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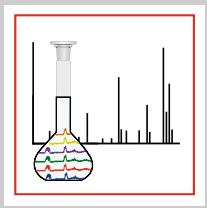

Highlights of Analytical Sciences in Switzerland

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^{1}H High-resolution Magic-Angle-Spinning NMR Spectroscopy to Determine Phosphate Sugars in Biological Tissues and Cell Cultures

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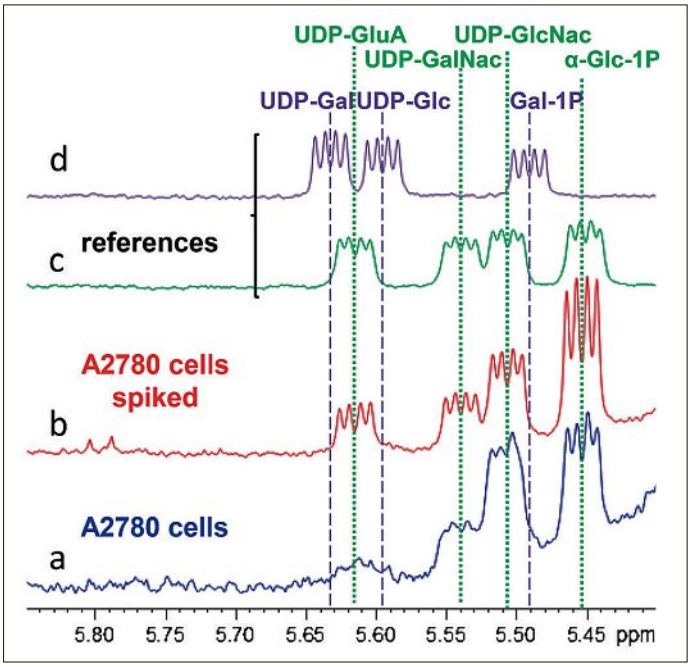
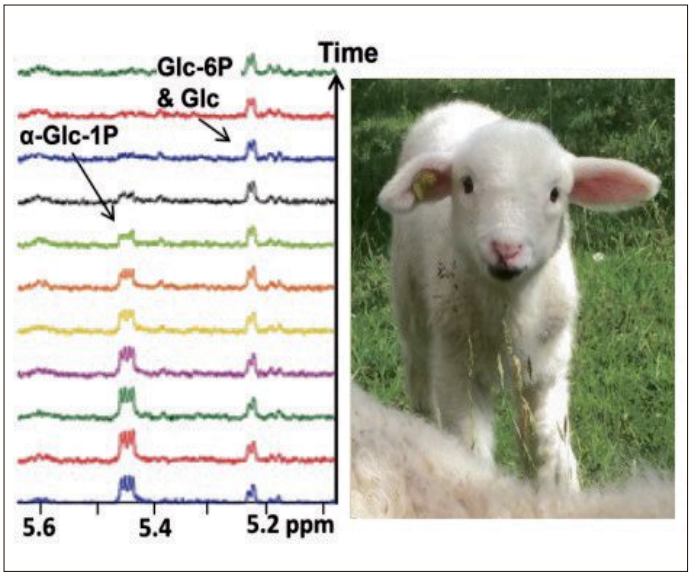
Nucleotide sugars, mainly those containing uridine diphosphate (UDP-X), are key players in glycosylation processes. Glucose phosphates (Glc-1P and Glc-6P) are intermediate metabolites of the glycogen cycle and as such important for storage and transfer of energy. Tissue biopsies and cells can be metabolically characterized by high-resolution magic-angle-spinning (HR-MAS) NMR. Temporal metabolite changes can be monitored by this technique, thus enabling metabolic pathway activities to be followed. ^{1}H HR-MAS NMR allows these phosphate sugars to be assessed qualitatively and quantitatively as a minimally invasive analytical tool, preserving the cell and biopsy integrity, as no extraction or separation steps are required.



Cell NMR spectra spiked with different phosphate sugars, confirming the presence of UDP-GluA, UDP-GalNAc, UDP-GlcNAc and α-Glc-1P. Reprinted with permission from Springer, Diserens *et al.*



Sheep cardiac muscle NMR spectra acquired over 3.5 h after biopsy, showing the evolution of the α-Glc-1P content.

Anomeric sugar protons bound to phosphate show the typical doublet of doublet resonances between 5.4 and 5.7 ppm. Due to similar patterns and only slight chemical shift differences of those peaks originating from different sugar phosphates, a correct assignment can be challenging. Therefore, the metabolite assignment was supported by spiking experiments.

The results of our study clearly demonstrated that sugar phosphates can be determined quickly and non-destructively in living cells and in biopsies by HR-MAS, including their quantitative estimation, without extraction processes. Considering the importance of phosphate sugars in cell metabolism for nucleic acid synthesis, HR-MAS measurements may prove valuable. Different phosphate sugars could be clearly separated from each other. In skeletal and cardiac muscle, the presence of α-Glc-1P and Glc-6P could be unambiguously assigned. The α-Glc-1P kinetics proves exemplarily the possibility of monitoring metabolic processes dynamically by ^{1}H HR-MAS NMR. As suggested by the kinetic analysis, the initial α-Glc-1P increase and subsequent decrease may be due to glycogen breakdown, followed by enzymatic conversion into Glc-6P and finally Glc through phosphoglucomutase. **^{1}H HR-MAS NMR allows the assessment of phosphate sugars contained *e.g.* in cells and skeletal and cardiac muscle biopsies, and facilitates the study of their kinetics for monitoring metabolic pathways.**

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Reference

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