COMPLETE SEPARATION OF EXTRA- AND INTRACELLULAR HYPERPOLARIZED ^{13}C METABOLITE SIGNAL WITH DIFFUSION WEIGHTED MR

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Introduction: Commonly employed proton diffusion weighting (DW) MR techniques are not capable of measuring rapid (sub-minute) changes in molecular motion. Thus, unlike hyperpolarized (HP) ^{13}C MR, proton DW MR is limited in monitoring highly dynamic processes. Recently, we validated the quantitative robustness of HP ^{13}C DW MR (*1*) and demonstrated the ability to monitor both real-time metabolism and translational motion. In this study, we extend HP ^{13}C DW to completely separate extra- and intracellular HP metabolite signal. Exploiting differences in the extra- and intracellular translational motion of HP molecules enables measurement of both the generation and the trans-membrane movement of HP ^{13}C metabolites. The translation of this methodology can allow for the non-invasive assessment of intra- and extracellular metabolite pools on the order of their generation, where differences for HP ^{13}C lactate have recently been correlated to cancer aggressiveness (*2*).

Experimental Methods: Studies were conducted on a 14.1T wide-bore microimaging spectrometer equipped with 100G/cm gradients and a 10mm broadband probe (Agilent Technologies). Pulsed gradient spin echo sequence (T_R=2s, T_E=28ms, δ=6ms,Δ=14ms ,G_{max}=40G/cm) was used for both spectroscopy and imaging studies (256x64, averages=4-100). [1-^{13}C] pyruvic acid and ^{13}C urea were hyperpolarized using a Hypersense (Oxford Instruments) and after dissolution, measurements were done using a double spin echo sequence (flip=10° or 90°, T_R=500ms, T_E=80ms, δ=10ms, Δ=30ms ,G=1.5-58G/cm). DW HP spectra were corrected for T_1 and multiple excitations according to $S/S_0 = exp(-bD)exp(t/T_1)cos^n(\theta)$ where $b = 2\gamma^2G^2\delta^2(\Delta-\delta/3)$. Proton and HP ^{13}C diffusion studies were conducted in an MR-compatible bioreactor using UOK262 renal cell carcinoma cell-filled alginate microspheres with media at 37°C and 0.5mL/min (*3*). Incremental diffusion weighting studies (**Fig1**) were done without flow while extra- and intracellular HP studies (**Fig2**) were done with flow.

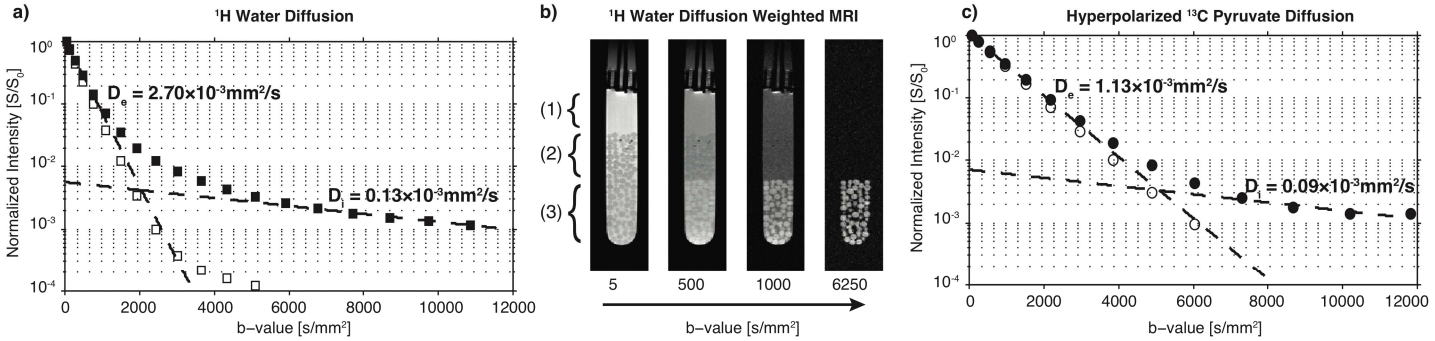

Figure 1: Diffusion weighting (b-value) in the absence of flow. a) Proton signal changes as a function of the b-value in empty (□) and cell-filled (■) alginate microspheres. b) Diffusion weighted proton images emphasize the effect of b-value's on intra and extracellular water; labeled regions (1) water, (2) empty microspheres, (3) cell-filled microspheres. c) HP ^{13}C pyruvate compares the drop in signal from empty alginate microspheres (○) and cell-filled microspheres (●), demonstrating the ability to localize solely intracellular HP signal at high b-values.

Results and Discussion: Proton diffusion studies of cell-filled alginate microspheres in the absence of flow show the DW necessary to suppress extracellular water signal. In agreement with prior studies (*4*), **Fig1a** shows a biexponential curve (■) where the signal at high b-values is dominated by intracellular water signal. The extra- and intracellular diffusion coefficients of water, as represented by the dotted lines, are D_e=2.7×10^{-3}mm^2/s and D_i=0.13×10^{-3}mm^2/s. As expected, empty microspheres show no signal at high b-values (>6,000 s/mm^2) (**Fig1b**). Extending this experiment for HP ^{13}C pyruvate (**Fig 1c**) clearly shows the need to use high b-values (>8,000 s/mm^2) to suppress extracellular HP ^{13}C metabolite signal. Extra- and intracellular diffusion coefficients for HP ^{13}C pyruvate were D_e=1.13×10^{-3}mm^2/ and D_i=0.09×10^{-3}mm^2/s. Next, we injected HP pyruvate into a bioreactor containing perfused UOK262 cells, and using diffusion gradients of b=10 and 8150 s/mm^2 obtained the total and intracellular HP ^{13}C metabolite signals (**Fig2**). The Lac/Pyr ratio for the total signal and intracellular signal are 0.05 and 0.63, respectively. The lack of urea signal, which is transported slowly across cell membranes, reinforces that the measured signal comes predominantly from the cellular compartment. Furthermore, monitoring signal changes between the total and intracellular spectra over time will allow for real-time membrane transport measurements of HP metabolites. Previously, we showed in the bioreactor that UOK262 cells rapidly transport HP ^{13}C lactate out during the relatively short HP experiment time (*2*).

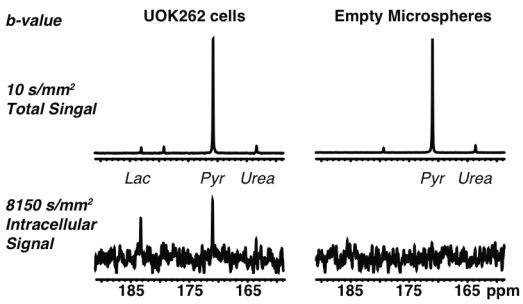

Figure 2: Spectra of HP metabolites showing the total signal (top) and only the intracellular signal (bottom). Data for the UOK262 cells were acquired about at the time point of maximum lactate signal in the bioreactor under flow. Intracellular signal loss do use of the high b-value is ~50%, as seen in **Fig1c**.

Conclusion: This study exploits the large signal intensities of HP ^{13}C MR to assess intracellular metabolites. By using HP DW, it will be possible to probe both the real-time production and membrane transport of ^{13}C lactate *in vitro* in bioreactors. Measuring the HP lactate's cellular efflux could help differentiate benign from metastasizing cancerous tissue (*5*). The technique developed here can also be used to study the membrane transport of various other HP ^{13}C compounds, such as dehydroascorbic acid and ascorbic acid, which are compartmentalized as they are metabolized (*6*). Also, *in vivo* HP diffusion weighted techniques can be use to understand the tissue/vascular distribution of HP metabolites, and determine their associated relaxation parameters for improved modeling and quantification.

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