Microcoil MRI of plants and algae at ultra-high field : an exploration of metabolic imaging

Schadewijk, R. van

APPENDIX

DW-CSI MATERIALS & METHODS

A phantom was constructed to evaluate sequence performance, consisting of a 5 mm NMR tube filled with demineralised water and three flame-sealed capillaries. The three capillaries contained hexadecane, oleic acid (Sigma-Aldrich, St. Louis, Missouri, USA) and a generic extra virgin olive oil, respectively. Hexadecane was chosen due to its property of being the longest chain alkane to have a freezing point below room temperature (293K).

DIFFUSION WEIGHTED CHEMICAL SHIFT IMAGING

CSI employs two orthogonal phase encoding steps with pulsed gradients to record a pure spectroscopic echo upon acquisition, instead of a conventional readout gradient used in imaging. DW-CSI adds to this by introducing a pair of diffusion sensitising gradients interspersed around the first excitation pulse and second refocusing pulse. Thus the gradient separation is linked to the interpulse timing of the CSI itself.

For the reference sample experiments, a diffusion gradient duration (δ) of 4 ms used in combination with a diffusion gradient separation of 6.92 ms. Gradient strength was increased from 5% to 80% of maximum gradient strength in 16 steps, achieving a maximum gradient strength of 11,516 s mm^{-2}. A Hanning function weighted *k*-space acquisition scheme was utilised, as implemented by the Bruker *'weighted'* measuring method, for an improved Spatial Response Function (SRF). Basic parameters were as follows: TR = 1150 ms; TE = 15 ms; Matrix = 16 × 16; FOV = 5 × 5 mm^2; slice thickness = 1 mm; resolution = 312 × 312 × 1000 μm^3; number of scans = 2,048; dummy scans = 4. Acquisition time was 39 m 19 s for each diffusion strength increment. Data were reconstructed into a 32 × 32 matrix with linear smoothing for display. Excitation and refocusing were achieved using Sinc3 pulses with a bandwidth of 8000 Hz, centred on the water resonance. Echoes were captured into 4096 points in 511 ms and spectral width was 8012 Hz (10.68 ppm). Magnetic field homogeneity in the selected volume was optimised by shimming the water resonance. Metabolite heatmaps were generated using a quadratic smoothing function.

DOSY NMR

For each compound, a separate sample was prepared and measured on a 600 MHz Avance III NMR spectrometer equipped with a TXI broadband probe. A stimulated echo sequence incorporating bi-polar gradients was used with the following basic parameters: spectral width was set 9.0 ppm or 5411 Hz; 16384 points were acquired with a dwell time of 92.4 µs in digital quadrature mode (DQD); total acquisition time was 1.51 s; number of averages was 8, as well as 8 dummy scans. The diffusion gradient duration was set individually for each compound to ensure sufficient attenuation at 95% diffusion sensitising gradient strength. The following parameters were used for each compound.

Distilled Water: 32 gradient strength increments, Δ = 0.2 s, δ = 2.4 ms, TR = 2 s; Hexadecane: 128 increments, Δ = 0.2 s, δ = 5.0 ms, TR = 10 s; Oleic acid: 64 increments, Δ = 0.5 s, δ = 8.0 ms, TR = 2 s; Olive Oil: 32 increments Δ = 0.5 s, δ = 16.0 ms, TR = 2 s.

DATA PROCESSING

For each reference compound, a series of spectra were extracted from the DW-CSI results using the 'CsiExtract1dSer' macro present in Paravision 5.1 (Bruker Biospin GmbH, Ettlingen, Germany). Stacking of spectra from DW-CSI and DOSY took place in MestReNova (MestreLab Research S.L., Santiago de Compostela, Spain) where peak integrals were calculated for each gradient strength increment. Spectra were zero-filled to 16,384 points and manually phased. Line broadening was 5 Hz. Consequently, tabulated data was exported to Origin Pro for fitting and normalised to facilitate comparison. Because the reference samples used are pure compounds, containing only one faction, *i.e.*, water (w) or hydrocarbons (h), only mono-exponential fitting is required to accurately describe the system: $S(b) = f_{\mathrm{h}} e^{-bD_{\mathrm{h}}}$, where b is the B-value used and D_{h} the apparent diffusion coefficient (ADC). The mono exponential fitting was performed in OriginPro 9.1.0 with Levenberg-Marquardt algorithm iteration (OriginLab Corporation, Northampton, Massachusetts, USA).