JOURNAL OF MAGNETIC RESONANCE 54, 170–173 (1983)

The 1–1 Hard Pulse: A Simple and Effective Method of Water Resonance Suppression in FT ^{1}H NMR

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Received March 21, 1983; revised April 27, 1983

In protein and nucleic acid NMR, the study of exchangeable protons can provide a wealth of structural information. In general, however, such protons undergo rapid deuterium exchange so that ^{1}H NMR spectra must be recorded in H_2O rather than D_2O. Because the concentration of solute protons ($\sim$1 mM) is so much smaller than that of the water protons ($\sim$110 M), methods of solvent suppression must be used to overcome the dynamic range and digitization problems imposed by a limited computer word length. Of the methods of water resonance suppression available, in general the only ones that are appropriate in the study of rapidly exchangeable protons are those that involve the application of rf excitation which has a zero spectral density at the water resonance position. This is because such methods do not involve perturbation of the exchangeable resonances by processes such as magnetization transfer, cross-relaxation, or intermolecular interactions with excited water protons. Examples of the selective excitation methods include the long Alexander (*1*) and 2–1–4 Redfield (*2*) pulses and the hard time-shared Redfield (*3, 4*), jump–return (*5*), and 1–2–1 pulses (*6*). The hard time-shared pulse sequences have the significant advantage over the long pulses that they do not require any hardware modification to existing FT spectrometers and are less sensitive to long-term drift in pulse amplitude. In the present paper we present a novel, very simple, and highly effective time-shared hard pulse method for water resonance suppression, the 1–1 pulse.

The 1–1 pulse is given by

$$\theta_x\text{–}\tau\text{–}\theta_x. \qquad [1]$$

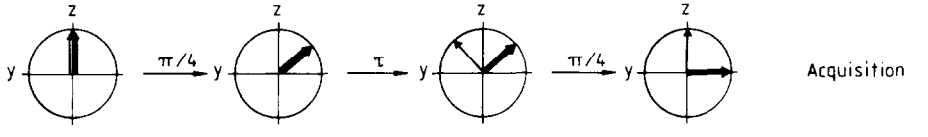

FIG. 1. Vector representation of the magnetization at the carrier frequency (thick arrow) and at $1/2\tau$ from the carrier frequency (thin arrow) in the yz plane of the rotating frame during excitation by the 1–1 pulse [1]. In this figure $\theta_x = \pi/4$. Prior to the first pulse, all magnetization lies along the $0z$ axis. The first pulse flips all magnetization by an angle $\pi/4$. In the interval τ, the magnetization at the carrier frequency remains stationary whereas that at $1/2\tau$ from the carrier frequency precesses 180°. The second $\pi/4$ pulse brings the magnetization at the carrier along the $0y$ axis and returns that $1/2\tau$ from the carrier to the $0z$ axis.

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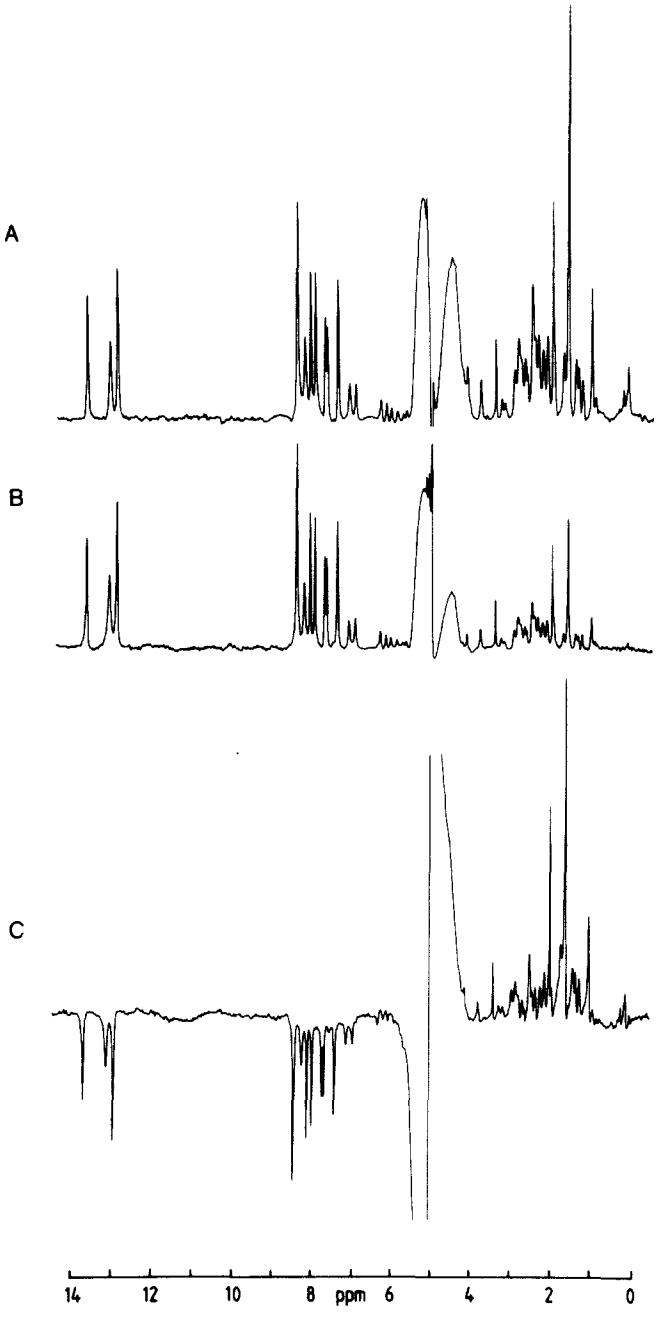

FIG. 2. 500 MHz ^{1}H NMR spectra of a 0.35 mM solution of the double stranded self-complementary DNA hexamer d(CGTACG)$_2$ in 90% H_2O obtained using the 1–1 (A), time-shared Redfield (B), and 1–2–1 (C) pulses. In all three cases the following instrumental conditions were used: carrier position, 3048 Hz downfield from the water resonance position; sweep width, 12,195 Hz; acquisition time, 0.366 sec; data points, 8096; interpulse delay, 0.5 sec; number of transients, 960; detection, in quadrature. In (A), (P–τ–P), P = 4.5 μsec and τ = 160.5 μsec; in (B), $(P\text{–}\tau)_{10}$, P = 0.9 μsec and τ = 31.8 μsec; in (C), (P_1–τ_1–P_2–

The carrier is placed near the region of interest—for example, in the case of an oligonucleotide, in the region between the imino and aromatic proton resonances—and τ is the time required for the water protons to precess 180° in the rotating frame (namely $1/2\delta\nu$ where $\delta\nu$ is the difference in frequencies between the carrier and the resonance position of the water protons). The total flip angle is $2\theta_x$. The vector representation of the magnetization behavior in the yz plane during excitation by the 1–1 pulse sequence is shown in Fig. 1.

Figure 2 compares the 500 MHz ^{1}H NMR spectra of a 0.35 mM solution of the self-complementary DNA hexamer d(CGTACG)$_2$ in 90% H_2O obtained in 960 transients using the 1–1 (Fig. 2A), time-shared Redfield (Fig. 2B), and 1–2–1 (Fig. 2C) pulses. In all three cases the total flip angle was 90°, the carrier was placed 3048 Hz to low field of the water proton resonance, and the spectral width was 12,195 Hz. Because in all three techniques the Fourier-transformed and phase-corrected spectra exhibit baseline distortions, arising from the still considerable intensity of the wings of the water peak in the spectral regions of interest, all three free induction decays (FID) were subjected to data shift manipulation (*7, 8*). In this manner, the intensity of the water peak is further reduced so that the Fourier transformed and phase corrected spectra no longer exhibit baseline distortions. It should be noted that this procedure is cosmetic and produces a slight additional amplitude distortion (*7*) which is minimal in practice in relation to the amplitude distortion already introduced by the nature of the excitation function of the three pulse sequences.

In Fig. 2, it is clear that to low field of the water peak the S/N ratios obtained using the 1–1 (Fig. 2A) and time-shared Redfield (Fig. 2B) pulses are the same and about 1.5 times greater than that using the 1–2–1 pulse (Fig. 2C). To high field of the water peak, however, the S/N ratio obtained using the 1–1 pulse is about 1.5 times greater than that using the 1–2–1 pulse and about 3 times greater than that using the time-shared Redfield pulse. In addition, it should be noted that whereas the peaks to low and high field of the water resonance have the same phase in the case of the 1–1 and time-shared Redfield pulses, they have opposite phases in the case of the 1–2–1 pulse.

One should note that the 1–1 pulse, like all hard pulse sequences for water resonance suppression, requires a high degree of accuracy of both delays and pulse lengths, typically of the order of 0.1 μsec which may not be achieved on some older spectrometers. In carrying out these experiments, it is therefore essential to optimize the receiver gain and pulse parameters so as to achieve maximum signal-to-noise. This can be done interactively while displaying the FID. Care should be taken, however,

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τ_2–P_3), P_1 = 2.4 μsec, P_2 = 4.7 μsec, P_3 = 2.2 μsec, τ_1 = 163.8 μsec, and τ_2 = 160.8 μsec. (It should be noted that in the case of the 1–1 and 1–2–1 pulses, the delay τ is the time required for the water protons to precess 180°, whereas in the time shared Redfield pulse 10τ is the time required for the water protons to precess 360°.) In all three cases prior to Fourier transformation and phase correction, the acquired FID was left shifted four times and added to the unshifted FID resulting in nulls at positions $1/4W$ from the carrier where W is the total sweep width; the resulting FID was then multiplied by an exponential equivalent to a line broadening of 2 Hz. The spectra were recorded on a Bruker AM500 spectrometer. The experimental conditions were 0.35 mM (in duplex) d(CGTACG)$_2$ in 90% H_2O, 10% D_2O, 50 mM potassium phosphate pH 6.5, 1 M KCl; temperature, 5°C. Chemical shifts are given with respect to external 4,4-dimethylsilapentane-1-sulfonate.

since the software for interactively changing parameters may itself add an extra delay which would not be present when the experiment is finally run. In addition, it is essential to ensure that neither the real nor imaginary FIDs are clipped; often only the real part of the FID is displayed unless specified.

The 1–1 pulse is similar to the jump–return (JR) sequence 90°_x–τ–$90^\circ_{\bar{x}}$ (*5*). In the JR sequence, however, the carrier is placed at the position of the water resonance. Although this has the advantage that a smaller spectral width and, hence, fewer data points can be employed, it has the disadvantage that the setting up of optimal conditions requires one not only to minimize the intensity of the water resonance by finely adjusting the carrier position and pulse phase, but also to maximize the intensity of the signals in the region of interest by adjusting the value of the delay τ. Given the small concentration of solute usually employed in biological NMR experiments, the latter requirement has an obvious disadvantage in terms of the length of time required to obtain the optimal parameter settings.

In conclusion, the advantages of the 1–1 pulse in the suppression of the water proton resonance may be summarized as follows. (i) The 1–1 pulse is very easy to set up in practice. The two pulses always have identical lengths so that any desired flip angle can be used, and only the delay τ need be optimized for any particular carrier position. Furthermore, no phase shifting is required. (ii) The degree of water resonance suppression obtained using the 1–1 pulse is between 1000 to 2000, and the S/N ratio obtained is superior to that of the time-shared Redfield and 1–2–1 pulses as judged over the whole spectrum. (iii) No lowering of the rf power is required to achieve optimal suppression. (iv) The total duration of the 1–1 pulse is half that of both the time-shared Redfield and 1–2–1 pulses, thus allowing greater potential time resolution to be achieved in T_1 and T_2 measurements.

ACKNOWLEDGMENTS

This work was supported by the Medical Research Council (G.M.C., A.M.G., and B.J.K.) and the Lister Institute for Preventive Medicine (G.M.C.). G.M.C. is a Lister Institute Research Fellow. The 500 MHz ^{1}H NMR spectra were obtained using the AM500 spectrometer of the MRC Biomedical NMR Centre at the National Institute for Medical Research.

REFERENCES

1. S. ALEXANDER, *Rev. Sci. Instrum.* **32,** 1066 (1961).
2. A. G. REDFIELD, S. D. KUNZ, AND E. K. RALPH, *J. Magn. Reson.* **19,** 114 (1975).
3. A. G. REDFIELD AND S. D. KUNZ, "NMR and Biochemistry" (S. J. Opella and P. Lu, Eds.), p. 225, Dekker, New York, 1981.
4. J. M. WRIGHT, J. FEIGON, W. DENNY, W. LEUPIN, AND D. R. KEARNS, *J. Magn. Reson.* **45,** 514 (1981).
5. P. PLATEAU AND M. GUÉRON, *J. Am. Chem. Soc.* **104,** 7310 (1982).
6. V. SKLENÁŘ AND Z. STARČUK, *J. Magn. Reson.* **50,** 495 (1982).
7. K. ROTH, B. J. KIMBER, AND J. FEENEY, *J. Magn. Reson.* **41,** 302 (1980).
8. C. A. G. HAASNOOT AND C. W. HILBERS, *Biopolymers,* in press (1983).