Could Lipids Contribute to the Exchange-Induced Resonance Frequency Contrast in Brain Tissue?

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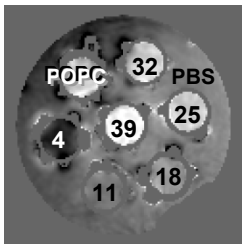
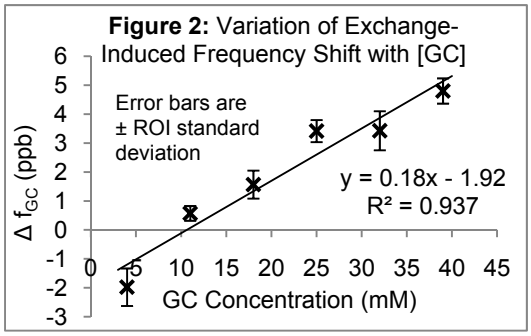
Introduction: Gradient-echo frequency images (directly proportional to the signal phase) are increasingly utilized because they provide high contrast that is complementary to conventional magnitude image contrast. Magnetic susceptibility is widely postulated to contribute to tissue frequency contrast but recent measurements in fixed human and fresh pig brain tissues [**1**] show a substantial contribution to white-gray matter (WM-GM) frequency contrast from chemical exchange of protons between water and macromolecules. Studies in protein solutions [**2,3**] found an exchange-induced frequency shift (f_x) that was directly proportional to the protein concentration. Therefore, f_x contrast has been attributed to exchanging NH and OH protons on proteins [**2**]. However, observations that myelin-rich WM has a larger f_x than GM suggest that there may be a contribution from sites of exchange in myelin, which contains more lipids than proteins [**4**]. Galactocerebrosides (GC), in particular, are a major lipid component of WM and are 4-fold more abundant in human WM than GM [**4,5**]. These lipids, whose OH groups are exposed at the surface of cell membranes, cause large magnetization transfer (MT) effects that are most likely due to chemical exchange [**6**]. Therefore, we tested the hypothesis that GC cause f_x, using an in-vitro model for WM cell membranes to investigate whether GC could contribute to the WM-GM f_x contrast in brain tissue.

Methods: To measure any f_x due to GC (Δf_{GC}), 6 GC + Palmitoyl oleoyl phosphatidyl choline (POPC) samples (with GC concentrations shown in Figs 1&2) and a POPC control (54 mM) were prepared. POPC was included in all samples because pure GC does not form stable vesicles and a constant 2:1 POPC:GC molar ratio was chosen to approximate the phospholipid to cerebroside ratio in human WM [**4-6**]. Lipid stock solutions (Avanti Polar Lipids Inc.) in chloroform / methanol were combined to achieve this ratio and the desired GC concentrations. The solvents were removed by slow evaporation under a vacuum. To form multi-lamellar vesicles (MLVs), an authentic model for WM cell membranes, the lipid films were rehydrated in phosphate-buffered saline (PBS) with 5 freeze-thaw cycles as in [**6**]. As in previous experiments [**1,3**], 1,4 dioxane was used as a reference chemical whose protons are assumed not to exchange; dioxane (15% v/v) was added to all the lipid samples and the surrounding PBS (see Fig. 1). Because local susceptibility-induced frequency shifts are identical for both water and dioxane protons, f_x can be measured by subtracting the dioxane frequency from the water frequency in every voxel [**1**]. Single-slice chemical shift MR imaging (CSI) was performed at constant room temperature using a 600 MHz vertical bore spectrometer (Bruker). CSI had 202 x 202 x 300 μm voxels, matrix size = 124 x 124, spectral width = 10 kHz, 1024 time points, TR = 1 s and flip angle = 45°. The data were band-pass filtered (FWHM 550 Hz) and centered to generate water and dioxane time-domain signals. These were spatially Fourier-transformed, giving water and dioxane magnitude and phase images at each time point. f_x was obtained from a linear fit of the phase difference between the dioxane and water signals in every voxel over time. Only timepoints having a magnitude signal-to-noise ratio greater than 10 were included in the fit. Regions of interest (ROIs) were drawn in the lipid tubes and surrounding fluid to calculate Δf_{GC}. Because the fluid had negligible macromolecule content, any apparent f_x in the fluid ROI was subtracted from the raw f_x map to correct for inaccurate centering of band-pass filters on the resonance peaks [**1**].

Results: Fig. 1 shows a map of f_x and the graph in Fig. 2 shows that Δf_{GC} increased linearly with GC concentration (at 0.18 ppb/mM). This measured Δf_{GC}, together with the ~31.5 mM WM-GM difference in cerebroside content in human brain [**5**], suggests that GC could lead to ~5.7 ppb WM-GM Δf_x in human brain tissue. This agrees with brain tissue measurements: WM-GM Δf_x = 6.3 to 13.5 ppb [**1**]. The POPC exchange-induced frequency shift was 3.14 ± 0.63 ppb.

Figure 1: f_x (-2 to +3Hz). Numbers are GC concentrations (mM). Air bubbles and tube glass have been masked out





Discussion and Conclusions: Here, exchange-induced frequency shifts were measured in MLVs formed from GC and POPC to model WM cell membranes. f_x increased linearly with GC concentration. The agreement between the positive WM-GM Δf_x measured in brain tissue and the Δf_x shift predicted from Δf_{GC} measured here (together with literature tissue cerebroside concentrations) suggests that GC could account for much of the exchange-induced contrast measured in brain tissue. Δf_{GC} (0.18 ppb/mM) is about 50 times smaller than f_x measured in BSA protein solutions (~8-11 ppb/mM) [**3**], perhaps reflecting the relative molecular sizes of BSA (~67 kg/mol) and GC (~812 g/mol) (or the number of exchanging protons on each macromolecule). The observed increase in f_x is not attributed to the increase in POPC concentration because two pilot experiments showed that f_x in POPC was negative and less than f_x in a GC sample with the same total lipid concentration (and a 2:1 POPC:GC ratio). In contrast to GC, POPC has only one exchangeable OH proton and almost no MT effect [**6**], suggesting that it does not contribute to chemical exchange processes. To confirm this, and to further understand any contribution of POPC to the f_x measured here, we plan to measure f_x over a range of POPC concentrations. The results presented here show that f_x increased with GC concentration in a WM model and suggest that GC could contribute to the WM-GM exchange-induced frequency contrast in brain tissue. These findings should aid the interpretation of contrast in MR frequency images. GC are essential for axonal myelin membrane integrity [**7-8**], therefore GC-based f_x contrast is likely to be applicable to the study of neurological diseases.

References: **1.** Shmueli et al. *MRM* **2010** **2.** Zhong et al. *Neuroimage* **2008**, 40:4:1561-6 **3.** Luo et al. *J. Magn Reson* **2009**, 202:102-8 **4.** Ed Siegel et al. *Basic Neurochemistry* **2006**, 56 **5.** O'Brien & Sampson *J Lipid Res* **1965**, 6:537-544 **6.** Kucharczyk et al. *Radiology* **1994**, 192:521-9 **7.** Bosio et al. *PNAS* **1996**, 93:13280-5 **8.** Boggs et al. *Biochimica et Biophysica Acta* **2008**, 1780:445-55