresponding to non-proline residues by dephasing the ^{15}N magnetization by the one-bond ^{15}N–^1H coupling ($^1J_{H^N,N}$). Figure 1 depicts the pulse sequence for the proline-edited HACA-(CO)(N) experiment. Magnetization begins on H^{α} and is transferred to C^{α}, C′, and N by a series of refocused INEPT steps. While on the nitrogen, the magnetization is dephased by $^1J_{H^N,N}$ and converted to unobservable multiple quantum coherence for all of the signals except those of proline which lack an amide proton and are thus unaffected during the $^1J_{H^N,N}$ dephasing period. Subsequently, the magnetization transfer pathway is reversed and follows the path $N_i \rightarrow C'_{i-1} \rightarrow C^{\alpha}_{i-1} \rightarrow H^{\alpha}_{i-1}$. Gradients (g1 and g3) are used to select the coherence transfer pathway[10-12] from C^{α} to H^{α} as shown in Figure 1. Although the signal to noise ratio using coherence selection is less than that obtained with other schemes employing gradients,[13] this approach was necessary to eliminate the large solvent signal.

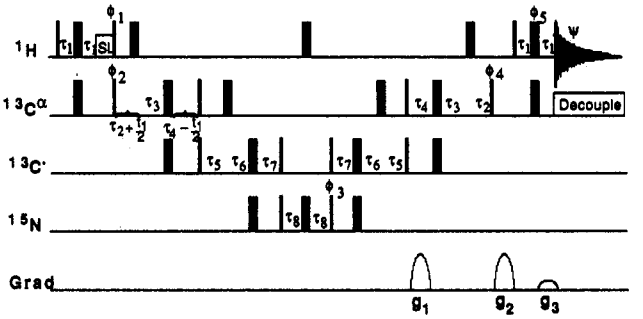

Figure 1. Pulse sequence of the proline-edited HACA(CO)(N) experiment. The thin and thick lines correspond to 90° and 180° pulses, respectively. The 90° and 180° pulses of C^{α} and C′ were adjusted by changing the power to provide a null in the excitation profiles at the C′ and C^{α} frequency. A spin lock pulse of 1 ms was applied along the x-axis. The gradients were applied as shaped sine pulses with a duration of 1 ms followed by a recovery delay of 0.5 ms. The gradient strengths for g_1–g_3 were 30, 3, and 7.5 G/cm, respectively. The phase-cycling of the pulses and receiver was as follows: $\phi_1 = 8y,8(-y)$; $\phi_2 = x,-x$; $\phi_3 = 2(x),2(-x)$; $\phi_4 = 4(x),4(-x)$; $\phi_5 = 16(x),16(-x),16(y),16(-y)$; $\psi = 2(x,-x,-x,x,2(-x,x,x,-x),x,-x,-x,x),2(-x,x,x,-x,2(x,-x,-x,x),-x,x,x,-x)$. All other pulses were along the x-axis. The ^1H, ^{15}N, $^{13}C^{\alpha}$, and $^{13}C'$ carrier frequencies were set at 4.75, 117.4, 59.6, and 175 ppm, respectively. The C^{α} chemical shifts were obtained in a constant time period by incrementing τ_2 and decrementing τ_4 by (dwell width)/2. Quadrature detection was achieved in t_1 using the States–TPPI method[18] by incrementing ϕ_2. The following delays were used in the experiment: $\tau_1 = \tau_2 = 1.7$ ms; $\tau_3 = 1.8$ ms; $\tau_4 = \tau_2 + \tau_3 = 3.5$ ms; $\tau_5 = 4.5$ ms; $\tau_6 = 6.5$ ms; $\tau_7 = \tau_5 + \tau_6 = 11$ ms; $\tau_8 = 2.7$ ms.

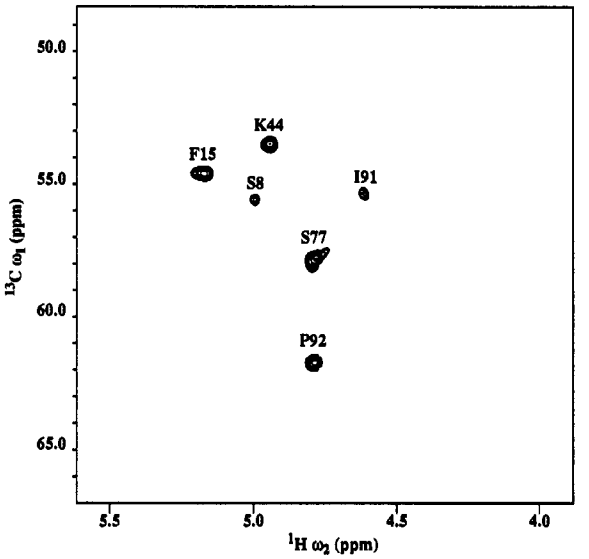

Figure 2. Proline-edited HACA(CO)(N) spectrum of the [U-^{15}N,^{13}C]-FKBP/ascomycin complex in H_2O recorded on a Bruker AMX600 NMR spectrometer at 30 °C. Thirty-two complex points were collected in t_1 with 128 scans per t_1. The H^{α}/C^{α} cross peaks of the FKBP residues prededing the prolines are labeled in the spectrum.

The technique is illustrated using a 3 mM sample of ^{15}N-, ^{13}C-labeled FK506 binding protein (FKBP)[14,15] complexed to the immunosuppressant, ascomycin,[16] dissolved in H_2O. There are seven prolines in FKBP located at positions 9, 16, 45, 78, 88, 92, and 93. Thus, seven H^{α}/C^{α} signals are expected in the 2D proline-edited HACA(CO)(N) spectrum. As shown in Figure 2, six of

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the seven expected H^{α}/C^{α} peaks corresponding to the amino acid residues preceding the prolines were observed in the 2D NMR spectrum. Only the H^{α}/C^{α} peak of H87 is missing from the spectrum, which is presumably due to the broad signals of this residue located in a large loop of the protein.[17] No signals corresponding to other residues or solvent are present in the spectrum, indicating a high level of suppression. Indeed, the H^{α} signals of S77 and P92 are nearly degenerate with the H_2O frequency, yet these signals are readily detected in the experiment. These results are in contrast to our earlier studies that employed a different gradient scheme[13] combined with other techniques for solvent suppression in which these signals were obscured by the solvent signal (data not shown).

A particularly challenging task can be the assignment of resonances in regions of the protein containing multiple prolines. For example in FKBP, the sequence I-P-P (residues 91-93) is present, which is difficult to assign. However, as shown in Figure 2, the H^{α}/C^{α} signals for I91 and P92 are readily identified in the spectrum, facilitating the assignment of these signals.

In summary, a 2D NMR method is described for identifying the H^{α}/C^{α} signals of amino acid residues preceding proline. The experiment can be performed in a short period of time (2 h in the example shown here) and provides information that has been difficult to obtain by other methods. Thus, this technique complements other triple-resonance experiments[7,8] for aiding in the assignment of isotopically labeled proteins.

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