High field MR imaging of proteins and peptides based on the amine-water proton exchange effect

Tao Jin[1], and Seong-Gi Kim[1]

[1]*Department of Radiology, University of Pittsburgh, Pittsburgh, Pennsylvania, United States*

Introduction Pathological changes in the protein or peptide concentrations occur in many diseases including tumor or Alzheimer's disease. The amide proton transfer (APT) effect has been proposed as a non-invasive imaging biomarker to detect changes in mobile protein levels [1-2]. APT is a variant of the chemical exchange saturation transfer (CEST) technique targeting the proton exchange between water and labile amide proton from protein and peptides. However, the *in vivo* imaging contrast is only a few percent of the water signal because the amide water exchange is quite slow. Beside the backbone amides, there are many other labile protons, such as amine and hydroxyl protons in the side chains of proteins and peptides. Although the concentration of these labile protons may be less than the amides, their exchange rates with water are much faster. Recently, it has been proposed that the amine-water proton exchange (APEX) dependent contrast may be exploited to improve the sensitivity of protein/peptide imaging at a high magnetic field [3]. In this preliminary study, we investigated the exchangeable amide, amine, and hydroxyl protons from several protein and peptide phantoms at 9.4 T, and examined their sensitivities for protein/peptide imaging and pH dependence.

Materials and methods MRI experiments were performed on a 9.4T Varian MRI system. A 3.8-diameter volume coil was used for excitation and reception. Four types of protein/peptide phantom were prepared: (1). 5mg/ml poly-l-lysine (PLL) were dissolved in phosphate buffered saline (PBS) and titrated to pH = 3.8, and 7.3; (2) 6 mg/ml protamine was dissolved in PBS and titrated to pH = 6.0, 6.8, 7.4, and 8.0; (3) 5% Bovine serum albumin (BSA) were dissolved in de-ionized water and titrated to pH = 6.2, 6.6, 7.0, 7.5, and 8.0; (4). 5% egg white albumin (EWA) were dissolved in PBS and titrated to pH = 6.2, 6.8, 7.4, and 8.0. The PLL, protamine, BSA phantoms were measured at 21°C, while the EWA phantoms were measured at both 21°C and 37°C. B_0 and B_1 maps were obtained to examine the homogeneity of the static magnetic field and RF field, and a T_1 map was measured with an inversion recovery pulse sequence. An off-resonance spin-locking imaging sequence, which is similar to the CEST approach but less direct water saturation effect, was used for the protein/peptide experiments [4]. The Z-spectra were measured from a frequency offset range of Ω = 8 to -8 ppm from water, with a 75 Hz (low power) and 5 s continuous wave irradiation pulse. For EWA phantoms, Z-spectra were also measured with a 150 Hz (medium power) and 5 s irradiation, as well as a 500 Hz (high power) and 2 s irradiation pulse. Control images were acquired at a frequency offset of 300 ppm for signal normalization. Immediately after the irradiation preparation, spin-echo EPI images were acquired on a 6 cm slice. The field of view was 4 cm × 4 cm, and the matrix size was 64 × 64. The repetition time was set at about five times of T_1. For data analysis, area of interests were selected on each phantom based on the B_0 map, where minimal field inhomogeneity (<3 Hz) was observed. From the Z-spectra, SLR_{asym} were calculated from these ROIs using $SLR_{asym} = M(\Omega) - M(-\Omega)/M_0$.

Results and discussions With a 75 Hz irradiation pulse, PLL samples showed a 3.6 ppm amide peak for pH = 7.3, and a 2.8 ppm amine peak for pH = 3.8 (Fig. A). PLL is a polypeptide with backbone amide groups as well as the ε-NH_2 from lysine residues. While the amide water proton exchange is very slow and can only be detected at a pH of 7.3, the amine proton exchange is too fast at this pH and can only be observed when the exchange is greatly slowed down, *e. g.*, for the pH = 3.8 sample. The spectra of protamine were quite different, showing two peaks at 3.6 and 1.9 ppm but none at 2.8 ppm (Fig. B). As identified by a recent protamine CEST study [4], the 3.6 ppm peak is from the backbone amide groups while the 1.9 ppm peak is due to the guanidino amine from the arginine side chains. Note that the 1.9 ppm guanidino amine is also present in Creatine and has been observed in many previous CEST studies. BSA showed an asymmetry peak at 2.8 ppm for low pH samples, while the spectra for pH = 7.4 and 8.0 samples were broader and the peak magnitude was reduced (Fig. C), suggesting that the amine-water proton exchange has deviated from the slow exchange regime at these higher pH values. The difference in BSA and protamine may likely be attributed to their compositions: while ~2/3 of protamine's amino acid residue is arginine, BSA has ~10% lysine but only ~4% arginine residues. In the EWA phantoms (Fig. D), which has ~ 6% lysine and also ~6% arginine residues, four different peaks can be identified: 3.6 ppm for amide, 2.8 ppm for amine from lysine residue, ~2.0 ppm for amine from arginine residue, and 0.9 ppm for hydroxyl, respectively. While the EWA data were shown for 37°C, these four peaks can also bee observed at 21°C with different peak intensities due to slower exchanges (results not shown).

One important feature of the off-resonance spin-locking technique is its B_1-tuning capability [3], *i. e.*, when the labile protons have a range of exchange rates (or pH values), the chemical exchange contrast will be maximized for those protons when the exchange rate k, matches $2\pi\omega_1$ (= γB_1). Interestingly, for EWA, the maximum of each peak is reached at different pH values (Fig. D). Assuming the exchanges are base-catalyzed in the pH range studies, the exchange rates of the labile protons we observed can be determined as: amide < arginine amine < hydroxyl < lysine amine. This is in line with a previous study which reported an exchange rate of 700-1200 s^{-1} for arginine amines and 4000 s^{-1} for lysine amines [5].

At physiological pH, the irradiation pulse power that maximizes the *in vivo* APT effect is on the order of 1 μT (≈ 42 Hz) [6]. While such a low power irradiation is optimal for slow chemical exchanges, higher irradiation pulse power would be necessary to tune the chemical exchange imaging contrast to the faster APEX effect [3]. As shown in Fig. E, irradiation with a medium power of 150 Hz gave higher SLR_{asym} for all the four peaks, but the peaks became broader because the faster chemical exchanges reduced the specificity. Note that the saturation efficiency for amide protons is already close to 1 even with a 75 Hz irradiation; thus the elevation of the amide peaks at 3.6 ppm with higher power pulses (Fig. E and F) is due to the contributions from neighboring faster exchanging protons, especially the amines from lysine residues. With a high irradiation power of 500 Hz (Fig. F), the peaks merge together and become indistinguishable. In a relative wide offset range of 2.0 – 3.5ppm, a 33-42% contrast can be detected for pH values of 6.2 to 7.4, which may have contributions from all of the exchangeable protons with different weightings, but the majority should come from amines of lysine and arginine residues.

In summary, faster exchanging protons, in particular the amines from lysine and arginine residues, can be exploited at high fields for the imaging of proteins and peptides. While the specificity to a certain type of labile proton may be reduced with a higher irradiation pulse power tuning to the APEX, the sensitivity enhancement can be as high as several times greater than the APT effect, depending on the magnetic field strength and the water R_1 and R_2 values.

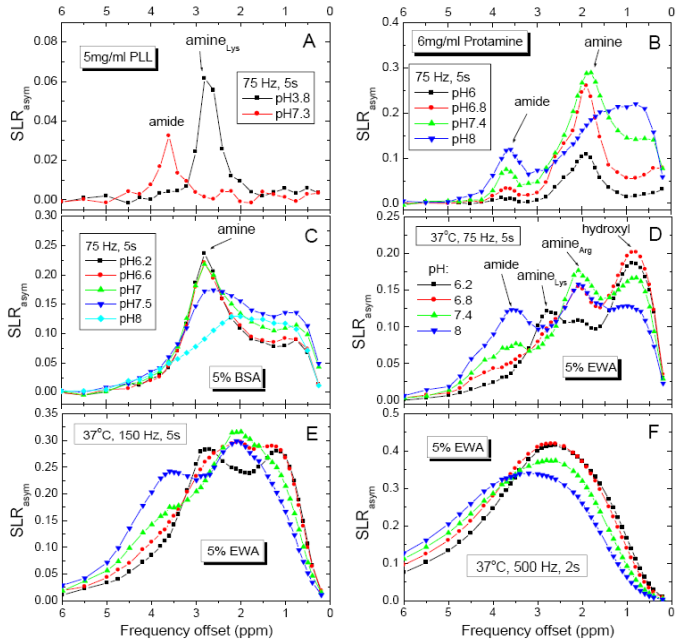

Fig. (A). The SLR_{asym} spectra for 5mg/ml PLL (A), 6mg/ml protamine (B), and 5% BSA (C) were measured at 21°C with a low power irradiation pulse. The 5% egg while albumin (EWA) spectra were measured at 37°C, with low power (D), medium power (E) and high power (F) irradiation pulses.

References [1]. Zhou JY et al. Nat Med (2003). [2]. Zhou JY et al. Nat Med (2011). [3]. Jin T et al. NeuroImage (in press). [4]. McMahon MT et al. MRM (2008). [5]. Liepinsh E et al, MRM (1996). [6]. Sun PZ et al. MRM (2007).