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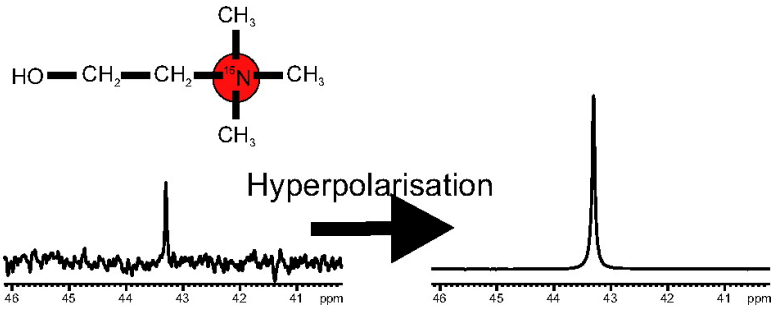

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Therapeutic Target Metabolism Observed Using Hyperpolarized ^{15}N Choline

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Choline is a precursor of cellular phospholipid metabolism that provides Magnetic Resonance (MR) and Positron Emission Tomography (PET) biomarkers for cancer detection and response assessment.[1] Employing Dynamic Nuclear Polarization we show that the MR signal of ^{15}N in choline can be enhanced by at least 4 orders of magnitude with a relaxation time T_1 of ca. 4 min, providing a method to observe the action of choline kinase, an important target for novel cancer therapeutics.[2]

Choline is an important substrate for cellular phospholipid metabolism. It is incorporated into the cell membrane via the Kennedy pathway, through phosphocholine (PCho) and CDP-choline synthesis producing phosphatidylcholine. Malignant transformation of cells is characterized by increased levels of phosphocholine, and both ^{1}H and ^{31}P magnetic resonance spectroscopy reveal elevated levels in many different forms of cancer compared to normal tissues.[3] Both choline transport into cells and phosphorylation to PCho catalyzed by choline kinase have been found to be upregulated in breast cancer,[4] and increasing evidence recognizes a decrease in PCho levels in cancer cells as a positive response to some treatments.[5] Choline kinase is an important target for anticancer drugs,[6] and identification of noninvasive methods to monitor therapeutic activity is crucial for the clinical development and evaluation of novel anticancer drugs.

Compared to PET, magnetic resonance offers excellent temporal and spatial resolution, no exposure to ionizing radiation, and is also chemically specific, able to differentiate between parent compounds and metabolic products. However, the main drawback of MR is the low sensitivity. Recent developments in Dynamic Nuclear Polarization (DNP) can enable the MR signal of nuclei such as ^{13}C and ^{15}N to be increased by up to 4 orders of magnitude.[7] This offers the potential to perform real-time imaging of the metabolic products shortly after introducing the hyperpolarized agent, with negligible background signal. Pyruvate is a substrate for several key metabolic processes that are implicated in cellular energy homeostasis and has been proposed as a new tool for metabolic imaging[8] using hyperpolarized ^{13}C. Recent advances have demonstrated the utility of this approach for detection of tumors in vivo,[9] for diagnostic MR in a transgenic mouse model of prostate cancer,[10] or for assessment of treatment response.[11]

We here demonstrate the potential to hyperpolarize the ^{15}N in isotopically labeled choline, measure the lifetime of the polarized signal governed by the longitudinal relaxation time constant, and demonstrate in real-time the metabolic conversion of hyperpolarized choline to phosphocholine employing DNP and purified human choline kinase. Choline chloride (98% ^{15}N enriched choline chloride, Sigma Aldrich, United Kingdom) was dissolved in a solution containing 15 mM of trityl free radical OX63 in DMSO/H_2O. The sample was polarized in a HyperSense DNP polarizer (Oxford Instruments Molecular Biotools, United Kingdom) at low temperature, 1.4 K, for 2 h in a superconducting magnet (3.35T) with microwave irradiation at 94 GHz. The polarized sample was dissolved in a 4 mL aqueous buffer solution prior to recording the spectrum in a 9.4T NMR spectrometer. Enzyme kinetics was followed by adding hyperpolarized ^{15}N choline to an NMR tube containing purified human choline kinase. Final concentrations were 100 mM Tris, 100 mM KCl, 50 mM $MgCl_2$, 20 mM Choline, 10 mM ATP, 1 mM EDTA, purified human choline kinase 2 μM, pH = 8.[12] The reaction was followed at 37 °C in a 9.4T NMR spectrometer.

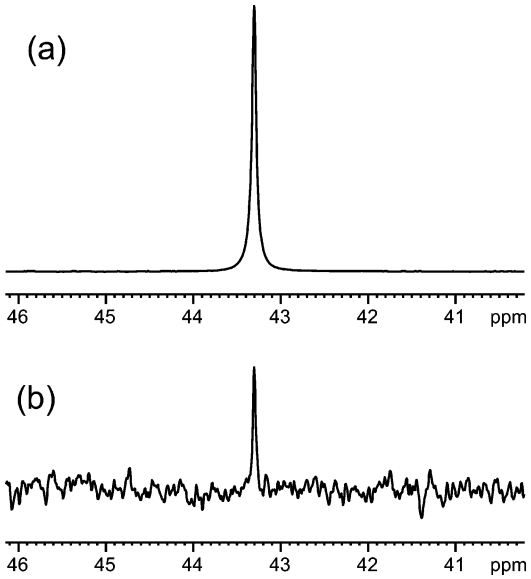

Figure 1. (a) ^{15}N NMR spectrum of hyperpolarized choline after dissolution employing a 10° flip angle and 1 scan, final concentration 30 mM. (b) Thermal equilibrium spectrum of the same sample recorded under Ernst angle conditions, 10° flip angle, 4096 scans, repetition time 6 s, acquisition time 6 h 48 min.

Employing a standard inversion recovery experiment and susceptibility-matched plugs to restrict sample volume, the longitudinal relaxation time T_1 of ^{15}N in a 100 mM sample of choline (90% H_2O/10% D_2O) was measured to be 285 ± 12 s at 11.7T and 25 °C. In human blood the T_1 was found to be 120 ± 10 s at 37 °C. The T_1 in the presence of a free radical (OX63) was measured from the decay of the hyperpolarized signal by recording a spectrum every 30 s employing a 10° flip angle and fitted taking into account the loss of polarization from the RF pulse was found to be 203 ± 10 s. We have also measured the T_2 relaxation time in aqueous solution employing a standard Carr−Purcell (CPMG)

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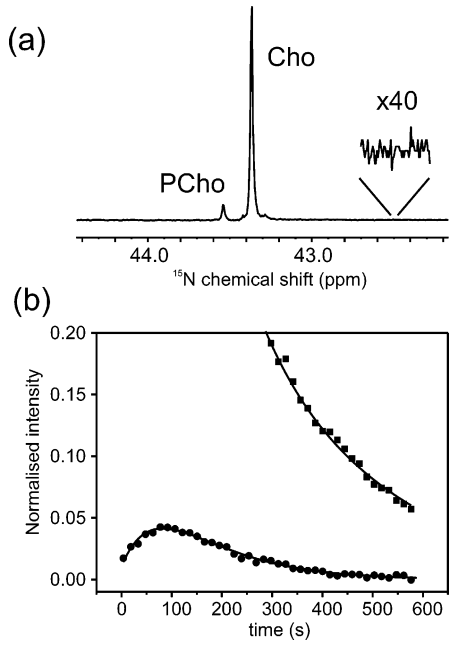

Figure 2. ATP-dependent conversion of hyperpolarized ^{15}N choline into hyperpolarized ^{15}N phosphocholine in the presence of Mg^{2+}. (a) Extracted spectrum at the maximum of the metabolic conversion. (b) Peak integral plotted as a function of time and fitted to the analytical expression derived from the Bloch equations and assuming a one-way reaction. Filled squares correspond to the decay of parent choline peak, whereas filled circles correspond to the buildup of the product phosphocholine peak.

spin−echo sequence and found it to be 42 ± 3 s (83 mM ^{15}N labeled choline in water, 16% v/v D_2O). The inherent symmetry of the quaternary nitrogen in choline results in remarkably long relaxation times of ^{15}N in aqueous solution.

The polarization level was estimated by comparing the signal amplitude of the polarized spectrum with that of the thermal signal. Figure 1a shows the ^{15}N spectrum of hyperpolarized choline recorded with a single acquisition employing a 10° radio frequency flip angle. This contrasts with the thermal spectrum in Figure 1b acquired with 4096 scans and the same flip angle under Ernst angle conditions, repetition time 6 s, and an experimental duration of 6 h 48 m. Final polarization is estimated by comparing the two peak areas and was found to be $P = 4.6 \pm 1\%$ corresponding to an enhancement factor of 13 940 compared to the thermal signal ($P = 3.3 \times 10^{-6}$ at 9.4T and 298 K). The polarization level that can be achieved for ^{15}N is typically less than that of ^{13}C where values of 20% are readily achieved.[7]

Figure 2 shows the ATP-dependent metabolic conversion of hyperpolarized ^{15}N choline into hyperpolarized ^{15}N phosphocholine. Figure 2a shows the spectrum recorded at the maximum of the buildup of PCho at $t = 114$ s, displaying signals from both the parent choline and product phosphocholine (∼0.2 ppm difference). The spectral integrals of the decay of Cho (filled squares) and buildup of PCho (filled circles) are displayed in Figure 2b employing a 10° radio frequency pulse every 15 s. The peak integrals were fitted as a function of time assuming a one-way metabolic reaction from choline to phosphocholine occurring at rate k (see Supporting Information). The initial rate of [PCho] buildup was found to be 1.45 mM/min with 2 μM purified choline kinase. It is remarkable to note that a signal still remains after 10 min, substantially exceeding the longevity found for other hyperpolarized tracers.

Hyperpolarization of metabolites with relaxation times on the order of minutes, similar to some PET isotopes, opens up an exciting new range of applications in medical imaging usually hindered by the intrinsic low sensitivity of MR. The remarkably long relaxation time of ^{15}N labeled choline suggests it may have considerable potential as a target molecule for hyperpolarization techniques to study tumor metabolism and the effects of novel therapeutics targeting cellular signaling pathways. Results from ^{11}C-Choline PET show that the initial uptake of ^{11}C choline in patients with uterine cervical cancer reached a maximum level within 3 min after injection.[13] Hyperpolarized ^{15}N choline may thus provide an MR alternative to ^{11}C choline and ^{18}F choline PET tracers, with the benefit of providing chemically specific metabolic information in real time with good spatial resolution.

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Supporting Information Available: Modified Bloch equations for the case of a one-way metabolic reaction. This material is available free of charge via the Internet at http://pubs.acs.org.

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