Genetically encoded biosensor for detecting Protein Kinase A (PKA) activity using CEST MRI

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INTRODUCTION

Protein kinases have crucial roles in a multitude of clinically important signaling pathways, making them of great interest for drug development in a wide range of diseases. Protein Kinase A (PKA) is of particular interest due to its central role in signaling pathways underlying cancer, diabetes, and inflammation (*1*). One of the major challenges is to monitor these changes directly in real-time in live subjects. Therefore, Molecular MRI, with tools like chemical exchange saturation transfer (CEST) reporter genes (*2-4*), is attractive for *in vivo* probe development as it may track metabolite dynamics in real-time with better spatial resolution and anatomic co-registration than traditional techniques. We have designed a genetically encoded biosensor whose proton exchange rates change sufficiently with phosphorylation by PKA to be detected with CEST MRI (**Figure 1a**). Such an MRI reporter may have applications in monitoring stem cell and transplant activity, and screening therapeutics that target this central signaling pathway.

METHODS

MRI: CEST images were acquired using an upright Bruker 500 MHz (11.7T) scanner with a modified RARE sequence (TR/TE=8000/9.4 ms, RARE factor=16, 1 mm slice thickness, FOV=11x11 mm, matrix size=64x32, resolution=0.17x0.34 mm, and NA=1-2) including a magnetization transfer module (B_1=5.0 μT/4000 ms) for frequencies -5 ppm to 5 ppm (step=0.2 ppm) from water resonance (0 ppm). Z-spectra were calculated from ROIs after B_0 correction for each voxel using WASSR (*5*). $MTR_{Asymmetry}=100\%*(S^{-\Delta\omega}-S^{+\Delta\omega})/S^0$ was computed at each offset Δω.

Probe design: We designed a peptide base sequence, LRRASLG, following the known PKA recognition sequence of RRX(S/T)Y (*6*), where X is any amino acid, S/T represents either serine or threonine, and Y is a hydrophobic amino acid; L= leucine, R= arginine, A= alanine, G= glycine. Peptides were custom synthesized by NeoBioSci (Cambridge, MA). Reporter genes were designed to include repeats of the base probe sequence, followed by a His6 tag (**Figure 3a**), and optimized for *E. coli* expression. Genes were custom synthesized by GenScript (Piscataway, NJ).

Phosphorylation assay: PKA, 10x stock reaction buffer, and ATP were purchased from New England Biolabs (Ipswich, MA). Reactions were initiated with a peptide concentration of 1 mM and 4 mM ATP, and completed at 30°C in 50 μL 1x PKA buffer for the indicated time period using a BioRad Thermal Cycler. The enzyme was heat inactivated by a 20 min, 65°C incubation prior to collection for downstream analysis.

RESULTS AND DISCUSSION

Phosphorylation of a PKA-specific peptide substrate, LRRASLG, produced >50% decrease in CEST contrast (quantified by $MTR_{Asymmetry}$) at 1.8 ppm and 3.6 ppm, the expected peaks for amine and amide protons of arginine residues (*3*) (**Figure 1b**). This contrast change has an apparent discrimination sensitivity near 250 μM and likely results from replacement of the serine hydroxyl and coordination of nearby arginines by the negatively charged phosphate group. This CEST MR probe can be used to track *in vitro* PKA activity dynamics, with the majority of signal change occurring in the first minutes of PKA action (**Figure 2**), similar to optical reporters of PKA activity (*6*). When expressed in live cells, an artificial reporter gene designed with 8 tandem repeats of this sequence showed the expected peaks at 1.8 ppm and 3.6 ppm at baseline (**Figure 3**). This biosensor may be used for *in vivo* preclinical screening of novel therapeutics and for monitoring the therapeutic efficacy of gene therapy and stem cell interventions.

CONCLUSION

This study demonstrates that an MRI-based genetically encoded biosensor may monitor real-time Protein Kinase A activity dynamics.

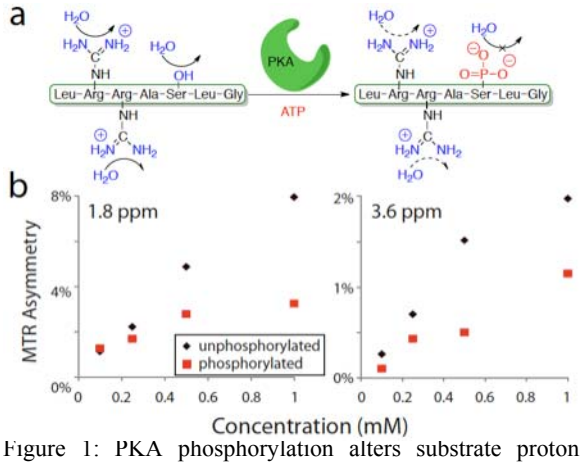

Figure 1: PKA phosphorylation alters substrate proton exchange rates sufficiently for CEST MRI detection at submillimolar substrate concentrations.

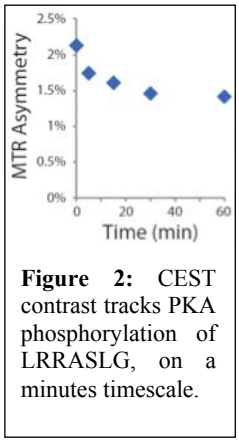

Figure 2: CEST contrast tracks PKA phosphorylation of LRRASLG, on a minutes timescale.

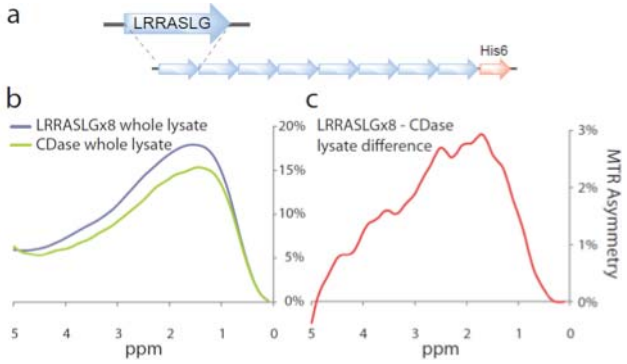

Figure 3: (a) Design of an MR reporter gene for PKA activity. CEST contrast of lysates (b) of cells expressing the MR PKA sensor (blue) and cells expressing a control protein, cytosine deaminase (CDase, green) and their difference (c, red).

REFERENCES

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