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^{1}H MRS detection of glycine residue of reduced glutathione *in vivo*

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ABSTRACT

Glutathione (GSH) is a powerful antioxidant found inside different kinds of cells, including those of the central nervous system. Detection of GSH in the human brain using ^{1}H MR spectroscopy is hindered by low concentration and spectral overlap with other metabolites. Previous MRS methods focused mainly on the detection of the cysteine residue (GSH-Cys) via editing schemes. This study focuses on the detection of the glycine residue (GSH-Gly), which is overlapped by glutamate and glutamine (Glx) under physiological pH and temperature. The first goal of the study was to obtain the spectral parameters for characterization of the GSH-Gly signal under physiological conditions. The second goal was to investigate a new method of separating GSH-Gly from Glx *in vivo*. The characterization of the signal was carried out by utilization of numerical simulations as well as experiments over a wide range of magnetic fields (4.0–14 T). The proposed separation scheme utilizes *J*-difference editing to quantify the Glx contribution to separate it from the GSH-Gly signal. The presented method retains 100% of the GSH-Gly signal. The overall increase in signal to noise ratio of the targeted resonance is calculated to yield a significant SNR improvement compared to previously used methods that target GSH-Cys residue. This allows shorter acquisition times for *in vivo* human clinical studies.

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1. Introduction

Glutathione (GSH), a tripeptide of the glutamate (GSH-Glu), glycine (GSH-Gly) and cysteine residues (GSH-Cys), is a primary source for antioxidant protection in various types of living cells due to its unique capacity as a reducing agent [1]. There is a growing amount of evidence that in the human brain, in addition to performing its well-established free radical scavenger role, it is also involved in the processes underlying memory [2]. It has been established in several different animal models, as well as in humans, that a decrease in GSH concentration may be associated with normal aging and the pathogenesis of several diseases, including Amyotrophic Lateral Sclerosis, AIDS, Alzheimer's and Parkinson's diseases [3].

NMR is an ideal tool for studying amino acids and has been frequently used to observe changes in GSH concentration in whole cells *ex vivo* [4,5]. In most cells, including those of the central nervous system, it exists primarily in its reduced form, while a much smaller fraction (~1%) can be found in the oxidized forms—primarily as a disulfide (GSSG) [1]. Since oxidized forms of glutathione have been reported in the brain tissue and blood cells in a concentration range virtually undetectable by *in vivo* MR methods (~0.01 mM), the GSH nomenclature in this manuscript will be used to refer to its reduced form. There are only a few clinical studies of *in vivo* MRS measurements [6–8] due primarily to two reasons: the severe spectral overlap of GSH resonances with resonances from other metabolites and its relatively low concentration in brain tissue (1–2 mM). Spectral editing techniques that target the *J*-coupled cysteine residue have been developed to overcome the problem of spectral overlap with singlet resonance of creatine at 3 ppm. These methods include multiple quantum filtering developed by Trabesinger et al. [9,10], *J*-difference based on MEGA-PRESS by Terpstra et al. [11,12] and polarization transfer [13]. However, the long acquisition times required by these methods remain prohibitively long for routine clinical studies. At higher fields where spectral

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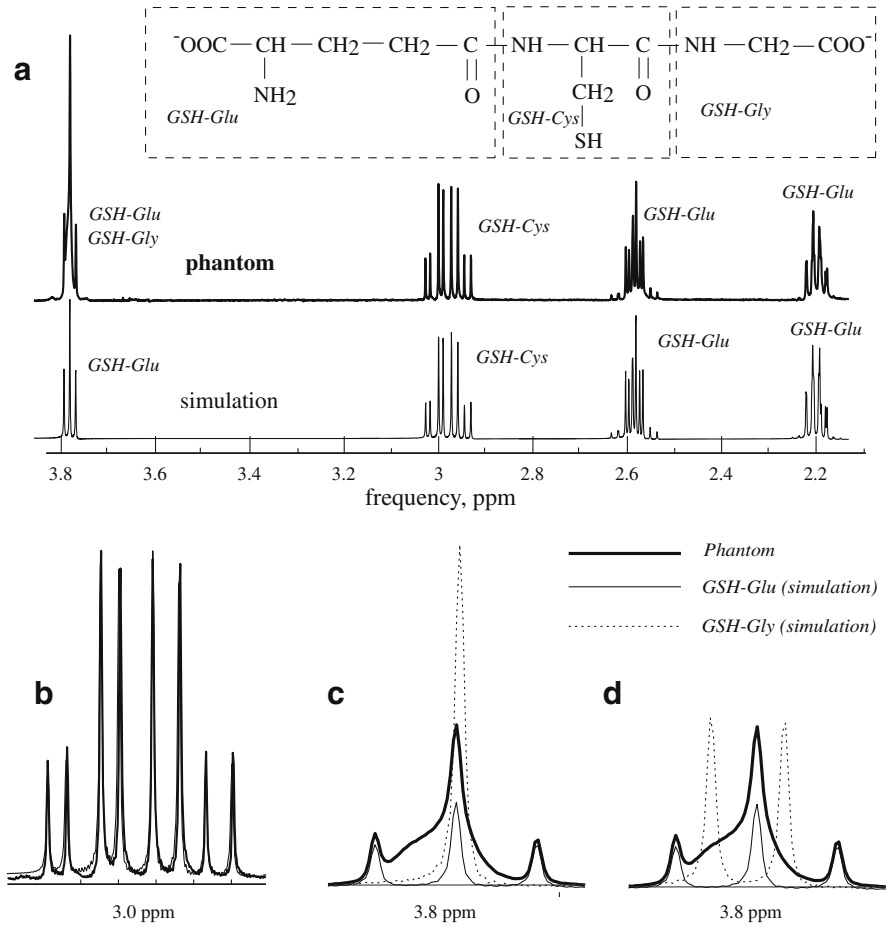

Fig. 1. High resolution experimental NMR spectrum (top) of reduced glutathione (B_0 = 11.7 T, T = 37 °C, pH 7.2) and simulations (bottom) (a). The simulations show a good agreement with an experiment (e.g. expanded GSH-Cys residue region (b)). However when the simulation of GSH-Gly is carried out using previously reported parameters, either a singlet (c) or a doublet (J = 5 Hz) (d) (with the same line width used for simulations of GSH-Glu and GSH-Gly), there is a large discrepancy with the experimental data. This discrepancy appears to be not just due to a small difference in line width or J-value.

overlap is reduced, an alternative approach was demonstrated in the study of schizophrenia, where prior knowledge was created from solution metabolites to fit the entire spectral content of the GSH in the short echo time spectrum [8]. The advantage of this method over cysteine residue editing methods is that it targets the entire spectral content of GSH, which should improve fitting accuracy. Additionally, this is a "one shot" technique that is less sensitive to artifacts due to experimental frequency and phase drifts than J-difference methods. However, this approach relies solely on the robustness and accuracy of the fitting routine, which is strongly hampered at short echo times by contributions of overlapping metabolites and macromolecules resonances. Additionally, inherent SNR of the STEAM localization is reduced by a factor of two compared to other techniques that are more suitable for detection of metabolites with low signal intensity such as GSH.

Under physiological conditions, GSH-Gly resonance is overlapped with resonances of other compounds, primarily glutamate (Glu) and glutamine (Gln), and also with its own glutamate residue (GSH-Glu). In order to devise an optimal strategy for spectral separation of those resonances it is important to have detailed information about the chemical shifts and J-coupling values of all those compounds. Unlike Glu, Gln, and other metabolites for which chemical shifts and J-coupling values are available in the literature [14], there is no consensus about spectral properties of GSH-Gly signal under physiological conditions. The previous NMR studies of the GSH-Gly signal considered it either as a singlet [14,15] or as a doublet (two glycine protons split by an amide proton) [16,17]. Fig. 1a (top) compares the high resolution NMR spectrum of GSH to the numerical simulation of GSH-Glu and GSH-Cys residues (Fig. 1a, bottom). This comparison demonstrates a good agreement between the NMR parameters used to simulate [14] the cysteine and glutamate residues and the experimental data (see for example the expanded cysteine residue signal at 3 ppm—Fig. 1b). However the GSH-Gly model of either the singlet or doublet deviates significantly from the experimental data (see Fig. 1c and d, respectively).

The overall aim of this study is to investigate the effectiveness of detecting the GSH signal at 3.77 ppm *in vivo*, which originates from two methylene protons of the GSH-Gly residue. The first goal of this study is to produce a spectral model for GSH-Gly signal that closely fits the experimental data. The second goal is to investigate the possibility of using spectral editing to separate GSH-Gly from other metabolites. To achieve these goals, experimental spectra were obtained at several field strengths in phantoms and *in vivo* and numerical simulations were performed. The performance of this new strategy for detection of GSH *in vivo*, as well as the advantages and disadvantages of this new strategy are compared to existing methods.

2. Methods

2.1. Phantom preparation and phantom data acquisition

2.1.1. High resolution (11.7 and 14.0 T) vertical bore instruments

A phosphate buffer (0.1 M) mixed with reduced GSH (Fisher Scientic) was used to prepare the sample in an oxygen free compartment (glovebox) at pH 7.1 and concentration 10 mM. High resolution NMR spectra (non-localized) were collected at 11.7 and 14.0 T on Bruker systems (pulse-acquire sequence with water presaturation) at $T = 37$ °C.

2.1.2. Horizontal bore MRI instruments

9.4 T data acquisition. Spectra from GSH phantom (70 mM GSH, 2 mM DSS, 200 mM formate in phosphate buffer) were acquired at physiological pH (~7.2) and temperature (37 °C) using a 9.4 T, 31 cm horizontal bore magnet (Magnex Scientific, Oxford, UK) interfaced with a Varian INOVA console (Varian Inc., Walnut Creek, CA). A quadrature 400-MHz ^{1}H RF surface coil was used to transmit and receive. Spectra were acquired from 9 μL voxel with localization by adiabatic refocusing (LASER) [18]. Parameters for spectral acquisition were: TR = 10 s, TE = 19, 60, 100, 200 ms, NEX = 16, sw = 5 kHz, number of acquired points = 20 k.

4 T data acquisition. A phosphate buffer mixed with reduced GSH (Fisher Scientific) was used (pH 7.1, concentration 100 mM).The data was collected on a Bruker/Siemens 4.0 T system equipped with an 8-channel array coil at $T = 37$ °C. The following localization sequences were used: MEGA-PRESS [19] (TE = 68 ms, TR = 3.5 s, NEX = 512, sw = 4 kHz) and PRESS + 4 [20] (TE = 72, TR = 3.5 s, NEX = 256, sw = 4 kHz). PRESS + 4 is a modified version of the PRESS sequence, which utilizes additional 4th pulse (non-selective 180°) to reduce "4 compartment" artifact.

4.7 T data acquisition. A phosphate buffer mixed with reduced GSH (Fisher Scientific) was prepared (pH 7.1, 100 mM GSH, 5 mM DSS). The data was collected on a Varian 4.7 T system equipped with 1 channel volume transmit and receive coil. Spectra were acquired from 27 μL voxel with (LASER) [18]. Parameters for spectral acquisition were: TR = 5 s, TE = 19, 60, 100 ms, NEX = 64, spectral width = 4 kHz, number of points = 8 k. All sequences on the horizontal bore scanners were preceded with 7 variable power RF pulses with optimized relaxation delays [21] (VAPOR water suppression scheme).

2.2. In vivo spectroscopy

All subjects ($N = 3$) provided written informed consent before participating in the study which was approved by the Committees of Human Research at the University of California and the VA Medical Center in San Francisco. The localized spectra were collected on a Bruker/Siemens MedSpec 4.0 T system equipped with an 8-channel array coil. Two different pulse sequences were used to obtain the data.

2.2.1. GSH-Cys residue detection

A MEGA-PRESS sequence (TE = 68 ms, TR = 3.5 s) was used to place a voxel (27 mL) in the precuneus (Fig. 5b). The size of the voxel of interest and the experimental parameters were consistent with those reported by Terpstra et al. [11]. After localized shimming, the unsuppressed water spectrum was collected for phase reference and eddy current correction. A total of 512 scans with editing pulses alternating between 4.56 (edit on scan) and 7.5 ppm (edit off scan) were collected during the acquisition time of ~30 min.

2.2.2. GSH-Gly residue detection

Following the completion of the MEGA-PRESS acquisition, the same volume location was used to acquire data with PRESS + 4 sequence [20] (TE = 72 ms, TR = 3.5 s), with 256 scans with editing pulses alternating between 2.1 ppm (edit on scan) and 1.3 ppm (edit off scan), total acquisition time ~15 min. Additional macromolecular (MM) baseline information was obtained with double inversion pulses applied prior to the PRESS + 4 localization sequence to null metabolic signals (TR = 2 s, TI1 = 1.1 s, TI2 = 0.070 s), total acquisition time ~ 8 min. This was necessary to evaluate MM content in the region of interest (3.77 ppm) and in the overall spectral range at TE = 72 ms. Both sequences (MEGA-PRESS) and (PRESS + 4) utilized the same RF editing pulse (duration = 19 ms) designed to minimize both pass band and rejection band ripples.

2.3. Data processing

The spectra were processed with MATLAB software routines developed in our laboratory, which have the flexibility to fit groups within a particular metabolite with separate line width parameter for each group. Prior knowledge based on numerical simulations [22] was generated for both MEGA-PRESS and PRESS + 4 sequences. A total of 14 metabolites were used in the simulations, including vitamin-C (ascorbic acid) and glucose, which also have resonances close to ~3.75 ppm The MEGA-PRESS difference spectrum was fitted to quantify the cysteine residue of GSH. PRESS + 4 difference spectrum (edit on − edit off) was first fitted to calculate glutamate and glutamine and GSH-Glu contribution (Glx). Following this, the Glx contribution was used as a constraint in the prior knowledge fitting of the PRESS + 4 summed spectrum (edit off + edit on). The resultant summed spectrum was used for final GSH-Gly fitting after MM contribution was subtracted.

3. Results

3.1. Investigation of the spectral pattern of GSH-Gly

Fig. 2 demonstrates GSH signal evolution at 9.4 T as a function of the echo time (TE = 19–200 ms). The insets show the expanded region of GSH-Gly at 3.77 ppm which is overlapped with the triplet from GSH-Glu residue. The prominent singlet-like signal at 3.77 ppm can be easily mistaken for a singlet pattern of GSH-Gly. However the expanded spectrum shows a broader structure underneath a triplet of the GSH-Glu residue. At longer echo time (TE = 60, 100 ms), no *J*-evolution of GSH-Gly signal is observed (only that of GSH-Glu residue). The GSH-Gly signal contribution at TE = 200 ms is weak, while the intensity of GSH-Glu (at 3.77 and 2.55 ppm) and GSH-Cys at 3.0 ppm remain strong (also notice significant signal reduction of GSH-Glu at 2.1 ppm). The broadened spectral pattern of GSH-Gly and a lack of *J*-evolution at longer TE indicate that the chemical exchange most likely plays a role in appearance of GSH-Gly spectrum.

A detailed examination of GSH-Gly signal pattern as a function of magnetic field strength and temperature is described in Fig. 3. Fig. 3a–d shows expanded GSH-Gly region of the experimental spectra collected at four different magnetic field strengths (14.0, 11.0, 9.4 and 4.7 T). Numerical simulations of the GSH-Gly (blue line)[1] and GSH-Glu (green line) allow the visualization of the individual contributions of those residues to the overall signal at 3.77 ppm (red line). Within experimental line widths of 1–2 Hz, it was possible to resolve GSH-Gly as a doublet with a *J* value of ~3.4 Hz at all four fields. The splitting between two GSH-Gly peaks originates from the *J*-coupling with an amide proton, which in turn undergoes an exchange with solvent H_2O protons. This exchange is slow enough

[1] For interpretation of color mentioned in this figure the reader is referred to the web version of the article.

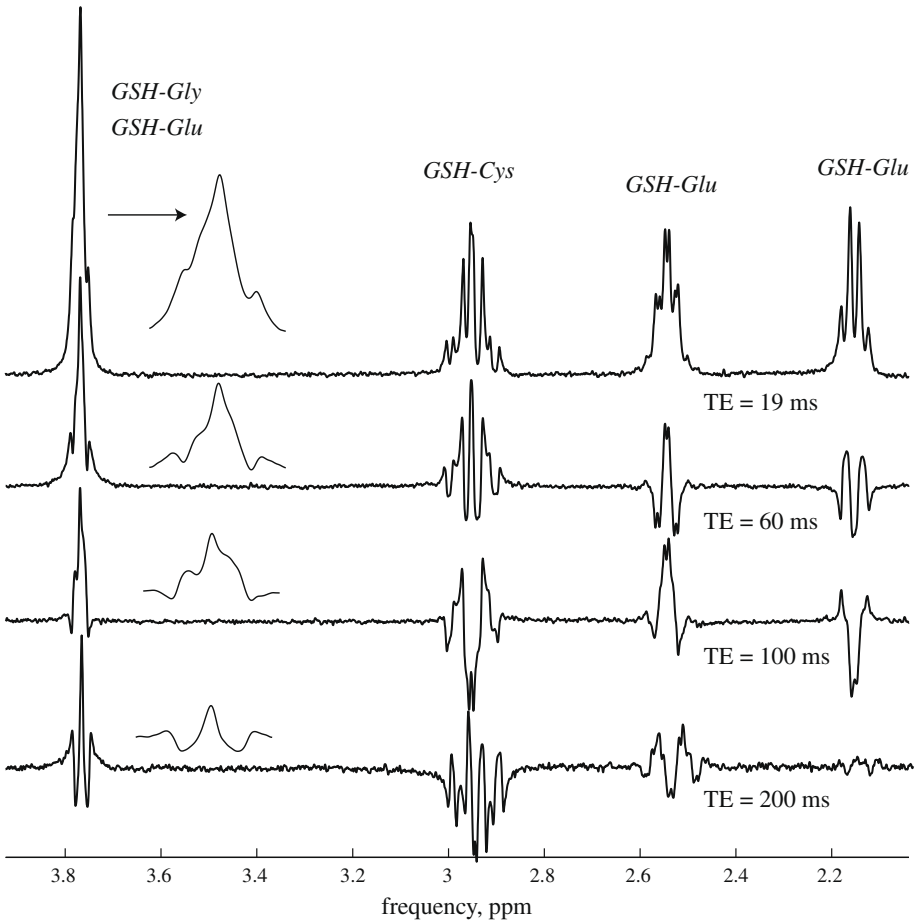

Fig. 2. Experimental GSH spectra (B_0 = 9.4 T, T = 37 °C, pH 7.2) as a function of the echo time. Note the absence of J-evolution for GSH-Gly signal and that at TE = 200 ms, GSH-Gly signal is significantly reduced, with most of the signal at 3.77 ppm remaining due to GSH-Glu residue.

on the NMR scale to detect a small splitting. It is confirmed by the cooling of the sample by a few degrees, which effectively slows down the exchange process and the J-coupling becomes apparent at an intermediate temperature of 30 °C with peaks separated by ~5.3 Hz (Fig. 3e). At room temperature (20 °C) the splitting is calculated to be on the order of 6.5 Hz and the line width becomes comparable to the line widths calculated for GSH-Glu and GSH-Cys residues. Based on the spectra obtained at different fields, the proposed GSH-Gly model under physiological conditions is described as two equivalent protons at 3.775 ppm split by the NH proton with J = 3.4 Hz. However, this should not be used directly as a model for prior knowledge for density matrix calculations with J-coupling and chemical shift Hamiltonian. A lack of J-evolution as a function of the echo time (most likely due to effective "decoupling" via the exchange of NH protons with H_2O solvent) and broader line widths require a modified "basis set" approach. In this case, a "two singlets" model separated by 3.4 Hz with a moderate Gaussian broadening (~100 ms) should approximate very well the GSH-Gly pattern *in vivo*. For example, at 4.7 T, the two singlet peaks were simulated at 3.767 and 3.784 ppm. For higher magnetic field strengths, the separation described in ppm should be reduced to account for higher Hz/ppm factor.

3.2. Separation of GSH-Gly residue from Glx signal in vivo

Fig. 4 displays *in vivo* spectra and numerical simulations of Glu, Gln and GSH-gly resonances acquired with PRESS + 4 sequence (TE = 72 ms) at 4 T. It illustrates the results of the editing method that allows the quantitative separation of GSH-Gly from Glu + Gln (Glx) resonances. This method is similar to GABA J-difference editing [19,23], except that it targets Glx resonance at 3.77 ppm and also uses the summed spectrum for quantification in addition to the difference spectrum. The experiment consists of the acquisition of two spectra, one with the editing pulse centered at 2.1 ppm (the spectral location of the Glx) during the edit on scan, and a second scan with editing pulse applied away from Glx during the edit off scan. In the edit on scan (Fig. 4a), Glu and Gln outer peaks at 3.77 ppm are refocused by the editing pulse and appear "phased up". In contrast, if the editing pulse is applied away from the coupled spin location, the non-refocused J-evolution of the Glu and Gln results in the spectral pattern with outer peaks of the triplet "phased down" (Fig. 4b). The difference spectrum (Fig. 4c) contains the contribution from Glu and Gln at 3.77 ppm, but no signal from GSH-Gly. In the first step of the quantification routine, this contribution is spectrally fitted (Fig. 4c, thick line) to obtain the amount of Glx. The spectral fitting for this step is restricted to the region around the Glx signal at 3.77 ppm (to avoid the residual MM contamination around 2 ppm and baseline distortion in the same area from the editing pulse in the edit on scan).

In the next step, Glx contribution in the summed spectrum (Fig. 4d) is reconstructed using numerical simulations based on the concentration obtained in the difference spectrum. The summed spectrum is used for fitting of GSH-Gly after accounting for Glu + Gln contributions. The summed spectrum is also used for fitting other metabolites because it has higher SNR compared to edit off or edit on spectrum.

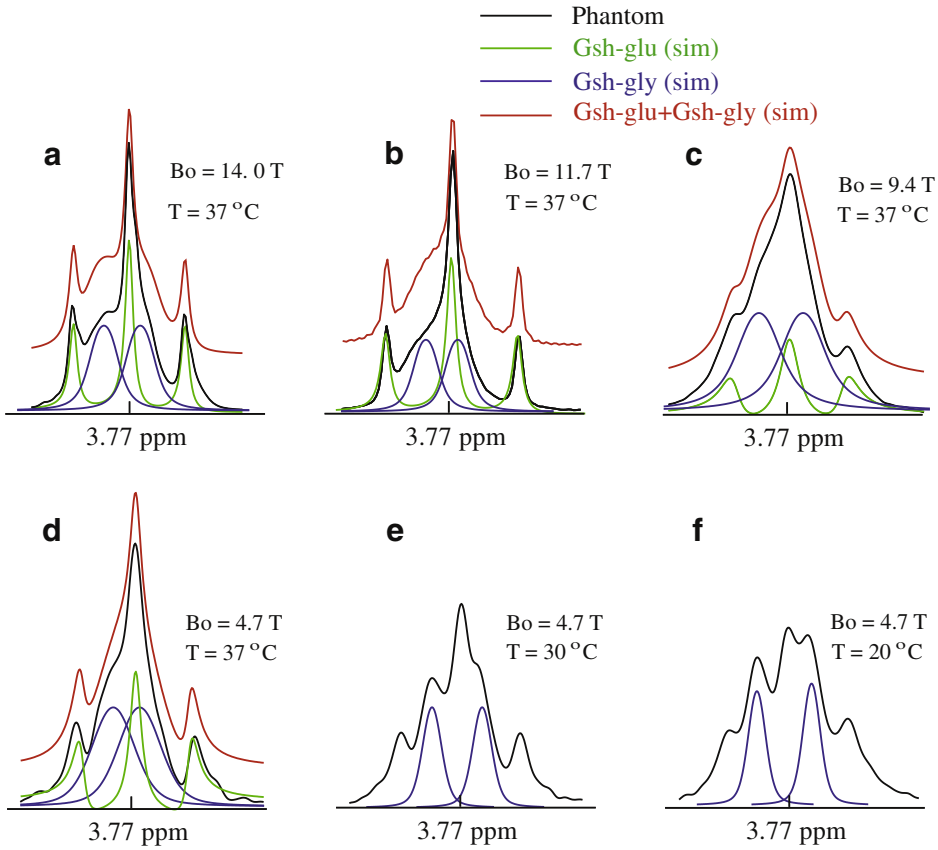

Fig. 3. Expanded experimental GSH-Gly and GSH-Glu signal region and simulations at 3.77 ppm as a function of magnetic field strength (a–d) and as a function of the temperature (d–f). All the spectra were collected at pH 7.1–7.2. The spectra at higher field were acquired with pulse-acquire (11.7 and 14 T), whereas the spectra at lower field were acquired with spin-echo (4.7 and 9.4 T, echo time = 19 ms). The model for GSH-Gly was based on the two singlet peaks separated by 3.4 Hz (T = 37 °C). The additional line broadening (Gaussian LB = 100 ms) due to the loss of the coherence during the exchange process is used to simulate GSH-Gly peaks for all four field strengths (4.7–14 T). After a small temperature decrease (~7 °C), the GSH-Gly pattern shows distinct separation of the glycine peaks (~5.3 Hz) (e), and at room temperature the separation is increased further (~ 6.5 Hz) (f).

Fig. 5a compares the simulated GSH-Cys signal in the difference spectrum of MEGA-PRESS (TE = 68 ms) and the simulated GSH-Gly signal in the summed spectrum of PRESS + 4 (TE = 72 ms). Both spectra are generated using density matrix simulations at 4.0 T. The intensity ratio of GSH-Gly:GSH-Cys is ~3.5 (using the line width of 7 Hz—typical conditions *in vivo* for 4 T). Preliminary *in vivo* spectra demonstrating both methods were obtained in the precuneus region of the brain of a healthy volunteer (Fig. 5b). Fig. 5c shows the editing of cysteine residue using *J*-difference MEGA-PRESS method [11] (acquisition duration = 30 min, Gaussian apodization of 2 Hz). The difference spectrum contains the edited GSH-Cys and residual NAA multiplet signal. Fig. 5d demonstrates PRESS + 4 edited spectra (acquisition duration = 15 min, Gaussian apodization of 1 Hz). Additional metabolite-nulled spectra containing macromolecules (MM) are shown underneath the metabolic spectra (thin line). The difference spectrum contains only the contribution from Glx at 3.77 ppm and GSH-Glu signal (the co-edited GABA signal is also present at 3 ppm). The GSH-Glu signal is included in the prior knowledge of the total Glx pattern during the initial fitting step. The difference spectrum is fitted (thick line) using numerical simulations to obtain the amount of Glx to constrain a prior knowledge for the subsequent fitting of the summed spectrum. The lower spectrum in Fig. 5d represents the sum of two scans (edit off + edit on). The spectrum below (dotted line) demonstrates the residual summed spectrum after subtracting MM and Glx contribution. This region also contains signal contribution from ascorbic acid (vitamin C), which is included in the final prior knowledge for fitting of the summed spectrum. The mean GSH concentration was calculated to be 1.6 ± 0.2 mM for both methods (N = 3 subjects), consistent with the values reported in the literature (6). This calculation was based on the Cr resonance as a reference (using 8 mM concentration for Cr).

4. Discussion

This study investigated the possibility of improving the sensitivity and reducing acquisition times for *in vivo* detection of GSH. The experimental data sets obtained at a wide range of field strengths (4–14 T) were analyzed to create a spectral model for GSH-Gly signal which closely fits the experimental data at physiological pH and temperature. The findings indicated that the GSH-Gly spectral pattern was affected by chemical exchange. Despite the exchange effects, it was possible to construct a prior knowledge model of the GSH-Gly pattern. Based on the constructed model of two singlet resonances for GSH-Gly, a *J*-difference method was designed to separate GSH-Gly from Glx *in vivo*.

The characterization of the spectral pattern under physiological conditions for GSH-Gly residue is difficult due to the sensitivity of the NMR parameters (chemical shifts, *J* values and line widths) to exact pH and temperature. While previous NMR studies are not in agreement about the specifics of the spectral pattern of GSH-Gly, for the most part they point to the specific exchange mechanism with a kinetic rate comparable to NMR time scales: *J*-coupling to NH proton that undergoes exchange with H_2O. In deuterated solvent at pD 7.4 (where NH proton is exchanged with D_2O and

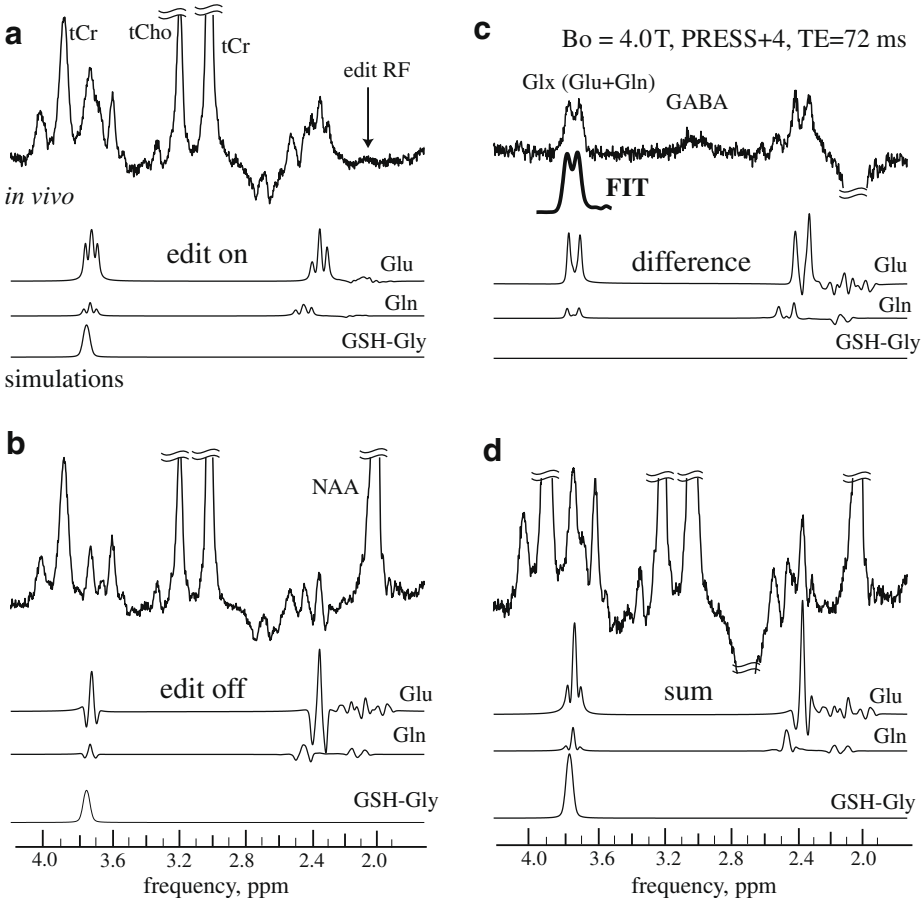

Fig. 4. Demonstration of the *J*-difference editing method for separating GSH-Gly from Glu and Gln. The editing pulse is applied at 2.1 ppm for the edit on scan (a), which refocuses Glu and Gln resonances at 3.77 ppm. In the edit off scan (b) Glu and Gln resonances undergo non-refocused *J*-evolution. In both edit on and edit off scans, GSH-Gly resonance is not affected. The difference spectrum (c) is fitted to extract Glu and Gln contribution, which is subsequently used as a constraint in the fitting of the summed spectrum (d). The individual contributions of Glu, GSH-Gly and Gln were simulated with 5:1:1 concentration ratios (with line broadening factors of 150 ms and 200 ms for Lorentz and Gauss, respectively and additional Gauss line broadening of 100 ms for GSH-Gly). *In vivo* spectra are shown without using apodization.

therefore *J*-coupling is not observable), the glycine protons give rise to a singlet [17]. At pH 7.25, at magnetic field of 9.4 T, Rabenstein et al. [17] report a doublet (J = 6.04 Hz). York et al. [16] also report a doublet (J = 5.2 Hz, pH 7.3, B_0 = 9.4 T). These observations are confirmed by the kinetic studies of the exchange rates of NH with GSH-Gly CH_2 protons, which demonstrate a much slower rate of exchange of NH with solution H_2O compared to GSH-Cys residue [17]. In the latter two reports only one chemical shift is given for both glycine protons in addition to a J value (A_2X pattern), indicating that ABX pattern is not observed. An ABX pattern from two non-equivalent GSH-Gly protons observed at much lower pH values [16] indicates that conformational changes may play a role (lower pH slows down the rate of exchange between conformations). While the separation between two GSH-Gly peaks may be attributed to two different conformations of GSH-Gly, this is unlikely since the separation between the two peaks would increase with magnetic field strength, which was not observed in our study at physiological pH. Our studies find GSH-Gly to exhibit A_2X pattern, in agreement with others [16,17]. Some of those differences in the *J*-coupling values reported at physiological (or near to it) pH can be attributed specifically to the sensitivity of GSH-Gly signal to the temperature, where the difference of only 5–10 °C changes J value by ~2–3 Hz.

There are a few studies describing GSH-Gly as a singlet not just in solvent with deuterium, but also in H_2O solution. Fujiwara et al. [24] report a singlet pattern at pH >7.5 (B_0 = 2.4 T). In another report, at a much higher field of 14 T, a singlet in H_2O solution (pH 7.0) is observed [14]. Therefore the previous NMR reports on GSH-Gly can be divided into two patterns: a doublet due to coupling with the amide proton or a singlet. The data collected for this study did not confirm the existence of the singlet pattern in the range of 4–14 T at physiological pH and temperature. The doublet pattern was observed throughout the range of the magnetic fields studied. However, the model of two equivalent protons *J*-coupled to an amide proton would not constitute an adequate prior knowledge for spectral fitting. First, no *J*-evolution was observed at longer echo time in our study, therefore inclusion of *J*-coupling into prior knowledge would compromise simulations with longer echo time (unless the simulations can account for the exchange phenomenon). Second, our studies indicate a need for additional line broadening contribution to the GSH-Gly pattern compared to line widths of GSH-Glu or GSH-Cys residues. Therefore a more accurate model for constructing prior knowledge would be two singlets separated by ~3.4 Hz with an additional line broadening.

The importance of using correct prior knowledge for GSH-Gly pattern for *in vivo* quantification cannot be overstated. This is important not only for reliable measurements of GSH, but also for quantification of other metabolites (e.g. Glx and vitamin-C) that overlap with GSH at several spectral positions. Despite the clearly discernible edited GSH-Cys signal at 3 ppm collected with MEGA-PRESS (Fig. 5c), the long acquisition time (~30 min for 1 voxel) precludes this method from being used routinely for clinical research in patients. The *J*-difference method for separating Glx from GSH-Gly signal *in vivo* examined in this study is very similar to GABA

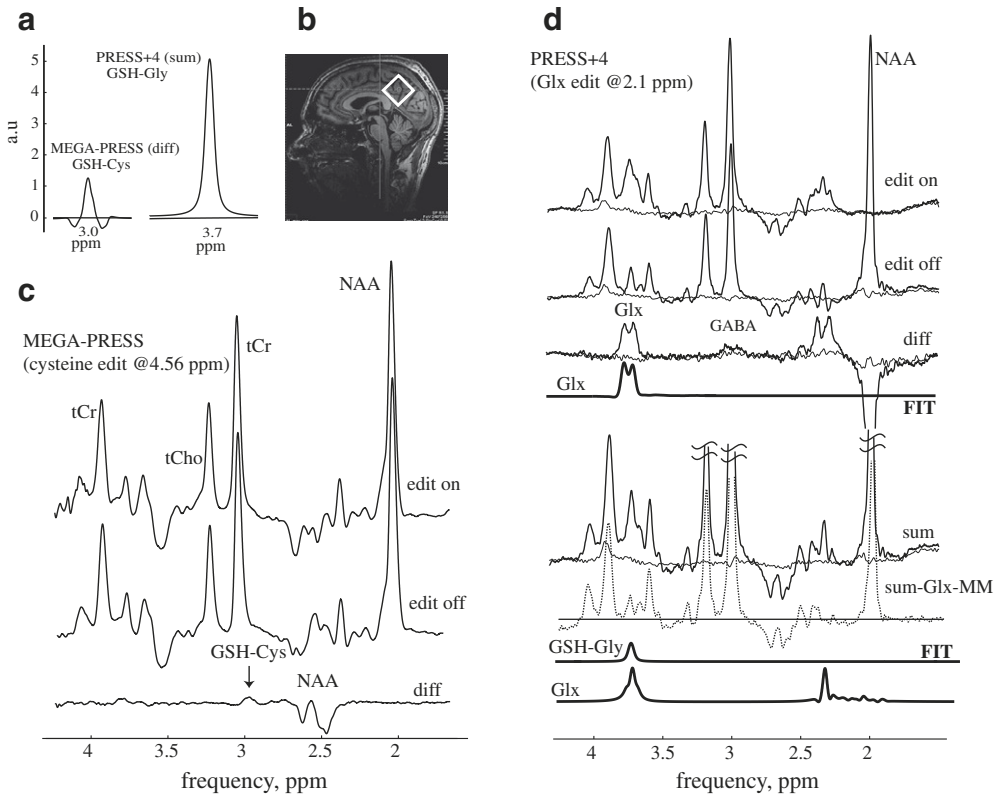

Fig. 5. Comparison of the *J*-difference editing methods for GSH-Gly and GSH-Cys residues: (a) The simulation of GSH-Cys (difference spectrum, MEGA-PRESS, TE = 68 ms, exponential LB = 45 ms) vs. GSH-Gly signal (summed spectrum, PRESS + 4, TE = 72 ms, exponential LB = 45 ms, Gaussian LB = 100 ms) at 4 T. *In vivo* spectra were collected in the precuneus brain region of a healthy volunteer (b). The editing of cysteine residue using *J*-difference MEGA-PRESS is demonstrated in (c). PRESS + 4 editing results are shown in (d). The difference spectrum contains only the contribution from Glx at 3.77 ppm (co-edited GABA signal is also present at 3 ppm). This contribution calculated from the difference spectrum is used as a constraint in the fitting of the summed spectrum. The residual summed spectrum (dotted line) is shown after subtracting MM and Glx.

editing technique suggested previously [23]. The proposed approach retains 100% of the GSH glycine residue and allows for the simultaneous detection of other metabolites, including Glu, Gln, ascorbic acid, myo-inositol, NAA, and GABA in the same experiment.

The disadvantage of this method compared to GSH-Cys editing is a more complicated post-processing (quantification) routine. The post-processing involves extra steps to quantify the Glx contribution from the difference spectrum. Also it relies on collection of two acquisitions, and is susceptible to subject motion and instrument instabilities, as is every *J*-difference method (including cysteine editing), which can cause degradation in the difference spectrum quality. Although this can be partly compensated by the post-processing routines and real time navigation schemes, a one shot acquisition would probably yield better reliability in data quantification. Additional studies are needed to obtain the reliability measurements for the performance of this method *in vivo*. Specifically, an optimal SNR needs to be evaluated for this method, so that the data can be collected from smaller voxel size regions. A substantial SNR increase can be achieved by additional signal averaging and omitting the acquisition of MM baseline, but this needs further investigation when the data are available for a larger number of subjects. The additional MM baseline information may become important if simultaneous GABA quantification is required [25].

In summary, an alternative strategy for GSH detection *in vivo* was investigated. The study estimated spectral parameters of GSH-Gly residue under physiological conditions as a function of the magnetic field strength, echo times and small temperature variations. Based on those parameters, a model of GSH-Gly was constructed and the *J*-difference method of separating GSH-Gly from Glx *in vivo* was proposed. The outlined method retained 100% of the GSH-Gly signal. Overall increase in signal to noise ratio of the targeted resonance was calculated to yield a significant SNR improvement (a factor of 3.5 at 4 T) compared to the method that targets GSH-Cys residue. This allows faster acquisition times for clinical studies *in vivo*.

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