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RESEARCH PAPER

Direct determination of phosphate sugars in biological material by ^{1}H high-resolution magic-angle-spinning NMR spectroscopy

Gaëlle Diserens[1,2] · Martina Vermathen[3] · Ilche Gjuroski[3] · Sandra Eggimann[4] · Christina Precht[5] · Chris Boesch[1] · Peter Vermathen[1]

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Abstract The study aim was to unambiguously assign nucleotide sugars, mainly UDP-X that are known to be important in glycosylation processes as sugar donors, and glucose-phosphates that are important intermediate metabolites for storage and transfer of energy directly in spectra of intact cells, as well as in skeletal muscle biopsies by ^{1}H high-resolution magic-angle-spinning (HR-MAS) NMR. The results 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. As proof of principle, an example of phosphate-sugar reaction and degradation kinetics after unfreezing the sample is shown for a cardiac muscle, suggesting the possibility to follow by HR-MAS NMR some metabolic pathways.

Keywords ^{1}H HR-MAS NMR · Glucose-phosphates · UDP-X · Nucleotide sugars

Introduction

Sugar phosphates are very important metabolites for energy metabolism, as well as for nucleic acid synthesis in cells. While nucleotide sugars, mainly those containing uridine diphosphate (UDP-X), are known to be key players in glycosylation processes, acting as a cosubstrate of the enzymatic reaction [1], glucose-phosphates (Glc-1P and Glc-6P) are important intermediate metabolites for storage and transfer of energy, being part of the glycogen cycle.

Due to their key role in muscle and cell metabolism, the determination of phosphate sugars is of great interest. Different techniques have been used to assess these phosphate sugars in cells [1, 2]. However, extraction procedures, needed for some analysis techniques such as LCMS or HPLC [1], are tedious and most importantly may induce unwanted hydrolysis or other modifications of the target compounds, if not following a strict protocol [2].

High-resolution magic-angle-spinning (HR-MAS) NMR is increasingly being used to metabolically characterize intact tissue biopsies [3, 4], as well as cells [5, 6]. Fast spinning around an axis inclined at the magic angle (54.7°) averages orientation-dependent effects, thereby reducing the spectral line width [3]. The technique can be used to monitor temporal metabolite changes, enabling to follow metabolic pathway activities [3].

^{1}H HR-MAS NMR offers a good alternative method to assess 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.

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✉ Gaëlle Diserens
gaelle.diserens@insel.ch

[1] Departments of Radiology and Clinical Research, University of Bern, Murtenstrasse 35, 3008 Bern, Switzerland

[2] Graduate School for Cellular and Biomedical Sciences, University of Bern, Freiestrasse 1, 3012 Bern, Switzerland

[3] Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, 3012 Bern, Switzerland

[4] University Institute of Clinical Chemistry, Bern University Hospital, F/INO, 3010 Bern, Switzerland

[5] Department of Clinical Veterinary Medicine, University of Bern, Länggassstrasse 124, 3001 Bern, Switzerland

Anomeric sugar protons bound to phosphate show typical doublet of doublet (dd) ^{1}H NMR 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 frequency shifts due to ion strength, pH, or temperature. Metabolic spiking is a common method for supporting metabolite assignment [7, 8]. UDP-*N*-acetyl-glucosamine (UDP-GlcNAc) and UDP-*N*-acetyl-galactosamine (UDP-GalNAc) have been previously assigned in cells either based on NMR of cell extracts [9] or liquid NMR of whole cells [10]. Statistical total correlation spectroscopy (STOCSY), a post-processing method generating a pseudo-2D-NMR spectrum based on correlations between resonances, was previously used for assignment of phosphate sugars [9, 11]. While the aforementioned studies used solely ^{1}H NMR, Wehrli et al. studied UDP-galactose (UDP-Gal) in cell extracts by ^{1}H, ^{13}C, and ^{31}P NMR [12].

Phosphate sugar content of muscle, specifically glucose 6-phosphate (Glc-6P), was previously investigated in vivo by ^{31}P NMR spectroscopy in rats [13] and humans [14] and was regarded as a valuable tool for metabolic investigations in humans. Glc-6P was assigned in the ^{1}H NMR spectrum of a cardiac muscle biopsy from a mouse [15]. To the best of our knowledge, the content of glucose 1-phosphate (Glc-1P) in biopsies has not been investigated by NMR spectroscopy.

The aim of our study was to unambiguously assign phosphate sugars directly in spectra of intact living cells, as well as in skeletal muscle biopsies, by ^{1}H HR-MAS NMR. As proof of principle, an example of phosphate-sugar reaction and degradation kinetics after unfreezing the sample is shown for a cardiac muscle biopsy, suggesting the possibility to follow some important metabolic pathways.

Materials and methods

Chemicals

The reference compounds D-glucose (Glc), α-D-galactose 1-phosphate dipotassium salt (Gal-1P), α-D-glucose 1-phosphate disodium salt hydrate (Glc-1P), D-glucose 6-phosphate disodium salt hydrate (Glc-6P), D-ribose 5-phosphate disodium salt hydrate (Rib-5P), uridine 5′-diphosphogalactose disodium salt (UDP-Gal), uridine 5′-diphosphoglucose disodium salt hydrate (UDP-Glc), uridine 5′-diphospho-*N*-acetylgalactosamine disodium salt (UDP-GalNAc), uridine 5′-diphospho-*N*-acetylglucosamine sodium salt (UDP-GlcNAc), uridine 5′-diphosphoglucuronic acid trisodium salt (UDP-GluA), and creatine were all obtained from Sigma-Aldrich®, Switzerland.

Twenty-millimolar reference solutions were prepared in D_2O and D_2O-based phosphate-buffered saline (PBS) containing 10 mM creatine as internal reference for chemical shift calibration.

Cell material

Human ovarian carcinoma cells A2780 were obtained from the European Centre of Cell Cultures (ECACC, Salisbury, UK; catalogue no. 93112519). The A2780 cells were routinely grown in RPMI-1640 medium, which contained 10 % fetal calf serum (FCS), 2 mM glutamine (Gln), and 1 % antibiotics (penicillin/streptomycin), at 37 °C and 5 % CO_2. After 72 h, the cells were harvested by trypsinization [16] and centrifuged at 2000 rcf for 5 min.

Muscle biopsies

Human skeletal muscle biopsies and sheep cardiac biopsies included in this study were stored at −80 °C until the NMR measurement. Ethical approval was obtained for getting biopsies. Sheep cardiac biopsies were collected from healthy sheep directly after euthanasia being part of a parallel clinical study.

Sample preparation for NMR

The harvested ovarian carcinoma cells (cell number 10^6–10^7) grown for 72 h (when P-sugars are more expressed [17]) were washed three times with 1 mL PBS. After the last washing step, the cells were taken up in 20 μL 10 mM D_2O-based PBS (pH 7.4). The cell suspension was transferred into a standard 4-mm MAS rotor using a 50-μL insert and submitted to ^{1}H HR-MAS NMR measurement (3 kHz MAS).

Human muscle (m. vastus lateralis) biopsies (~10 mg) and sheep cardiac biopsies (~10 mg) were washed and placed into a standard 4-mm MAS rotor using a 50-μL insert with the addition of PBS and submitted to ^{1}H HR-MAS NMR measurement.

Spiking

For spiking, 1–1.5 μL of a mixture containing either Glc-1P, UDP-GlcNAc, UDP-GalNAc, and UDP-GluA (4 mM each) or UDP-Gal, UDP-Glc, and Gal-1P (4 mM each) as well as 2 mM creatine were added to the cell suspension directly in the MAS rotor.

For muscle spiking, 1 μL of a mixture containing Glc-1P and Glc-6P (4 mM each) with 2 mM creatine and 5 μL Glc (4 mM) with 2 mM creatine were added, respectively, to one of the two muscle biopsies directly in the MAS rotor.

^{1}H HR-MAS NMR spectroscopy

The ^{1}H HR-MAS NMR experiments were performed on a 500-MHz Bruker Avance II spectrometer using a 4-mm HR-

MAS dual inverse $^{1}H/^{13}C$ probe with a magic angle gradient. The Bruker Topspin software (version 3.2, patch level 5) was used to acquire the NMR data. 1D ^{1}H NMR spectra of unspiked samples were acquired with water presaturation.

For cell samples, a 1D ^{1}H HR-MAS nuclear Overhauser effect (NOE) experiment for improved water suppression [18] was applied (*noesypr1d* from the Bruker pulse-program library) at a spinning speed of 3 kHz. Two hundred fifty-six transients, a spectral width of 6002.4 Hz, a data size of 32 K points, an acquisition time of 2.73 s, and a relaxation delay of 4 s were applied. The 90° pulse length was adjusted for each measurement and the NOESY mixing time was 10 ms. Additionally, the commonly applied 1D ^{1}H Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence was used [19] with water presaturation of the water resonance (*cpmgpr1d* from the Bruker pulse-program library). It is based on relaxation-editing for suppression of broad components arising from high molecular weight compounds which typically have shorter T2 relaxation times than the small metabolites of interest [20]. Acquisition parameters were 1024 transients, spectral width 6009.6 Hz, data size 32 K points, acquisition time 2.73 s, relaxation delay 2 s, and echo time 9.6 ms.

For muscle biopsy samples, ^{1}H HR-MAS 1D CPMG spectra were recorded with presaturation of the water resonance at a spinning speed of 5 kHz. Sixty-four transients, a spectral width of 7002.8 Hz, a data size of 32 K points, an acquisition time of 2.34 s, and a relaxation delay of 4 s were applied. The 90° pulse length was adjusted for each measurement and the echo time was 76.8 ms.

Spectral processing included slight line broadening, Fourier transform, phasing, and chemical shift calibration to the creatine *N*-methyl peak (3.02 ppm). No baseline correction was applied.

As part of ongoing metabolomic studies, the cell spectra were acquired at a temperature of 310 K and the biopsy spectra at a temperature of 275–277 K to ensure long stability of the samples.

Reference spectra containing the pure sugar compounds and creatine as an internal chemical shift calibration reference were acquired at 310 K and 3 kHz MAS rate and at 275 K and 5 kHz MAS rate. The resonance of the creatine *N*-methyl group was set to 3.02 ppm. These included the phosphate sugars α-galactose-1P (α-Gal-1P), α-glucose-1P (α-Glc-1P), glucose-6P (Glc-6P), ribose-5P (Rib-5P), UDP-galactose (UDP-Gal), UDP-glucose (UDP-Glc), UDP-*N*-acetylgalactosamine (UDP-GalNAc), UDP-*N*-acetylglucosamine (UDP-GlcNAc), UDP-glucuronic acid (UDP-GluA) as well as glucose (Glc). The ^{1}H HR-MAS NMR spectra of the reference compounds with signal assignments are shown in the Electronic Supplementary Material (ESM, Figs. S1–S9). Signal assignments were based on additional 2D $^{1}H^{1}H$-COSY and 2D $^{1}H^{13}C$-HSQC spectra as well as on data obtained from the Human Metabolome Database (HMDB) [21] and reported in the literature [22].

Subsequently, cells were spiked with α-Glc-1P, UDP-GalNAc, UDP-GluA, and UDP-GlcNAc. One skeletal muscle biopsy was spiked with α-Glc-1P and Glc-6P and a second skeletal muscle with Glc to distinguish from Glc-6P, since the doublets at 4.65 and 5.22 ppm are present in both Glc and Glc-6P.

The temporal change of α-Glc-1P in a sheep cardiac muscle biopsy in a 50-μL rotor filled with PBS was studied for 3.5 h at 277 K (5 kHz MAS) acquiring 20 1D CPMG spectra of 5 min duration with a 5-min break between each experiment. The integral of the 5.46-ppm peak was calculated for each spectrum in the range between 5.426 and 5.526 ppm.

Results

An example of a CPMG spectrum in the ranges between 0.5 and 4.4 ppm and between 5.1 and 8.1 ppm from A2780 cells is shown in Fig. 1, with the region between 5.4 and 5.75 ppm enlarged. Figure 2 shows 1D NOESY A2780 cell spectra in the spectral region between 5.4 and 5.85 ppm without spiking (Fig. 2a) and with phosphate sugar spiking (Fig. 2b), as well as the reference spectra of the phosphate sugars (Fig. 2c, d). This spectral region shows the characteristic doublet of doublet pattern of the anomeric sugar protons bound to phosphate. The comparisons of chemical shift positions in combination with the spiking confirmed the presence and detectability of several sugar phosphates in A2780 cells.

The presence of UDP-GalNAc (Fig. 2c) is confirmed by the spiking, with the anomeric dd resonance at 5.54 ppm, as well as α-Glc-1P in higher quantity with the dd resonance at 5.46 ppm (Fig. 2c). The spiking also confirmed the presence of UDP-GlcNAc (Fig. 2c) in A2780 cells with the dd resonance at 5.51 ppm. In addition, a small amount of UDP-GluA (Fig. 2c, 5.62 ppm) is present in A2780 cells (Fig. 2a), as was confirmed by the spiking experiment (Fig. 2b) and as was confirmed in an averaged spectrum from eight separate A2780 cell samples (see ESM Fig. S10) [17]. The presence of UDP-Gal (Fig. 2d) could not be confirmed. The anomeric dd resonance of UDP-Gal is slightly shifted downfield in comparison to UDP-GluA. Similarly, UDP-Glc and α-Gal-1P (Fig. 2d) were not detected in the investigated cells. UDP-Glc and UDP-Gal were also not detected directly in ^{1}H spectra of lymphoblast extracts due to low concentrations [12].

CPMG spectra from human skeletal muscle are shown in Fig. 3A. Figure 3A (a) shows the initial muscle biopsy spectrum, while Fig. 3A (b) shows the spectrum from muscle spiked with α-Glc-1P and Glc-6P. Pure solution spectra of α-Glc-1P and Glc-6P (with

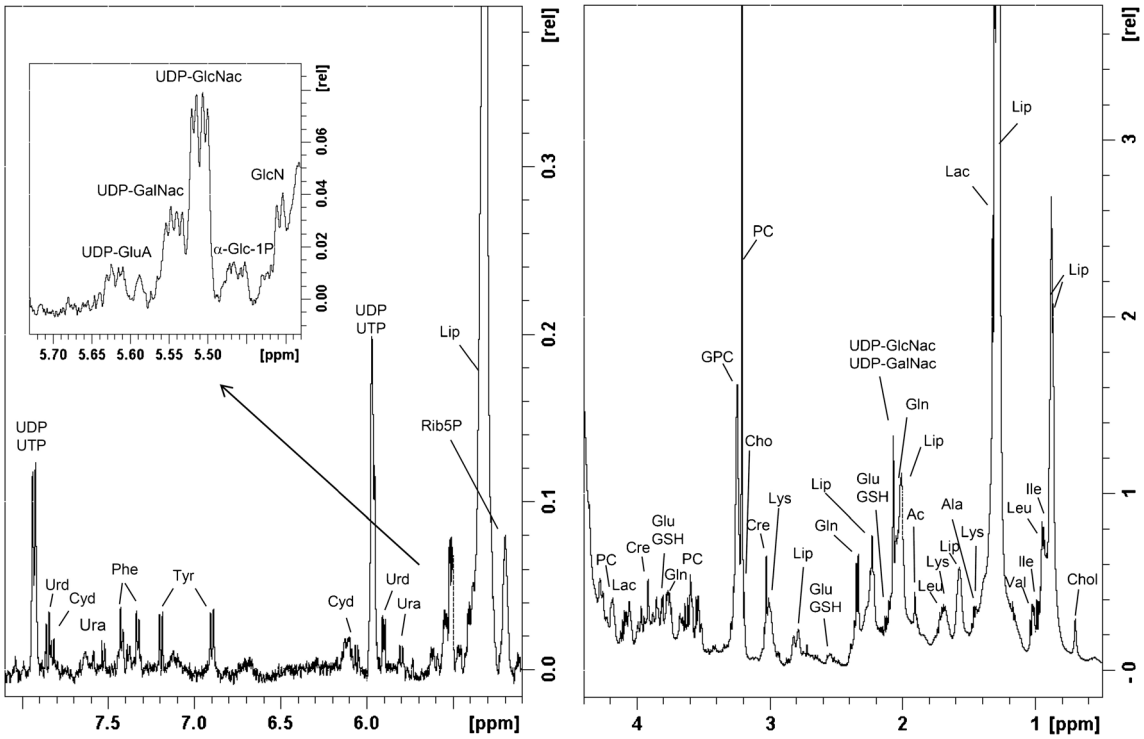

Fig. 1 Example of a CPMG spectrum in the ranges between 0.5 and 4.4 ppm and between 5.1 and 8.1 ppm from A2780 cells, with the *region between 5.4 and 5.75 ppm* enlarged. Signal assignments were based on 2D spectra and data obtained from the HMDB [21] and reported in the literature [17, 22]. For assignment abbreviations, see [17]

added creatine as a chemical shift reference) are displayed in Fig. 3A (c) and (d) respectively. α-Glc-1P and Glc-6P, with their respective characteristic peaks at 5.46 ppm and at 4.65 and 5.22 ppm, are clearly present in the muscle spectrum, and the exact chemical shift positions are confirmed by the spiking. The H-1 doublets of Glc-6P at 4.65 and 5.22 ppm (β- and α-form) are overlapping with the corresponding resonances of glucose (Fig. 3A (g)); however, the dd pattern at 4.01 ppm, representing the coupling of the H-6 protons and phosphorus of Glc-6P, is clearly present and not present in the glucose spectrum. The additional presence of glucose was also confirmed by spiking (Fig. 3A (e–g)), as detected also previously in another ^{1}H HR-MAS NMR study of muscle biopsies [23]. Other phosphate sugars were not detected in this muscle.

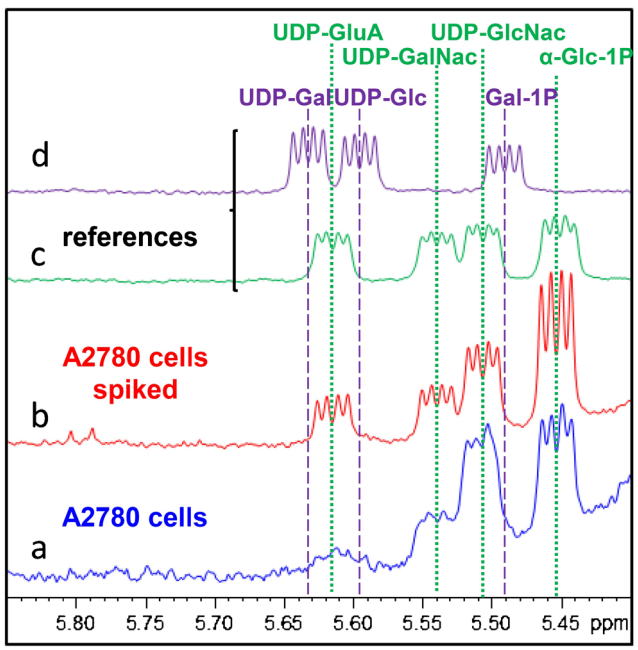

Fig. 2 1D NOESY cell spectra spiked with phosphate sugars (spectral region between 5.4 and 5.85 ppm). **a** A2780 cell spectrum; **b** A2780 cells spiked with α-Glc-1P, UDP-GlcNAc, UDP-GalNAc, and UDP-GluA. Reference spectra: **c** solution with UDP-GluA, UDP-GalNAc, UDP-GlcNAc, and α-Glc-1P and **d** solution with UDP-Gal, UDP-Glc, and α-Gal-1P

The results of the investigation of the reaction and degradation kinetics of α-Glc-1P in a cardiac muscle of a healthy sheep are shown in Fig. 3B. The α-Glc-1P dd peak at 5.46 ppm slightly increased for the first 0.5 h, before decreasing and disappearing within 2.5 h for this biopsy (Fig. 3C). Other α-Glc-1P peaks showed a similar temporal pattern change with an initial increase followed by a decrease. The overlapping peaks of Glc-6P and Glc at 4.65 and 5.22 ppm showed an initial slight increase before stabilization, while the Glc-6P peak at 4.01 ppm demonstrated an increase and a subsequent decrease. Other peaks remained relatively constant over the time period investigated.

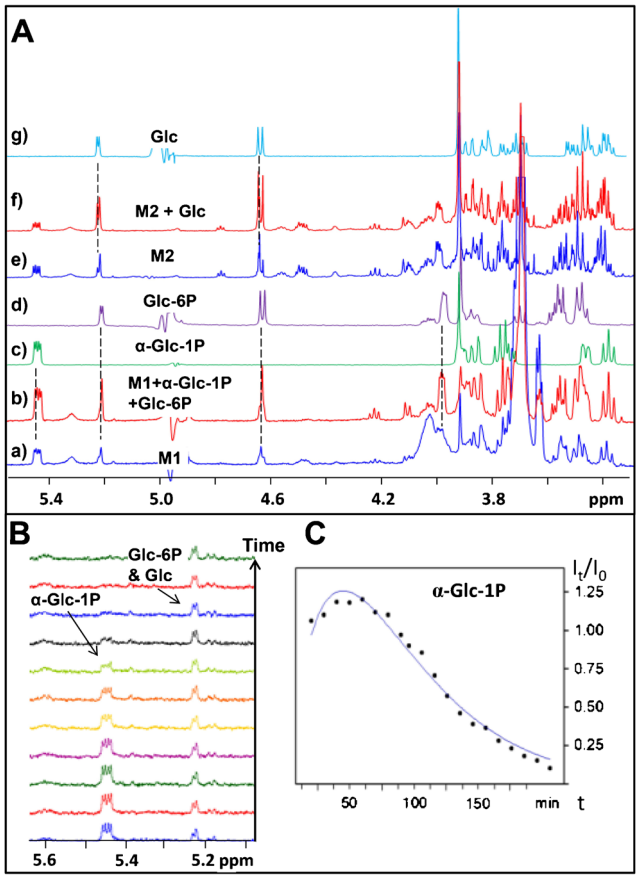

Fig. 3 **A** CPMG human skeletal muscle biopsy spiked with α-Glc-1P and Glc-6P: **a** first muscle biopsy (M1) spectrum; **b** first muscle (M1) spiked with α-Glc-1P and Glc-6P; **c** α-Glc-1P; **d** Glc-6P; **e** second muscle biopsy (M2) spectrum; **f** second muscle (M2) spiked with Glc; **g** Glc. **B** Reaction kinetics of α-Glc-1P in sheep cardiac muscle spectra taken over 3.5 h; **C** evolution over time of the α-Glc-1P 5.45-ppm peak with fit

Discussion

The results clearly demonstrate that sugar phosphates can be determined quickly and non-destructively in cells and muscle biopsies by HR-MAS, which may prove valuable considering the importance of phosphate sugars in cell metabolism for nucleic acid synthesis and for energy metabolism. The different phosphate sugars can be distinguished from each other by the chemical shift differences of the anomeric dd resonances of the sugar protons bound to phosphate. In skeletal and cardiac muscle, the presence of α-Glc-1P, Glc-6P, and Glc could be unambiguously assigned. As estimated from the spiking solutions, the concentration of phosphate sugars detected in the present study were in the micromolar range. While HR-MAS NMR and HR-NMR spectroscopy in general are inherently less sensitive than other analytical techniques such as MS, the HR-MAS NMR method provides a snapshot of the living system with minimal interference imposed from sample preparation. Besides the reliable qualitative detection of various P-sugars simultaneously, the NMR visibility comprises additional information on the mobility and hence microenvironment of the corresponding compounds. This means that the detected compounds exhibit sufficient mobility (as in semi-solid materials) to be observed by HR MAS. If, however, the mobility is strongly reduced as it may be the case in more rigid surroundings such as membranes, dipolar interactions can usually not be averaged out by MAS speeds commonly applied in HR MAS NMR [24]. This feature may allow, for example, monitoring the cellular release of metabolites like P-sugars in response to extrinsic stimuli such as drugs or specific growth conditions. HR-MAS NMR likewise has the potential for quantitative estimation in living cell and tissue measurements, without extraction processes, and thus may allow for monitoring reaction processes. For this purpose, techniques, such as the CPMG method (as applied and shown in Fig. 3 for tissue and in ESM Fig. S10 for cells), for suppression of broad components are well suited to ensure a flat baseline for reliable resonance integration. While the CPMG sequence, due to its T_2 filtering, also results in small signal attenuations of metabolites with long T_2 relaxation times, relative metabolite concentrations are typically not substantially affected [19], especially at the relatively short echo time of 76.8 ms used in this study. Thus, relative amounts—as often addressed in metabolomic studies—are directly accessible, while reference standards would be required for absolute quantitation.

The investigation of the reaction kinetics of α-Glc-1P from the sheep cardiac muscle after thawing the sample provides 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 analysis of the temporal changes.

Furthermore, from a technical point of view, it is important to consider the temporal changes of specific metabolites. If studies are performed only at one defined time point, they only represent a metabolic snapshot.

In conclusion, ^{1}H HR-MAS NMR allows the direct assessment of phosphate sugars contained in cells and skeletal and cardiac muscle biopsies, and facilitates the study of their reaction kinetics. Therefore, this technique has potential 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.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Ethical approval and informed consent of all patients were obtained for getting biopsies.

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References

1. del Val IJ, Kyriakopoulos S, Polizzi KM, Kontoravdi C. An optimized method for extraction and quantification of nucleotides and nucleotide sugars from mammalian cells. Anal Biochem. 2013;443(2):172–80.
2. Dietmair S, Timmins NE, Gray PP, Nielsen LK, Kromer JO. Towards quantitative metabolomics of mammalian cells: development of a metabolite extraction protocol. Anal Biochem. 2010;404(2):155–64.
3. Lindon JC, Beckonert OP, Holmes E, Nicholson JK. High-resolution magic angle spinning NMR spectroscopy: application to biomedical studies. Prog Nucl Magn Reson Spectrosc. 2009;55:79–100.
4. Giskeodegard GF, Cao MD, Bathen TF. High-resolution magic-angle-spinning NMR spectroscopy of intact tissue. Methods Mol Biol. 2015;1277:37–50.
5. Griffin JL, Shockcor JP. Metabolic profiles of cancer cells. Nat Rev Cancer. 2004;4(7):551–61.
6. Maria RM, Altei WF, Andricopulo AD, Becceneri AB, Cominetti MR, Venancio T, et al. Characterization of metabolic profile of intact non-tumor and tumor breast cells by high-resolution magic angle spinning nuclear magnetic resonance spectroscopy. Anal Biochem. 2015;488:14–8.
7. Louis E, Bervoets L, Reekmans G, De Jonge E, Mesotten L, Thomeer M, et al. Phenotyping human blood plasma by 1H-NMR: a robust protocol based on metabolite spiking and its evaluation in breast cancer. Metabolomics. 2014;11(1):225–36.
8. Le Gall G. NMR spectroscopy of biofluids and extracts. Methods Mol Biol. 2015;1277:29–36.
9. Pan X, Wilson M, Mirbahai L, McConville C, Arvanitis TN, Griffin JL, et al. In vitro metabonomic study detects increases in UDP-GlcNAc and UDP-GalNAc, as early phase markers of cisplatin treatment response in brain tumor cells. J Proteome Res. 2011;10(8):3493–500.
10. Grande S, Palma A, Luciani AM, Rosi A, Guidoni L, Viti V. Glycosidic intermediates identified in 1H MR spectra of intact tumour cells may contribute to the clarification of aspects of glycosylation pathways. NMR Biomed. 2011;24(1):68–79.
11. Duarte IF, Ladeirinha AF, Lamego I, Gil AM, Carvalho L, Carreira IM, et al. Potential markers of cisplatin treatment response unveiled by NMR metabolomics of human lung cells. Mol Pharm. 2013;10(11):4242–51.
12. Wehrli SL, Reynolds R, Chen J, Yager C, Segal S. Galactose metabolism in normal human lymphoblasts studied by (1)H, (13)C and (31)P NMR spectroscopy of extracts. NMR Biomed. 2001;14(3):192–8.
13. Bloch G, Chase JR, Avison MJ, Shulman RG. In vivo 31P NMR measurement of glucose-6-Phosphate in the rat muscle after exercise. Magn Reson Med. 1993;30:347–50.
14. Rothman DL, Shulman RG, Shulman GI. 31P nuclear magnetic resonance measurements of muscle glucose-6- phosphate: evidence for reduced insulin-dependent muscle glucose transport or phosphorylation activity in non-insulin-dependent diabetes mellitus. J Clin Invest. 1992;89:1069–75.
15. Griffin JL, Williams HJ, Sang E, Nicholson JK. Abnormal lipid profile of dystrophic cardiac tissue as demonstrated by one- and two-dimensional magic-angle spinning (1)H NMR spectroscopy. Magn Reson Med. 2001;46(2):249–55.
16. Phelan K, May KM. Basic techniques in mammalian cell tissue culture. Curr Protoc Cell Biol. 2015;66:1.
17. Vermathen M, Paul LEH, Diserens G, Vermathen P, Furrer J. 1H HR-MAS NMR based metabolic profiling of cells in response to treatment with a hexacationic ruthenium metallaprism as potential anticancer drug. PLoS One. 2015;10(5):e0128478.
18. Mckay RT. How the 1-NOESY suppresses solvent signal in metabonomics NMR spectroscopy: an examination of the pulse sequence components and evolution. Concepts Magn Reson Part A. 2011;38(5):197–220.
19. Piotto M, Moussallieh FM, Imperiale A, Detour J, Bellocq JP, Namer IJ. Reproducible sample preparation and spectrum acquisition techniques for metabolic profiling of human tissues by 1H HRMAS NMR. In: Lutz NW, Sweedler JV, Wevers RA, editors. Methodologies in metabolomics: experimental strategies and techniques for metabolomics research. 2013. p 496–524.
20. Tang H, Wang Y, Nicholson JK, Lindon JC. Use of relaxation-edited one-dimensional and two dimensional nuclear magnetic resonance spectroscopy to improve detection of small metabolites in blood plasma. Anal Biochem. 2004;325(2):260–72.
21. Wishart DS, Jewison T, Guo AC, Wilson M, Knox C, Liu Y, et al. HMDB 3.0—the human metabolome database in 2013. Nucleic Acids Res. 2013;41(Database issue):D801–7.
22. Yang T, Bar-Peled M. Identification of a novel UDP-sugar pyrophosphorylase with a broad substrate specificity in Trypanosoma cruzi. Biochem J. 2010;429(3):533–43.
23. Righi V, Andronesi O, Mintzopoulos D, Tzika AA. Molecular characterization and quantification using state of the art solid-state adiabatic TOBSY NMR in burn trauma. Int J Mol Med. 2009;24(6):749–57.
24. Beckonert O, Coen M, Keun HC, Wang Y, Ebbels TM, Holmes E, et al. High-resolution magic-angle-spinning NMR spectroscopy for metabolic profiling of intact tissues. Nat Protoc. 2010;5(6):1019–32.