J|A|C|S
COMMUNICATIONS

Sensitive Through-Space Dipolar Correlations between Nuclei of Small Organic Molecules by Partial Alignment in a Deuterated Liquid Solvent

Brad Bendiak
University of Colorado Health Sciences Center, Department of Cellular and Structural Biology,
Campus Box B111, 4200 East Ninth Avenue, Denver, Colorado 80262

Received September 4, 2002

NMR spectroscopy is now a routine tool for the structural elucidation of small molecules of natural isotopic abundance, where proton chemical shifts and *J*-couplings measured under isotropic tumbling conditions remain a cornerstone for deduction of their structure. Direct through-space interactions between nuclei can be observed via the nuclear Overhauser effect (NOE) and more recently through residual dipolar couplings[1] (D_{ij}) that require molecules to be partially aligned with respect to the magnetic field. These couplings can be observed for water-soluble molecules of large or intermediate size, weakly oriented using lipid bicelles or bacteriophage in aqueous solution.[1] However, a great many molecules of interest to chemists and biochemists are soluble in organic solvents. Measurement of residual D_{ij} in this class of molecules has not been possible as they align too strongly in bicellar systems. Here, a new procedure for their partial alignment is reported that employs a fully deuterated, essentially pure liquid crystalline solvent *above* its liquid-crystalline/isotropic (LC/iso) transition temperature. This permits more sensitive through-space interactions to be detected between nuclei via coherence transfer though residual *D*-couplings.

The alignment of molecules in a magnetic field results in additional effects in NMR spectra.[2] These include *D*-couplings between nuclei, chemical shift anisotropy (CSA), anisotropy of *J* values,[3] and anisotropy of intra- and intermolecular relaxation events.[2] A predominant effect results from dipolar couplings. The intensity of these couplings is dependent on the distance between nuclei, r, and the angle, θ, between the direction of the magnetic field and the vector connecting the nuclei, according to eq 1, where ξ_{ij} is a constant made up of other physical constants and is dependent on the type of nuclei involved in the coupling:[2]

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

In principle, *D*-couplings offer a more sensitive means to detect through-space interactions between nuclei than NOESY or ROESY[4] experiments, as they have a r^{-3} rather than a r^{-6} distance dependence. In practice, molecules aligned too strongly have D_{ij} up to thousands of hertz that result in complex multiplets with numerous splittings, often larger than chemical shift differences. With weakly aligned molecules, multiplets are restricted to narrow spectral regions. Unique structural information about rigid subunits of molecules and their relative orientation can be derived from residual D_{ij}. This requires knowledge of at least five angular parameters (i.e., from D_{ij} measurements) provided by each subunit, which can be used to constrain the orientations of subunit order tensor frames.[5]

The orienting solvent, 4-*n*-pentyl-4′-cyanobiphenyl-d_{19} (PCBP) is shown in Figure 1d and is usually used undiluted. In the liquid state it induces partial alignment of dissolved molecules, the extent of which can be controlled through a temperature change. The solvent itself has been the topic of extensive studies in the liquid crystalline state.[7] Even above the LC/iso transition temperature, however, a slight ordering of the compound has been reported, which has been examined by measurements of magnetic-field-induced birefringence via the Cotton−Mouton coefficient[8] and quadrupolar splittings of the fully deuterated compound.[9] The temperature dependence of ^{2}H NMR spectra of PCBP in the liquid state, above the LC/iso transition temperature[10] is shown in Figure 1. Changes occur during the approach to the transition in that quadrupolar splittings increase in magnitude, chemical shifts change, and line width at half-height is increased. Quadrupolar splittings increase up to nearly 20 Hz; the upfield $-CD_3$ signal is the best for quantitatively monitoring the effect (Figure 1a−c). These splittings result from weak anisotropic orientation, as do chemical shift changes. Line width increases reflect a lowering of the rates of molecular reorientation and conformational changes due to slower fluctuations of components of the ^{2}H quadrupolar tensor.

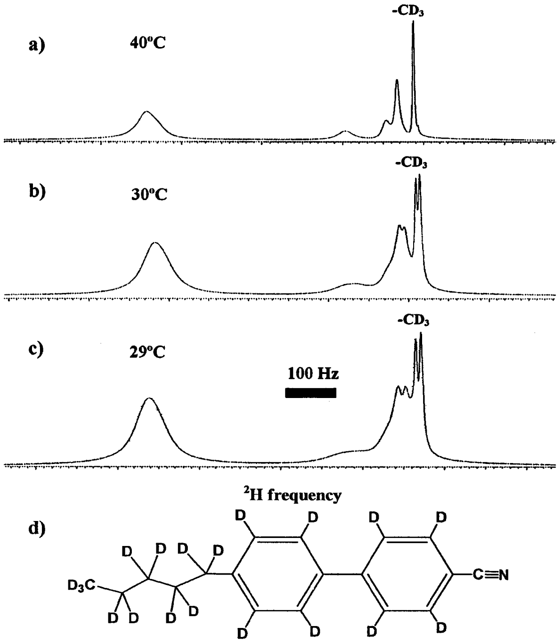

Figure 1. Temperature dependence of ^{2}H NMR spectra of the orienting solvent, PCBP[6] (structure in d). Spectra were recorded with the material in the liquid state at (a) 40 °C, (b) 30 °C, and (c) 29 °C. A 100-Hz chemical shift width and the signals corresponding to the $-CD_3$ group are shown.

Dissolving small protonated molecules in the fully deuterated solvent and recording ^{1}H NMR spectra in the liquid state between 45 and 50 °C gave essentially the same results as observed in other isotropic solvents, indicating negligible alignment under these conditions. However, nearer to the LC/iso transition temperature modified multiplet patterns were observed in 1D spectra, and additional cross-peaks were observed in 2D COSY- and TOCSY-type experiments. Spectra of the molecule 2-acetylphenanthrene

---

* To whom correspondence should be addressed. E-mail: brad.bendiak@uchsc.edu.

10.1021/ja028412q CCC: $22.00 © 2002 American Chemical Society

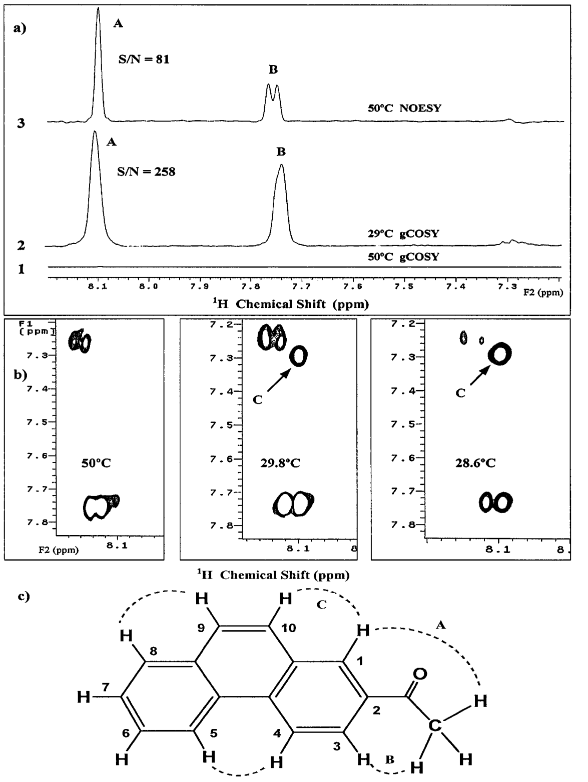

Figure 2. ^{1}H NMR spectra[6] of 2-acetylphenanthrene (structure in c) in PCBP-d_{19}. (a) Spectra are 1D traces parallel to the F_2 axis from 2D ^{1}H−^{1}H gCOSY or NOESY experiments. The F_1 frequency corresponds to the methyl protons at 2.23 ppm, and the F_2 region shown is that of the aromatic signals. The temperature and type of each 2D spectrum is indicated above each trace. Peaks A and B either represent the through-space residual D-coupling correlations under weakly aligned conditions (trace 2) or NOE correlations under isotropic conditions (trace 3) between protons of 2-acetylphenanthrene shown in panel c. Signal-to-noise measurements (S/N) are shown for peak A. (b) The three strips shown are from 2D gCOSY spectra accumulated at the temperatures indicated. The cross-peak "C" shown with an arrow corresponds to a $D_{H,H}$ between protons 1 and 10, indicated in panel c. Other residual $D_{H,H}$ are indicated in c between ^{1}H that showed no J-couplings under isotropic conditions.

(Figure 2) are illustrative of the effects of orientation that were observed with other small molecules. In Figure 2a, comparisons are shown between traces of 2D spectra at the F_1 frequency of the methyl group, parallel to the F_2 axis in the region containing aromatic proton signals. Under isotropic conditions (50 °C, trace 1), no cross-peaks were observed in COSY spectra between the acetyl methyl protons and H-1 or H-3 protons. These protons are five bonds distant; consequently, through-bond J-couplings would be expected to be negligible. However, closer to the LC/iso transition temperature (29 °C, trace 2, shown at the same noise level as trace 1), intense cross-peaks were observed in COSY experiments and in purely absorptive in-phase TOCSY spectra[11] (not shown) as the sample partially oriented. These resulted from coherence transfer through-space via $D_{1,Me}$ and $D_{3,Me}$ (labeled as peaks A and B, interactions shown in Figure 2c). These cross-peaks were also observed in a NOESY experiment accumulated for the same time period and optimized under isotropic conditions (50 °C, trace 3), but the signal/noise measured for correlation A, for example, was about 3.2-fold higher in the COSY experiment, despite broadening of the signal somewhat along the F_2 axis due to additional residual $D_{H,H}$. Other cross-peaks appeared among aryl protons that were not J-coupled under isotropic tumbling conditions. For example, in COSY experiments shown in Figure 2b, a new correlation appeared between H-1 and H-10 that was orientation-dependent (correlation C, marked with an arrow). Chemical shifts also varied with temperature. Caution should be taken in evaluation of any CSA effects in this system, however, as samples are intimately associated with the (alignable) solvent.

Partially oriented samples actually contain a great deal more structural information *inherent in their multiplet patterns* than isotropic samples. However, the contribution of more couplings (J and D) to multiplet patterns requires greater demands of methods for extraction of their coupling constants, as has been pointed out.[12] Despite issues of quantification, observing coherent through-space cross-peaks at sensitivities higher than NOESY experiments is valuable in itself, as correlations between nuclei at further distances than observed by NOEs[1] will be useful to detect.

Multiplet patterns are reproducible in this system, provided the quadrupolar splitting of the solvent $-CD_3$ group is temperature-tuned to the same value and the sample quantity is small ($\lesssim$2%) compared to the solvent quantity. The transition temperature can also be modified by adding small quantities of an isotropic solvent such as dimethylformamide-d_7, which lowers and broadens the transition zone for tuning the molecular alignment tensor.

In summary, the major advantage of this system is that liquid-phase NMR spectra of small nonpolar molecules can be conveniently compared between the isotropic and weakly aligned states. This provides access of a large class of small molecules to structural information via through-space nuclear interactions that are fundamentally different and often more sensitive than NOE correlations and should enable homonuclear and heteronuclear D-couplings to be observed, as reported for water-soluble macromolecules.[1,13] There will be advantages to using simple spin systems rather than macromolecules to test efficiencies of different magnetization-transfer processes between nuclei in molecules in the partially aligned state.

Acknowledgment. I thank A. Pardi for reviewing the manuscript and J. W. Emsley for a useful discussion concerning cosolvents. Supported in part by the Mizutani Foundation for Glycosciences, the University of Colorado Health Sciences Center, an NCI supplement to NIH5-P30-CA46934-10, and spectrometers from the Howard Hughes Medical Institute.

References

(1) (a) Tjandra, N.; Bax, A. *Science* **1997**, *278*, 1111−1114. (b) Bolon, P. J.; Prestegard, J. H. *J. Am. Chem. Soc.* **1998**, *120*, 9366−9367. (c) Hansen, M. R.; Rance, M.; Pardi, A. *J. Am. Chem. Soc.* **1998**, *120*, 11210−11211.
(2) Diehl, P. In *Nuclear Magnetic Resonance of Liquid Crystals*; Emsley, J. W., Ed.; Reidel: Dordrecht, the Netherlands, 1985; pp 147−180.
(3) The effects of J-anisotropy on splittings is typically 2−3 orders of magnitude less than the effects of dipolar coupling; therefore, in weakly oriented samples, J-anisotropy contributes negligibly to multiplet patterns.
(4) (a) Bothner-By, A. A.; Stephens, R. L.; Lee, J.-M.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811−813. (b) Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *63*, 207−213.
(5) (a) Losonczi, J. A.; Andrec, M.; Fischer, M. W. F.; Prestegard, J. H. *J. Magn. Reson.* **1999**, *138*, 334−342. (b) Ramirez, B. E.; Bax, A. *J. Am. Chem. Soc.* **1998**, *120*, 9106−9107.
(6) Spectra were recorded at a field strength of 11.74 T (76.7 MHz ^{2}H, 500 MHz ^{1}H). PCBP-d_{19} (100 μL, CDN isotopes) was placed in a 3-mm tube susceptibility-matched to chloroform (Shigemi). ^{2}H spectra were recorded on the lock channel using a Nalorac 3-mm triple resonance probe. Protonated samples (0.5 mg) were used.
(7) Emsley, J. W. In *Nuclear Magnetic Resonance of Liquid Crystals*; Emsley, J. W., Ed.; Reidel: Dordrecht, the Netherlands, 1985; pp 379−412.
(8) Zhang, S.-L.; Peng, Z.-Y.; Wu, J.; Shen, T.-H.; Wu, N.-Q.; *Mol. Cryst. Liq. Cryst.* **1983**, *91*, 295−307.
(9) Attard, G. S.; Beckmann, P. A.; Emsley, J. W.; Luckhurst, G. R.; Turner, D. L. *Mol. Phys.* **1982**, *45*, 1125−1129.
(10) For PCBP-d_{19} containing samples up to about 1% w/w, the transition was near 27 °C, as recorded on a Varian Inova instrument.
(11) Davis, A. L.; Estcourt, G.; Keeler, J.; Laue, E. D.; Titman, J. J. *J. Magn. Reson.* **1993**, *A105*, 167−183.
(12) Tian, F.; Bolon, P. J.; Prestegard, J. H. *J. Am. Chem. Soc.* **1999**, *121*, 7712−7713.
(13) Hennig, M.; Carlomagno, T.; Williamson, J. R. *J. Am. Chem. Soc.* **2001**, *123*, 3395−3396.

JA028412Q