Indirect Detection of Phosphorus-31 Signals in Cells by 2D-Heteronuclear Methods

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Introduction: Indirect detection (ID) is a method of observing the signals of a less sensitive NMR nucleus through cross-polarization or spin-spin (J) coupling with a more sensitive nucleus. ^{1}H and ^{31}P are the most common nuclei used for observation of biochemical and metabolic processes *in vitro* and *in vivo*, and consequently there might be some advantage in using these ID approaches for ^{31}P observation. Generally the only coupling between ^{1}H and ^{31}P is through the phosphate ester 3 bond couplings (J_{POCH} = 8-10 Hz). While this is a small coupling its presence is enough to obtain ^{31}P spectra at proton sensitivity. However, difficulties arise in carrying out these experiments due to the interference of ^{1}H-^{1}H couplings with similar values. A means of overcoming these difficulties and to obtain ^{31}P data at higher sensitivity is to use heteronuclear multiple quantum correlation (HMQC) spectroscopy as the POCH fragment requires multiple quantum transitions. In this project we evaluate the use of ID 2D-heteronuclear methods for the detection of phosphate metabolites in live cells.

Material and Methods: HL-60 cells (acute myeloid leukemia) and A2780 cells (human ovarian carcinoma) were grown according to established procedures. Spectra of intact cells were determined using the gel perfusion method were cells are embedded in alginate beads (Cohen and Kaplan, Immunomethods 4:139,1994). Cytoplasmic components were extracted according to Teleman et al (Anal. Biochem., 272:71, 1999) . NMR spectra were obtained with Varian Inova 500. 1mM methylphosphonic acid was used as an internal standard for method development and optimization (at 10°C, 1.1 ppm)

Results: ATP (50 mM) in D_2O standard was used for method development and optimization. The chemical shift was stable under pH of 7.0 ±0.1 at temp. of 10ºC and peak volume compared to the peak of methylphosphonic acid at constant concentration was reproducible. Chemical shifts of ^{1}H and ^{31}P correlate with the cross peak obtained by HMQC (**Fig. 1,2**). Peak volume was found to be linear with concentration (**Fig. 2**) using the methylphosphonic (MeP) acid cross peak as an internal standard.

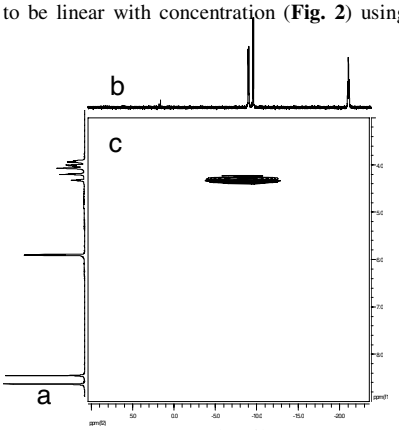

Fig 1 (left): ATP NMR spectra: a-^{1}H; b-^{31}P; and c-HMQC

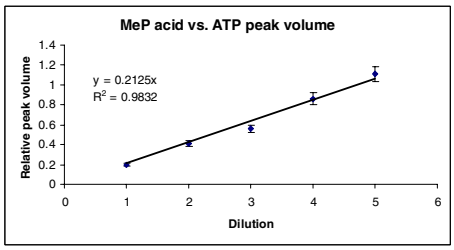

Fig. 2 (right) : Methylphosphonic acid cross peak vs. ATP cross peak relative volume.10mM ATP standard was diluted relatively to constant 10mM MeP acid concentration, and peak volume change relative to the constant MeP peak volume was measured.

HMQC of cell extracts (not shown) and perfused intact cells (Fig 3) revealed that ^{1}H -^{31}P HMQC provides improved resolution and sensitivity compared to direct ^{31}P MR detection. The UDPS peak is resolved into UDP-glucose and UDP-galactose. Additional phosphosugars mono- and diesters (fructose 6 phosphate, fructose 1,6 bisphosphate, glucose 6 phosphate, glucose 1,6 bisphosphate) peaks are also resolved.

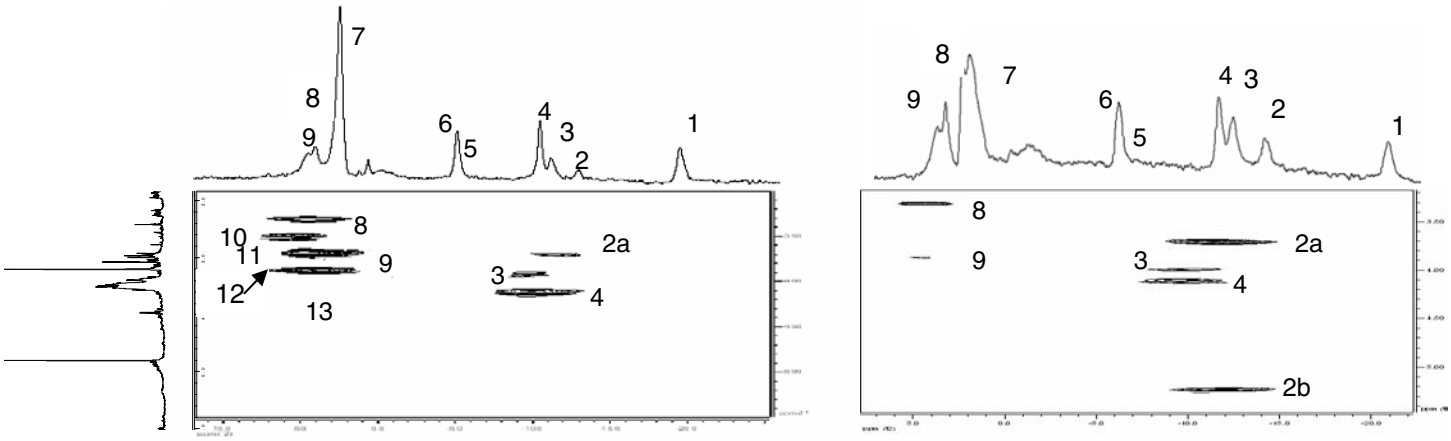

Figure 3: HMQC spectra of perfused live HL-60 (left) and A2780 (right) cells . Peak reference: 1- β-ATP, 2- UDPs, 2a- UDP-glucose, 2b- UDP-galactose, 3- α-ATP, 4- α-ADP, 5- γ-ATP, 6 -β-ADP, 7- Pi, 8- PC, 9-PE, 10- fructose 1,6 bisphosphate, 11- glucose 1,6 bisphosphate, 12- fructose 6 phosphate, 13- glucose 1,6 bisphosphate.

Conclusions: ^{1}H -^{31}P HMQC provides improved resolution and sensitivity compared to direct ^{31}P MR detection. The UDPS peak is resolved into UDP-glucose and UDP-galactose. Additional phosphosugars mono- and diesters (fructose 6 phosphate, fructose 1,6 bisphosphate, glucose 6 phosphate, glucose 1,6 bisphosphate) peaks are also resolved. Spectra of A2780 and HL-60 intact cells are well resolved, reproducible and peak chemical shifts and relative volumes correlate well with cell extract spectra.