In vivo ^{13}C NMR detection of labeled serine and glycolytic intermediates in the rat brain during ^{13}C glucose infusion

P. G. Henry[1], G. Oz[1], R. Gruetter[1]

[1]Center for Magnetic Resonance Research, University of Minnesota, Minneapolis, MN, United States

Recent improvements in ^{1}H-localized ^{13}C spectroscopy allowed localized broadband detection of ^{13}C resonances over a 85ppm bandwidth in the rat brain *in vivo* with excellent sensitivity. These advances were exploited to detect of several resonances not previously observed *in vivo*, and their tentative assignment to [3-^{13}C]serine, [1-^{13}C]fructose and [1-^{13}C]glycerol-3-phosphate using high-resolution 1D and 2D NMR spectroscopy (HSQC-TOCSY) of brain extracts. These compounds are labeled due to reactions closely associated with glycolysis and thus open a new non-invasive window on glycolytic reactions.

Introduction

It was recently reported that ^{1}H-localized ^{13}C detection using semi-adiabatic polarization transfer results in improved sensitivity and minimal chemical-shift displacement error when measuring ^{13}C spectra in the rat brain at 9.4T (1). In particular, good localization performance resulted in the complete elimination of natural abundance extra-cerebral glycerol signals in the 60-70ppm region. The goal of this study was to examine if the improved sensitivity, localization and spectral range in ^{13}C spectra allowed detection of previously undetected resonances in this spectral region and to assign these resonances using two-dimensional NMR methods.

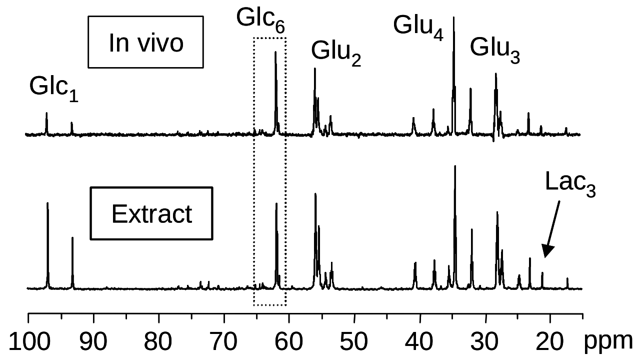

Fig. 1. Localized *in vivo* ^{13}C spectrum (top) and extract spectrum after funnel-freezing from the same animal. The extract spectrum was line-broadened to match the *in vivo* linewidth. Note the low lactate signal and the high Glc$_6$ signal on the extract spectrum, indicating minimal post-mortem metabolism. Glc$_1$ appears lower *in vivo* due to off-resonance effects.

Methods

In vivo ^{13}C spectra were acquired at 9.4T from the rat brain using a previously described ^{1}H-localized ^{13}C polarization transfer sequence (1) during an infusion of 70%-enriched [1,6-$^{13}C_2$]glucose under α–chloralose anesthesia. Immediately after the *in vivo* acquisition, brains were funnel-frozen and dissected under intermittent liquid nitrogen to minimize post-mortem metabolic changes, and metabolites were extracted with perchloric acid. 1D ^{13}C spectra and 2D ^{1}H{^{13}C} HSQC-TOCSY spectra were measured from brain extracts at 600MHz.

Results and Discussion

In vivo ^{13}C spectra were strikingly similar to extract spectra (Fig. 1). Signals detected *in vivo* included not only strong multiplets from glutamate, glutamine, aspartate and glucose, but also weaker signals from NAA, GABA, myo-inositol and glutathione, the assignment of which was confirmed by 2D-NMR. Three hitherto unreported resonances at 61.3ppm, 63.8ppm and 64.9 ppm were also detected when infusing ^{13}C-labeled glucose. These resonances were not detectable in natural abundance spectra (not shown). Based on HSQC-TOCSY and published chemical-shift values (2,3), the carbon at 61.3ppm was tentatively assigned to [3-^{13}C]serine ($\delta(^{1}H)$ = 3.95ppm and 3.83ppm). The ^{13}C resonance at 63.8ppm coupled to a ^{1}H spin system with $\delta(^{1}H)$= 3.57ppm and 3.67ppm, consistent with the glycolytic intermediate [1-^{13}C]glycerol-3P. The ^{13}C resonance at 64.9ppm coupled to ^{1}H at 3.5ppm and 3.71ppm consistent with [1-^{13}C]fructose. A fourth, currently unassigned resonance was detected at 64.2 ppm. In conclusion, the non-invasive detection of serine and glycolytic intermediates opens new perspectives for measuring brain metabolism at the level of glycolysis.

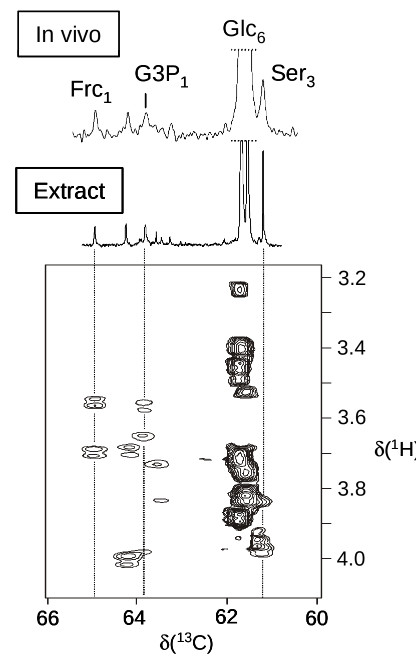

Fig. 2. Expansion of 1D spectra around 63ppm (top 2 spectra) and corresponding region in a 2D ^{1}H{^{13}C} HSQC-TOCSY (bottom) at 600 MHz.

References

1) Henry et al., *Proc. Intl. Soc. Magn. Reson. Med.* 10, (2002)
2) Willker et al., *J. Magn. Reson. Anal.* 2,21 (1996)
3) http://www2.swmed.edu/rogersmr/chemical_shifts.htm

Acknowledgements

S. Crawford and K. Yue for technical assistance. Supported by NIH R01NS38672, NIH P41RR08079 and the Keck Foundation. Funding for the high-resolution NMR facility was provided by the University of Minnesota Medical School, NSF (BIR-961477) and MMF.