^{13}C MRI Reporter Probe system with Dynamic Nuclear Polarization

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Introduction: Detecting activity of an enzyme *in vivo* due to the expression of a reporter gene or an antibody localized enzyme complex has been demonstrated with optical or radio-labeled imaging modalities (1-2). With the development of methods to retain highly polarized ^{13}C spins in solution, sensitivity of ^{13}C MRI can be elevated by several orders of magnitude (3). Thus it may be feasible to develop an agent/enzyme combination for a MRI reporter probe system with the utilization of dynamic nuclear polarization (DNP). Such a system would allow direct correlation of the molecular imaging data from the targeted enzyme to high-resolution anatomical and/or functional MRI data. We report the development of and preliminary results for such a system using pre-polarized [1-^{13}C] N-acetyl-L-methionine and Acylase I.

Methods and materials:

Hyperpolarized agent and hardware: [1-^{13}C] N-acetyl-L-methionine (99%, Sigma Aldridge, Miamisburg, OH) was prepared in dimethylacetymide for a final solution of ~2.6M [1-^{13}C] N-acetyl-L-methionine and 15mM OX63 trityl radical. 50µl of the formulation was polarized each time in a Hypersense DNP polarizer (Oxford instrument, Abingdon UK). Dissolutions of the DNP samples were performed with 5 ml of 100mM phosphate buffer (pH 7.2). Polarization enhancement [1-^{13}C] N-acetyl-L-methionine in solution was estimated at 3T (n = 2) by measuring the NMR signals of the solution at hyperpolarized state as well as at thermal equilibrium polarized state. T1 relaxation time of hyperpolarized [1-^{13}C] N-acetyl-L-methionine in solution was also estimated (n=2). 5 minutes prior to each of the *in vitro* enzyme experiments, 1mg of lyophilized Acylase I (from porcine kidney, Sigma Aldrich) was prepared in 1ml of the same buffer used for dissolution. All experiments were performed using a GE Signa 3T Scanner (GE Healthcare, Waukesha WI) equipped with the multinuclear spectroscopy package. A custom-built dual-tuned ^{13}C/^{1}H rat coil (Magvale, Palo Alto, CA) based on a micro-strip design was used in all experiments. All data were processed using SAGE software (GE Healthcare).

In vitro experiments: A double spin-echo pulse sequence was used in all experiments (4). *In vitro* dynamic MRS (n=3) as well as 2D CSI (n=2) experiments were performed by first injecting 1.5ml of the hyperpolarized [1-^{13}C] N-acetyl-L-methionine solution into a syringe then followed by injection of the enzyme solution into the same syringe (or phosphate buffer as control in the 2D CSI) inside the RF coil. The dynamic MRS experiments were started 10s prior to injection of the enzyme solution (5 degree tip angle, 3s TR). The 2D CSI experiments were started 10s after the acylase enzyme solution and phosphate buffer were simultaneously injecting into syringes containing hyperpolarized [1-^{13}C] N-acetyl-L-methionine (10x10 matrix, 50mm FOV, 10mm slice thickness).

In vivo experiments: Preparation and physiological monitoring of the animals in the ^{13}C experiment followed the protocol approved by the local Institutional Animal Care and Use Committee. The *in vivo* non-localized dynamic MRS and 3D CSI experiments were performed in normal Sprague-Dawley rats (n=2) after injection of ~3ml (over 12s) of the hyperpolarized [1-^{13}C] N-acetyl-L-methionine via a tail vein catheter. The *in vivo* dynamic MRS experiment (5 degree tip angle, 3s TR) was used to determine the imaging window for the 3D CSI experiment. *In vivo* 3D CSI was performed 15s after the start of the injection with 8x8x16 matrix (echo-planar readout on Z-axis) and (10mm)^3 spatial resolution over 14s.

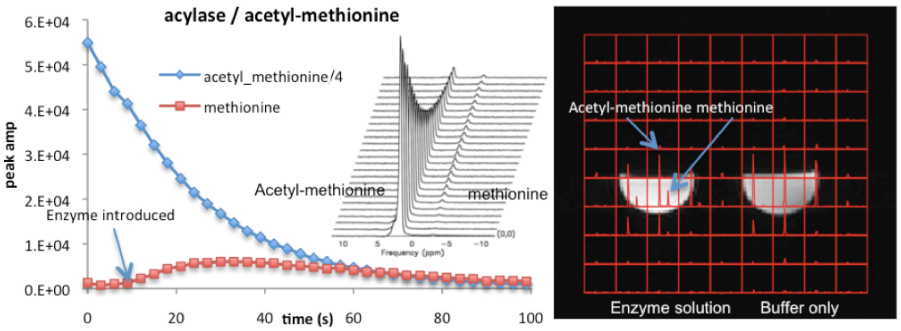

Figure1. *In vitro* dynamic MRS (left) and 2D CSI (right) data of [1-13C] N-acetyl-methionine and acylase I enzyme. [1-^{13}C] methionine were observed as the result of de-acetylation reaction of the pre-polarized substrate by the enzyme.

Results: A ~15,000 fold polarization enhancement in solution was observed for the pre-polarized [1-^{13}C] N-acetyl-L-methionine at 3T. T1 relaxation time of hyperpolarized [1-^{13}C] N-acetyl-methionine in solution was ~ 29s. Representative in vitro dynamic MRS data as well as 2D CSI are shown in Figure 1. [1-^{13}C] methionine was observed in these spectra from de-acetylation of the [1-^{13}C] N-acetyl-L-methionine via acylase I. The activity of 1mg of acylase I enzyme was estimated to be 9.6 (± 5.3) µmol/min for N-acetyl-methionine from the *in vitro* dynamic MRS data (Figure1, left). The *in vitro* 2D CSI (Figure1, right) demonstrated the feasibility of performing MR imaging of the acylase I activity (SNR of [1-^{13}C] methionine: ~17) at the current level of substrate polarization. Only [1-^{13}C] N-acetyl-L-methionine and no [1-^{13}C] methionine was observed in the control syringe (buffer only). The *in vivo* 3D CSI data (Figure2) acquired in normal rats following injection of pre-polarized [1-^{13}C] N-acetyl-L-methionine did not demonstrate observable de-acetylation of the substrate while the substrate was widely distributed in body of the animal.

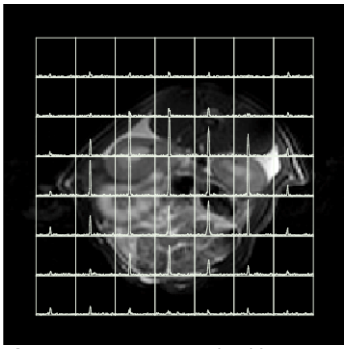

Figure2. 3D CSI acquired in a normal rat following injection of per-polarized [1-^{13}C]N-acetyl-L-methionine. No observable de-actylation was observed.

Discussion: A hyperpolarized ^{13}C MR reporter probe/enzyme system was presented in this study. The *in vitro* dynamic MRS and 2D csi data demonstrated the feasibility of using MR to image the acylase I activity with pre-polarized [1-^{13}C] N-acetyl-L-methionine with a clinical MR system. The absence of observable de-acetylation of the [1-^{13}C] N-acetyl-L-methionine *in vivo* in normal rats indicated that back ground acylase I activity in normal tissues is very low, further suggested the potential of this reporter probe/enzyme system for targeted MR imaging *in vivo*.

References:

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