Molecular Size Dependency of Water Macromolecule Exchange Induced Frequency Shift

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Introduction: A recent study suggested that water macromolecule exchange (WME) processes can contribute to the *in vivo* gray matter (GM) and white matter (WM) contrast in high field phase imaging [1]. Due to the complex nature of macromolecule-water interaction, bovine serum albumin (BSA) was used as a model system to demonstrate this effect. It is assumed that the WME interaction depends on the macromolecule size, conformation, and the surrounding chemical environment. The goal of this work was to study systematically the WME shift abilities of several proteins with different molecular weight (Mw) under neutral pH and to evaluate the possible relationship between WME shifts of different macromolecules and their potential contributions to *in vivo* phase contrast.

Theory: The WME frequency shift arises from the fast exchange of bulk water with the hydro-layer of macromolecules that have a different chemical environment. This effect can be characterized by the standard two-site exchange model that has been well described [2]. The exchange is on the fast side (10^{-9} s) and the frequency shift of the bulk water *f* can be determined as $f = f_0 + p \cdot \Delta f$ (f_0: bulk water frequency without macromolecules; Δf: chemical shift separation; *p*: time proportion for water molecules reside in the macromolecular pool). However, the two-site exchange model can not directly be applied for calculating the WME shift since the exact retention time of water on the macromolecule can not be measured precisely and the chemical shift difference also depends strongly on the macromolecule conformation and molecular motion, e.g. molecule rotation. For globular proteins, the molecule weight is correlated with its Stokes radius R_S (Fig. 1A), suggesting that the chemical behavior of the outer hydro-layer is similar for different molecular weight. For low protein concentration in the mM range, the WME between water and globular proteins can be expressed using the first term from a virial expansion as $\Delta f = k_a \cdot M \cdot \exp(bM)$, where M is the macromolecule concentration and k_a the WME interaction strength. Conceptually the WME process could also be described by Brownian motion of water molecules around the macromolecule (Fig.1B). The mean translational diffusion length D_L of free water and the rotational diffusion length D_R for macromolecules can be used to characterize the interaction strength by the correlation scale, e.g. $k_a \sim \exp(-D_R/D_L)$.

Methods: The experimental details are equivalent to [1]. Dulbecco's Phosphate Buffered Saline (DPBS) solution (pH = 7) with 5% (v/v) D_2O was used to simulate the *in vivo* physiological conditions. TMSP (tetramethyl-silyl-propionate, 25 mM) was used for NMR frequency reference (zero ppm) and water frequency shifts are reported as the difference from the water frequency in bulk buffer solution (no proteins). Four proteins (Ribonuclease A: 13.7 kDa; BSA: 67 kDa; Aldolase: 158 kDa; Thyroglobulin: 669 kDa) were used for the study. All measurements were performed on a Bruker DRX-400 400 MHz NMR spectrometer. Samples were stabilized at 298, 303, and 308 K and the respective water frequencies were determined. In addition, BSA with different sucrose concentrations (0 to 100 mM) was used to investigate the effect of D_L on the WME shift.

Results and Discussion: The Mw dependency on Rs for selected globular proteins is shown in Fig. 1. The power order for Rs from fitting is 2.83 and deviates slightly from 3 for an ideal sphere. The high correlation (R = 0.998) suggests that the hydro-layer property for different molecular weight is very similar. The dependency of k_a on Rs for four different globular proteins is shown in Fig. 2. For small protein size such as Ribonuclease A (13.7 kDa), k_a is about 20 times smaller compared to that of BSA. This effect can be attributed to the faster tumbling rate of small molecules ($D_R \gg D_L$) that reduces the frequency shift due to dipole averaging (motional narrowing) (Fig. 1B). For $D_R \le D_L$ (slow regime) k_a will be dominated by water translational diffusion and the macromolecule conformation. This is indeed the observation for macromolecules with higher Mw and Rs. Fig.2 suggests that the fast to slow regime transition occurs around 60 kDa, and interestingly, BSA seems to produce the strongest k_a among the proteins studied so far. Fig. 3 shows k_a of BSA for different sucrose concentrations and temperatures. The BSA k_a increases with high temperature (from 298 to 308 K) and decreases with high sucrose concentration due to alternation in D_L. The trend of BSA k_a changes is very similar for all three temperature conditions, suggesting that the BSA conformation is not significantly modified in this temperature range. The BSA/sucrose data support further the model that k_a of different macromolecules could be characterized by their corresponding Mw and Rs, using a calibration curve similar to that shown in Fig. 2. This result can be readily extended to *in vivo* situations: As long the distribution of macromolecules for a given tissue type could be determined, the total *in vivo* frequency shift from all macromolecules can be quantitated by a single parameter that depends only on Mw and macromolecule fraction. This result will assist the quantification of frequency shifts for different tissue types and help to establish a relationship between the macromolecule content and the phase contrast changes for *in vivo* phasing imaging.

Conclusion: The WME shift ability of macromolecules depends strongly on their size and has been determined quantitatively. A strong relationship between the macromolecule distribution and the corresponding *in vivo* frequency shift for phase imaging is thus predicted. This finding paves the way for phase imaging applications in pathologies with altered *in vivo* macromolecule content.

Reference: [1] Zhong K, et al. Neuroimage. 40(4):1561-6 (2008)
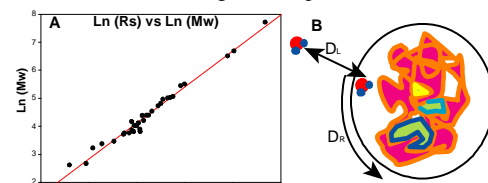   [2] Protein NMR Spectroscopy, Elsevier (2007)

Fig.1: A) Relationship between Mw and Rs for selected globular proteins (17 – 2200 kDa; source: CRC Handbook). B) Depiction of WME process through Brownian motion. For $D_R \gg D_L$, the WME is dominated by dipole averaging (fast regime). The transition occurs at $D_R \le D_L$ (slow regime).
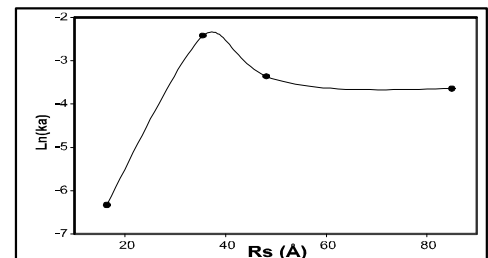

Fig. 2: ka as a function of protein size. For Rs < 20 Å the shift ability is significantly smaller due to fast molecule tumbling. ka decreases slowly for Rs > 40 Å, probably due to conformational difference for larger macromolecules.
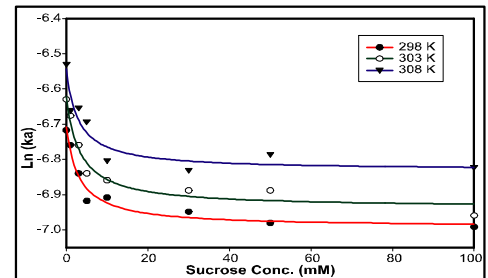

Fig. 3: BSA ka changes for different sucrose conc. (0-100 mM) at 298, 303, and 308 K. The change is correlated to translational diffusion (D_L) of free water modified by different sucrose conc. (viscosity) and temperatures.