Spectral editing for in vivo ^{13}C MRS

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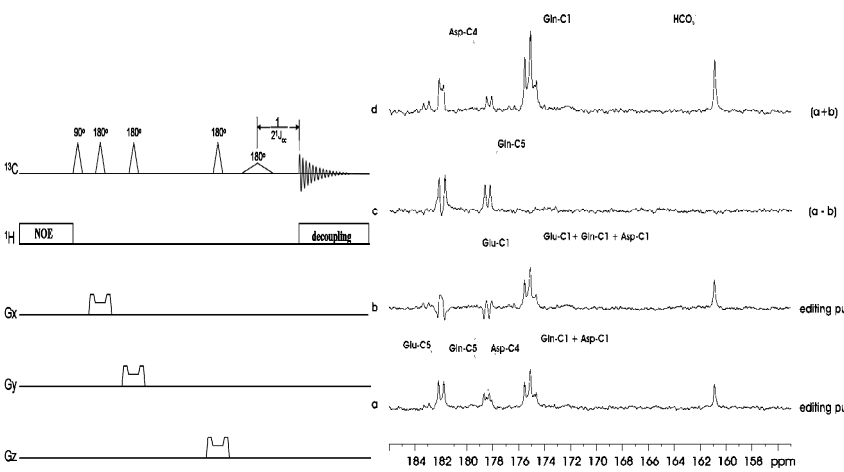
Introduction In vivo proton spectra contain many overlapping peaks resonating over a small chemical shift range [1]. Spectral editing techniques have been developed to limit the detection to specific metabolite signal(s) [2]. Many proton spectral editing methods rely on scalar couplings between metabolite signals of interest. There have been no reports in the MRS literature of spectral editing in experiments with direct detection of heteronuclei. In the carboxylic/amide spectral region where proton decoupling requires very low RF power there is spectral overlapping between glutamine C5 at 178.5 ppm and aspartate C4 at 178.4 ppm and between glutamate C5 at 182.0 ppm and acetate C1 at 182.15 ppm. When [1-^{13}C] acetate is used care has to been taken to ensure that tissue concentration of acetate is sufficiently low so as not to contaminate the glutamtate C5 signal [3]. Although acetate is a valuable specific substrate for cerebral astroglial metabolism the severe spectral overlapping between glutamate C5 and acetate C1 limits its usefulness. Since glutamate C5 and acetate C1 only generate singlets when [1-^{13}C] acetate is administered spectral editing techniques based on homonuclear couplings cannot be used to resolve them. In this work, we propose a ^{13}C-^{13}C spectral editing method for in vivo ^{13}C MRS. We introduce homonuclear ^{13}C-^{13}C couplings by infusing [1,2-$^{13}C_2$]acetate in order to spectrally separate glutamate C5 from acetate C1 by editing. We demonstrate that the same approach can also be used to spectrally separate the glutamine C5 and aspartate C4.

Methods All experiments were performed on a Bruker AVANCE spectrometer interfaced to an 11.7 Tesla 89-mm bore vertical magnet. A home-built RF surface coils/head holder system mounted on a half-cylindrical plastic cradle was used. A train of non-selective hard pulses with a nominal flip angle of 180^o spaced at 100 ms apart was used for generation of broadband ^{1}H→^{13}C heteronuclear Overhauser enhancement. Direct three-dimensional spatial localization of ^{13}C spins in the carboxylic/amide region was employed (see Fig. 1). The ^{13}C 180^o spin echo pulses also refocus the long-range heteronuclear ^{1}H-^{13}C couplings. The ^{13}C carrier frequency was placed around 180.4 ppm. A single spectrally selective editing pulse (180^o hyperbolic secant pulse, phase factor = 3 and truncation level = 1%, pulse length = 10~12 ms, bandwidth = 1012~842 Hz) was placed either at 24.5 ppm for separating glutamate C5 from acetate C1 (10 ms) or at 31.7 ppm for separating Gln C5 (178.5 ppm) from aspartate C4 (178.4 ppm, 12 ms). The proton decoupler frequency was at 4.82 ppm. The experimental animals (male rat, 200–251 g) were orally intubated and mechanically ventilated with a mixture of 70% N_2O, 30% O_2 and 1.5% isoflurane. All animals were randomly divided into two groups subject to intravenous infusions of 0.75 M [$^{13}C_6$]-D-glucose and 0.9 M [1,2-$^{13}C_2$]acetate, respectively. Throughout all experiments, normal physiological conditions were maintained. In vivo ^{13}C MRS data were acquired in an interleaved fashion during the steady state of metabolism with NS = 16 per spectrum, TR = 20s, SI = 16K and LB = 10 Hz.

Results and Discussion Fig. 2 (a) & (b) show the interleaved brain ^{13}C MRS spectra acquired from one animal during intravenous infusion of [1,2-$^{13}C_2$]acetate. Infusion of [1,2-$^{13}C_2$]acetate leads to both a doublet and a singlet at glutamate C5 due to pyruvate recycling [4]. With the editing pulse on, the spectral pattern is complicated due to the inversion of the acetate doublet. The smaller acetate doublet signal was revealed in (c) after subtracting (b) from (a). The pure glutamate triplet signal is obtained in (d) by adding (b) to (a) which cancels the acetate doublet. The complete removal of acetate in (d) was verified using a phantom containing 0.1 M [1,2-$^{13}C_2$]acetate (pH = 7.4; data not shown). Using a separate in vivo experiment with intravenous infusion of [$^{13}C_6$]-D-glucose the editing pulse was found to have no effect on the signal intensity of glutamate C5 (data not shown). There have been no known in vivo MRS techniques that can spectrally resolve acetate C1 and glutamate C5 singlets. The strategy proposed here achieves spectral separation of acetate C1 from glutamate C5 by two-step J-editing after introducing homonuclear ^{13}C-^{13}C scalar coupling between carboxylic/amide carbons and aliphatic carbons. By infusing [1,2-$^{13}C_2$]acetate instead of [1-^{13}C]acetate the acetate doublet can be spectrally edited because of the large separation between acetate C2 and glutamate C4 in the aliphatic region. The results of spectral editing of glutamine C5 and aspartate C4 were illustrated in Fig. 3. The interleaved brain ^{13}C MRS spectra acquired from one animal during intravenous infusion of [$^{13}C_6$]-D-glucose were shown in Fig. 3 (a) and (b). The 12 ms editing pulse was placed at glutamine C4 at 31.7 ppm. With the editing pulse off (a), the glutamine C5 doublet at 178.5 ppm partially overlaps with the aspartate C4 doublet at 178.4 ppm. With the editing pulse on (b), the glutamine C5 doublet is inverted while the aspartate C4 doublet is not affected because aspartate C3 resonates at 37.1 ppm while glutamate C4 resonates at 34.1 ppm. Primarily due to its couplings to the methylene protons part of the undecoupled glutamate C4 multiplets is spanned by the transition band of the editing pulse, leading to partial inversion as well as phase distortion of the glutamate C5 doublet signal. In the subtracted spectrum (c), the pure glutamine C5 doublet was detected. In the summed spectrum (d), only the aspartate C4 doublet signal was seen. ^{13}C spectral editing leads to complete separation of glutamine C5 from aspartate C4 (at the expense of distorted glutamate C5 signal). These results demonstrate that this in vivo ^{13}C MRS spectral editing technique can be used to resolve overlapping resonances in in vivo ^{13}C MRS. In particular, it can be applied to studying acetate transport and metabolism in brain in the carboxylic/amide region without spectral interference.



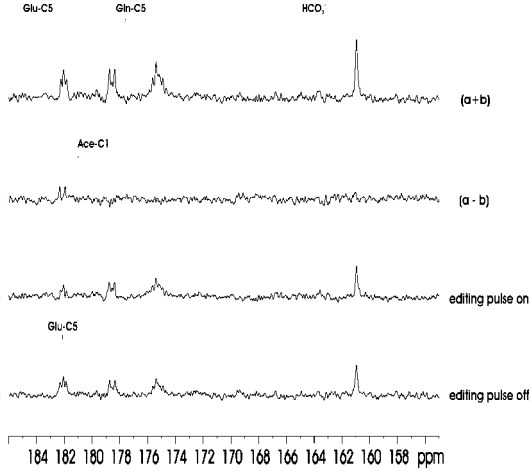


Fig. 1 **Fig. 2** **Fig. 3**

Fig.1. Schematic diagram of the pulse sequence designed for in vivo ^{13}C editing. **Fig. 2.** In vivo ^{13}C NMR spectra acquired during infusion of [1,2-$^{13}C_2$]acetate. (a) without the editing pulse; (b) with the editing pulse on acetate C2 (24.5 ppm); (c) (a-b); (d) (a+b). **Fig. 3.** In vivo ^{13}C NMR spectra acquired during infusion of [$^{13}C_6$]-D-glucose. (a) without the editing pulse; (b) with the editing pulse on glutamine C4 (31.7 pp); (c) (a-b); (d) (a+b).

References: [1] Oayyum A. Radiographics 2009; 29:1653-64. [2] Jung WI. *et al*. MAGMA1993; 1:5-9. [3] Xiang Y. *et al*. J Neurosci Methods 2011; 198:8-15. [4] Cerdán S, *et al*, J. Biol. Chem 1990; 265:12916–12926.