A nanoemulsion based CEST agent for hyperpolarized ^{129}Xe

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Introduction

There are a growing number of potential *in vivo* applications that would benefit from sensitive molecular recognition. High sensitivity has been demonstrated for chemical exchange saturation transfer (CEST) agents that utilize molecular cryptophane cages to functionalize hyperpolarized ^{129}Xe (1), with reported detection thresholds as low as 230 fM (2). A drawback of these cryptophane-based agents is the expertise required for their chemical synthesis. In this study, a novel type of ^{129}Xe CEST agent based on readily made nanoemulsions is presented. At the core of these nanoemulsions is perfluorooctyl bromide (PFOB), a short linear perfluorocarbon that has excellent gas solubility and has been previously employed in oil-in-water (o/w) type emulsions as an artificial blood substitute. In addition to the high solubility of xenon in PFOB, ^{129}Xe has long T_1 relaxation times and has displayed a unique resonance frequency when solvated in PFOB ($^{129}Xe_P$ ~ 100 ppm [referenced to ^{129}Xe gas at 0 ppm]) that is well separated from ^{129}Xe solvated in aqueous solutions ($^{129}Xe_W$ ~ 190 ppm) (3). As xenon has previously been shown to chemically exchange in and out of similar emulsified droplets (3,4), it was hypothesized that a CEST detection scheme that (i) benefits from the large number of xenon atoms solvated within each droplet at a given time, and (ii) takes advantage of the more rapid xenon exchange dynamics compared to those of cryptophane hosts, would be able to produce large negative contrasts due to the nanoemulsion droplets. *The purpose of this study was to investigate the utility of using PFOB-in-water nanoemulsions as CEST agents for ^{129}Xe, and to determine the xenon exchange dynamics and detection thresholds associated with these agents.*

Methods

Nanoemulsions were prepared from 1 mL of PFOB and 750 uL of 100 mg/mL solution of a nonionic triblock copolymer (poloxamer) surfactant with a high hydrophilic-lipophilic balance (HLB) (molar mass = 8.4 kD, HLB = 29) in 23.75 mL of purified, deionized water. Samples were processed with a small volume, high shear microfluidizer, and were subsequently diluted 20X in water for storage prior to use. Dynamic light scattering (DLS) was used to evaluate the size distribution and temporal stability of each nanoemulsion sample over the course of several weeks. Slow droplet growth coupled with continued small size polydisperisty allowed for the successive selection of nanoemulsions with increasing average droplet diameters (160 nm, 210 nm, 265 nm, 310 nm), which were subsequently used at varying dilutions. Prior to NMR experimentation, a small amount (0.01% v/v) of a low HLB poloxamer (molar mass = 2.75 kD, HLB = 2) was added to the diluted solution to help stabilize the droplets during bubbling and to aid in defoaming. Pressurized xenon gas (2% Xe [natural abundance], 10% nitrogen, 88% helium at 65 psi[g]) was hyperpolarized with a MITI XenoSpin polarizer and was solvated by bubbling through a small capillary into a 5mm sample tube containing 1 mL of the diluted nanoemulsion solution. Xenon was bubbled for 15 sec at a flow rate of 0.5 SLM to saturate the solution with hyperpolarized ^{129}Xe, followed by a 4 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 dual-tuned (^{1}H/^{129}Xe) RF saddle coil (i.d. = 5 mm), and all data was collected with a 25 kHz spectral width over an acquisition time of 0.5 s at temperatures of 37°C and 20°C (data not shown). To compare the xenon exchange dynamics for the different sized droplets (collected with matched total PFOB volume), CEST spectra were acquired with a 20 uT continuous wave saturation pulse applied at frequencies that varied over 35 kHz. The experimental CEST spectra where modeled with McConnell-Bloch equations to determine the xenon exchange rates, and the probability that a xenon atom arriving at the droplet boundary would cross the surfactant monolayer was determined by comparing these rates to the xenon diffusion transit times associated with the different sized PFOB droplets. For modeling input, chemically shifted resonance frequencies, and T_1 and T_2 relaxation times were determined experimentally for non-emulsion samples of (i) water with 0.01% L-81 and (ii) pure PFOB. The chemical shift of $^{129}Xe_P$ was allowed to vary downfield from the pure PFOB resonance to reflect the nature of frequency shifts in spheres of linear media surrounded by a magnetically distinct linear media. To determine some representative detection thresholds for 210 nm and 310 nm diameter droplets, CEST contrast was plotted against varying saturation times for several further dilutions.

Results

The CEST spectra for the increasingly large droplets clearly slowed the effective xenon exchange rate getting slower due to increases in the diffusion limited xenon residence time in the droplets (Fig. 1). This data was accurately modeled with the modified Bloch equations, and the probability that a xenon atom arriving at the droplet boundary would cross the surfactant monolayer was found to be 1.3% at 37°C (1.1% at 20°C). As shown in the saturation time curves, the 210 nm droplets were clearly detected down to a concentration to 1 pM (Fig. 2a), and the 310 nm droplets were detected down to a concentration of 100 fM (Fig. 2b).

Discussion

A novel and highly sensitive class of CEST contrast agent based on nanoemulsion for hyperpolarized ^{129}Xe has been demonstrated. The minimal material and time costs for nanoemulsion preparation will simplify further development of these agents, and will allow for easy scale-up for future applications in which larger quantities and time-sensitive samples are required (e.g. in vivo applications). Further work will aim to extend control over the droplet sizing, and will leverage developments in targeted hydrophobic drug delivery to create nanoemulsions that can specifically bind to analytes of interest.

References

(1) Schroder L et al. Science 2006, 314: 446-449. (2) Stevens TK et al. Proceedings ISMRM 2010, # 453. (3) Wolber J et al. MRM 1999, 41: 442-449. (4) Gherase MR et al. J Chem Phys 2006, 125: 044906.

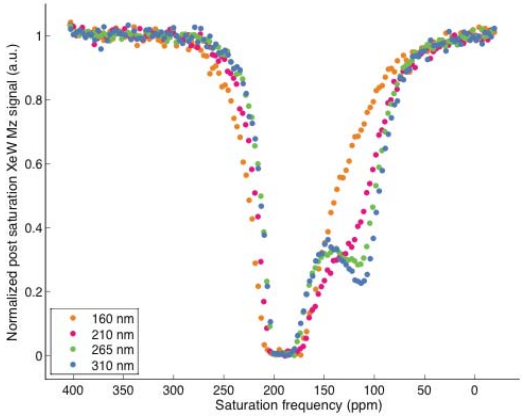

Fig. 1. CEST spectra for various sizes of nanoemulsion droplets. As witnessed by the transition between fast-intermediate to slow-intermediate regimes, diffusion with the droplets dictates the effective ^{129}Xe exchange rates.

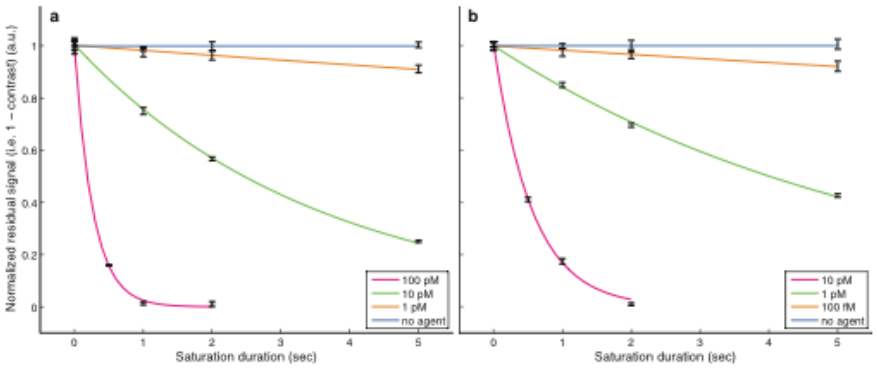

Fig. 2. Plots of normalized residual $^{129}Xe_W$ signal following RF saturation vs. droplet concentration for (a) 210 nm droplets and (b) 310 nm droplets. The residual signal is defined as (1 – contrast), where the contrast is equal to the difference in the off-resonance post-saturation signal and on-resonance post-saturation signal, normalized by the off-resonance post-saturation signal.