J|A|C|S COMMUNICATIONS

Published on Web 10/22/2009

Proton NMR of ^{15}N-Choline Metabolites Enhanced by Dynamic Nuclear Polarization

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Dynamic Nuclear Polarization (DNP) by the so-called 'dissolution' procedure[1] is rapidly gaining momentum as a novel method to enhance weak nuclear magnetic resonance (NMR) signals from molecular tracers, so that one can visualize their biodistribution and metabolism *in vivo*.[2] The major limitation of the technique arises from the short lifetimes of hyperpolarized spin states in liquids. In particular, longitudinal relaxation times T_1 of protons in solutions of biomolecules are too short to allow for transport and *in vivo* injection of hyperpolarized compounds. Most applications of the technique have therefore focused on ^{13}C NMR of ^{13}C-enriched tracers containing nonprotonated carbons with $T_1(^{13}C) \approx 20-40$ s. Choline $(CH_3)_3N^+CH_2CH_2OH$ plays a key role in several critical biological processes, in particular in the synthesis and metabolism of phospholipids in cell membranes, and in cholinergic neurotransmission. Although the choline molecule does not contain any slowly relaxing carbons, it possesses a quaternary nitrogen with $T_1(^{15}N) > 120$ s, which lends itself to hyperpolarization.[3] The conversion of choline to phosphocholine catalyzed by choline kinase has recently been monitored by ^{15}N NMR *in vitro*,[3] employing hyperpolarization of ^{15}N spins. *In vivo* measurements using this method may, however, be hampered by insufficient ^{15}N peak separation of choline metabolites ($\sim$ 0.2 ppm for phosphocholine vs choline, i.e., only 6 Hz in a field $B_0 = 7$ T) and by poor sensitivity of ^{15}N NMR. A sensitivity improvement by at least an order of magnitude would be required, e.g., to monitor phosphocholine accumulation in tumor cell cultures.[4] The above limitations may be overcome by transferring the hyperpolarization from ^{15}N to protons, as in recent heteronuclear 2D DNP-NMR experiments.[5] A similar concept was recently used for ^{13}C enhanced by PASADENA.[6] In this work, we show that one can transfer the long-lived ^{15}N hyperpolarization to remote methylene CH_2O protons in choline across three bonds via $^3J(^{15}N,^1H)$, which significantly improves both the sensitivity and the spectral dispersion of choline metabolites. We also show that $T_1(^{15}N)$ in choline can be considerably increased by deuteration of the methyl groups.

The conventional ^{1}H spectrum of ^{15}N-enriched choline is shown in Figure 1A. The peak at 3.19 ppm, which stems from the nine magnetically equivalent methyl protons, is commonly used for *in vivo* quantification of choline-containing compounds,[7] whereas the multiplets due to the NCH_2 (3.50 ppm) and CH_2O protons (4.05 ppm)[8] exhibit an AA′XX′ pattern.[9] The CH_2O and methyl peaks have additional doublet structures due to $^3J(^{15}N,^1H) \approx 3.7$ Hz and $^2J(^{15}N,^1H) \approx 0.8$ Hz. (In nonenriched choline, one observes triplets due to $^3J(^{14}N,^1H) = 2.7$ Hz and $^2J(^{14}N,^1H) = 0.6$ Hz.[8,9]) As shown in Figure 1B, the small $^nJ(^{15}N,^1H)$ couplings in choline can be used to transfer hyperpolarization from ^{15}N to CH_2O and methyl protons, using a reversed INEPT pulse sequence.[10,11] While the NCH_2 signal is absent in the ^{1}H DNP spectrum because the relevant coupling constant is too small, the CH_2O and $N(CH_3)_3$ signals are remarkably enhanced (>2 × 10^3 times), compared to the inverse-INEPT spectrum in thermal equilibrium (not shown). Among the major choline metabolites, the CH_2O peak exhibits the largest ^{1}H chemical-

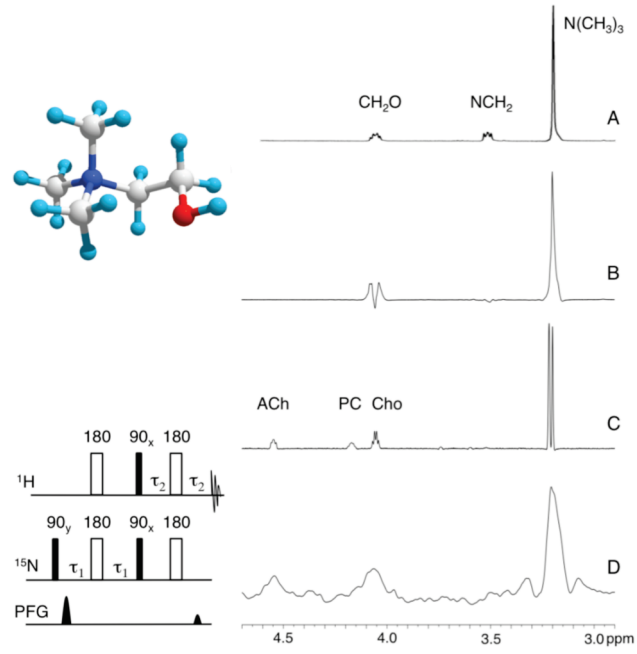

Figure 1. (A) Conventional ^{1}H NMR of ^{15}N-enriched choline in H_2O. (B) ^{1}H DNP-NMR of ^{15}N-choline, using inverse-INEPT. (C) ^{15}N-filtered ^{1}H spectrum of a mixture of 0.3 M natural-abundance acetylcholine (ACh) and phosphocholine (PC) and 1.3 mM ^{15}N-choline (Cho), 256 scans. (D) The inverse-INEPT ^{1}H DNP-NMR spectrum of a mixture of natural-abundance Cho and ACh. *Left:* Molecular structure of choline and inverse-INEPT pulse sequence. Proton signals that do not originate from ^{15}N magnetization were suppressed by two pulsed field gradients (PFG) in a 10:1 ratio.

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10.1021/ja9021304 CCC: $40.75

shift dispersion,[8] whereas the ^3J(N,H) coupling is preserved,[8,9] which ensures a nearly uniform efficiency of polarization transfer (Figure 1C).

To imitate less favorable *in vivo* conditions, we have recorded the ^{1}H DNP spectrum of a mixture of choline and acetylcholine at the natural abundance of ^{15}N and performed the experiment 90 s after dissolution. The obtained spectrum, plotted in Figure 1D using a line broadening of 8 Hz, exhibits noticeable CH_2O peaks from both compounds.

For the DNP experiments, 6 M ^{15}N-enriched choline chloride (SigmaAldrich), or a mixture of 3 M choline chloride and acetylcholine chloride (SigmaAldrich), were dissolved in 60/40 v/v glycerol-d_8/D_2O containing 50 mM TEMPO. Frozen beads were placed in a home-built prepolarizer[12] and irradiated with 30 mW of microwave power at 94 GHz for 3 h at 1.2 K and 3.35 T. The sample was rapidly dissolved in 5 mL of D_2O (at *ca.* 190 °C and 12 bar) to a final choline concentration of 50 ± 5 mM. The hyperpolarized solution was transferred through a thin tube into an injection device located in the bore of a high-resolution 7 T magnet (Bruker) and maintained at 25 °C. After a few seconds of mixing and thermal equilibration, part of the solution was injected into a 5 mm tube positioned in the NMR coil and kept at 25 °C using a thermostated air flow. The delay between dissolution and acquisition was 8 s for the ^{15}N-choline sample and 90 s for the mixed sample.

Figure 2A shows the DNP-enhanced ^{15}N-detected spectrum of the same solution as used for the experiment in Figure 1B. The enhancement factor was 13 400 compared to a thermal-equilibrium spectrum obtained with 140 scans, 90° pulses, and a repetition time of 1000 s (not shown). Nearly the same enhancement factor was reported by Gabellieri et al.[3] at 9.4 T, using a commercial DNP polarizer working at 3.35 T and 1.4 K with trityl radicals as the polarizing agent. The thermal-equilibrium (Boltzmann) polarization can be estimated to be $P(^{15}N) = \tanh(h\nu_0/(2k_BT)) = 2.45 \times 10^{-6}$ at $T = 298$ K and $B_0 = 7$ T. The enhanced polarization $P(^{15}N)$ was thus 3.3%, which is consistent with the polarization level measured in the 6 M solid sample prior to dissolution.

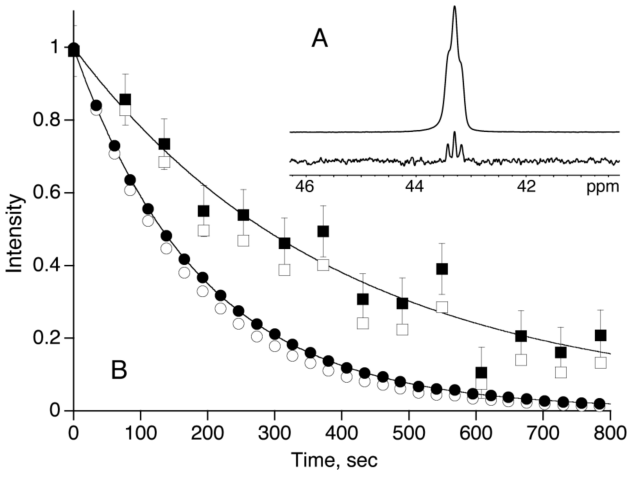

Figure 2. (A) Proton-coupled ^{15}N spectra of hyperpolarized solutions of ^{15}N-enriched choline (top; 10° pulse), and of choline-d_9 (bottom; 15° pulse). (B) Decays of ^{15}N hyperpolarization in ^{15}N-choline (○) and choline-d_9 (□), obtained using a series of 10° and 15° pulses, respectively. The curves represent three-parameter exponential fits to signal amplitudes that were corrected for magnetization losses due to the small-angle pulses (filled symbols).

To explore the effect of partial deuteration on the relaxation of ^{15}N spins in choline, we have measured the relaxation time constants $T_1(^{15}N)$ of hyperpolarized ^{15}N-choline and natural-abundance choline-trimethyl-d_9 chloride (SigmaAldrich). The longitudinal ^{15}N relaxation in choline is dominated by dipolar interactions[13] with $N(CH_3)_3$ and NCH_2 protons, with smaller contributions from the more distant CH_2O protons, solvent spins, and free radicals. Figure 2B shows the decays of hyperpolarized magnetization for ^{15}N-choline and choline-d_9 (where 9 of the 11 hydrogen nuclei nearest to ^{15}N are replaced by deuterium) in D_2O. For ^{15}N-choline, we obtained $T_1(^{15}N) = 189 \pm 2$ s. A similar value of $T_1(^{15}N) = 203 \pm 10$ s was reported by Gabellieri et al. in the presence of free radicals.[3] For choline-d_9, we obtained $T_1(^{15}N) = 390 \pm 110$ s. Despite a high noise level for the latter sample (with only 0.37% ^{15}N), the results indicate a nearly two-fold increase of $T_1(^{15}N)$ for the deuterated molecule.

In conclusion, the present study shows that the ^{15}N relaxation time in choline is prolonged by deuteration of the methyl groups and that the ^{15}N hyperpolarization can be readily transferred via small scalar couplings to the distant CH_2O protons. One can therefore use the long-lived ^{15}N spin states in deuterated choline compounds for the storage of hyperpolarization and recur to ^{1}H detection to achieve better sensitivity and spectral resolution of metabolites. Kinetic measurements using this approach may open the way to monitor biodistribution and metabolism of choline and structurally related compounds by DNP-enhanced ^{1}H NMR.

Acknowledgment. We are deeply grateful to Dr. Jacques van der Klink for initiating the DNP projects in Lausanne and for his invaluable technical help. The work was supported by the Swedish Research Council (Project No. K2009-61P-20945-03-1), the Swedish Foundation for Strategic Research, the Swiss National Science Foundation (Grants No. 200020_124901, PZOOP2_121928, and 200020_124694), by the Centre d'Imagerie Biomedicale (CIBM) of the UNIL, EPFL, UNIGE, CHUV, and HUG, and by the Leenards and Jeantet Foundations.

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JA9021304