Editing through Multiple Bonds: Threonine Detection

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Introduction

In *in vivo* ^{1}H spectroscopy, the signal at 1.32 ppm is usually assigned to lactate. This resonance position at physiological pH is shared with threonine, which has similar *J*-coupling and whose coupling partner resonates at 4.24 ppm, very close to that of lactate (4.11 ppm). The close proximity of coupling partners of lactate and threonine renders the measurement of threonine with and without editing technically challenging. The aim of this study was to exploit multiple-bond editing and quantify the threonine signal *in vivo*.

Methods

Threonine has five non-labile protons from a CH_3 and two CH groups (Figure 1). The ^{2}CH proton gives a doublet at 3.58 ppm, and the ^{3}CH proton resonates at 4.24 ppm to give an eight-line multiplet. The $^4CH_3$ protons give a doublet at 1.32 ppm.

In vivo ^{1}H NMR edited spectra were obtained using a previously described 1D-TOCSY-LASER sequence[1]. MR experiments were performed using a 9.4-T (31-cm horizontal bore) magnet interfaced with Varian INOVA console. A quadrature 400-MHz ^{1}H RF surface coil was used to transmit and receive. Localizer T_2-weighted multislice rapid acquisition with relaxation enhancement images were acquired to select 133 µL voxel of interest in the rat brain.

The pulse sequence was optimized for the detection of the threonine proton bound to ^{2}C (3.58 ppm) by using multiple-bond coherence transfer. The center of the frequency-selective 4 ms gauss pulse was placed at 1.32 ppm to selectively refocus the source spins, protons bound to ^{4}C and all the signals outside of the bandwidth of the gauss pulse obliterated. The mixing time of 72 ms was used based on the optimization performed on the phantom.

Five male Sprague-Dawley rats were intubated and femoral veins were cannulated for blood sampling. Blood gases were measured every 30 minutes to ensure stable physiological conditions. Rats were anesthetized using 1.0%-1.5% isoflurane and O_2/NO_2 and positioned in a custom-build holder to immobilize the head during the experiments.

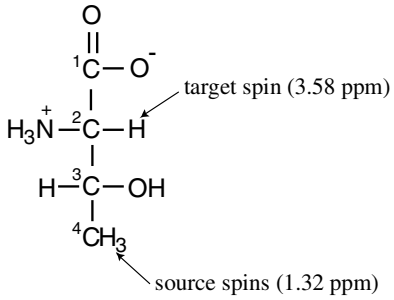

Figure 1. Chemical structure of threonine with source spins and target spin indicated.

Results and Discussion

The ^{2}CH resonance was unequivocally and consistently detected at 3.58 ppm in all five *in vivo* studies as judged from the consistent spectral pattern at 3.58 ppm similar to the spectral pattern measured in an accordingly line-broadened phantom spectrum (Figure 2). The assignment of the resonance was reinforced by the inability to detect it when the train of the adiabatic TOCSY pulses was turned off (not shown).

The polarization was transferred from protons resonating at 1.32 ppm (both lactate and threonine) to all other protons. The signals at 3.58 ppm for threonine and at 4.11 ppm were detected. The other threonine signal at 4.24 ppm could not be resolved from the macromolecule background signal. In edited spectra, additional expected intensities were observed since other resonances such as N-acetylaspartate, glutamate, glutamine and macromolecules resonate within the bandwidth of the refocusing pulse.

Based on the lactate concentration and the expected recovery of the signal obtained from phantom data, the concentration of threonine was estimated to be ~0.5 mM in excellent agreement with brain extract data.

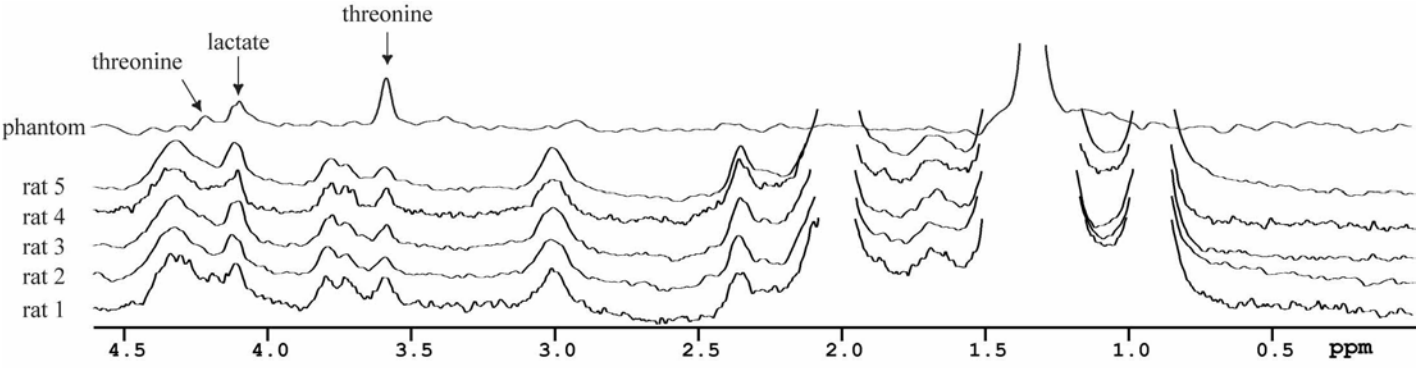

Figure 2. Consistent detection of threonine in 1D-TOCSY-LASER spectra. Edited *in vivo* spectra from five different rats and one edited phantom spectrum containing equal molar concentrations of lactate and threonine. The *in vivo* spectra were obtained from 133 µL voxel placed in the rat brains. The *in vivo* spectra are shown with the linewidths adjusted using NAA peak at 2.01 ppm (repetition time used = 3 s, 1024 scans).

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References

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