Enhanced sensitivity for multidimensional high-resolution magic-angle-spinning 1H-MR spectroscopy

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Abstract:

We present a solid-state NMR method that maximizes the advantages of high-resolution magic-angle-spinning (HRMAS) ^{1}H-MRS applied to intact tissue biopsies when compared to more conventional liquid-state NMR approaches. Numerical simulations and experimental results of an optimized adiabatic TOBSY (TOtal through Bond correlation SpectroscopY) solid-state NMR pulse sequence for two-dimensional ^{1}H-^{1}H homonuclear scalar-coupling mixing indicate that a significant SNR gain (>100% theoretically and 20-50% experimentally) relative to its liquid-state analogue TOCSY (TOtal Correlation SpectroscopY) sequence is attainable. Multidimensional ^{1}H-MRS is crucial for unambiguous assignment and quantification of overlapping ^{1}H spectra of tissues. Hence, ensuring the best sensitivity is highly desirable. To the best of our knowledge, this is the first demonstration of such a concept for HRMAS metabolic profiling of disease processes, including cancer, from tissue biopsies requiring reduced sample degradation for further genomic analysis.

Introduction:

HRMAS ^{1}H-MRS on tissue biopsies, currently, employs conventional liquid-state pulse sequences and assumes that MAS alone is sufficient to remove the residual anisotropic interactions present in partially immobilized samples. However, in experiments that rely on ^{1}H-^{1}H homonuclear scalar-coupling (J) mediated magnetization transfer, the residual anisotropic interactions such as chemical shielding anisotropy (CSA) or dipolar couplings (D) can be reintroduced unintentionally [1,2] by pulse sequences not designed to eliminate them. Hence, the interference between the MAS and the r.f. field can alter dramatically the transfer efficiency. The effect is pronounced in cases of slower molecular dynamics such as for macromolecular biomarkers (phospholipids, proteins) and at low temperatures (< 0 °C) necessary to preserve sample integrity for further gene expression analysis [3] or to clamp tissues in a certain metabolic state. As of to date, HRMAS ^{1}H-MRS experiments are performed at temperatures above 0 °C where samples are susceptible to metabolic degradation. In addition to increased sensitivity, our approach at lower temperatures can help to alleviate these problems and minimizes implications of the freeze-thawing for tissues previously collected and cryogenically stored.

Pulse sequence:

We refined [4] the C9$^1_{15}$ (TOBSY) symmetry-based ^{13}C MAS solid-state NMR pulse sequence [5] for 2D HRMAS ^{1}H-MRS use and compared the magnetization transfer efficiency and SNR to MLEV-16 (TOCSY) [6] on brain biopsy specimens. C9$^1_{15}$ cancels the 1st order average Hamiltonian and minimizes the higher orders contributions from CSA, D and offset terms, retaining only the isotropic J-coupling. In both cases, WURST-8 [7] adiabatic inversion pulses were employed for their efficient use of r.f. power to compensate pulse offsets, inhomogeneity and miscalibration with reduced r.f. heating. By design C9$^1_{15}$ is rotor-synchronized, and we rotor-synchronized MLEV-16 according to [8]. The 2D TOBSY pulse sequence using C9$^1_{15}$ is shown in Figure 1 (a cw water suppression block is omitted).

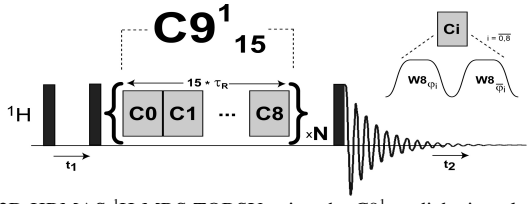

Figure 1. 2D HRMAS ^{1}H-MRS TOBSY using the C9$^1_{15}$ adiabatic pulse sequence. Nine C elements span 15 rotor periods, each C element is constructed from two 180° phase-shifted WURST-8 pulses, black rectangles represent hard 90° pulses.

Results:

Prior to measurements, we performed quantum mechanical numerical simulations using GAMMA [9] environment to predict the magnetization transfer buildups of C9$^1_{15}$ and MLEV-16. A two-spin system was assumed having a J-coupling of 10 Hz and: (a) offsets of ±1500 Hz (±2.5 ppm at 600 MHz ^{1}H, S = 0), or dipolar couplings of (b) 70 Hz (corresponding to an order parameter S = 0.01 for vicinal protons at a distance of 2.5 Å), (c) 2100 Hz (S = 0.3) and (d) 7000 Hz (S = 1), respectively. Simulations shown in Figure 2 indicate that C9$^1_{15}$ can more than double the transfer efficiency of MLEV-16. Tumor (from 6 patients diagnosed with Glioblastoma Multiforme, GBM) and control (from 4 epilepsy patients) brain biopsies were measured experimentally. Representative 2D spectra obtained on a 14.1 T Bruker Avance wide-bore spectrometer equipped with a 4 mm double-channel HRMAS probehead (Bruker), using MAS of 3 kHz and a temperature of -8 °C, are shown in Figure 3. The same experimental (200 t_1 increments (TPPI), 45 ms mixing time, 2 s repetition time (1 s cw water suppression), 8 scans, 2 dummy scans, 50 min total acquisition time) and processing parameters (zero filling to 1k in F1, QSINE = 3 window function, base line correction) were used. Similar contour levels are chosen for 2D spectra. The 1D slices extracted along the indirect dimension for several metabolites prove the increase in SNR for C9$^1_{15}$. 1D slices are scaled to the same noise levels and integral values are given (diagonal peaks are normalized to 1).

Discussions:

The proposed method provides increased SNR for high-molecular weight biomarkers such as phospholipids (GPC, PUFA ~ ↑ 50% in SNR), as expected, but also for low-molecular weight metabolites (NAA, Lactate ~ ↑ 20% in SNR). Although significant, the observed SNR gain was lower than predicted by simulations for the two-spin system under ideal conditions. Experimentally, this might be due to a variety of factors, such as variation in the size of interaction parameters (scalar couplings, order parameters), multispin effects, relaxation in the rotating frame, or slight errors in generation of the adiabatic pulses.

Our results suggest that the novel concept can be used to detect new biomarkers (proteins), decrease acquisition times and reduce variability for metabolite quantification of physiological and disease processes in tissue biopsies or stem cells. We envision that performing measurements at low temperatures to protect or metabolically clamp tissues can be further coupled with dynamic nuclear polarization (DNP) [10] for further signal enhancement. Moreover, in the broader context of life sciences our approach can be extended into structural biology (membrane proteins, protein fibrils) complementary to the existing NMR methods.

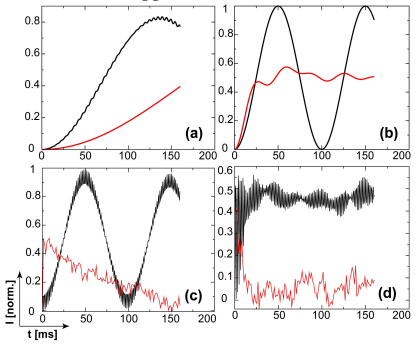

Figure 2. Simulations of magnetization transfer buildup curves for C9$^1_{15}$ (black) and MLEV-16 (red).

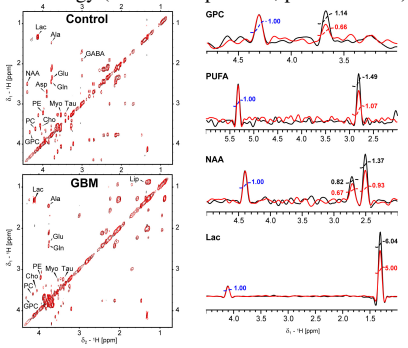

Figure 3. 2D spectra and 1D slices for control and tumor (GBM) biopsies of human brain. Overlay of C9$^1_{15}$ (black) and MLEV-16 (red).

References:

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