NMR Measurement of Signs and Magnitudes of C−H Dipolar Couplings in Lecithin

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Received November 2, 1994

The investigation of lipids, polymers, and other organic materials has been greatly advanced by solid-state NMR utilizing anisotropic nuclear interactions, such as dipolar and quadrupolar couplings. In particular, the quadrupolar ^2H interaction has proved to be a useful local probe of molecular order, structure, and dynamics.[1−3] In rigid solids, the ^2H quadrupole coupling reflects the angle between the C−^2H bond and the external magnetic field, while in mobile systems, the motionally narrowed spectrum provides the order parameter of the C−^2H bond.[1]

The necessity of ^2H labeling can be avoided by measuring $^{13}C-^1H$ dipolar couplings, which yield similar orientational information. Overlapping dipolar spectra of the various sites in a complex molecule can be separated by two-dimensional (2D) NMR techniques, specifically separated local field (SLF) NMR.[4−7] However, traditional SLF techniques do not produce dipolar powder spectra with simple splittings for CH_2 nor CH_3 groups.[6] Only recently, proton-detected local field (PDLF) NMR, which involves the detection of proton spins evolving in the dipolar field of a single ^{13}C nucleus, has made it possible to obtain clear Pake-type C−H dipolar powder spectra.[8,9]

In this Communication, we demonstrate the measurement of C−H dipolar coupling constants $\bar{\delta}_D$, including the signs, for a number of sites in a liquid crystalline lipid, lecithin. 2D PDLF NMR with switched-angle spinning (SAS) provides the magnitudes of the couplings and their signs by comparison of spectra taken at different spinner axis orientations. More directly, the signs are observed as the signs of peak amplitudes in a novel 1D SAS technique using J couplings to make sign-dependent dipolar PDLF coherences observable. The signs of the C−H couplings, so far determined only in macroscopically oriented systems,[10−15] are related to the average segmental orientations in liquid crystalline systems. For instance, C−H bonds oriented preferentially perpendicular to the director exhibit a negative narrowing factor $\bar{\delta}_D/\delta_D$, corresponding to a positive $\bar{\delta}_D$ (the narrowing factor and $\bar{\delta}_D$ have opposite signs[11] since the rigid value dipolar coupling δ_D is negative). Lecithin, a lipid abundant in biological membranes, was chosen in this study since ^2H couplings have been measured for >20 sites[1,16] in phospho-

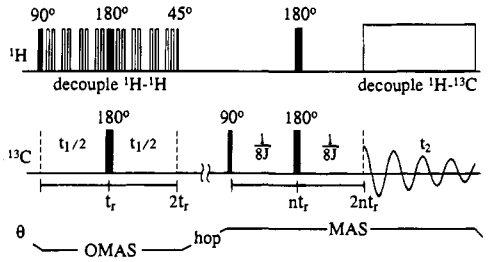

Figure 1. DISTINCT pulse sequence for sign determination of C−H couplings. Initially, at $\theta \neq 54.74°$, MREV-8 $^1H-^1H$ decoupling and a pair of simultaneous 180° pulses remove all interactions except heteronuclear couplings, which transform ^1H magnetization into sine-modulated heteronuclear coherences. These coherences are converted into two-spin order I_zS_z by the 45° pulse before the hopping period. At $\theta = 54.74°$, they are transformed into detectable ^{13}C magnetization by a 90° ^{13}C pulse and a rotor-synchronized J coupling period, in which 180° pulses reverse the chemical shift while magic-angle spinning refocuses the dipolar couplings at full rotor periods. The sign-modulated MAS signals are detected under high-power proton decoupling. ^1H and ^{13}C 90° pulse lengths range from 6 to 9 μs according to the spinner orientation. The MREV-8 cycle time of 120 μs was one-quarter of the sample rotation period t_r.

choline and can thus be used to estimate the accuracy of the dipolar couplings. Egg yolk lecithin, a phosphocholine with 16:0, 18:1, and 18:2 acyl chains, was hydrated with D_2O [70:30 (w/w)] and freeze−thawed to yield a uniform aqueous dispersion.

Figure 1 displays the pulse sequence designed to exhibit the sign of the heteronuclear coupling as the sign of the corresponding ^{13}C magic-angle spinning (MAS) peak intensity. At sufficiently short t_1, due to $\sin(\omega t_1) \approx \omega t_1$, the sign of the heteronuclear coupling ω is manifested as the sign of the signal. The technique is termed DISTINCT (*DI*polar *S*ine *T*erm by *IN*direct-*C*oupling *T*ransformation). In the first part of the experiment, rapid off-magic-angle spinning (OMAS) scales the dipolar couplings by a factor $P_2 = {}^1/_2(3\cos^2\theta - 1)$, where θ is the angle between the spinner axis and the external field. Scaled C−H couplings are selectively retained under homonuclear ^1H decoupling and a pair of ^1H and ^{13}C 180° pulses. From ^1H magnetization, the C−H interactions create heteronuclear coherences that are modulated with the sine of the sum of the J and dipolar C−H couplings. Having survived the hop time in the form of heteronuclear two-spin order I_zS_z, these sine-modulated terms are converted, following a ^{13}C 90° pulse, into observable ^{13}C magnetization under the action of the isotropic heteronuclear J coupling and then detected under ^1H decoupling. Thus, the sine-modulated C−H couplings determine the sign of the peaks in the resulting ^{13}C MAS spectrum.

Figure 2 shows DISTINCT spectra of lecithin taken at $P_2 = \pm 0.2$ and with a fixed dipolar evolution time of two rotor periods. The positive reference for the signs is provided by the CH_3 (ω and γ site) signals[17] that are dominated by the positive[18] ^1J couplings, which are +120 to +150 Hz as measured by MAS in the absence of proton decoupling. The spectrum for $P_2 = +0.2$ exhibits negative peaks for the acyl and glycerol residues, indicating that their dipolar couplings have the same signs. At $P_2 = -0.2$, C_α exhibits a negative signal, which shows that the dipolar sign of C_α is opposite to that of the acyl and glycerol sites. This demonstrates an unusual orientation of the C_α methylene group, consistent with the bend of the molecule at the phosphate junction.[19] For C_β, DISTINCT spectra with $P_2 = \pm 0.4$ exhibit only positive peaks, indicating that the C_β

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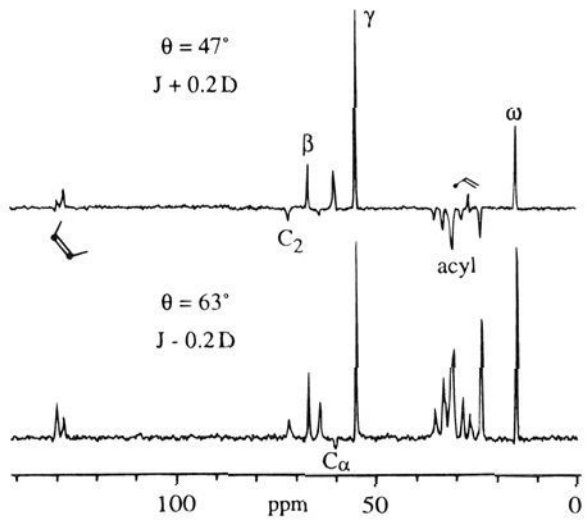

Figure 2. DISTINCT spectra of lecithin in the L_α phase at scaling factors (a) $P_2 = +0.2$ and (b) $P_2 = -0.2$. Each peak sign reflects the *sign of the sum of the corresponding J coupling and P_2-scaled dipolar* coupling constant. The ^{13}C MAS signals have been assigned by Husted et al.[17] The experiments were carried out at 75.74 MHz for ^{13}C using a 7-mm sample rotor spinning at 2080 Hz in a home-built SAS probehead. Measuring times were 8 and 4 h.

coupling strength is significantly smaller (narrowing factor < 0.02) than expected from ^2H NMR (0.045) of fully hydrated bilayers.[20] This may be due to lower hydration of our sample.

Since the dipolar frequency depends on the angle β between the director and the rotor axis according to $^1/_2(3\cos^2\beta - 1)$, both positive and negative dipolar frequencies occur due to the isotropic distribution of bilayer orientations. By incrementing t_1 in the pulse sequence of Figure 1, we obtained a 2D DISTINCT spectrum (not shown) which establishes that the powder pattern maximum determines the sign in the 1D DISTINCT spectrum. Taking into account that the frequency of the powder-pattern maximum, $-^1/_2\delta_D$, has a sign opposite that of the dipolar coupling constant, the δ_D of the acyl sites is found to be positive, i.e., their narrowing factors are negative.

To measure the strengths of the dipolar couplings δ_D and complete the sign determination, we have taken 2D PDLF SAS spectra with the pulse sequence of Nakai and Terao,[8] where initially ^1H magnetization evolves under $^{13}C-^1H$ dipolar and J couplings at $\theta \neq 54.74°$. After cross-polarization (CP) from ^1H to ^{13}C and a hop to $\theta = 54.74°$, the ^{13}C MAS spectrum is observed during the detection period. This results in a 2D spectrum where the C−H Pake patterns are separated according to the isotropic ^{13}C chemical shifts. In the spectra of Figure 3, taken with P_2 scaling factors of ±0.2, C−H splittings between 200 and 1300 Hz are observed. Upon inversion of P_2 from −0.2 to +0.2, *splittings increase or decrease according to the* relative signs of J and δ_D. For splittings exceeding twice the J coupling, the average of the splittings at opposite P_2 values is $|\delta_D|$, while their difference is the J coupling. In Figure 4, the dipolar couplings with their signs are summarized and compared with ^2H NMR order parameters from the literature.[1] Due to scaling factors from sample rotation and homonuclear decoupling, the dipolar couplings are less precise than the ^2H results. The C−H couplings are hardly affected by the acyl chain composition, except in the double bond region and at the end of the acyl chains. This can be concluded from C−H dipolar couplings measured in DMPC, as well as from ^2H data.[1,3,21−24] The signs of the S_{C-H} values determined here are important for testing structural models of the lipid[25] and providing complete order tensor information.[12,24]

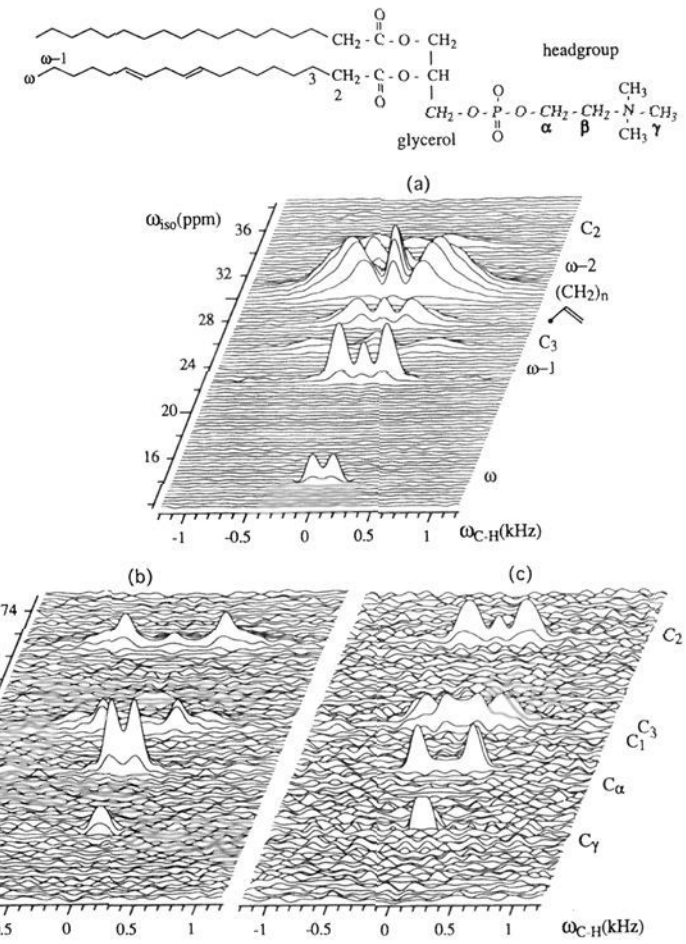

Figure 3. PDLF 2D $^{13}C-^1H$ spectra of lecithin with a schematic of the lecithin molecule. (a) and (b) Spectrum taken with $P_2 = -0.2$; measuring time, 12 h. (a) Acyl region. (b) Headgroup and glycerol regions. (c) Similar to (b), but with $P_2 = +0.2$. The increase of the C_α splitting relative to that in (c) verifies the positive dipolar order parameter (narrowing factor) of C_α, while the decrease for C_1, C_2, and C_3 indicates negative order parameters. The peak in the center of the $(CH_2)_n$ dipolar pattern, which is enhanced at longer CP times, results from the weak dipolar interactions of the specific methylene carbon with the protons of neighboring CH_2 groups. CP time was 0.5 ms; MREV-8 scaling factor, from the J splittings, was 0.5 ± 0.05.

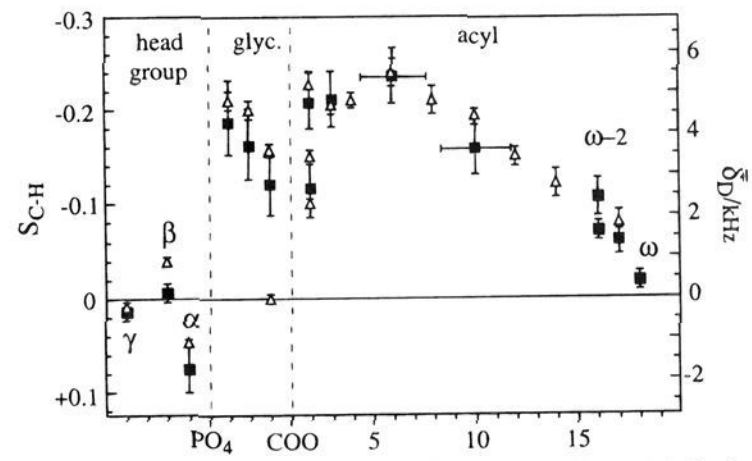

Figure 4. Motionally averaged C−H dipolar couplings (■), including signs, for various segments in the lecithin molecule, compared with values of ^2H quadrupolar couplings (△) from the literature. For clarity, *the ^2H couplings, whose signs are unknown, are shown with the same* signs as those of the corresponding dipolar couplings. On the right, the dipolar coupling constants are given (without scaling by sample rotation). The narrowing factors on the left axis are equivalent to S_{C-H} bond order parameters.[1] Most CH_2 groups display only one splitting, but for certain CH_2 groups at the junction of the glycerol and acyl regions, inequivalent C−H couplings can be observed. In the acyl region, only the order parameters for the saturated chains are shown for clarity.

The methods described here are expected to increase the amount of structural and dynamical NMR data on lipids, liquid crystals, and oriented polymers, supplementing deuterium NMR.

Acknowledgment. This work was supported by the Director, Office of Energy Research, Office of Basic Energy Sciences, Materials Science Division of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098. K.S.-R. gratefully acknowledges a fellowship from the BASF AG and the German National Scholarship Foundation. We thank R. G. Larsen for his help.

JA9435596

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