Identification of signals from glycosylation precursors in ^{1}H MR spectra of intact tumour cells

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INTRODUCTION

The glycosylation process, either in the secretory pathway or in the nucleocytoplasmatic compartment, is a major post translation modification of proteins [1]. MRS is a technique able to observe cell metabolites directly in intact cells as well as in vivo. On the other hand, carbohydrate metabolism is not fully exploited with this technique in intact systems due to the difficulty of identifying signals in very crowded regions in the NMR spectrum. In a previous study [2] we identified some relevant signals of glycosylation precursors in the low field region in intact tumor cells spectra. The present study deals with identification of signals from GalNAc in a cancer cells from adenocarcinoma of the human cervix. Treatment of cells with ammonium chloride allowed to confirm signal assignment, to follow metabolic changes of related molecules and even to get insight into some aspects of glycosylation.

MATERIALS AND METHODS

HeLa cells were grown as adherent cells as described elsewhere [3]. Cells were treated with 20 and 40 mM NH_4Cl for 24 hours before NMR experiments. ^{1}H MR spectra were run at 400.14 MHz on a digital Avance spectrometer (Bruker, AG, Darmstadt, Germany) equipped with a 1mm 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

Spectra of HeLa cells are particularly rich of signals at frequencies of glycosylation precursors. Besides the signals G_{NH}, U_5; U_6 of Fig 1a, previously assigned to UDP-GlcNAc [2], 1D spectrum shows a doublet, labelled Gal_{NH}, at 8.02 ppm (Fig. 1a) crossing with a peak at 4.16 ppm in 2D COSY spectrum (Fig. 1b). Its coupling constant is 8.6 Hz. To identify which sugar residue gives rise to the signal, spectra of GalNAc and GlcNAc in H_2O solution containing 10% ^2H_2O were run. On the basis of chemical shifts reported in the Table, the Gal_{NH} signal could be tentatively attributed to the NH of GalNAc. Moreover, spectrum of GalNAc in H_2O shows a very intense CH_3 signal at 2.058 ppm (Table); a similar signal at 2.06 ppm was observed in Hela cells spectra (Fig. 2b). When HeLa cells were treated with NH_4Cl, a strong decrease (dose dependent) was observed for the signal Gal_{NH} at 8.02 ppm, paralleled by a decrease of the signal at 2.06 ppm (Fig. 2a', b'). Chemical shift of the COSY cross peak at 8.00-4.16 ppm are compatible with NH signals of free GalNAc in solution (see Table) and with of GalNAc bound to Ser in mucin derived O-linked glycopeptides (7.93 ppm) and G_2 (4.15 ppm). These signals were absent in spectra of unrelated tumor cells, namely MCF-7 from breast cancer and A172 and T98G from gliomas (not shown).

Mucin is a high molecular weight transmembrane glycoprotein widely distributed on numerous epitehlia. It contains a range of (GalNAc)-SerThr O-linked oligosaccharides that comprises more than 50% of the mucin molecule. A large family of GalNAc-transferases (ppGalNAcT) in the Golgi catalyses the first step of O-glycan synthesis. The arrangement of biosynthetic enzymes in the assembly line in the Golgi can vary among cell types. Particularly in HeLa cells, ppGalNAcT1, ppGalNAcT2 and ppGalNAcT3 have a broad Golgi localization because are present through the cis-, medial- and trans-Golgi compartments [4]. If the first sugar (GalNAc) is added in a later compartment than the one in which extension enzymes reside, then the glycoprotein cannot be a substrate for extension reactions. This would be consistent with the presence of partly processed and structurally heterogeneous O-glycans. The presence of the intense doublet at 8.02 ppm in HeLa cell spectra can be assigned to GalNAc unprocessed residues, being therefore a marker of abnormal glycosylation.

Axelsson and coworkers [5] showed that the neutralization of pH in the Golgi apparatus causes i) an increased number and size of Golgi stacks, while the essential Golgi architecture was preserved; ii) a redistribution of glycosyltransferases. Particularly GalNAc-T2 was partly relocalized to endosomal vesicles, but the relocation was compensated by production of new enzymes maintaining the density over the Golgi stacks. Authors, therefore, indicated an increased amount of GalNAc-T2 in HeLa cells. The decrease of the GalNAc signals (Fig. 2) after NH_4Cl treatment points to a decrease of free GalNAc and is consistent with an increase of mucin glycosylation, that may be produced by larger quantity of glycosyltransferases.

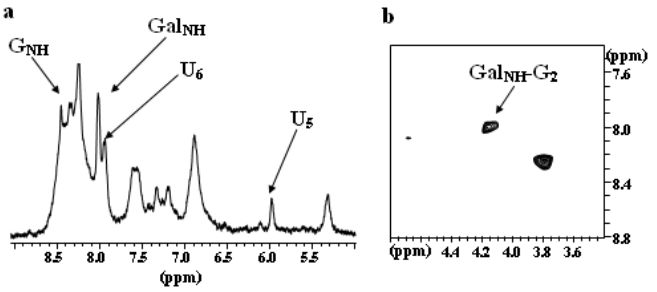

Figure 1. *1D (panel a) and 2D COSY (panel b) ^{1}H MR spectrum (low field region) of HeLa cells.*

Table	δ (ppm)	J (Hz)	COSY cross peaks δ(1)- δ(2) (ppm)
HeLa cells			
Gal_{NH}	8.02±0.01	8.6±0.5	8.00-4.16 (±0.02)
G_{CH3}	2.06±0.01		
GalNAc in H_2O			
Gal_{NH}	8.041±0.002	9.3±0.1	8.04-4.14 (±0.01)
G_1	5.241±0.002	3.6±0.1	5.24-4.13 (±0.01)
G_2	4.14±0.01		
CH_3	2.058±0.002		

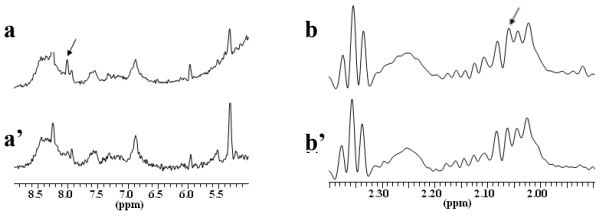

Figure 2. *1D ^{1}H NMR spectra of HeLa cells at confluency. Traces a', b': cells treated with 40 mM NH_4Cl for 24 h, (low and high field respectively). Traces a, b are relative to the corresponding control sample.*

CONCLUSIONS

The existence of a signal from GalNAc molecule and its behaviour after treatment with NH_4Cl, in Hela cells, may be related to the presence of a broad Golgi localization of glycosyltransferases, specific of this cell line, as well as the increased number and size of Golgi stacks after NH_4Cl treatment. The low field region of NMR spectra may be exploited to study aberrantly processed mucins such as in HeLa cells and to characterize changes in the glycosylation pathway. It is worth noting that other MRS techniques such as MAS or the use of PCA extracts, did not observe these signals, pointing to a selected use of NMR microprobes for these studies in whole cells.

REFERENCES

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