CHEMICAL EXCHANGE SATURATION TRANSFER AND R1RHO DISPERSIONS OF POLYPEPTIDES WITH VARYING COMPLEXITIES

K. Li[1,2], J. G. Cobb[1,3], J. Xie[1,2], Z. Zu[1,2], D. F. Gochberg[1,2], and J. C. Gore[1,2]

[1]Institute of Imaging Science, Vanderbilt University, Nashville, TN, United States, [2]Department of Radiology, Vanderbilt University, Nashville, TN, United States, [3]Department of Biomedical Engineering, Vanderbilt University, Nashville, TN, United States

Introduction: Chemical exchange saturation transfer (CEST) imaging techniques have been developed to detect the proton exchange between water and mobile solute molecules which contain exchangeable protons. These techniques have been applied to detect endogenous and exogenous proteins and peptides with very low concentrations. In CEST, the measured contrast is sensitive to pH, temperature, concentration etc. To obtain CEST contrast, low-power saturation pulses are applied at opposite side of the water resonance at particular offsets, such as 3.5 ppm for amide proton transfer (APT) imaging [1]. The increase of APT image intensity in brain tumors was tentatively explained by increase of cytosolic protein and peptide content [1]. As an alternative, the measured relaxation rates in the rotating frame by using spin-locking techniques are also sensitive to exchanges, which are reflected by dispersions in measured $T_{1\rho}$ values [2]. We hypothesize that CEST contrast and $T_{1\rho}$ relaxation rates are sensitive to the complexity of exchanging molecule and not just the amide content. To test this idea, preliminary studies were performed on a poly-L-Lysine (PLL) model system with different molecular weights, thus different complexities. It is demonstrated that the CEST effects, $T_{1\rho}$ dispersions and relaxation properties of such model systems are strongly related with the integrity of polypeptides, as pointed out in our previous studies [3].

Methods: The data were acquired on a 9.4T Varian magnet with a Litz 38 quadrature coil. PLL samples with low, medium and high molecular weights of 0.5-2 (LW), 15-30 (MW), and 30-70 (HW) kDA were prepared in 0.6 ml plastic tubes, in 1X phosphate-buffered saline (PBS) and titrated to pH 7.4 with either HCl or NaOH solution as necessary. All samples have concentrations of 10mg/ml. In addition, a control tube with same volume of 1x PBS alone was also measured, as a reference. All four samples were put into a 50 ml plastic tube full of 3mM $MnCl_2$ solutions. The temperature of the samples was controlled at 37°C during the measurements. The CEST data were acquired using an 8 s continuous-wave saturation pulse (~1 μT, offset varied between -6 and 6 ppm, step by 0.1 ppm), followed by a single-shot spin-echo echo-planer imaging (EPI) readout (TR = 30 s, TE = 38 ms). Other imaging parameters include: field of view 16×16 mm^2, slice thickness 1 mm. In addition to CEST data, R_1 values were measured using a ten-point inversion recovery sequence (inversion recovery time spaced from 5ms to 10s, a constant pre-delay of 3.5s) with fast-spin-echo (FSE) readout; and R_2 values were measured using a multi-echo sequence (TR = 6 s, TE =10 ms, 80 echoes). To correct for B_0 inhomogeneities, a spoiled-gradient echo sequence with two echo times was acquired (TR = 28 ms, TE = 5 and 8 ms). $T_{1\rho}$ dispersions were measured with a spectroscopic spin-locking sequence consisting of an adiabatic 90-degree pulse (AHP), followed by on-resonance spin-lock (SL) pulse and FSE readout. The SL pulse was varied in 10 time increments (SLT) between 20 ms and 1 sec, and also in amplitude between $2\pi \times$ [150 and 10 kHz]. $T_{1\rho}$ values were calculated by fitting signal decay to a three-parameter mono-exponential decay function in MATLAB.

Results: The CEST z-spectra, MTR asymmetry (MTR_{asym}) spectra, and $T_{1\rho}$ dispersion curves of the PBS, LW, MW, and HW PLL samples are shown in Fig. 1 (a), (b) and (c), respectively. The MTR_{asym} was estimated by $MTR_{asym} = [S(-\Delta\omega) - S(\Delta\omega)] / S_0$, where $\Delta\omega$ is the offset from the central water frequency. $S(\Delta\omega)$ and $S(-\Delta\omega)$ are two signals at opposite frequency offsets and S_0 is taken at 6 ppm. R_1 values are determined as 0.247, 0.258, 0.253, and 0.253 Hz for PBS, LW, MW, and HW, respectively; while R_2 values are 1.214, 1.487, 1.749, and 2.030 Hz.

Discussion: It was found that the R_1 values of the PLL samples are very close to that of the PBS sample. However, R_2 increases linearly with molecular weights. From the z-spectra and MTR_{asym} spectra, as given in Fig. 1 (a) and (b), it was found that these PLL samples have different chemical shifts and CEST effects. For the LW, MW and HW PLL samples, the chemical shifts are 2.8, 3.3, and 3.7 ppm, respectively. The MTR_{asym} of the LW sample is approximately two times of that of the HW sample. However, the MTR_{asym} of the MW sample is much smaller. Since all samples have same amide proton concentrations, it indicates that the chemical exchange rates in these samples are different from each other. This point is supported by the $T_{1\rho}$ dispersion measurements, as shown in the dispersion curves. In summary, the complexities of polypeptides play important roles in relaxation properties and contrast mechanism in tissue model systems.

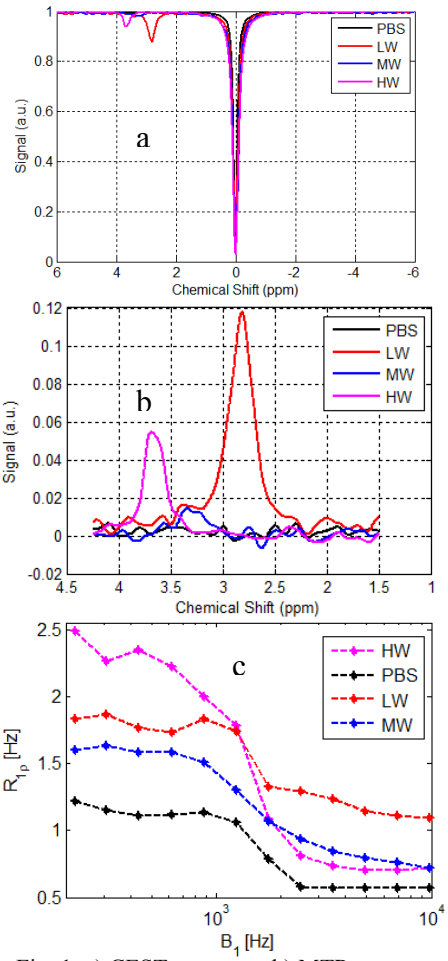

Fig. 1. a) CEST z-spectra, b) MTR_{asym} spectra, and c) R1rho dispersion curves of PBS, LW, MW, and HW PLL samples.

References: [1] Zhou J et al., MRM 2008;60;842-849. [2] Duvvuri U et al. MRM 1997;38;863-867. [3] Gore JC et al. MRM 1986;3(3):463-466.

Acknowledgements: This research is supported by NIH EB000214.