Quantitative Magic Angle Spinning Detection of Deuteration in Small Biopsies of Rat Brain

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Introduction: The replacement of the ^{12}C present in cerebral metabolites by ^{13}C derived from appropriate ^{13}C enriched precursors, as detected by ^{13}C NMR, has allowed the determination of the cerebral tricarboxylic acid and glutamine cycle fluxes. However, the ^{13}C NMR method is relatively insensitive and requires significantly long acquisitions, a circumstance limiting the time resolution of the method. Up to now, only fluxes slower than the tricarboxylic acid cycle flux have been determined after fitting the ^{13}C turnover curves to appropriate models of cerebral metabolism. To overcome this limitation we proposed earlier the investigation of the ^{1}H-^{2}H exchange of specific metabolite protons from ^{13}C isotopomers, a process depicting faster kinetics and thus potentially able to investigate faster reaction rates. However, the High Resolution ^{13}C NMR approach we proposed earlier, requires the use of relatively large amounts of brain samples for the preparation of extracts. More recently, it has become possible to obtain high resolution ^{13}C and ^{1}H NMR spectra directly from the tissue biopsies avoiding extract preparation, by using High Resolution-Magic Angle Spinning (HR-MAS) Spectroscopy. Here, we report on the use of 1D and 2D ^{2}H, ^{1}H-^{2}H and ^{1}H-^{13}C HR MAS methodologies to investigate quantitatively ^{1}H-^{2}H turnover in small (< 10 mg), intact and unprocessed, biopsy samples prepared from the brain of deuterated rats.

Materials and Methods: All animal protocols were approved by the appropriate institutional bioethical committee and follow the guidelines of the responsible governmental agency. Male Wistar rats (180-200g, n=3) fed *ad libitum,* drunk 50% D_2O for nine days. On day ten, the animals were anesthetized (1% isofluorane in 95% oxygen), a solution of (1-^{13}C) glucose infused through the right jugular vein for sixty minutes and the brain fixed with high power microwaves (5 kW, Muromatsu Inst., Tokio, Japan). Microwave fixed brains were divided in two parts, one used to obtain HR-MAS and the other to acquire High Resolution spectra from a perchloric acid extract. Biopsies smaller than 20 mg were introduced in 50 μL zirconium rotors and 1D ^{1}H or ^{2}H and 2D COSY ^{1}H-^{2}H HR-MAS ($4^{0}C$, 4000 Hz) acquired either at 9,4 Tesla or 11,7 Tesla using Bruker AVANCE instruments equipped with HR MAS and high resolution probes. ^{2}H High resolution and HR-MAS spectra were routinely acquired through the lock channel after deactivating the lock frequency sweep. High resolution 1D ^{1}H, and 2D HSQC spectra from extracts were acquired using commercial 5 mm triple (^{1}H, ^{13}C, ^{31}P) probes.

Results: Figure 1 compares ^{2}H NMR spectra obtained under high resolution conditions from a conventional PCA extract from brain (1A) and under HR-MAS conditions from the contralateral biopsy (1B). The fractional deuteration of animals under these feeding conditions is 16% as revealed previously by the deuteration of the urine, yielding values of absolute deuteron content of ca. 9 M $^{2}H_20$ in the heavy water resonance, 1.08 mM in Lactate H3, 0.98 mM in NAA H_6, 0.46 mM Glux H_3, 0.31 mM Glux H_4 and 0.55 mM in the Cr resonance of Fig. 1B. Fractional deuterations are, however metabolically more informative. These can be derived from either 2D ^{1}H-^{2}H correlations or ^{1}H-^{13}C correlations as follows. Figure 2 shows a ^{1}H-^{2}H 2D COSY HR-MAS spectrum obtained from the same brain biopsy. Only the correlations of the most prominent resonances are observed like; Lac H_3-$^{2}H_2$ (1.35-4.07 ppm) or NAA H_6-$^{2}H_6$ (2.05-2.05 ppm), since no correlations can be obtained for perprotonated or perdeuterated species. Under these conditions, the volume of the corresponding ^{1}H-^{2}H correlation peak reaches a maximum for 50% fractional deuteration (Figure 3), becoming undetectable either at natural abundance or 100% deuterations. To improve this situation, we used 2D ^{1}H-^{13}C HSQC spectra, a method in which the volume of the ^{1}H-^{13}C cross peak is linearly inversely proportional to the 2H enrichment of the attached carbon. We demonstrated this (Figure 4) using samples of (U-^{13}C) glucose containing 97% deuteration over the entire molecule, 50% deuteration over the entire molecule or natural abundance deuterium, respectively. The same methodology made it possible to resolve the presence of the differently deuterated methyl isotopomers (CH_3, CDH_2 and CD_2H) of acetate in an acetate sample with 50% deuteration in a ^{1}H-^{13}C (Figure 5). Under these conditions, the presence of one or two deuterons in the methyl group originates the shifted triplet or quintet structures in the HSQC spectrum.

Conclusion: In summary, we implemented several 1D (^{1}H, ^{2}H) and 2D (^{1}H-^{2}H, ^{1}H-^{13}C) methods allowing the determination of the absolute or fractional deuteration in specific metabolite carbons of small brain biopsies using HR MAS methodology.

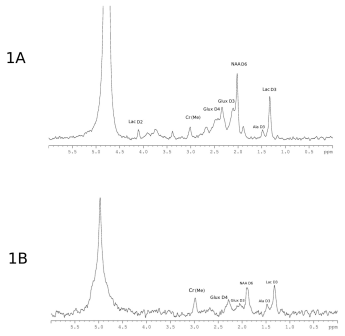

Figure 1. Deuterium spectra of brain extract (upper) and tissue (bottom), both acquired at 400 MHz

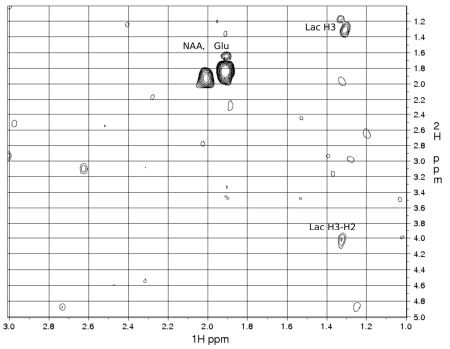

Figure 2. ^{1}H-^{2}H 2D COSY HR MAS spectrum of brain biopsy (400 MHz)

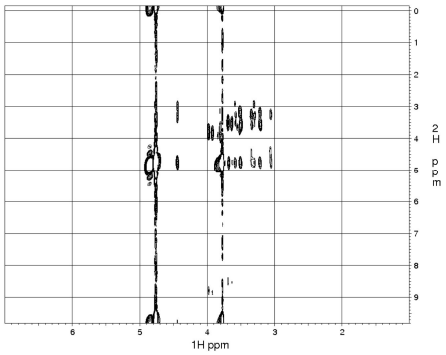

Figure 3. Proton-deuterium HMBC correlation spectrum of 50% ^{2}H, 99% ^{13}C labelled Glucose (500 MHz)

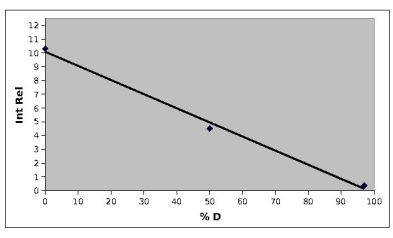

Figure 4. Linearity in Glucose ^{1}H-^{13}C cross-peak volume versus percentage of deuteration as measured in C_1-βH1 correlation.

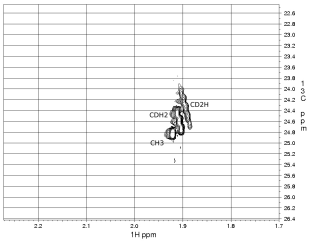

Figure 5. ^{1}H-^{13}C HSQC of 50% deuterated (2-^{13}C) acetate sample. Note resolution of perprotonated, monodeuterated and bideuterated methyl groups