Identification of amide protons of GSH in MR spectra of tumor cells

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INTRODUCTION

Magnetic Resonance Spectroscopy (MRS) is a non invasive technique that can be used to monitor intracellular pH in *in vitro* and *in vivo* samples. In particular, 1H MRS was used to investigate pH effects in rat brain, focusing the attention on amide proton signals [1]. These signals were chosen because their intensity is related to pH value due to the exchanging nature of these protons with solvent water [2]. On the other hand, the origin of these signals is not yet clear. Some authors [2] suggested that nucleosides and their breakdown products may contribute to peak at 8.3 ppm, but they concluded that stoichiometrically these molecules cannot be the only source of this signal.

In the present work, we examine the 1H MRS spectra of two different tumor cell lines, MCF-7 from mammary carcinoma and T98G from glioma. We observed in the past that both these cell lines are characterized by intense signals from GSH in the aliphatic region of the 1H MR spectra [3]. Through the comparison of spectra from cells and from GSH solution, we now assign two main peaks in the low field region of the 1H MR spectra to NH protons of GSH. Assignment is confirmed by treatment of cells with buthionine sulfoximine (BSO), that inhibits the activity of the enzyme γ-glutamyl cysteine synthetase, the rate limiting step in GSH synthesis.

MATERIALS AND METHODS

MCF-7 cells and T98G cells were grown as adherent cells, as described elsewhere [3]. MCF-7 and T98G cells were treated with BSO (1 mM) for 18 h . The medium was then removed, cells washed and fresh medium added. GSH solutions were prepared by dissolving GSH in 10% $2H_2O$ to a final concentration of 10 mM .

1H MR spectra were run at 400.14 MHz on a digital Avance spectrometer (Bruker, AG, Darmstadt, Germany) equipped with a 1 mm microprobe. 1D spectra were acquired with a 90° RF pulse and a sweep width of 4006.4 Hz. The number of scans was 512 with a repetition time of 4.36 s. Water suppression was obtained by irradiating water signal. 2D 1H COSY spectra of cells were acquired with a 90°–tl–90°–t2 pulse sequence, by summing 16 free induction decays for each of 256 increments in t1. Spectra were acquired as a matrix of 512 • 256 data points in the time domain. To perform MRS measurements on cells, a pellet of approximately $5x10^6$ cells was suspended in phosphate-buffered saline with 10% $2H_2O$ and inserted in a 1 mm tube.

RESULTS AND DISCUSSION

Figure 1a shows the 1D and Figure 2a the 2D COSY 1H MR spectra of GSH solution while Figure 1b and 2b corresponding spectra of T98G cells.

According to previous peak assignments in the high field spectral regions of GSH solution and tumour cells [3], peaks of glu in GSH resonate at 2.17, 2.55 and 3.82 ppm (β-CH_2, γ-CH_2 and α-CH protons, respectively) in 1D (Figure 1ab), and corresponding correlations at 2.53–2.17 and 3.76–2.17 in 2D COSY spectra (not shown). Peaks at 2.97 and 4.57 ppm are from cys (β -CH_2 and α -CH protons, respectively) and corresponding cross peak 2.97–4.59 ppm (not shown); finally, peak at 3.87 is from the two protons of gly in GSH, about superimposed on the α-CH of glu (Figures1ab).

In the low field spectral regions of GSH (Figure 1a), we could observe the signal from NH of gly in GSH at 8.27 ppm (triplet) and from NH of cys in GSH at 8.48 ppm (doublet), and corresponding correlations in 2D COSY spectra (Figure 2a) were found at 8.27-3.87 ppm (gly NH-CH_2) and at 8.48-4.54 ppm (cys NH-α-CH), respectively. Signals originating from the amine group of glu in GSH were not observed.

In the low field spectral region of tumour cells, a signal was clearly observable at 8.27 ppm (Figure 1b) and could be attributed to the NH proton of gly in GSH, while peak attributable to the NH-cys was present at 8.49 ppm (Figure 1b). The attribution was confirmed by the presence of the cross peaks at 8.27-3.78 ppm and at 8.49-4.56 ppm in 2D COSY spectra of cells (Figure 2b).

Figure 1c shows the 1D 1H MR spectrum of BSO-treated T98G cell samples. The intensity decrease of signals at 2.17, 2.55, 2.97 and 3.78 ppm after BSO treatment is accompanied by an equivalent intensity decrease of signals at 8.27 ppm, and 8.49 ppm. 2D COSY spectra show a similar decrease for cross peaks in the low field region (not shown). In particular, the ratio between the cross peak attributed to the NH-CH_2 of gly and that of β-CH_2-γ-CH_2 of glu of GSH, equal to 0.25 in control sample, remained constant after BSO treatment when GSH signals were strongly depressed.

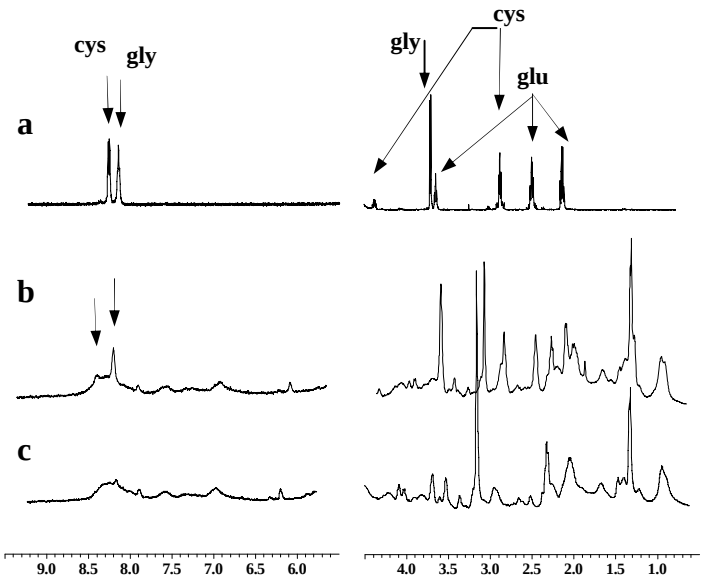

Figure 1. 1D spectra of (a). GSH solution; (b). T98G cells; (c). BSO treated T98G cells

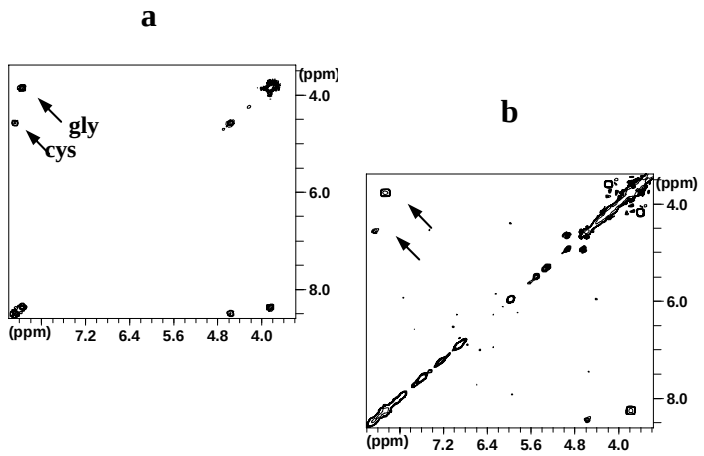

Figure 2. 2D COSY spectra of (a).GSH solution; (b) T98G cells

CONCLUSIONS

From the comparison of 1D and 2D COSY spectra of GSH in solution with those of tumour cells and from the examination of spectra of cells treated with BSO, it appears that some of the amide signals, clearly evident in tumor cell spectra, derive from gly and cys residues of GSH. Further studies are in progress to relate chemical shift and intensity of these signals to intracellular pH.

REFERENCES

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