The use of heteronuclear cross-polarization for backbone assignment of $^2$H-, $^{15}$N- and $^{13}$C-labeled proteins: A pulse scheme for triple-resonance 4D correlation of sequential amide protons and $^{15}$N

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Summary

A new four-dimensional pulse scheme is described for the main-chain assignment of proteins by means of the J connectivity of the amide proton and nitrogen resonances of adjacent residues. Since the new experiment, 4D CP-HN(COCA)NH, involves heteronuclear cross-polarization for magnetization transfer from $^{13}$C=O to $^{15}$N via $^{13}$C, a relatively strong WALTZ-16 decoupling rf field is applied to $^{13}$C during magnetization transfer. Consequently, $^{13}$C is effectively decoupled from its attached $^2$H in the case of deuterated proteins, in the absence of a decoupling rf field for $^2$H. This efficiently improves the sensitivity of the experiment through $^{13}$C line narrowing. The experiment was performed on a randomly 60% deuterated protein, and the sensitivity of the final 4D spectrum was found to be excellent.

The development of multidimensional triple-resonance NMR techniques has enabled studies of the solution structures of 'medium-size' proteins with molecular masses smaller than 25 kDa (Ikura et al., 1990; Kay et al., 1990; Bax and Grzesiek, 1993). The highest barrier for their application to larger proteins is the short transverse relaxation time, $T_2$, of $^{13}$C, since magnetization transfer passes $^{13}$C in most multidimensional triple-resonance NMR experiments. The $^{13}$C transverse relaxation is dominated by a strong dipolar interaction with $^1$H. Since the gyromagnetic ratio of $^2$H is 6.5 times smaller than that of $^1$H, the transverse relaxation of $^{13}$C due to the dipole interaction is reduced by deuteration at the $^1$H site. However, due to scalar relaxation of the second kind (Abragam, 1961), the $^{13}$C line width is still broadened by deuteration at magnetic fields of 10–15 T. Several groups have shown that $^2$H decoupling during the periods of transverse $^{13}$C magnetization efficiently eliminates the effect of scalar relaxation of the second kind, and thereby results in $^{13}$C line narrowing (Grzesiek et al., 1993; Kuslan and LeMaster, 1993; Yamazaki et al., 1994).

However, high-power deuterium decoupling (more than several hundred Hz) leads to additional hardware demands. Another radiofrequency channel is required, in addition to the three rf channels for $^1$H, $^{15}$N and $^{13}$C for triple-resonance NMR experiments. Moreover, the $^2$H channel causes instabilities of the $^2$H lock, unless $^2$H lock-holding and -blanking of the $^2$H channel for decoupling are used. A $^2$H lock coil in the NMR probe, sufficiently stable for high-power $^2$H decoupling, is also required.

Recently, several groups have shown that heteronuclear cross-polarization (CP) in solution is as practical for magnetization transfer as pulsed sequences, such as INEPT or DEPT (Zuiderweg, 1990; Ernst et al., 1991; Majumdar et al., 1993; Richardson et al., 1993; Schleucher et al., 1994). It has also been shown to be beneficial under some circumstances, in comparison with corresponding pulsed transfers, such as INEPT (Majumdar et al., 1993; Richardson et al., 1993).

In this communication, we present a new four-dimensional triple-resonance experiment which directly correlates the amide proton and nitrogen resonances of adjacent residues. In contrast to a related experiment introduced by Grzesiek et al. (1993), our experiment involves

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heteronuclear cross-polarization in solution (Ernst et al., 1991) instead of INEPT for the magnetization transfer from $^{13}$C=O to $^{15}$N via $^{13}$C$. Since a strong WALTZ-16 decoupling rf field (more than 1.9 kHz) is applied to $^{13}$C during the magnetization transfer, $^{13}$C is effectively decoupled from its attached $^2$H. Thus, applying this pulse sequence with heteronuclear CP to deuterated proteins, we can expect a longer transverse relaxation time for $^{13}$C attached to $^2$H, which results in more efficient magnetization transfer from $^{13}$C=O to $^{15}$N through $^{13}$C in the absence of $^2$H decoupling.

Figure 1 illustrates the pulse sequence employed in the experiment. According to the convention introduced by Kay and co-workers (1990), we name the experiment CP-HN(COCA)NH. The flow of magnetization is the same as in the HN(COCA)NH experiment introduced by Grzesiek et al. (1993). After $^1$H(i+1) chemical shift evolution during $t_1$, magnetization is transferred to the attached amide nitrogen $^{15}$N(i+1) through INEPT transfer at time point a. In the constant-time evolution period, $2T$, and during the subsequent $^{15}$N and $^{13}$C=O 90° pulses, the magnetization is relayed to $^{13}$C=O(i) at time b, and in-phase $^{13}$C=O(i) magnetization appears at time c, after rephasing caused by $^1J(13^{N}(i+1)-13^{C}=O(i))$. From time c to time d, a WALTZ-16 rf field is applied to $^{13}$C=O and $^{13}$C at the same field strength, using double selective rectangular pulses, generated by a single rf channel (Vincent et al., 1993; Ito, Y. and Laue, E.D., personal communication). During this period, transverse in-phase $^{13}$C=O(i) magnetization is transferred to in-phase $^{13}$C(i) magnetization by heteronuclear CP (Ernst et al., 1991). Just after the field gradient pulse $z$-filter, $^{13}$C=O-$^{15}$N CP takes place by the application of a WALTZ-16 decoupling field from time e to time f. In-phase $^{13}$C(i) magnetization is transferred either to $^{15}$N(i), which relies on the intraresidue $^1J_{CN}$ ($\sim$ 11 Hz) coupling and gives a larger signal in the final spectrum, or to $^{13}$N(i+1), which relies on the interresidue $^2J_{CN}$ ($\sim$ 7 Hz) coupling and gives a smaller signal. Since the WALTZ-16 decoupling rf field, which is applied to $^{13}$C at the same field strength, during both CP periods, is sufficiently high (more than 1.9 kHz, which is several times larger than the $T_1(2$H$)$ relaxation rate) (Grzesiek et al., 1993), the scalar relaxation of the second kind of $^{13}$C by its attached $^2$H is efficiently removed, without an additional $^2$H decoupling field. The $^{15}$N(i) and $^{13}$N(i+1) magnetization is dephased relative to their attached protons, and relayed to $^1$H(i) and $^1$H(i+1), respectively, at time g. Finally, the $^1$H(i) and $^1$H(i+1) transverse magnetization is refocused and