High-Sensitivity ^{2}H NMR in Solids by ^{1}H Detection

Klaus Schmidt-Rohr,*,† Kay Saalwächter,‡ Shu-Feng Liu,† and Mei Hong†

Department of Chemistry and Ames Laboratory
Iowa State University, Ames Iowa 50011
Max-Planck-Institut für Polymerforschung
Postfach 3148, D-55021 Mainz, Germany

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The ^{2}H quadrupolar coupling is an excellent nuclear magnetic resonance (NMR) probe of segmental orientation, molecular dynamics, or hydrogen-bonding in solids and liquid crystals.[1,2] It has provided information on protein dynamics[3] and structure,[4] on motions in polymers,[5] on bond-order parameters in liquid crystals,[6] on dynamics of guest molecules in zeolites,[7] clays,[8] and inclusion compounds,[9] and on hydrogen-bond lengths.[2,10] However, due to large line widths and the relatively small magnetic moment of ^{2}H (15% of that of ^{1}H), the sensitivity of traditional ^{2}H NMR in solids is relatively low, except for the special case of C^2H_3 groups.

We report here a novel method, *p*roton *i*nverse-detected *de*uteron (PRIDE) NMR, that can provide sensitivity enhancement in ^{2}H NMR by an order of magnitude or more. It can be regarded as the solid-state NMR analogue of "inverse-detection" schemes in solution NMR,[11,12] and recently demonstrated in fast-magic-angle-spinning solid-state NMR.[13] In these experiments, the spectra of low-sensitivity nuclei are detected indirectly, in the first dimension of a two-dimensional spectrum, via the modulation of the strong ^{1}H signals. The sensitivity gain in the PRIDE NMR experiment comes primarily from the sensitivity of ^{1}H detection, which is higher than that of ^{2}H (=D) by a factor of $(\gamma_H/\gamma_D)^{5/2} = 108$, where γ_H and γ_D are the gyromagnetic ratios of ^{1}H and ^{2}H, respectively.[14]

Other factors also contribute to sensitivity enhancement, in particular ^{1}H line-narrowing by a solid-echo train (pulsed spinlock) used during detection.[15] Due to the longer persistence of the ^{1}H signal under the spinlock, where it decays with a time constant $T_{1\rho,H}$, the line width $\Delta\nu_{1H} \sim 1/T_{1\rho,H}$ in the ^{1}H spectrum is decreased, and the signal height increased. Combined with the indirect (two-dimensional) detection scheme, this results in a signal-to-noise gain of $(T_{1\rho,H}/\tau_D)^{1/2}(t_{det}/t_{dw,2})^{1/2}$, where τ_D is the maximum acquisition time of the ^{2}H time signal,[13] $t_{dw,2}$ is the dwell time between acquired points, and t_{det} is the detection window length. Typically, the sensitivity gain from this factor, relative to ^{2}H detection, is ∼2−10. Compared to standard ^{1}H wide-line detection, a more than 5-fold sensitivity enhancement is achieved. The larger electronic quality factor of the ^{1}H resonance circuit also increases the relative sensitivity of the PRIDE experiment (∼2-fold). Other effects decrease it, e.g. the ratio of the spin-dependent prefactors in the magnetization expression,[16] $I(I+1)/[S(S+1)] = {}^3/_8$, with the spin quantum numbers $I = {}^1/_2$ for ^{1}H and $S = 1$ for ^{2}H. The detection efficiency per ^{2}H, which combines several factors, was measured to be 0.7. The use of a double-resonance probe results in an estimated sensitivity reduction by 0.5−0.2. The combined factors of ∼600 * 0.08 = 48 will still result in a large sensitivity enhancement.

Instead of double cross polarization[13] with its high ^{2}H radio-frequency power requirements,[17] we use a heteronuclear multiple-quantum coherence (HMQC) approach[11,12] with only two or three ^{2}H pulses of ∼10 μs total duration.[18] The pulse sequence is shown in Figure 1. After the initial ^{1}H 90°-pulse, the ^{1}H−^{2}H dipolar coupling generates ^{1}H−^{2}H coherence. During this period τ_{HD}, ^{1}H homonuclear decoupling must be applied; for simplicity, the MREV-8 cycle[19] was used. Then, a "magic sandwich" consisting of four pulses[20] is applied, so that ^{1}H homonuclear dipolar evolution in the following long window is refocused into a magic-sandwich echo (MSE).[20] A ^{1}H 180° pulse at the center of the window refocuses the chemical-shift evolution for the entire sequence until the start of signal detection. A ^{2}H 90° pulse near the start of the window makes the heteronuclear coherence transverse in the ^{2}H term, which is then modulated by the ^{2}H quadrupolar coupling. The duration of the quadrupolar evolution time t_1 and the lengths of the two long inner pulses of the magic-sandwich are incremented synchronously to fulfill the MSE condition.[20] The quadrupolar evolution is terminated by a 90° ^{2}H pulse that makes the coherence again longitudinal on ^{2}H. A third ^{2}H pulse, shown dashed in Figure 1, partially compensates for the finite duration of the two other ^{2}H pulses, by effectively creating a solid echo. This composite-pulse[21] scheme provides efficient ^{2}H excitation. Then MREV-8 decoupling is resumed, and the ^{1}H−^{2}H dipolar coupling converts the heteronuclear coherence modulated by the ^{2}H quadrupolar coupling back into observable ^{1}H magnetization.

For detection of the ^{1}H magnetization with enhanced sensitivity, we use a pulsed-spinlock sequence of ^{1}H 90° pulses of the same phase as the magnetization,[15] separated by windows of ∼8 μs, with a 1.5-μs data sampling interval at the end. The modulation by the ^{2}H quadrupolar coupling is detected indirectly by systematic incrementation of t_1.[14,22] After two-dimensional (2D) Fourier

* To whom correspondence should be addressed. Telephone: 515-294-6105. Fax: 515-294-0105. E-mail: srohr@iastate.edu.
† Iowa State University.
‡ Max-Planck-Institut für Polymerforschung.

(1) Spiess, H. W. In *Deuteron NMR: A New Tool for Studying Chain Mobility and Orientation in Polymers*; Spiess, H. W., Ed.; Springer: Berlin, 1985; Vol. 66, pp 24−57.
(2) Hunt, M. J.; McKay, A. L. *J. Magn. Reson.* **1976**, *22*, 295−301.
(3) (a) Mack, J. W.; Torchia, D. A.; Steinert, P. M. *Biochemistry* **1988**, *27*, 5418−5426. (b) Lee, K.-C.; Hu, W.; Cross, T. A. *Biophys. J.* **1993**, *65*, 1162−1167. (c) Williams, J. C.; McDermott, A. E. *Biochemistry* **1995**, *34*, 8309−8319. (d) Palmer, A. G.; Williams, J.; McDermott, A. *J. Phys. Chem.* **1996**, *100*, 13293−13310.
(4) (a) Copie, V.; McDermott, A. E.; Beshah, K.; Williams, J. C.; Spijker-Assink, M.; Gebhard, R.; Lugtenburg, J.; Herzfeld, J.; Griffin, R. G. *Biochemistry* **1994**, *33*, 3280−3286. (b) Gröbner, G.; Burnett, I. J.; Glaubitz, C.; Choi, G.; Mason, A. J.; Watts, A. *Nature* **2000**, *405*, 810−813.
(5) (a) Pschorn, U.; Rössler, E.; Sillescu, H.; Kaufmann, S.; Schaefer, D.; Spiess, H. W. *Macromolecules* **1991**, *24*, 398−402. (b) Miura, H.; Hirschinger, J.; English, A. D. *Macromolecules* **1990**, *23*, 2169−2182. (c) Blum, F. D.; Xu, G.; Liang, M.-H.; Wade, C. G. *Macromolecules* **1996**, *29*, 8740−8745.
(6) (a) Bloom, M.; Burnell, E. E.; Roeder, S. B. W.; Valic, M. I. *J. Chem. Phys.* **1977**, *66*, 3012−3020. (b) Seelig, J.; Seelig, A. *Q. Rev. Biophys.* **1980**, *13*, 19−61. (c) Hodgkinson, P.; Auger, C.; Emsley, L. *J. Chem. Phys.* **1998**, *109*, 1873−1884.
(7) Isfort, O.; Boddenberg, B.; Fujara, F.; Grosse, R. *Chem. Phys. Lett.* **1998**, *288*, 71−76.
(8) Wong, S.; Vaia, R. A.; Giannelis, E. P.; Zax, D. B. *Solid State Ionics* **1996**, *86*−*88*, 547−557.
(9) Sozzani, P.; Behling, R. W.; Schilling, F. C.; Brückner, S.; Helfand, E.; Bovey, F. A.; Jelinski, L. W. *Macromolecules* **1989**, *22*, 3318−3322.
(10) Malyarenko, D.; Vold, R. L.; Hoatson, G. L. *Macromolecules* **2000**, *33*, 1268−1279.
(11) Mueller, L. *J. Am. Chem. Soc.* **1979**, *101*, 4481−4484.
(12) Bax, A. *Curr. Opin. Struct. Biol.* **1994**, *4*, 738−744.
(13) Ishii, Y.; Tycko, R. *J. Magn. Reson.* **2000**, *142*, 199−204.
(14) Ernst, R. R.; Bodenhausen, G.; Wokaun, A. *Principles of Nuclear Magnetic Resonance in One and Two Dimensions*; Clarendon Press: Oxford, 1987.
(15) Ostroff, E. D.; Waugh, J. S. *Phys. Rev. Lett.* **1966**, *16*, 1097.
(16) Mehring, M. *Principles of high-resolution NMR in solids*, 2nd ed.; Springer-Verlag: Heidelberg, 1983.
(17) Takegoshi, K.; Ito, M.; Terao, T. *Chem. Phys. Lett.* **1996**, *260*, 159.
(18) Sandström, D.; Hong, M.; Schmidt-Rohr, K. *Chem. Phys. Lett.* **1999**, *300*, 213−220.
(19) Rhim, W.-K.; Elleman, D. D.; Vaughan, R. W. *J. Chem. Phys.* **1973**, *59*, 3740−3749.
(20) Rhim, W.-K.; Pines, A.; Waugh, J. S. *Phys. Rev. B* **1971**, *3*, 684−695.
(21) Levitt, M. H. *Prog. Nucl. Magn. Reson. Spectrosc.* **1986**, *18*, 61.
(22) Schmidt-Rohr, K.; Spiess, H. W. *Multidimensional Solid-State NMR and Polymers*, 1st ed.; Academic Press: London, 1994.

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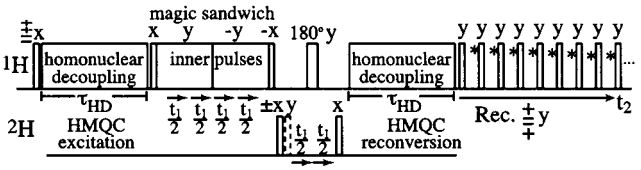

Figure 1. Pulse sequence for the PRIDE NMR experiment, with ^{1}H 90° excitation pulse, homonuclear decoupled excitation of the ^{1}H−^{2}H heteronuclear coherence, magic-sandwich echo on ^{1}H with ^{2}H quadrupolar evolution, reconversion of the heteronuclear coherence, and ^{1}H detection under pulsed spin lock (at the times labeled by *). A third ^{2}H pulse (shown dashed) is used to greatly reduce distortions of ^{2}H line shapes by finite-pulse-length effects.

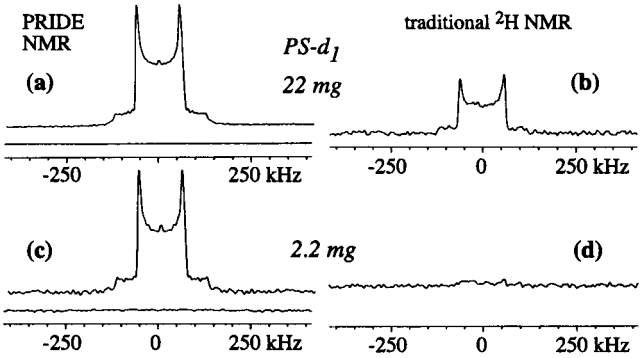

Figure 2. Proton inverse-detected and regular ^{2}H NMR of glassy polystyrene-d_1, [−CH_2−CD{C_6H_5}−]$_n$ (Cambridge Isotopes). (a) PRIDE spectrum of 22 mg of sample, maximum t_1-time of τ_D = 160 μs, total number of scans = 1280, recycle delay = 4 s (86 min total). The smooth line above the frequency axis is a cross section at $\omega_2 \neq 0$, showing that the random noise is very low; the main noise component is signal-proportional t_1 noise. (b) Regular ^{2}H spectrum acquired under essentially the same conditions (number of scans = 1024, recycle delay = 5 s). (c) PRIDE spectrum of 2.2 mg of sample, conditions as in (a). (d) Regular ^{2}H spectrum of 2.2 mg of sample, as in (b). The spectra in (b) and (d) are plotted on the same scale chosen such that the noise levels in (b), (c), and (d) are matched. The filling factor in (c) and (d) was only ∼0.05. Experiments were performed on a Bruker DSX-400 spectrometer at a ^{1}H frequency of 400 MHz (61 MHz for ^{2}H), in a stationary Bruker double-resonance probehead with a 5-mm diameter radio frequency coil. 160 time increments of 1 μs were recorded in the ^{2}H dimension. ^{2}H 90° pulse length: ∼3 μs. The central ^{2}H pulse (dashed in Figure 1) was optimized to a duration of 3.5 μs by maximizing the signal at t_1 = 0. The indirectly detected ^{2}H spectra are displayed without phasing or time extrapolation. Eight scans were averaged per t_1 slice, preceded by two "dummy scans" without acquisition. A 6.5 μs deadtime delay was used before detection of each ^{1}H signal point (1.5-μs detection window, 625 kHz filter width. Proton 90° pulse lengths of 2.9 or 3.5 μs were used, with similar results. Eight MREV-8 cycles of 12*4.5 μs = 54 μs duration each were applied for the excitation and for the reconversion of the heteronuclear coherence (total of 860 μs). MREV-8 with 103°-pulses gave the highest echo signal. The initial duration of the two long inner pulses in the magic sandwich was 21 μs each.

transformation,[14,22] the PRIDE spectrum is extracted from the 2D spectrum as the cross-section at $\omega_2 = 0$, parallel to the ω_1-axis.

The experiment was tested on polystyrene [−CH_2−CD-{C_6H_5}−]$_n$, PS-d_1, which yields a typical ^{2}H powder spectrum (Pake pattern) with a 120-kHz splitting between the two sharp horns.[1,22] This line shape is obtained without any phasing in the PRIDE spectra of Figure 2a and c, proving that the three ^{2}H pulses, after their lengths have been set so as to maximize the signal for $t_1 = 0$, provide good compensation of finite ^{2}H pulse-lengths. The traditional ^{2}H solid-echo spectrum in Figure 2b exhibits the same line shape, but with inferior signal-to-noise ratio (S/N). The traditional ^{2}H spectrum of the 2.2-mg sample, Figure 2d, is hardly observable within the 86-min measuring time, while the corresponding PRIDE spectrum, Figure 2c, has a good S/N. These data indicate that the PRIDE NMR experiment provides a 20-fold enhanced S/N. If PRIDE spectra are compared with symmetrized traditional spectra, the enhancement factor of the PRIDE is still $20/(2^{1/2}) = 14$. The detection of 20 μmol of deuterons demonstrated here is by no means the lower limit for the PRIDE technique. With a small coil matched to the 20-times smaller sample volume, the (S/N) would be enhanced ca. 20-fold.

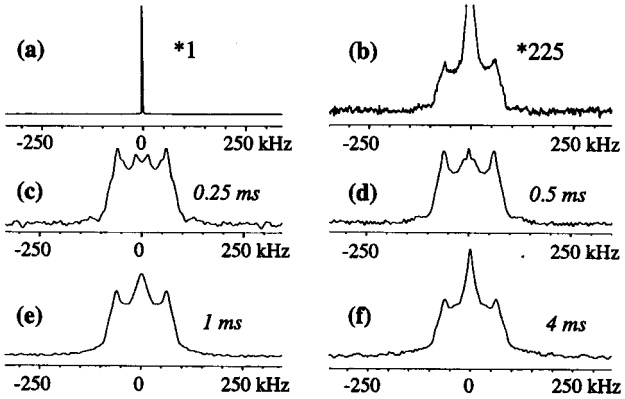

Figure 3. Proton inverse-detected and regular ^{2}H NMR of 66 mg of chicken egg-white lysozyme (Sigma-Aldrich) deuterated by dissolving 275 mg of the protein in 3 mL of D_2O at 30 °C. After drying, the protein was rehydrated with ∼20% D_2O by weight. (a) Regular ^{2}H spectrum, dominated by the narrow and high D_2O signal. (b) Signal of (a) after 225-fold vertical expansion (measured in 20 min). (c−f) Series of PRIDE spectra with increasing HMQC excitation times. Total excitation plus reconversion times 2 τ_{HD}: (c) 0.24 ms; (d) 0.48 ms; (e) 0.96 ms; (f) 3.8 ms. Each PRIDE spectrum was recorded in a total time of 80 min.

The comparison of the noise in the signal-containing slices at $\omega_2 = 0$ with slices away from all signal (shown in Figure 2, a and c) suggests that much of the noise in the PRIDE spectrum is associated with the NMR signal; this is known as "t_1-noise", which arises from spectrometer instabilities that lead to fluctuations in the signal.[14] Since all the protons contribute to the signal in an individual scan, PRIDE is particularly sensitive to these instabilities.

In addition to the increased sensitivity, the PRIDE approach also provides suppression of the sometimes dominant sharp and high signal of D_2O or other mobile deuterated species. We demonstrated this on lysozyme deuterated by ^{1}H/^{2}H exchange from D_2O; this produced ND, OD, and ND_3 groups. The traditional ^{2}H spectrum of this sample, Figure 3a, is dominated by the high, narrow D_2O signal. Only after 225-fold vertical expansion does the protein signal become visible, Figure 3b. In the PRIDE spectrum of Figure 3c, the D_2O peak is completely suppressed. Two splittings of 117 and 31 kHz are observed. The large splitting and the deviation from the Pake pattern are typical of immobile ND and OD groups. The smaller splitting may be due to ND_3 groups of lysine; a 33-kHz splitting has been observed for ND_3 groups.[23] At longer excitation times, Figure 3, d−f, a narrow central peak due to mobile deuterated segments slowly grows in; their identification is in progress.

PRIDE spectra are not affected by inhomogeneous linebroadening; thus, PRIDE NMR is applicable to rigid noncrystalline materials, of which glassy polystyrene is a typical example. PRIDE NMR is less useful for highly mobile samples such as elastomers or polymer melts, where the ^{1}H−^{2}H dipolar couplings are weakened, and where motional narrowing of the ^{2}H spectrum increases the direct ^{2}H sensitivity. Nevertheless, the two-component motionally narrowed signals in the lysozyme spectra of Figure 3 indicate that signal from partially mobile segments can be observed. Since no high ^{1}H resolution is required, the multiple-pulse sequences in PRIDE are more robust than those in high-resolution solid-state ^{1}H NMR. In conclusion, the high sensitivity, tunable selectivity, and combination of ^{1}H and ^{2}H NMR make the PRIDE NMR technique a promising new tool for studying dynamics and structure of complex organic materials.

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(23) Datema, K. P.; Puls, K. P.; Bloom, M. *Biochemistry* **1986**, *25*, 3796.