Contribution of protein-induced magnetic susceptibility and ^{1}H exchange effects to water MR frequency shifts

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Introduction: Conventional gradient recalled echo (GRE) MR phase images acquired at high field show superior contrast between gray matter (GM) and white matter (WM) in human brain (1). Possible origins of this shift, such as magnetic susceptibility effects due to tissue lipids, non-heme iron, deoxyhemoglobin (1) as well as water-protein ^{1}H exchange (2) have been suggested. However, none of these factors fully account for the observed frequency shifts. Previously, the magnetic susceptibility of tissue protein has also been discussed in conjunction with MR signal frequency shifts (3, 4). Herein the protein-susceptibility-induced frequency shifts are separated from those of water-protein ^{1}H exchange. In native protein solution, the water ^{1}H MR frequency shift is dominated by magnetic susceptibility effects, while the contribution from water-protein ^{1}H exchange effects is rather small.

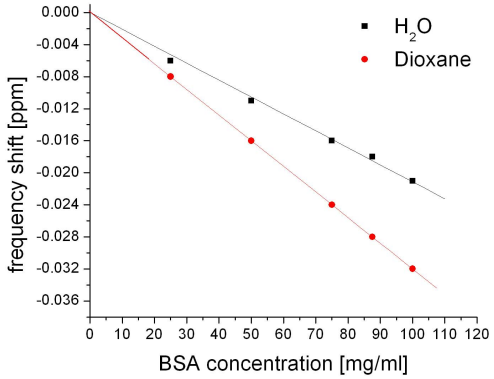
Methods: Phosphate buffered saline (PBS) solution (PH=7.4) with various concentrations of bovine serum albumin (BSA, 66 kDa, CAS No. [9048-46-8]) simulated *in vivo* conditions. TSP-d_4 (2,2,3,3-tetradeuterio-3-trimethylsilyl-propanoate, 50mM) and dioxane (1%) were employed as NMR frequency references. Two sample sets were prepared, in 99% D_2O (v/v) and in 10%D_2O (v/v), at concentrations of BSA 0, 25, 50, 75, 87.5, 100 mg/ml. Radiation damping was eliminated in the 10% D_2O solutions by detuning the receiver coil and reducing its filling factor. A coaxial set of two NMR tubes was used. The inner-most coaxial tube was filled with protein solution; the outer tube was used as a reference without protein. All measurements were performed on a Varian Inova 500-MHz (11.74–T) spectrometer. Samples were stabilized at 298 K before each measurement. The D_2O ^{2}H resonance provided a field/frequency lock signal. Sixteen transients were acquired for each sample with 128 K complex data points, providing digital resolution of 0.15 Hz/pt. Samples in 10% D_2O solution were additionally examined at 310K (37°C). The frequency shift of each resonance (water, dioxane, and TSP) caused by the presence of protein was determined as the corresponding frequency difference between inner and outer tubes.

Results and Discussion: The figure at right shows the MR frequency shift of water and dioxane at different protein concentrations, measured at 25°C in 99% D_2O solution. Since dioxane does not interact with BSA (*vide infra*), the frequency shift of dioxane can be attributed solely to a susceptibility effect:

$$(\Delta\nu/\nu_0)_{susceptibility} = (\Delta\nu/\nu_0)_{Dioxane} = (-0.32 \pm 0.01) \times 10^{-3}\, ppm/(mg(BSA)/ml)$$

The frequency shift of water is the sum of susceptibility and water-protein ^{1}H exchange effects. The frequency shift due to water-BSA ^{1}H exchange can be estimated by subtracting the dioxane-reported susceptibility shift:

$$(\Delta\nu/\nu)_{exchange} = (0.11 \pm 0.02) \times 10^{-3}\, ppm/(mg(BSA)/ml)$$



Protein susceptibility decreases the ^{1}H resonance frequency; ^{1}H exchange with labile sites on BSA increases the ^{1}H frequency. However, magnetic susceptibility clearly dominants the water MR frequency shift in native protein solution. Because the majority of protein in brain tissue is cross-linked as cytoskeleton fibers (5), the effect of ^{1}H exchange *in vivo* may be even smaller than in native protein solution. Experiments conducted using 10% D_2O at 25°C produced essentially identical results, consistent with the similar volume magnetic susceptibility of D_2O and H_2O (6), and the fast ^{1}H exchange between water and hydrophilic labile groups on the protein surface (7).

Comparing studies conducted at 25°C *vs.* 37°C, the dioxane frequency linear dependence on protein concentration is temperature independent, while the water frequency linear dependence is shifted by 4.12E-5 ppm/(mg(BSA)/ml).

In our experiments, we also included the commonly used ^{1}H MR reference TSP, whose frequency shift is expected to report only magnetic susceptibility effects (like dioxane). However, we found that (a) the TSP frequency shift is temperature dependent and (b) its resonance linewidth ($\Delta\nu = 1/\pi T_2^*$) is strongly dependent on BSA concentration (mg BSA / ml solution): $\Delta\nu$ (TSP) = $\Delta\nu_0$ + (0.0641±0.0076)×[BSA]×$\Delta\nu_0$, where $\Delta\nu_0$=1.57Hz, is the TSP linewidth in solution without BSA. These two observations indicate the presence of an interaction or association between TSP and BSA. Such dependences were not observed for dioxane. Hence, using TSP as a ^{1}H frequency reference to quantify magnetic susceptibility effects induced by proteins could lead to significant systematic errors.

Conclusion: We have demonstrated that the MR frequency shift of water is affected by the presence of protein through two effects – changes in the magnetic susceptibility of the solution and water-protein ^{1}H exchange. The water frequency shift due to protein susceptibility is about three times larger (and with opposite sign) than the frequency shift due ^{1}H exchange. In the biological tissues (for example brain) significant protein content is cross-linked, eliminating sites of exchangeable ^{1}H and thus possibly further reducing frequency shift contributions from water-protein ^{1}H exchange.

References: 1. Duyn J., et al, PNAS 2007, 104:11796-11801; 2. Zhong K. et al. NeuroImage 2008, 40:1561-1566; 3. Spees WM, et al, MRM 2001, 45:533-542; 4. He X and Yablonskiy DA, MRM 2007, 57:115-126; 5. Koenig S.H. et al. MRM 1993, 29:311-316; 6. Ergin YV, et al, J. Struct. Chem. 1970, 11:481-482; 7. Liepinsh E and Otting G, MRM 1996, 35:30-42.