JOURNAL OF MAGNETIC RESONANCE **126,** 256–265 (1997)
ARTICLE NO. MN971166

Optimization of the Water-PRESS Pulse Sequence and Its Integration into Pulse Sequences for Studying Biological Macromolecules

William S. Price,*,† Kikuko Hayamizu,‡ and Yoji Arata*

Water Research Institute, Sengen 2-1-6; and ‡National Institute of Materials and Chemical Research, Higashi 1-1, Tsukuba, Ibaraki 305, Japan

Received December 19, 1996; revised March 11, 1997

In this paper, the recently developed "Water-PRESS" method of water suppression [W. S. Price and Y. Arata (1996), *J. Magn. Reson. B* **112, 190] in which homospoil pulses are used to manipulate the effects of radiation damping on the water resonance and thereby selectively alter the effective relaxation times of the water resonance with respect to the solute (e.g., biological macromolecules) resonances is further developed and applied. In the present work, methods for optimization in terms of degree of water suppression and in temporal terms (important for the application of Water-PRESS to multidimensional experiments) are considered so that recycle delays of less than 2.3 s (including the acquisition time) are possible. Also, a simple modification which allows the observation of solute resonances with relaxation times similar to that of the water resonance is presented. Finally, the inclusion into more complicated pulse sequences is also discussed. Experimental examples using aqueous samples of lysozyme and immunoglobulin are given. Compared to most other NMR water suppression techniques, this method is extremely simple to implement and optimize and does not require accurately calibrated RF pulses or perfect lineshape.**

INTRODUCTION

In protein NMR studies, it is often desirable to observe the (exchangeable) NH or OH protons and also perhaps bound water molecules [e.g., Ref. (*1*)]; this entails conducting the NMR experiments in a solvent consisting of ~90% H_2O. The large difference between the protein (~m*M* range) and the water–proton (~100 *M*) concentrations directly impedes efficient spectral acquisition of the protein resonances in two ways. First, the receiver gain must be set to a small value ill-suited to efficiently digitizing the weak protein resonances, and second, the water resonance may conceal many protein resonances (especially α protons). This second problem is compounded by the effects of radiation damping (*2*) which cause the water resonance to be artificially broadened. The single largest problem in water suppression is that it is not generally possible to manipulate the water resonance independently of the solute resonances in the frequency domain.

Traditional Means of Water Suppression and Complications

Many water-suppression methods exist, including presaturation, relaxation-based techniques, selective-excitation, gradient, and combined selective-excitation–gradient-based methods. Most of these methods have been recently reviewed (*3–7*). The selectivity to the water resonance and the degree of suppression of these methods are diminished by a combination of five factors: (1) radiation damping, (2) selective pulses are not perfectly selective and generally do not have a pure phase, (3) inhomogeneity of the RF (i.e., B_1) and static magnetic (i.e., B_0) fields, (4) spin diffusion, and (5) due to radiation damping, the water resonance has an effective relaxation time close to that of the protein resonances.

The use of selective-excitation (*3, 4*) and gradient-based methods involving selective RF pulses [e.g., WATERGATE (*8, 9*), flipback (*10*), WET (*11, 12*), and RAW (*13*)] complicates pulse sequences. It has been shown in a recent work that a double PFG echo obviates the phase problems usually associated with selective RF pulses (*14*); however, the sequence requires more time. The efficiency of selective RF pulses is also compromised by the effects of radiation damping (*15*). Although the diffusion coefficient of proteins is typically at least an order of magnitude below that of water (*7*), diffusion-based gradient methods such as DRYCLEAN (*16*) are only applicable when the solute and the solvent (e.g., water) have very different diffusion coefficients, and such sequences generally result in significant attenuation of the solute resonances, especially those of exchangeable protons. Further, any echo-based system such as DRYCLEAN will result in unequal losses of the protein resonances according to the T_2 relaxation rates of the individual spins.

Thus, the traditional methods of water suppression are not totally specific to water and may also introduce additional complications into the intended pulse sequence and resulting spectrum. Solvent suppression in samples with very large

† To whom correspondence should be addressed.

1090-7807/97 $25.00

macromolecules (i.e., MW $\gg$ 10,000) such as immunoglobulins (*17*) presents special difficulties due to the unfortunate combination of short relaxation times and high susceptibility to spin diffusion (*18–20*). Thus, irradiation at the water frequency can result in a severe loss of intensity of the protein resonances. Further, the short T_2 relaxation times of the resonances (*20*) severely restrict the possible methods that can be used, especially those involving echoes. Jump–return and binomial sequences (*21*) have considerable advantages for use with high-molecular-weight species; however, they introduce complicated phase relationships through the spectrum, and they preclude the observation of resonances at the solvent frequency.

An elegant method of water suppression is to use the difference in relaxation time between the water and the protein resonances in an inversion-recovery experiment to suppress the water resonance (also known by the acronym WEFT) (*22–24*). WEFT-based techniques are particularly attractive for protein studies as they allow the observation of solute resonances close to the water resonance (*25*). However, radiation damping causes the observed water relaxation time to become very close to that of the protein resonances, which makes the WEFT method ineffective. We have recently presented a variation of the WEFT method termed *w*ater-*pres*equence *s*uppression (Water-PRESS) in which magnetic-field-gradient pulses are used to inhibit the effects of radiation damping and thereby selectively alter the effective relaxation time of the water resonance so that it becomes much longer than that of the protein resonances (*26*). Although in the present examples the solvent is water and the solute is protein, the Water-PRESS method is quite general and the solute and solvent can be any species with suitable relaxation characteristics.

In the present work we first briefly review the basic Water-PRESS sequence and then develop strategies for optimizing the time required and the degree of water suppression. A detailed description of how to use the Water-PRESS sequence is presented. Some emphasis is placed on the use of this method for suppressing water in samples of very large macromolecules, and a simple modification to the pulse sequence is presented which allows the unperturbed observation of protein resonances having spin–lattice relaxation times of similar order to that of the water resonance. Examples are also given of ways in which this method can be used to specify the magnitude of the water resonance for different applications such as protein–water NOE measurements. Finally, an example of combining the Water-PRESS sequence with a COSY sequence is presented.

THEORY

The Basic Idea

Radiation damping results from the precessing spin magnetization generating an oscillating current in the receiver coil which in turn generates an oscillating magnetic field which tends to rotate the magnetization back to its equilibrium position. The effect of radiation damping can be characterized by a time constant (in CGS units) (*27, 28*)

$$\tau_{\mathrm{rd}}^{-1} = 2\pi\gamma\eta Q M_0, \qquad [1]$$

where γ is the gyromagnetic ratio of the spins, η is the filling factor of the probe, $Q = \omega L/R$ is the quality factor of the probe (where ω, L, and R are the resonance frequency, inductance, and resistance of the coil, respectively), and M_0 is the equilibrium magnetization per unit volume. Thus, the effects of radiation damping are much more prevalent in modern spectrometers with their larger static fields and more sensitive probes.

In biological NMR samples, the solute (e.g., protein) concentration is typically four orders of magnitude smaller than the water concentration. As a result the solute resonances are almost totally unaffected by radiation damping. With water, however, the situation is entirely different and the effects of radiation damping, especially at higher magnetic fields and with sensitive probes, dominate the effects of the inherent relaxation mechanisms of the water protons. This results in the water resonance having an effective relaxation time very similar to that of the protein resonances. In the Water-PRESS sequence (see Fig. 1A), the π RF pulse inverts all of the magnetization (i.e., protein and water resonances) to the $-z$ axis. A series of weak gradient pulses oriented along the z axis (i.e., homospoil pulses) is then applied to remove any transverse magnetization and consequently prevents the initiation of radiation damping of the water resonance so that the water relaxes back to equilibrium near its inherent longitudinal relaxation rate during the period D_{NP}. As the natural longitudinal relaxation rate of water, $T_{1\,\mathrm{nat}}^{\mathrm{H_2O}}$, is generally much longer than that of the protein resonances, T_1^{P}, it is easily possible to choose an appropriate delay (i.e., D_{NP}) such that the water magnetization is $\sim$zero while the protein resonances are (nearly) fully relaxed (see Fig. 1B). An observe pulse (e.g., $\pi/2$) can then be applied to observe the protein resonances with near complete suppression of the water resonance. Also because of the long water relaxation time, a small error in the setting of the duration of D_{NP} will not result in a large water signal. If $D_{\mathrm{H_2O}} > 5 \times T_1^{\mathrm{H_2O}}$, where $D_{\mathrm{H_2O}}$ is the recycle delay and $T_1^{\mathrm{H_2O}}$ is the effective relaxation time of the water, then the optimal condition for water suppression will be given by $D_{\mathrm{NP}} = \ln(2) \times T_1^{\mathrm{H_2O}}$. In Fig. 2, an aqueous solution of lysozyme is used to demonstrate water suppression obtained using the Water-PRESS subunit. In our previous paper, we compared the suppression obtained with Water-PRESS to that obtained using presaturation and WATERGATE.

Strategies for Optimization

Optimization can be taken in two senses: (1) minimizing the residual water resonance or (2) minimizing the time

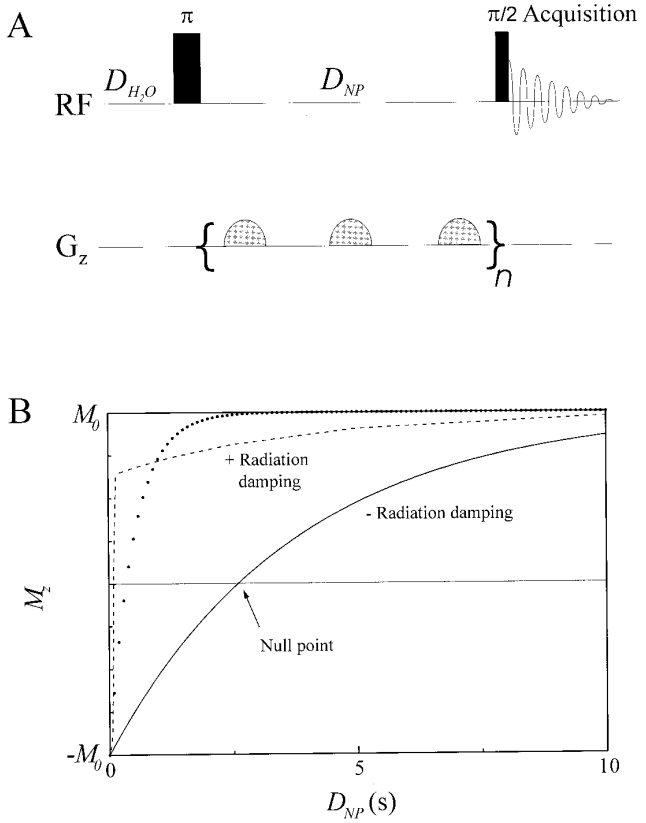


FIG. 1. A schematic representation of (A) the Water-PRESS subunit and how it produces water suppression. The Water-PRESS subunit consists of a relaxation delay (D_{H_2O}) to allow the water magnetization to reach thermal equilibrium. Next, a π pulse is applied to rotate the magnetization to the $-z$ axis. A delay (D_{NP}) of sufficient length is used to allow the water magnetization to relax to the origin. In the presence of radiation damping (i.e., without gradient pulses), the water (---) quickly returns to the equilibrium position at a rate similar to that of the protein nuclei ($\cdots$). However, if during D_{NP}, a series of n very weak and evenly spaced homospoil pulses is applied so as to inhibit the effects of radiation damping, the water relaxes according to its natural spin–lattice relaxation rate (—). To effect water suppression, D_{NP} would be set to the value so that the water resonance is nulled (see B), and the protein resonances, by virtue of their faster relaxation rate, have achieved thermal equilibrium. If an observe pulse, a nonselective $\pi/2$ pulse in the present example, is applied, an almost water-free protein spectrum is acquired.

required to conduct the experiment while still obtaining acceptable suppression. In the following subsections, we consider both types of optimization.

Composite pulses, B_0 and B_1 homogeneity. Due to inhomogeneity of the B_1 field, not all of the water protons in the sample will receive exactly the same tip angle, and consequently no single value of D_{NP} will result in the water magnetization being null over the entire sample volume. Hence, the net magnetization may be null when integrated over the linewidth of the water resonance, but at a particular frequency the water resonance may not be null. The simplest approach is to restrict the sample size. The sequence can also be altered to better cope with B_1 inhomogeneity by, for example, using a composite π pulse (*29*). Further, the inhomogeneity in the B_1 field is often coincident with the inhomogeneity in the B_0 field, and this can be used as a basis for volume selection (*30*). Using this idea the $\pi/2$ pulse can be replaced, for example, by a suitably phase-cycled, φ—180°—180° (φ can be any angle but for the purposes of our discussion it is conveniently taken as a 90° pulse) as in the FLIPSY sequence (*30*). However, since all the experimental results of the present work were obtained with the sample contained in a susceptibility matched microtube, we found that these two pulse sequence approaches provided negligible benefit and did not justify the additional complication.

Variable angle and soft RF pulses. If the π pulse is replaced by a pulse in the range $\pi/2 < \theta \leqslant \pi$ (see Fig. 3A) and a value of D_{NP} ($0 < D_{NP} \leqslant \ln(2)T_1^{H_2O}$) is chosen, then the value of θ which will give a null point is

$$\theta = \cos^{-1}\left[1 - \exp\left(\frac{D_{NP}}{T_1^{H_2O}}\right)\right]. \qquad [2]$$

However, as θ approaches $\pi/2$, D_{NP} becomes shorter and the solute signals may become T_1-weighted. The $\pi/2$ pulse can be replaced by a pulse of any angle since the water is nulled at the time of the application of this pulse (see Fig. 3A). Thus, if $D_{NP} \ll 5 \times T_1^P$, the optimum signal-to-noise for the protein resonances will be given when the $\pi/2$ pulse is replaced by one at the ''Ernst angle'' (*31*), i.e.,

$$\phi = \cos^{-1}\left[\exp\left(-\frac{D_{NP}}{T_1^P}\right)\right]. \qquad [3]$$

Although the ^{1}H resonances of proteins typically have subsecond relaxation times, some resonances (e.g., met-δ and some aromatics) have particularly long relaxation times. If a hard π pulse is used in the Water-PRESS sequence, the intensity of these resonances will be considerably reduced because the recycle delay is insufficient to allow full relaxation. One method of circumventing this problem is to replace the hard π pulse with a frequency-selective π pulse (see Fig. 3B). Since we do not wish to observe the water resonance, the phase purity of the selective pulse is not important. Also, protein resonances with long relaxation times are typically well separated from the water resonance, and the frequency-selectivity requirements for the selective pulse are not severe. Some protein resonances close to the water frequency will be affected by the selective pulse but since these typically have shorter relaxation times they will recover during D_{NP}. Hence, the soft pulse is only required to rotate the water magnetization below the x–y plane; the precise angle is not important. Thus, in contradistinction to virtually all other water-suppression methods involving soft

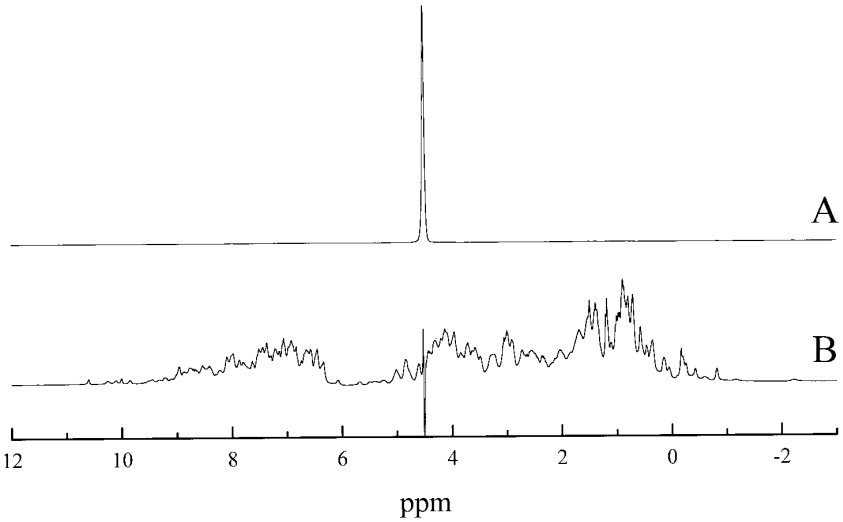


FIG. 2. ^{1}H NMR spectra of a lysozyme solution (10 mM in 10:90 ^2H_2O:H_2O, pH 3.7) at 310 K (A) without water suppression and (B) with the water resonance suppressed using the Water-PRESS sequence. Both spectra were acquired at 300 MHz, and in both cases, a 90° observe pulse was used. To avoid saturation of the analog-to-digital converter, the receiver gain used in acquiring spectrum (A) was 28 dB less than that used in acquiring spectrum (B). The parameters used in the Water-PRESS sequence were D_{H_2O} = 10 s and D_{NP} = 2.24 s which included a series of half-sine-shaped homospoil pulses of 0.5 ms duration with a maximum amplitude of 1 G cm^{-1}. The sharp spike at ~4.5 ppm results from a combination of the effects of radiation damping on the water resonance during the acquisition time and the transmitter offset.

pulses, the performance requirements of the soft pulse are minimal. In the present case, a simple Gaussian soft pulse was used, but almost any shape will suffice.

Water steady state. The sequence can be greatly shortened if the requirement for full water relaxation prior to the inversion pulse (*32*) is removed. Instead, the only requirement is for the water magnetization to be nulled at the time of the observe pulse (see Fig. 4A). During D_{H_2O}, which includes the acquisition time, the water magnetization starts from 0 (N.B., for water suppression the water signal must be 0 at the beginning of signal acquisition) and relaxes toward a maximum of M_0 with time constant $T_1^{H_2O}$, thus,

$$M_Z^{D_{H_2O}} = M_0\left[1 - \exp\left(-\frac{D_{H_2O}}{T_1^{H_2O}}\right)\right]. \qquad [4]$$

The ''inversion'' pulse rotates $M_Z^{D_{H_2O}}$ about the origin so that the magnetization after the pulse is given by $M_Z^{D_{H_2O}}\cos(\theta)$. During D_{NP}, the water magnetization relaxes toward M_0; thus,

$$M_Z^{D_{NP}} = M_0 - [M_0 + M_Z^{D_{H_2O}}\cos(\theta)]\exp\left(-\frac{D_{NP}}{T_1^{H_2O}}\right). \qquad [5]$$

To achieve a null point at the time we apply the observe pulse, we require $M_Z^{D_{NP}} = 0$. We solve Eq. [5] for D_{H_2O}, since D_{NP} will normally be determined by the solute relaxation time, to obtain

$$D_{H_2O} = \ln\left\{\frac{\cos(\theta)\exp(-D_{NP}/T_1^{H_2O})}{1 + [\cos(\theta) - 1]\exp(-D_{NP}/T_1^{H_2O})}\right\}T_1^{H_2O}. \qquad [6]$$

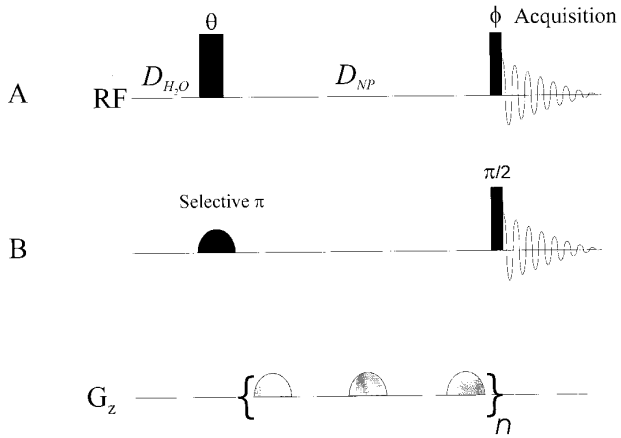


FIG. 3. Simple modifications to the Water-PRESS sequence. In the pulse-sequence diagrams, it is understood that the series of gradient pulses are applied during the delay D_{NP} (as in Fig. 1). In (A), the π pulse in the Water-PRESS subunit has been replaced by $\pi/2 < \theta \leqslant \pi$ and the $\pi/2$ pulse replaced by a variable angle pulse, ϕ. In (B), the π pulse is replaced by a selective π pulse (e.g., a Gaussian). The performance required (i.e., selectivity and phase) for the soft pulse is not high as we do not wish to observe the water signal, and the solute resonances that are affected by the soft pulse will have largely relaxed to their equilibrium value by the end of D_{NP}.

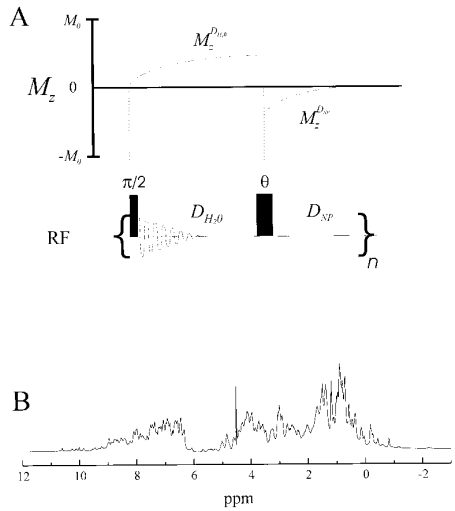


FIG. 4. Steady-state Water-PRESS. The change in the longitudinal magnetization of the water protons when $D_{H_2O} \ll 5 \times T_1^{H_2O}$ is schematically shown in (A). We have chosen to present the sequence in this order to make it more obvious that the acquisition time is part of D_{H_2O}. As before (e.g., Fig. 1A), there is a series of *z*-gradient pulses applied during D_{NP} (not shown). The sequence is shown contained in brackets with a subscript *n* (integer) to emphasize the repetition of the sequence which in combination with $D_{H_2O} \ll 5 \times T_1^{H_2O}$ maintains the water in a steady state. An example of water suppression using the Water-PRESS sequence using an aqueous sample of lysozyme while keeping the water in the steady state is shown in (B). The relevant experimental parameters were $D_{H_2O} = 2.3$ s (N.B., this includes the acquisition time of 0.8 s) and $D_{NP} = 1.36$ s. The other experimental details are given in the legend to Fig. 2. Because the Water-PRESS sequence was run in the steady state, the effects of radiation damping are largely eliminated throughout the entire sequence including the acquisition time, and the only remaining evidence of the water (including a contribution from the transmitter spike) is the small narrow spike at ~4.5 ppm.

For Eq. [6] to be valid, we require that

$$[\cos(\theta) - 1]\exp\left(-\frac{D_{NP}}{T_1^{H_2O}}\right) < -1. \qquad [7]$$

If $\theta = \pi$, then the limit given in Eq. [7] becomes, as expected,

$$D_{NP} < \ln(2) \times T_1^{H_2O}, \qquad [8]$$

and Eq. [6] simplifies to the solution given by Benz *et al.* (*22*).

The above equations were derived assuming that the effective $T_1^{H_2O}$ is the same throughout the pulse sequence, which will not occur if the radiation-damping mechanism is present. Thus, D_{H_2O} as given by Eq. [6] represents a maximum value.

A spectrum of lysozyme acquired with the water in the steady state is given in Fig. 4B. Excellent water suppression could be obtained when using a recycle delay (i.e., D_{H_2O} + acquisition time) of only 2.3 s and $D_{NP} = 1.37$ s. Of particular interest is the degree to which radiation-damping effects are eliminated not only during D_{NP} but also during the acquisition period. When the Water-PRESS sequence is performed with $D_{H_2O} \geqslant 5 \times T_1^{H_2O}$, the water magnetization relaxes along the *z* axis toward $+z$ during D_{NP}. Thus, as soon as gradient pulses are no longer applied, there is still a large coherent-water-magnetization vector with a possibility of being stimulated into producing radiation damping (see Discussion). However, when D_{H_2O} is shorter such that the water magnetization is in a steady state, the water magnetization still relaxes toward $+z$ during D_{NP}, but instead of its ''relaxation path'' comprising only the *z* axis (i.e., one line), much of the magnetization can be imagined to sweep out the volume of a sphere. As a result, there is much less coherent magnetization available for evoking/producing radiation damping. This is clearly seen in Fig. 4B where there is only a very small spike-like resonance resulting from the induction of radiation damping during the acquisition period and the transmitter offset. This spectrum should be compared to those in the previous paper (*26*) obtained with the same sample using WATERGATE, presaturation, and Water-PRESS run with a recycle delay allowing full relaxation of the water resonance. When the water resonance is kept in the steady state, better suppression is achieved with nearly an order of magnitude time saving (i.e., $D_{H_2O} = 2.3$ s and $D_{NP} = 1.4$ s, compared with $D_{H_2O} = 20.8$ s and $D_{NP} = 2.6$ s where full relaxation was allowed). In both cases, D_{H_2O} includes the acquisition time of 0.8 s.

Temperature control. Accurate temperature control is critical for ensuring that all of the water protons have the same longitudinal relaxation rate. In fact, the use of a small sample volume helps to meet this requirement.

EXPERIMENTAL

Chicken-egg lysozyme was obtained from Seikagaku Corporation (Tokyo). Human myeloma immunoglobulin IgG1 (κ) Ike-N (IgG) was prepared as described previously (*17*). Millipore water was used in all experiments. The lysozyme NMR sample was prepared by dissolving the protein in H_2O:D_2O 90:10 and adjusting the pH with HCl. The immunoglobulin NMR sample was prepared by dissolving 10 mg of protein in 0.4 ml of H_2O:D_2O 90:10. The pH of the lysozyme and IgG samples were 3.7 and 6.9 (uncorrected for the deuterium isotope effect), respectively. NMR samples were dispensed into 5 mm NMR microtubes (Shigemi, Tokyo).

All NMR experiments were performed at 300 MHz on a Bruker DRX 300 NMR spectrometer using a 5 mm inverse gradient probe. Typical acquisition parameters were a 90° pulse width of 9 μs and a spectral width of 6 kHz digitized

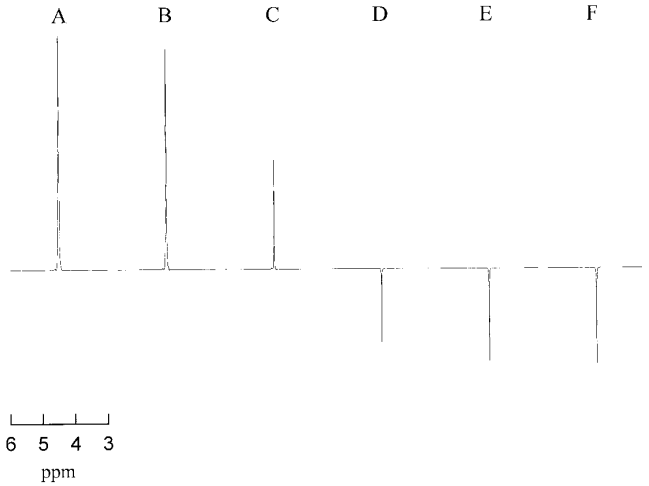


FIG. 5. An example of the use of the Water-PRESS pulse sequence to control the effective relaxation rate of the water resonance in a lysozyme solution (10 m*M* in 10:90 ^2H_2O:H_2O, pH 3.7) acquired at 310 K at 300 MHz. In this experiment, D_{NP} was set to 2 s. The number of homospoil pulses (0.5 ms, half sine with a maximum strength of 1 G cm^{-1}) used in acquiring the spectra were (A) 1, (B) 21, (C) 31, (D) 41, (E) 51, and (F) 61. As can be clearly seen, after the interval between homospoil pulses is decreased to about 40 ms (i.e., spectrum E) there is no further increase in the effective water relaxation time. Since the effect of radiation damping is diminished with increasing number of gradient pulses, the linewidth of the water signal decreases.

into 16 K data points. Prior to Fourier transformation, 0.3 Hz of linebroadening was added to improve the apparent signal-to-noise ratio. Since in the absence of radiation damping at 310 K, $T_{1=nat}^{H_2O}$ is nearly 4 s, a recycle delay of 20 s was used unless otherwise noted to allow fair comparisons. However, as noted in Fig. 4B, a much shorter (nearly an order of magnitude) relaxation delay is possible. Further experimental conditions are given in the figure legends.

APPLICATIONS

Controlling the Effective Water Relaxation Rate and the Magnitude of the Water Resonance

In some applications, it can be desirable to control the magnitude of the water resonance. For example, by starting a sequence with a different value of the water magnetization, it might be possible to discriminate between NOE or exchange effects. Typically, the water resonance, due to the effects of radiation damping, relaxes in a nonexponential manner [actually a hyperbolic tangent profile (*33*)] on a time scale of several hundred milliseconds or less (see Fig. 1). However, if the Water-PRESS pulse sequence is used, $T_1^{H_2O}$ can be varied in the range $T_{1=rd}^{H_2O} \leqslant T_1^{H_2O} \leqslant T_{1=nat}^{H_2O}$ where $T_{1=rd}^{H_2O}$ is the relaxation time in the presence of radiation damping, by changing the ratio (number of gradient pulses):(duration of D_{NP}) as shown in Fig. 5.

A series of weak gradient pulses is experimentally more convenient than one long weak gradient pulse for selectively altering the effective water relaxation time as additional RF pulses can be inserted into the gradient off periods and also the effective water relaxation rate can be altered while keeping D_{NP} constant.

Water Suppression

Experimental setup. The experimental parameters needed to effect water suppression with the Water-PRESS subunit are quickly and easily obtained using the following procedure. First, a trial value of D_{NP} is set (e.g., 2.4 s) and the number of gradient pulses for the delay D_{NP} are selected, for example, 120 half-sine-shaped pulses of 0.5 ms duration with a maximum strength of ~1 G cm^{-1}. The exact number of gradient pulses is not important as after a certain ratio of (number of gradient pulses):(duration of D_{NP}) there is no further change in the effective water relaxation rate (see Fig. 5). A spectrum is then recorded and phased, then by repeatedly changing D_{NP} and recording a spectrum, a value of D_{NP} providing better water suppression can be found [it is easier to vary D_{NP} than the number of gradient pulses (n) to achieve high degrees of suppression]. If the Water-PRESS subunit is to be used in the steady state, a number of dummy scans (e.g., 8) must be used.

Water suppression in solutions containing high-molecular-weight solutes. Spectra of IgG (MW ~ 150,000) ac-

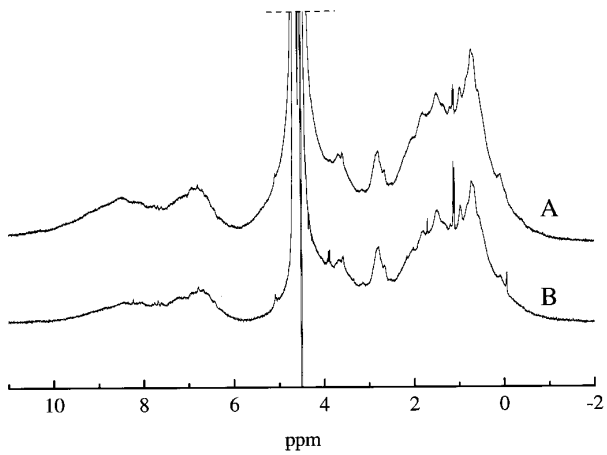


FIG. 6. One-dimensional ^1H spectra of IgG solution (10 mg of protein in 0.4 ml of H_2O:D_2O 90:10, pH 6.9) acquired using (A) the Water-PRESS method and (B) presaturation. Both spectra were acquired using the same receiver gain and averaging 160 transients. Spectrum (B) was acquired using 1.5 s of presaturation (included as part of the recycle delay) at a field strength of ~35 Hz at the water frequency. Many of the resonances in the spectrum acquired with presaturation have lost more intensity due to the effects of spin diffusion than in the spectrum acquired using the Water-PRESS sequences. This is particularly clear from observing the differences in intensity in the amide region. The spectra are presented with the noise set to the same level.

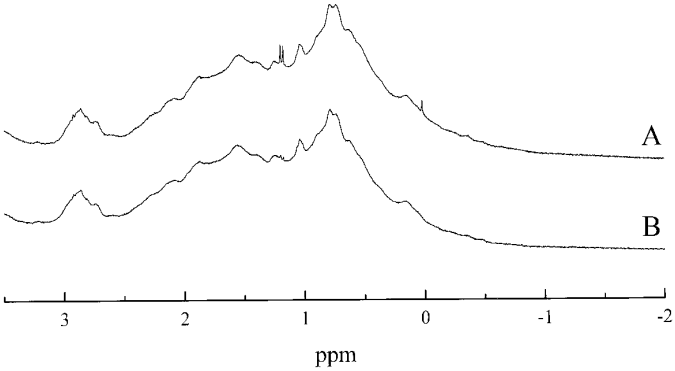


FIG. 7. The aliphatic regions of one-dimensional ^{1}H spectra of IgG acquired using the Water-PRESS method in the steady state with (A) a selective π (60 ms Gaussian) pulse and (B) a hard π pulse. The experimental parameters were $D_{H_2O} = 1.5$ s in both cases and $D_{NP} = 1.455$ s for (A) and 1.46 s for (B). The different D_{NP} values result from the different lengths of the π pulses. The other experimental details are given in the legend to Fig. 6. The spectra are presented with the same absolute intensity. From comparison of the two spectra, it can be clearly seen that the selective π pulse allows larger contributions from the more slower relaxing resonances (i.e., the impurities at ~0.1 and ~1.3 ppm).

quired using presaturation and the Water-PRESS sequence are shown in Fig. 6. It was observed that relatively small increases in the irradiation strength when observing the spectrum using presaturation resulted in very large losses of spectral intensity (data not shown). In Fig. 7, the use of a soft π pulse in the Water-PRESS subunit is demonstrated. Since the selective pulse only inverts those resonances near or under the water resonance, the resonances of nuclei with longer relaxation times, which are generally distal to the water, have more time to relax. The use of a selective pulse also provides a means of acquiring spectra of low-molecular-weight species with long relaxation times, so long as the peaks of interest are not too close to the water resonance.

Combining with more-complicated pulse sequences. The requirements for water suppression in multidimensional experiments are generally less stringent than those for one-dimensional experiments (*4*), since the nature of many experiments is to filter and cancel out the effects of strong signals (e.g., experiments containing double-quantum filters). There are so many variations of multidimensional sequences that it is not possible to present individual solutions for finding optimal conditions for water suppression using the Water-PRESS subunit. However, here we present some guiding principles for combining Water-PRESS into multidimensional sequences. The first approach is to incorporate the ''nonwater suppression'' part of the sequence on a temporal basis into D_{NP} as noted by Patt and Sykes (*23*) for the WEFT pulse sequence. However, a prerequisite for this approach is that the nonwater suppression part of the sequence does not affect the water magnetization in a complicated manner so that the delays cannot be set so as to achieve a null point of the water resonance at the beginning of acquisition. The second approach, which is more applicable to more-complicated pulse sequences, is to prefix the desired sequence with the Water-PRESS sequence and set D_{NP} so that the water is nulled at the beginning of the ''real'' part of the sequence. We can then take advantage of the water magnetization being ''orthogonal'' (i.e., the water magnetization is nulled) while the protein magnetization is close to its thermal equilibrium value at the start of the sequence. Hence, it may be possible to maintain the water magnetization ''90° out of phase'' with the solute magnetization. For this purpose, it should be noted that most protein resonances of interest are *J*-coupled while the water protons are not.

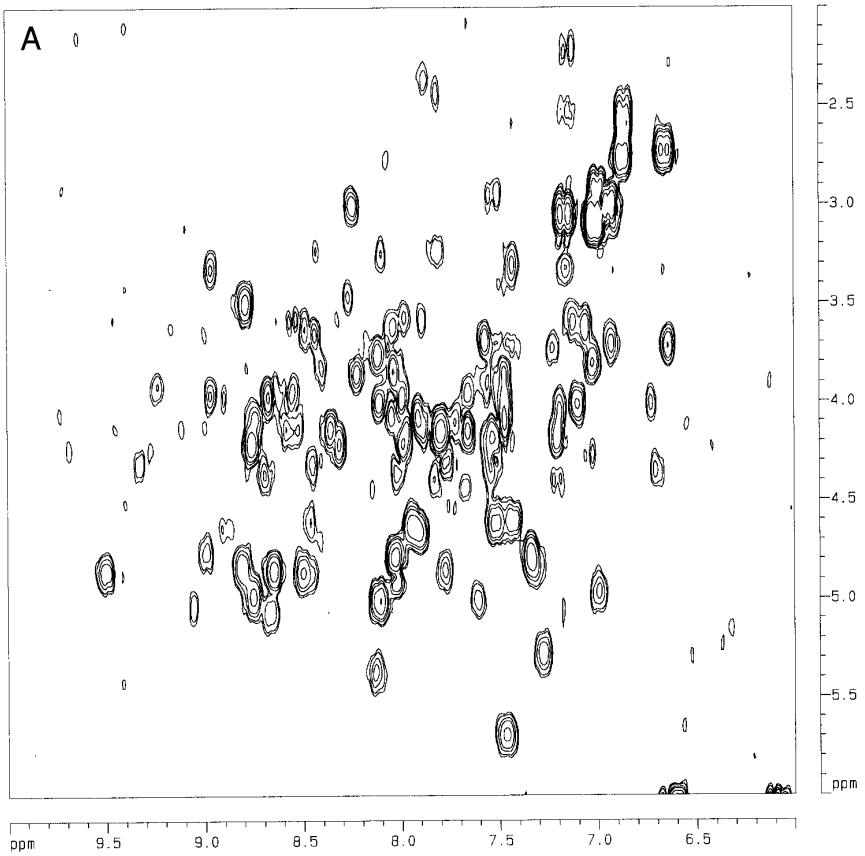
Experimentally, the optimum conditions for water suppression when the Water-PRESS sequence is combined with a more complicated sequence are best found using a 1D analogue of the multidimensional sequence and adjusting D_{NP} as described above. In some circumstances and if the multidimensional sequence involves large increments in a variable delay, it may be possible to adjust D_{NP} in accordance with the changes of the variable delay to maintain optimal water-suppression conditions.

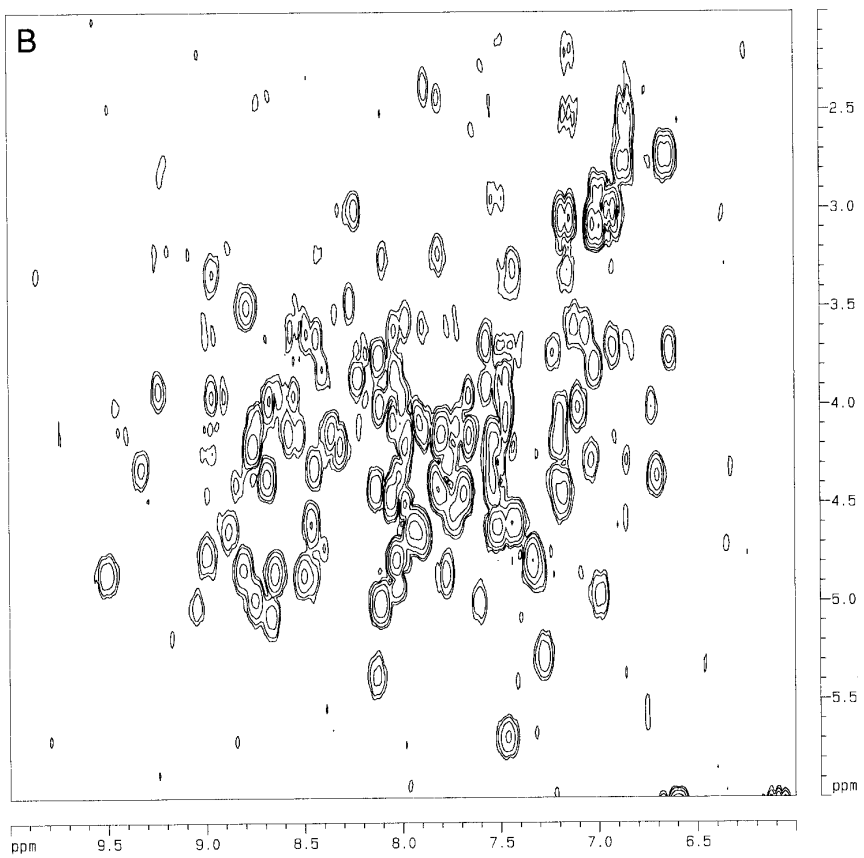
As a simple example of water suppression in a multidimensional sequence, the amide regions of COSY spectra of lysozyme acquired using presaturation and the Water-PRESS method are given in Fig. 8. Since the Water-PRESS method, unlike presaturation, does not bleach out the α protons under the water resonance, there are numerous amide cross peaks close to the water frequency that are only visible in the COSY acquired using Water-PRESS.

DISCUSSION

The Water-PRESS subunit is an extremely simple and technically undemanding means of suppressing water. Compared to the WEFT method (*22–24*), the Water-PRESS subunit is not susceptible to the effects of radiation damping

FIG. 8. Amide regions of COSY spectra of the lysozyme solution (10 m*M* in 10:90 ^2H_2O:H_2O, pH 3.7) acquired using (A) presaturation of the water resonance prior to the COSY sequence and (B) the Water-PRESS sequence prefixed to the COSY sequence. The parameters used in acquiring the presaturation COSY were the following: a relaxation delay of 20 s including 3 s of presaturation at a field strength of ~35 Hz at the water frequency. In this experiment, D_{NP} was set to 2.59 s which included a series of half-sine-shaped homospoil pulses of 0.5 ms duration with a maximum amplitude of 1 G cm^{-1}. Much shorter values of D_{H_2O} and D_{NP} could have been used with no loss of water suppression (see Fig. 4B). The water resonance was at 4.51 ppm. The effects of presaturation on the resonances near and under the water resonance are clearly seen by the absence/diminution of the cross peaks having α-proton resonances around 4.5 ppm.


A
2.5
3.0
3.5
4.0
4.5
5.0
5.5
ppm
ppm
9.5
9.0
8.5
8.0
7.5
7.0
6.5


B
2.5
3.0
3.5
4.0
4.5
5.0
5.5
ppm
ppm
9.5
9.0
8.5
8.0
7.5
7.0
6.5

and is consequently suited for use at higher magnetic fields and with more sensitive probes. Radiation damping can be initiated in magnetization stored along the $-z$ axis by residual RF leakage and even thermal noise from the RF coil (*33*). The train of gradient pulses in the Water-PRESS sequence stops this induction by eliminating any net transverse magnetization. The problem of radiation damping will be greatly magnified by the usage of superconducting RF coils with their extremely large Q values. Many methods for the suppression of radiation damping exist, including those requiring sophisticated electronics (*34–38*) or (not commonly available) B_1 gradients (*39*); however, the use of a train of homospoil pulses to manipulate the radiation-damping effects (see Fig. 5) is possible with little or no modification on many current spectrometers. Zhang and Gorenstein have proposed the idea of interleaving pairs of opposed z-gradient pulses between acquisition of FID data points (*40*). However, this method only addresses the problem of radiation damping during acquisition. The Water-PRESS method, especially when the water magnetization is kept in the steady state, largely removes the effects of radiation damping throughout the entire sequence including acquisition.

The suppression of radiation damping in multidimensional NOE and ROE experiments for studying protein hydration is particularly desirable since radiation damping reduces the amount of water magnetization available for transfer to the protein resonances during the mixing time. Further, since the lineshape in the indirectly detected domains is that of the bulk water, the cross peaks will be significantly broadened. Sklenář has proposed the use of alternating gradient pulses during the evolution periods of multidimensional experiments and NOE mixing periods as a means for suppressing the effects of radiation damping (*41*). While using the Water-PRESS method, it is possible to control the radiation damping at the start of the sequence as well as set the water magnetization to a certain level (see Fig. 5).

Both the water relaxation, by definition, and the effects of the radiation damping are totally frequency selective to the water resonance, and thus the Water-PRESS method is totally frequency selective to the water resonance. This is particularly important in its implications for spin diffusion. First, it means that the transfer of magnetization resulting directly from the water-suppression method (i.e., that resulting from the nonequilibrium water magnetization) only originates at the water frequency; second, the upper limit for the amount of magnetization transferred is determined by the amount of water magnetization. This is in contradistinction to presaturation in which the saturating field is neither exactly frequency selective nor limited in magnitude as it depends on the experimental parameters used in acquiring the spectrum. By inhibiting the effects of radiation damping, any residual water resonance in the observed spectrum is much closer to the inherent linewidth, thereby allowing observation of resonances near or under the water resonance, which also reduces the frequency range for magnetization transfer from the nonequilibrium water magnetization. These aspects were clearly shown in Fig. 6.

The Water-PRESS method obviates many of the problems associated with previous water-suppression schemes; for example, lineshape is not a prerequisite for good water suppression. This method is suitable for samples with large molecules, such as IgG, which have short T_2 values; it allows observation of signals near or under the water resonance, and the method does not cause any diffusional loss of intensity. In this work, we have addressed the temporal efficiency problem that would otherwise preclude the use of Water-PRESS from (already) time-intensive multidimensional NMR experiments.

CONCLUDING REMARKS

In the present work, we have shown that the Water-PRESS subunit is an experimentally and conceptually easy method for providing extremely high degrees of water suppression. It does not introduce any complicated phase relationships into the resulting spectrum and it allows observation of solute resonance at or near the water resonance frequency. An additional advantage of this method is that the suppression of radiation damping can extend into the acquisition period when the water magnetization is kept in the steady state. Similarly, by keeping the water in the steady state, it is possible to suppress water in multidimensional spectra without any additional time requirement than that ordinarily required for solute relaxation since the Water-PRESS subunit is substituted for the usual relaxation delay. By replacing the hard inversion pulse with a selective inversion pulse, Water-PRESS can be adapted to suppression of water in samples where the solute(s) contain a large range of relaxation times.

ACKNOWLEDGMENT

Dr. Denis Sandoz (Nalorac) is thanked for useful discussions.

REFERENCES

1. G. Otting and E. Liepinsh, *Acc. Chem. Res.* **28,** 171 (1995).
2. W. S. Warren and W. Richter, *in* ''Encyclopedia of Nuclear Magnetic Resonance'' (D. M. Grant and R. K. Harris, Eds.), p. 1417, Wiley, New York, 1996.
3. P. J. Hore, *Methods Enzymol.* **176,** 64 (1989).
4. M. Guéron, P. Plateau, and M. Decorps, *Prog. NMR Spectrosc.* **23,** 135 (1991).
5. P. C. M. Van Zijl and C. T. W. Moonen, *NMR Basic Princ. Prog.* **26,** 67 (1992).
6. M. Guéron and P. Plateau, *in* ''Encyclopedia of Nuclear Magnetic Resonance'' (D. M. Grant and R. K. Harris, Eds.), p. 4931, Wiley, New York, 1996.
7. W. S. Price, *in* ''Annual Reports on NMR Spectroscopy'' (G. A. Webb, Ed.), p. 51, Academic Press, London, 1996.

8. M. Piotto, V. Saudek, and V. Sklenář, *J. Biomol. NMR* **2,** 661 (1992).

9. V. Sklenář, M. Piotto, R. Leppik, and V. Saudek, *J. Magn. Reson. A* **102,** 241 (1993).

10. S. Grzesiek and A. Bax, *J. Am. Chem. Soc.* **115,** 12593 (1993).

11. R. J. Ogg, P. B. Kingsley, and J. S. Taylor, *J. Magn. Reson. B* **104,** 1 (1994).

12. S. H. Smallcombe, S. L. Patt, and P. A. Keifer, *J. Magn. Reson. A* **117,** 295 (1995).

13. A. S. Altieri and R. A. Byrd, *J. Magn. Reson. B* **107,** 260 (1995).

14. T.-L. Hwang and A. J. Shaka, *J. Magn. Reson. A* **112,** 275 (1995).

15. X.-A. Mao and J.-H. Chen, *Chem. Phys.* **202,** 57 (1996).

16. P. C. M. Van Zijl and C. T. W. Moonen, *J. Magn. Reson.* **87,** 18 (1990).

17. S. Endo and Y. Arata, *Biochemistry* **24,** 1561 (1985).

18. A. Kalk and H. J. C. Berendsen, *J. Magn. Reson.* **24,** 343 (1976).

19. K. Akasaka, M. Konrad, and R. S. Gordy, *FEBS Lett.* **96,** 287 (1978).

20. P. Tsang and M. Rance, *J. Magn. Reson. B* **111,** 135 (1996).

21. J. P. K. Tong and G. Kotovych, *J. Magn. Reson.* **69,** 511 (1986).

22. F. W. Benz, J. Feeney, and G. C. K. Roberts, *J. Magn. Reson.* **8,** 114 (1972).

23. S. L. Patt and B. D. Sykes, *J. Chem. Phys.* **56,** 3182 (1972).

24. R. K. Gupta, *J. Magn. Reson.* **24,** 461 (1976).

25. R. Freeman, ''A Handbook of Nuclear Magnetic Resonance,'' Longman, Essex, 1988.

26. W. S. Price and Y. Arata, *J. Magn. Reson. B* **112,** 190 (1996).

27. A. Abragam, ''The Principles of Nuclear Magnetism,'' Clarendon Press, Oxford, 1961.

28. W. S. Warren, S. L. Hammes, and L. J. Bates, *J. Chem. Phys.* **91,** 5895 (1989).

29. M. H. Levitt and R. Freeman, *J. Magn. Reson.* **33,** 473 (1979).

30. D. Neuhaus, M. I. Ismail, and C.-W. Chung, *J. Magn. Reson. A* **118,** 256 (1996).

31. R. R. Ernst, *Adv. Magn. Reson.* **2,** 1 (1966).

32. L.-F. Kao and V. J. Hruby, *J. Magn. Reson.* **70,** 394 (1986).

33. A. Sodickson, W. E. Maas, and D. G. Cory, *J. Magn. Reson. B* **110,** 298 (1996).

34. D. Abergel, C. Carlotti, A. Louis-Joseph, and J. Lallemand, *J. Magn. Reson. B* **109,** 218 (1995).

35. C. Anklin, M. Rindlisbacher, G. Otting, and F. H. Laukien, *J. Magn. Reson. B* **106,** 199 (1995).

36. P. Broekaert and J. Jeener, *J. Magn. Reson. A* **113,** 60 (1995).

37. A. Louis-Joseph, D. Abergel, and J.-Y. Lallemand, *J. Biomol. NMR* **5,** 212 (1995).

38. L. Picard, M. von Kienlin, and M. Décorps, *J. Magn. Reson. A* **117,** 262 (1995).

39. W. E. Maas, F. H. Laukien, and D. G. Cory, *J. Magn. Reson. A* **113,** 274 (1995).

40. S. Zhang and D. G. Gorenstein, *J. Magn. Reson. A* **118,** 291 (1996).

41. V. Sklenář, *J. Magn. Reson. A* **114,** 132 (1995).