

Phase-Sensitive Spectra in a Single Scan with Coherence Selection by Pulsed Field Gradients

VOLKER DÖTSCH, GERHARD WIDER, AND KURT WÜTHRICH

Institut für Molekularbiologie und Biophysik, Eidgenössische Technische Hochschule-Hönggerberg, CH-8093 Zurich, Switzerland

Received April 20, 1994

Coherence selection with pulsed field gradients (PFG) yields a phase-modulated signal with concomitant mixed lineshapes (1–4). Phase-sensitive spectra have been obtained by recording a second data set with inverted sign of the refocusing gradient and the use of rather complicated data-processing schemes (5–8). This Communication now describes an avenue to phase-sensitive spectra with a single scan for each experiment in t_1 , using a PFG-based experimental scheme, conventional data acquisition, and conventional data processing. Potential applications are in all areas where optimal sensitivity is not a concern and workable data can be accumulated with a single scan per t_1 increment and for selected multiple-scan experiments, for example, in combination with certain solvent-suppression schemes (see below).

Figure 1 shows the experiment used to record single-scan, phase-sensitive NOESY spectra with PFG-based coherence selection. Before and after the mixing period, τ_m , a self-compensating "PFG sandwich" is applied, which consists of two PFGs with inverted sign separated by a $\pi(^1\text{H})$ pulse and ensures rapid recovery after the PFG as well as refocusing of the chemical-shift evolution during the PFG (9). An additional PFG in the middle of τ_m destroys all coherences present after the second 90° pulse (a in Fig. 1). After the second PFG sandwich (b in Fig. 1) one has the magnetization (7, 8)

$$\sigma_b = \frac{1}{2}I_x \sin(\omega t_1) + \frac{1}{2}I_y \cos(\omega t_1). \quad [1]$$

The new feature introduced in this paper is to select one of the two terms in Eq. [1] either with a trim pulse (Fig. 1) or an additional PFG framed by two $(\pi/2)^{(^1\text{H})}$ pulses. For example, a trim pulse along the x axis at time b (Fig. 1) destroys the y component of the magnetization by the inhomogeneity of the radiofrequency field and thus leads to a pure sine dependence of the signal with respect to t_1 . Incrementation of the phase ϕ_1 by $\pi/2$ gives the corresponding cosine term. Therefore, pure phases can be obtained using standard complex or real data collection in t_1 and with standard processing (10).

The result obtained is shown in Figs. 2 and 3, which display NOESY spectra recorded using the experiment in Fig. 1 with and without the SL pulse. The spectrum acquired without

the SL pulse shows strong phase distortions (Figs. 2B and 3B), whereas a spectrum with absorptive lineshapes resulted when the complete experimental scheme in Fig. 1 was used with the parameters given in the legend to Fig. 2. This approach to obtaining pure lineshapes is not limited to NOESY, but can also be used in combination with other pulse sequences, e.g., in HSQC experiments. If use of a high-power trim pulse is detrimental to the outcome of the experiment, the SL pulse in Fig. 1 can be replaced by a PFG preceded and followed by a $(\pi/2)^{(^1\text{H})}$ pulse.

It is clear from Eq. [1] that the approach used leads to a loss in sensitivity by a factor $\sqrt{2}$ compared to phase-sensitive spectra obtained from a combination of recordings using PFGs with inverted sign (5–7). Practical applications can therefore be envisaged mainly in situations where sensitivity is not a limiting factor, but the time savings with single-scan experiments and the convenience of obtaining phase-sensitive spectra with standard data handling are appreciated. This could include in the future many routine applications in chemistry laboratories. However, there are also low-sensitivity experiments using extensive data accumulation for which the standard scheme already includes a purge pulse corre-

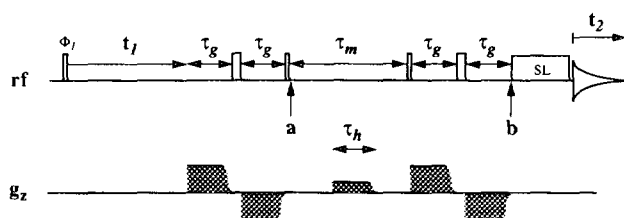


FIG. 1. Experimental scheme for phase-sensitive 2D NOESY with PFG-based coherence pathway selection. The evolution time is t_1 , τ_m is the NOESY mixing period, t_2 is the acquisition time, τ_g is the length of the PFGs, τ_h is the length of the PFG used as a "homospoil" pulse during τ_m , and SL is a spin-lock purge pulse. ^1H radiofrequency pulses are given by vertical bars on the line RF, where $\pi/2$ and π pulses are indicated by thin and thick bars. PFGs are indicated by the shaded shapes on the line g_z . Each half-gradient in the two PFG sandwiches (9) has a duration τ_g and a modified rectangular shape, with the last quarter having a \cos^2 dependence. The PFG in the middle of τ_m eliminates coherences present at point a. SL is applied between point b and the start of the acquisition. All ^1H pulses are applied with the same phase, except that quadrature detection in t_1 is achieved by altering the phase ϕ_1 according to the States-TPPI scheme (14).

sponding to SL in Fig. 1 (or, as an alternative, an equivalent PFG), and the resulting loss in sensitivity is accepted for other reasons. An example is the use of spin-lock purge pulses for solvent suppression (11, 12). In these experiments the absorptive lineshape obtained for PFG-based coherence selection would come as an extra benefit of the solvent suppression scheme which is used, without additional loss of sensitivity. We have actually developed the experiment in Fig. 1 when working on measurements of the exchange rates of labile protons (4) and intact water molecules (13) between interior sites in proteins and the bulk water, which depend critically on solvent suppression schemes that can be applied immediately before the acquisition period (Fig. 1).

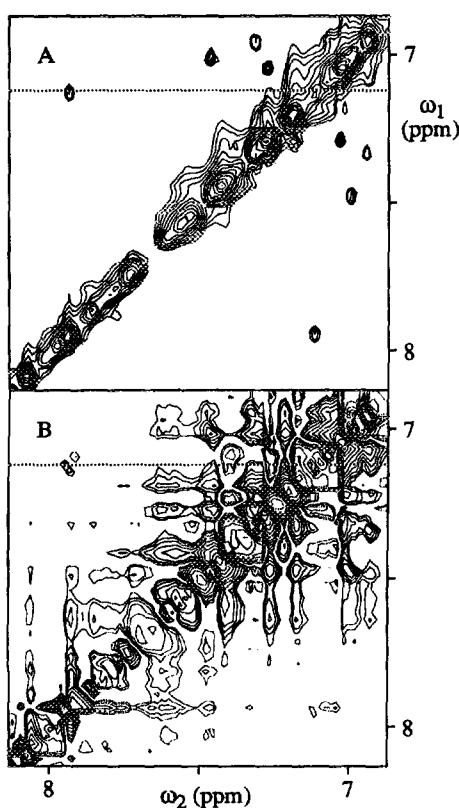


FIG. 2. Contour plots showing the region ($\omega_1 = 6.8\text{--}8.2$ ppm, $\omega_2 = 6.8\text{--}8.2$ ppm) from two ^1H , ^1H NOESY spectra recorded using the experiment in Fig. 1 (A) with or (B) without a purge pulse SL of length 1 ms (20 mM solution of BPTI in 90% $\text{H}_2\text{O}/10\%$ D_2O at pH 3.5 and $T = 298$ K, $\tau_m = 80$ ms); $\tau_g = \tau_h = 1$ ms, and the field strength was 36 G/cm in the PFG sandwiches and 6 G/cm for the PFG applied during the mixing time. The delay between PFG and the following ($\pi/2$)(^1H) or SL(^1H) pulse was 100 μs , and the delay between PFGs and π (^1H) in the PFG sandwiches was 10 μs . One scan was acquired per t_1 increment, and no presaturation for solvent suppression was applied; $t_{1\text{max}} = 80$ ms, $t_{2\text{max}} = 164$ ms, complex time domain data size = 500×1024 points, and zero-filling was applied in t_1 only to a final size of 512 complex points. The data were multiplied with a cosine function along t_1 and t_2 . Complex acquisition with the States-TPPI scheme (14) and standard complex Fourier transformation were used. The dotted lines indicate the position of the cross sections in Fig. 3.

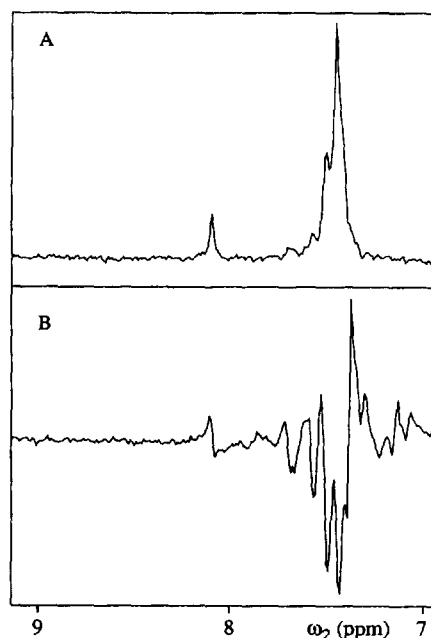


FIG. 3. (A and B) Cross sections along ω_2 at the position of the dotted line in the corresponding parts (A) and (B) in Fig. 2.

ACKNOWLEDGMENTS

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

REFERENCES

1. 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).
2. A. L. Davis, R. Boelens, and R. Kaptein, *J. Biomol. NMR* **2**, 395 (1992).
3. G. W. Vuister, R. Boelens, R. Kaptein, M. Burgering, and P. C. M. van Zijl, *J. Biomol. NMR* **2**, 301 (1992).
4. C. T. W. Moonen, P. van Gelderen, G. W. Vuister, and P. C. M. van Zijl, *J. Magn. Reson.* **97**, 419 (1992).
5. A. L. Davis, J. Keeler, E. D. Laue, and D. Moskau, *J. Magn. Reson.* **98**, 207 (1992).
6. J. Boyd, N. Soffe, B. K. John, D. Plant, and R. Hurd, *J. Magn. Reson.* **98**, 660 (1992).
7. J. R. Tolman, J. Chung, and J. P. Prestegard, *J. Magn. Reson.* **98**, 462 (1992).
8. L. E. Kay, P. Keifer, and T. Saarinen, *J. Am. Chem. Soc.* **114**, 10663 (1992).
9. G. Wider, V. Dötsch, and K. Wüthrich, *J. Magn. Reson. A* **108**, 255 (1994).
10. D. Marion and K. Wüthrich, *Biochem. Biophys. Res. Commun.* **113**, 967 (1983).
11. B. A. Messerle, G. Wider, G. Otting, C. Weber, and K. Wüthrich, *J. Magn. Reson.* **85**, 608 (1989).
12. G. Otting, E. Liepinsh, B. T. Farmer II, and K. Wüthrich, *J. Biomol. NMR* **1**, 209 (1991).
13. R. W. Kriwacki, R. B. Hill, J. M. Flanagan, J. P. Caradonna, and J. H. Prestegard, *J. Am. Chem. Soc.* **115**, 8907 (1993).
14. D. Marion, K. Ikura, R. Tschudin, and A. Bax, *J. Magn. Reson.* **85**, 393 (1989).