A New Strategy to Measure Reduced Glutathione (GSH) at 3 and 4 Tesla Using an Optimized STEAM Sequence

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Introduction

Glutathione is present in living tissue in two forms, the reduced form (GSH) and oxidized form (GSSG), with a ratio of greater than 500:1 in normal cells [1]. As the most concentrated form of glutathione, GSH is a major antioxidant in the central nervous system and plays a significant role in the detoxification of reactive oxygen species. It is generally difficult to measure GSH using one-dimensional ^{1}H-MRS because the proton resonances of the three (glycine, cysteine, and glutamate) moieties of GSH overlap with other metabolite resonances [2]. Spectral editing methods, such as J-difference editing [1] or multiple quantum filtering [3-4], have been employed to measure GSH by distinguishing the CH_2 proton resonances of the cysteine moiety of GSH at 2.95 ppm from the upfield slope of the prominent singlet resonance of creatine (Cr) at 3.02 ppm. A few recent reports [5-7] suggested the measurement of GSH by LCModel analysis of short-echo PRESS or STEAM spectra. However, as revealed by the correlation matrix, fully overlapping metabolites can not be reliably retrieved by any quantification algorithm [8]. To improve the quantification of GSH, we propose here a new strategy to resolve the spectral overlap between GSH and other metabolites by optimizing the timing parameters of a standard STEAM sequence through spectral simulation. This single-shot method is insensitive to motion and is simple for implementation. We performed spectral simulation using the GAMMA NMR library [9] and optimized the timing parameters of a standard STEAM sequence, i.e., the echo time and mixing time (TE, TM), for suppressing those overlapping resonances from *N*-acetylaspartate (NAA), glutamine (Gln), and aspartate (Asp) around 2.54 ppm at 3T and 4T.

Methods

Spectral Simulation. The C4 proton resonance of the glutamate (Glu) moiety of GSH (glu-GSH) at 2.54 ppm was chosen as the target resonance, since the other proton resonances of GSH are more difficult to resolve from the overlap in a standard STEAM spectrum without spectral editing. The major resonances from other metabolites that overlap the target C4 proton resonance of glu-GSH are the multiplet proton resonance of NAA around 2.48 ppm, of Gln at 2.45 ppm and of aspartate (Asp) at 2.65 ppm. The spectral response of each metabolite to a standard STEAM sequence was calculated throughout the two-dimensional (TE, TM) parameter space in a range of 0 to 200 ms for TE with a step size of 2 ms and a range of 0 to 140 ms for TM with the same step size. The chemical shifts and coupling constants used in the spectral simulation were taken from the literature [2]. The three 90º pulses in STEAM were simulated using ideal pulses for computational efficiency, since a realistic selective 90º pulse does not deviate far from an ideal pulse. As the response of a metabolite spin system to a STEAM sequence is sensitive to the design of spoiler gradients in the two TE/2 periods and the TM period, the simulation on the effects of spoiler gradients was more complete, in which a cubic voxel of 20x20x20 mm^3 was divided into 1000 sub-voxels (10 steps each in the x-, y-, or z-direction), and the signals from the sub-voxels were summed up. The spoiler gradient parameters were derived from a realistic Siemens STEAM sequence.

Search for Optimal (TE, TM) Timing Sets. For each metabolite, 7000 spectra were simulated at various (TE, TM) timing sets, from which a full search was performed to find the optimal timings. An index or cost function was defined for the optimization procedure. The left column of Fig. 1 shows the simulated STEAM spectra of Asp, Gln, NAA, and GSH at 3T and 4T at (TE, TM) = (10 ms, 20 ms), with a line broadening of 5 Hz. Only the region of 1.9-2.85 ppm is shown, for simplicity. The shaded bars indicate the region of the central peak of the target C4 proton resonance (a pseudo-triplet) of glu-GSH. Extensive spectral overlap is shown in the region around 2.54 ppm. To distinguish the target GSH peak form the overlapping peaks, one needs to suppress the other metabolite resonances while keeping the target GSH resonance as strong as possible. As such, an index was designed as $\boldsymbol{F} = \boldsymbol{C}_{GSH}[[\boldsymbol{b}]^2/([\boldsymbol{a}]+[\boldsymbol{c}])] - \boldsymbol{C}_{Asp}\boldsymbol{A}_{Asp} - \boldsymbol{C}_{Gln}\boldsymbol{A}_{Gln} - \boldsymbol{C}_{NAA}\boldsymbol{A}_{NAA}$, where $\boldsymbol{C}$, as a constant weighting factor in the index, stands for the metabolite concentrations (according to the literature [2], $\boldsymbol{C}_{GSH}$:$\boldsymbol{C}_{Asp}$:$\boldsymbol{C}_{Gln}$:$\boldsymbol{C}_{NAA}$ =2:1.2:4.4:10), $[\boldsymbol{b}]$ is the peak area of the central peak of the target pseudo-triplet GSH proton resonance, $[\boldsymbol{a}]$ and $[\boldsymbol{c}]$ are the peak areas of the two outer-wings of the psedo-triplet, and $\boldsymbol{A}$ stands for the peak area of the proton resonances of Asp, Gln, or NAA in the region of the target GSH proton resonance. The boundaries of the $[\boldsymbol{a}]$, $[\boldsymbol{b}]$, and $[\boldsymbol{c}]$ regions were determined based on the simulated GSH spectra at (10ms, 20ms). The index combined two factors: 1) maintaining the intensity of the central peak of the target C4 proton resonance of GSH while suppressing the outer-wings; and 2) suppressing the contaminating resonances of Asp, Gln, and NAA around 2.54 ppm.

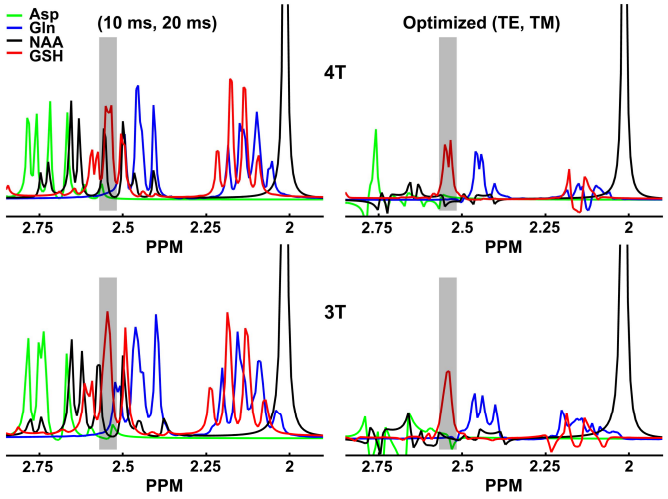

Fig. 1. Simulated STEAM spectra of Asp, Gln, NAA, and GSH at 3T and 4T at (TE, TM) = (10ms, 20ms) in the left column and at optimized (TE, TM) in the right column, respectively, with a 5-Hz line broadening. The shaded bars indicate the regions of the central peaks of the target C4 proton resonances (a pseudo-triplet) of glu-GSH at 2.54 ppm.

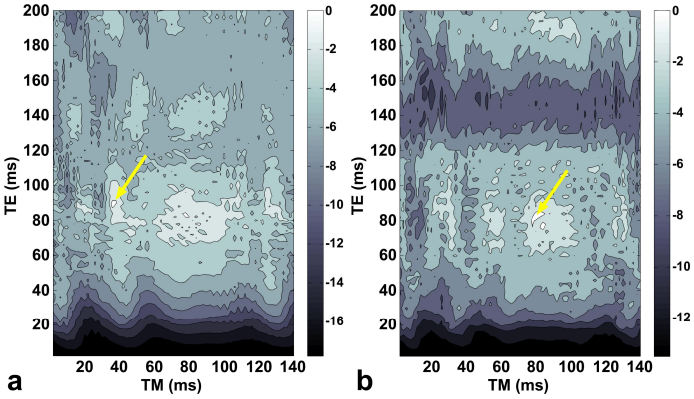

Fig. 2. Contour diagrams, in (TE, TM) parameter space, of the index calculated at (a) 3T and (b) 4T, normalized to the maximum in each data set. The maximum index, as indicated by the yellow arrow, resides at (90 ms, 36 ms) at 3T and (82 ms, 80 ms) at 4T.

Results and Discussion

Fig. 2 shows the contour diagrams, in the (TE, TM) parameter space, of the index at (a) 3T and (b) 4T. The contours were individually normalized to the maximum at individual field strength. The maximum index, as indicated by the yellow arrow, resided at (90 ms, 36 ms) at 3T and (82 ms, 80 ms) at 4T, respectively. The right column of Fig. 1 shows the simulated spectra at the above optimized sequence timing sets at 3T and 4T. The spectra at the short and optimized timing sets were displayed with the same scale at each field strength. Compared to the corresponding spectra at (10ms, 20ms), the target GSH resonance at the optimal timings appear resolved from the overlapping metabolite resonances. The resolved GSH peak at 2.54 ppm provides a potential to significantly improve the quantification of GSH levels.

References

[1] Terpstra M et al., MRM 2003; 50:19-23. [2] Govindaraju V et al., NMR Biomed 2000;13:129-153. [3] Trabesinger AH et al., MRM 2001;45:708-710. [4] Zhao T et al., MRM 2006; 55:676-680. [5] Kreis R et al., MRM 2002; 48:949-958 [6] Opstad KS et al., MRM 2003, 49; 632-637. [7] Terpstra M, et al., MAGMA 2005; 18: 276-282. [8] Ratiney H et al., NMR Biomedicine 2005; 18:1-13. [9] Smith SA et al., J Magn Reson 1994; 106A: 75-105.