Natural Abundance ^{17}O NMR Spectroscopy of Rat Brain *In Vivo*

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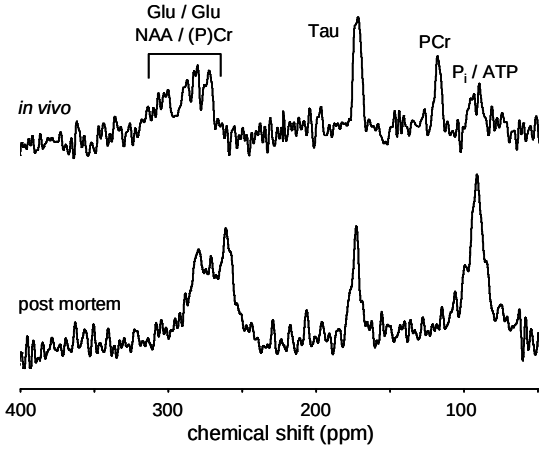
Introduction – Oxygen is an abundant element that is present in almost all biologically relevant molecules. NMR observation of oxygen has been relatively limited since the NMR-active isotope, oxygen-17, is only present at a 0.037% natural abundance (e.g. see [1] for review). Furthermore, as a spin 5/2 nucleus oxygen-17 has a moderately strong quadrupole moment which leads to fairly broad resonances (T_2 = 1 – 5 ms). However, the similarly short T_1 relaxation constants (1 – 5 ms) allow substantial signal averaging, whereas the large chemical shift range (> 500 ppm) improves the spectral resolution of ^{17}O NMR. Here it is shown that high-quality, natural abundance ^{17}O NMR spectra can be obtained from rat brain *in vivo* at 11.74 T.

Methods - All *in vivo* experiments were performed on a 11.74 T Magnex magnet equipped with Magnex gradients (395 mT/m in 180 μs) interfaced to a Bruker console. ^{17}O signal transmission and reception was performed with a double-loop 12 mm diameter surface coil tuned to 67.76 MHz. ^{17}O NMR spectra were acquired with a pulse-acquire sequence (20 us 90º pulse) over 60,000 Hz (TR = 15 ms) in blocks of 8,192 averages. All *in vitro* experiments were performed on a vertical-bore 500 MHz Bruker magnet and console. *In vitro* ^{17}O NMR spectra were acquired on 100 mM solutions in water (no D_2O) at room temperature, unless specified otherwise.

Results – Extensive *in vitro* experiments established a chemical shift and line width library. With water placed at 0 ppm, the biologically relevant nuclei roughly grouped in three areas; oxygen in phosphate groups (90 – 120 ppm), sulfate groups (170 – 180 ppm) and carbonyl groups (260 – 290 ppm). Oxygen in hydroxyl groups (e.g. glucose) were not observable. Spectral line widths varied strongly, from <150 Hz for acetate, inorganic phosphate, phosphocreatine and taurine to >250 Hz for glutamate, glutamine and NAA. The chemical shift of inorganic phosphate showed a strong pH dependence from 86 ppm (pH 4) to 98 ppm (pH 9). The temperature dependence of ^{17}O-water is circa –0.025 ppm/ºC. Figure 1 shows a surface coil localized *in vivo* ^{17}O-NMR spectrum acquired from rat brain in 6 hours. Clear resonances from phosphocreatine, taurine and carbonyl groups were observed. The post-mortem spectrum shows the disappearance of phosphocreatine and the build-up of inorganic phosphate. Given the relatively high signal-to-noise ratio and the low natural abundance of ^{17}O (the taurine signal originates from circa 1.5 uM), the sensitivity of ^{17}O NMR can be of the same order as that of ^{1}H NMR.

Conclusions – Natural abundance ^{17}O NMR spectroscopy on rat brain *in vivo* has been demonstrated. Despite the broad line widths, a large number of resonances could be separately detected. When used in combination with infusions of ^{17}O-enriched substrates, ^{17}O NMR spectroscopy could be developed into a powerful method to study *in vivo* metabolism.



[1] J.-P. Kintzinger, Oxygen NMR. Characteristic Parameters and Applications, NMR Basic Principles and Progress. (Eds. P. Diehl, E. Fluck, R. Kosfeld), Vol 17, pp 1-64, Springer-Verlag, Berlin (1981)

Figure 1: Pulse acquire ^{17}O NMR spectra from rat brain *in vivo* (top) and post mortem (bottom). The spectra represent the total of circa 1.5 million (*in vivo*) and 3.5 million (post mortem) averages, totaling 6 and 14 hours, respectively. Besides the limited active volume of the surface coil no additional localization was applied. The post mortem spectrum is acquired from pure brain tissue, following the removal of all extracranial tissues and skull.