Measurement of Hyperpolarized ^{15}N-choline using Polarization Transfer with [1]H Detection

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Introduction:

Choline ($(CH_3)_3N^+CH_2CH_2OH$) is an essential nutrient and an important precursor of acetylcholine, betaine and phosphatidylcholine. Since choline phospholipid metabolism is altered in a variety of cancers, levels of choline-containing compounds represent an important biomarker in oncological diseases. The metabolic conversion of ^{15}N-choline to phosphocholine has recently been visualized *in vitro* using hyperpolarized ^{15}N MRS [1]. The ^{15}N spin-lattice relaxation time in aqueous choline solution was reported to be as long as 285 s [1], suggesting ^{15}N-choline as a useful probe for liquid-state DNP MRS [2]. However, the small ^{15}N chemical shift difference between choline and its metabolites (*ca* 0.2 ppm), combined with the small magnetogyric ratio of [15]N (10 times smaller than for proton) can hinder the use of direct ^{15}N MRS detection for *in vivo* imaging of choline metabolism. In the present work we investigated possibility of the transfer of ^{15}N-choline hyperpolarizarion from nitrogen to neighbouring protons with subsequent ^{1}H MRS detection.

Methods:

The ^{15}N and ^{1}H spectra were recorded at 7 T on a Bruker 300 WB spectrometer using a 5 mm inverse broadband probe. ^{15}N-choline chloride and TEMPO radical were dissolved in a glycerol-d_8/D_2O mixture at concentrations of 6 M and 50 mM, respectively. The sample was polarized for *ca* 3 hrs at 3.35 T and 1.2 K using a prepolarizer described in [3], and rapidly dissolved into 5 ml of D_2O to the final ^{15}N-choline concentration of ca 50 mM. Two 5 mm tubes were filled with 800 µl of the solution and placed in the magnet within *ca* 30 and 100 s after dissolution, respectively. For each sample, the ^{15}N signal was recorded using a single 10° pulse. This experiment indicated the DNP enhancement factors of up to 5000, compared to the thermal equilibrium state. Thereafter, a single ^{1}H scan was performed using the inverse INEPT sequence [4] that encodes exclusively the [15]N - [1]H transfer pathway using two gradients in a 10:1 ratio. The proton signals that do not originate from nitrogen magnetisation (including the water signal) are suppressed.

Results and Discussion:

Although the choline nitrogen lacks directly attached protons, its ^{15}N peak exhibits small splittings due to two-bond J-couplings to the methylene and methyl protons, respectively. Thanks to the narrow homogeneous linewidth of the ^{15}N peak, these couplings can be used for ^{15}N-to-^{1}H polarization transfer. Figures 1 & 2 show the ^{1}H spectra of hyperpolarized ^{15}N-choline solution observed using inverse INEPT. In Figure 1, the primary methylene anti-phase peak (4.07 ppm) was more than 2000 times larger compared to the thermal-equilibrium spectrum, whereas the second-methylene (3.52 ppm) and the residual water (4.79 ppm) peaks were similar in size to the ^{13}C satellites of the primary methylene peak. The relative efficiency of polarization transfer to the methylene (4.07 ppm) and methyl (3.2 ppm) protons depended strongly on the value of the inter-pulse delay (unequal for Figures 1 and 2).

The results indicate that the polarization transfer methods can be used to utilize the long relaxation time of ^{15}N-choline for the storage of nuclear hyperpolarization (thus allowing more time for the sample delivery, biodistribution and metabolic conversion), and subsequently obtain an improved spectral resolution of metabolites together with superior sensitivity, provided by the ^{1}H detection.
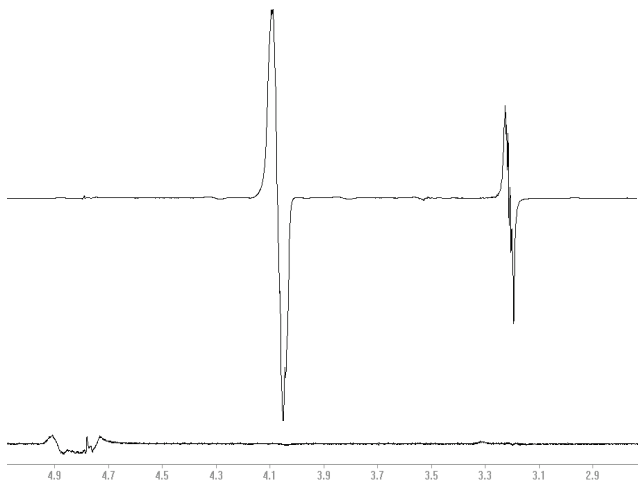

Figure 1. *Top*: The ^{1}H spectrum resulting from the non-refocused INEPT pulse sequence, applied *ca* 35 s after dissolution of the hyperpolarized ^{15}N-choline sample. *Bottom:* a thermal-equilibrium scan using the same sequence (magnified).
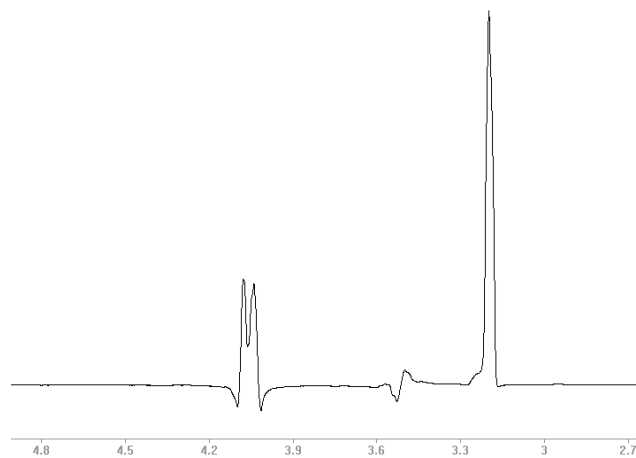

Figure 2. The ^{1}H spectrum resulting from the refocused INEPT pulse sequence, applied *ca* 110 s after dissolution of the hyperpolarized ^{15}N-choline sample.

References: [1] Gabellieri C. et al., J. Am. Chem. Soc. 130 (2008) 4598. [2] Ardenkjaer-Larsen J.H. et al, Proc. Nat. Acad. Sci. 100 (2003) 10158. [3] Comment et.al., Conc Magn Reson 31B (2007) 255; Comment et.al., Appl. Magn. Res. 34 (2008) 313. [4] Morris G.A. & Freeman R., J. Am. Chem. Soc. 101 (1979) 760; Burum D.P. & Ernst R.R., J. Magn. Res. 39 (1980) 163.