A 13C HR-MAS Technique for Studying the Cellular Bioenergetics Associated with Prostate Cancer

M. J. Albers[1], A. Zektzer[2], J. Kurhanewicz[2]

[1]Bioengineering, University of California - San Francisco, San Francisco, CA, United States, [2]Department of Radiology, University of California - San Francisco, San Francisco, CA, United States

Introduction

The motivation for using ^{13}C labeled substrates as probes for prostate cancer detection and characterization arises from the unique metabolism of healthy prostate epithelial cells and the extensive literature on the changes in cellular bioenergetics that occur with prostate cancer evolution and progression (1). The use of ^{13}C labeled pyruvate and glucose as metabolic tracers provides the potential to noninvasively assess changes in metabolic intermediates from multiple biochemical pathways (glycolysis, citric acid cycle and fatty acid synthesis) simultaneously. Since Swanson et. al. (2) have already detected changes in citrate by acquiring ^{1}H High Resolution – Magic Angle Spinning (HR-MAS) spectra from prostate tissue samples, it would be valuable to be able to study these ^{13}C tracers by performing HR-MAS on cellular samples and animal tissue samples prior to starting in-vivo studies. Unfortunately, ^{13}C HR-MAS spectroscopy is limited by the low natural abundance of ^{13}C (1.1%), its low magnetogyric ratio (γ of ^{13}C is 1/4 of ^{1}H), and the relatively small sample size used for HR-MAS (5-40mg). To address this sensitivity problem, a carbon decoupled heteronuclear single quantum coherence (HSQC) sequence (3) was used to indirectly detect ^{13}C through directly coupled protons, which provides a theoretical 32 fold increase in SNR. In addition, the HSQC provides both ^{13}C and ^{1}H chemical shift data. In order to use technique, this study first focused on optimization of carbon decoupling and non-labeled proton suppression (TANGO and BIRD) and developing a method for estimating ^{13}C enrichment.

Methods

All data were collected from 19-25mg samples using a 11.7Tesla Varian HR-MAS spectrometer equipped with a ^{1}H nanoprobe. GARP decoupling and several permutations of Adiabatic Decoupling were compared. A TANGO - gradient and a BIRD pulse were added to the HSQC and evaluated for suppression of ^{1}H's attached and ^{1}H's NOT attached to ^{13}C nuclei. For each two-dimensional HSQC, 4096 complex points were collected in the ^{1}H dimension and 256 in the ^{13}C dimension. A TR of 1.8sec and averaging 64 scans (16 scans for solution samples) created a 17hour (4.25hrs for solution) experiment. The technique was applied to tissue samples collected from a mouse prostate tumor harvested 30 minutes after an intraperitoneal injection of 3-^{13}C Pyruvate. In order to quantify the ^{13}C enrichment present in the tissue, a 1mM 2-^{13}C Glycine reference was added, and a $^{1}H[^{13}C]$ spectrum was acquired before and after each HSQC experiment. A ratio was computed for the metabolites in the ^{13}C enriched HSQC spectrum relative to a non-enriched HSQC. A similar ratio was computed for the metabolites in the $^{1}H[^{13}C]$ spectrum. Finally, the calculated ratios were divided for the metabolites of interest to estimate the percentage of ^{13}C enrichment.

Results

The GARP decoupling bandwidth was 14kHz at 41dB. Whereas, the Adiabatic decoupling bandwidth was 21-26kHz, depending on the pulse period and phase modulation. The $^{1}H[^{13}C]$ linewidth and peak height were slightly better for Adiabatic decoupling with a 444µsec pulse period. The decoupling noise was least for Adiabatic Decoupling with 444µsec pulse period and an m64 phase modulation. The BIRD pulse attenuated the ^{13}C labeled signal by 50% and the unlabeled signal by 36%. While the TANGO-gradient pulse yielded 18% and 70%, respectively.

Discussion

Synchronizing the Adiabatic pulses to the HR-MAS rotation period (444µsec) provided the best decoupling performance. While TANGO-gradient pulses are superior to the BIRD pulse, the HSQC on ^{13}C enriched samples had few artifacts from ^{1}H's not attached to ^{13}C (Figure 1). However, in natural abundance ^{13}C studies the TANGO-gradient will help remove these artifacts from the HSQC. By adding the ^{13}C Glycine reference and combining information from the $^{1}H[^{13}C]$ spectra and the HSQC, it was possible to estimate the amount of ^{13}C enrichment in a mouse prostate tumor after injection of 3-^{13}C Pyruvate (Table 1). The 2% estimated ^{13}C enrichment for GPC+PC and Choline (data not shown in Table 1) indicate that these estimates have slight errors. As a result, the estimate will be improved with correction factors for J-coupling and other differences. Finally, the optimized HSQC sequence and ^{13}C enrichment estimation will be utilized in human prostate cancer cell line and transgenic mouse models to determine the ^{13}C labeled metabolites of glucose and pyruvate that best identify the presence of prostate cancer and characterize its aggressiveness.

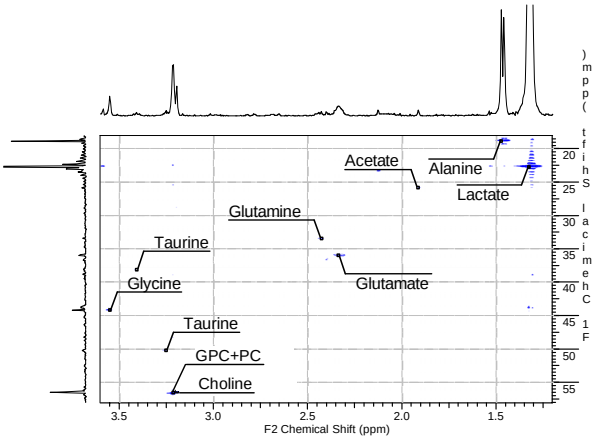

Figure 1 An HSQC performed on a 19mg mouse tissue sample after ^{13}C enrichment with 3-^{13}C Pyruvate.

Metabolite	^{13}C Ratio	^{1}H Ratio	^{13}C Enrichment
Lactate	63	1.4	44%
Alanine	134	2.5	54%
Glutamate	9	1.7	6%

Table 1 Metabolite ratios of ^{13}C enriched to non-enriched samples from the HSQC (^{13}C Ratio) and the $^{1}H[^{13}C]$ spectra (^{1}H Ratio) along with the calculated ^{13}C enrichment.

References

1. Costello LC, Franklin RB. Prostate 1998; 35:285-296.
2. Swanson MG et al. J. Mag. Res Med. 2003;50:944-954.
3. Bodenhausen G, Ruben, DJ. Chem. Phys. Lett. 1980; 69:185-189.

Acknowledgements

Robert Bok is acknowledge for assistance with the animal studies and Paul DiCamillo for processing the $^{1}H[^{13}C]$ spectra in LC Model.
This work was supported by NIH CA079980 and LSIT 10107.