

# Simultaneous detection of metabolism of different substrates in the carboxylic/amide region using *in vivo* $^{13}\text{C}$ MRS

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## Introduction

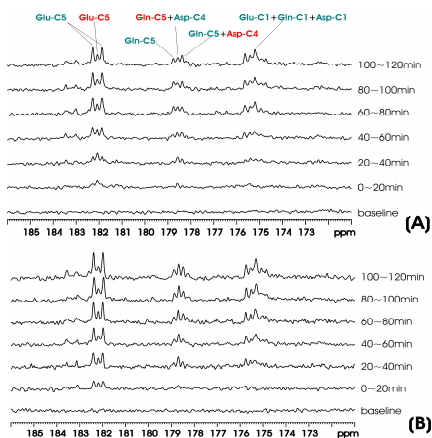
*In vivo*  $^{13}\text{C}$  NMR spectroscopy, due to its noninvasive features, has been widely used to investigate cerebral metabolism and neurotransmission [1]. As  $^{13}\text{C}$  NMR spectra characterized by the distinct  $^{13}\text{C}$ - $^{13}\text{C}$  homonuclear splitting could be followed through various stages of the substrate metabolism and the  $^{13}\text{C}$ - $^{13}\text{C}$  bond remains intact, thus yielding structural information which would be difficult to obtain otherwise, they have been exploited in some *ex vivo* and *in vivo* studies on the metabolism of certain  $^{13}\text{C}$ -labeled chemicals [2]. With the advances in high field *in vivo* MRS technology, this kind of *in vivo*  $^{13}\text{C}$  NMR spectroscopy has increasingly been used to observe neurochemical metabolism and especially that involves neuronal-glial interactions in the brain after the infusion of  $^{13}\text{C}$ -enriched substrates [3-5]. In the present study, we show that co-infusion of [ $^{13}\text{C}_6$ ]-D-glucose with [ $1\text{-}^{13}\text{C}$ ] acetate or with [ $1,3\text{-}^{13}\text{C}_2$ ]hydroxybutyrate sodium salt (BHB) can be used to simultaneously detect metabolism of two different substrates *in vivo* in the carboxylic/amide region.

## Methods

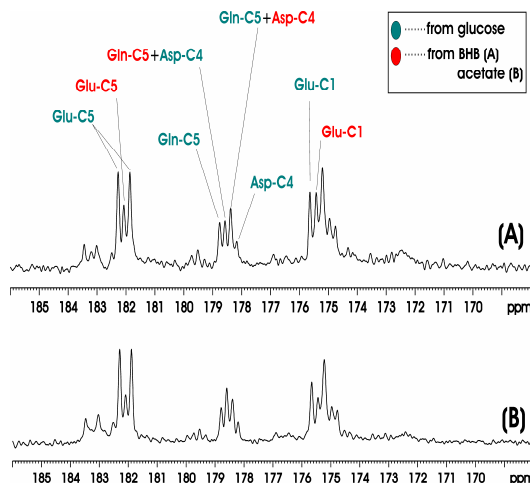
All experiments were performed on an 11.7 Tesla Bruker spectrometer equipped with a Mini 0.5 gradient insert using a  $^{13}\text{C}\{^1\text{H}\}$  RF coil assembly made of single-sided printed circuit board according to a previously published protocol [1]. The overnight-fasted male adult SD rats (177-264g) were divided into five groups and were subjected to intravenous infusion of  $^{13}\text{C}_6$ -D-glucose (0.75M, 10min bolus: 75.5mg/min/Kg BW, 10~270min: 28.5mg/min/Kg BW)(n=5), [ $1\text{-}^{13}\text{C}$ ] acetate (0.9M, pH=7.0, 10min bolus: 18.7mg/min/Kg BW, 10~270min: 7.1mg/min/Kg BW)(n=5), [ $1,3\text{-}^{13}\text{C}_2$ ]BHB(0.8M, pH=7.0, 10min bolus: 112.5mg/min/Kg BW, 10~270min: 12.5mg/min/Kg BW)(n=1), co-infusions of  $^{13}\text{C}_6$ -D-glucose and [ $1\text{-}^{13}\text{C}$ ]acetate (n=10), and co-infusion of [ $^{13}\text{C}_6$ ]-D-glucose and [ $1,3\text{-}^{13}\text{C}_2$ ]BHB (n=1), respectively. After coil tuning and matching, MR images were acquired for proper positioning of the animals in the MR scanner. The gradient isocenter was about 0-1 mm posterior to bregma. Mixture of 70%  $\text{N}_2\text{O}$ , 30%  $\text{O}_2$ , and 1.5% isoflurane was used for anesthesia. One femoral artery was cannulated for periodically sampling of arterial blood to monitor blood gases ( $p\text{O}_2$ ,  $p\text{CO}_2$ ), pH, blood glucose concentration (12~15mM) and continuously monitoring the arterial blood pressure level.  $^{13}\text{C}$ -labeled substrates were infused intravenously after cannulation of vein(s). Normal physiological conditions were maintained throughout the experiment (pH ~7.4,  $\text{PCO}_2$  ~35mm Hg and  $\text{PO}_2$  >100mmHg).

## Results and Discussion

All spectra were processed using  $\text{lb} = -5$ ,  $\text{gb} = 0.1$  and zero-order phase correction. Fig. 1A shows the *in vivo* proton decoupled  $^{13}\text{C}$  NMR time course spectra from the rat brain in the 168-186 ppm region with co-infusion of [ $1,3\text{-}^{13}\text{C}_2$ ]BHB and [ $^{13}\text{C}_6$ ]-D-glucose. Fig 1B shows the corresponding spectra with co-infusion of [ $1\text{-}^{13}\text{C}$ ] acetate and [ $^{13}\text{C}_6$ ]-D-glucose. Glutamate C5 originated from [ $^{13}\text{C}_6$ ]-D-glucose appears as a doublet with a J coupling constant of 51 Hz while glutamate C5 originated from [ $1\text{-}^{13}\text{C}$ ] acetate or [ $1,3\text{-}^{13}\text{C}_2$ ]BHB appears as a singlet. The large homonuclear  $^{13}\text{C}$ - $^{13}\text{C}$  between an aliphatic carbon and a carboxylic or amide carbon and the lack of interference from other one-bond couplings allow a clean separation of signals originated from different substrates as clearly shown in Figs 1 and 2. Interference from [ $1\text{-}^{13}\text{C}$ ] acetate was negligible based on separate infusion experiment using [ $2\text{-}^{13}\text{C}$ ] acetate and the same infusion protocol. At 11.7 Tesla, the chemical shift separation between glutamine C5 and aspartate C4 is coincidentally one half of the one-bond J coupling between an aliphatic carbon and a carboxylic or amide carbon. As a result, a pseudo quartet was detected in the 178-179 ppm region, allowing easy separation of contributions to glutamine C5 and aspartate C4 from different substrates. Since glutamate C1 is widely separated from glutamine C1, aspartate C1 and NAA C5, contributions from different substrates to glutamate C1 are also separable. In addition, the features of the spectra acquired during co-infusion matches the sum of the corresponding single substrate infusion spectra (data not shown). Interestingly, a comparison of Fig 2A and 2B reveals a significantly higher contribution to glutamine C5 and lower contribution to glutamate C1 from [ $1\text{-}^{13}\text{C}$ ] acetate than from [ $1,3\text{-}^{13}\text{C}_2$ ]BHB, consistent with previous observations that acetate is a glial substrate while BHB enters both neuronal and glial compartments.



**Fig.1.** (A) Time-course spectra from an individual rat brain after co-infusion of [ $1,3\text{-}^{13}\text{C}_2$ ] BHB and [ $^{13}\text{C}_6$ ]-D-glucose. (B) Spectra after co-infusion of [ $1\text{-}^{13}\text{C}$ ] acetate and [ $^{13}\text{C}_6$ ]-D-glucose. Each individual spectrum was averaged for 20 min. Green: signals originated from  $^{13}\text{C}_6$ -D-glucose; Red: signals originated from [ $1\text{-}^{13}\text{C}$ ] acetate or [ $1,3\text{-}^{13}\text{C}_2$ ] BHB.



**Fig.2.** (A) Steady state spectrum during co-infusion of [ $1,3\text{-}^{13}\text{C}_2$ ]BHB and [ $^{13}\text{C}_6$ ]-D-glucose from an individual rat brain. (B) Steady state spectrum during co-infusion of [ $1\text{-}^{13}\text{C}$ ] acetate and [ $^{13}\text{C}_6$ ]-D-glucose. Both spectra were accumulated from 120-260 min after the start of infusion. Spectra were processed using the same parameters as in Fig. 1.

## References

- [1] Li S, *et al.* NMR Biomed 2005; 18:560-9. [2] Taylor A, *et al.* Dev. Neurosci 1996; 18:434-42. [3] Deelchand K, *et al.* J Mag Reson 2009; 196:157-63. [4] Cerdan S, *et al.* J Biol Chem 1990; 265:12916-26. [5] de Graaf RA, *et al.* NMR Biomed 2003; 16:339-57.