Proton NMR Analysis of Human Prostate Tissue with Slow Rate High Resolution Magic Angle Spinning: (A+B-|A-B|)

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Synopsis. Applying high resolution magic angle spinning (HRMAS) NMR spectroscopy to investigate cellular metabolism of diseases has encouraged exploration of slow spinning methodologies to better preserve tissue pathology structures against HRMAS centrifuging damages. Spinning sidebands (SSB) resulting from slow spinning must be eliminated to prevent complications to metabolite spectra. A novel scheme employing two spectra (A, B) of different slow spinning rates is evaluated with human prostate tissue. By editing these spectra according to the formula A+B-|A-B|, SSB free spectra can be obtained for metabolite quantification, resulting in spectra that are comparable to those measured with high rate spinning.

Background. The use of ex vivo HRMAS in studying the proton NMR of intact tissue results in highly resolved spectra comparable to what in the past was observed only with solutions of tissue chemical extractions. However, unlike destructive chemical extractions, the HRMAS method provides a more complete chemical signature, as well as largely preserves histopathological structures and grants the opportunity for samples to be histopathologically examined after NMR measurement. The HRMAS method hence increases the accuracy of associating observed changes in cellular metabolites with pathological changes. The ability to measure NMR and histopathology on the same specimens is critical to establishing correlations between individual NMR-visible metabolic alterations and pathogenic processes, particularly for studies of human neoplastic diseases since there are well-known heterogeneous characteristics within individual tumors. Spinning of samples at the magic angle splits the broad resonance into a center peak at the isotropic resonance frequency and a number of SSB separated by the spinning rate. To eliminate the SSB complication on spectra of cellular metabolites, spinning rates that are equal to or above 5 ppm (a few kHz, depending on the magnetic field strength utilized) have commonly been employed in studies of biological samples.

Since spectral broadening observed in biological tissue is predominantly caused by the bulk magnetic susceptibility, it has thus been noted experimentally that for most of the analyzed intact tissues, if the complex issue of SSB could be resolved, HRMAS proton NMR spectra could be measured at a spinning rate at least an order of magnitude lower than is currently used. A reduction in sample spinning rates by 5-10 fold represents a 25-100 fold decrease in spinning-induced centrifugal stress that may damage the organizational structure of connective tissue, although may not disrupt cells. Reducing spinning centrifugal stress can better preserve tissue pathological structures, which will translate into a more accurate evaluation of their pathologies. This consideration has prompted a number of recent HRMAS studies aimed at SSB suppression with the assistance of somewhat complicated pulse sequence schemes [1].

Methods & Materials. Here, we propose to achieve this aim by applying a simple but novel mathematical scheme to edit two simple spectra (A, B) obtained at different slow spinning rates with A+B-|A-B| [2]. The success of this scheme depends on the following conditions that are usually met: 1) these two spectra are obtained from the same sample, other than spinning rates, under the same experimental conditions; 2) the line-width for an individual resonance is the same (within the measurement error) in the two spectra; and 3) the two spinning rates are decided such that there are no SSB overlap points in the regions-of-interest in the two spectra.

Nine samples of human prostate intact tissue have thus far been analyzed at spinning rates of 600, 700 Hz and 3 kHz. The spectra were acquired with tissue water signal being suppressed by presaturation, which also significantly reduced water SSB, followed by a single 90º excitation pules. Among these nine samples, the shimming conditions for the two samples were not optimal. These two samples were excluded, and only data from the remaining seven samples were further analyzed and reported here.

Results & Discussions. **Figure 1** presents results of one sample that had the largest residual water SSB after presaturation. Spectra (A) and (B) were measured at 600 and 700 Hz spinning rates, respectively, where SSB from water and external standard were clearly observable. Spectrum (D) was measured at 3 kHz spinning rate where all of the SSB were pushed away from the region of interest. It is clear that there is no visible difference between (D) and (C), where (C) represents the result of A+B-|A-B| edited spectrum. Furthermore, with examples in **Figure 2**, we demonstrate that the line-widths measured at 600-700 Hz spinning rates are the same as those obtained at 3 kHz for mobile metabolites, such as lactate, while, as expected, the line-widths are slightly narrower at higher spinning rate for less mobile molecules, such as lipids. Nevertheless, our results in **Table 1** indicate that, for a number of metabolites, their intensities measured from the edited spectra can accurately represent their true values as determined with 3 kHz spinning rate (i.e. a linear correlation with slope = 1, and intercept = 0.) Additional measurements are currently underway to evaluate the other metabolites with which differences are listed in the Table. All the aspects including the physical and chemical states of these cellular metabolites, and the applicability of the scheme in clinical evaluation will be discussed in details in our presentation.

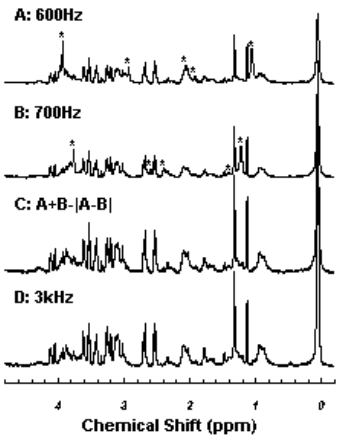

Figure 1. Human prostate tissue HRMAS proton spectra measured at (A) 600, (B) 700 Hz, and (D) 3 kHz. (C) edited spectrum from (A) and (B) according to A+B-|A-B|. Spectra were collected on a 600MHz (Bruker) spectrometer at 3º C. Spinning sidebands are denoted by *.

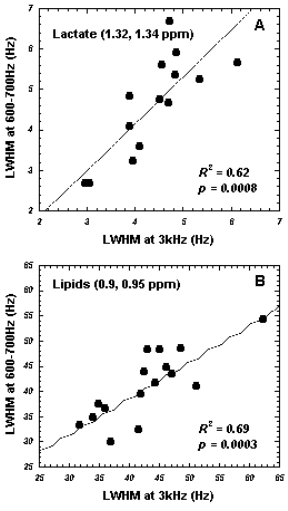

Figure 2. Comparison of resonance line-widths at slow and 3 kHz spinning for (A) lactate, and (B) lipids.

Met. Reson. (ppm)	P value	R^2	Slope		Intercept	
			Mean	SE	Mean	SE
Lac(4.10-4.14)	0.0087	0.71	**1.03**	**0.27**	**-0.02**	**0.59**
MI(4.06)	0.016	0.64	**1.10**	**0.33**	**-0.01**	**0.48**
3.60-3.63	0.082	0.42	**0.75**	**0.36**	**0.70**	**1.07**
Gly(3.55)	0.043	0.52	**0.96**	**0.38**	**0.07**	**0.31**
3.52-3.54	0.0055	0.75	0.78	0.18	1.10	0.67
3.41-3.43	0.0028	0.80	**1.09**	**0.22**	**-0.18**	**0.45**
3.34	0.033	0.56	0.63	0.23	0.21	0.13
3.29-3.25	0.0027	0.80	1.30	0.26	-2.50	1.17
Pch(3.22)	0.0002	0.92	1.46	0.18	-0.70	0.29
Chol(3.20)	0.011	0.69	0.69	0.19	0.32	0.27
Spm(3.09-3.14)	0.0002	0.92	0.80	0.10	**0.43**	**0.44**
Cr(3.03)	0.067	0.45	0.40	0.18	0.66	0.26
Cit(2.52-2.71)	0.0007	0.87	0.71	0.11	2.45	0.88
Glu(2.33-2.36)	0.0015	0.83	**1.19**	**0.22**	**-0.05**	**0.17**
Ala(1.47-1.49)	0.013	0.67	0.38	0.11	0.27	0.08
Lac(1.32-1.34)	0.0051	0.76	**0.96**	**0.22**	**0.29**	**1.85**
Lipid(0.95)	0.056	0.48	**0.85**	**0.35**	**0.14**	**1.49**
1.13-1.14	<0.0001	0.99	0.92	0.03	0.11	0.10
STD(0.06)	0.0046	0.76	0.77	0.17	**-3.81**	**5.98**

Table 1. Linear correlations (p and r^2 values) between metabolite intensities measured at slow HRMAS with those at 3 kHz (n=8). Bold faced data indicate either slopes are statistically indifferent (considering the range of Mean based on +/- SE) from 1, or the intercepts are 0.

References. (1) Wind R.A. etal MRM 2001;46:213; Hu J.Z. etal MRM 2002;47:829; Taylor J. L. etal PISMRM 2002;10:135. (2) Patt S. Private Communication.

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