Identification of glycine spin systems in ^{1}H NMR spectra of proteins using multiple quantum coherences

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Double-quantum filtered COSY and triple-quantum filtered COSY techniques have been compared for the tripeptide Gly-Tyr-Gly and for human lysozyme. The insertion of a triple-quantum filter in the COSY experiment leads to dramatic spectral simplification in the fingerprint region of the spectrum and permits the specific identification of glycine spin systems in the complex ^{1}H NMR spectra of proteins. The assignment of these peaks to glycine H^α can be confirmed using 2D double-quantum correlated spectroscopy.

^{1}H NMR *Assignment* *Glycine* *Human lysozyme* *Multiple-quantum NMR*

1. INTRODUCTION

Multiple-quantum coherences have proved particularly useful for the interpretation of the ^{1}H NMR spectra of proteins [1–5]. Two-dimensional double-quantum spectroscopy has been applied to the identification of spin systems in the aromatic and methyl regions of the NMR spectrum of hen lysozyme [1,2]. An important advantage of this 2D technique is the spectral simplification which is achieved by proper selection of the preparation period τ in the INADEQUATE sequence [6,7]. Recently, double-quantum filtration has been used in the COSY experiment to reduce the intensity of the diagonal [8,9]. This reduction arises because, in contrast to the COSY spectrum, diagonal peaks have antiphase fine structure in the DQF COSY spectrum and because singlet resonances are removed from the diagonal by the double-quantum filter. Using this technique is it possible to identify pairs of coupled resonances which give rise to cross peaks close to the diagonal. The double-quantum filtered COSY experiment does not, however, give rise to any spectral simplification in the off-diagonal regions of the spectrum because any pair of spins A and X which give rise to a cross peak in the COSY spectrum will also give rise to a double-quantum coherence A + X which leads to a cross peak in the DQF COSY spectrum.

The extension of the multiple-quantum filtered COSY technique to higher order coherences should, however, lead to simplification both for the diagonal peaks and also for the cross peaks. This follows from the multiple quantum selection rules described by Braunschweiler et al. [10]. Systems of less than p coupled spins will be removed by a p-order filter [11]. In addition, a complete set of diagonal and cross peaks will only be observed if all the couplings between the p spins directly involved in the p-order coherence are resolved. Recently, other more complex pulse sequences aimed at the recognition of specific spin patterns in complex NMR spectra have been described [12].

One of the simplest spin systems in protein NMR spectra is that given by glycine. The H^N and two H^α resonances of glycine form a 3-spin system in which all 3 couplings are resolved. Thus, glycine is expected to give rise to both cross peaks and

Abbreviations: DQF, double-quantum filtered; TQF, triple-quantum filtered

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diagonal peaks in the TQF COSY spectrum. In all the other common amino acids the coupling between H^N and H^β is vanishingly small and the H^N, H^α and H^β resonances form linear 3-spin systems in which extensive cancellation of both diagonal peak and cross peak multiplets is expected in the TQF COSY spectrum. This cancellation will give rise to spectral simplification in the fingerprint region of the spectrum. Here we demonstrate that the triple-quantum filtered COSY experiment can be used for the specific detection of glycine spin systems in the complex NMR spectra of proteins.

2. MATERIALS AND METHODS

The present experiments were performed using home-built 470 MHz and 500 MHz NMR spectrometers employing Oxford Instrument Co. magnets and a GE/NICOLET 1280 data acquisition system. Human lysozyme (Sigma) was dissolved in D_2O, pH 4.5, at 7 mM. The tripeptide Gly-Tyr-Gly (Vega Biochemicals) was dissolved in d_6-DMSO at 50 mM.

The multiple-quantum filtered COSY experiments were carried out using the pulse sequence

$$90°-t_1-90°-\tau-90°-t_2$$

with τ set to 15 μs [8]. Double-quantum or triple-quantum coherences were selected using the phase cycling scheme described by Wokaun and Ernst [14]. The appropriate phase cycling for N-peak selection [11] and CYCLOPS [13] were superimposed on this scheme giving a minimum of 32 and 48 transients for the DQF COSY and TQF COSY experiments, respectively. The $\pi/3$ phase shifts necessary for the TQF COSY experiment were obtained using an analogue phase shifter [15].

The time-domain data matrix for the tripeptide DQF COSY and TQF COSY experiments was 1024 × 2048 and sweep widths of ± 2000 Hz were used in both the F_1 and F_2 dimensions. A total of 32 and 48 transients were collected for the DQF and TQF COSY experiments, respectively.

The time-domain data matrix for the lysozyme DQF and TQF COSY experiments was 1024 × 2048 and sweep widths of ± 3012 Hz were used in both dimensions. A total of 64 and 96 transients were collected for the DQF and TQF COSY experiments, respectively.

The double-quantum correlated experiment was carried out using the INADEQUATE pulse sequence [6,7]

$$90°-\tau-180°-\tau-90°-t_1-\alpha-t_2$$

as described in [1]. Preparation period delays of τ = 15 and 30 ms were used and the detection pulse, α, was set to 135° to minimize correlations arising from remote connectivities [7]. Sweep widths of ± 5263 and ± 10526 Hz were used in F_2 and F_1, respectively. The time-domain data matrix was 512 × 4096 and a total of 64 transients was collected.

3. RESULTS AND DISCUSSION

The double- and triple-quantum filtered COSY experiments have been compared for the tripeptide Gly-Tyr-Gly which contains a variety of spin systems. The 1D NMR spectrum of the tripeptide is shown in fig.1A. In this tripeptide the two H^α resonances of both glycine residues are non-equivalent. Each H^α resonance of Gly-3 is composed of 4 lines arising from coupling to H^N and the other H^α. The H^N, $H^{\alpha 1}$ and $H^{\alpha 2}$ resonances of Gly-3 form a 3-spin ABX system in which none of the couplings is zero. The H^α resonances of Gly-1 appear as doublets and no resonance is observed for the NH_2 or NH_3^+ group. These protons may exchange with a small amount of water present in the sample leading to decoupling of the two α resonances [16]. Thus, the $H^{\alpha 1}$ and $H^{\alpha 2}$ resonances of Gly-1 form a 2-spin AB system. The H^N, H^α, $H^{\beta 1}$ and $H^{\beta 2}$ resonances of Tyr-2 form a 4-spin AMQX system in which the couplings between H^N and $H^{\beta 1}$ or $H^{\beta 2}$ ($^4J_{N\beta 1}$, $^4J_{N\beta 2}$) are vanishingly small and cannot be detected in the 1D spectrum.

The DQF COSY spectrum of the tripeptide is shown in fig.1A. All cross peaks expected on the basis of the coupling patterns in the tripeptide are observed. Cross peaks between Tyr-2 H^N and $H^{\beta 1}$ or $H^{\beta 2}$ are not observed because the active couplings, $^4J_{N\beta 1}$ and $^4J_{N\beta 2}$, are vanishingly small. The TQF COSY spectrum of the tripeptide is shown in fig.1B. Comparison of fig.1A and B indicates that 2 groups of peaks present in the DQF COSY spectrum are absent in the TQF COSY spectrum. First, the diagonal and cross peaks of Gly-1 $H^{\alpha 1}$ and $H^{\alpha 2}$ are completely absent from the 2D spectrum. These protons form a 2-spin system which is not

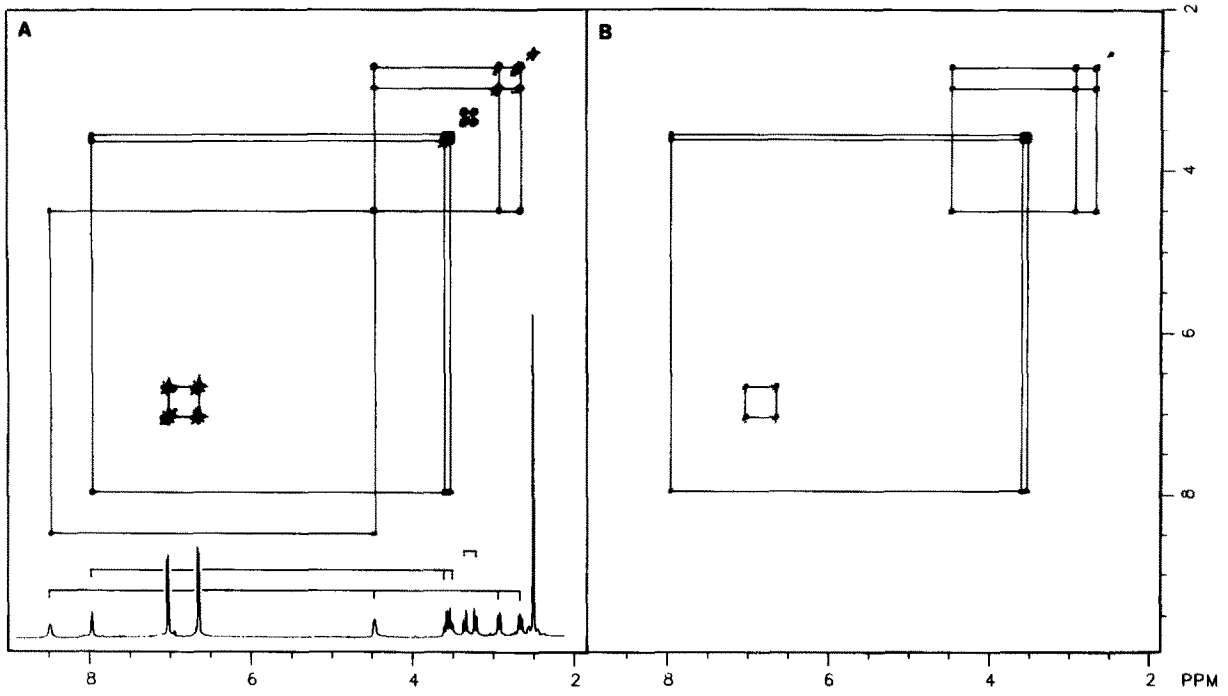

Fig.1. (A) 500 MHz 2D double-quantum filtered COSY spectrum of the tripeptide Gly-Tyr-Gly. Groups of coupled resonances are indicated in both the 1D and 2D spectra. (B) 500 MHz 2D triple-quantum filtered COSY spectrum of the tripeptide. Note the disappearance of the diagonal and cross peaks of Gly-1 and Tyr-2 H^N and H^α.

expected to give rise to triple-quantum coherence. Second, the diagonal peak of H^N and the cross peak between H^N and H^α of Tyr-2 are absent. The observation of a cross peak between a pair of resonances A and X in the TQF COSY spectrum requires that the active coupling between these two resonances, J_{AX}, is resolved and that the active couplings between A, X and a third spin M, J_{AM} and J_{MX}, are also resolved. In tyrosine the couplings $J_{N\alpha}$, $J_{\alpha\beta_1}$ and $J_{\alpha\beta_2}$ are non-zero but $J_{N\beta_1}$ and $J_{N\beta_2}$ are vanishingly small and no cross peak is observed between H^N and H^α. The H^α, H^{β_1} and H^{β_2} protons of Tyr-2 do, on the other hand, form a 3-spin system with all non-zero couplings which gives rise to cross peaks as expected. The experiments carried out on the tripeptide demonstrate that a triple-quantum filter can lead to spectral simplification both on the basis of the number of coupled spins and on the nature of the coupling within the spin systems.

The fingerprint region of the double-quantum filtered COSY spectrum of human lysozyme is shown in fig.2A. Cross peaks arising from H^N and H^α of more than 85% of the amino acid residues can be identified. Glycine residues should give a characteristic pattern in the COSY spectrum. Two cross peaks with F_1 values equal to the two H^α chemical shifts should be found for each of the H^N chemical shifts in F_2. However, the significant degree of overlap in the H^N and H^α regions of the human lysozyme spectrum makes the identification of glycine spin systems difficult. The triple-quantum filtered COSY spectrum of human lysozyme is shown in fig.2B. Application of the triple-quantum filter leads to a dramatic simplification of the fingerprint region of the spectrum. The majority of peaks present in the DQF COSY spectrum are absent in the TQF COSY spectrum of human lysozyme. Eight pairs of cross peaks with the characteristic glycine pattern can be identified in the TQF COSY spectrum. The remaining 3 glycine residues could be absent from the spectrum for a number of reasons. For example, the H^N or H^α resonances could be saturated by the 1 s presaturation pulse used to suppress the H_2O signal, the H^N or H^α resonances could be

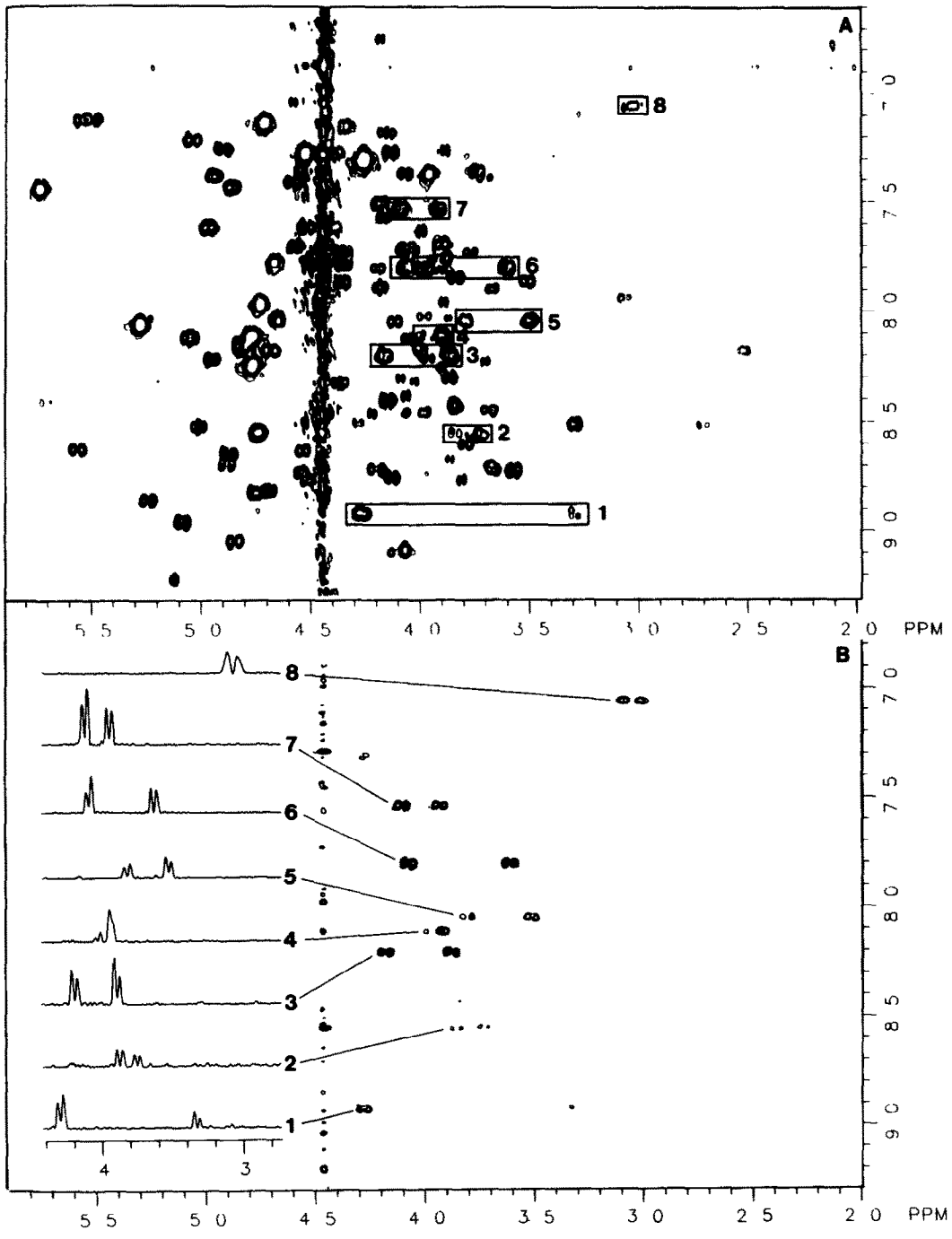

Fig.2. (A) 500 MHz 2D double-quantum filtered COSY spectrum of human lysozyme. (B) 500 MHz 2D triple-quantum filtered COSY spectrum of human lysozyme. Eight cross sections parallel to F_1 showing the H^{α} resonances of the 8 glycine spin systems identified in the TQF COSY spectrum are shown. These glycine spin systems are indicated in (A).

broad under these conditions of pH and temperature or the magnetic equivalence of the two H^{α} resonances could lead to peak cancellation.

The assignment of the peaks in the TQF COSY spectrum to glycine can be confirmed using 2D double-quantum correlated spectroscopy. In the absence of H^{N} protons the H^{α} protons of glycine form a 2-spin system. It has been shown that for an AX system double-quantum coherence is created with maximum efficiency when a preparation period delay of $\tau = 1/4\ J$ is used in the INADEQUATE pulse sequence. When a τ value of $1/2\ J$ is used no double-quantum coherence is created [6,7]. The coupling between the H^{α} protons of glycine is approx. -16 Hz. The double-quantum spectra of human lysozyme obtained with τ values of $1/4\ J$ (15 ms) and $1/2\ J$ (30 ms) are shown in fig.3. Several pairs of peaks symmetrically disposed about the skew diagonal can be seen in the $1/4\ J$ spectrum. The F_2 chemical shift values of 8 of these pairs of peaks corresponded to the values found in the H^{α} region of the TQF COSY spectrum. The double-lobed shape of the peaks in the double-quantum spectrum reflects the -16 Hz coupling constant of the glycine H^{α} doublets. The 8 pairs of symmetric double-lobed peaks are absent from the $1/2\ J$ spectrum as expected for AX spin systems.

The insertion of a triple-quantum filter in the COSY experiment leads to a dramatic simplification of the 2D spectrum. This simplification has been demonstrated for the fingerprint region of the human lysozyme spectrum where only glycine H^{N}-H^{α} cross peaks are observed in the TQF COSY experiment. Similar spectral simplification is expected to occur, for example, in the H^{α} and H^{β} region of the 2D spectrum [14,15]. Using the basic method for excitation of multiple quantum coherences, where all the transitions are excited approximately to an equal extent, there will be a reduction in sensitivity as higher order coherences are selected [17]. However, although there is some sensitivity reduction between the double and triple-quantum filtration experiments this reduction does not significantly degrade the signal-to-noise ratio of the final 2D spectrum.

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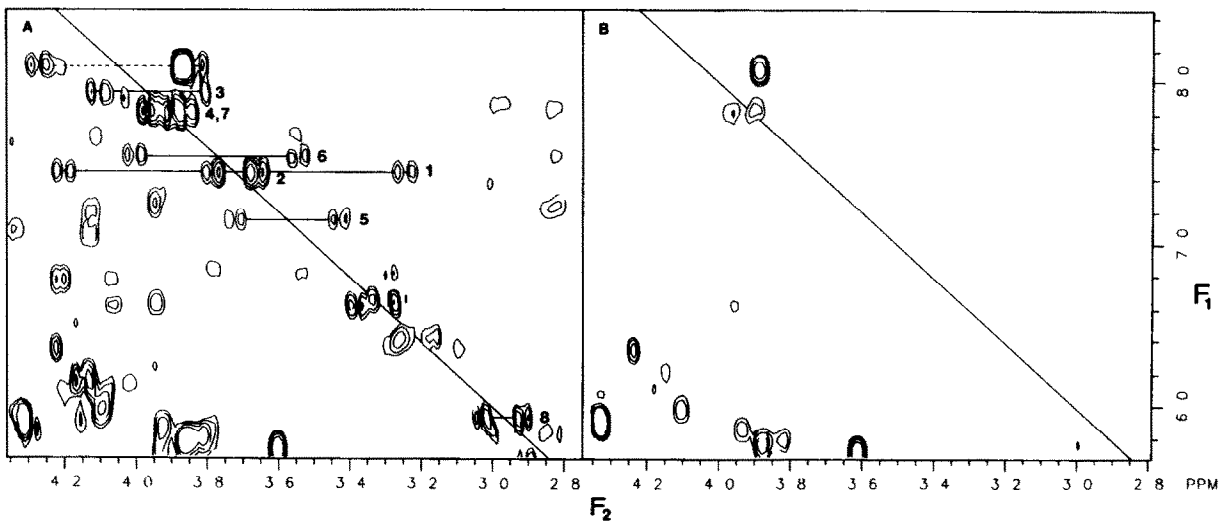

Fig.3. 470 MHz 2D double-quantum correlated spectra of human lysozyme obtained with preparation periods delays of (A) $1/4\ J$ and (B) $1/2\ J$. The skew diagonal is shown. Double-quantum frequencies are found in F_1 and single-quantum frequencies in F_2. The numbering of pairs of peaks in (A) corresponds to that used in fig.2.

REFERENCES

[1] Boyd, J., Dobson, C.M. and Redfield, C. (1983) J. Magn. Reson. 55, 170–176.
[2] Boyd, J., Dobson, C.M. and Redfield, C. (1985) J. Magn. Reson., in press.
[3] Wagner, G. and Zuiderweg, E.R.P. (1983) Biochem. Biophys. Res. Commun. 113, 854–860.
[4] Rance, M., Sørensen, O.W., Leupin, W., Kogler, H., Wüthrich, K. and Ernst, R.R. (1985) J. Magn. Reson. 61, 67–80.
[5] Hore, P.J., Scheek, R.N. and Kaptein, R. (1983) J. Magn. Reson. 52, 339–342.
[6] Bax, A., Freeman, R., Frenkiel, T.A. and Levitt, M.H. (1981) J. Magn. Reson. 43, 478–483.
[7] Mareci, T.H. and Freeman, R. (1983) J. Magn. Reson. 51, 531–535.
[8] Piantini, U., Sørensen, O.W. and Ernst, R.R. (1982) J. Am. Chem. Soc. 104, 6800–6801.
[9] Rance, M., Sørensen, O.W., Bodenhausen, G., Wagner, G., Ernst, R.R. and Wüthrich, K. (1983) Biochem. Biophys. Res. Commun. 117, 479–485.
[10] Braunschweiler, L., Bodenhausen, G. and Ernst, R.R. (1983) Mol. Phys. 48, 535–560.
[11] Shaka, A.J. and Freeman, R. (1983) J. Magn. Reson. 51, 169–173.
[12] Levitt, M.H. and Ernst, R.R. (1983) Chem. Phys. Lett. 100, 119–123.
[13] Hoult, D.I. and Richards, R.E. (1975) Proc. R. Soc. Lond. A 344, 311–340.
[14] Wokaun, A. and Ernst, R.R. (1977) Chem. Phys. Lett. 52, 407–412.
[15] Boyd, J., Porteus, R., Redfield, C. and Soffe, N. (1985) J. Magn. Reson., in press.
[16] Frankel, L.S. (1970) J. Chem. Phys. 50, 943–950.
[17] Bax, A. (1982) in: Two-Dimensional Nuclear Magnetic Resonance in Liquids, D. Reidel, Dordrecht, The Netherlands.