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COMMUNICATIONS

Offset Profiles of Selective Pulses in Isotopically Labeled Macromolecules

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The experimental verification of offset profiles and calibration of selective pulses in NMR is usually carried out with doped water samples but not under conditions typical of macromolecules with short T_2, long T_1, and possibly homo- and heteronuclear couplings. A new method for selective excitation in isotopically labeled macromolecules is shown to be particularly suited to this purpose. This is illustrated for a backbone amide resonance in a sample of ^{15}N-labeled human ubiquitin. © 1999 Academic Press

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Selective pulses have become a ubiquitous tool in the design of novel experiments in solution-state NMR (*1*). The design of amplitude-modulated pulses which exhibit ideal offset profiles and minimal phase dispersion has been extremely successful (*2–5*). In order to achieve a high degree of selectivity it is necessary to use pulses of long duration, so that the magnetization is subject to T_1 and T_2 relaxation which may cause a degradation of the offset profile (*6*). In biological macromolecules, due to substantial spectral overlap, it has been hitherto impossible to record frequency profiles of a single resonance. Pulse calibration was therefore usually carried out with doped water samples.

In this Communication a method for selective excitation using solution-state cross-polarization, as applied recently to NOE measurements (*7*), is demonstrated to be particularly suited to the measurement of frequency-offset profiles. Figure 1 displays the pulse sequence used in this experiment. In macromolecules labeled with ^{15}N or ^{13}C it is possible to excite selectively protons which overlap in the proton domain provided that they are bound to heteronuclei with differing resonance frequencies. Transfer of magnetization from H^N to ^{15}N and back again is achieved by cross-polarization using two weak radiofrequency fields with amplitudes smaller than the heteronuclear J coupling (*8*). Following doubly selective transfer the ^{1}H magnetization is stored along the z axis with subsequent application of a spoil gradient G_3. The selective pulse that is to be characterized is then applied. The sequence displayed is applicable to selective inversion pulses, but may be adapted to evaluate selective excitation pulses if the final hard $\pi/2$ pulse is removed.

Figure 2 shows frequency profiles for the I-Burp2 inversion pulse (*4*). All experiments have been acquired on a Bruker 300 MHz DRX spectrometer and are recorded by stepping the carrier frequency of the soft pulse while maintaining the transmitter frequency on resonance during all other pulses. One can observe a degradation near the edges of the profiles for the protein sample, compared to the water sample, which is primarily due to the short T_2 ($\approx$34 ms). The transverse components relax during the pulse and the magnitude of M_z after the pulse will therefore be reduced. This would affect the efficiency of various schemes that have been designed to suppress spin diffusion in Overhauser studies (*9*). Despite the slight flattening at the edges of the profile, the I-Burp2 pulse remains very efficient as the magnetization resides mostly along the z axis during the pulse. Pulses which cause more protracted deviations of the magnetization from the z axis will give rise to more pronounced distortions.

Figure 3 shows the effect of removing ^{15}N decoupling during the selective proton pulse, which results in a severe degradation of the entire offset profile due to evolution under the scalar coupling during the pulse. This coupling becomes active whenever the magnetization deviates from the z axis. The effects of scalar coupling become more pronounced as the pulse length increases. For very long selective pulses, scalar coupling leads to some curious effects even on resonance (Fig. 3b). Compared are simulations using the magnetic resonance platform GAMMA (*10*). A coupling constant $J(^1\text{H}–^{15}\text{N}) = 95$ Hz was assumed. No relaxation effects during the selective pulse were included in these simulations.

This method is believed to be the only practical approach for

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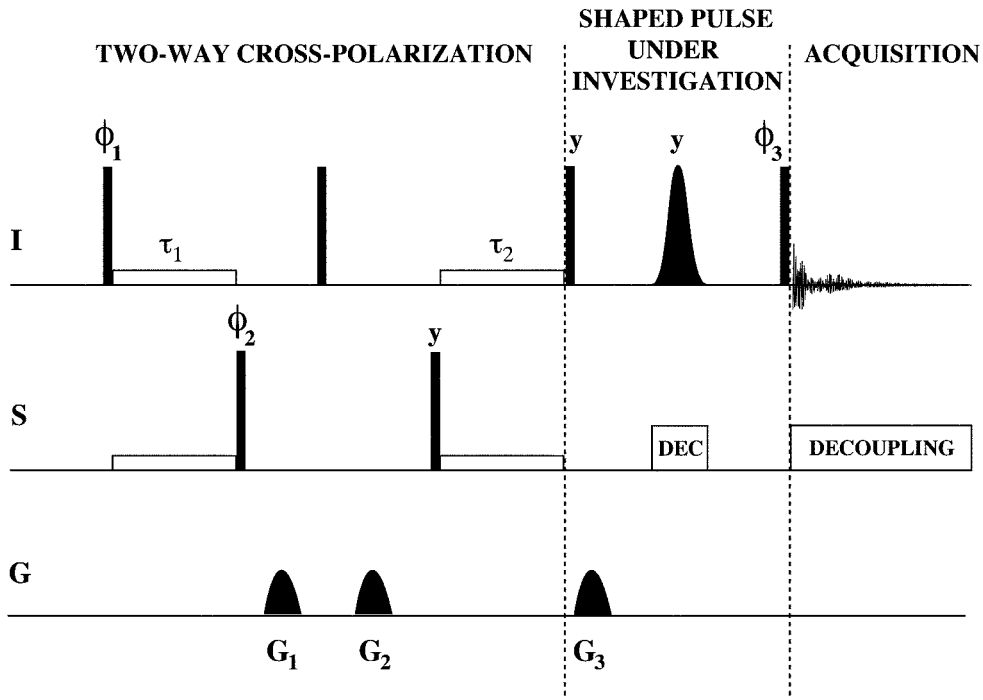

FIG. 1. Pulse sequence for selective excitation using two-way cross-polarization. The duration of the spin-lock intervals is set to $\tau_1 = \tau_2 = ({}^1J_{IS})^{-1}$ (i.e., 10.9 ms for amide H^N) with the carrier frequencies set to the chemical shifts of the selected proton and nitrogen. The amplitudes of the RF fields are set to approximately ${}^1J_{IS}/2$ (ca. 40 Hz). An 8 step phase cycle was used where ϕ_1, ϕ_2, and ϕ_3 were alternated independently with a concomitant alternation of the receiver phase. The alternation in Φ_3 removes spurious I_x or I_y magnetization created during the soft pulse.

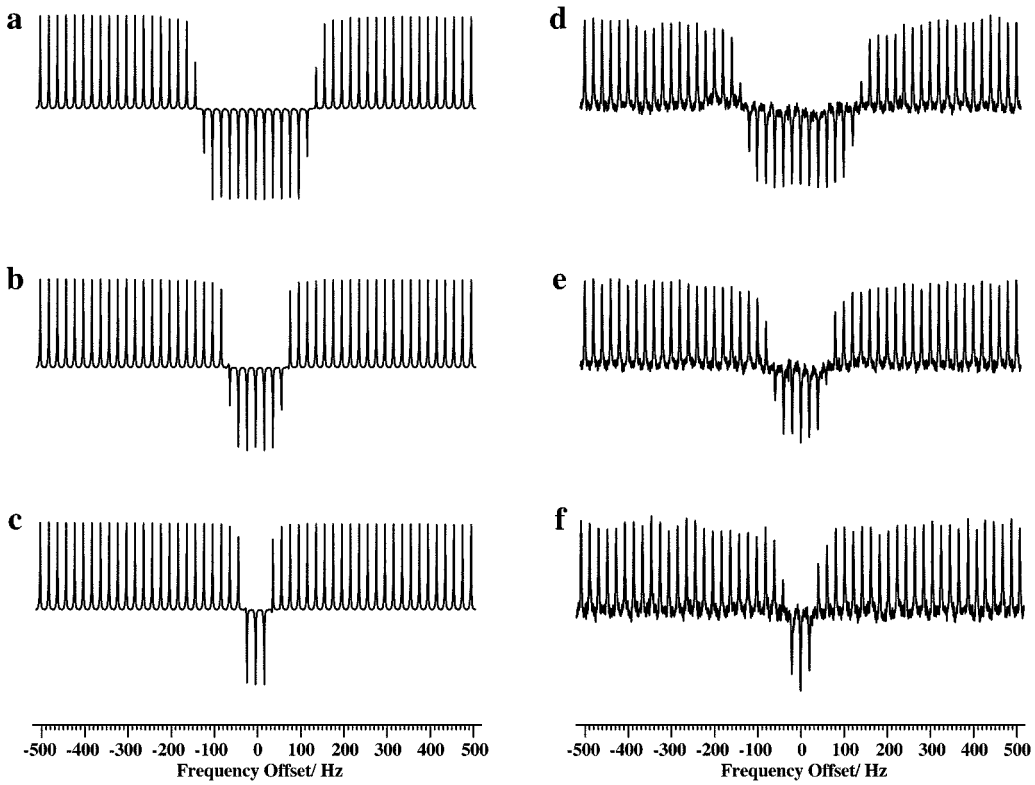

FIG. 2. (a)–(c) Offset profiles of shaped inversion pulses for a sample of 1% H_2O in D_2O doped with $Cr(acac)_2$. (d)–(f) Offset profiles for the A46 H^N amide proton in a 1.5 mM sample of ^{15}N-labeled human ubiquitin (8.5 kDa, $\tau_c = 4$ ns at 303 K) in H_2O:D_2O = 9:1 buffered at pH 4.5 with 20 mM perdeuterated acetic acid, using the same pulses as in (a)–(c). Each point is acquired in 32 scans. The inversion profiles are displayed for I-Burp2 pulses with durations of 20, 40, and 80 ms from top to bottom and corresponding bandwidths (separation between offsets where 50% inversion is achieved in the absence of relaxation) of 240, 120, and 60 Hz. Amplitudes have been calibrated for a 180° flip angle on resonance.

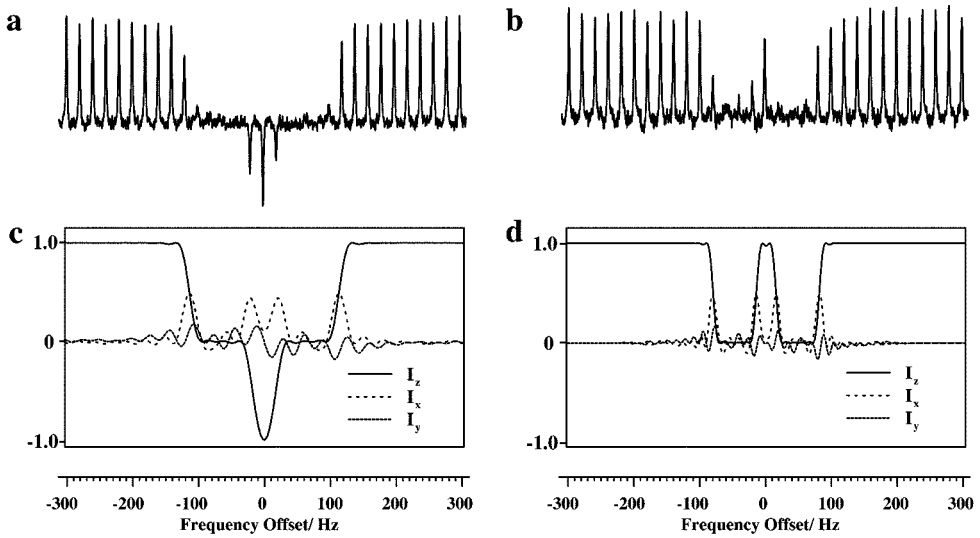

FIG. 3. (a)–(b) I-Burp2 inversion offset profiles for 40 and 80 ms pulses without ^{15}N decoupling during the shaped proton pulse. (c)–(d) Corresponding simulations incorporating a ^{1}H–^{15}N coupling of $J = 95$ Hz.

recording offset profiles in labeled proteins. The method of excitation lends itself to more detailed investigations of the effects of relaxation and scalar coupling during soft pulses applied to proteins.

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