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COMMUNICATIONS

Multidimensional NMR in Lipid Systems. Coherence Transfer through *J* Couplings under MAS

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Multidimensional chemical-shift-correlation spectroscopy is a standard analytical tool for unraveling complicated NMR spectra of biomolecules in solution. These techniques are useful for establishing connectivity relationships and resonance assignments which are subsequently required for interpretation of NOESY spectra that contain information related to internuclear distances (*1*). In general, chemical-shift correlations are established by coherence transfer between nuclear spins which may proceed through either dipolar (through space) or scalar *J* (through bond) couplings. However, in isotropic liquids, molecular tumbling averages spatially dependent interactions such as dipolar couplings and chemical-shift anisotropies (CSAs) to zero, leaving behind the rotationally invariant scalar couplings and isotropic chemical shifts.

The situation in rigid solids is quite different. CSAs and dipolar couplings are not averaged due to the absence of molecular motions, and these orientation-dependent interactions give rise to characteristic powder lineshapes. Nevertheless, high-resolution liquid-like spectra are obtained routinely by application of magic-angle spinning (MAS), which averages inhomogeneous interactions such as CSA, heteronuclear, and, in certain cases, homonuclear dipolar couplings (*2*).

Recently, there has been a resurgence of interest in chemical-shift correlation spectroscopy in rigid solid and semisolid materials. Most schemes employ MAS along with a mixing period where dipolar couplings are partially restored (*3–6*). Such mixing schemes have reduced transfer efficiency due to the orientation dependence of the recoupled dipolar interaction; therefore, transfer through the isotropic *J* coupling could potentially offer improved signal strengths. However, coherence transfer through *J* couplings in MAS of rigid solids is precluded in most biomolecules since the magnitude of the *J* coupling is smaller than the homogeneous linewidth. In addition, ^{13}C–^{1}H transfer is complicated by ^{1}H–^{1}H interactions that render the proton reservoir at least partially homogeneous (*2*).

In contrast to rigid solids, lipid/water systems in the L_α and H_{II} phases—present in biological membranes under physiological conditions—undergo high-frequency motions such as gauche–trans isomerization and lateral and axial diffusion which attenuate both inter- and intramolecular dipolar interactions (*7, 8*). Furthermore, as illustrated by Forbes *et al.*, such motions render the proton reservoir inhomogeneous (*9*); thus MAS of lipid/water systems in the liquid-crystalline phase attenuates residual anisotropic interactions, thereby producing high-resolution ^{1}H spectra (*9*). This fact, plus the observation that lipid systems yield ^{13}C spectra with linewidths of a few hertz (*10*), permits observation of both J_{CH} and J_{CC} in spinning samples.

We report here the use of *J* couplings for coherence transfer in a glycolipid/water system under MAS. In general, solution-style transfer schemes such as INEPT (*11*) and TOCSY (*12, 13*) may be employed without modification provided that the sample rotation is greater than the motionally averaged CSA and dipolar couplings. Otherwise, one must pay attention to rotor synchronization.

For the following, all spectra were recorded at room temperature on a homebuilt solid-state spectrometer operating at 397.7 MHz for ^{1}H and 100.0 MHz for ^{13}C. The MAS probe was also homebuilt and utilized rotors and stators purchased from Doty Scientific, Inc. (Columbia, South Carolina). Typical ^{1}H and ^{13}C pulse lengths were 4.5 and 5 μs, respectively. Natural-abundance monogalactosyl diacyldiglyceride (MGDG) (primarily 1,2-di[9*Z*,12*Z*,15*Z*]octadec-9,12,15-trienoyl]-3-beta-D-galactopyranosyl-*sn*-glycerol) was purchased from Lipid Products (South Nutfield, Surrey, UK)

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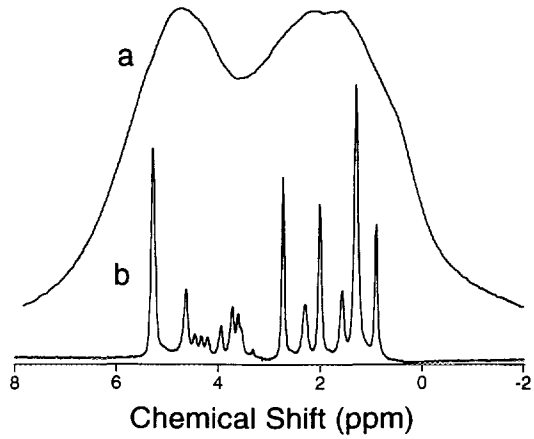

FIG. 1. ^{1}H Bloch decay of the natural-abundance MGDG/D_2O mixture described in text. (a) Static and (b) MAS spectra with $\omega_r/2\pi$ = 6.6 kHz.

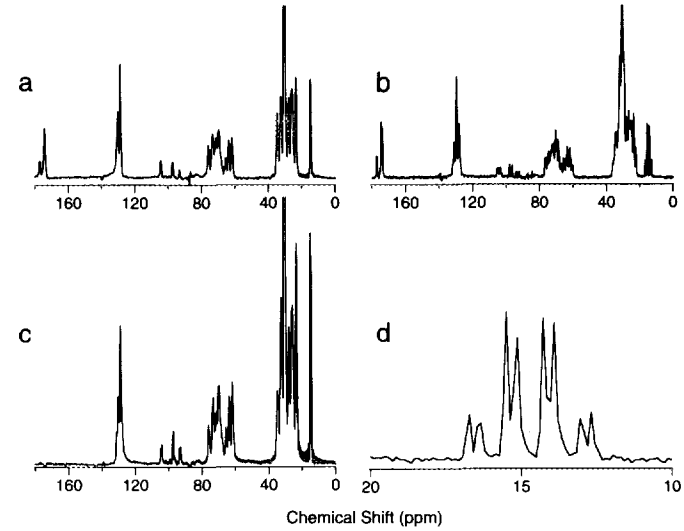

FIG. 2. ^{13}C MAS Bloch decay of the U-(^{13}C)-MGDG/D_2O mixture described in text with $\omega_r/2\pi$ = 5.3 kHz (a) with and (b) without ^{1}H decoupling. (c) ^{1}H–^{13}C MAS/refocused INEPT with CW ^{1}H decoupling on the U-(^{13}C)-MGDG/D_2O mixture described in text with a spinning speed of 5.3 kHz. The pulse sequence employed was $90^\circ_x(^1H)$—δ_1—$180^\circ_x(^1H,^{13}C)$—δ_1—$90^\circ_y(^1H)$, $90^\circ_x(^{13}C)$—δ_2—$180^\circ_x(^1H,^{13}C)$—δ_2—acquire. The first delay, δ_1, is 1.85 ms, while δ_2 is 1.11 ms. (d) Expansion of terminal methyl region in b.

and used without further purification. Uniformly ^{13}C-labeled MGDG (U-(^{13}C)-MGDG) was isolated from a crude lipid mixture that was extracted with acetone/chloroform/methanol (2/1/1) from Spirulina Maxima grown on ^{13}C sodium carbonate as the sole carbon source. The U-(^{13}C)-MGDG fraction was separated using chromatography on silica gel columns eluted with (chloroform/acetone) (9:1) and purified by TLC on silica gel plates. ^{13}C enrichment was estimated at 98% using mass spectrophotometry. The sample is a mixture containing different levels of saturation in the acyl chains which are 16 and 18 carbons in length as revealed by GLC. The 16-carbon chain is roughly 84% saturated and 16% delta 7. The 18-carbon chain is 77% delta 6:9:12, 10% delta 9:12, 10% delta 9, and 3% saturated.

Figure 1a is a static ^{1}H spectrum of a natural-abundance mixture of MGDG as a 1:1 (w/w) ratio dispersion in D_2O at room temperature. MGDG is thought to be in the H_{II} phase at ~22°C under these conditions (*14*). Figure 1b is the same sample spinning at 6.6 kHz about the magic angle and shows resolved isotropic chemical shifts consistent with spectra obtained by Adebodun *et al.* (*15*).

Figure 2a is a ^{13}C (^{1}H decoupled) MAS spectrum of U-(^{13}C)-MGDG as a 1:1 (w/w) ratio dispersion in D_2O spinning at 5 kHz at room temperature. Figure 2b is the same as Fig. 2a but without ^{1}H decoupling. Here, one is able to observe resolvable heteronuclear J couplings which may be used for heteronuclear coherence transfer. That the heteronuclear J couplings are observable is consistent with the fact that the proton reservoir is inhomogeneous and that ^{13}C–^{1}H dipole couplings are motionally averaged while any residual couplings are attenuated by MAS.

Figure 2c is a refocused-INEPT (*16*) spectrum of the U-(^{13}C)-MGDG/D_2O mixture under the same experimental conditions as in Fig. 2a. The spectra in Figs. 2a and 2c have the same intensity scale. As in solution NMR, INEPT-style transfers from ^{1}H to ^{13}C increase the overall sensitivity for ^{13}C detection. These results imply that 2D heteronuclear ^{13}C–^{1}H NMR experiments such as HMQC (*17*) and HSQC (*18*) are feasible in lipid/water systems in the liquid-crystalline

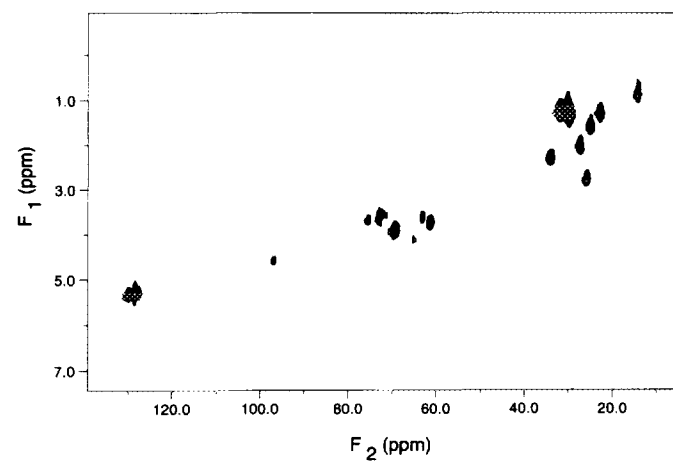

FIG. 3. MAS/^{13}C-detected 2D ^{1}H–^{13}C chemical-shift correlation on the U-(^{13}C)-MGDG/D_2O mixture described in text with a spinning speed of 5 kHz. The pulse sequence employed was $90^\circ_x(^1H)$—$t_1/2$—$180^\circ_x(^{13}C)$—$t_1/2$—δ_1—$180^\circ_x(^1H,^{13}C)$—δ_1—$90^\circ_y(^1H)$,$90^\circ_x(^{13}C)$—δ_2—$180^\circ_x(^1H,^{13}C)$—δ_2—acquire (t_2). Timings are the same as for refocused INEPT. Data were acquired using the method of States *et al.* (*26*). Sixteen transients were collected for each t_1 point with a recycle delay of 3 s. The 2D matrix of 128 × 1024 points was zero filled to 512 × 2048 points. Line broadenings of 5 and 15 Hz were applied in F_1 and F_2, respectively.

phases. Figure 3 is a ^{13}C-detected heteronuclear chemical-shift-correlation spectrum of the U-(^{13}C)-MGDG/D_2O mixture.

Finally, the expansion of the aliphatic region depicted in Fig. 2d shows resolvable J_{CC} couplings. Since these couplings are often between 35 and 45 Hz while the homogeneous ^{13}C linewidths are often less than 10 Hz, it is possible to perform ^{13}C–^{13}C homonuclear chemical-shift correlation using J_{CC}. Figure 4 is a ^{13}C–^{13}C TOCSY spectrum of the U-(^{13}C)-MGDG/D_2O mixture. The acyl chains, glycerol backbone, and galactosyl group show up as independent spin systems, and preliminary assignments have been made. Additional experiments such as 3D HCCH–TOCSY (*19*) and 3D CCH–TOCSY–REV–INEPT (*20*) may be employed to assign completely the U-(^{13}C)-MGDG/D_2O) mixture.

These results demonstrate the feasibility of obtaining high-resolution multidimensional chemical-shift-correlation spectra in lipid/water systems by employing coherence-transfer schemes routinely followed in solution NMR experiments, together with MAS. In addition, these results have implications for CP/MAS (*21*) in lipid/water systems. It is well known that ^{1}H–^{13}C CP/MAS intensities become extremely sensitive to Hartmann–Hahn mismatch when spinning speeds exceed the strength of the relevant dipolar couplings (*22*). This dependence of the Hartmann–Hahn match on spinning speed for lipid/water systems in the liquid-crystalline phase can be especially acute since dipolar couplings are reduced significantly by motional averaging. Therefore, optimal CP intensities may be difficult to achieve and maintain for long times even at moderate spinning speeds. Typ-

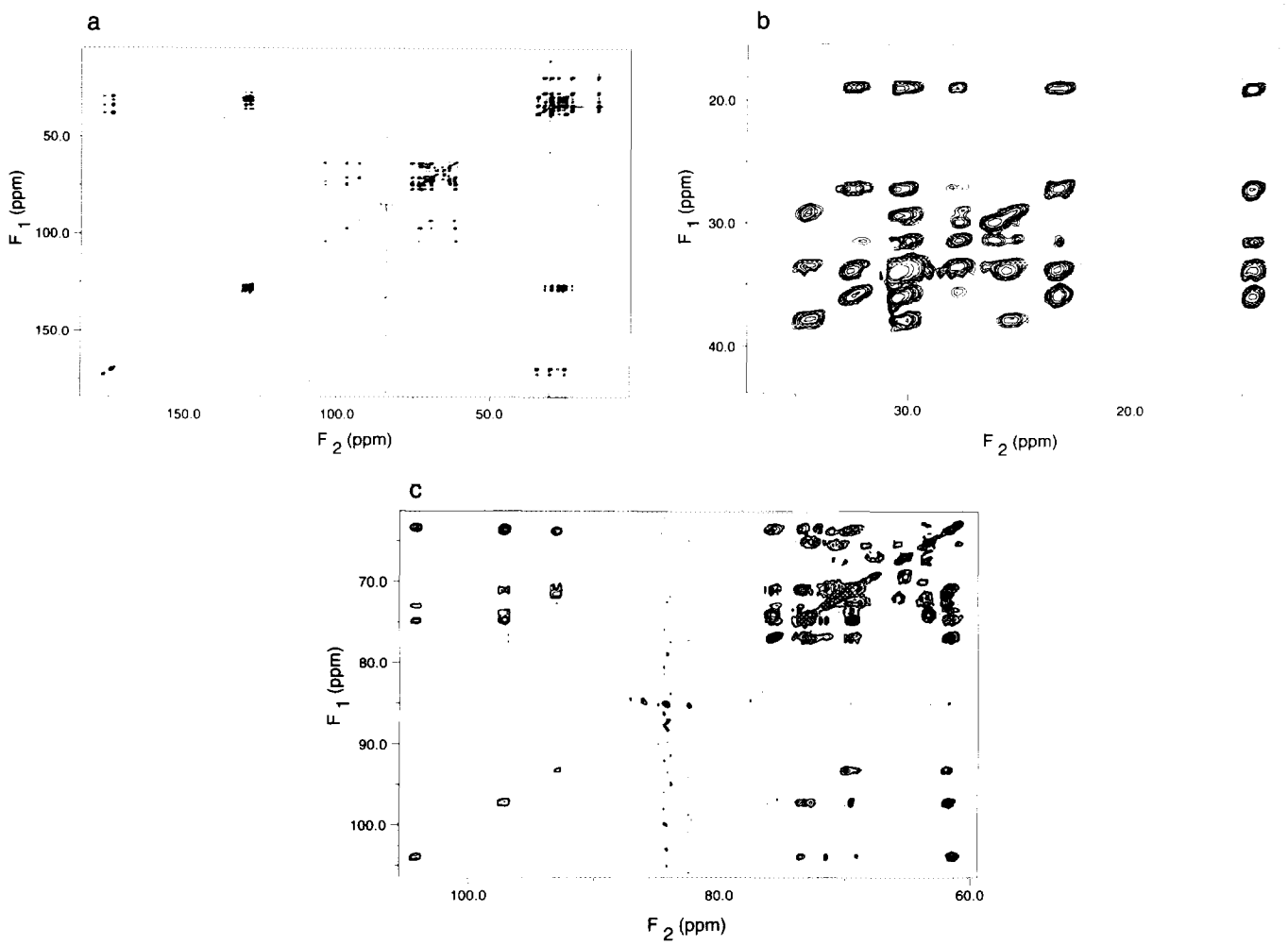

FIG. 4. MAS 2D ^{13}C–^{13}C TOCSY on the U-(^{13}C)-MGDG/D_2O mixture described in text with a spinning speed of 5 kHz: (a) full spectrum; (b) expansion of aliphatic region; and (c) expansion of glycerol/galactosyl region. The MLEV-17 mixing scheme of Bax *et al.* was employed (*13*). The total mixing time was 20 ms while trim pulses were 2.5 ms. The ^{13}C carrier was placed in the middle of the ^{13}C spectrum and an RF field strength of 40 kHz was employed to minimize offset effects. Proton decoupling during the evolution period was achieved by placing a 180° pulse on the ^{1}H channel in the middle of t_1. CW proton decoupling was applied during detection. Sixteen transients were collected for each t_1 point, and a recycle delay of 3 s was employed. Data were acquired using the method of States *et al.* (*26*). The 2D matrix of 256 × 1024 points was zero filled to 1024 × 2048 points.

ically the problem of CP at high spinning speeds is circumvented by any of a wealth of new phase-switched and amplitude-modulated techniques (*23–25*). INEPT provides an alternative for polarization transfer in lipid/water systems.

We have shown that MAS combined with multidimensional chemical-shift-correlation techniques are useful for obtaining high-resolution spectra of lipids in their biologically relevant state. Individual components of membranes may be traced out using the connectivity information provided by these experiments. It is possible that chemical-shift correlation, in conjunction with dipolar mixing, will provide a probe for studies of lipid structure and membrane organization.

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