Quantitative evaluation of 31P MRS of Tissue Samples using Magic Angle Spinning

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Introduction: There is much current interest in understanding mechanisms responsible for large choline peaks seen in ^{1}H spectra of most cancers. ^{31}P MRS measurements in patients show elevated phosphomonoesters (PME) and phosphodiesters (PDE), and early changes in response to treatment (1). Work in cells suggests some changes may relate to specific signalling pathways (e.g. (2)), while a switch from PC/GPC < 1 to PC/GPC >> 1 is seen with increasing malignancy in human breast epithelial cells (3). Clinical studies would be aided by histology-validated measurements on biopsy tissue samples, but *ex vivo* MRS measurements of tissue extracts excludes this possiblity. Following the introduction of high-resolution ^{1}H magic-angle spinning (MAS) spectroscopy to study small (~ 10-50 mg) tissue samples directly at high field (4), we investigated the potential of high-resolution ^{31}P MAS of tissue samples (5). Having established the visibility and relative stability of peaks from phosphoethanolamine (PE), phosphocholine (PC), glycerophosphoethanolamine (GPE) and glycerophosphocholine (GPC) we present here a comparison of methods to evaluate the tissue concentrations in MAS measurements, and compare with high-resolution MR studies of extracts of the same tissue samples.

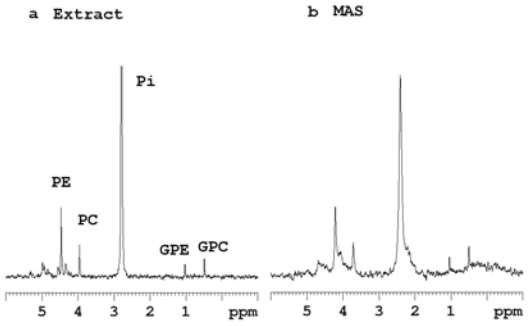
Methods

MAS: The radiation-induced fibrosarcoma (RIF-1) tumour (grown on a C3H mouse) was used, being relatively homogeneous with few necrotic or cystic regions (6). Tissue samples (approx 48 mg, N=15) were weighed in 4 mm ZrO_2 rotors. 10 µl of 10 mM methylene diphosphoric acid (MDPA) was added as a concentration and chemical shift reference (16.8 ppm) and 5 µl D_2O was added as a field frequency lock. Measurements were performed in a high-resolution MAS probe in a Bruker Avance 14 T spectrometer (242 MHz for ^{31}P). Rotors were spun at 3 kHz and maintained at 4ºC. ^{1}H-decoupled ^{31}P HR-MAS spectra were obtained with 512 transients, TR = 3.35 s and ^{1}H decoupling. Peak areas were measured using the AMARES algorithm (7) included in the jMRUI software package (8). Estimates of metabolite concentrations were calculated using as a reference (i) the signals from MDPA in individual samples, (ii) the average MDPA signal from all the samples, (iii) signal from a separate measurement of a rotor filled with 50 µl of 10 mM MDPA. Our previous study has shown that no correction was required for partial relaxation with this TR.

Tissue Extracts. Water-soluble metabolites were extracted from each sample using 1 ml of 6% perchloric acid. The tumour extracts were freeze-dried and re-dissolved in 0.6 ml of D_2O with 50 µl of 5mM 3amino-propylphosphonate added as a concentration reference. Samples were measured in a 5mm QNP probe (^{1}H and ^{31}P with deuterium lock) at 14T, using TR = 6.35 s, and 5500 scans. ^{31}P spectra were processed with 1 Hz exponential apodisation, Fourier transformation, and phase correction.

Results and Discussion:

Appearance. Fig 1 shows example high resolution ^{31}P MAS spectra of a RIF tumour sample using (a) MAS with NS = 512, and (b) tissue extract with NS = 5500. The MAS spectrum shows well-resolved peaks are from PE, PC, Pi, GPE and GPC. The linewidths achieved (2.70±0.62 Hz for GPC & GPE; 9.78± 4.09 Hz for PE & PC) are only slightly larger than obtained in the extract spectra (2.0±0.4 Hz for GPC & GPE; 4.2±1.9Hz for PE & PC). The relatively larger linewidths in tissue of PE and PC reflect their sensitivity to intracellular pH. As expected there are no significant signals from high-energy phosphates (ATP or PCr).



Estimate of Signal-to-noise ratio of MAS relative to extracts. The relative SNR for MAS (28 minutes) vs extract measurements (585 minutes) for a subset of data was estimated (Table 1), showing an approximate 3-4 fold improvement in sensitivity by study of the tissue sample directly. Since different repetition times were used for the MAS (3.35s) and extract (6.35s) measurements, owing to the anticipated difference in T_1 values, experiments with accurately optimised repetition times might lead to slightly different values for the relative sensitivity of the two techniques. One factor contributing to the lower SNR of the extract spectrum is that the metabolites in the sample are diluted into a larger volume.

	PE	PC	GPE	GPC
MAS	12 ±2	6 ±2	4±1	5±1
Extracts	4.2 ±0.9	1.75± 0.4	1.1 ±0.2	1.1 ±0.4

Table 1. Comparison of signal-to-noise ratio achieved from MAS and tissue extracts of the same samples. Results for extracts have been scaled to account for the longer acquisition time.

Concentration estimates. Concentration calculations are summarised in Table 2. The large sd when using the peak area of MDPA from individual rotors (row 1) is much reduced when averaging MDPA peaks from all samples (row 2). This suggests that non-uniform distribution of MDPA with tissue leads to significantly non-uniform detection. This is supported by the data of row 3, where a separate measurement of MDPA in a rotor without tissue sample leads to good agreement with the results of the extract analysis (row 4). The correlation coefficient is reported below.

	PE	PC	GPE	GPC
1. MAS – individual MDPA	3.53 ± 3.43	1.44 ± 1.49	0.73 ± 0.89	1.30 ± 1.29
2. MAS – average MDPA	2.32 ± 0.75	0.94 ± 0.34	0.48 ± 0.22	0.89 ± 0.27
3. MAS – separate ref measurement	1.19 ± 0.39	0.48 ± 0.18	0.24 ± 0.11	0.46 ± 0.14
4. Extract measurement	1.49 ± 0.34	0.62 ± 0.13	0.27 ± 0.16	0.39 ± 0.18
Corr between MAS(3) and extracts	0.56 (p=0.03)	0.46 (p=0.08)	0.53 (p=0.04)	0.47 (p=0.07)

Table 2. Calculated concentrations (µmol/g wet wt) in tissue samples (mean ± sd; N=15), with correlation coefficient between MAS and extract results.

Conclusion: ^{31}P MAS of tissue samples produce spectra of superior SNR and with concentration estimates in good agreement with those obtained from MRS of extracts from the same samples. Data from a reference compound acquired in a separate measurement is more reliable than adding compound to tissue samples within the rotor. The method is well suited to study of tumour biopsies where only small tissue samples are available and where histological verification is required.

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Acknowledgement: This work was supported by Cancer Research UK [CUK] grant numbers C1060/A808 and C12/A1212)