COSY Cross-Peaks from $^1H-^1H$ Dipolar Couplings in NMR Spectra of Field Oriented Oligosaccharides

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Through space dipole−dipole interactions in oriented media, as first described by Saupe and Englert,[1] have recently been combined with other advances in high-resolution NMR spectroscopy to provide structural chemists with powerful tools for unraveling the complexities of biomolecular systems. For example, Tjandra and Bax have described the use of heteronuclear dipole interactions in ubiquitin[2] in a dilute bicelle system as a means for refining protein structures, and Sanders et al have described the use of heteronuclear dipole interactions in the structural characterization of membrane anchored glycolipids.[3] We would like to illustrate here use of similar data from an experiment that is homonuclear in nature and does not rely on isotope labeling. A very simple homonuclear proton−proton correlation spectroscopy (COSY) experiment is applied to an oligosaccharide in a field oriented medium. Oligosaccharides are members of a class of compounds for which structural determinations have traditionally been difficult to do. The experiment presented shows pure dipole−dipole coupling information as a cross-peak between protons for which a dipole interaction exists but no scalar coupling exists. For cases where scalar couplings are present the cross-peak arises as a result of the sum of the scalar and dipole couplings.

Three-dimensional structures of oligosaccharides and other moderate size natural products are often determined with nuclear Overhauser effects (NOEs) and three-bond scalar couplings from high-resolution proton NMR spectra.[4,5] While there are numerous examples of successful applications, these are not always straightforward because of complexities in interpretation of NOE data. NOEs, which arise from dipole−dipole interactions between pairs of protons, have both an interproton distance dependence ($1/r^6$) and a correlation time dependence (τ_c). The correlation time dependence is often eliminated by assuming that it arises from the tumbling of a rigid, approximately spherical molecule; the distance dependence then becomes a useful constraint in molecular structure determination. However, for molecules which are not rigid, one is never sure of the validity of simplifying assumptions regarding correlation times. Also, for moderate sized molecules such as oligosaccharides, the correlation time dependence can make NOEs go to zero independently of distance, making NOEs unmeasurable. We show here that the dipolar interaction can be measured in an alternate way, one that does not suffer from the above complexities. The method is based on the direct measurement of homonuclear dipolar couplings using a dilute aqueous liquid crystal medium (bicelle medium) that has recently been introduced for the investigation of protein structure and dynamics using heteronuclear NMR experiments.[2] We illustrate potential applications to oligosaccharides with data on a trisaccharide, methyl 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside, the molecule shown in Figure 1.

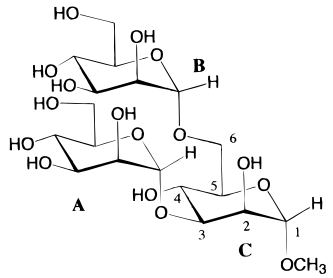

Figure 1. Structure of methyl 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside. For clarity, only anomeric protons are shown on the rings.

Dipolar couplings depend on both an internuclear distance, r, and an angle between the magnetic field and the internuclear vector, θ, as shown in eq 1:

$$D_{ij} = \xi_{ij}(3\cos^2\theta - 1)(1/r^3) \qquad (1)$$

where ξ_{ij} is an interaction constant that depends on the nuclear properties of nuclei i and j. Under normal solution NMR conditions molecules tumble, sampling orientations nearly isotropically. The θ-dependent term then averages to zero and the interaction can only be measured indirectly via relaxation phenomena such as the NOE. In the bicelle medium molecules still tumble rapidly, but because the medium orders with respect to the magnetic field, sampling of orientations is anisotropic. The θ-dependent term then does not average to zero and residual dipolar interactions enter as contributions to splittings of multiplets. Where through bond scalar couplings already exist, apparent coupling constants change; where they do not, new couplings arise. In either case the new contributions contain valuable distance- and angular-dependent structural information.

One of the easiest ways to demonstrate coupling between pairs of spins in complex molecules is through coupling correlated spectroscopy (COSY) data. A normal double quantum filtered COSY spectrum[6] of the trimannoside of Figure 1 is shown in Figure 2a. The sample is 2 mM in D_2O and the spectrum was collected at 39° on a Varian Inova 600 spectrometer. Figure 2a shows a single cross-peak to an autopeak from the corresponding H2 ring proton for each of the three anomeric protons, A−H1, B−H1, and C−H1. Note that there is no peak near 3.6 ppm for the *O*-methyl where the four-bond scalar coupling to the anomeric proton is too small to produce a cross-peak. Figure 2b shows a corresponding section from a double quantum filtered COSY experiment collected on a 2 mM trimannoside sample prepared in a 20 wt %/vol DMPC-DHPC/D_2O (1,2-ditetradecanoyl-*sn*-glycero-3-phosphocholine and 1,2-dicaproyl-*sn*-glycero-3-phosphocholine, 3:1 mol ratio) liquid crystal taken at 41 °C. Cross-peaks between anomeric protons and H2 ring protons still appear. They appear larger partly because resonances are broadened in this rather concentrated bicelle medium. However, we note that additional cross-peaks have appeared on the columns above each of the anomeric proton cross-peaks. They are at positions that correspond to A−H1 to C−H3, B−H1 to C−H6, and C−H1 to C−OMe connectivities.[7] There are also a number of other changes in the cross-peak patterns seen in simple solution (Figure 2b) that can be attributed to changes in apparent sizes of scalar

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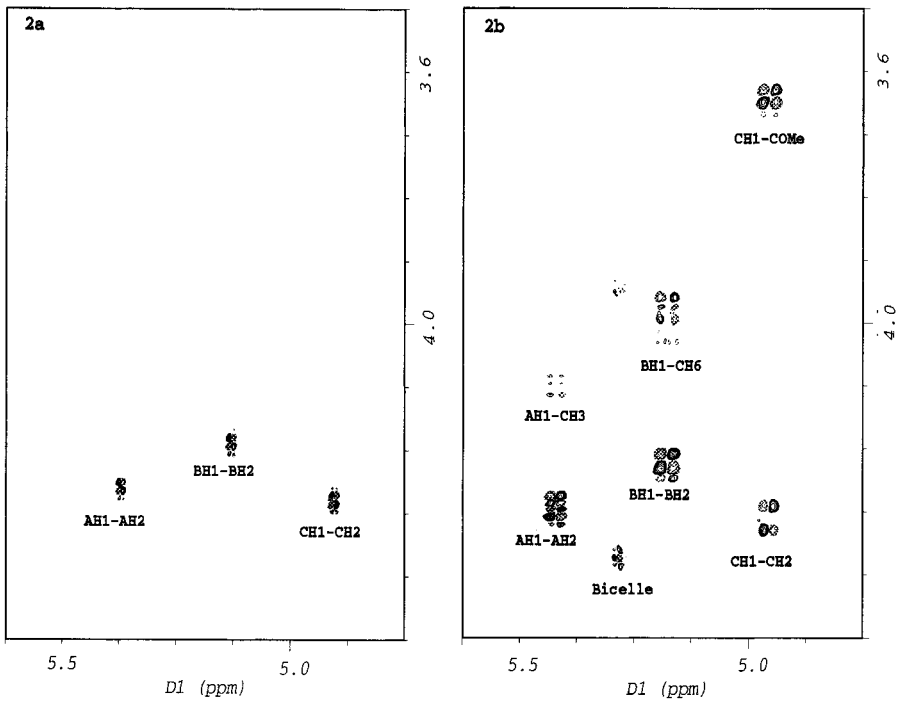

Figure 2. (a) DQF-COSY expansion of anomeric to H2 cross-peaks for residues A, B, and C. (b) DQF-COSY expansion of the same region showing the addition of cross-peaks due to through space dipolar couplings between anomeric and neighboring interresidue protons. Acquisition parameters for both data sets: 32 transients were collected for each direct dimension FID of 1024 complex points, with 256 complex points acquired in the indirect dimension. Spectral width was 3600 Hz with transmitter offset at 3.0 ppm. Each direct dimension FID was apodized with a 90° shifted squared sinebell and zero filled to 2048. Indirect FIDs were apodized with a 30° shifted squared sinebell and zero filled to 512 points, creating a 2048 × 512 matrix.

couplings. Another point of note is that there are almost no lipid signals apparent in the spectrum, despite the fact that the lipids are protonated and represent 20% of the weight of the sample. These lipids are actually part of bilayer disks that are very strongly oriented as a part of the liquid crystal. Large and numerous residual dipolar couplings in the lipid array make the resonances from the lipid very broad, and signals from these resonances do not survive the relatively long transfer periods required for a double quantum filtered COSY. Resonances from the weakly oriented soluble molecules remain sharp and survive the COSY transfer. Modified COSY experiments which introduce a coupling evolution delay prior to the t_1 evolution period can take advantage of the different T_2 behavior of the lipid and solute to further suppress lipid background.[8]

The new cross-peaks in Figure 2b represent pairs of protons that are spatially proximate, but that are not normally scalar coupled. Spatial proximity makes the appearance of dipolar couplings in the partially oriented sample likely (they depend on $1/r^3$). The couplings are relatively large in some cases, estimated to be 10 Hz for the CH1 to *O*-methyl pair. They also vary substantially, a fact that is reflected in the cross-peak intensity for similar pairs such as the AH1−CH3 and BH1−CH6 cross-peaks. Since these pairs lie on opposite sides of glycosidic bonds between residues, quantitation of the couplings could provide useful constrains on the allowed glycosidic torsion angles.

We do not attempt quantitative measurement of residual dipolar couplings here, nor will we attempt interpretation on a structural basis in this communication. Techniques for doing analogous measurements and interpretation are being worked out for heteronuclear applications to biomolecules.[9−11] However, we do wish to emphasize the feasibility of making measurements on soluble intermediate sized molecules, and point to the possible advantages of this type of measurement. First, the distance dependence of the coupling is $1/r^3$, rather than $1/r^6$, as in an NOE. This may make the measurements longer in range. Second, angular as well as distance information is available. For some interactions the presence increases complexity of analysis, but for intra ring connectivities, where interproton distances are known, there is a unique opportunity to constrain the orientation of one ring relative to another, even when they are spatially remote. We believe this and other factors point to substantial potential for these measurements, and we look forward to future applications.

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