JOURNAL OF MAGNETIC RESONANCE 75, 534–539 (1987)

Application of the z-COSY Technique with a Modified Pulse Sequence to Measurement of Coupling Constants in Macromolecules

HARTMUT OSCHKINAT, G. MARIUS CLORE, MICHAEL NILGES, AND ANGELA M. GRONENBORN

Max-Planck Institut für Biochemie, D-8033 Martinsried bei Munich, Federal Republic of Germany

Received July 15, 1987

Coupling constants can provide useful supplementary information in the structural analysis of nucleic acids and proteins. It is often difficult, however, to determine their values as the large linewidths tend to obscure the multiplet patterns. For this reason it is desirable to interpret reduced rather than full multiplet effects in COSY spectra (*1, 2*). To this end several alternative methods have been developed. These include β-COSY (*2*), bilinear COSY (*3*), E.COSY (*4*), and z-COSY (*5*). The z-COSY and E.COSY techniques both yield spectra with reduced multiplet patterns and pure absorption lineshapes for all peaks in the spectrum. While full suppression of the undesired multiplet components can be achieved with E.COSY, the extent of suppression in z-COSY depends on the choice of flip angle for the two mixing pulses. The effects of strong coupling, however, are not as severe for z-COSY as it uses only small pulses (*6*).

In this paper we demonstrate the application of z-COSY to macromolecules using the DNA hexamer 5'd(GCATGC)$_2$ as an example and discuss the effects resulting from incomplete suppression of the undesired multiplet components of the cross peaks. Further, we show that in order to obtain a spectrum of sufficient quality for the measurement of coupling constant, the z-COSY technique should be applied in a modified manner with respect to the suppression of zero-quantum coherences.

The basic pulse sequence for z-COSY is $90°-t_1-\beta-\tau_m-\beta-t_2$. The phase cycling must be designed in such a manner that coherences of the order $p = \pm 1$ are retained during t_1 and only populations and zero-quantum coherences are selected during the delay τ_m. In the case of macromolecules, the application of the normal NOESY phase-cycle selecting coherences of the order $p = 0 \pm 4n$ (n = integer) during the delay τ_m is sufficient.

To obtain a z-COSY spectrum with pure absorption lineshapes it is necessary to suppress the zero-quantum transitions (ZQT) appropriately. Because of the intrinsic similarity of the pulse sequences, the methods used for NOESY experiment (*7–9*) can also be applied to z-COSY. In the original description of the z-COSY experiment (*5*), zero-quantum suppression was achieved by a random variation of the delay between the two mixing pulses. Unfortunately, this introduces noise into the spectrum due to the effective smearing of the zero-quantum peaks along the ω_1 axis and the generation of t_1 noise arising from the random variation in the relaxation of the diagonal peaks

0022-2364/87 $3.00

during τ_m. Further, techniques using a π pulse within the mixing delay are not appropriate as they destroy the linear properties of the z-COSY pulse sequence (*6*). Consequently, rather than attempt to suppress the ZQTs, we decided to shift them away from the positions of the cross peaks by the systematic incrementation of the delay between the two mixing pulses by a factor χt_1 using the pulse sequence $90°$–t_1–β–$\tau_m(t_1)$–β–t_2, where $\tau_m(t_1) = \tau_0 + \chi t_1$.

The measurement of coupling constants from cross-peak multiplets is achieved in the manner illustrated in Fig. 1. A theoretical cross peak of a z-COSY spectrum, similar to one expected between the H1′ and H2″ protons of the deoxyribose unit of a nucleotide, is shown. The dominant multiplet components are indicated by large circles and the suppressed components by the sign of the amplitude they would have with larger flip angles for β. Two groups of dominant peaks appear. Both consist of two squares shifted along ω_1 by the value of the coupling $J_{H2'-H3'}$. The two groups are displaced with respect to each other by $J_{H1'-H2'}$ in ω_2 and $J_{H2'-H2''}$ in ω_1. The value for the coupling $J_{H1'-H2'}$ is extracted by comparing the frequency at the center of the doublets appearing in the rows marked with arrows on the left-hand side of the figure *providing* the frequencies of the two doublets are not distorted by interference from the residual undesired multiplet components. For a z-COSY experiment with $\beta = 20°$, their amplitude is 6% of the dominant components. This is still small compared to the noise level in a typical spectrum of a macromolecule.

To determine that distortions caused by interference of the dominant and recessive multiplet components are less than 1 Hz, which represents a typical experimental

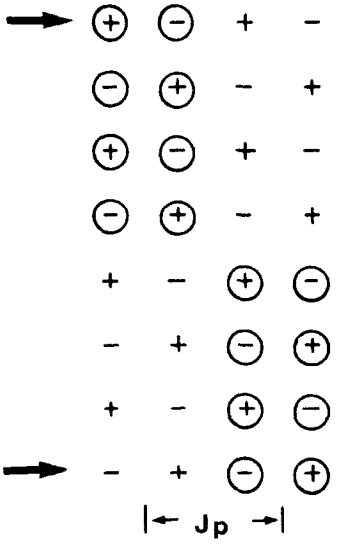

FIG. 1. Schematical diagram of a cross peak in a z-COSY spectrum between the H1′ and H2″ protons of the deoxyribose unit of a nucleotide. The dominant components are indicated by circles, while the positions of the suppressed components are given by the signs that they would have for $\beta = 90°$, assuming positive progressive signals. The ratio of the couplings is $J_{H2'-H2''} = 2J_{H1'-H2'} = 2J_{H2'-H3'} = 4J_{H1'-H2''}$. The coupling $J_{H1'-H2'}$ appears as a passive coupling in this multiplet and leads to the splitting indicated by J_p. It can be evaluated by comparison of the positions of the doublets in the traces indicated by arrows.

digital resolution for such experiments at 500 MHz, a series of simulations were carried out. An antiphase doublet with a splitting of 5.4 Hz and a linewidth of 3 Hz was created and "undermined" step by step with a doublet having the same splitting and linewidth but only 6% intensity. The results indicate that the deviations in the position of the antiphase doublet arising from the presence of the 6% residual signal are $\leq$0.2 Hz.

The region comprising all the H1′–H2′ and H1′–H2″ *z*-COSY cross peaks for the hexamer 5′d(GCATGC)$_2$ is shown in Fig. 2. The appearance of the cross peaks differs from the idealized scheme in Fig. 1 owing to the different magnitude of the couplings to the third spin (i.e., the H3′ proton). The $J_{1'2'}$ and $J_{1'2''}$ coupling constants, however, can still be obtained by comparing the upper or lower trace of each cross peak, as indicated at the H1′–H2″ cross peak of residue C_2 at 2.49/5.68 ppm. The $J_{1'2'}$ coupling, given by the splitting shown in Fig. 2, is 9.9 $\pm$ 0.5 Hz, and the $J_{1'2'}$ coupling is 4.1 $\pm$ 0.5 Hz. For residue T_4 the corresponding values are 9.0 $\pm$ 0.5 and 5.8 $\pm$ 0.5 Hz. Using the Karplus relations given in (*10*), these values correspond to sugar pucker conformations in the C2′-endo and O1′-endo ranges, respectively, consistent with the findings from restrained molecular dynamics calculations on the basis of NOE data (*11*).

Some care has to be taken when the signals arising from zero-quantum coherences precessing in the delay τ_m are left in the spectrum but shifted with respect to the cross and diagonal peaks. In the case of the conventional NOESY experiment, that is, for $\beta = 90°$, a variety of ZQTs are observed due to different excitation and detection pathways. In an AMX spin system for example, a single quantum coherence of spin A can be converted into the ZQT involving A and one of the two coupling partners by the direct excitation pathway $\underline{A}MX \rightarrow \underline{AM}X, \underline{AM}\underline{X}$. In addition, the remote excitation pathway $\underline{A}MX \rightarrow A\underline{MX}$ leads to a ZQT between the M and X spins. Similarly, there are direct and remote detection pathways. In contrast to the normal NOESY experiment, however, some of the ZQTs in a *z*-COSY spectrum have a smaller apparent amplitude due to the small mixing pulses.

The number of ZQTs appears rather large, but most can be shifted into regions of the spectrum not normally occupied by proton resonances. This is particularly so in the case of nucleic acid and protein spectra, where the principal chemical-shift ranges of the resonances are known beforehand. There are, for example, 10 different signals due to ZQTs grouped around a cross peak between the resonances of the H2″ and H1′ protons of a sugar residue. These 10 signals are the echo and anti-echo components of five different ZQTs, which can be excited from the single-quantum coherence of H2″ and converted into the single-quantum coherence of H1′, with the frequencies ω(H1′)–ω(H2′), ω(H1′)–ω(H2″), ω(H2′)–ω(H2″), ω(H2′)–ω(H3′), and ω(H2″)–ω(H3′). As there is no resolved coupling between the H1′ and H3′ protons, the ZQT between these protons cannot be reconverted into observable magnetization of the H1′ proton. The ZQTs appear in the spectrum at frequencies $\omega_1 = \omega(H2'') \pm \chi\omega(ZQT)$. With a value of $\frac{1}{3}$ for χ nearly all the ZQTs appear in the regions between 3.3 and 3.7 ppm and 0.8 and 1.17 ppm, quite far away from the H1′–H2″ and H1′–H2′ cross peaks. Only the ZQT with the frequency $\chi(\omega(H_2')-\omega(H_2''))$ appears close to the H1′–H2″ and H1′–H2′ cross peaks. Its amplitude, however, is small as it arises through remote detection.

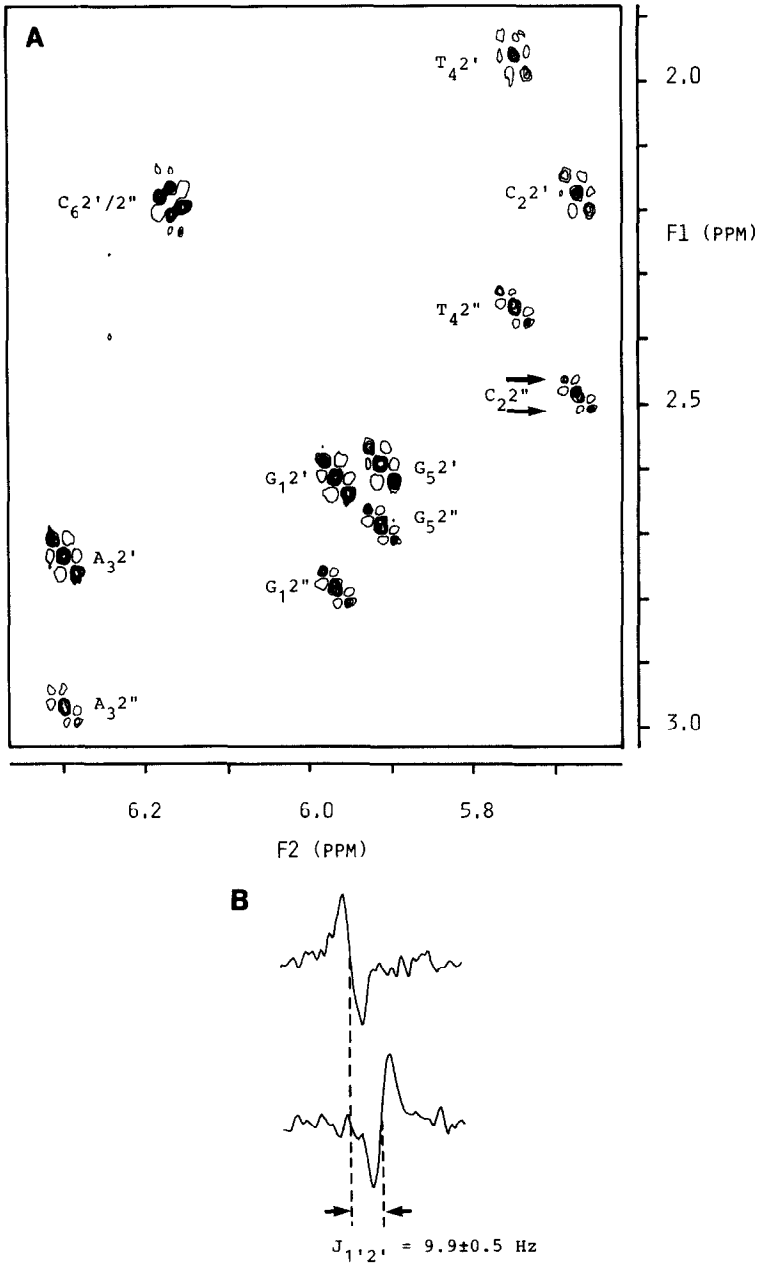

FIG. 2. (A) H1′ (ω_2 axis)–H2′/H2″ (ω_1 axis) region of the *z*-COSY spectrum of 5′d(GCATGC)$_2$. (B) Sections along the ω_2 axis at the positions of the upper and lower traces of the H1′–H2″ cross peak of residue C_2 (indicated by arrows in (A)). The pulse sequence used was $90°$–t_1–$20°$–$\tau_m(t_1)$–$20°$–t_2, where $\tau_m(t_1) = \tau_0 + \frac{1}{3}t_1$ with a value of 2 μs for τ_0. The spectrum was processed with smooth Gaussian weighting functions in both dimensions and the digital resolution, obtained by appropriate zero-filling, is 0.45 Hz/point. Progressive signals have been filled in with contours (plotted on a logarithmic scale). Note that three small signals have not been filled in due to their weaker amplitude. The assignments are taken from (*11*). Experimental conditions: 2 m*M* oligonucleotide in 99.96% D_2O containing 500 m*M* KCl, 50 m*M* phosphate buffer, pH 6.8, and 0.02 m*M* ethylenediamine tetraacetate; the spectrum was recorded at 20°C.

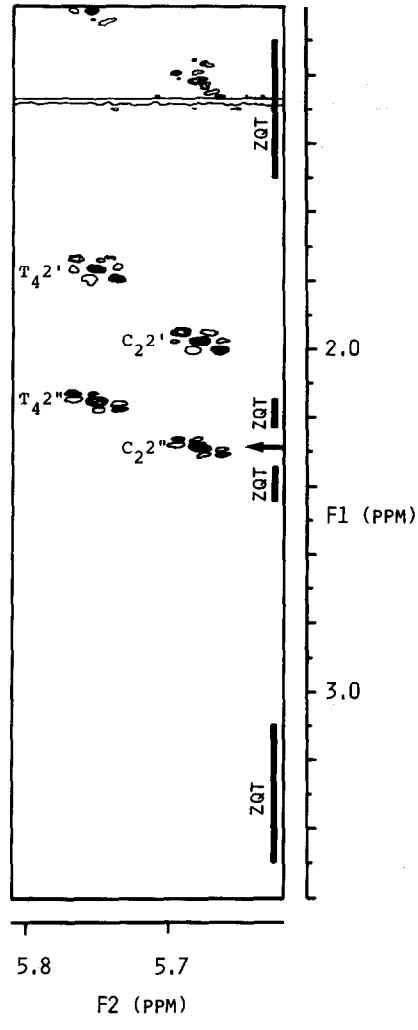

FIG. 3. The region of the *z*-COSY spectrum of 5'd(GCATGC)$_2$ comprising cross peaks between the H1' protons of residues C_2 (5.68 ppm) and T_4 (5.75 ppm) and the corresponding H2' and H2" protons. Signals representing progressive connections are filled with contours. The ZQTs are shifted in the spectrum by a factor of $\frac{1}{2}$ of their frequency with respect to the chemical shift of the nucleus from which they originate (see text). All ZQTs that originate from single-quantum coherence of the H2" proton (arrow) of residue C_2 in ω_1 and that can be converted into single-quantum coherence of the H1' proton (see text) have been calculated from the known chemical-shift values. The ranges in which they appear are indicated at the left edge of the figure. Noticeably, only one ZQT is of significant amplitude, visible at ω_1 of $\sim$1.4 parts per million. The experimental conditions are the same as in Fig. 2.

These features are illustrated in Fig. 3, which presents a strip of a *z*-COSY spectrum containing the H1'–H2' and H1'–H2" cross peaks of residues C_2 and T_4 together with their associated ZQTs. The positions for the ZQTs belonging to the H1'–H2" cross peak of residue C_2 (marked by an arrow) are indicated at the right of the spectrum. As can be seen, no complications arise in this case. The multiplet structures of the

H1′–H2′ and H1′–H2″ cross peaks belonging to the same sugar residue can be readily analyzed, as exactly the same pattern of ZQTs is grouped around the H1′–H2′ cross peak. The only ZQT with significant amplitude originating from single-quantum coherence of H2″ and observed in ω_2 at the frequency of the H1′ resonance occurs at $\sim$1.4 parts per million with the frequency $\omega(H2'')-\frac{1}{3}(\omega(H1')-\omega(H2''))$. All other ZQTs have much smaller amplitudes. Obviously, the situation becomes more complicated, when several H1′ resonances appear at the same chemical shift. It is also evident that most of the ZQTs can be eliminated by symmetrization, although we recommend that they be identified rather than removed.

In conclusion we have shown that *z*-COSY is a useful experiment to determine coupling constants in macromolecules providing suitable care is taken to shift the ZQTs appropriately. Further the accuracy is principally limited by the digital resolution providing a relatively small value of β ($\leqslant 20°$) is used for the two mixing pulses.

ACKNOWLEDGMENTS

This work was supported by the Max-Planck Gesellschaft and Grant 321/4003/0318909A from the Bundesministerium für Forschung und Technologie (G.M.C. and A.M.G.).

REFERENCES

1. W. P. Aue, E. Bartholdi, and R. R. Ernst, *J. Chem. Phys.* **64,** 2229 (1976).
2. A. Bax and R. Freeman, *J. Magn. Reson.* **44,** 542 (1981).
3. M. H. Levitt, C. Radloff, and R. R. Ernst, *Chem. Phys. Lett.* **114,** 435 (1985).
4. C. Griesinger, O. W. Sørensen, and R. R. Ernst, *J. Am. Chem. Soc.* **104,** 6800 (1982).
5. H. Oschkinat, A. Pastore, P. Pfändler, and G. Bodenhausen, *J. Magn. Reson.* **69,** 559 (1986).
6. P. Pfändler and G. Bodenhausen, *J. Magn. Reson.* **72,** 475 (1987).
7. S. Macura, Y. Huang, D. Suter, and R. R. Ernst, *J. Magn. Reson.* **43,** 259 (1981).
8. S. Macura, K. Wüthrich, and R. R. Ernst, *J. Magn. Reson.* **46,** 269 (1982).
9. M. Rance, G. Bodenhausen, G. Wagner, K. Wüthrich, and R. R. Ernst, *J. Magn. Reson.* **62,** 497 (1985).
10. D. B. Davies, *Prog. NMR Spectrosc.* **12,** 135 (1985).
11. M. Nilges, G. M. Clore, A. M. Gronenborn, A. T. Brunger, M. Karplus, and L. Nilsson, *Biochemistry* **26,** 3718 (1987).