P.COSY, a Sensitive Alternative for Double-Quantum-Filtered COSY

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In recent years, the recording of phase-sensitive two-dimensional NMR spectra has become increasingly popular. Both the hypercomplex Fourier transformation approach (*1, 2*) and the TPPI method (*3, 4*) can be applied to nearly all 2D NMR experiments, permitting the recording of absorption-mode spectra in the presence of two-dimensional quadrature detection. One fundamental problem in applying either approach to the widely used COSY experiment is that the diagonal resonances have a phase that differs from the phase of the cross peaks (*5*) making it fundamentally impossible to simultaneously phase all resonances to the absorption mode. A large number of variations on the original COSY experiment have since been proposed that can solve this problem. Such methods vary from complete suppression of diagonal peaks (*6*) to *z* filtering (*7*) and multiple-quantum filtering (*8*). In particular, the double-quantum-filtered COSY experiment has become accepted as one of the standard advanced tools of the NMR spectroscopist.

Here, we demonstrate that a minor variation of the P.E.COSY experiment, recently proposed by Mueller (*9*), can be used to purge the dispersive character of the diagonal in a regular COSY experiment. Experimental results indicate that this purged COSY (P.COSY) method yields spectra of a quality comparable to the double-quantum-filtered COSY method, but with twice the sensitivity.

The P.E.COSY experiment was designed to offer a simple alternative to the complex but powerful E.COSY (*10, 11*) method. As proposed by Mueller, a 2D spectrum quite similar in appearance to an E.COSY spectrum can be obtained if one subtracts the results of a "COSY spectrum" recorded with 0° mixing pulse from a COSY spectrum recorded with a small-flip-angle mixing pulse. Instead of recording an entire 2D data matrix for the 0° COSY spectrum, the same data can be obtained from a single FID (*12*). By left shifting the data of this single FID, the time-domain data for successive t_1 values of the 0° COSY experiment are obtained. By recording the single FID with an N times larger number of scans (typically, $N = 16$, followed by appropriate scaling before subtraction), the signal-to-noise ratio of the cross peaks in the difference spectrum is decreased by only a very small amount, $\sqrt{(N^2 + 1)}/N$. As shown in

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this note, this purging procedure is not limited to small-flip-angle COSY spectra and can be used equally well for the regular COSY experiment.

The simple vector diagram of Fig. 1 serves to illustrate the purging character of the signal of a single isolated spin. The vector $\mathbf{M_r}$ is the orientation of the magnetization vector just before the mixing pulse. If a 90°_x mixing pulse is applied, only the x component of this vector ($\mathbf{M_d}$) remains, giving rise to the dispersive diagonal resonance in the COSY spectrum. The difference of $\mathbf{M_d}$ and $\mathbf{M_r}$ (90° COSY $- 0^\circ$ COSY) yields a signal, $\mathbf{M_p}$, that is aligned along the y axis, in-phase with the cross-peak multiplet components.

To analyze the behavior of weakly coupled spins, operator formalism is the most convenient method (13). At the end of the evolution period, the density operator for two coupled spins, I and S, is given by

$$\sigma(t_1) = -I_y c_I c_J - S_y c_S c_J \qquad [1a]$$

$$+I_x s_I c_J + S_x s_S c_J \qquad [1b]$$

$$+2I_x S_z c_I s_J + 2I_z S_x c_S s_J \qquad [1c]$$

$$+2I_y S_z s_I s_J + 2I_z S_y s_S s_J \qquad [1d]$$

with $c_I = \cos(\Omega_I t_1)$, $s_I = \sin(\Omega_I t_1)$, $c_S = \cos(\Omega_S t_1)$, $s_S = \sin(\Omega_S t_1)$, $c_J = \cos(\pi J t_1)$, and $s_J = \sin(\pi J t_1)$. After a subsequent 90°_x pulse, the operator is given by

$$\sigma(t_1, 0) = -I_z c_I c_J - S_z c_S c_J \qquad [2a]$$

$$+I_x s_I c_J + S_x s_S c_J \qquad [2b]$$

$$-2I_x S_y c_I s_J - 2I_y S_x c_S s_J \qquad [2c]$$

$$-2I_z S_y s_I s_J - 2I_y S_z s_S s_J. \qquad [2d]$$

The difference, $\sigma(t_1, 0) - \sigma(t_1)$, gives cancellation of terms [1b] and [2b]. Of the remaining terms that contribute to the diagonal, [1a], [1c], and [1d], only [1a] has net integrated intensity and is in-phase with the cross peaks (term [2d]). The other two terms, [1c] and [1d], correspond to antiphase dispersive components. As shown
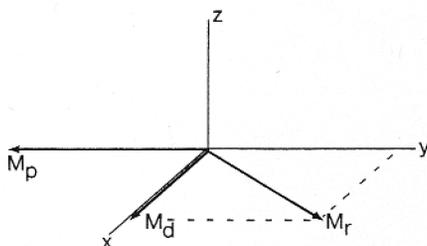

FIG. 1. Vector picture of the transverse magnetization of an isolated spin in the P.COSY experiment. $\mathbf{M_r}$ is the magnetization vector just before the mixing pulse. $\mathbf{M_d}$ is the transverse magnetization after the 90°_x mixing pulse and gives rise to the diagonal resonance. $\mathbf{M_p}$ is the vector that is obtained if the vector $\mathbf{M_r}$ is subtracted from $\mathbf{M_d}$. Note that this vector is aligned along the y axis and therefore has the same phase as the cross peaks observed for coupled spins. The signal corresponding to $\mathbf{M_r}$ can be obtained for all t_1 durations from a single FID by left shifting of the data.

by Mueller (9), the summed intensity of these antiphase dispersive resonances falls off like a regular absorption-mode peak.

A few minor practical problems remain to be discussed. The pulse scheme of the COSY experiment is

$$90_\phi^\circ\!-\!t_1\!-\!90_x^\circ\!-\!\mathrm{Acq.}(t_2).$$

For the hypercomplex variant of the COSY experiment, two sets of data are acquired. The first set uses the phase cycling $\phi = x, -x$; Acq. $= x, -x$. The second set uses $\phi = y, -y$; Acq. $= x, -x$. The reference signal to be subtracted from the second data set must be increased in phase by 90° relative to the reference signal recorded for the first data set. This can be done either by manipulation of the free induction decay of this one-dimensional experiment, or by recording two separate reference signals, with the phase of the excitation pulse incremented by 90°. Alternatively, a phase-cycling procedure of the COSY experiment can be used where the phase of the second pulse instead of the first pulse is phase cycled (9), but this requires different phasing by 90° of the two sets of acquired data. The number of complex data points acquired in the one-dimensional reference spectrum must be at least equal to the sum of the number of t_1 increments and the number of complex t_2 data points. Therefore, the data acquisition period is longer for the reference experiment than for the 2D experiment. To ensure proper purging and avoid differences caused by relaxation, the delay time between scans in the reference experiment should be corrected for this longer data acquisition period, such that the time between the start of data acquisition and the next 90° pulse is identical in the COSY and in the reference experiment. A fixed delay corresponding to $2/3\tau_{90^\circ}$ should be inserted prior to the start of data acquisition in the reference experiment to correct for the duration, τ_{90°, of the 90° pulse width. Phasing of the data in the F_2 dimension should be done on the first FID of the 2D experiment, after the reference signal has been subtracted from the COSY signal.

As an example, we have applied the P.COSY method to a sample of the antimicrobial peptide, magainin-2 (23 amino acids), in $2/1$ (v/v) H_2O/CF_3CD_2OH, 25°C, 100 mM NaCl, pH 4.0, prior to the addition of trifluoroethanol. Under these conditions the peptide assumes an α-helical conformation (14). The fingerprint region of the P.COSY spectrum is shown in Fig. 2a. The same region of the double-quantum-filtered COSY spectrum (recorded with four times the number of scans) is shown in Fig. 2b. The sensitivity of the two spectra (relative to the noise, which is not visible in this contour plot) is identical, despite the four times longer measuring time used for the double-quantum-filtered COSY spectrum. This is easily understood by considering the pulse scheme of the double-quantum-filtered COSY experiment,

$$90_\phi^\circ\!-\!t_1\!-\!90_x^\circ 90_\psi^\circ\!-\!\mathrm{Acq.}(t_2),$$

where the double-quantum filtering is obtained by incrementing the phase ψ by 90° in four consecutive experiments ($7, 15$). However, two of the four steps (with $\psi = x, -x$, corresponding to mixing pulses of 180° and 0°, respectively) do not contribute any signal to the cross peaks of the COSY spectrum (16); they only add noise and subtract the dispersive character from the diagonal.

Of course, unlike double-quantum-filtered COSY, the P.COSY experiment does not remove singlet signals from the diagonal. In practice this makes little difference,
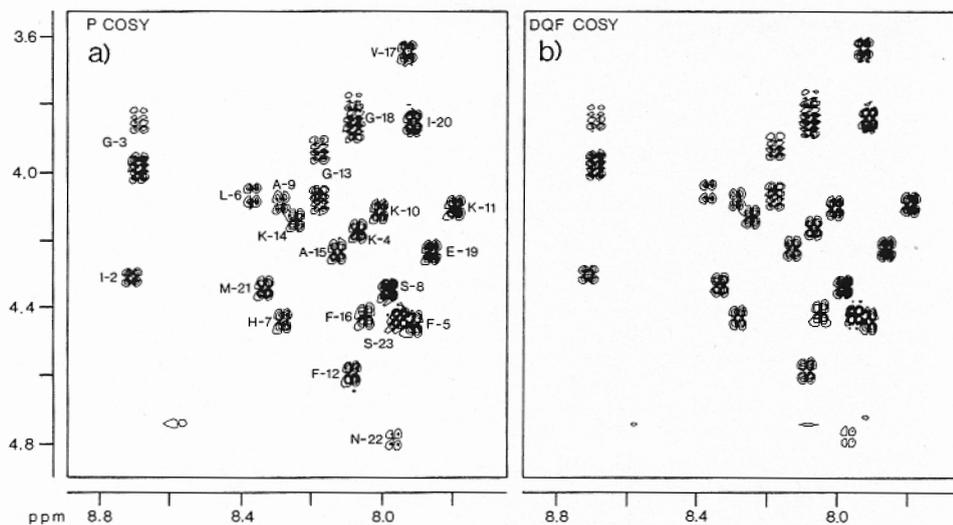
FIG. 2. Fingerprint regions of the COSY spectra of a sample of the peptide magainin-2 in 2/1 (v/v) H_2O/trifluoroethanol-d_3, recorded at 500 MHz. (a) Obtained with the P.COSY method, 16 scans per t_1 value, total measuring time 3 h; and (b) obtained with the double-quantum-filtered COSY experiment, 64 scans per t_1 value, total measuring time 12 h. Both spectra result from $2 \times 400 \times 1024$ data matrices, with acquisition times of 102 and 80 ms in the t_2 and t_1 dimensions, respectively.

unless long-term stability of the spectrometer is poor or an erroneous use of the Fourier transform algorithm introduces ridges for strong diagonal peaks (17). A comparison of the aliphatic regions of the P.COSY (Fig. 3) and the double-quantum-filtered COSY spectra again shows very little difference. The tails of the narrow (and truncated) diagonal singlet resonance of the methyl group of Met-21 can be seen in the P.COSY spectrum (at 2.07 ppm) whereas it is completely suppressed in the double-quantum-filtered COSY spectrum. Inspection of individual slices through the 2D data matrices showed that both the cross peak/noise and the cross peak/t_1-noise ratios are identical for the two spectra, despite the difference in measuring time of a factor of four. The cross peaks in the upper right-hand corner of the double-quantum-filtered COSY spectrum are attenuated relative to the P.COSY spectrum. This attenuation is caused by resonance offset effects; the ^1H RF field was only 8 kHz and the double-quantum frequencies for these cross peaks also are about 8 kHz.

We have shown that the P.COSY method provides a more sensitive alternative for the widely used double-quantum-filtered COSY experiment. The diagonal resonances in the P.COSY experiment have dispersive components that are in antiphase. Such antiphase diagonal multiplet components are also present in double-quantum-filtered COSY spectra (for systems of more than two spins), but to a lesser degree. Since the dispersive components are antiphase, they only have a small effect on the appearance of the spectra. The intensity of diagonal resonances is higher in the COSY spectrum than in the double-quantum-filtered spectrum. Because the integrated intensity of the diagonal in the P.COSY spectrum is not zero, phasing in the F_2 dimension of the 2D spectrum can be done using the spectrum for the first t_1 increment,
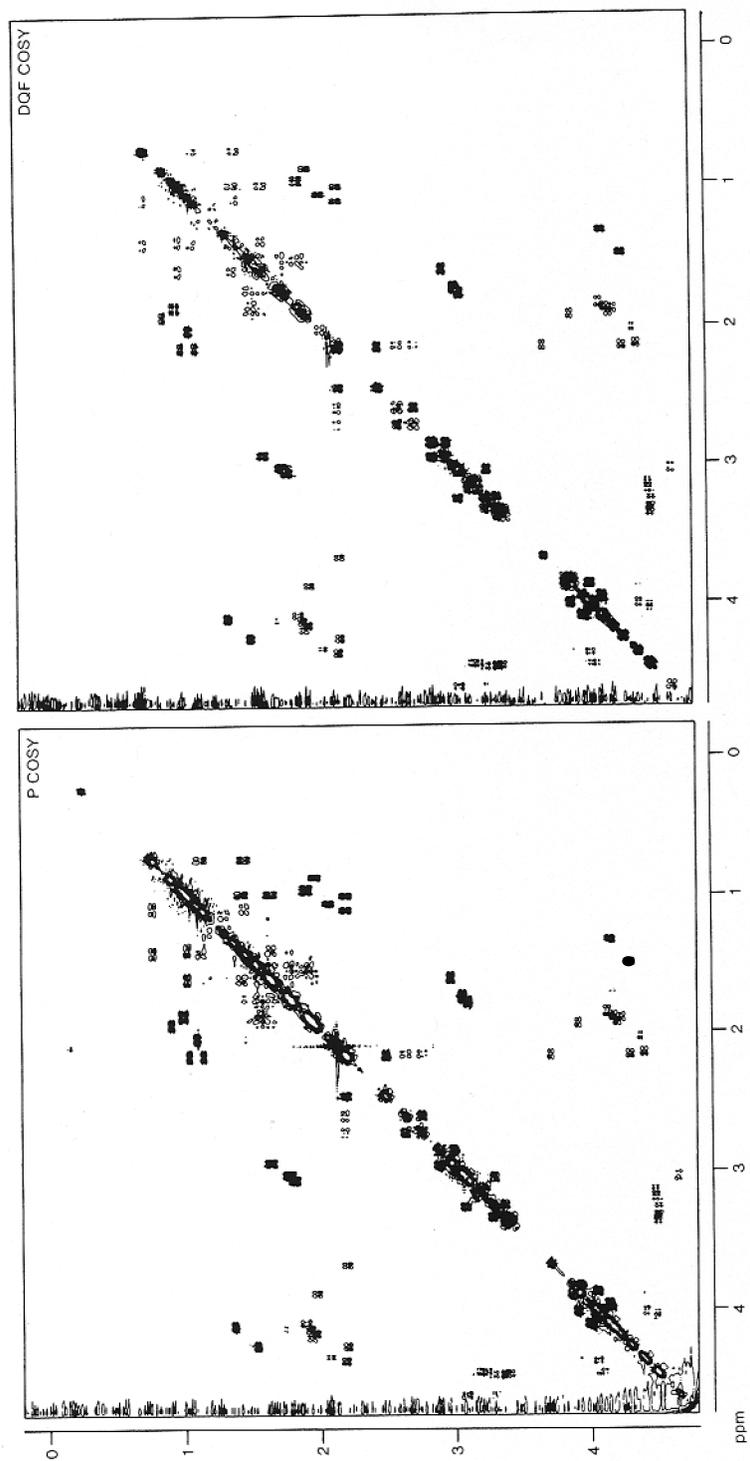
FIG. 3. Aliphatic regions of the COSY spectra of magainin-2, obtained from the same data sets as Fig. 2. The water resonance at 4.75 ppm was suppressed by presaturation during the preparation period.

making the phasing procedure simpler than it is for the double-quantum-filtered method, where a reference spectrum is required for convenient F_2 phasing.

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