The Molecular Basis for Gray and White Matter Contrast in Phase Imaging

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Introduction: Phase images acquired at 7 Tesla (Figure 1) showed superior gray matter (GM) and white matter (WM) contrast with a contrast-to-noise ratio (CNR) gain of up to 10 compared to conventional magnitude images (1). A multi-field GM/WM phase contrast study also showed that the GM/WM phase difference is a pure frequency effect (2). Possible sources of susceptibility differences between GM and WM, such as blood deoxy-hemoglobin, tissue myelin content, and tissue iron content have been discussed (1). However, none of those factors can fully explain the observed *in vivo* phase difference between GM and WM. We proposed here that the GM/WM phase contrast originates from the *in vivo* water-macromolecule exchange processes. The exchange induced direct water frequency shifts due to different mobile macromolecule concentrations are observed in our study and this effect could explain well the *in vivo* GM/WM phase contrast (5.5 Hz). Such a direct frequency effect has not been assessed in previous water-macromolecule exchange studies despite the overwhelming number of publications in this field.

Methods: Dulbecco's Phosphate Buffered Saline (DPBS) solution (pH = 7) was used to simulate the *in vivo* physiological conditions. D_2O (99.9%) was added to the buffer solution resulting in a D_2O concentration of 5% (v/v). TMSP (tetramethyl-silyl-propionate, 50 mM) was used for NMR frequency reference. BSA (bovine serum albumin, 67kDa, CAS-No. [9048-46-8]) was used to study the water-macromolecule exchange. Samples were prepared with concentrations of 0, 10, 20, 40, 60, 75, 100, 120 and 150 mg /ml. All BSA measurements were performed on a Bruker DRX-400 400 MHz NMR spectrometer. All samples were stabilized at 303 K before each measurement. D_2O resonance was used to lock the field frequency. Standard FID with 48 averages was acquired for each sample with 64K data size and a digital resolution of 0.125 Hz/Pt. In addition, three samples (0, 20, 75 mg/ml) were stabilized at 288, 293, 298, 303, 308 and 313 K for the temperature dependence measurements. Water frequency was determined as the frequency separation between the water resonance and TMSP (zero ppm). The BSA-related water frequency shifts are reported as the difference from the water frequency in bulk buffer solution (no BSA).

Results and Discussion: Due to the high complexity of tissue macromolecule compositions *in vivo*, the water shift du to different macromolecule concentration is difficult to quantitate. Therefore as a model the water-BSA system was used. This forms the basis for the discussion of the *in vivo* GM/WM phase contrast. The dependence of the water frequency shift on BSA concentration is plotted in Figure 2. A linear relation between the water frequency shift and the BSA concentration is observed. The frequency shift is 0.040 ppm per mM of BSA. Water-BSA temperature dependence was determined as 0.00067 ppm per mM•K. To our knowledge, this is the first demonstration of a direct water frequency shift due to water-macromolecule (BSA) exchange.

The dependence of the transverse relaxitivity R_2 on BSA was determined in (3) as 0.087 s^{-1}/mg/ml at 7 Tesla (300 MHz). Assuming that both frequency and R_2 differences between GM/WM are induced solely by macromolecules (in BSA equivalent units), the CNR gain (CNR_g) for phase images compared to magnitude images can be derived using the equation given in (1), e.g. $CNR_g \approx 2 \cdot \pi \cdot TE \cdot \Delta F/(\exp(-TE \cdot \Delta R_2)-1)$, where ΔF is the GM/WM frequency separation and ΔR_2 is the R_2 difference between GM/WM. For a GM/WM frequency separation of 5.5 Hz with 21.5 ms echo time, CNRg is around 13.2. This value is close to the *in vivo* measurement at 7 T in this study (CNRg = 10.5). Therefore the water-macromolecule exchange processes are the first proposed mechanism that can fully explain the *in vivo* GM/WM phase contrast. The CNRg difference between *in vivo* and calculated results might be due to different *in vivo* macromolecule compositions and other contributions such as blood deoxy-hemoglobin which is not included in the BSA model.

The small water frequency shift due to macromolecules (0.04 ppm/mM BSA) explains why the effect was largely omitted in most exchange studies. However, this effect is still on the same order of magnitude (12 Hz per mM BSA) compared with the 6 Hz maximum GM/WM frequency separation at 7 T. Therefore, an accurate knowledge of macromolecule concentration in brain tissue will help to understand the *in vivo* GM/WM phase contrast behavior. Recent magnetization transfer studies (4) suggested that the predominant fast water exchange sites on macromolecules are assigned to amide and hydroxyl groups of proteins and peptides which are MR observable mobile macromolecules. Spectroscopic studies have reported relative *in vivo* mobile macromolecule concentrations on the order of millimolar and a higher mobile macromolecule level (5) in normal GM (~ 40%) compared to WM. In addition, the macromolecule fraction of brain cytosol in rats and humans and their NMR resonances were also characterized (6). The macromolecule concentration is around 40 mg/g tissue wet weight and is consistent with a recent study using tuberculous brain abscess cell culture (7) showing a total protein concentration around 60 mg/ml. The chromatographic separation of the cytosolic macromolecule fraction suggested an average macromolecule molecular weight between 48 ~ 63 kDa in the brain cytosol and therefore a protein concentration on the order of 1 mM. Based on the water-BSA frequency shift, the mobile macromolecule *in vivo* concentration in GM and WM can be estimated from the difference in spectroscopic measurements (40%) and the *in vivo* frequency separation of 0.02 ppm (≈ 0.45 mM BSA, 37 °C). This results in a WM concentration of 1.1 mM (in BSA equivalent unit) and 1.6 mM for GM. These values are in good agreement with the above mentioned results from cell culture and spectroscopic measurements.

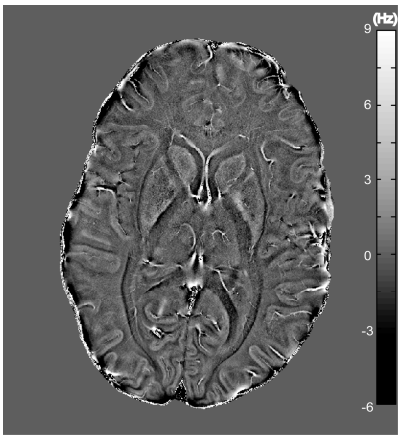

Figure 1: Phase images acquired at 7 T from this study showing superior GM/WM contrast.

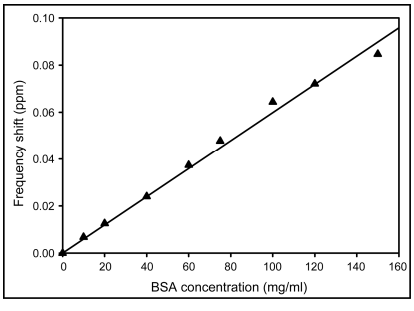

Figure 2: Water Frequency shift due to water-macromolecule (BSA) exchange. The frequency shift is 0.04 ppm/mM.

Conclusion: Phase contrast determined by water macromolecule exchange provides a potentially novel method to study molecular processes *in vivo*. This is especially promising in neurodegenerative diseases such as multiple sclerosis (MS) or Alzheimer disease. A recent report suggested that MS lesions could have different contrast in phase images. This observation could be explained by the water-macromolecule exchange model since a spectroscopic study in MS (8) showed that acute MS lesions have a significantly higher macromolecule concentration compared to chronic lesions or normal tissue. Therefore, phase imaging might open a new diagnostic window to evaluate numerous pathologies involving macromolecule alterations.

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