Detecting the invisible: DNP-enhanced detection of ^{13}C in carboxyl resonances of rat brain extracts

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Introduction

^{13}C spectra are largely used in MRS to investigate brain metabolism *in vivo*. Due to the low sensitivity of ^{13}C it is possible to measure mainly the highly concentrated metabolites. So far, hyperpolarization has been used to enhance the signal of a specific ^{13}C tracer with the aim to observe metabolism. The advantage of long T_1, required for such metabolic studies, is a disadvantage for detecting label in the carboxyl resonances in brain extracts, which may yield important additional insight into the measurement of metabolic rates [1]. The aim of the current study was to establish hyperpolarization of ^{13}C in brain extract for the detection of low concentration ^{13}C with intrinsically low sensitivity.

Materials and Methods

The brain extract was obtained from a male Sprague-Dawley rat (382 g) which was infused with a solution of 67% U-$^{13}C_6$ glucose over a period of 7h. After fixation by microwave the brain was carefully removed from the skull and frozen immediately under liquid nitrogen, after which the metabolites were extracted with standard PCA procedure [2]. The extract was successively dissolved in a 40/60 mixture of D_2O/glycerol, 0.50 ml of this mixture was then polarized at 3.35 T and 1.2 K using the polarizer described in reference [3]. After dissolution into 5 ml of D_2O, the solution was automatically transferred within 6 s to a phase separator placed in the bore of a Varian Inova actively-shielded 9.4 T scanner. The first spectrum was acquired within 7 seconds from dissolution using a 3 ms 45° BIR4 pulse with 1.5 s interpulse delay. The FIDs were post processed with a 0.045 Hz Gaussian and a 5 Hz line broadening.

Results and Discussion

To the best of our knowledge, for the first time a brain extract solution has been successfully hyperpolarized. In figure 1a, the first acquired spectrum is shown; the three low intensity peaks on the left correspond to hyperpolarized metabolites. Due to the low sample concentration and to the loss in magnetization due to both relaxation and the applied RF pulse, the signal from the metabolites was detectable only in the first four spectra. To improve the signal to noise ratio, the corresponding FIDs were summed (figure 1b). The detected resonances at 182.4(m), 178.8(m), 176.9 and 175.4(m) ppm respectively correspond to the Glutamate C5 and GABA C1, Glutamine C5, Alanine C1, Glutamate C1 and Glutamine C1 based on published chemical shifts. Due to label scrambling the carboxyl resonances are, for the same component, singlet and doublets and this increased the apparent linewidth of the resonances. Two unassigned resonances were observed at 171 and 162 ppm, which were tentatively attributed to a fold-over artifact of the glycerol solvent. Glutamate and glutamine estimated effective concentrations in rat brain are 10 and 5 µmol/g respectively [4], while the concentration of enriched glutamate can reach up to up 60% for the conditions of the present study. As a result, the estimated concentration of ^{13}C in the final solution was about 2 nM. Using identical conditions, a ^{13}C spectrum of the unpolarized solution was acquired for 14 hours, 1280 scans (figure 2). The main peak is due to the ^{13}C C1 acetate solution that was added for internal initial calibration (3 mM). Resolution and detection of signal will benefit from the use of another type of radical and another radical concentration that strongly affect the T_1 of the nuclei.

In conclusion, the present study shows that PCA extracts of rat brain can be hyperpolarized for the detection of ^{13}C resonances with unfavorable signal characteristics, such as the carboxyl resonances of amino acids and related compounds.

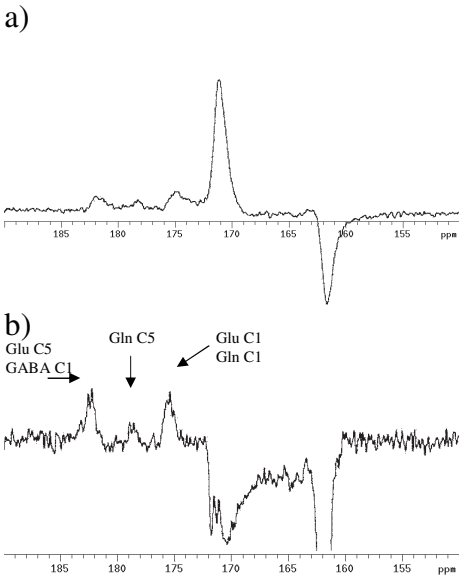

Figure 1 a) Carboxyl region of the spectra acquired 7 s after dissolution. **b)** Sum of the first three spectra.

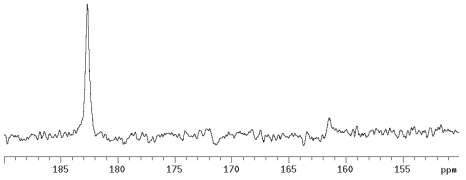

Figure 2: Unpolarized ^{13}C brain extract spectra obtained with 1280 scans, experiment time 14 hours. The peak at 182.6 ppm is the acetate which was added for internal initial calibration.

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