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Gradient-tailored excitation for single-quantum NMR spectroscopy of aqueous solutions

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SUMMARY

A novel approach to tailored selective excitation for the measurement of NMR spectra in non-deuterated aqueous solutions (WATERGATE, WATER suppression by GrAdient-Tailored Excitation) is described. The gradient echo sequence, which effectively combines one selective 180° radiofrequency pulse and two field gradient pulses, achieves highly selective and effective water suppression. This technique is ideally suited for the rapid collection of multi-dimensional data since a single-scan acquisition produces a pure phase NMR spectrum with a perfectly flat baseline, at the highest possible sensitivity. Application to the fast measurement of 2D NOE data of a 2.2. mM solution of a double-stranded DNA fragment in 90% H_2O at 5 °C is presented.

^1H single-quantum coherences supply the information necessary for the determination of the spatial structure of biological macromolecules by multidimensional (mD) NMR spectroscopy (Wüthrich, 1986). The need to work in aqueous non-deuterated solutions has led to the development of a variety of methods for suppressing the large water resonance (Hore, 1989; Sklenář, 1990; Guéron et al., 1991). The most common approach is water presaturation by applying a weak radiofrequency (RF) perturbation during the relaxation delay (Jesson et al., 1973; Hoult, 1976). However, the intensities of many important resonances are perturbed by cross-relaxation or chemical exchange, and observation of hydrating water is a priori precluded. For these cases, techniques which selectively prevent the excitation of water (Hore, 1989; Sklenář, 1990; Guéron et al., 1991) or achieve its saturation within milliseconds before detection (Sklenář and Bax, 1987; Otting and Wüthrich, 1989; Otting et al., 1991) must be applied. Unfortunately, the majority of corresponding pulse schemes suffer from non-uniform excitation, baseline distortions, and a sub-

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stantially lower degree of water suppression. Recently emerged technology for the application of short-pulsed magnetic field gradients has paved the way for the development of water suppression techniques which are applicable in cases where a coherence transfer pathway involves periods with evolution of double-quantum or higher-order coherences (Hurd, 1990; Hurd and John, 1991; Vuister et al., 1991; Davis et al., 1991, 1992; John et al., 1992) or where the difference in diffusion properties of water and the molecules of interest can be exploited (Zilj and Moonen, 1990). In this communication, we show that a combination of tailored excitation with pulsed magnetic field gradients provides highly selective and extremely effective water suppression, even in cases where no multiple-quantum transitions are generated. The excitation scheme, which we propose to refer to as WATERGATE (WATER suppression by GrAdient-Tailored Excitation), can be easily incorporated into most mD NMR experiments designed to detect single-quantum proton coherences in all dimensions. Since high-quality spectra are obtained from a single scan, this technique is ideally suited for rapid measurement of mD NMR spectra.

Essentially, the pulse scheme we propose is a gradient echo sequence composed of two parts (Fig. 1). In the first part, a non-selective 90° RF pulse excites all resonances uniformly, regardless of their chemical shift. The subsequent symmetrical echo segment is formed by two short field-gradient pulses of the same amplitude and sign with a centrally placed 180° selective RF pulse. All coherences dephased by the first field gradient are rephased by the second one, provided they experienced 180° rotation by the selective RF pulse. If the selective sandwich is designed such that the net rotation at the water resonance frequency approaches zero while the rest of the spectrum is flipped by 180°, no water signal is left at the moment when acquisition starts. There are several ways to produce such an excitation (Sklenář, unpublished results). Here we present a simple approach in which the non-selective 180°(x) pulse is flanked by two rectangular selective 90°(−x) pulses which rotate the water magnetization in the opposite direction and prevent its rephasing during the subsequent gradient. In order to minimize the effects of J-modulation and spin–spin relaxation, the echo length is kept as short as possible. The width of the non-excited region is determined by the length of selective 90° pulses, τ, while the excitation bandwidth is limited primarily by the RF field intensity of the non-selective 90° and 180° pulses. To a first approximation, the spectral range with reduced intensities is restricted to frequencies $\pm 1/\tau$ from the carrier, centered on the position of the water resonance.

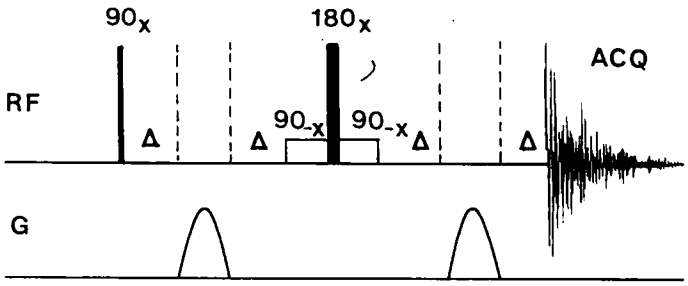

Fig. 1. Pulse scheme for gradient-tailored water suppression. The radiofrequency and field-gradient pulses are shown on separate lines. In addition to a standard non-selective spin-echo pulse pair, two selective 90° pulses with the opposite direction of rotation and two shaped magnetic field gradients are placed symmetrically to the non-selective 180° pulse. Four delays (Δ) are inserted to allow for gradient recovery. For experimental details see Fig. 2.

As an example, the single-scan 2D NOE spectrum of a 2.2 mM solution of a double-stranded DNA fragment composed of two complementary strands $d(G_1T_2G_3A_4C_5T_6C_7A_8G_9)$ and $d(C_{10}T_{11}G_{12}A_{13}G_{14}T_{15}C_{16}A_{17}C_{18})$ measured in 90% H_2O at 5 °C, pH 6.1, is shown in Fig. 2. The spectrum was obtained using a standard approach (Macura and Ernst, 1980; Kumar et al. 1980)

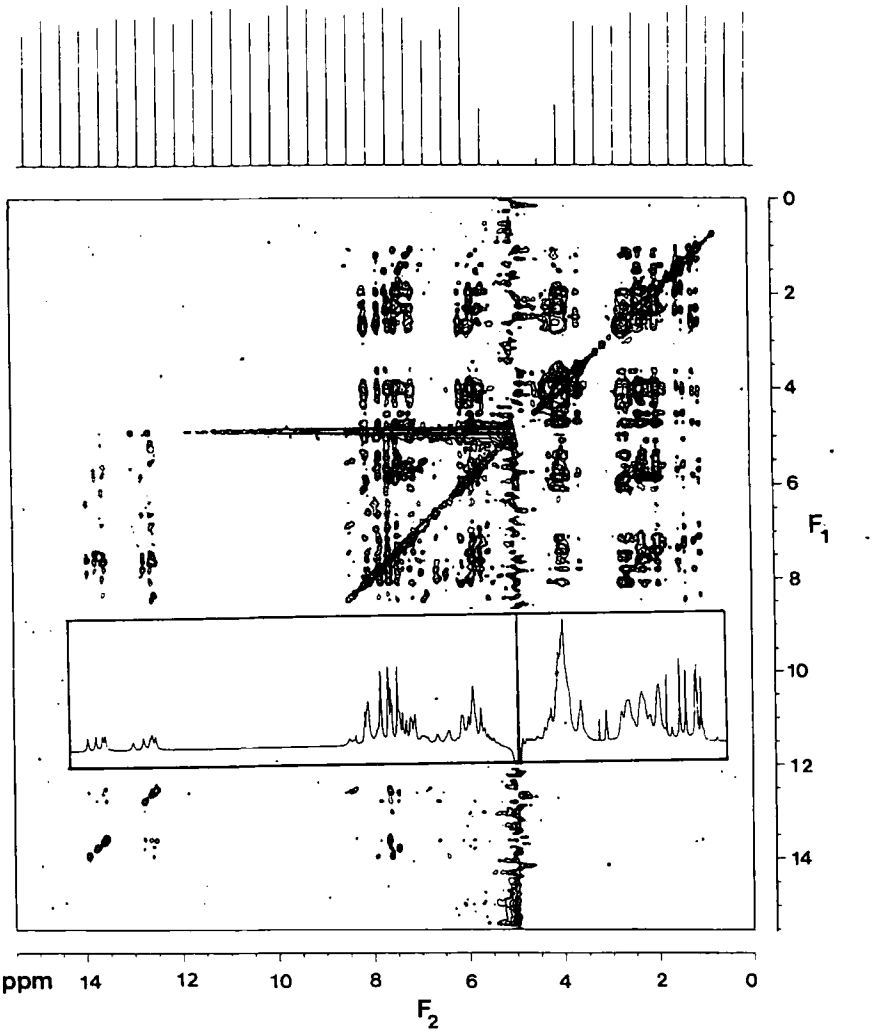

Fig. 2. Phase-sensitive 2D NOE spectrum of double-stranded DNA formed by d(GTGACTCAG) and d(CTGAGTCAC) oligonucleotides. Sample conditions: 100 mM NaCl, 12 mM $MgCl_2$, pH 6.1, and a 2.2 mM solution of double-stranded DNA in 500 µl, T = 5 °C. The spectrum was obtained using a Bruker AMX 500 spectrometer operating at 500 MHz. Acquisition parameters: 2k complex points in t_2 and 300 t_1 increments, spectral width 11364 Hz in both dimensions, 1 scan per t_1 value, a recycle delay of 1.5 s, and 180 ms mixing time; 17.5 kHz non-selective and 165 Hz selective RF fields were applied at the water resonance frequency. The 20 G/cm field-gradient pulses, 1 ms each, were shaped to a 1% truncated *sine* envelope. A 'triple resonance' probe with a built in self-shielded z-gradient coil was used. Gradient-recovery delays were set to 125 µs. No baseline flattening routine other than the dc-offset correction in F_2 dimension was applied. The experimental phase-sensitive excitation spectrum shown along the F_2 axis was obtained by increasing the offset in steps of 200 Hz. The inset shows the 1D spectrum (64 scans) measured with identical excitation parameters.

with the new sequence incorporated as a read pulse. Phase cycling for the States-TPPI F_1-quadrature detection (Marion et al., 1989b) and post-acquisition convolution difference treatment of data in the F_1-domain (Marion et al., 1989a) were applied to remove the axial peaks. A gradient pulse was inserted at the end of the mixing interval, to eliminate the undesirable effects of radiation damping. These effects would otherwise be observed in one out of every four t_1-increments when the water was inverted during the mixing period. We found that even for short mixing times (about 50 ms) this was sufficient to avoid interference of the residual transversal water magnetization at the start of a read pulse. The experimental excitation profile is shown along the F_2 frequency axis in Fig. 2. As demonstrated, two 1.5-ms selective 90° pulses and two 1-ms gradients with an intensity of 20 G/cm were enough to achieve highly selective suppression of the water resonance. The width of the non-excited region enabled resonances as close as 1.2 ppm to the water resonance to be observed at full intensity. The amplitude variation introduced by two rectangular selective 90° pulses was less than 10% and could be removed by shaping the envelope of these component pulses (Sklenář, unpublished results). The overall length of the gradient echo,

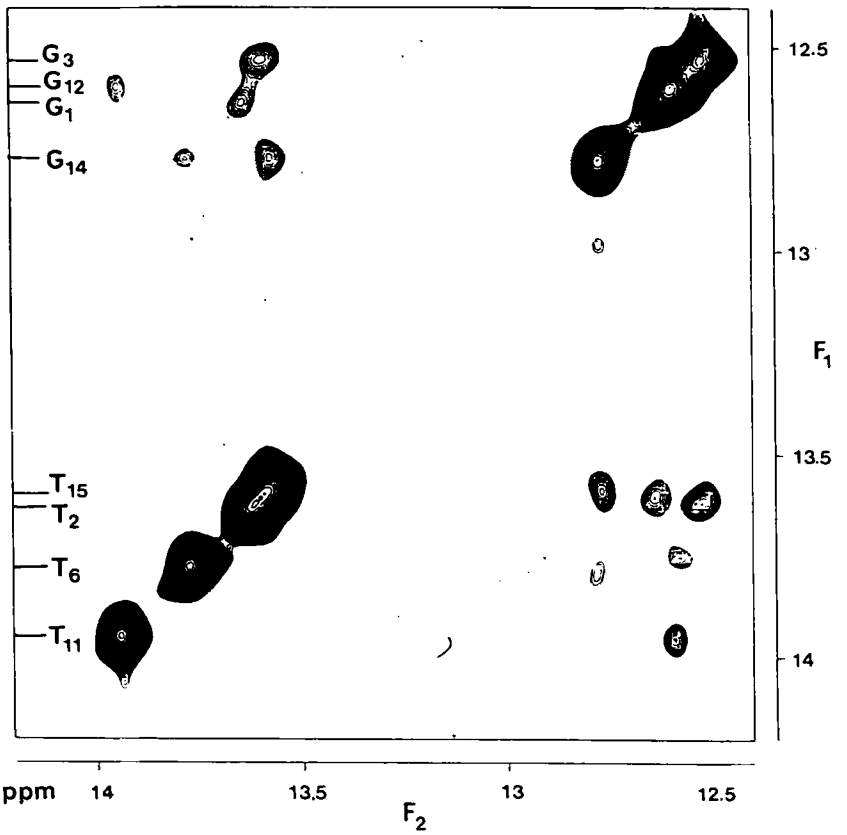

Fig. 3. Portion of the 2D NOE spectrum (Fig. 2) showing the imino–imino proton connectivities. The cross peak T_6/G_{12} was observed only on one side of the diagonal because of its low intensity. This connectivity was clearly visible in a larger number of scans. The terminal imino resonance G_9 at 12.93 ppm was not seen along the diagonal since it exchanged rapidly with water during the relatively long mixing time. It was detected at mixing times < 100 ms and with a higher number of scans.

including gradient recovery delays of $\Delta = 125$ μs, was 5.61 ms. Sequential connectivities and assignments of all imino protons could be obtained from this spectrum (Fig. 3).

The approach described above makes it possible to record NMR spectra in water with the highest possible sensitivity since the receiver gain is limited only by signals from solute protons and not by the residual water. The experiment is not particularly sensitive to the fine-tuning of excitation parameters and can be run in a routine fashion. A very high degree of water suppression ($10^4 - 10^5$) obtained from a single scan, no linear phase gradient, and simple experimental adjustment make this technique ideally suited for incorporation in many mD NMR experiments.

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REFERENCES

Davis, A.L., Keeler, J., Laue, E.D. and Moskau, D. (1992) *J. Magn. Reson.*, **98**, 207–216.
Davis, A.L., Laue, E.D., Keeler, J., Moskau, D. and Lohman, J. (1991) *J. Magn. Reson.*, **94**, 637–644.
Guéron, M., Plateau, P. and Decorps, M. (1991) *Prog. NMR Spectr.*, **23**, 135–209.
Hore, P. (1989) *Methods Enzymol.*, **176**, 64–77.
Hoult, D.I. (1976) *J. Magn. Reson.*, **21**, 337–347.
Hurd, R.E. (1990) *J. Magn. Reson.*, **87**, 422–428.
Hurd, R.E. and John, B.K. (1991) *J. Magn. Reson.*, **91**, 648–653.
Jesson, J.P., Meakin, P. and Kneissel, G. (1973) *J. Am. Chem. Soc.*, **95**, 618–620.
John, B.K., Plant, D., Webb, P. and Hurd, R.E. (1992) *J. Magn. Reson.*, **98**, 200–206.
Kumar, A., Ernst, R.R. and Wüthrich, K. (1980) *Biochem. Biophys. Res. Commun.*, **95**, 1–6.
Macura, S. and Ernst, R.R. (1980) *Mol. Phys.*, **41**, 95–117.
Marion, D., Ikura, M. and Bax, A. (1989a) *J. Magn. Reson.*, **84**, 425–430.
Marion, D., Ikura, M., Tschudin, R. and Bax, A. (1989b) *J. Magn. Reson.*, **85**, 393–399.
Otting, G. and Wüthrich, K. (1989) *J. Am. Chem. Soc.*, **111**, 1871–1875.
Otting, G., Liepinsh, E., Farmer, B.T. and Wüthrich, K. (1991) *J. Biomol. NMR*, **1**, 209–215.
Sklenář, V. and Bax, A. (1987) *J. Magn. Reson.*, **75**, 378–383.
Sklenář, V. (1990) In *NMR Application in Biopolymers* (Eds, Finley, J., Schmidt, S.J. and Seriani, A.S.) Academic Press, San Diego, pp. 63–84.
Vuister, G.W., Boelens, R., Kaptein, R., Hurd, R.E., John, B. and van Zilj, P.C.M. (1991) *J. Am. Chem. Soc.*, **113**, 9688–9690.
Wüthrich, K. (1986) *NMR of Proteins and Nucleic Acids*, Wiley, New York.
Zilj, P. and Moonen, C.T.W. (1990) *J. Magn. Reson.*, **87**, 18–25.