Direct determination of phosphate sugars in biological material by ^{1}H High Resolution-Magic Angle Spinning (HR-MAS) NMR spectroscopy

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PURPOSE

While nucleotide sugars, mainly UDP-X, are known to be key players in glycosylation processes[1], glucose-phosphates (Glc-1P and Glc-6P) are important intermediate metabolites for storage and transfer of energy, being part of the glycogen cycle[2].

High resolution magic angle spinning (HR-MAS) NMR is increasingly being used to metabolically characterize tissue biopsies[3], as well as cells[4]. The technique can be used to monitor temporal metabolite changes, enabling to follow metabolic pathway activities. ^{1}H HR-MAS NMR allows to assess these phosphate sugars qualitatively and quantitatively as a minimally invasive analytical tool without the need of extraction or separation steps, conserving the cell and biopsy integrity.

Anomeric sugar protons bound to phosphate show typical doublet of doublet (dd) resonances between 5.4 and 5.7 ppm. As those peaks show the same pattern and are located very close to each other, a correct assignment can be challenging, especially considering potential small shifts due to ion strength, pH or temperature. Spiking might help for supporting metabolite assignment. UDPNAc-Glc and -Gal have been previously assigned in cells either based on NMR of cell extracts[5], statistical data[4,5] or liquid NMR of whole cells[6]. The aim of our study was to unambiguously assign phosphate sugars directly in spectra of intact cells, as well as in skeletal and heart muscle biopsies by ^{1}H HR-MAS NMR. A second aim was to study the kinetic of α-Glc-1P, identified in a cardiac muscle biopsy.

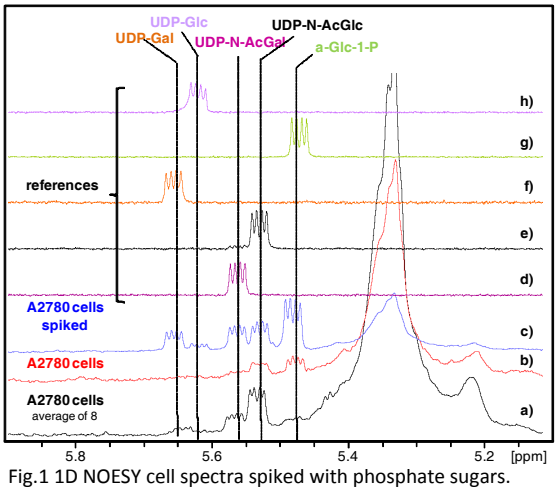

Fig.1 1D NOESY cell spectra spiked with phosphate sugars.

METHODS ^{1}H HR-MAS NMR experiments were performed on a 500MHz Bruker Avance II spectrometer. Cancer cells (A2780) grown for 72h and human skeletal muscle biopsies were each measured in a 50µl rotor filled with PBS at 310K (3kHz MAS) and 277K (5kHz MAS), respectively. 1D NOESY and CPMG spectra of unspiked samples and pure phosphate sugars using creatine as internal chemical shift reference were acquired. Subsequently, cells were spiked with α-Glc-1P, UDP-NAc-Gal, UDP-Gal and UDP-NAc-Glc and muscle with α-Glc-1P and Glc-6P. The kinetic of α-Glc-1P in a sheep heart muscle biopsy was studied during 3.5h at 277K (5kHz MAS) acquiring 20 1D CPMG spectra of 5 min duration with a 5 min break between each experiment. The integral of the 5.46ppm peak was calculated for each spectrum.

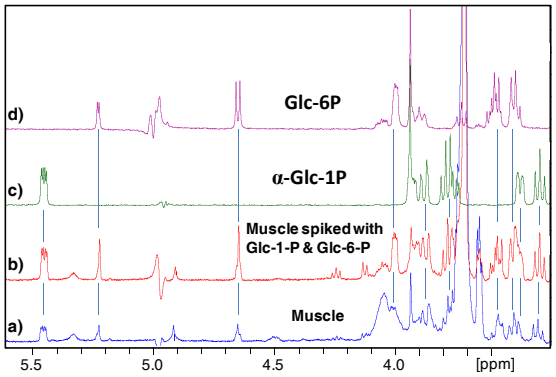

Fig.2 CPMG human muscle biopsy spiked with α-Glc-1P &Glc-6P.

RESULTS Fig.1 shows 1D NOESY cell spectra with and without spiking with phosphate sugars. The comparisons of chemical shift positions in combination with the spiking confirmed the presence of α-Glc-1-P, UDP-N-AcGal, UDP-N-AcGlc, and UDP-Gal in cells. UDP-Glc appeared after the spiking at 5.62ppm, probably resulting from a degradation process.

Fig.2 shows human skeletal muscle spectra. α-Glc-1P and Glc-6P, with their respective characteristic peaks at 5.45ppm and 4.65-5.22ppm, are clearly present in the muscle spectrum, as confirmed by the spiking. Other phosphate sugars were not detected in this muscle.

The kinetic of α-Glc-1P in a cardiac muscle of a sheep is shown in Fig.3. The α-Glc-1P dd peak at 5.46ppm slightly increased for the first 0.5h, before decreasing and disappearing within 2.5h (Fig.3b). Other α-Glc-1P peaks showed a similar kinetic pattern with initial increase followed by a decrease. Peaks of both Glc-6P and Glc, showed an initial slight increase before stabilization. Other peaks remained relatively constant over the time period investigated.

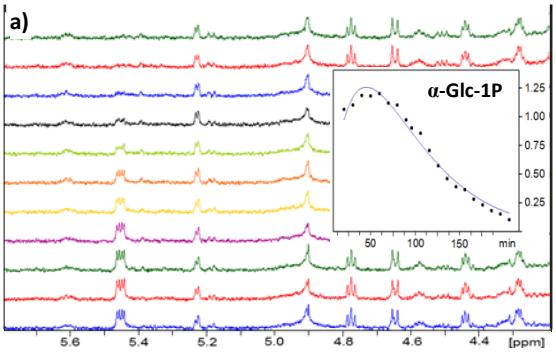

Fig.1 Glc-1P kinetic a) Sheep cardiac muscle spectra taken over 3.5h. b) Evolution of the α-Glc-1P 5.45ppm peak with fit.

DISCUSSION The results clearly demonstrate that sugar phosphates can be determined quickly and non-destructively in cells and biopsies by HR-MAS, which may prove valuable considering the importance of phosphate sugars in cell metabolism for nucleic acid synthesis. It allows their quantitative estimation in living cell measurements, without extraction processes. The different phosphate sugars can be clearly separated from each other. In skeletal and cardiac muscle, the presence of α-Glc-1P (to our knowledge for the first time) and Glc-6P could be unambiguously assigned. The α-Glc-1P kinetics proves exemplarily the possibility of measuring the dynamics of specific metabolic processes by ^{1}H HR-MAS NMR. The initial α-Glc-1P increase may be due to glycogen breakdown. The subsequent Glc-1P decrease may be a result of enzymatic conversion into Glc-6P and finally Glc through phosphoglucomutase, as suggested by the kinetic analysis.

CONCLUSION In conclusion ^{1}H HR-MAS NMR allows the assessment of phosphate sugars contained in cells and skeletal and cardiac muscle biopsies, and facilitates the study of their kinetics. Therefore this technique can be used for studying phosphate sugar metabolic pathways, compounds that are physiologically very important in nucleic acid synthesis, glycosylation processes, and the glycogen cycle. The quantitative assessment of α-Glc-1P and Glc-6P being key players in energy metabolism by ^{1}H HR-MAS NMR may prove important for metabolic studies in biopsies.

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