

Self-Compensating Pulsed Magnetic-Field Gradients for Short Recovery Times

GERHARD WIDER, VOLKER DÖTSCH, AND KURT WÜTHRICH*

Institut für Molekularbiologie und Biophysik, ETH-Hönggerberg, CH-8093 Zurich, Switzerland

Received January 28, 1994

The use of pulsed magnetic-field gradients (PFG) in high-resolution NMR is a major recent advance (1-16) which was enabled by the availability of shielded gradient coils. These resulted in greatly improved performance of PFGs, with substantial reduction of the recovery time needed to reach a stable homogeneous static magnetic field after the application of a gradient. Among the many potential applications of PFGs in high-resolution NMR, use to reduce the extent of phase cycling needed to define the coherence pathway and to cancel artifacts has attracted special interest. For such experiments, gradients up to about 30 G/cm are needed, for which satisfactory recovery times can be obtained. A different application of PFGs is for studies of the exchange times of labile protons and intact water molecules, for example, between the interior of proteins and the bulk solvent, by evaluation of differences in the respective diffusion constants. For such experiments, high gradient strengths with short recovery times are needed to prevent substantial exchange during the application of the gradient and the dead time. For a particular exchange experiment, a gradient field strength of 180 G/cm was available in our laboratory, but since the concomitant dead time of several milliseconds was long compared to the expected exchange times of less than one millisecond, no differences in individual diffusion constants could be measured. Kriwacki *et al.* (17) recently proposed the use of moderate PFG strength for similar measurements, but no evaluation of individual exchange rates was given.

Preemphasis units have been introduced to reduce long waiting times after intense gradients, which is of general interest since upon application of gradients with long recovery times to transverse magnetization, both relaxation and exchange processes can lead to substantial signal loss. A preemphasis unit shapes the gradient pulses, for example, with exponentials of different time constants and amplitudes, resulting in compensation of the transient response induced in the system when magnetic-field gradients are switched on or off (18). Drawbacks of this approach are that the adjust-

ment of analog preemphasis units requires manipulations which are similar to the shimming of a magnet and may require substantial time and experience. Furthermore, the induced transient signals have a complex spatial and time dependence and cannot usually be fully corrected by a preemphasis unit.

In this Communication we propose a self-compensating PFG sequence ("PFG sandwich") which allows one to greatly reduce transient responses without tedious adjustments and without the need of additional hardware. Since successive PFGs are highly reproducible, the application of a pair of sign-inverted but otherwise identical PFGs will result in transient responses which are also opposite in sign and thereby compensate imperfections of the individual pulses. To prevent refocusing of the effect of the first gradient pulse, a 180° radiofrequency pulse is applied between the two gradients, so that the location-dependent phase of the individual magnetization components obtained during the first gradient will further develop during the second gradient. Figures 1A and 1B show the implementation of a PFG sandwich into a pulse sequence designed to measure the recovery time after the application of a gradient. In Fig. 1B, the gradient with length $2\tau_g$ in Fig. 2A is substituted by two gradients with length τ_g and opposite sign, which are separated by a 180° RF pulse. Figures 1C and 1D show the use of a PFG sandwich in a pulse sequence for diffusion measurements, where we want to compensate transient responses due to the application of strong gradients. A similar pulse sequence has been proposed to compensate for strong static background magnetic-field gradients in solids (19).

Figure 2 compares ¹H NMR spectra of a 20 mM solution of the protein bovine pancreatic trypsin inhibitor (BPTI) in 90% H₂O/10% D₂O which were recorded with conventional PFGs (Figs. 2A and 2C) or with the presently proposed, self-compensating PFG sandwiches (Figs. 2B and 2D). The experiments used for Figs. 2A and 2B (Figs. 1A and 1B) or for Figs. 2C and 2D (Figs. 1C and 1D) were otherwise identical. The measurements were performed on a Bruker AMX-500 NMR spectrometer equipped with shielded gradient coils

* To whom correspondence should be addressed.

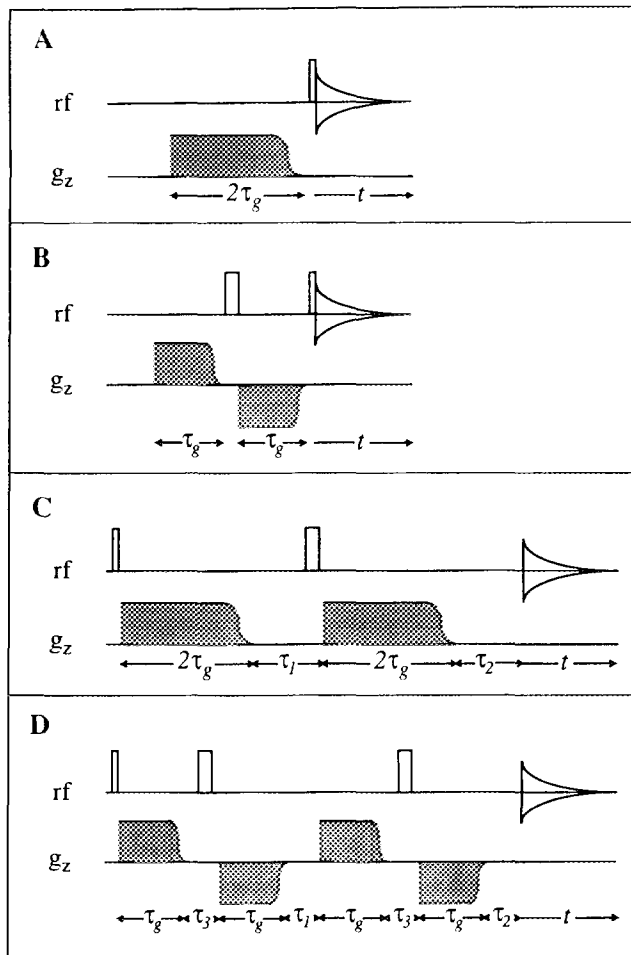


FIG. 1. Pulse sequences for one-dimensional ^1H NMR experiments with pulsed magnetic-field gradients (PFG) using conventional PFGs or the newly proposed PFG sandwich, respectively. RF stands for radiofrequency, g_z for pulsed magnetic-field gradient, and t for the acquisition time. Vertical bars represent radiofrequency pulses, where the different pulse lengths for $\pi/2$ and π pulses are indicated by the width of the bars. Gradient pulses are indicated by the shaded shapes. The duration of the PFGs is indicated in units of τ_g . The delays between subsequent gradient pulses, or between gradient and radiofrequency pulses, are typically a few microseconds, unless indicated by a delay time τ_1 , τ_2 , or τ_3 (see Fig. 2). (A) Pulse sequence used to measure the recovery time after the application of a PFG. (B) Same as (A), but with a self-compensating PFG sandwich. (C) Pulse sequence used for diffusion measurements. (D) Same as (C), but with PFG sandwiches.

and a 10 A gradient amplifier, no preemphasis was used, and the lock signal was not switched off during the application of the gradients. With the experimental parameters given in the legend to Fig. 2, the spectrum in Fig. 2A was distorted by the slow recovery of the field homogeneity. The spectrum in Fig. 2B corresponds to a spectrum measured in a homogeneous field, which was rapidly attained with the PFG sandwich. If more than one gradient is applied in the same pulse sequence (Figs. 1C and 1D), even more dramatic

differences can be observed. The high quality of the spectrum in Fig. 2D and the absence of an interpretable spectrum in Fig. 2C are an impressive illustration of the performance of self-compensating PFGs.

Although the experiments in Fig. 2 provide a clearcut, qualitative demonstration of the potential of PFG sandwiches, measurements of diffusion constants with PFGs provide a more quantitative picture of the recovery of field homogeneity, because the evaluation of such data depends critically on good suppression of induced transient responses (20). Figure 3 shows data on H_2O self-diffusion in a 20 mM solution of basic pancreatic trypsin inhibitor in a mixed solvent of 90% H_2O /10% D_2O . [The experiment was performed with a solution of BPTI to obtain a reference for measurements of exchange rates of labile protons and intact water molecules between the interior of this protein and the bulk

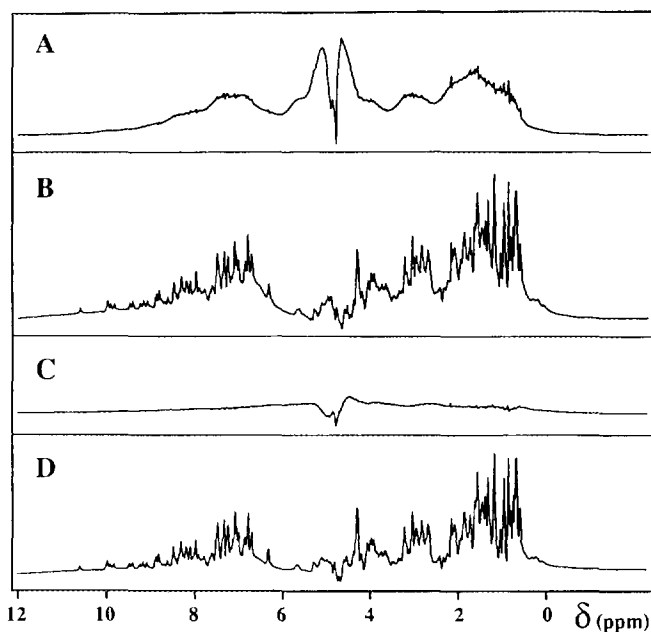


FIG. 2. One-dimensional ^1H NMR spectra recorded with a 20 mM solution of BPTI in 90% H_2O /10% D_2O at pH 3.5 and $T = 293$ K using the pulse sequences indicated in Fig. 1. To reduce the residual H_2O signal, a moving time average of the FID taken over 50 complex points was subtracted from the original FID (24). No baseline correction was used. All spectra were plotted with identical noise levels. All gradient pulses had a length of 2 ms for single PFGs and 1 ms for each individual pulse in the PFG sandwiches. The individual gradient pulses had a modified rectangular shape, with the last quarter of the rectangle attenuated by multiplication with cosine squared. The gradient strength was 60 G/cm throughout. (A) Spectrum acquired using the conventional pulse sequence shown in Fig. 1A, with a 4 μs delay between switching off of the gradient and the 90° excitation pulse. (B) Same as (A), but using self-compensating PFGs (Fig. 1B) with delays of 4 μs between RF pulses and gradients. (C) Scheme in Fig. 1C for diffusion measurements, with $\tau_1 = 32$ μs , $\tau_2 = 4$ μs , and $\tau_3 = 4$ μs delays separating gradients and RF pulses. (D) Same as (C) but using gradient sandwiches (Fig. 1D) with $\tau_1 = 32$ μs , $\tau_2 = 4$ μs , and $\tau_3 = 24$ μs .

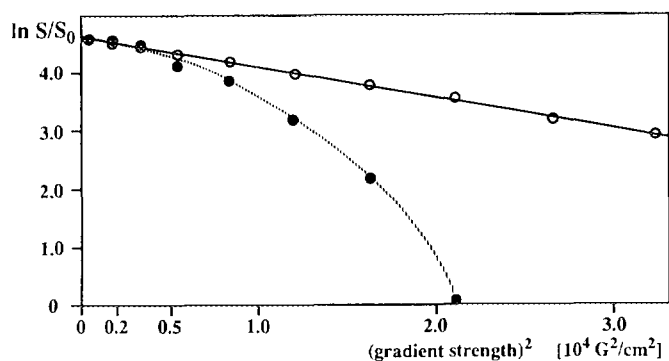


FIG. 3. Two self-diffusion measurements of H₂O in a 20 mM solution of BPTI in 90% H₂O/10% D₂O at pH 3.5 and $T = 277$ K using the pulse sequences indicated in Fig. 1C (●) and Fig. 1D (○), respectively. In the scheme in Fig. 1C the lengths of the pulse gradients were 2 ms each, with $\tau_1 = \tau_2 = 1$ ms. In the experiment in Fig. 1D all gradients had a length of 1 ms, with $\tau_1 = \tau_2 = 1$ ms and $\tau_3 = 24$ μ s. All gradient pulses had a modified rectangular shape, with the last quarter of the rectangle attenuated by multiplication with a cosine squared. To determine the self-diffusion constants, different experiments with identical time intervals τ_1 , τ_2 , and τ_3 were performed with variable gradient strengths of 18, 36, 54, 72, 90, 108, 126, 144, 126, and 180 G/cm. The straight line through the open circles represents a fit of a linear function to $\ln(S/S_0)$. The self-diffusion constant D for H₂O in 20 mM BPTI at 277 K was thus determined to be 1.0×10^{-5} cm²/s (see text). The curved dotted line through the filled circles was drawn manually to guide the eye.

solvent (unpublished results)]. Two series of spectra were recorded, one with conventional PFGs (Fig. 1C) and the other one using PFG sandwiches as shown in Fig. 1D. The data were collected using constant time intervals τ_1 , τ_2 , and τ_3 , while the gradient strength was incremented stepwise from 18 to 180 G/cm using a Bruker 30 A gradient amplifier without a preemphasis unit. The delays $\tau_1 = \tau_2 = 1$ ms were chosen sufficiently long to enable quantitative measurements with the conventional PFGs for small gradient strengths up to about 50 G/cm. For higher gradient strengths, the spectrum resulting from the scheme in Fig. 1C was distorted to the extent that an evaluation was no longer possible above 120 G/cm. For the experiments in Figs. 1C and 1D, with a rectangular gradient shape, the formula

$$\ln(S/S_0) = -4\gamma^2\tau_g^2g_z^2(5\tau_g/3 + \tau_1)D \quad [1]$$

describes the signal attenuation, S/S_0 , due to diffusion (21), where D is the diffusion constant and g_z the gradient strength. For nonrectangular gradient pulse shapes one obtains a more complicated dependence on the gradient length, τ_g , and the delay between the gradients, τ_1 (19, 22). Instead of calculating the actual dependence for the gradient shape applied in our measurements, we used the known self-diffusion constant of H₂O in a pure mixture of 90% H₂O/10% D₂O [$D = 1.2 \times 10^{-5}$ cm²/s at 277 K (23)] to calibrate the mea-

surements in Fig. 3. With this calibration the self-diffusion constant for H₂O in 20 mM BPTI was found to be 1.0×10^{-5} cm²/s at 277 K.

In conclusion, the results obtained with the experimental schemes in Fig. 1 demonstrate that the application of a self-compensating PFG sandwich for reduction of unwanted transient distortions after switching the gradients on or off enables much shorter recovery times than would be possible with a single PFG. We compared the improvements achieved with self-compensating PFG sandwiches for different hardware configurations. Measurements with and without preemphasis with different gradient shapes, or with the use of a shielded gradient coil or the z shim coil to deliver the gradients, gave corresponding improvements, confirming the general applicability of the technique. (Of course, the recovery time increases when unshielded coils, e.g., the z shim, are used, or for rectangular pulse shapes, whereas it gets shorter when a preemphasis unit is used.) The radiofrequency inhomogeneity of the 180° pulse will usually lead to a small signal loss for each PFG sandwich used. It depends on the particular experiment whether transverse relaxation during a long recovery delay or the application of a 180° pulse per PFG sandwich will result in a greater loss of sensitivity. For example, whenever chemical exchange during the recovery delay is of concern, selection of a short recovery delay is crucial for all types of experiments. On the other hand, if the magnetization of interest is either transverse with a long value of the T_2 relaxation time or longitudinal, the length of the recovery delay is usually not critical and the use of PFG sandwiches may not be indicated.

ACKNOWLEDGMENTS

We thank Spectrospin AG (Fällanden, Switzerland) for making a self-shielded gradient accessory available to us and Mrs. E. Huber for the careful processing of the manuscript. Financial support was obtained from the Kommission zur Förderung der wissenschaftlichen Forschung (Project 2223.1).

REFERENCES

1. R. E. Hurd, *J. Magn. Reson.* **87**, 422 (1990).
2. A. L. Davis, E. D. Laue, J. Keeler, D. Moskau, and J. Lohman, *J. Magn. Reson.* **94**, 637 (1991).
3. A. L. Davis, J. Keeler, E. D. Laue, and D. Moskau, *J. Magn. Reson.* **98**, 207 (1992).
4. J. R. Tolman, J. Chung, and J. H. Prestegard, *J. Magn. Reson.* **98**, 462 (1992).
5. J. Boyd, N. Soffe, B. John, D. Plant, and R. Hurd, *J. Magn. Reson.* **98**, 660 (1992).
6. A. D. Davis, R. Boelens, and R. Kaptein, *J. Biomol. NMR* **2**, 395 (1992).
7. G. W. Vuister, J. Ruiz-Cabello, and P. van Zijl, *J. Magn. Reson.* **100**, 215 (1992).
8. J. Ruiz-Cabello, G. W. Vuister, C. T. W. Moonen, P. van Gelderen, J. S. Cohen, and P. van Zijl, *J. Magn. Reson.* **100**, 282 (1992).

9. A. Bax and S. Pochapsky, *J. Magn. Reson.* **99**, 638 (1992).
10. B. John, D. Plant, and R. E. Hurd, *J. Magn. Reson. A* **101**, 113 (1993).
11. G. W. Vuister, G. M. Clore, A. M. Gronenborn, R. Powers, D. S. Garrett, R. Tschudin, and A. Bax, *J. Magn. Reson. B* **101**, 210 (1993).
12. D. R. Muhandiram, G. Yi Xu, and L. E. Kay, *J. Biomol. NMR* **3**, 463 (1993).
13. L. E. Kay, G. Y. Xu, A. U. Singer, D. R. Muhandiram, and J. D. Forman-Kay, *J. Magn. Reson. B* **101**, 333 (1993).
14. L. E. Kay, *J. Am. Chem. Soc.* **115**, 2055 (1993).
15. P. W. Kuchel and B. E. Chapman, *J. Magn. Reson. A* **101**, 53 (1993).
16. G. Wider and K. Wüthrich, *J. Magn. Reson. B* **102**, 227 (1993).
17. R. W. Kriwacki, R. B. Hill, J. M. Flanagan, J. P. Caradonna, and J. H. Prestegard, *J. Am. Chem. Soc.* **115**, 8907 (1993).
18. P. D. Majors, J. L. Blackley, S. A. Altobelli, A. Caprihan, and E. Fukushima, *J. Magn. Reson.* **87**, 548 (1990).
19. R. F. Karlicek and I. J. Lowe, *J. Magn. Reson.* **37**, 75 (1980).
20. S. J. Gibbs and C. S. Johnson, Jr., *J. Magn. Reson.* **93**, 395 (1991).
21. E. O. Stejskal and J. E. Tanner, *J. Chem. Phys.* **42**, 228 (1966).
22. M. R. Merrill, *J. Magn. Reson. A* **103**, 223 (1993).
23. K. T. Gillen, D. C. Douglas, and M. J. R. Hoch, *J. Chem. Phys.* **57**, 5117 (1972).
24. D. Marion, I. Mitsuhiro, and A. Bax, *J. Magn. Reson.* **84**, 425 (1989).