Development of an activatable MRI T2 agent sensitive to NADH

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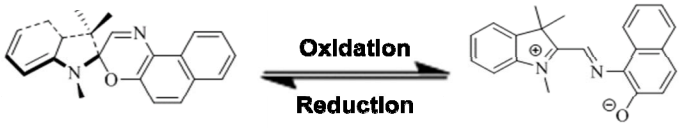
Oxidative stress has been implicated in various mechanisms of illness and injury and recognized as a contributor to harmful disease processes. For instance, elevated NADH levels have been correlated with an increase in harmful reactive oxygen species in tissues affected by cancer, neurodegeneration, and vascular disease.[1] The ability to detect regions with abnormal redox conditions could facilitate early detection of pathology and potentially assist the evaluation of treatment efficacy. The aim of this work was to develop an activatable magnetic resonance imaging (MRI) probe that can non-invasively identify areas of elevated NADH. This contrast agent consists of a biocompatible paramagnetic nanoparticle that is conjugated with a redox active "molecular switch" (Fig. 1). The "molecular switch", spirooxazine (SO), was chosen as the surface functionalization because it has well-established conformation switching behavior,[2] and we have previously demonstrated its ability to respond to NADH/peroxide.[3] SO changes conformation between hydrophilic and hydrophobic isomers in response to oxidation and reduction (Scheme 1). We hypothesized that the presence of NADH will trigger isomerization of spirooxazine, from the hydrophilic to the hydrophobic isomer. This isomerization, and its reverse elicited by an oxidizer such as peroxide, directs the aggregation or dispersion of nanoparticles in aqueous medium thus modulating the MR signal.

Scheme 1. Isomerization of Spirooxazine



The dextran coated iron oxide nanoparticles were prepared in our lab using previously developed methods.[4] In order to stabilize the dextran coating and allow for coupling with spirooxazine, the dextran has been cross-linked and substituted with amines.[5] Early attempts to attach spirooxazine to iron oxide nanoparticles through a substitution reaction were unsuccessful, presumably due to steric hinderance imposed by a short linker. Therefore, the synthesis of spirooxazine with a longer linker was undertaken. The modified spirooxazine was then appended to the nanoparticle using previously developed methods.[6] The attachment was confirmed by infrared spectroscopy. To characterize the agents redox response, the T2 relaxation time and hydrodynamic size was measured upon reaction with NADH and hydrogen peroxide (approx 3:1 ratio NADH:SO and H_2O_2: SO). Relaxation time was measured on a Bruker Minispec mq60 relaxometer at 60 MHz and 37 °C. Particle size in solution (expressed as the volume weighted diameter) was determined by dynamic light scattering (DLS) with a Nanotrac 150 size analyzer.

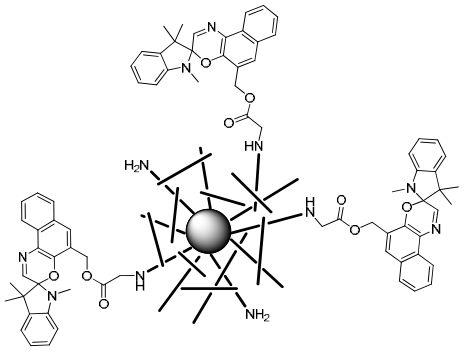


Figure 1. Cross-linked, aminated dextran coated iron oxide nanoparticles terminated with spirooxazine (CLIO-NH-SO)

Table 1. T2 relaxation time and hydrodynamic size of CLIO-NH-SO upon reaction with NADH and hydrogen peroxide

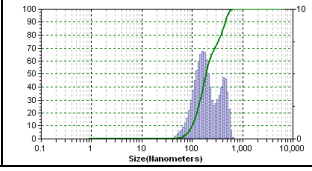
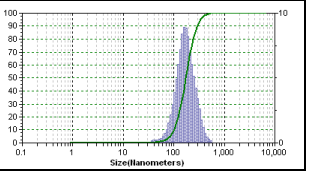
	NADH	H_2O_2
T2 (msec)	60.8 ± 8.9	77.4 ± 9.7
Size (nm)		

Table 1 shows an average relaxation time of 60.8 msec for the samples (n=3) when reacted with NADH and 77.4 msec upon subsequent reaction with peroxide. The spin-spin relaxation time (T2) is lengthened by 27% with oxidation of the probe which is statistically significant with a p-value < 0.01. Representative DLS graphs for a single measurement are presented in Table 1 which demonstrate the proposed aggregation/dispersion. Reduction with NADH causes aggregation of the hydrophobic probe yielding size measurements of 154 nm and 415 nm, while oxidation leads to dispersion of the nanoparticles and a size distribution at 172 nm. It is our hope to optimize the sensitivity of the probe through the spirooxazine moiety such that complete aggregation and dispersion can be achieved with reliable reversibility.

In conclusion, these results indicate successful synthesis and preliminary characterization of the probe, CLIO-NH-SO. Upon reduction with NADH, the molecular switches convert to the hydrophobic spirooxazine isomer causing aggregation of the iron oxide nanoparticles, thus producing a measured size increase and shortened T2 relaxation time. Likewise, oxidation of the molecular switches causes dispersion and lengthened T2 due to the shift to the hydrophilic isomer. Ultimately, our goal is to characterize the ability of these probes to provide *in vivo* MR contrast enhancement, indicative of tissue NADH redox environment, through local variation in iron concentration due to nanoparticle aggregation or dispersion. This novel MRI contrast agent has promising potential to respond to NADH related biochemical activities in living systems.

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