Insight into neural cell metabolism by NMR – employing UDP-GlcNAc as a unique metabolic marker

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Introduction

2-5% of the intracellular glucose is used to generate UDP-N-acetylglucosamine (UDP-GlcNAc), an activated sugar, produced via the hexosamine biosynthetic pathway (HBP). The sugar-scaffold is provided by this glucose and the amide-group of intracellular glutamine together with acetyl-CoA build up the N-acetyl-group of UDP-GlcNAc [1]. This activated sugar is the key substrate for O-GlcNAcylation of nuclear and cytosolic proteins. O-GlcNAcylation is a stress- and nutrient-sensitive dynamic posttranslational modification of proteins where a single GlcNAc residue is covalently bound to a serin or threonin residue similar to phosphorylation [2]. Changes in flux through the HBP either increase or decrease UDP-GlcNAc levels, affecting the O-GlcNAcylation of many proteins. This modification not only plays an important role in many fundamental cellular processes, but also its dysregulation can be associated with human diseases such as diabetes, Alzheimer's disease and cancer [3,4,5,6]. Alterations of UDP-GlcNAc levels may provide an indication of the development of metabolic disorders, making UDP-GlcNAc an ideal metabolic marker.

We are currently exploring the potential of UDP-GlcNAc as a Nuclear Magnetic Resonance (NMR) detectable metabolic marker in neuronal cells which are known to have high levels of UDP-GlcNAc making them well suitable for our experiments [3]. In our study we used [1-$^{13}C_1$] glucose and [^{15}N-amide] glutamine to follow the incorporation of these labels into UDP-GlcNAc as detected by $^{1}H,^{13}C$-HSQC and 1D-$^{1}H,^{15}N$-HSQC NMR spectra. As a proof of principle we treated the cells with azide, an inhibitor of complex IV of the respiratory chain, to examine the impact of cellular stress on the labeling pattern of UDP-GlcNAc.

Methods

For experiments with labeled glucose, we incubated HT-22 cells, a murine neuronal cell line, with 11 mM [1-$^{13}C_1$] glucose, 4 mM glutamine in serum free media for 24 h with or without 10 mM sodium azide. During incubation times media samples were taken. Cell metabolites were extracted with perchloric acid (PCA). Protein content was determined with BCA protein assay using bovine serum albumin as standard. For NMR measurements samples were dissolved in D_2O containing 1 mM TSP as internal standard.

For experiments with labeled glutamine, HT-22 cells were incubated with different concentrations of [^{15}N-amide] glutamine (2-6 mM) and glucose (6-25 mM) for different time periods (4h-4d). Media samples were taken during incubation. Cell metabolites were extracted with PCA and protein pellets were hydrolyzed using a slightly modified method after Flögel et al. [7]. For NMR measurements samples were dissolved in PBS (pH 6.8) + 10 % D_2O containing 1 mM TSP as internal standard. All measurements were performed on a Bruker Avance AV-500 spectrometer equipped with a TCI-cryoprobe.

Results

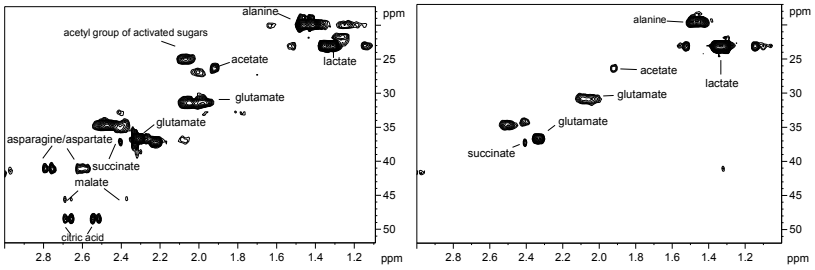

Figure 1: $^{1}H,^{13}C$-HSQC spectra of cell extracts. left: incubation with 11 mM [1-$^{13}C_1$] glucose, right: incubation with 11 mM [1-$^{13}C_1$] glucose and 10 mM azide

It could be shown that HT-22 cells metabolize [1-$^{13}C_1$] glucose via glycolysis, tricarboxylic acid (TCA) cycle and HBP (fig 1, right). The acetyl-group of UDP-GlcNAc is labeled via acetyl-CoA which becomes labeled via TCA cycle. Incubating the cells with sodium azide turns their metabolism into a glycolytic state. This is reflected in glucose uptake that increases from 1.16 µmol glucose/h/mg protein to 2.08 µmol glucose/h/mg protein during incubation. Furthermore, $^{1}H,^{13}C$-HSQC spectra clearly show that peaks of metabolites of the TCA cycle (malate, citric acid) vanish. But the most important finding is that the peak of the acetyl-group of UDP-GlcNAc disappears (fig. 1, left) however the C_1 atom which is also labeled is not affected (data not shown).

HT-22 cells metabolize the [^{15}N-amide] glutamine added to the incubation medium as seen by a pronounced decrease of the glutamine concentration in the medium after completion of the incubation time (data not shown). However, we could not detect the ^{15}N-label in any acid-soluble metabolites of the cell extracts under different incubation methods. 1D-$^{1}H,^{15}N$-HSQC NMR spectra of hydrolyzed protein fractions revealed that the ^{15}N-label of glutamine is almost completely incorporated into cellular proteins of the HT-22 cells (fig. 2).

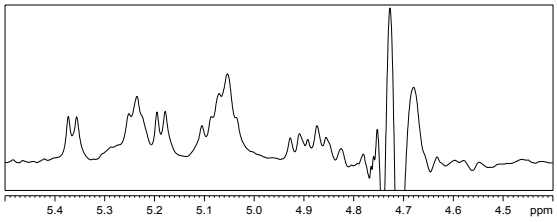

Figure 2: 1D-$^{1}H,^{15}N$-HSQC NMR spectrum of the hydrolyzed protein fraction of HT-22 cells after incubation with 6 mM [^{15}N-amide] glutamine and 25 mM glucose for 3 days.

Discussion

We could clearly demonstrate that the labeling pattern of UDP-GlcNAc reflects the metabolic activity of TCA cycle (labeling of acetyl-group) vs. HBP (labeling of C_1 atom). Using sodium azide as an inhibitor of the respiratory chain the TCA cycle ceases and labeling of acetyl-group switches off while C_1 still carries the label. Experiments with rotenone, a more specific inhibitor of the respiratory chain, can show if there is a further potential of UDP-GlcNAc as a metabolic marker. Quantification of different metabolites in NMR spectra will allow us to compare energy metabolism (glycolysis, TCA cycle) with fluxes through the HBP. This can offer valuable clues to pathological states of the cells during diabetes, Alzheimer's disease or cancer.

Although the amide side chain of glutamine is the sole source of nitrogen for the biosynthesis of the N-acetyl-group in UDP-GlcNAc, we could not show an incorporation of the ^{15}N-label into the N-acetyl-group of UDP-GlcNAc in the cell extracts under these incubation conditions. Interestingly, almost all ^{15}N-label was incorporated into cellular proteins. Experiments identifying if the ^{15}N-label in the cellular proteins belongs to O-linked GlcNAcylation and experiments increasing the amount of ^{15}N-labeled metabolites resulting from [^{15}N-amide] glutamine in the cell extracts by modifying the incubation conditions are currently underway.

References

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