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Water Suppression Using a Combination of Hard and Soft Pulses

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Numerous methods are available for suppression of the H_2O resonance in the NMR spectra of water-soluble compounds. These methods include presaturation (*1, 2*), excitation with a soft pulse (*3*) or a Redfield pulse (*4*), rapid scan correlation (*5*), binomial hard-pulse sequences (*6–9*), and methods that exploit the different relaxation characteristics of H_2O and solute protons (*10–13*). In recent years, the binomial hard-pulse sequences and variations thereof (*14, 15*) have become most widely used. Morris and Smith (*16*) showed that the binomial sequences can be combined with a single nonselective 90° pulse to provide an excitation spectrum which has nearly maximal excitation over a wide frequency range except for a narrow region around the H_2O resonance which has near-zero excitation. The phase distortions resulting from this type of excitation are severe which leads to problems when this type of composite pulse is used in phase-sensitive two-dimensional NMR experiments. Levitt and Roberts (*17*) very recently proposed a new hard-pulse sequence which has a much weaker phase dependence across the spectrum while still providing good water suppression. However, the remaining small phase distortions, which present no problem in 1D NMR, can present difficulties in 2D NMR spectra. For example, cross peaks in a NOESY spectrum are typically two orders of magnitude weaker than diagonal peaks and even small phase distortions of the diagonal resonances give rise to serious distortions in the 2D spectrum. A recently proposed echo scheme (*18*) yields pure absorptive resonances across the entire spectrum but gives poor excitation close to the water resonance.

Here we demonstrate the use of a hard/soft pulse combination first suggested by Gupta and Redfield (*19*). In principle, this type of hard/soft pulse combination can solve both the excitation window and the phasing problems mentioned above. However, it should be noted that the hard/soft pulse combinations described below require very stable spectrometer hardware.

Conceptually the method is extremely simple. With the carrier placed on the H_2O resonance, a weak $90°_x$ pulse of duration τ ($\tau \approx 5$ ms) rotates the H_2O magnetization from the z axis to the $-y$ axis (Fig. 1a), while leaving all resonances more than about 50 Hz removed from the H_2O resonance along the z axis. A subsequent nonselective $90°_x$ pulse rotates the H_2O magnetization back to the z axis and rotates the resonances

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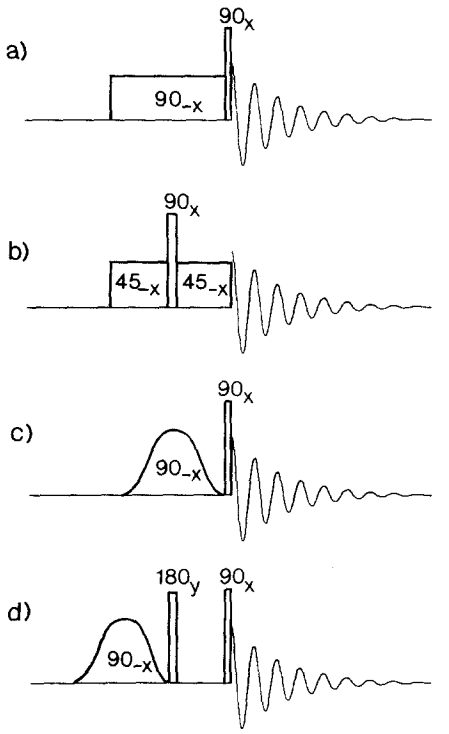

FIG. 1. Various hard/soft pulse combinations for suppression of H_2O. The carrier has to be positioned exactly on the center of the H_2O resonance.

of interest to the y axis. This pulse sequence has an excitation and phase profile as sketched in Fig. 2a. A wider near-zero excitation region at the cost of a deteriorating phase profile can be obtained by positioning the hard 90°_x pulse in the center of the soft pulse (Fig. 1b). The phase profile of sequence 1b (Fig. 2b) is very poor because the excitation of the resonances of interest by the hard 90°_x pulse occurs at a time $\tau/2$ before data acquisition is started. The much smaller oscillations in phase observed far off-resonance with sequence 1a are caused by the the small amount of excitation far off-resonance by the rectangular soft pulse. The x components of off-resonance magnetization vectors are not returned to the z axis by the nonselective 90°_x pulse and create the small phase deviations shown in Fig. 2b. Although in 1D NMR spectra these small variations in phase are difficult to observe, they cause serious problems when this type of excitation is incorporated in phase-sensitive 2D experiments.

A better phase profile can be obtained by shaping the soft pulse to, for example, a Gaussian (Fig. 1c), which provides minimal excitation far off-resonance. In principle, an excellent phase and intensity profile can be obtained by using the sequence of Fig. 1d. This scheme employs a shaped 90°_{-x} pulse followed by a nonselective 180°_y pulse followed by a short delay, needed for all transverse magnetization components to refocus to the $-y$ axis, followed by the nonselective 90°_x pulse which brings all transverse water magnetization back to the z axis and excites the rest of the spectrum. Unfortunately, in contrast to the other excitation schemes, this last sequence is quite sensitive

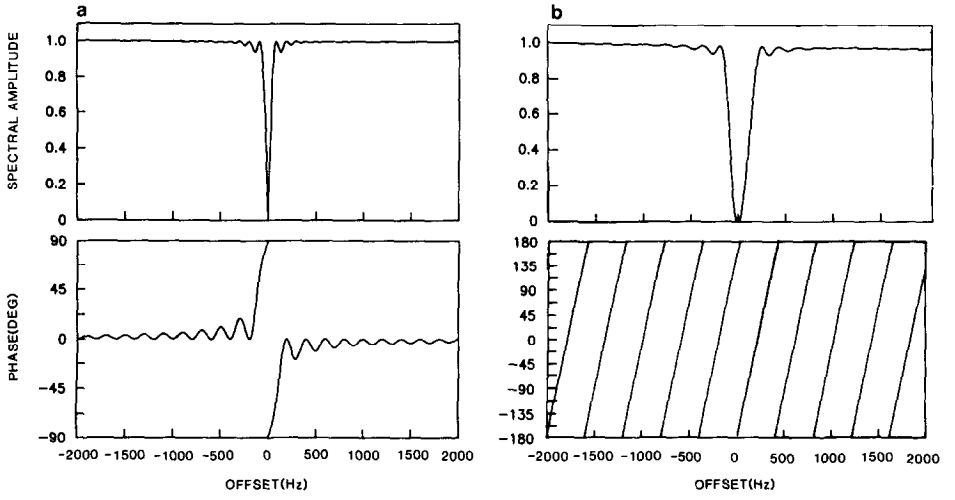

FIG. 2. Amplitude and phase profiles of the excitation scheme of (a) Fig. 1a and (b) Fig. 1b.

to inhomogeneity of the RF field and on our spectrometer the obtainable water suppression per single scan is significantly lower than with schemes 1a–1c.

Incorporation of the hard/soft pulse excitation scheme in 2D NMR pulse schemes is straightforward and three examples are shown in Fig. 3. Figure 3a shows the simplest kind of implementation, where both 90° pulses of the regular COSY experiment are replaced by the pulse of Fig. 1b. With a duration τ of the soft pulse, the first τ ms of

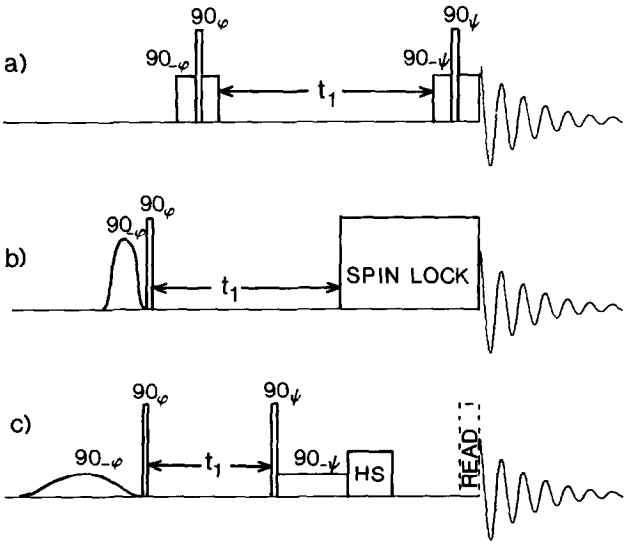

FIG. 3. Examples of how the hard–soft pulse sequences can be incorporated into (a) a COSY experiment, (b) a ROESY or HOHAHA experiment, or (c) a NOESY experiment. In the NOESY experiment the actual suppression is accomplished by a "water suppression read pulse" of arbitrary type. The soft $90°_\varphi$ and $90°_\psi$ pulses merely serve to avoid inversion of the H_2O resonance.

the t_1 evolution and the first $\tau/2$ ms of the t_2 detection periods are lost in this COSY experiment. Since the observed signal of magnetization transferred from proton A to protons X is modulated by $\sin(\pi J_{AX} t_1)\sin(\pi J_{AX} t_2)$, very little cross-peak signal is lost because of these extra delays provided that $\tau \ll 1/J_{AX}$. Because this type of COSY spectrum is usually recorded in the absolute-value mode, the phase distortions generated by the soft/hard/soft 90° pulses do not affect the final presentation of the spectrum.

Figure 3b shows the incorporation of pulse 1c in the spin-locked 2D NOE experiment (*20, 21*). To avoid phase distortions of the intense diagonal resonances it is essential in the spin-locked NOE experiment to use a shaped soft pulse. Incorporation in the 2D HOHAHA experiment (*22*) can be accomplished in an identical manner. All longitudinal H_2O magnetization that is present at the end of the evolution period is removed from the spectrum by the long spin-lock pulse. Since the spin-lock field applied during the mixing period is exactly perpendicular to the on-resonance H_2O magnetization, RF inhomogeneity defocuses the H_2O longitudinal magnetization very rapidly.

Figure 3c shows how the hard/soft pulse can be incorporated in the NOESY experiment to reduce the effects of radiation damping. Previously proposed schemes for NOESY in H_2O utilized a water-suppression read pulse, preferably of the 1–1 type (*6, 23*) or of the "1–1 echo" type (*18*). Because of the phase cycling used in this type of phase-sensitive NOESY experiment (*24*), in one out of every four scans the H_2O magnetization is inverted after the second 90° pulse. A very slight excitation of this inverted H_2O resonance by the final read pulse can be sufficient to start the stimulated emission process, commonly known as radiation damping (*25*). By incorporating the soft, Gaussian-shaped pulses before the first and after the second 90° pulse (Fig. 3c) the H_2O magnetization never gets inverted and the radiation damping effect which can cause serious dynamic range problems is absent. Because the final water suppression is obtained by the read sequence at the end of the mixing period, the soft pulses can be made very long, yielding a very narrow region of nonexcitation. Radiation damping (and T_1 relaxation) occurs during the soft pulses and requires the first soft pulse to have a flip angle larger than 90° and the second soft pulse to have a flip angle less than 90°.

As an example, Fig. 4 shows a COSY spectrum of 10 mg of the decapeptide LH-RH in 0.5 ml 90% H_2O/10% D_2O, containing 100 m*M* NaCl, pH 5.8, 11°C. The spectrum results from a 512 × 1024 data matrix (102 ms acquisition times in both dimensions) with 16 scans per t_1 value. The total measuring time was 5 h. The spectrum has been recorded with the scheme of Fig. 3a, using a 90° soft-pulse width of 4.8 ms and a 90° hard-pulse width of 35 μs. The 1D spectrum obtained with scheme 1a, is shown along the F_1 axis of the 2D spectrum.

The approach described here offers an attractive alternative for the popular "hard-pulse-water-suppression" methods. The spectral window where no excitation is obtained can be set to arbitrary width by varying the power and duration of the soft pulse. Phase distortions of the hard/soft pulse excitation scheme can be kept to a minimum by using amplitude-modulated soft 90° pulses. Of course, the price one pays is that the hard/soft pulse experiments are more critical to exact adjustment than the hard-pulse sequences. On our system fast switches (10 μs) for changing between the high- and the low-power mode of the observe channel of our spectrometer were

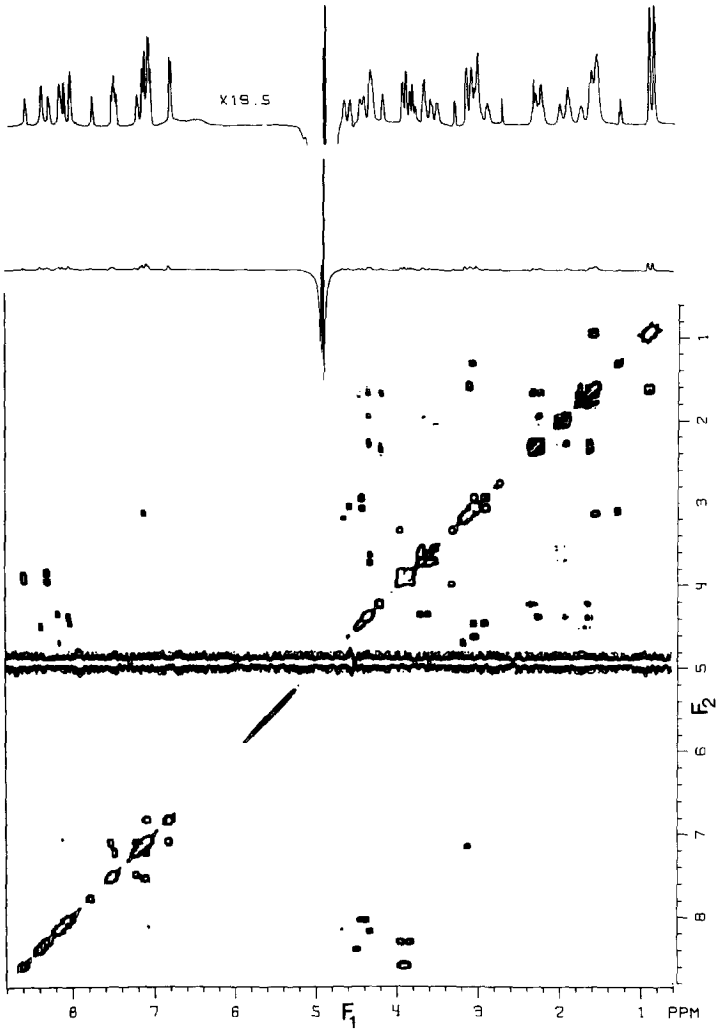

FIG. 4. Example of a COSY spectrum of the peptide hormone LH-RH (pGlu-His-Trp-SER-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2). 10 mg LH-RH was dissolved in 0.5 ml 90% H_2O/10% D_2O, pH 5.8, 11°C. The 1D spectrum shown along the top of the 2D spectrum was recorded with the sequence of Fig. 1a, with 4.8 ms and 35 μs for the durations of the soft and hard 90° pulses, respectively. Identical power settings were used for the 2D spectrum, using the sequence of Fig. 3a. Experiments were recorded on a modified NT-500 spectrometer. The 2D spectrum results from a 512 × 1024 data matrix with 16 scans per t_1 value.

used to replace the original slow relay type switches. It is important that the phase relationships between the high- and low-power channels be absolutely constant as a function of time. On our spectrometer, small instabilities (±1°) in the phase of the decoupler vs the observe channel prohibited the use of the decoupler channel for generating the soft pulses. It is critical to make the phase of the soft 90°_{-x} pulse exactly opposite to the phase of the nonselective 90°_x pulse. Since these pulses are generated via separate pathways (or different attenuator settings) the phases generally will not

differ by 180° unless an adjustable phase shifter is inserted in one of the two pathways.

Other combinations of shaped soft pulses and hard pulses for water suppression have been developed independently by Warren (*26*).

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