

Towards hyperpolarized ^{13}C succinate for biomedical MR imaging

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The PASADENA (parahydrogen and synthesis allow dramatically enhanced nuclear alignment) method used for polarization enhancement requires a double or triple chemical bond for molecular hydrogen addition [1]. While it was demonstrated that nuclear polarization of order of unity can be achieved for ^{13}C by the polarization transfer to ^{13}C sites, PASADENA application was limited to a small set of acrylate based precursors for many years. Here we apply PASADENA to hyperpolarization of ^{13}C C1 carbon in carboxylic acids on example of succinic acid, intermediate of TCA cycle. Additionally, we demonstrate that succinic acid can be used in biomedicine. Specifically, when succinic acid injected in rat artery, it crosses the blood brain barrier into the tumor where it is metabolized to glutamate and glutamine and acts as metabolic biomarkers.

Two different chemical pathways were investigated. In the first, disodium acetylenedicarboxylate (ADC) (Isotec) is employed as the unsaturated precursor for the molecular addition of dihydrogen in PASADENA to produce maleic acid and succinic acid when hydrogenation is complete, Fig. 1 [2]. Alternatively, we used 1- ^{13}C -fumaric acid- d_2 (CIL, Andover, MA) to produce hyperpolarized succinic acid. In both cases, the chemical goal was to achieve this reaction in a timescale which is small compared to spin lattice relaxation times. In order to break the symmetry to achieve PASADENA hyperpolarization, the ^{13}C label is confined to only one carbon nucleus (C1) in this symmetric molecule. The choice of carbonyl C1 label and additional deuteration in the second pathway are also to maximize the T_1 relaxation for the hyperpolarized species. The ^{13}C spectrum in Figure 1, acquired 15 s after hydrogenation and polarization transfer at 1.76 mT, illustrates dramatically increased polarization of C1 atom in 1- ^{13}C -succinic acid- d_2 .

In a separate experiment utilizing ADC pathway, the resulting mixture (16 mM of 1- ^{13}C -maleate and 8 mM 1- ^{13}C -succinate), 3 ml, was injected in the carotid artery of a 9L tumor-bearing rat. We anticipate that 1- ^{13}C -succinate is taken up differently by brain and brain tumor and would then be metabolized primarily in TCA cycle of glia and neurons. One hour was allowed to metabolize succinate before brain and tumor tissues were collected for *ex vivo* high resolution NMR. Using *ex vivo* ^{13}C spectroscopy at 11.7T we find that glutamine and glutamate C1 and C5 carbons are enriched with ^{13}C label from C1 of succinate in tumors, Figure 2. Our results suggest that hyperpolarized 1- ^{13}C -succinate could be a highly potent molecular imaging agent to assess the differential metabolism of normal and diseased tissue by non-invasive NMR and MRI methods. Most likely, succinate enters the tumor through the dysfunctional blood brain barrier, but is substantially excluded by the normal brain tissue. These experiments indicate that it may be possible to perform *in vivo* dynamic MR spectral-spatial imaging and spectroscopy of tumor metabolism following injection of millimolar concentrations of hyperpolarized 1- ^{13}C -succinate with high contrast and high SNR (work in progress).

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References

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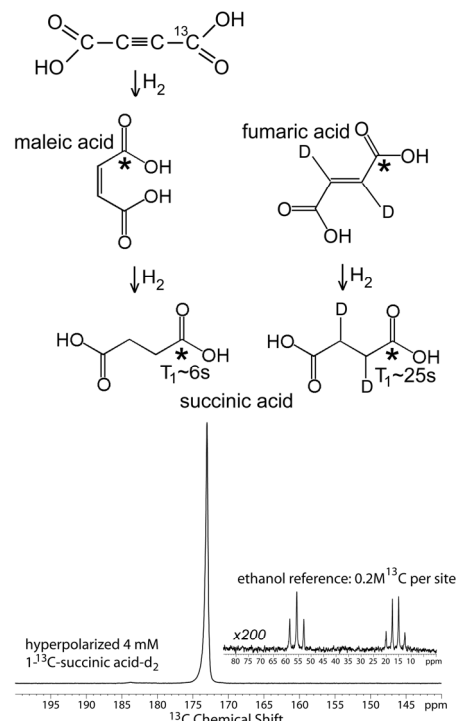


Figure 1. PASADENA hydrogenation schemes and *in vitro* ^{13}C spectrum of hyperpolarized 1- ^{13}C -succinic acid- d_2 . Ethanol spectrum is provided for reference purpose. ^{13}C spectra are acquired using a double tuned $^1\text{H}/^{13}\text{C}$ circuit at 4.7T.

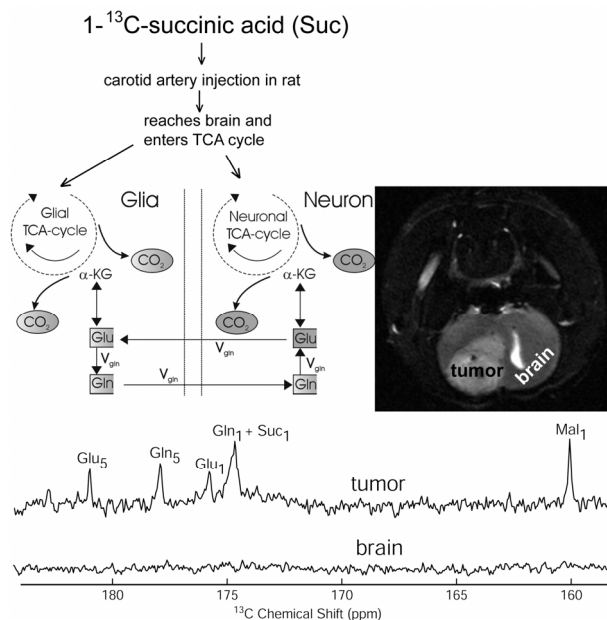


Figure 2. *Ex vivo* MAS ^{13}C spectra of brain and brain tumor tissues. 80 mg of tissue was used in each experiment. Spectra were acquired using 11.7T Bruker NMR console at 4°C.