Genetic Engineering of Human Protamine-1 for use as MRI Reporter Gene Based on Proton Exchange

A. Bar-Shir[1,2], G. Liu[1,3], X. Song[1,2], P. Walczak[1,2], M. T. McMahon[1,3], P. C. van Zijl[1,3], J. W. Bulte[1,2], and A. A. Gilad[1,2]
[1]Division of MR Research, the Russel H. Morgan Department of Radiology, The Johns Hopkins University School of Medicine, Baltimore, MD, United States, [2]Cellular Imaging Section, Institute for Cell Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD, United States, [3]F.M. Kirby Research Center for Functional Brain Imaging, Kennedy Krieger Institute, Baltimore, MD, United States

Introduction

Recent advances in molecular MR imaging have revolutionized our ability to monitor gene expression (*1-4*). One such example is the Lysine-Rich-Protein (LRP) which is a prototype of artificial reporter gene that produces MRI contrast based on the chemical exchange saturation transfer (CEST) mechanism (*5*). However, the sensitivity and stability of such reporters can be further improved. Protamine sulfate, purified from salmon sperm, provides a considerably higher CEST contrast than poly-L-Lysine, a synthetic analog of LRP, due to its high arginine content (*6*). We therefore hypothesized that human Protamine 1 (hPRM1), which encodes to 51 amino acids with 47% arginine residues, should be a more sensitive CEST reporter gene. As a first step to confirm this, hPRM1 was engineered for expression in *E. coli* and its CEST MRI contrast was compared to control expressed proteins. We found that hPRM1 generates high CEST-MRI contrast thus appears to be a good candidate for improved imaging of gene expression based on chemical exchange.

Materials and Methods:

Cloning: The gene encoding to hPRM1 (NM_002761) was obtained from Origene (Rockville, MD). A synthetic gene encoding *E. coli* optimized hPRM1 was obtained from Blue Heron Biotechnology (Bothell, WA). Both genes, as well as Cytosine Deaminase (CD) and human calmodulin like 3 (CALM3) genes were sub-cloned into the pEXP5-CT expression vector (Invitrogen) and were expressed in-frame with a 6-histidine tag under a T7 promoter.

A modified RARE (TR/TE=6000/9.4ms, RARE factor=16, 2mm slice thickness, FOV=14x14 mm, matrix size=128x64, resolution= 0.11x022mm, and NA=2) including a magnetization transfer (MT) module (B1 = 4.7 μT, 4000ms) was used to acquire CEST weighted images from -5ppm to 5ppm (step=0.2ppm) around the water resonance (0ppm). The absolute water resonant frequency shift was measured using a modified Water Saturation Shift Reference (WASSR) method (*7*), using the same parameters as in CEST imaging except TR=1500ms, T_{sat}=250 ms, B1 =0.5 μT, and sweeping from -3ppm to 3ppm (step= 0.1ppm). Data processing was performed using custom-written scripts in Matlab. Z-spectra were calculated from the mean of ROI for each sample after B_0 correction. MTRasym =$(S_{-\Delta\omega} - S_{+\Delta\omega})/ S_{-\Delta\omega}$ was computed at different offsets Δω (i.e. +1.5 ppm and +3.6 ppm).

Result and Discussion

Although *E. coli* is the preferred host for the production of recombinant proteins, poor expression levels of arginine-rich protein are obtained. Therefore, we designed a synthetic gene encoding *E. coli* optimized hPRM1, which does not contain the arginine rare-codons for *E. coli*, and cloned it under the T7 promoter. Figure 1 shows an arginine-rich segment of hPRM1. The codons that encoded for arginine in the human gene were replaced by codons that are present in *E. coli* in order to maximize the hPRM1 expression level. Indeed, as can be seen from the Western Blot analysis (Figure 1), high levels of the optimized hPRM1 were detected in *E. coli* lysate but not when the human gene was used. The ability to express the protein in *E. coli* is crucial for the determination of its potential as CEST-MRI reporter gene as compare to control proteins.

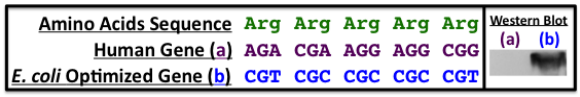

Figure 1: Arginine (**Arg**) rich hPRM1 segment. The amino acids sequence and the relevant codons used in the human (**a**) and *E. coli* optimized (**b**) gene. On the right, the Western Blot analysis of the expressed proteins showing the expression of the *E. coli* optimized hPRM1 (**b**) as compare to Human gene (**a**, lack of expression).

The optimized hPRM1 was extracted after induced expression in *E. coli* along with two control proteins that were expressed separately, CD and CALM3. Following dialysis, the optimized hPRM1, CD and CALM3 were transferred to capillaries at equal concentrations for measuring CEST contrast. We found that hPRM1 has higher MTR asymmetry values compared to control proteins (Figure 2). The optimized hPRM1 sample showed the typical MTR asymmetry profile reported previously *(6)* for protamine sulfate with high CEST contrast at 1.5 (guanidyl NH_2 proton) and 3.6 (amide NH) ppm. The higher CEST contrast is demonstrated on the CEST MTR asymmetry maps obtained at 1.5 and 3.6 ppm (Figure 2).

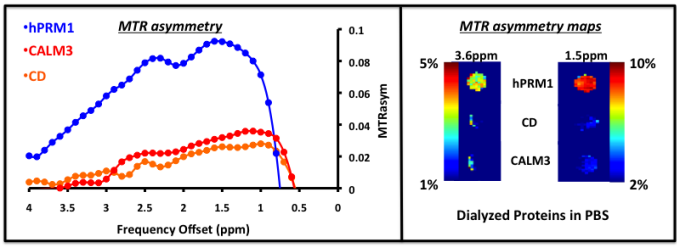

Figure 2: CEST MRI data of dialyzed proteins expressed in *E. coli*. MTR asymmetry graphs as well as maps obtained at 1.5 and 3.6 ppm are shown for optimized hPRM1, CALM3 and CD.

Conclusion

This study shows that hPRM1 protein generates considerably higher CEST -MRI contrast than control proteins. High levels of expressed hPRM1 protein were obtained after optimization of the hPRM1 gene for *E. coli* by replacing the arginine rare codons with *E. coli* commonly used codons. The expressed recombinant arginine-rich hPRM1 protein showed superior CEST MRI contrasts to CD and CALM3, making it a potential new reporter gene for MRI based on the chemical exchange contrast mechanism.

References

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