Therapeutic target metabolism observed using hyperpolarized ^{15}N choline

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Introduction

Choline is widely used as a diagnostic marker in oncology where malignant transformations are associated with altered phospholipid metabolism and increased choline kinase activity, resulting in increased levels of phosphocholine (PCho) in almost all forms of cancer [(1)]. Both of the processes involved in using choline as an exogenous marker - choline transport into cells and phosphorylation to PCho catalyzed by choline kinase – have been found to be upregulated in breast cancer [(2)]. Inhibitors of choline kinase are being investigated as therapeutic agents in cancer research [(3)]. Increasing evidence recognises a decrease in PCho level in cancer cells and *in vivo* as a positive response to some treatments. Identification of non-invasive endpoints to monitor their activity is crucial for the clinical development and evaluation of novel anticancer drugs. We previously showed the feasibility of hyperpolarizing ^{15}N in choline [(4)] by employing Dynamic Nuclear Polarization (DNP), a recent and powerful method to enhance the magnetic resonance signal by up to a factor 10,000 above equilibrium in a variety of molecules in solution state [(5).] Here we report the real-time metabolic conversion of hyperpolarized ^{15}N choline to hyperpolarized ^{15}N phosphocholine catalyzed by choline kinase.

Methods and Results

Choline chloride (98% 15N, Sigma Aldrich) was polarised in a HyperSense® DNP polariser. The T_1 in solution was measured in a 9.4T NMR spectrometer and was found to be 203s in the presence of OX63 free radical at 9.4T [(4)]. Final polarization attained in the liquid state was 4.6 ±1 % corresponding to an enhancement factor of the order of 14,000. Hyperpolarised choline was dissolved rapidly in Tris buffer and added to the enzymatic buffer solution. Final concentrations were 100mM Tris, 100mM KCl, 50mM $MgCl_2$, 20mM Choline, 10mM ATP, purified human choline kinase [(6)] 2µM, pH=8. The reaction was followed at 37°C in a 9.4T NMR spectrometer by a 5 degree flip angle RF pulse every 15s.

In Fig. 1 we show the ATP-dependent metabolic conversion of hyperpolarised ^{15}N choline into hyperpolarised phosphocholine. The data were fitted taking into account the one-way metabolic reaction from choline to phosphocholine occurring at rate k and the decay due to relaxation rates $\rho_{Cho} = 1/T_{1Cho}$ and $\rho_{PCho} = 1/T_{1PCho}$. The rate constant from the non-linear curve fit is k=0.006±0.003s^{-1}. The initial rate of PCho build-up is 7.2 mM/min with 2µM purified choline kinase.

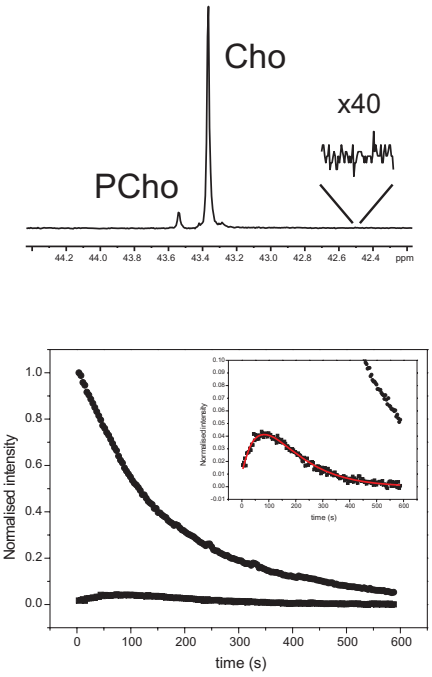

Fig. 1 In vitro conversion of the ATP-dependent metabolism of hyperpolarized ^{15}N choline into hyperpolarized ^{15}N phosphocholine in the presence of Mg^{2+}. Top shows the extracted ^{15}N NMR spectrum at the maximum of the metabolic conversion after 114s from the start of the experiment. Bottom shows the 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.

Conclusion

The exceptionally long lifetime of the hyperpolarized signal of both parent ^{15}N choline and daughter ^{15}N PCho combined with the potential to observe a metabolic process implicated in the pathology of cancer make these preliminary experiments highly encouraging. The ability to follow the metabolic process shows the future potential for using hyperpolarized ^{15}N choline as a non invasive biomarker to monitor treatment response. Hyperpolarization of metabolites with relaxation times of the order of minutes will open up an exciting new range of applications in medical imaging usually hindered by the intrinsic low sensitivity of MR.

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