

The nucleotide sugar UDP-GlcNAc, a major glycosylation precursor, can be observed in intact tumour cells by ^1H NMR in the low field spectral region

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INTRODUCTION

Alterations in glycan metabolism have been found associated with various human diseases including diabetes mellitus and neurodegeneration. Besides the congenital disorders of glycosylation pathways, some aspects of sugar metabolism are involved in tumour progression. Protein glycosylation is species- and cell-specific and it is determined by the protein backbone and sugar attachment site. The dynamic changes in glycosylation levels in response to extracellular stimuli suggest a key role of signal transduction pathways, with important effects on cell life [1]. With this respect, nucleotide sugars have elicited the attention in many studies due to the fact that they are donor substrates for glycosylation of proteins and lipids.

^1H NMR can be used to observe sugar-related metabolites in intact cells, though identification and quantitation of carbohydrate signals are difficult, due to the crowding of the spectral region and the proximity to the suppressed water signal. In the present study, we aim at observing major hexosamine precursors in two different tumour cell lines.

METHODS

All cells were grown as adherent cells as described elsewhere [2]. Cells were treated with 20 mM NH_4Cl for 24 hours before NMR experiments. ^1H NMR spectra were run at 400.14 MHz on a digital Avance spectrometer (Bruker, AG, Darmstadt, Germany) equipped with a 1 mm microprobe. Signals were acquired with a 90° RF pulse and a sweep width of 4006.4 Hz. Water suppression was obtained by irradiating water signal.

RESULTS AND DISCUSSION

^1H NMR spectra of cells from breast adenocarcinoma (MCF-7) and glioma (T98G) were run and compared to the spectra from solutions of UDP-N-acetylglucosamine (UDP-GlcNAc) and UDP-N-acetylgalactosamine (UDP-GalNAc). Fig.1 shows the spectral region where amide protons resonate. The signal from amide proton in N-acetylglucosamine is present at 8.35 ppm, while the amide proton in N-acetylgalactosamine resonates slightly upfield at 8.34 ppm and cannot be clearly assigned in 1D spectra. These signals are coupled to the proton on the sugar ring, at 3.99 and 4.22 ppm respectively. By comparison with 2D COSY spectra, the signal at 8.35 ppm is assigned to UDP-GlcNAc. Signals at 8.00 ppm and 5.90 ppm are from the uridine ring. Similar spectra were displayed by HeLa cells (from adenocarcinoma) and A172 cells from glioma (not shown).

Elevated ammonium concentration in the growing medium has been demonstrated to affect the metabolism and the intracellular level of these sugar nucleotides [3]. Cells were then treated with ammonium chloride, and ^1H NMR spectra were run. Increased UDP-GlcNAc signals at 8.35 ppm was observed in all cell lines (not shown). The effect was more relevant in MCF-7 cells (Fig 2). In these cells, the corresponding UDP-GalNAc signal, barely visible or absent in non-treated cells, is now detectable in 2D (Fig 2) and also 1D spectra (Fig 3).

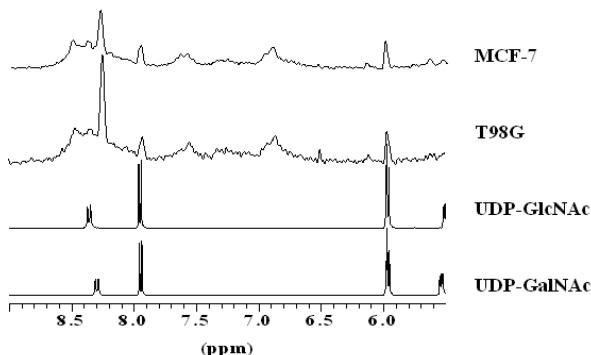


Figure 1: Low field region of representative ^1H NMR spectra of MCF-7 and T98G cells compared to spectra from solutions of UDP-GlcNAc and UDP-GalNAc. 10% D_2O was present for providing lock signal.

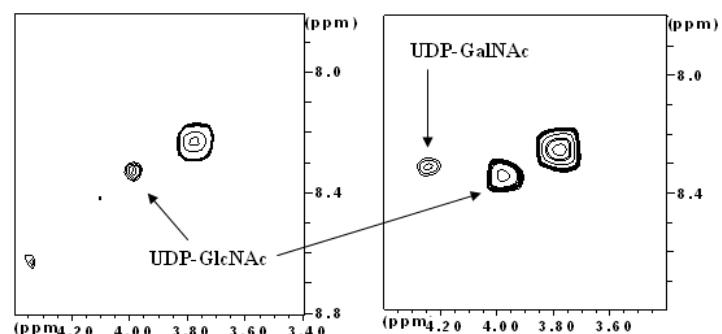


Figure 2: 2D COSY ^1H NMR spectra of MCF-7 cells - same experiment reported in Figure 1. Left: control sample; right: NH_4Cl treated cells

CONCLUSIONS

Sugar metabolism plays an important role in tumour progression, mainly for what concerns the involvement of nucleotide sugars that are donor substrates for glycosylation of proteins and lipids. Elevated ammonium concentration has been demonstrated to affect the metabolism and the intracellular level of these sugar nucleotides in tumour cells. Comparison of 1D and 2D ^1H NMR spectra of the two cell lines with the spectra of free compounds allowed spectral assignments. Modulation of signal intensities by NH_4Cl treatment allowed to confirm signal assignments, thus providing a useful tool for quantitation of these nucleotide sugars in intact cells and, possibly, in tumour tissues

REFERENCES

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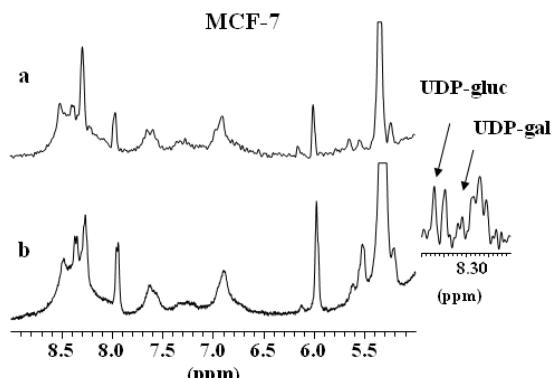


Figure 3: ^1H NMR spectra of MCF-7 cells treated with 20 mM NH_4Cl (b) compared to the corresponding control sample (a). Spectra are run after 24 hours from the treatment.