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Sign determination of dipolar couplings in field-oriented bicelles by variable angle sample spinning (VASS)

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Abstract

Residual dipolar couplings are being increasingly used as structural constraints for NMR studies of biomolecules. A problem arises when dipolar coupling contributions are larger than scalar contributions for a given spin pair, as is commonly observed in solid state NMR studies, in that signs of dipolar couplings cannot easily be determined. Here the sign ambiguities of dipolar couplings in field-oriented bicelles are resolved by variable angle sample spinning (VASS) techniques. The director behavior of field-oriented bicelles (DMPC/DHPC, DMPC/CHAPSO) in VASS is studied by ^{31}P NMR. A stable configuration occurs when the spinning angle is smaller than the magic angle, 54.7°, and the director (or bicelle normal) of the disks is mainly distributed in a plane perpendicular to the rotation axis. Since the dipolar couplings depend on how the bicelles are oriented with respect to the magnetic field, it is shown that the dipolar interaction can be scaled to the same order as the J-coupling by moving the spinning axis from 0° toward 54.7°. Thus the relative sign of dipolar and scalar couplings can be determined.

Introduction

Residual dipolar couplings are being increasingly used as structural constraints for NMR studies of biomolecules (Tolman et al., 1995; Tjandra and Bax, 1997; Clore and Gronenborn, 1998; Hansen et al., 1998; Kiddle and Homans, 1998; Prestegard, 1998). The angular dependence of the couplings is particularly pronounced, with couplings changing from positive to negative values as the preferred orientation of an internuclear vector relative to the magnetic field changes from parallel to perpendicular. Even a simple determination of the sign of the dipolar coupling would yield valuable structural information. Unfortunately, it is seldom possible to directly determine the sign of the dipolar couplings and ambiguities in determining orientations arise. An exception occurs when a small residual dipolar coupling adds to a large scalar coupling (J-couplings) of the same nuclear pair. This pertains to investigations of directly bound N-H or C-H pairs in dilute bicelle media, where the dipolar coupling produces small increases or decreases in splittings dominated by a scalar coupling of known sign. In many cases, however, the residual couplings are larger than the J-couplings. ^{1}H-^{1}H homonuclear dipolar couplings in an oligosaccharide oriented in a weakly aligned bicelle, such as were measured recently using CT-COSY experiments, are one such case (Tian et al., 1999). In highly ordered bicelle systems, typically used as membrane mimetics, the residual dipolar couplings are also much larger than the scalar couplings since the studied molecules associate strongly with the bicelles. In such cases it is difficult to unambiguously determine signs of the dipolar couplings (Sanders et al., 1994). Here we present one solution to that problem.

A few approaches to determine signs of dipolar couplings have been explored in the past. One approach employs media variation, such as varying the relative concentration of the short chain lipid, or changing the temperature, to gradually scale down

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the dipolar couplings. Scalar couplings remain constant during such processes. Therefore, if the splitting passes through a null before reaching the isotropic value, the scalar and the dipolar term must be of opposite sign (Sanders and Prestegard, 1991). A second method measures the rate of Hartmann–Hahn transfer between two spins in the rotating frame and compares this measurement to the splitting in directly observed 1D spectra. The dipolar and scalar coupling contributions are different in these two experiments, thus the relative sign can be determined (Tolman and Prestegard, 1995). It is also possible to determine relative signs of coupling in three-spin systems using an E-COSY style experiment (Griesinger et al., 1985), selective decoupling as well as analysis of second-order effects (Becker, 1980). All of the above suffer limitations in requiring media manipulation, the presence of unique spin properties, or the use of complex pulse sequences. Here a method is presented that may be generally applicable to liquid crystal media. The orientation of the liquid crystal director (here the bicelle normal) to the magnetic field is tuned by variable angle sample spinning. Since the dipolar couplings depend strongly on (bicelle) orientation while the scalar couplings do not, the sign of the dipolar couplings can be determined given the known signs of the J-couplings.

The dynamics of the liquid crystal director during variable angle sample spinning NMR experiments (VASS) have been extensively investigated (Courtieu et al., 1982, 1994). When a liquid crystal is spun about a particular axis that makes an angle, Ω, with respect to the magnetic field, the director does not have time to reorient itself if the spinning rate is larger than some critical speed. Instead, the director reorients in such a way that the time average of the potential energy is minimum. In general, for liquid crystals with negative diamagnetic anisotropy, $\Delta\chi < 0$, the director is distributed in a plane perpendicular to the rotation axis when $0° < \Omega < 54.7°$. Consequently the anisotropic interactions are scaled down by:

$$\Delta = -\frac{1}{4}\Delta_{\parallel}(3\cos^2\Omega - 1) \qquad (1)$$

where $\Delta_{\parallel}$ is the parallel component of the static anisotropic interaction. There is a minor complication in that the distribution of the directors generates spinning side bands. When $54.7° < \Omega < 90°$, the directors tend to align along the rotating axis, and the anisotropic interactions are scaled down by:

$$\Delta = \frac{1}{2}\Delta_{\parallel}(3\cos^2\Omega - 1) \qquad (2)$$

A single narrow line results with no spinning side bands.

The above experiments are quite different from magic-angle sample spinning (MASS) experiments. In VASS experiments the sample spinning is used to reorient the director to the magnetic field, and consequently all anisotropic interactions are scaled according to the director's new orientation. In contrast, in MASS experiments the mechanical spinning about the magic angle, 54.7°, is used to achieve a coherent average that reduces anisotropic contributions to zero. The application of VASS to structural studies has been shown in a few cases (Kimura et al., 1997, 1998; Zhou et al., 1998), but no applications to the 'bicelle' systems used for both high resolution NMR and membrane protein studies have appeared. Nor has a quantitative analysis of the spinning angle dependence of the bicelle spectra been reported.

Results and discussion

The bicelles used in work to be presented here exhibit a nematic liquid crystalline phase, and are an attractive model membrane system for the study of membrane proteins and membrane associated molecules by NMR (Sanders et al., 1994). They consist of short- and long-chain phospholipids (DMPC/DHPC) and are assumed to have a discoidal shape with a lipid bilayer-like internal structure. Phospholipid bilayers have a negative diamagnetic anisotropy. Thus, bicelles align in the magnet with the bilayer normal perpendicular to the field. This behavior is easily verified in ^{31}P NMR spectra of the constituent phospholipids. A static, oriented ^{31}P spectrum of 25% w/v DMPC/DHPC (molar ratio 2.8:1) bicelles is shown in Figure 1A. The peak at -4.5 ppm is from DHPC and the peak at -11 ppm is from DMPC. Both are measured relative to the isotropic chemical shift value for DMPC. The effective ^{31}P chemical shift tensor for a phospholipid is axially symmetric and has its most shielded direction perpendicular to the average acyl chain orientation (coincident with the bilayer normal) (Sander and Schwonek, 1992). This is manifested in powder patterns of randomly disperse samples being $\sim$35 ppm wide with maximum intensity upfield of the isotropic chemical shift because of the higher probabilities of finding orientations near 90°. In our case we have a single preferred orientation, and because its chemical shift is close to the most upfield value, the bicelle normal must orient close to 90°.

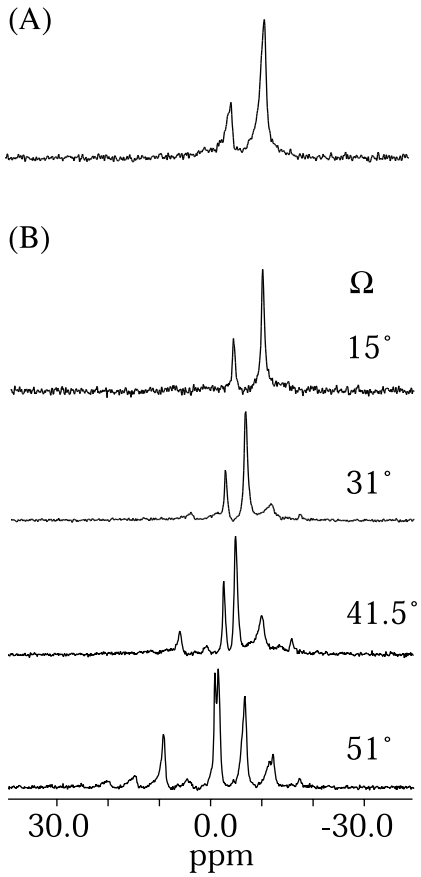

Figure 1. ^{31}P spectra of 25% w/v DMPC/DHPC (molar ratio 2.8:1) bicelles at 45 °C. (A) Static sample. (B) Bicelle sample spun at 1.08 kHz about an axis which makes an angle Ω with the external field. The preparation of bicelles is described elsewhere (Losonczi and Prestegard, 1998). Spectra were taken on a Varian Inova500 spectrometer with a Doty variable angle spinning probe. The static spectrum represents 32 acquisitions with 30 kHz spectral width, 3 s recycle delay and 20 kHz proton decoupling.

Figure 1B shows the ^{31}P spectrum when the bicelle sample is spun about an axis which makes an angle of less than 54.7° with the external field. In this region, the bicelle normals are expected to be distributed in a plane perpendicular to the rotation axis. The spectra should exhibit only even-order spinning side bands because the anisotropic interaction would return to its initial value in just half a rotor cycle (Courtieu et al., 1982). However, in these spectra, except at a spinning angle of $\Omega = 15°$, odd-order side bands also appear. This indicates that the bicelle normals do not perfectly orient at 90° with respect to the spinning axis. When off 90° orientation, the anisotropic interactions actually require a full rotor cycle to return to their initial value. These observed spinning side bands are mainly from DMPC since the DHPC is less abundant, and the CSA of DHPC is nearly averaged out at the employed spinning rate of 1.08 kHz. It should also be noted, that the center bands in these spectra remain sharp at all angles, suggesting that the bicelle normals are not randomly distributed. Random orientations of bicelle normals would result in broadening of the center band under off-magic angle sample spinning to produce a scaled powder-pattern-like shape.

The unexpected pattern and intensities of the spinning side bands can be analyzed in terms of models for the orientation and distribution of the bicelle normals (Emsley et al., 1981; Kumar et al., 1987, 1988). Following this lead, we have written a program using a combination of C and Mathematica routines to simulate expected spectra. The variable angle spinning spectrum for arbitrary distributions of bicelle normals can be calculated using two sets of Euler rotations (Munowitz and Griffin, 1982). The first transformation takes the chemical shift principal axes system (PAS) to the rotor frame by Euler angles (α, β, γ). Since the orientation of PAS in the molecular or bicelle frame is known (the most shielded direction is perpendicular to the bicelle normal), these Euler angles also define the orientation of the bicelle normals in the rotor frame. A subsequent rotation takes the rotor frame to the laboratory frame through (0, Ω, $-\omega$t), where ω is the spinning rate. For a spin (or bicelle) with defined orientation (α, β, γ) in the rotor frame, the instantaneous precession frequency is related to ωt and varies with time. The free induction decay (FID) with this time dependence superimposed is generated via numerical integration. For each bicelle orientation model, FIDs are summed based on assumed bicelle distributions. The resulting FIDs are transformed to produce simulated spectra.

A reasonable model for distributions of bicelle directors, illustrated in Figure 2A, was identified from spectral simulations, which qualitatively reproduced the experimental observations. In this model, the bicelle normals orient in a plane nearly perpendicular to the spinning axis, but with deviations uniformly spanning a range of $\pm\Delta\beta$ above and below. As an example, both simulated and experimental spectra with spinning angle $\Omega = 41.5°$ are shown in Figure 2B. The spread of the bicelle normals is best modeled by a uniform distribution $\pm$ 20° above and below the plane perpendicular to the spinning axis. While the distribution seems large, the line width contribution from this distribution is only about 4% given a ^{31}P DMPC CSA of 33 ppm. In contrast, the center band line width

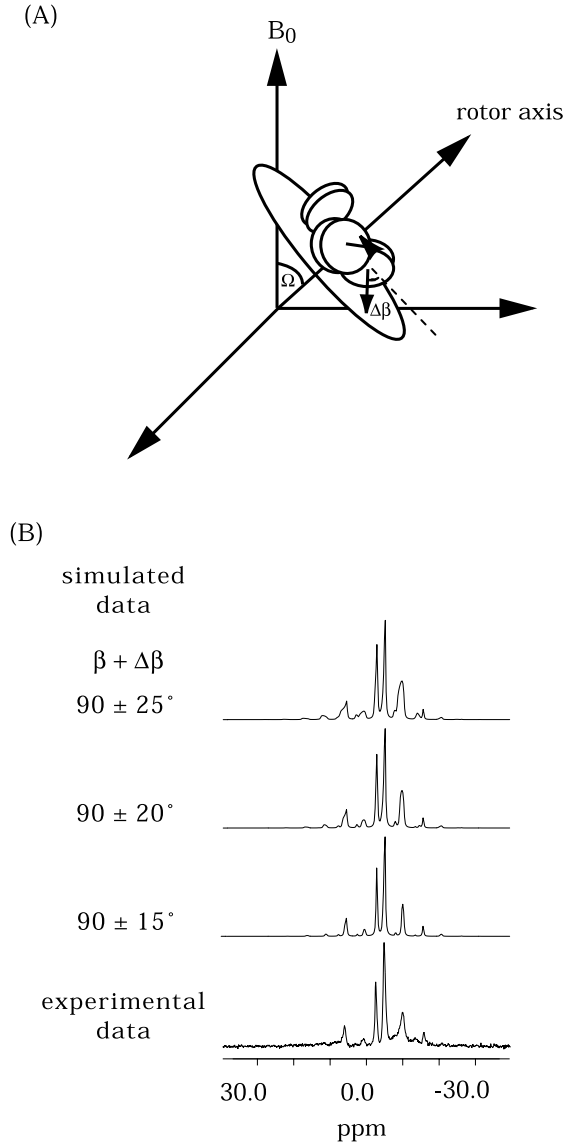

Figure 2. (A) A model for the orientation and distribution of the bicelle normals in VASS experiments. The bicelle normals orient in a plane nearly perpendicular to the spinning axis, but with deviations spanning a range of $\pm\Delta\beta$ above and below the plane. (B) A comparison of simulated and experimental VASS spectra at a spinning angle $\Omega = 41.5°$. A 33 ppm CSA for DMPC and 13.5 ppm CSA for DHPC were used for spectral simulations.

would experience a 35% contribution if the bicelles were randomly distributed. The large expected contribution from a random distribution is one of the reasons that off-magic angle spinning is not widely used for powder sample studies: in powder samples, the center band would be broadened dramatically. In spinning bicelles, however, the bicelle normals are mainly distributed in a plane perpendicular to the spinning axis, thus the center band remains sharp, allowing the scaled anisotropic interactions to be determined to high precision. Similar behavior was observed for 10%, 17%, lipid in buffer, DMPC/DHPC as well as 25%, lipid in buffer, DMPC/CHAPSO bicelles. Spinning speeds up to 3 kHz gave qualitatively similar results.

Spinning bicelle samples at angles larger than $\Omega = 54.7°$ was also pursued. Unfortunately, no stable configuration was found after extensive testing of spinning rates, temperatures and sample compositions. There may be entropic reasons for this: in non-spinning, perpendicularly aligned bicelles, the disk normals randomly orient in the transverse plane. The alignment of the bicelle normals parallel to the rotation axis requires organization in a second dimension and would result in an entropic penalty.

With disk systems that align with a normal parallel to the field, spinning to achieve organization with normals parallel to the spinning axis (here $\Omega < 54.7°$) imposes no such penalty. Hence disks such as cesium perfluorooctanoate disks have been observed to behave well in this angular range (Boden et al., 1987). It is possible to change the preferred orientation of phospholipid bicelles by adding paramagnetic ions and we might expect similar success with this system (Prosser et al., 1996). However, paramagnetic ions added to accomplish reorientation of bicelle normals often broaden lines and may not be compatible with many protein systems. Hence the emphasis here on the possibility of spinning native bicelles at angles less than 54.7°.

Given a successful demonstration of narrow center lines at $\Omega < 54.7°$, we go on to make applications to sign determination for dipolar couplings. This is demonstrated in Figure 3 for Myr($^{13}C_{1,2}$)-Gly(^{15}N)OMe in 25% w/v DMPC/CHAPSO bicelles. The myristoyl chain (Myr) is from a 14-carbon saturated fatty acid that is often found in membrane associated proteins and peptides and serves to anchor the peptide to the bicelle (Bhatnagar and Gordon, 1997; Silvius, 1999). A splitting of 1100 Hz was observed in the proton-coupled ^{15}N spectrum. This is a ^{15}N-^{1}H dipolar coupling since in this sample, the $^{13}C_1$-^{15}N coupling is very small, $\sim$20 Hz, and not resolved. Since the observed coupling is $|J + D|$, the ^{15}N-^{1}H dipolar coupling could be either 1195 or -1005 Hz based on addition or subtraction of the known -95 Hz scalar J-coupling. When this sample was spun at angles $\Omega = 32°$, 37° and 43°, the couplings were measured as 565, 405 and 241 Hz, respectively (Fig-

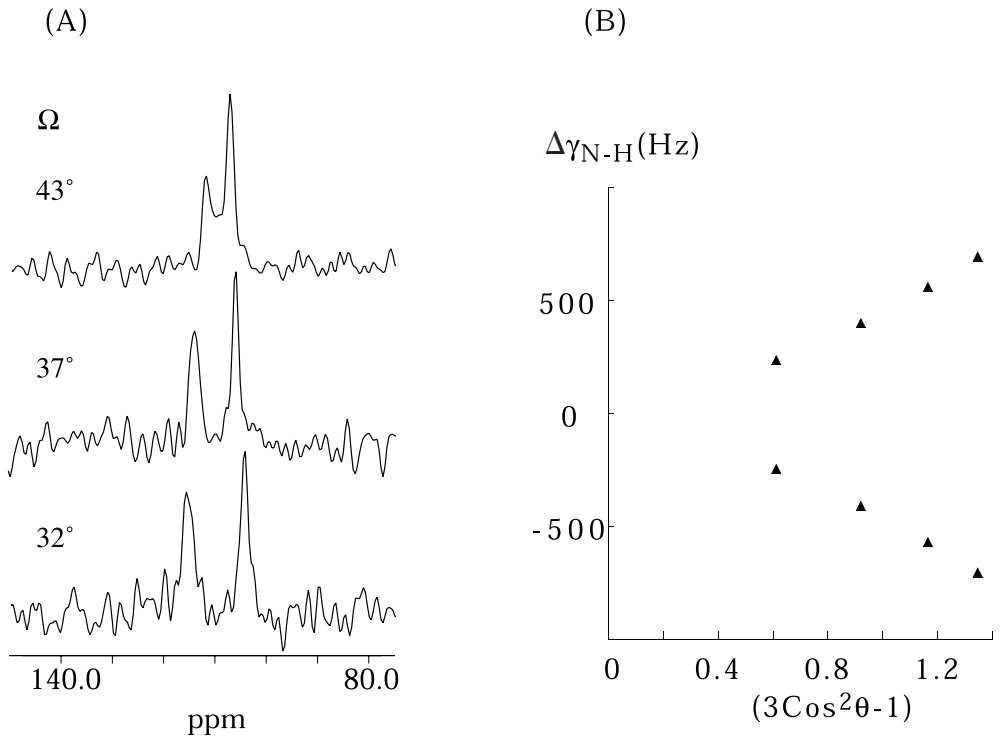

Figure 3. (A) Proton coupled ^{15}N spectra of Myr($^{13}C_{1,2}$)-Gly(^{15}N)OMe (5 mg) in 500 μl 25% w/v DMPC/CHAPSO bicelles under VASS conditions. (B) The observed ^{15}N-^{1}H splittings were plotted versus $(3\cos^2\theta - 1)$. The intercept corresponds to a proper negative J value only if the measured splittings were positive. The spectrum at $\Omega = 37°$ represents 6000 acquisitions with 20 kHz spectral width, 2 s recycle delay.

ure 3A). The spinning angles were calibrated using the ^{31}P chemical shift of the lipid and the data plotted versus $(3\cos^2\theta - 1)$ are shown in Figure 3B. The intercept corresponds to a proper negative J value only if the measured splittings were positive, hence the dipolar coupling is 1195 Hz.

Conclusions

In summary, the sign of dipolar couplings in a field-oriented bicelle can be determined by spinning the bicelles at angles smaller than the magic angle. In this region, the bicelle normals are mainly distributed in a plane perpendicular to the rotating axis. When the spinning angle is close to the magic angle, the bicelle normal deviates from that plane. However, at a spinning angle 41.5°, the deviation is small about 20°. Despite the heterogeneous line that one would expect, the center band of the resonance remains reasonably sharp. This allows the accurate measurement of the scaled anisotropic interactions. The technique presented here does not require high spinning speed, therefore homonuclear multi-pulse techniques can be easily incorporated for measuring dipolar couplings in 2D PDSLF and SLF VASS experiments (data not shown) (Hester et al., 1976; Caldarelli et al., 1996). The scaling down of the dipolar interactions can also convert second order spectra to first order spectra, making data analysis more straightforward. The simulation program is available through our Web site at www.ccrc.uga.edu.

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