Intensity-Based Measurement of Homonuclear Residual Dipolar Couplings from CT-COSY

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We present here a new procedure for measuring homonuclear spin−spin couplings based on analysis of a simple constant time COSY experiment. The measurement and assignment of homonuclear couplings in NMR spectra has, of course, been of interest to chemists and biochemists for a long time because of the useful structural information carried in the Karplus relationship between vicinal proton couplings and torsional angles defined by the intervening bonds.[1] The importance of measuring homonuclear couplings has, however, increased recently because of an interest in using structural information carried in residual dipolar contributions to spin−spin couplings when spectra are acquired in field-oriented media.[2−7] While most work has involved heteronuclear couplings in ^{13}C or ^{15}N enriched molecules, recent observations of residual couplings in proton spectra of unenriched molecules offer promise of much more general application.[8−10] The experiment described here will facilitate this application.

The development of methodology for measuring residual $^1H-^1H$ dipolar interactions is of interest because it avoids the necessity of isotopic labeling, and in the case of remote pairs of protons, distance information more akin to that available from NOEs might be obtained. In the latter case the measurements even have some potential advantages over NOEs in that the distance range may be longer because of the $1/r^3$ distance dependence of directly observed dipolar couplings as opposed to a $1/r^6$ distance dependence of NOEs. Additionally, direct dipolar coupling measurements for intermediate sized molecules may be more reliable because of a less explicit dependence on motional time scales.

With this new interest comes some additional problems: dipolar couplings occur by a through-space mechanism, so they are not confined to small groups of bonded spins. In general, more couplings and more complex multiplets occur. Couplings will also have a wide range of sizes, and often the smaller couplings are of most interest. This places extra demands on experiments for a clear identification of coupled pairs and quantitative extraction of small coupling values from complex multiplet structures. The experiment proposed offers solutions to these problems.

The problem of extracting coupling constants from multiplets that occur in simple isotropic solution spectra has existed for some time, and a number of very elegant procedures for the measurement of these couplings have been devised.[11−17] Most have involved analysis of frequency domain spectra and application is limited to small groups of coupled spins.

It is also possible to extract couplings based on intensities of cross-peaks in certain multidimensional experiments. This has become a very popular approach in the measurement of one-bond heteronuclear couplings,[18,19] as well as some three-bond proton−proton couplings in isotopically labeled proteins.[20] These approaches have however relied heavily on isotopic labeling with low abundance spins and most measurements have again been restricted to cases where coupling among rather small groups of spins is involved. In situations which involve larger numbers of interacting spins, as is frequently the case with homonuclear couplings, intensities transferred to cross-peaks are complex functions of many couplings. Below we describe an intensity based approach similar in concept to the heteronuclear HNHA experiment [20] that uses a constant time ^1H COSY spectrum on an unlabeled molecule to yield a simple function of the single active coupling in a given cross-peak. Thus, quantitative measures of couplings and an assignment of the coupling to a particular spin pair can be simultaneously achieved.

The constant time COSY is simply derived from early COSY experiments[21] by inserting a 180° pulse between the two 90° pulses in a standard COSY and moving it incrementally from the midpoint to one extreme of the 90° separation period, Δ. It has been highlighted recently based on the advantages it offers in terms of sensitivity improvement.[22] This improvement occurs partly because Δ can be chosen to optimize coherence transfer, and partly because the multiplets normally present in the indirect dimension collapse to a single resonance. Here we exploit a different property. In the absence of significant differential relaxation, cross-peak intensity for a simple pair of spins experiencing weak scalar (J) and weak dipolar coupling (D) is proportional to $\sin(\pi(J_{12} + D_{12})\Delta)$, and autopeak intensity under the same circumstances is proportional to $\cos(\pi(J_{12} + D_{12})\Delta)$. Hence the sum of dipolar and scalar coupling can be obtained from the arctan of the ratio of intensities all divided by $\pi\Delta$, that is $J + D = \arctan(I_{cross}/I_{auto})/\pi\Delta$. For more complex systems, for example a three-spin system, the additional couplings affect both types of peaks equally; $\sin(\pi(J_{12} + D_{12})\Delta)\cos(\pi(J_{13} + D_{13})\Delta)$ for the 1−2 cross-peak and $\cos(\pi(J_{12} + D_{12})\Delta)\cos(\pi(J_{13} + D_{13})\Delta)$ for the spin 1 autopeak. The ratio of intensities in the absence of differential relaxation of the two peaks is still given by $\tan(\pi(J_{12} + D_{12})\Delta)$. This basic relationship for a variety of spin systems can be verified by using product operator algebra as implemented in programs such as POMA.[23]

The intensities used in the above relationship are total intensities of magnetization, not phase-dependent amplitudes or integrals over frequency domains. Hence, at least the ratio of intensities must be determined independently of absolute or relative phase of multiplet components. The antiphase character of the cross-

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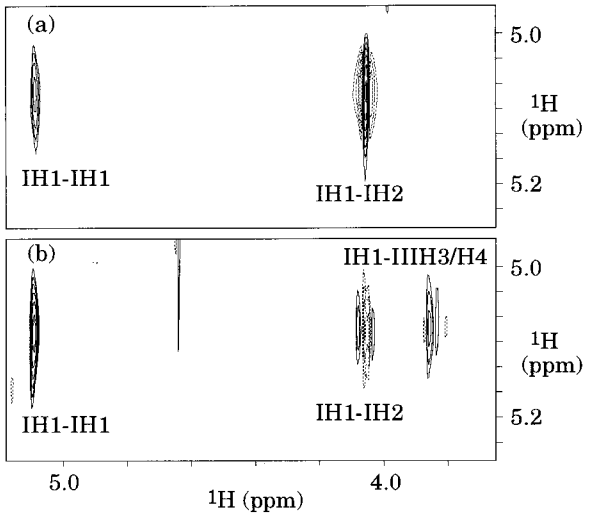

Figure 1. Sections of the CT-COSY spectra of trimannoside in dilute bicelle media at (a) 20 °C showing IH1 and IH2 isotropic connectivities and (b) 36 °C showing IH1, IH2, IIIH3, and IIIH4 oriented connectivities. The autopeaks (IH1) are shown absorptive in phase, consequently the cross-peaks are dispersive antiphase. Spectra were recorded on an Inova 500 with spectral widths of 4000 Hz in both dimensions, 1024 × 128 complex points which were apodized in both dimensions with a 90° shifted squared sinebell and zero filled to 2048 × 512.

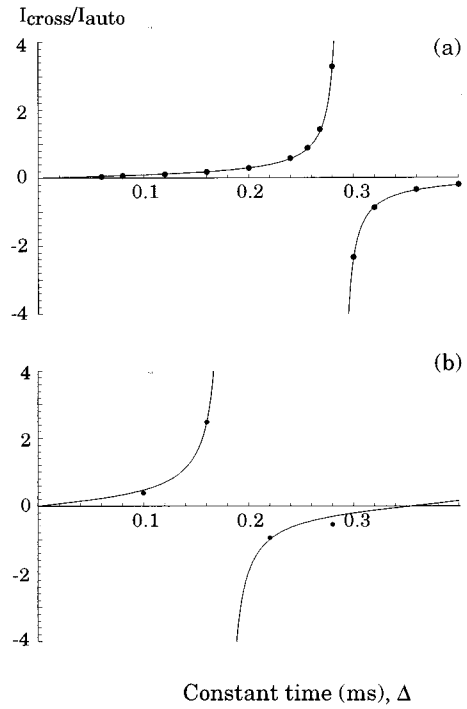

Figure 2. Plot of the intensity ratio I_{ross}/I_{auto} versus constant time value (Δ) for IH1−IH2, where the solid circle represents experimental data points. Best fit to the function $I_{cross}/I_{auto} = k \tan(\pi(J + D)\Delta)$ allows determination of $(J + D)$: istropic case: $(J + D) = 1.730 \pm 0.001$ Hz, scaling factor $k = 0.15 \pm 0.001$; oriented case: $(J + D) = 2.8 \pm 0.2$ Hz, scaling factor $k = 0.39 \pm 0.04$.

peak and phase-shifted character of the autopeak in the experiment described do present a problem in terms of direct determination of intensities by processes such as integration.

An approach that avoids this complication involves the collection of two or more CT-COSY spectra at different values of Δ. The transfer function of the last hard pulse in the proposed sequence ensures a line shape that is approximately independent of Δ for peaks in the directly detected domain. Plotting the observed ratio of cross-peak and autopeak amplitudes as a function of Δ, using any convenient directly detected frequency domain point as a measure of amplitude, then yields a graph that can be fit to extract the active coupling for the cross-peak. The use of measured frequency domain amplitude as opposed to actual full coherence intensity necessitates the introduction of a multiplet-dependent scaling factor that relates amplitude ratios to intensity ratios. The discontinuity in the plot of the tangent function at $\Delta = 1/(2(J + D))$ contributes significantly to the precision in the measurement of $(J + D)$. In practice data are used more effectively if integrals replace amplitudes in the above procedure. Integration can be performed separately on cross-peaks and autopeaks in a magnitude version of the data set. To achieve slightly better resolution the autopeak can be phased absorptive and integrated before phase shifting the rows of the two-dimensional set, changing the set to a magnitude spectrum, and integrating the cross-peak. The latter procedure was used in processing the data which follow.

Below we illustrate application of the procedure to a simple oligosaccharide in a liquid crystal "bicelle" medium that is ordered at 36 °C and isotropic at 20 °C.[24] At 36 °C this medium leads to a departure from isotropic distribution of molecular orientations of solutes and residual dipolar coupling contributions. The oligosaccharide is α(1-3)-mannosyl-[α(1-6)-mannosyl]-α-methylmannoside. It is dissolved at a concentration of 20 mM in a liquid crystal composed of 10% w/v dimyristoylphosphatidylcholine (DMPC)−dihexanoylphosphatidylcholine (DHPC), 3:1 molar ratio, in D_2O. We have reported previously the simple observation of through-space dipolar couplings in this molecule using a double quantum filtered COSY experiment, but quantification of couplings was difficult.[8] Figure 1 shows a section of a CT-COSY spectrum of the molecule in isotropic solution (a) and in the ordered liquid crystal phase (b). The data were acquired at 500 MHz with a Varian INOVA spectrometer using 128 t_1 points, each with 16 acquisitions and recovery delay of 2 s. Note the appearance of peaks that correspond to pure through-space connectivities in the ordered spectra (Figure 1b); for example, the peak between the H1 anomeric proton of the α(1-3) sugar, residue I, and the 3 and 4 trans glycosidic protons of residue III.

Figure 2 shows the ratio of cross-peak to autopeak intensity versus Δ for the IH1−IH2 pair. The plot is fit using the function $k \tan(\pi(J_{12} + D_{12})\Delta)$ where k is a scaling factor to account for misrepresentation of intensities in integrals. The excellent fit suggests that neglect of differential relaxation in our treatment is valid for this particular system. The derived splitting $(J_{12} + 0.0)$ for the isotropic phase (Figure 2a) was 1.730 ± 0.001 Hz, in agreement with a literature value of 1.6 ± 0.2 Hz.[25] The precision (root-mean-square deviation from the functional fit) for this set is high despite the fact that apparent line widths are large (∼10 Hz); the extensive cancellation of antiphase components in the cross-peak is reflected in the scaling factor of 0.15. The data for the oriented phase (Figure 2b) were collected with a much smaller number of Δ values and the scatter of points is larger, possibly due to additional line broadening from remote couplings. Nevertheless, a reasonably precise value of 2.8 ± 0.2 Hz, with a scaling factor of 0.39, is determined. It is larger due to the addition of a IH1−IH2 dipolar component to the splitting. In summary, we have presented a robust method for the assignment and determination of homonuclear splittings in cases where residual dipolar couplings abound.

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