A hyperpolarized xenon based contrast agent using a modified M13 bacteriophage scaffold

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Introduction

Molecular imaging aims to detect the presence and spatial distribution of specific biomarkers in tissue. However, for many diseases the detection of these biomarkers must be done at very low concentrations to maximize diagnostic and prognostic value. Due to lack of sensitivity in conventional MRI techniques, exogenous contrast agents (e.g. SPIO, PARACEST) are being widely studied to lower concentration detection thresholds. Recently, targeted hyperpolarized xenon-based biosensors that exploit the exchange of solvated ^{129}Xe between bulk solution (XeW) and cryptophane-A (CryA) molecular cages (XeC) have demonstrated high sensitivity (1). To build upon this work, a filamentous bacteriophage M13 was chosen as a scaffold upon which a large number of CryA copies could be assembled. M13 bacteriophage are routinely employed in phage display techniques used in panning for targeting moieties such as single chain fragment antibodies (scFv) (2), and thus can be straightforwardly targeted to biomarkers allowing for drastically increased CryA payloads per bound target. *The purpose of this study was to investigate the feasibility of using an M13 bacteriophage modified with cryptophane-A molecular cages as a sensitive xenon-based MR contrast agent and to determine the detection thresholds of CryA-modified phage.*

Methods

The M13 bacteriophage was selected as a scaffold because its single-stranded DNA is coated by ~2700 copies of an identical pVIII coat protein. To generate the CryA-modified phage, the M13 first underwent a pyridoxal 5'-phosphate (PLP) mediated biomimetic transamination reaction (3) (Fig. 1), followed by a reaction with a polyethylene glycol (PEG, MW 5000) to increase the hydrophilicity of the bacteriophage and to aid in biodistribution for future in vivo use. The PEG-modified M13 was then reacted with alkoxyamine derivatized CryA, coating the surface of the protein. The resulting M13-PEG-CryA conjugate was purified by centrifugation and characterized using both reverse phase high performance liquid chromatography and mass spectrometry. The pVIII coat proteins of the M13-PEG-CryA construct were found to be approximately 27% modified with PEG (730 copies) and approximately 30% modified with CryA (810 copies). Pressurized xenon gas (2% Xe [natural abundance], 10% nitrogen, 88% helium at 70 psi[g]) was hyperpolarized with a MITI XenoSpin polarizer (Nycomed Amersham) and was solvated by bubbling through a small capillary into a 5mm NMR tube containing 650 µL of the biosensor solution. Xenon was bubbled for 25 sec at a flow rate of 0.5 SLM to saturate the solution with hyperpolarized ^{129}Xe, followed by a 2 sec wait period to allow the solution to settle and bubbles to clear. MR experiments were performed on a 300 MHz Varian ^{UNITY}INOVA vertical bore spectrometer equipped with a 26 channel shim set and a dual-tuned (^{1}H, ^{129}Xe) RF saddle coil (i.d. = 5 mm). Data was collected using a CEST pulse sequence that applied a continuous wave saturation pulse prior to signal excitation (BW = 25 kHz, acquisition time = 0.5 s). A CEST spectrum in which the saturation frequency was varied over 300 ppm was acquired from an aqueous solution (phosphate-buffered saline w/ 5% v/v isopropanol) containing 3.1 nM M13-PEG-CryA (~2.5 µM CryA), as well as a control solution containing "isolated" 2.5 µM CryA with a short peptide chain but not on an M13 scaffold. These experiments were performed at 25ºC using a 10 sec saturation pulse of 89 µT. The resultant data was fit in the time domain using a nonlinear least-squares regression (MATLAB; MathWorks, Natick, MA), and the signal amplitudes were considered for subsequent analyses. Upon determining the chemically shifted frequency of the XeC signal, the M13 sample was diluted 500X to ~6.25 pM M13-PEG-CryA (~5 nM CryA) and its detection was attempted at 37ºC by collecting repeated points for saturation frequencies on-resonance with XeC (ν_{XeC} = 62 ppm; referenced to the ^{129}Xe gas frequency) and off-resonance (ν_{XeW} + [ν_{XeW} - ν_{XeC}] = 322 ppm). The on- and off-resonance data was analyzed with a one-tailed Student's t test.

Results

The CEST spectra clearly showed XeW signal loss corresponding to saturation at the XeC frequency (62 ppm) as well as direct saturation at the XeW frequency (192 ppm) for both the M13-PEG-CryA and isolated CryA samples (Fig. 2). The maximum negative CEST contrast was 20% for the M13-PEG-CryA sample and 30% for the CryA sample, showing that the CryA cages produce a similar order of effect whether or not they are conjugated to the M13 scaffold. At high temperature, a modest though statistically significant contrast of 2.4% was seen between the on-resonance saturation data and the off-resonance saturation control.

Discussion

The M13 bacteriophage was been demonstrated to be effective as a supramolecular scaffold for CryA assembly, resulting in a xenon-based contrast agent with a single digit pM concentration detection threshold. Continued optimization of the construct synthesis and pulse sequence detection is expected to lead to further sensitivity improvements of this contrast agent.

References

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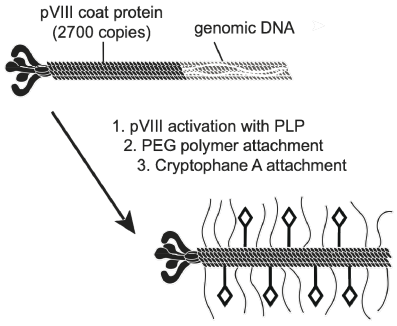

Fig. 1. Schematic representation of the M13 bacteriophage modification with polyethylene glycol and cryptophane-A molecular cages.

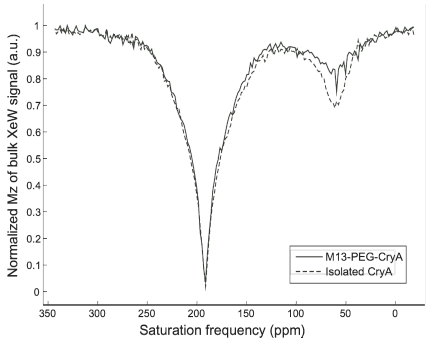

Fig. 2. CEST spectra for solutions containing 2.5 µM cryptophane-A: both as part of 3.1 nM M13-PEG-CryA (solid) and by itself (dashed).

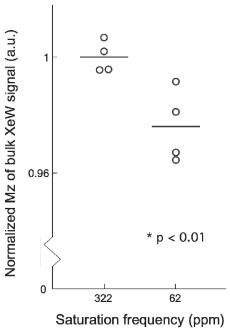

Fig. 3. CEST contrast for a 6.25 pM M13-PEG-CryA solution. Shown are repeated data points and their mean for off-resonance (322 ppm) and on-resonance (62) saturation.