

Detection of glycine residue of glutathione in vivo

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INTRODUCTION

Glutathione (GSH) is a powerful antioxidant in the human brain. Previous ¹H MRS methods focused mainly on the editing of the cysteine moiety of the GSH to overcome spectral overlap with other metabolites (1-3). The proposed method focuses on the glycine residue of GSH (at ~3.75 ppm), which is overlapped by glutamate and glutamine (Glx) under physiological pH and temperature. The scheme utilizes J-difference editing to quantify Glx contribution to separate it from a target resonance of glycine moiety of GSH. This method is developed and tested by utilizing numerical simulations, solution phantom and in vivo studies at 4 Tesla. In addition, this method is compared to previously proposed method (2), where cysteine residue of GSH is separated from creatine (Cr) via J-difference editing (2).

METHODS

Written consent approved by local institution IRB was obtained for the human subjects used for development of the presented method. **Data Acquisition:** The data was collected on the Bruker/Siemens MedSpec 4T system equipped with an 8-channel array coil. Two different pulse sequences were used to obtain the data. For cysteine residue of GSH detection: MEGA-PRESS sequence (TE=72 ms, TR= 3.5 s), with WET water suppression was used to place a voxel (27 mL) in the precuneus (Fig. 1a). The size of the voxel of interest and the experimental parameters were consistent with those reported by Terpstra and co-workers (2). After localized shimming, the unsuppressed water spectrum was collected for phase reference and eddy current correction. Total of 512 scans with editing pulses alternating between 4.56 (edit on scan) and 7.5 ppm (edit off scan) was collected during the acquisition time of ~ 30 min. For the detection of GSH glycine residue: following the completion of the MEGA-PRESS acquisition, the same volume location was used to acquire data with PRESS+4 sequence (4) (TE=72 ms, TR=3.5 s), with 256 scans with editing pulses alternating between 2.0 ppm (edit on) and 7.5 ppm (edit off), total acquisition time ~ 15 min. Additional macromolecular baseline information was obtained with double inversion pulses that null metabolites (TR=2 s, TI1=1.1 s, TI2=0.070 s). This facilitates the analysis of the summed spectrum (edit on+edit off), which contains some macromolecular contribution at ~3.75 ppm. **Data Processing:** The spectra were processed with MATLAB software routines developed in our laboratory. Prior knowledge based on numerical simulations (5) was generated for both sequences. Total of 14 metabolites were used in the simulations, including vitamin C (ascorbic acid), which also has a resonance close to ~3.75 ppm. MEGA-PRESS difference spectrum was fitted to quantify cysteine residue of GSH. PRESS+4 difference spectrum was fitted to extract contributions of glutamate and glutamine (Glx). After this, Glx contribution was subtracted from PRESS+4 summed spectra. The resultant summed spectrum was used for GSH fitting after MM contribution was subtracted.

RESULTS

Figure 1b shows in vivo spectra of GSH of a healthy volunteer acquired with the MEGA-PRESS sequence (duration=30 min) (2). The difference spectrum contains the GSH and residual NAA multiplet signal. Figure 1c shows the in vivo spectra from a healthy volunteer collected from the same volume in the precuneus (Fig.1a) in 15 minutes using PRESS+4 sequence (4). Figure 1c demonstrates "edit off" (second row), "edit on" (top row) and difference (third row) spectra. The difference spectrum contains only the contribution from Glx at 3.75 ppm (co-edited GABA signal is also present at 3 ppm). The difference spectrum is spectrally fitted (red line) to obtain the amount of Glx. The lower most spectrum in Figure 3 represents the sum of two scans (fourth row), where the Glx contribution to the signal at 3.75 ppm have been subtracted so that only the peak from glycine residue of GSH is left. This region (marked with an arrow) also contains signal contribution from ascorbic acid (vitamin C), which is included in the final prior knowledge for fitting of the summed spectrum. The GSH concentration was calculated to be ~1.6 mM for both methods, consistent with the values reported in the literature (6). This calculation was based on the use of the Cr resonance as a reference (using 8 mM concentration for Cr).

SUMMARY/DISCUSSION

Despite the clearly discernible edited cysteine signal of GSH at 3 ppm in Fig. 1b collected with MEGA-PRESS, the long acquisition time (~30 min for 1 voxel) precludes this method from being used routinely for clinical research in patients. Therefore, the detection of a different residue of GSH (glycine) was investigated in order to decrease experimental times. The traditional spectroscopic editing methods eliminate a singlet resonance (e.g. creatine) to uncover the "hidden" J-coupled resonances. In the proposed method, the overlapping GLX contribution is calculated from the difference spectrum instead of being eliminated. This calculated contribution is then subtracted from the summed spectrum (edit on+edit off). Resultant signal to noise ratio of the GSH signal yields a significant improvement compared to previously used methods. The proposed approach retains 100% of the GSH glycine moiety and allows for the simultaneous detection of other metabolites, including glutamate, glutamine, ascorbic acid, myo-inositol, NAA, γ -aminobutyric acid in the same experiment. Additional collection of the metabolite nulled spectrum facilitates the final data fitting analysis of the summed spectrum, since MM signal in the region of ~3.75 ppm is retained in the summed spectrum even at relatively long echo time (72 ms).

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