

Measuring glutamate metabolism *in vivo* in tumors using Dynamic Nuclear Polarization

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Introduction

Dynamic Nuclear Polarisation (DNP) has recently been shown to increase the sensitivity of ¹³C magnetic resonance spectroscopy (MRS) by several orders of magnitude [1]. The number of metabolites that can be hyperpolarized using this technique is increasing; here we show that [$1\text{-}^{13}\text{C}$]glutamic acid can also be polarized using DNP. The metabolism of hyperpolarized glutamate to α -ketoglutarate, catalyzed by the enzyme alanine transaminase (ALT), was detected *in vitro* and *in vivo* in tumor models. As non-polarized α -ketoglutarate is below the threshold for MRS detection, this presents a new method for probing tumor metabolism.

Methods

[$1\text{-}^{13}\text{C}$]glutamic acid was hyperpolarized in a prototype polarizer (GE Healthcare) using a method similar to that previously reported for other metabolites [1]; the final concentration of glutamate was 18 mM. Human hepatoma cells ($\sim 10^7$; HepG2) were grown on microcarrier beads as described previously [2] and for some experiments these were incubated with 20 mM sodium pyruvate prior to the experiment. The hyperpolarized glutamate (2 mls) was added to an NMR tube containing 2 mls of cell suspension. ¹³C spectra were acquired with proton-decoupling, 1 s repetition time, and 4 transients per spectrum. For the *in vivo* experiments, murine lymphoma EL4 cells were implanted subcutaneously 10 days prior to the experiment. A 1 cm surface coil was placed over the tumor and 0.2 ml of hyperpolarized glutamate was co-injected with 75 mM of non-polarized sodium pyruvate. A pulse-acquire sequence with a nominal flip angle of 30° was used and spectra were acquired every 2 s. Measurements were made at 9.4 T.

Results

[$1\text{-}^{13}\text{C}$]glutamic acid was polarized to 28% in the liquid state which represents a 35,000-fold increase above thermal polarization at 37°C and 9.4 T. When injected into HepG2 cells, the production of α -ketoglutarate was demonstrated. The production of this metabolite was enhanced by the addition of 20 mM of (unlabeled and non-hyperpolarized) sodium pyruvate (Fig. 1). α -ketoglutarate was also demonstrated *in vivo* following the intravenous injection of hyperpolarized glutamate and non-hyperpolarized sodium pyruvate (Fig. 2).

Discussion

The results demonstrate that the metabolism of glutamate to α -ketoglutarate can be measured using DNP. The enhancement demonstrated with the addition of unlabeled (and non-hyperpolarized) pyruvate confirms that the reaction is an exchange reaction catalyzed by the enzyme ALT. α -ketoglutarate cannot normally be measured using MRS and therefore this presents a new method for probing the citric acid cycle in tumors. Glutamate is the major excitatory neurotransmitter and abnormalities in glutamate neurotransmission are associated with a number of neurological disorders; however, it is not clear whether transport across the blood-brain barrier is sufficiently rapid for detection within the brain. Glutamate is central to cellular metabolism, is a significant nitrogen source, and is involved in nitrogen transport within the body; therefore the ability to measure glutamate and its metabolites could have wide-ranging applications in medical imaging.

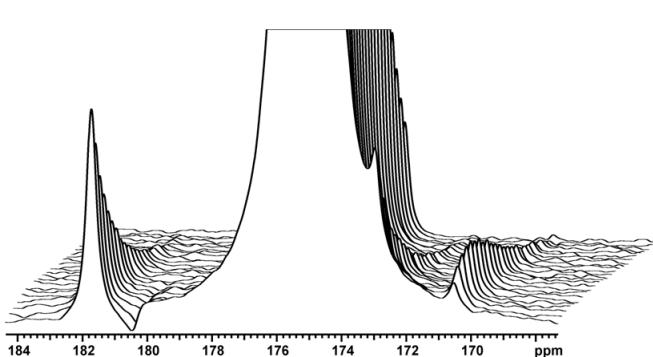


Figure 1. Series of *in vitro* ¹³C spectra acquired over 100 s following the injection of hyperpolarized glutamate into a tube containing HepG2 cells. The truncated peak at 175.1 ppm is the injected hyperpolarized [$1\text{-}^{13}\text{C}$]glutamate and the peak at 181.8 ppm is from the natural abundance [$5\text{-}^{13}\text{C}$]glutamate. The peak at 170.7 ppm is from α -ketoglutarate.

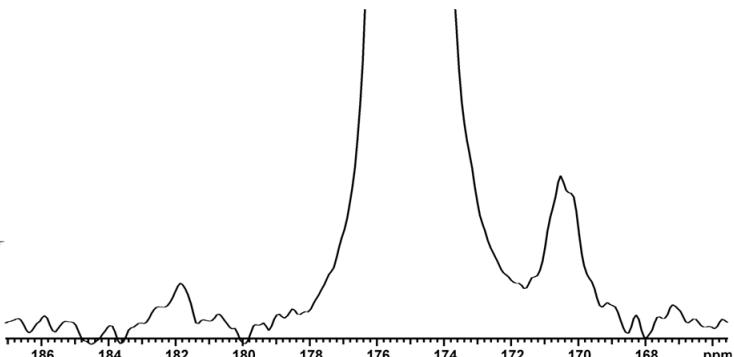


Figure 2. Single *in vivo* spectrum acquired using a ¹³C-tuned surface coil placed over a murine lymphoma tumor following injection of hyperpolarized glutamate and non-polarized pyruvate. The signal from α -ketoglutarate was observed at ~ 170.7 ppm.

Acknowledgments

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References

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2. Gallagher FA *et al.* (2008), *MRM* 60:253.