Water-protein NOEs: Optimized scheme for selective water excitation

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Abstract

An alternative scheme for selective water excitation is proposed. The pulse sequence saturates the resonances from the solute, allowing the observation of water-solute NOEs with low artifact levels. The water resonance is subsequently excited by a relatively non-selective 90° pulse. The scheme is compared to other selective water excitation schemes. 2D NOE-NOESY and ROE-NOESY pulse sequences are proposed which afford high sensitivity by efficient water excitation and flip-back by radiation damping, yet allow the use of short mixing times for the buildup of water-solute NOEs.

Sensitivity is a major limitation for the observation of weak intermolecular NOEs between water and biomolecules (Otting et al., 1991). Selective water excitation offers an important way of maximizing sensitivity and numerous schemes have been devised to overcome the complicating effects from radiation damping and demagnetizing field effects (Abergel, 1996; Otting, 1997 and references therein; Sobol et al., 1998). While phase distortions and spurious signals are frequently observed in difference experiments, where the water signal is excited with different flip angles in different scans, cleaner data are obtained with selective 90° pulse excitation in every scan (Otting and Liepinsh, 1995a; Mori et al., 1996; Sobol et al., 1998). Radiation damping during a selective pulse can be controlled by hardware accessories like Q-switches (Otting and Liepinsh, 1995a), electronic feedback loops (Broekaert and Jeener, 1995; Abergel et al., 1996) and pulsed field gradients (Dalvit and Hommel, 1995; Böckmann and Guittet, 1997). Here, an alternative selective 90° pulse water excitation scheme is proposed which performs well with respect to excitation efficiency, selectivity, and artifact suppression. The scheme requires no special hardware other than pulsed field gradients. Its excitation efficiency makes

it particularly attractive for use with 2D NOE-NOESY and ROE-NOESY schemes with water flip-back.

Experimental data were recorded with a sample of 35 mM oxytocin in 70/30 (v/v) water/d_6-acetone at $-25\,°C$. With the probehead used, radiation damping turned the water magnetization back to equilibrium within 85 ms after a non-selective 170° excitation pulse. Four different schemes which yield an effective 90° excitation of equilibrium water magnetization in a selective way (Figure 1A-D) were tested for excitation efficiency. The integral of the water signal measured after selective excitation was compared to the integral measured after a hard 90° pulse. A 90° Gaussian pulse (Bauer et al., 1984) of 50 ms duration, applied as a Q-switched pulse (Otting and Liepinsh, 1995b; Figure 1A), resulted in 83% excitation. A modified WANTED excitation scheme (Böckmann and Guittet, 1997; Figure 1B) of 52 ms duration resulted in 72% excitation. The e-PHOGSY excitation scheme (Dalvit and Hommel, 1995; Figure 1C) resulted in 79 and 72% excitation with a 25 and 50 ms selective pulse, respectively. The novel excitation scheme of Figure 1D uses a relatively short selective 90° pulse which excites the water magnetization with about 99% efficiency. To achieve selectivity, this pulse is preceded by the sequence [selective 90° pulse – hard 90° pulse of opposite phase – pulsed field gradient], repeated n times, which serves to saturate the solute

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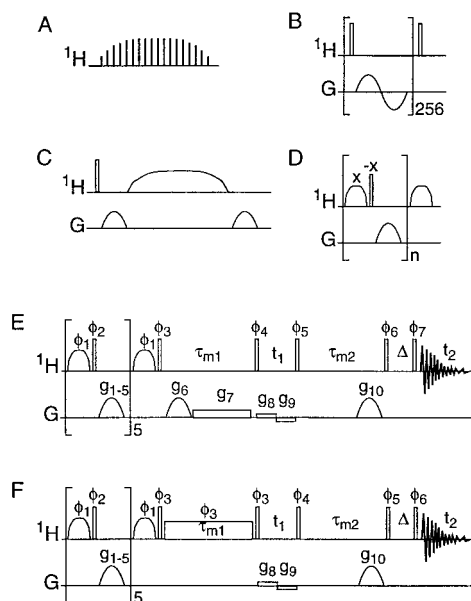
Figure 1. Pulse sequence elements for selective water excitation with minimum radiation damping and examples for NOE-NOESY and ROE-NOESY experiments. (A) Q-switched selective 90° pulse. The pulse shape is interrupted by short free-precession periods during which the quality factor Q of the radiofrequency coil is switched low. Parameters used here: 50 ms Gaussian pulse interrupted by 511 free-precession delays of 82 μs duration each. Throughout this work, shaped pulses were truncated at 1% of their maximum peak intensity. (B) 90° pulse applied in the WANTED fashion. Parameters used here: 257 pulses of 2 μs duration each, interspaced by 200 μs delays with bipolar gradients with sine-bell amplitude profile and 2.5 G/cm at maximum intensity. (C) e-PHOGSY excitation scheme. The first pulse is a hard 90° pulse. Parameters used here: 1 ms sine-bell shaped gradients with 7.5 G/cm maximum intensity, 50 ms 180° Gaussian pulse. (D) MS excitation scheme. A short selective 90° pulse, typically of a few ms duration, is followed by a hard 90° pulse of opposite phase and a gradient pulse. After n repetitions with different gradient amplitudes, the water magnetization is excited by a short selective 90° pulse. (E) NOE-NOESY pulse sequence with water flip-back by radiation damping used to record the spectrum of Figure 4. The jump-return sequence (Plateau and Guéron, 1982) at the end of the sequence minimizes magnetization loss by transverse relaxation, but more sophisticated sequences would yield a more uniform excitation profile (Sodano et al., 1998 and references therein). Parameters: MS excitation with six 7.5 ms 90° Gaussian pulses, τ_{m1} = 30 ms, τ_{m2} = 200 ms, Δ = 100 μs, t_{1max} = 19 ms, t_{2max} = 146 ms, interscan delay 1 s, total experimental time 9.5 h. Gradients: sine-bell shaped or rectangular as indicated in the figure, each of 1 ms duration except for G_7 (28 ms), G_{10} (1.5 ms), and the bipolar gradients during t_1 (Sklenář, 1995); maximum amplitudes: G_{1-10} = 40, 20, 10, 5, 2.5, 3.75, 0.5, 0.25, −0.25, 3.75 G/cm. Phase cycle: ϕ_1 = −ϕ_2 = x,−x; ϕ_3 = x,x,−x,−x; ϕ_4 = 4(x),4(−x); ϕ_5 = 16(π/4),16(5π/4); ϕ_6 = −ϕ_7 = 8(x),8(−x); receiver = x,−x,−x,x,−x,x,x,−x,2(−x,x,x,−x,x,−x,−x,x),x,−x,−x,x,−x,x,x, −x. (F) ROE-NOESY pulse sequence with water flip-back. Parameters: as in (E), except for the phase cycle: ϕ_1 = −ϕ_2 = x,−x; ϕ_3 = y,y,−y,−y; ϕ_4 = 8(π/4),8(5π/4); ϕ_5 = −ϕ_6 = 4(x),4(−x); receiver = x,−x,x,−x,2(−x,x,−x,x),x,−x,x,−x.
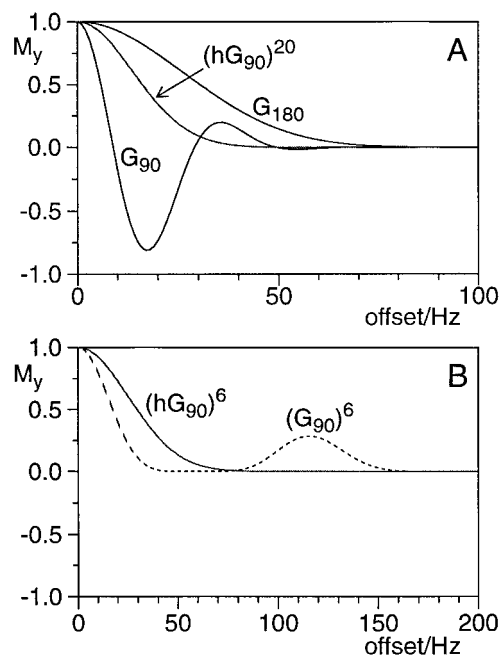
Figure 2. Frequency profiles of selective excitation schemes, starting from equilibrium magnetization M_z. The transverse magnetization M_y is plotted versus the offset from the carrier frequency. (A) Comparison between a 50 ms 90° Gaussian pulse (G_{90}), the MS excitation scheme (Figure 1D) with 20 7.5 ms 90° half-Gaussian pulses ((hG_{90})[20]), and the e-PHOGSY excitation scheme (Figure 1C), using a 25 ms 180° Gaussian pulse (G_{180}). (B) Comparison of MS excitation (Figure 1D) using six 90° Gaussian ((G_{90})[6]) or half-Gaussian ((hG_{90})[6]) pulses of 7.5 ms duration each.

resonances. The hard 90° pulse flips the water magnetization back to the z-axis, and the gradient defocuses the transverse magnetization of the solute. With n = 5 and Gaussian pulses of 7.5 ms duration, 90% water excitation efficiency was achieved. n = 5 and 15 ms half-Gaussian pulses (Friedrich et al., 1987), or n = 10 and 7.5 ms half-Gaussian pulses resulted in 88–89% excitation. This multiple selective pulse excitation scheme is henceforth referred to as MS excitation scheme.

Figure 2A compares the selectivity of a single Gaussian 90° pulse with that of the MS excitation scheme, using 20 half-Gaussian pulses of 7.5 ms duration each, and the e-PHOGSY excitation scheme, using a Gaussian 180° pulse of 25 ms duration for refocusing. All schemes yielded comparable excitation efficiency (∼80%). The long Gaussian pulse is very selective, but produces a pronounced negative sidelobe which can lead to confusion when positive and negative NOEs occur in the same spectrum. MS excitation with an even number of selective pulses
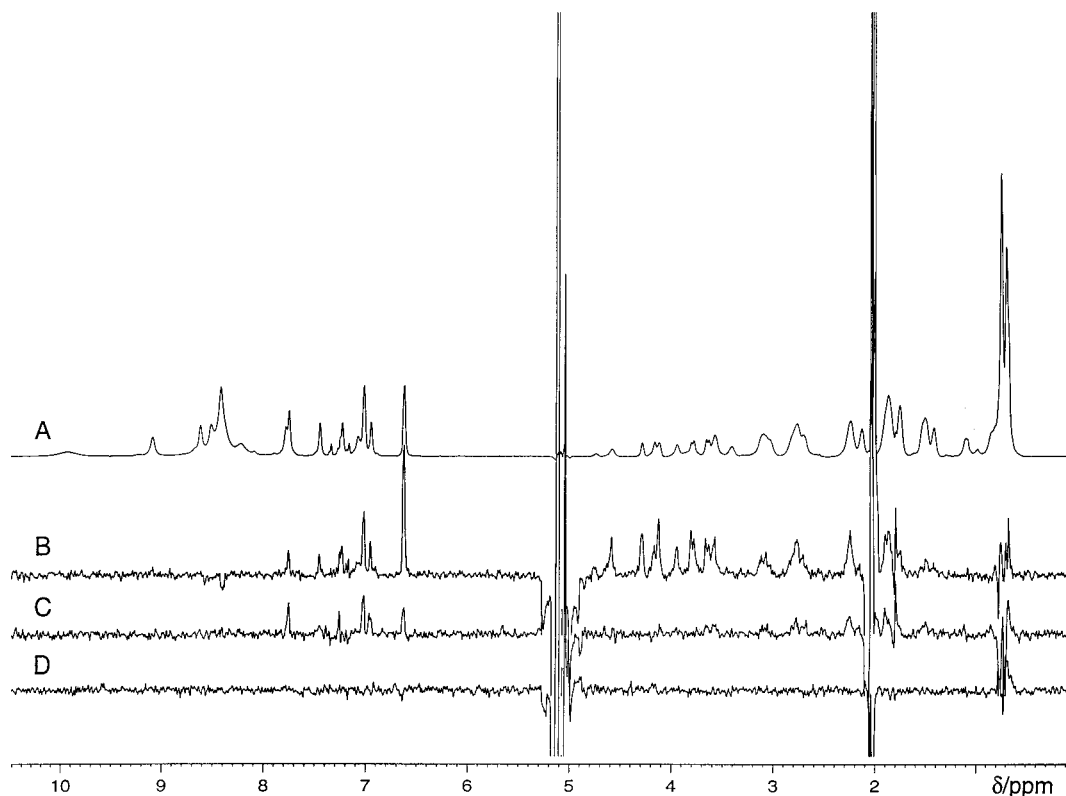
Figure 3. Artifacts generated by different water-selective excitation schemes. The spectra were recorded with a 35 mM solution of oxytocin in mixed water/d_6-acetone (2:1 v/v), pH 3.4, at 600 MHz ^{1}H frequency and $-25\,°$C, using a Bruker DMX 600 MHz NMR spectrometer. (A) 1D NMR spectrum. (B–D) Diagonal cross sections taken at $F_1 = F_2$ through 2D NOE-NOESY spectra recorded with the scheme of Figure 1E except that different selective water excitation schemes were used: 50 ms 90° Gaussian pulse (B), 50 ms Q-switched 90° Gaussian pulse (C), MS excitation with six 7.5 ms 90° half-Gaussian pulses (D). Other parameters were as described in Figure 1E, except that $\tau_{m1} = 2\,\mu$s and only 8 scans were used per FID, resulting in an experimental time of 40 min per spectrum.

does not produce negative sidelobes. Although the excitation profile achieved with 20 90° half-Gaussian pulses appears very acceptable, the total duration of the sequence may be prohibitively long for solutes with short T_1 relaxation times. Therefore, shorter MS excitation with reduced selectivity (and concomitantly enhanced excitation efficiency) may be preferred.

Figure 2B compares the selectivity of MS excitation, using six 7.5 ms 90° Gaussian or half-Gaussian pulses, respectively. While the Gaussian pulses yield better selectivity on-resonance, the negative sidelobes of the single Gaussian pulse result in excitation sidebands about 115 Hz from the carrier.

MS excitation suppresses the solute resonances which are not near the water signal in a way reminiscent of WET (Ogg et al., 1994; Smallcombe et al., 1995) and does not rely on difference spectroscopy. This property, also featured by the e-PHOGSY excitation scheme (Figure 1C), minimizes subtraction arti-

facts (Sobol et al., 1998). Figure 3 compares the cross sections taken along the diagonal of NOE-NOESY spectra recorded with different selective water excitation schemes. No signals would be expected at the short τ_{m1} value used (2 μs). Yet, a simple 90° Gaussian pulse excites many solute resonances, particularly near the water frequency (Figure 3B). The situation is improved by applying the 90° Gaussian pulse as a Q-switched pulse (Figure 3C), suggesting that most of the peaks in Figure 3B arise from truncation of the radiation damping field at the end of the selective pulse, when the pulse amplitude is low but the radiation damping field is maximum. Recording the spectrum with the MS excitation scheme results in artifactual peaks only at the positions of strong t_1-noise (Figures 3D and 4). The e-PHOGSY scheme yields similarly good results, albeit with lower sensitivity (data not shown). Experimentally we found that the excitation efficiency of the e-PHOGSY scheme
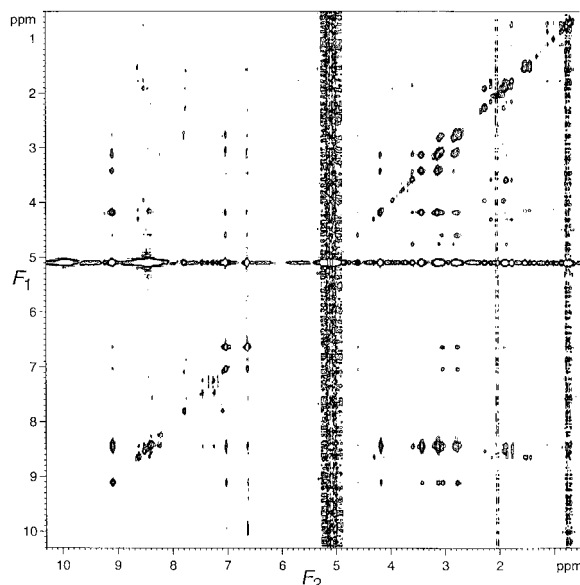
Figure 4. 2D NOE-NOESY spectrum of oxytocin recorded at $-25\,^\circ$C with the experimental scheme of Figure 1E and the sample of Figure 3.

improves significantly for dilute samples or solutes with few exchangeable protons, indicating that exchange broadening of the water signal is a major cause of sensitivity loss with that scheme.

Two-dimensional NOE-NOESY and ROE-NOESY experiments are readily performed with water flipback even for short water-solute contact times τ_{m1}, as the mixing time τ_{m2} can be chosen sufficiently long to take the water magnetization back to the equilibrium position by radiation damping (Figures 1E and F; Driscoll et al., 1989). The ratio of ROE and NOE water-solute cross relaxation rates, $\sigma^{ROE}/\sigma^{NOE}$, which is indicative of the water residence times (Otting et al., 1991), can thus be measured conveniently by recording the diagonal peaks of ROE-NOESY and NOE-NOESY spectra with short repetition delays, short τ_{m1} and short t_{1max} values. As an additional benefit, broad exchange cross peaks are absent from the diagonal (Jacobson et al., 1996). If the sensitivity is sufficient to observe off-diagonal peaks (Figure 4), $\sigma^{ROE}/\sigma^{NOE}$ can equally well be determined from those. Absolute water-solute magnetization transfer rates can be determined by comparison to a reference NOESY spectrum recorded in an identical way but without the selective excitation scheme and τ_{m1} (Figure 1E).

In principle, the solute suppression sequence $[90^\circ_x(\text{selective})\text{-}90^\circ_{-x}(\text{hard})\text{-gradient}]_n$ can improve the performance of any selective water excitation scheme.

If followed by a 160° pulse for water excitation in a difference experiment with and without radiation damping (Otting and Liepinsh, 1995a), artifact suppression can be combined with the near 100% water excitation efficiency afforded by this water excitation scheme. Although solute signal suppression does *not* compromise the sensitivity of this experiment (Sobol et al., 1998), it seems desirable to avoid the hardware dependency of water excitation by radiation damping and the off-resonance excitation resulting from truncated radiation damping fields. MS excitation with half-Gaussian pulses presents a way to control the trajectory of the water magnetization even in the presence of fairly strong radiation damping fields.

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