Negligible Dehydroascorbate and GSSG Signal Contributions to Human Brain ^{1}H NMR Spectra In Vivo

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Introduction

Although the concentrations of dehydroascorbate (DHA) and GSSG (the oxidized forms of vitamin C and glutathione (GSH) respectively) are below the detection threshold of in vivo ^{1}H MRS under physiologic conditions, it is of interest to understand the extent to which these compounds could contribute resonances to in vivo human brain ^{1}H MR spectra under diseased conditions. We measured ^{1}H NMR spectra from DHA and GSSG at physiologic pH and temperature and deduced chemical shifts and coupling constants. The goal of this project was to determine the extent to which DHA and GSSG contribute resonances to ^{1}H NMR spectra measured in the human brain in vivo.

Methods

^{1}H NMR spectra from solutions of DHA and GSSG were measured using a high resolution 600 MHz Varian INOVA spectrometer (5 mm probe). DHA spectra were initially measured at low temperature (5°C, neutral pH) whereby the characteristic rapid rate of degradation was substantially reduced (1). Spectra were then acquired throughout warming, and after neutralizing at 37°C. A second fresh DHA sample was quickly prepared at room temperature (pH = 7.11) then measured at 37°C. ^{1}H chemical shifts and coupling constants were deduced by visually matching simulated and measured spectra.

Results

The DHA spectrum measured at 5°C (Fig. 1a) clearly shows DHA resonances from ^{6}CH$_2$ (4.16 and 4.27 ppm), ^{5}CH (4.6 ppm) and ^{4}CH (4.8 ppm) and is in agreement with a previously published spectrum (2). Decreasing signal to noise for DHA resonances with increasing temperature (Fig. 1a-e) is evidence for degradation of DHA and assignment of resonances at physiologic pH and temperature. Concurrent buildup of resonances in the region spanning 3.6 to 4.1 ppm is evidence for assignment to degradation products. Figure 2 illustrates measured and simulated GSSG spectra. Deduced chemical shifts and coupling constants are reported in Table 1. Figure 3 illustrates Asc, GSH, and GSSG resonances simulated under double edited MEGA-PRESS at 4 T (3) (Figs. 3a-3c, equimolar) along with analogous resonances measured in solution (Fig. 3d) and in the human brain in vivo (Fig. 3e). Agreement between the simulated and measured resonances illustrates accurate simulation.

Table 1. Chemical shifts and coupling constants.

Metabolite	Proton	δ (ppm)	J (Hz)
GSSG	1	4.7408	1,2: 4.6
	2	3.2954	1,3: 9.3
	3	2.9840	2,3: -14.2
DHA	1	4.754	
	2	4.598	2,3: 5.4
	3	4.275	2,4: 2.4
	4	4.169	3,4: -10.5

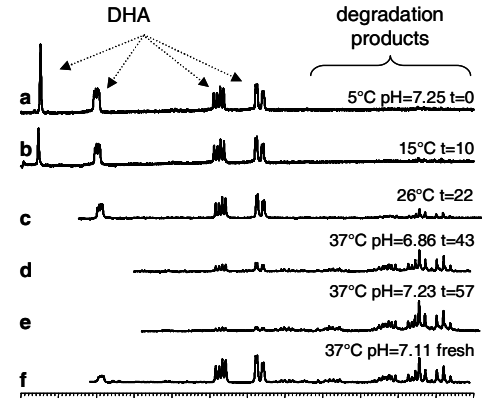

FIG. 1. ^{1}H NMR spectra of DHA. One sample (a-e) was measured at a range of temperatures because DHA degrades rapidly at physiologic pH and temperature. The fresh sample provided increased S/N for DHA.

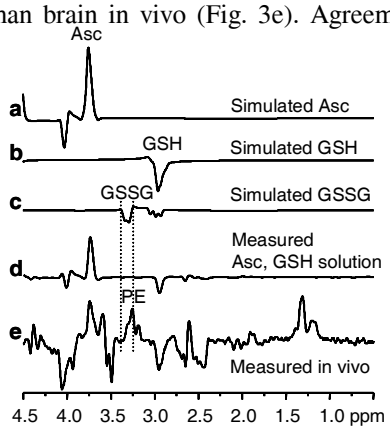

FIG. 3. Double edited MEGA-PRESS spectra: simulated for equimolar (**a**) Asc, (**b**) GSH and (**c**) GSSG, and measured (B_0 = 4 T, TE = 112 ms, TR = 4.5 s, NEX = 512) in (**d**) a solution of 13.5 mM GSH, 35.5 mM Asc and 35.5 mM NAA and (**e**) in vivo.

Discussion and Conclusions

Since DHA does not contribute a resonance in the vicinity of 3.73 ppm, DHA does not contribute to the double edited Asc signal measured in vivo. Given that the simulated intensity shown for GSSG (Fig. 3c) would be contributed by approximately 1 mM GSSG, which is 50 times more concentrated than expected in a live organism (4), contributions from GSSG to double-edited (Asc and GSH) MEGA-PRESS spectra are negligible in vivo. Furthermore, exceptionally high levels of GSSG would be evidenced by the GSSG resonance at 3.3 ppm in vivo. Chemical shifts and coupling constants reported herein are useful for understanding if and to what extent DHA and GSSG contribute signals to in vivo human brain ^{1}H MR spectra.

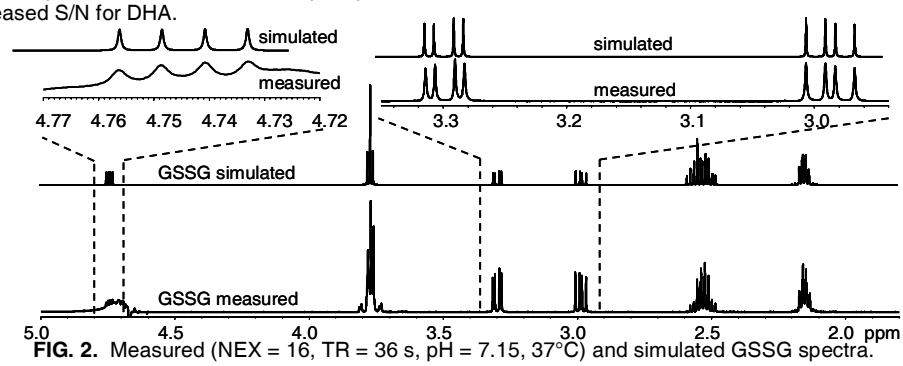

FIG. 2. Measured (NEX = 16, TR = 36 s, pH = 7.15, 37°C) and simulated GSSG spectra.

References and Acknowledgments: 1) Bode et al, Clin Chem, 36:1807, 1990. 2) Yazzie et al, Chem Res Toxicol, 16:524, 2003. 3) Terpstra et al, Proc ISMRM, 14:493, 2006. 4) Slivka et al, Neurosci Lett, 74:112, 1987. Financial sponsors: NIH R01NS038672, P41RR008079, Whitaker Foundation.