13C Line Narrowing by 2H Decoupling in H/13C/15N-Enriched Proteins. Application to Triple Resonance 4D J Connectivity of Sequential Amides

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Short transverse 13C relaxation times, T2, constitute the principal barrier for the application of heteronuclear J correlation NMR techniques to larger proteins uniformly enriched with 13C and 15N.1-6 The 13C T2 is dominated by the strong dipolar interaction with its attached protons.7 As the magnetogyric ratio of 1H is ~6.5 times lower than that of 13C, the heteronuclear dipolar interaction is greatly reduced by deuteration. Because of the large 1H quadrupolar interaction (~170 kHz), the 1H spin lattice relaxation time, T1, in proteins is in the millisecond range at a magnetic field strength of 14 T. Therefore, the 1H–13C J coupling (~22 Hz) does not result in the triplet shape, expected for a 13C nucleus coupled to a spin-1 nucleus, but gives rise to a collapsed singlet resonance that is broadened by scalar relaxation of the second kind.8,9 High-power (~2.5 W) 2H decoupling with an RF field strength much stronger than the inverse 1H T1 effectively removes this broadening and results in a 13C line width that is much narrower than for the protonated 13C.

One of the triple resonance J correlation experiments affected most by the 13C line width is the H(CA)NH experiment,10,11 which relies on magnetization transfer from Cα to the backbone 13C nucleus via the relatively small JαCα (~11 Hz) and JαCα (~5–8 Hz) couplings. Although experiments have been proposed to alleviate this difficult J correlation step,12,13 the sequential assignment procedure which is based on J correlation between the intraresidue 1H/15N and 1H/13C resonances and between the 1H/13C of residue i and 1H/15N of residue i + 1 is complicated by the high degree of overlap among 1H/13C sequences. Here we describe a procedure which allows J correlation between the much better resolved 1H/15N resonances of sequential residues, thereby bypassing the overlapping 1H/13C pairs. Efficient transfer of magnetization from 13Cα to 15N is possible in the present case because of the 13Cα line narrowing afforded by deuteration and 2H decoupling.

Briefly the scheme of Figure 1 functions as follows. After Hα+1 evolution during t1, magnetization is transferred to its attached nucleus Nα+1 (time a). Following a constant time evolution period, t2, during which 13C is decoupled from 15N, the 13Cα magnetization is relayed via 15CO (time b) and 13Cα magnetization (time c) to the 15N of residue i (time d). The effect of dephasing caused by the homonuclear 13C–13C J coupling during the period where C magnetization is transferred (η = η1 + η2 + η3) is effectively eliminated by setting η to ~1/JCC (27.6 ms). At time d, a fraction sin[π1(JαCα)] cos[π1(JαCα)] of the Cα magnetization is transferred to the intraresidue 15N, and a smaller fraction, sin[π1(JαCα)] cos[π1(JαCα)], is transferred back to the amide from which magnetization originated. After the second 13C constant time evolution period, t3, (time e), magnetization is transferred by means of a reverse INEPT sequence to HN for observation. In the 4D spectrum, the frequency coordinates of J correlations in the F1, F2, F3, and F4 dimensions then correspond to the chemical shifts of HN, HNα, HN, and HN, respectively. The “diagonal peaks” at HN, HN, HNα, and HNα, due to the above mentioned two-bond JαCα transfer process, are 2–4 times weaker.

Experiments are conducted on a Bruker AMX-600 spectrometer, modified such that the 1H lock receiver is disabled during 2H decoupling. Details regarding this hardware modification will be published elsewhere. The method is demonstrated for a sample containing ~1.4 mM of the protein calcineurin B (19.7 kD), uniformly enriched with 1H, 15N, and 13C to levels of 50%, 98%, and 88%, respectively. A second sample, with higher deuteration (~83%), was also prepared to illustrate directly the 13C line narrowing obtainable. The fact that the deuteration level of the sample used for the 4D experiment is only 50% lowers the sensitivity of this particular experiment, but it permits this sample to be used also for a range of other experiments that require partial side-chain protonation. Both samples also contain 20 mM CaCl2 and 20 mM CHAPS, a zwitterionic detergent which was shown not to significantly affect the structure or binding affinity of calcineurin B.14 Experiments were conducted at 37 °C, pH 5.6.

Figure 2 illustrates the 13C resolution enhancement obtained by deuteration and 2H decoupling for a small region of the 2D H(N)CA correlation spectrum,16 displaying connectivities between amide protons and their intraresidue Cα. In the absence of deuteration and 2H decoupling, the 13Cα resonances is a poorly resolved doublet, split by JαCα coupling with 13Cα, with a line width for the doublet components of ca. 25 Hz (Figure 2A). In the case of deuteration and 2H decoupling, the Cα doublet is well-resolved with line widths of ~10 Hz (Figure 2B). The 13Cα resonances in the deuterated protein are shifted upfield by ~0.35 ppm, caused primarily by the one-bond 1H isotope effect. The 10-Hz 13Cα line width is determined primarily by the limited acquisition time in the t1 dimension of the 2D H(N)CA experiment, and by incomplete deuteration of the amino acid side chains which results in a distribution of two- and three-bond isotope effects.

Figure 3 shows four cross sections through the 4D HN(COCA)-NH spectrum, illustrating J connectivities between the amides of residues F72–F75. Of all the sequential J connectivities expected on the basis of the backbone assignments,17 98% were observed, except for a stretch of residues close to the C154K mutation, which causes line broadening of the resonance.17 Previous attempts to demonstrate the 13Cα line narrowing

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under investigation. At the high magnetic field strength used in our present work successful because the rapid obtained by deuteration and of experiments that can benefit from deuteration is presently larger ]3C/l5N-enriched proteins. The present experiment is only a single example of the utility of this approach, but a large range of experiments that can benefit from deuteration is presently under investigation.

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