Hyperpolarized ^{13}C MR Reporter Probe System with *Acy-I* Gene and [1-^{13}C] N-acetyl-L-methionine

A. P. Chen[1], R. E. Hurd[2], Y-P. Gu[3], D. M. Wilson[4], and C. H. Cunningham[3]
[1]GE Healthcare, Toronto, ON, Canada, [2]GE Healthcare, Menlo Park, CA, United States, [3]Imaging Research, Sunnybrook Health Sciences Centre, Toronto, ON, Canada, [4]Radiology, UCSF, San Francisco, CA, United States

Introduction: Indirect labeling of cells with reporter genes allows a stable beacon for assessment of their survival, proliferation, migration and possibly function at the target site or tissue. Detection of reporter gene expression using ^{31}P and ^{19}F MRS techniques have been reported (1-2). One of the main drawbacks of magnetic resonance, and especially using non-proton nuclei for *in vivo* reporter detection, is the low sensitivity, and thus limited spatial resolution and long scan time required. Recently, techniques to achieve highly polarized nuclear spins states via dynamic nuclear polarization (DNP) and retain the polarization in solution were developed (3), and ^{13}C MR imaging and spectroscopy data depicting endogenous enzyme mediated reactions have been obtained *in vivo* and *in vitro* with high spatial and temporal resolution (4). Hence, it may also be feasible to develop a suitable hyperpolarized ^{13}C substrate to target an enzyme reporter gene (5-6). In this study, a hyperpolarized^{13}C MR reporter system is demonstrated in cells transfected to overexpress a reporter gene, aminoacylase I (*Acy-I*), using pre-polarized [1-^{13}C] N-acetyl-L-methionine as the reporter probe.

Methods: Hyperpolarized substrate and hardware: [1-^{13}C] N-acetyl-L-methionine (99%, Isotec, 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 sodium phosphate buffer (pH 7.6). The polarization level achieved and T1 of the substrate have been reported previously (~4% and 28s) (6). All experiments were performed using a GE MR750 3T Scanner (GE Healthcare, Waukesha WI) equipped with the multinuclear spectroscopy package. A custom-built dual-tuned $^{13}C/^{1}H$ birdcage coil (5 cm I.D.) was used in all experiments. All data were processed using SAGE software (GE Healthcare). Cell preparation: Purified plasmid DNA of human aminoacylase I gene was transfected to 293 cells (human kidney cells) with LipoD293 DNA in vitro Transfection Reagent (SignaGen Laboratories). 24 hours after transfection, cells were harvested by trypsinization, washed twice with DMEM without methionine and resuspended with 5ml of the same medium supplemented with 5µM ZnCl2 for the *in vitro* MRS experiment. 293 cells without transfection were used as control. A total of ~2.5×10^8 cells were used in each experiments. Immediately after the each cell experiment, 1×10^5 cells were used for apoptosis assay, while the rest of the cells were washed 3 times in PBS and lysed in cold lysis buffer. The MRS experiment was then repeated using the cell lysate. In vitro experiments: *In vitro* MR spectroscopy experiments were performed on intact cells (transfected cells: n=2, control cells: n=1) and their cell lyaste. A double spin-echo pulse sequence was used in all experiments. Dynamic MRS data acquisition (TE/TR = 40ms/3s, 10° excitation RF pulse) started after ~3.0 ml of the hyperpolarized [1-^{13}C] N-acetyl-L-methionine solution was inserted into the RF coil; the cells suspension (or the lysate) was added to the substrate solution ~10s after the start of the acquisition. Fraction of the substrate converted by the enzyme was calculated from the dynamic MRS data from 21s to 78s and fitted to a linear function to quantify the amount of enzyme activity in these experiments.

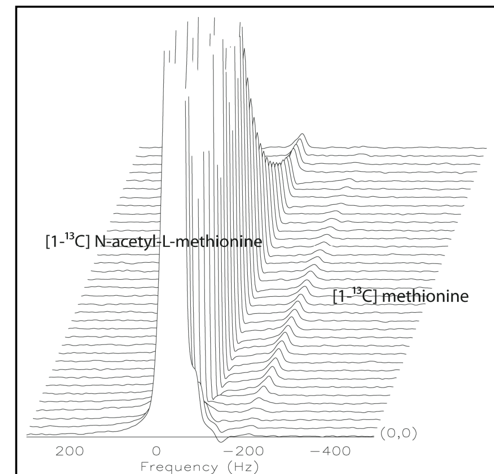

Figure 1. Representative dynamic MRS data from lysed cells that were transfected with the Acy-I reporter gene. The per polarized substrate[1-^{13}C] N-acetyl-L-methionine were de-acetylated by the aminoaylase-I enzyme and its product [1-^{13}C] methionine was observed. A small amount of [1-^{13}C] methionine imprurity (~0.2%) in the substrate solution was observed prior to addition of the lysate.

Results and Discussion: After the transfected cells (or lysate from the cells) were added to the pre-polarized substrate solution, an increase in [1-^{13}C] methionine signal was observed as the result of de-acetylation of the [1-^{13}C] N-acetyl-L-methionine substrate (Fig.1 and 2) by the aminoacylase-I enzyme. No observable de-acetylation of the substrate was measured in the experiment with control cells. Cell viability during the intact cell experiments was confirmed by the apoptosis assay. Enzyme activities of 1.9 U and 1.4 U were estimated from the two intact cell experiment, while 3.2 U and 4.8 U were estimated from the corresponding cell lysate. Membrane transport and/or insufficient mixing may have contributed to the lower observed activity in cells vs their lysate.

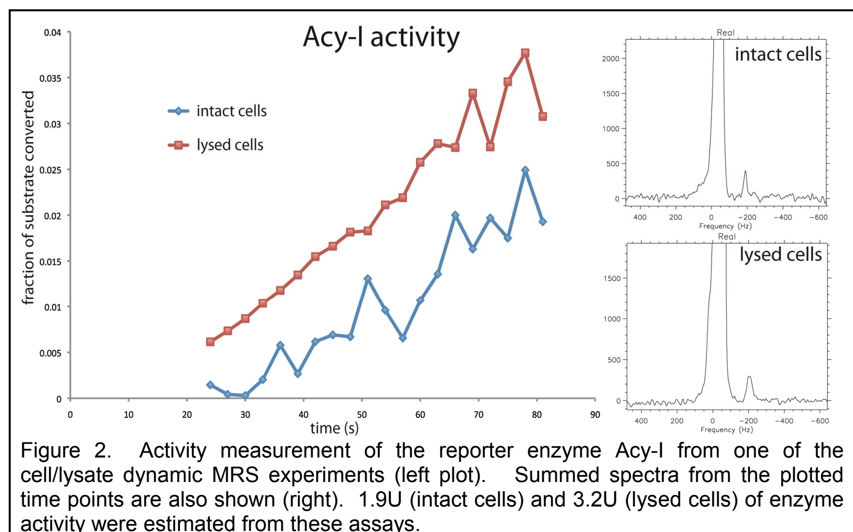

Figure 2. Activity measurement of the reporter enzyme Acy-I from one of the cell/lysate dynamic MRS experiments (left plot). Summed spectra from the plotted time points are also shown (right). 1.9U (intact cells) and 3.2U (lysed cells) of enzyme activity were estimated from these assays.

Conclusions: Detection of an enzyme reporter gene expression using a hyperpolarized ^{13}C reporter probe was demonstrated in live cells. The proposed system has potential for non-invasive targeted MR imaging *in vivo*.

References: 1. Auricchiom A. et al. Proc Nat Acad Sci 2001;98:5205-5210. 2. Stegman L. et al. Proc Nat Acad Sci 1999;96:9821-9826. 3. Ardenkjaer-Larsen JH. et al. Proc Natl Acad Sci 2003;100(18):10158-10163. 4. Day SE. et al. Nat Med 2007;13(11):1382-1387. 5. Jamin Y. et al. MRM 2009; Epub. Sept 24th. 6. Chen AP. et al. Proceedings, 17th ISMRM annul meeting, Honolulu, 2009. P2419.