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NMR Measurement of Resolved Heteronuclear Dipole Couplings in Liquid Crystals and Lipids

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Simplified nuclear magnetic resonance (NMR) spectra of heteronuclear dipolar couplings for molecules in an anisotropic environment, such as liquid crystals and lipids, are obtained by proton-detected local-field spectroscopy. The distance-dependent dipolar interactions between spins of magnetically active nuclei can be determined directly from the spectrum because many-body effects that complicate conventional dipolar NMR spectra are avoided by selectively probing local fields produced by rare spins at the location of abundant spins. We employ the technique to resolve the carbon–proton dipolar couplings of benzene dissolved in a nematic liquid crystal and to measure phosphorus–proton couplings in lecithin in the L_α phase to obtain constraints on the phosphocholine headgroup structure.

Internuclear distances in anisotropic samples, such as solids and liquid crystals, can be determined from dipolar couplings between magnetically active nuclei. However, this approach has been hampered in all but the simplest cases by the complexity of traditional nuclear magnetic resonance (NMR) spectra in the presence of dipolar couplings.[1,2] This complexity is a consequence of the fact that an ensemble of strongly coupled nuclear spins represents a many-body system. In this Letter we demonstrate that simple dipolar spectra can be obtained by the NMR method of proton-detected local-field spectroscopy.[3–5] Applied to liquid-crystalline samples or molecules oriented in a liquid-crystalline matrix, the method directly yields a pair of lines for each heteronuclear dipolar coupling constant. Not only bond lengths[5] but also long-range couplings can be determined. Applying the technique to lecithin in the bilayer L_α phase, we have obtained ^{31}P–^{1}H dipolar couplings, which, combined with NMR data from the literature, provide strong constraints on the possible phospholipid headgroup conformations.

Figure 1 shows conventional proton and carbon-13 NMR spectra of benzene dissolved in a nematic liquid crystal. The anisotropy of the solvent leads to orientational ordering of the solute which results in spectra that reflect partially averaged dipolar coupling constants. It is clear that the spectra of Figure 1 do not reflect the chemical structure in a straightforward manner. There are many more lines than the seven distinct dipolar interactions among the spins in the molecule. In general, the spectrum of an N-spin system contains up to $n_{\max} = \binom{2N}{N+1}$ lines.[6] For $N = 7$, this number is 3003. Even the very high symmetry of the benzene molecule does not simplify the spectra sufficiently for a straightforward analysis. Therefore, conventional studies employ numerical simulations to learn about structure and dynamics.[7–9] Although this approach has provided the most accurate structure of benzene to date,[10] it has been limited to molecules containing a maximum of a dozen interacting spins ($n_{\max} \approx 2.5 \times 10^6$).

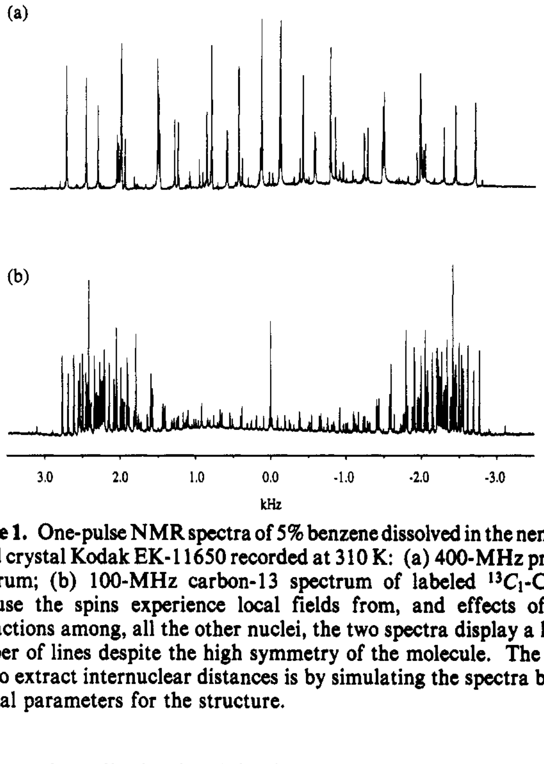

Figure 1. One-pulse NMR spectra of 5% benzene dissolved in the nematic liquid crystal Kodak EK-11650 recorded at 310 K: (a) 400-MHz proton spectrum; (b) 100-MHz carbon-13 spectrum of labeled $^{13}C_1$-C_5H_6. Because the spins experience local fields from, and effects of the interactions among, all the other nuclei, the two spectra display a large number of lines despite the high symmetry of the molecule. The only way to extract internuclear distances is by simulating the spectra based on trial parameters for the structure.

The problem arises from the fact that each proton (or carbon) spin gives rise to a spectrum reflecting the interactions to, and among, all the proton spins in the molecule. Some degree of simplification of the spectra can be achieved by observing ^{13}C spectra under homonuclear dipolar decoupling of the protons.[11–14] The ^{13}C spins then evolve only under the influence of the heteronuclear dipolar local fields produced by the N protons, which leads to a maximum of 2^N lines (i.e., 128 lines for $N = 7$).

An approach for a further reduction of the number of lines, without loss of information, was first proposed by Weitekamp, Garbow, and Pines[3] and applied by Nakai and Terao for studying one-bond couplings in spinning solids.[5] The effect was also noted in heteronuclear correlation spectroscopy of single crystals and exploited to distinguish selective transfer from neighboring protons and diffusive transfer from more remote protons.[4] The concept of spectral simplification involves detecting the *single* local field produced by the rare ^{13}C spin at the site of each proton and thus leads to a dramatic simplification of the spectrum. We have implemented the technique in two different ways, employing the pulse sequences shown in Figure 2. The method yields spectra containing a maximum of N doublets whose frequency splittings are proportional to the strengths of the heteronuclear dipolar couplings. Each splitting, ω_d, is directly related to the corresponding internuclear C–H distance r, according to

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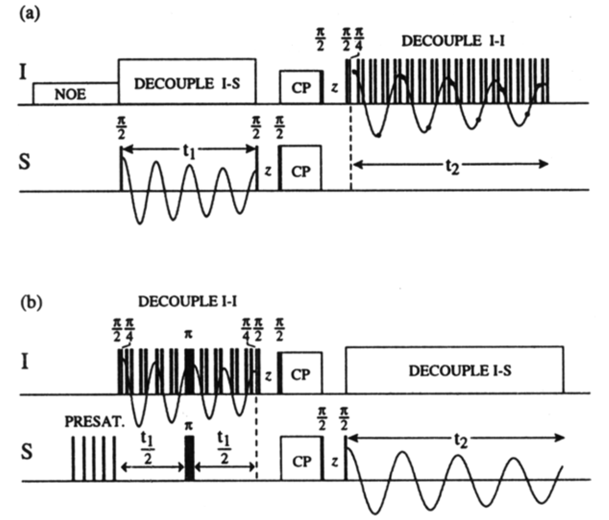

Figure 2. Two pulse sequences suitable for two-dimensional ^{1}H–^{13}C proton-detected local-field spectroscopy. In (a) carbon (S-spin) polarization is first enhanced through the nuclear Overhauser effect during saturation of the protons (I spins). The carbon magnetization is then rotated into the transverse plane by a 90° pulse and subsequently precesses with a characteristic frequency during the first incremented delay t_1. The proton couplings are removed during this period by heteronuclear decoupling. Following the t_1 period, one component of the carbon magnetization is stored along the z direction to allow time for power switching on the proton channel. Subsequently, the carbon magnetization is transferred to protons by Hartmann–Hahn cross-polarization.[15,16] The resulting proton magnetization is stored parallel to the field along the z axis to allow spin diffusion to occur among the protons, yielding more even intensities in the spectra. By a (fully phase-cycled) 90° pulse and a 45° y pulse, the proton magnetization is rotated into the plane perpendicular to the MREV-8[17–19] effective-field direction (1,0,1). In this plane, it oscillates during the acquisition period, t_2, under the influence of the heteronuclear dipolar couplings, with the MREV-8 sequence effecting homonuclear dipolar decoupling. Scheme (b) provides an alternative in which proton magnetization first evolves under homonuclear decoupling and is then transferred to ^{13}C spins for detection. It has the advantage that no signal detection occurs during the ^{1}H multiple-pulse sequence and that the ^{1}H chemical shift is refocused by a 180° pulse at $t_1/2$. Both experiments are repeated many times for different values of t_1, so that a data set is acquired as a function of t_1 and t_2. Two-dimensional Fourier transformation yields a spectrum which displays carbon–proton dipolar doublets resolved according to carbon chemical shifts. Thus, for each carbon we obtain a direct map of the dipolar couplings to nearby protons.

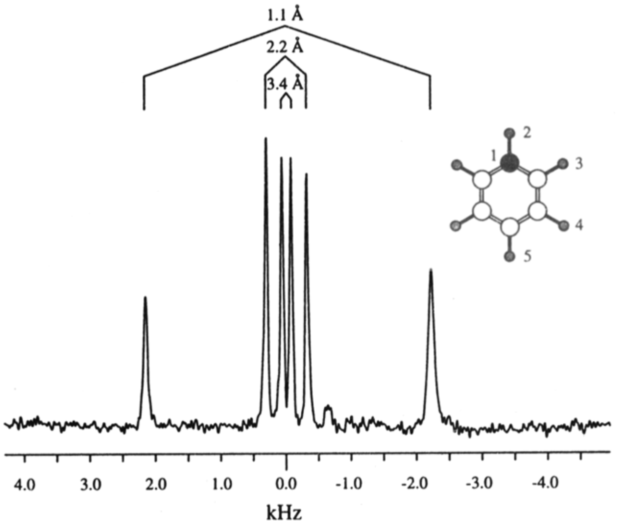

Figure 3. Proton-detected local-field spectrum of natural abundance benzene dissolved in a nematic liquid crystal (Kodak EK-11650). The spectrum was recorded with the pulse sequence of Figure 2a implemented on a Bruker AM-400 spectrometer using a standard Bruker 5-mm "inverse" solution NMR probe head. Multiple-pulse decoupling by means of the MREV-8 sequence was applied during t_2, with a cycle time of 234 μs (corresponding to a spectral width, after correction for scaling, of ~9 kHz). The proton 90° pulse length was 16 μs ($\gamma B_1 = 2\pi$ 15 kHz), and the cross-polarization time was 10 ms. Quadrature artifacts were removed by cycling the phase of the recall pulse after the storage period in concert with the receiver phase in 90° steps. The spectral width was 6 kHz in the ω_1 dimension. A total of 512 points were recorded in t_2 for each of 80 t_1 increments. The data were zero-filled to 512 × 2048 before two-dimensional Fourier transformation. The spectrum shown is a slice taken parallel to the ω_2 axis at the carbon peak maximum. The width of the lines near the center of the spectrum is about 15 Hz, with the resolution probably being limited by the presence of ^{1}H–^{1}H scalar couplings. The spectrum consists of three doublets which correspond to the 1–2, 1–3, and 1–4/1–5 distances in benzene. The 1–4 and 1–5 distances of 3.4 and 3.9 Å, respectively, do not give rise to resolved peak pairs since the separation of the corresponding dipolar lines would be only 20 Hz, which is obscured by J couplings.[3]

$$\omega_{\mathrm{d}} = \frac{\mu_0}{4\pi}\frac{\gamma_I\gamma_S\hbar}{\langle r^3\rangle}S \qquad (1)$$

where γ_I and γ_S are the gyromagnetic ratios of the proton and carbon spins, respectively, and S is an order parameter that depends on the degree to which the solute is ordered by the solvent. In molecules with low symmetry, S is replaced in eq 1 by an angle-dependent factor $S_{xx}\cos^2\phi\sin^2\theta + S_{yy}\sin^2\phi\sin^2\theta + S_{zz}\cos^2\theta$, where (θ,ϕ) denote the orientation of the C–H internuclear vector in a molecular axis system and $S_{\alpha\alpha}$ are the order parameters[1] for this axis system.

In the experiment, illustrated in Figure 2, polarization transfer techniques[15,16] allow proton magnetization from molecules containing a carbon-13 nucleus (1% natural abundance) to be detected selectively. Subject to homonuclear dipolar decoupling,[17–19] each proton spin experiences only one heteronuclear dipolar interaction with the one carbon-13 spin in the molecule. Due to the low abundance of ^{13}C, there is only a few percent probability of finding a second carbon-13 close enough to complicate the spectrum. The resulting spectra are further simplified by carrying out the experiment in two frequency dimensions,[20] in a manner similar to conventional separated local-field techniques[12,14] where the extra dimension displays the carbon chemical shifts. At each carbon chemical shift peak, a slice extracted parallel to the dipolar frequency dimension represents the proton-detected local-field spectrum of the corresponding carbon spin. It should be noted that, in the scheme of Figure 2b, the proton-detected local fields are probed by the proton magnetization in the evolution period, even though S-spin magnetization is observed in the detection period t_2. In the example shown in Figure 3, measured with the sequence of Figure 2a, we can easily determine the 1–2, 1–3, and 1–4 distances in benzene. The measurement of progressively larger distances requires improvements in the resolution of these spectra, which may be achieved by enhancing the efficiency of the homonuclear decoupling and by eliminating scalar couplings in higher-dimensional experiments.[21]

To further indicate the possibilities of the technique, Figure 4 displays the proton-detected ^{1}H–^{31}P local-field spectrum of lecithin (phosphatidylcholine) in its liquid-crystalline (L_α) bilayer phase, taken with the sequence of Figure 2b. The spectrum shown was obtained from the full 2D spectrum by taking a cross section at the maximum of the ^{31}P chemical shift powder line shape, which corresponds to director orientations perpendicular to the external field. Therefore, the cross section appears like the spectrum of an oriented sample, without the inhomogeneous broadening typical of a powder pattern. The ^{1}H–^{31}P dipolar couplings are of considerable value to constrain the torsion angles that define the headgroup structure. In particular, dipolar couplings exceeding 400 Hz are ruled out by the spectrum. This is a significant constraint since the dipole couplings between the ^{31}P spin and the nearest proton in the structure, at a distance of less than 0.3 nm, could exceed 1500 Hz if the internuclear vector was parallel to the director axis.

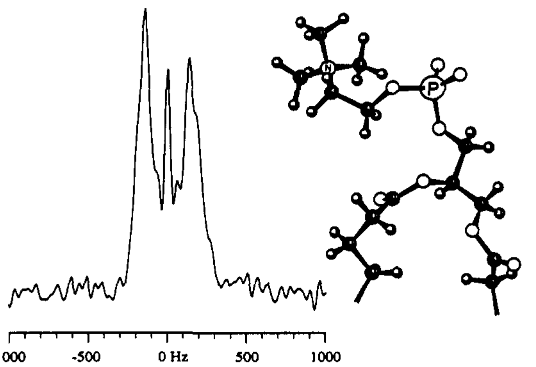

Figure 4. Proton-detected ^{31}P–^{1}H local-field spectrum of egg yolk lecithin (99% phosphatidylcholine) in the liquid-crystalline bilayer (L_α) phase. The spectrum was obtained using the pulse sequence of Figure 2b, with a ^{1}H 90° pulse length of 11 μs and a cross-polarization time of 200 μs, at a ^{31}P Larmor frequency of 162 MHz. In this case, phosphorus-31 (spin $S = {}^1/_2$, 100% natural abundance, one per lipid molecule) is the relevant rare S spin, instead of ^{13}C. The spectrum shown is a slice taken at the maximum of the ^{31}P signal in the 2D spectrum, which corresponds to director orientations perpendicular to the external magnetic field. On the right, a portion of a phosphocholine molecule is shown, in a conformation that is consistent with the NMR data if one assumes fast interconversion with the structure that is obtained by inversion of the headgroup torsion angles.

From the four spectral splittings available from conventional ^{2}H and ^{31}P NMR spectroscopy,[22,23] the headgroup structure cannot be unequivocally determined.[24,25] Even in a simple model with fast rotation around the glycerol C(2)–C(3) axis and rapid interconversion of only two mirror-symmetric headgroup conformations,[22] the ^{2}H and ^{31}P NMR data allow four ranges of conformations.[24] Of these, only one (structure III of ref 24) is consistent with our proton-detected local-field spectrum, while the three other structures produce spectra with significantly different shapes and a main spectral splitting which is much larger than observed experimentally. However, since the model includes a number of untested assumptions, the full determination of the dynamic phospholipid headgroup structure requires further constraints from NMR spectroscopy. Such work is currently under way in our laboratory. In the search for a dynamic headgroup structure that is consistent with all NMR data, the ^{1}H–^{31}P dipolar spectrum will represent an important check.

In summary, NMR spectroscopy based on proton-detected local fields represents a promising step toward the three-dimensional structure determination of lipids, liquid crystals, and oriented solids.

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