Developing New Multicolor Protein MRI Contrast Agents

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INTRODUCTION

Recently, Sherry, Aime and coworkers produced a series of small paramagnetic agents that can be distinguished in-vitro based on frequency-dependent contrast[1-4], called Paramagnetic Chemical Exchange Saturation Transfer (PARACEST) agents. We are interested in developing non-metallic, protein MRI contrast agents which may be expressed via a reporter gene and have the potential to be distinguished based on frequency too. We have shown that contrast enhancements by factors as large as 500,000 can be achieved for the simple protein sequence PLL, in which a large concentration of rapidly exchangeable amide protons (k_{ex} values of about 100-300 Hz) is present per molecular weight unit (e.g. 4.78 amide protons/kD for PLL) [5]. In this study, as a first demonstration, we tested four different polypeptide sequences that can be distinguished from one another using CEST due to a difference in chemical shift for their exchangeable protons. In addition, previous studies by Englander, Wüthrich, Rosenberg and coworkers have produced equations for *a priori* prediction of exchange rates in proteins [6-9], allowing us to optimize contrast in CEST agents. We tested these by verifying the predictions with experimental data taken on a set of peptides using the Quantifying Exchange with Saturation Time (QUEST) experiment[10,11].

METHODS

Theory: HX (X = O or N) exchange rates were predicted based left (l) and right (r) neighboring peptides using the combined base-catalyzed and acid-catalyzed equation[6] :

$$k_{ex} = k_{A,ref}(A_l \times A_r)[H+] + k_{B,ref}(B_l \times B_r)[OH-] + k_{W,ref}(B_l \times B_r) \quad [1]$$

where: $k_{A,ref}$, $k_{B,ref}$, and $k_{W,ref}$ are the acid, base and water rate constants for the appropriate reference peptide, A is the specific acid rate factor for the neighboring peptides, and B is the specific base factor for the neighbor. This equation can be used for both backbone amide protons (HN) and sidechain exchangeable groups (NH_2, OH, SH), by adjusting the reference rates. The temperature dependence of these rates can be added as well, by adjusting each rate according to the expression:[7]

$$k_{A,B,W}(T) = k_{A,B,W}(293)\exp(-Ea[1/T-1/293]/R) \quad [2]$$

where Ea is the activation energy for each rate. CEST contrast increases exponentially with chemical exchange rate (expression in Snoussi et al. [12]), provided a sufficiently strong saturation field is present to fully saturate the exchangeable group. This saturation strength will need to increase for higher exchange rates. *Materials:* Poly-L-Lys (PLL, 30 kD), Poly-L-Arg (PLR, 35 kDa), Poly-L-Asn (PLN, 10.7 kD), and Poly-L-Thr (PLT, 7.6 kD) were purchased from Sigma and diluted to the equivalent concentration of amino acids (amide protons), which for 35kD polypeptides is 100uM in pH 7.4 0.01M PBS buffer. In addition, the 12 residue peptides d-$(LysSer)_6$, d-$(LysLys)_6$, d-$(LysGly)_6$, d-$(LysHis)_6$, and d-$(ArgSer)_6$, d-$(ArgArg)_6$, d-$(ArgGly)_6$, d-$(Arg\ His)_6$ were synthesized by Sigma-Genosys. *.NMR Experiments:*The experiments were all run at 310 K on an 11.7T Bruker Avance system using a triple axis gradient NMR probe. Z spectra (relative water saturation as a function of saturation frequency) were taken with the saturation transfer sequence consisting of a saturation pulse with variable offset, power and time followed by a 10 μs π/2 pulse,-Grad-20μs π pulse-Grad- acquire. The hard 10μs and 20μs pulses were centered on the water resonance. QUEST [10,11]experimental data were collected using a 200Hz saturation pulse, with saturation frequencies of +/- 3.65 ppm from water, varying the saturation time between 1 – 10 s.

RESULTS AND DISCUSSION

In Figure 1 on the left the contrast generated by four peptide sequences is plotted as a function of irradiation frequency, displaying the four characteristic frequency dependencies or "colors" of the agents. The data was taken with a saturation power of 200Hz, and 10s of saturation. For all peptides but PLL, multiple exchangeable proton types are detected. We have also tested this concept out on an imaging phantom, and were able to discriminate between the different "color" agents in the resulting images.

For these prototypes, faster chemical exchange rates would result in larger contrast, and so we were interested in determining a way to predict which peptide sequence modifications would increase these rates. In Figure 2 on the left, we used the equation described above to show how changing the amino acid sequence of PLL will affect the exchange rate and compared the predictions to the experimental measurements. A good correlation was found between the predictions and the empirical results (R^2= 0.7, n=5). For example we were able to double the exchange rate of PLL by altering every second Lys with Ser in the sequence. We are interested in optimizing the other three "colors" as well.

Figure 1. Experimental Frequency Dependence of Contrast

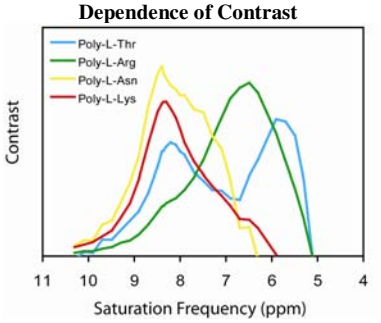
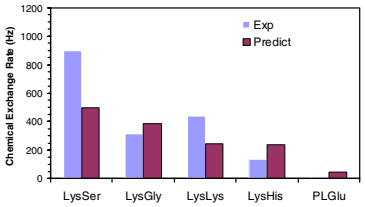


Figure 2. Predicted and Experimental Chemical Exchange Rates



CONCLUSIONS

In this study we defined four prototype polypeptide sequences that can be distinguished from one another by their CEST irradiation frequency and exchange properties. Mathematical modeling was found to be useful as a tool for predicting the proton exchange rates and designing a second generation of more sensitive CEST contrast agents.

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