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Modulation of T2 Relaxation Time by Light-Induced, Reversible Aggregation of Magnetic Nanoparticles

Elizabeth A. Osborne,[†] Benjamin R. Jarrett,[‡] Chuqiao Tu,[‡] and Angelique Y. Louie*[,‡]

Department of Chemistry, Department of Biomedical Engineering, University of California, Davis, One Shields Avenue, Davis, California 95616

Received January 11, 2010; E-mail: aylouie@ucdavis.edu

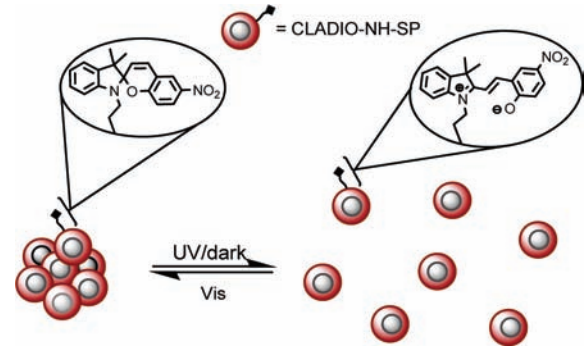
Magnetic resonance imaging (MRI) is a premier diagnostic tool for noninvasive, deep tissue anatomical imaging. In recent years, advancements in this imaging field have been aimed toward developing MRI contrast agents that are activatable.[1] These agents offer the ability to report on biochemical processes rather than strictly anatomy. Researchers have reported T1 agents that are sensitive to pH,[2,3] calcium ions,[4] pO_2,[5,6] zinc ions,[7] and specific enzyme activity.[8,9] The mechanism of activation for these "smart" agents has been due to their ability to modify either the number of water molecules bound to the paramagnetic ion (q), the lifetime of bound water (τ_m), or the rotational correlation time of the complex (τ_r).

While the majority of the activatable contrast agents under investigation affect the spin−lattice (T1) relaxation time, a few activatable T2 agents have also been developed. Weissleder and colleagues[10] have developed an *in vitro* MRI probe, termed a magnetic relaxation switch (MRS), which employs cross-linked iron oxide nanoparticles that are functionalized with DNA sequences complementary to that of the target oligonucleotides. Upon recognition of the target sequence, the nanoparticles aggregate into clusters resulting in decreased T2 relaxation time for adjacent water protons. MRS aggregates can subsequently be separated into their constituent nanoparticles through the action of a DNA-cleaving enzyme, thus resulting in an increased T2 relaxation time. In other research, Schellenberger et al.[11] developed an MRI agent that reports on matrix metalloproteinase 9 (MMP-9) activity. These agents consist of peptide-mPEG copolymers adsorbed to citrate stabilized iron oxide nanoparticles. Upon contact with MMP-9, the sterically bulky mPEG coat is cleaved resulting in irreversible aggregation of the particles and an R2 relaxivity increase.

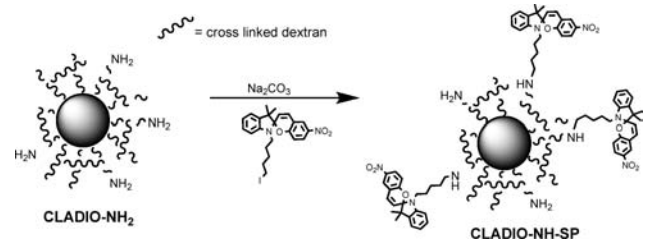
Recently, our lab developed reversible, small-molecule Gd(III) chelates that act as light[12,13] and NADH[14] responsive MRI contrast agents. These probes employ "molecular switches", from the spiropyran and spirooxazine family, that change conformation between hydrophilic and hydrophobic isomers in the presence of NADH and peroxide or with visible and UV light irradiation. Visible light or reduction produces a shift to the nonpolar, hydrophobic spiropyran/oxazine isomer, and UV light or oxidation produces a shift to the polar, hydrophilic merocyanine isomer. Isomerization of these switches alters the accessibility of water molecules to Gd(III), thus altering the T1 relaxation time. While investigating the properties of these probes, we envisioned producing reversible T2 agents by attaching these molecular switches to magnetic nanoparticles.

Herein, we report the synthesis of a reversible T2 agent that is capable of modulating the relaxation time in response to light irradiation. Similar to our previously developed agents, these employ a spiropyran (SP) derivative that changes conformation between hydrophilic and hydrophobic isomers in response to light. This spiropyran derivative is covalently attached to dextran sulfate coated iron oxide nanoparticles (ADIO). We hypothesized that, attached in sufficient number, the molecular switches could direct the aggregation or dispersion of the nanoparticles by way of the differing charge properties of the isomers, thus altering T2 relaxation time. Scheme 1 illustrates the proposed mechanism of aggregation/dispersion in an aqueous medium.

Scheme 1. Proposed Mechanism of Reversible Aggregation



Scheme 2. Preparation of CLADIO-NH-SP



In recent years, ADIO have been synthesized and characterized in our lab.[15,16] To stabilize the dextran coating and provide functionalization in which to tether spiropyran, the nanoparticles were cross-linked and aminated via literature methods.[17] Amine termination allows the nanoparticles to undertake nucleophilic substitution with iodinated spiropyran. A brominated spiropyran derivative was prepared by a known method,[18] and then the bromide was converted to iodide by stirring with potassium iodide in acetone overnight. CLADIO-NH-SP was then prepared by mixing aqueous CLADIO-NH_2 particles with a sodium carbonate solution followed by addition of iodinated SP in dimethylformamide (Scheme 2). The mixture was heated at 55−60 °C overnight. The resulting nanoparticle probes were purified by size-exclusion column chromatography to remove excess, unbound spiropyran.

To ensure successful coupling, absorption spectrum were measured for each sample. The representative spectrum in Figure 1a

[†] Department of Chemistry.
[‡] Department of Biomedical Engineering.

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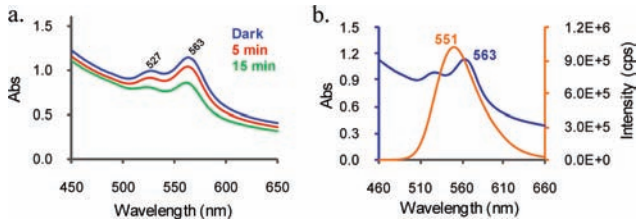

Figure 1. Absorption properties of CLADIO-NH-SP (a) in the dark and with visible light irradiation (5 and 15 min) and (b) in comparison with luciferin emission wavelength (orange).

Table 1. Relaxation Time, Size, And Image Data for Aqueous CLADIO-NH-SP in the Dark and with Visible Light Stimulus

	T1 (msec)	T2 (msec)	Size (nm)	T2W MR image
dark	505	37.09 ± 3.09	70, 540	
light	496	24.55 ± 1.86	520	

shows the absorbance profile of the SP terminated probe in the dark (blue) and with 5 (red) min and 15 (green) min of visible light irradiation. The characteristic decrease in absorbance at 563 nm corresponding with light induced isomerization from the merocyanine (MC) to the spiropyran (SP) form is observed. In addition, IR spectroscopy (Supporting Information) confirmed the presence of spiropyran in the agent by the aromatic C=C vibrations.

The effect of light triggered isomerization of CLADIO-NH-SP on relaxation time and size was evaluated. The particle size in solution (expressed as the volume weighted diameter) was determined by dynamic light scattering (DLS) while longitudinal (T1) and transverse (T2) relaxation times were measured on a Bruker Minispec relaxometer at 37 °C. The MR image was obtained on a Bruker Biospec 7T system. All aqueous samples were kept in the dark at least 1 h prior to measurement to maximize conversion to the hydrophilic MC form. Particle size, T1, and T2 were measured prior to and then following white light irradiation (150 W) for 10 min. Table 1 shows the average relaxation times for the samples in the dark and after visible light irradiation. The spin−spin relaxation time (T2) is shortened by 33.7 ± 2.4% ($n = 4$) with light irradiation, and the size by DLS correspondingly shifts from two size populations at 70 and 540 nm to a single average distribution of 520 nm ($n = 2$) indicating aggregation. Representative DLS graphs for a single measurement are presented in Table 1. The decrease in T2 relaxation time observed for light stimulus is statistically significant with a p-value < 0.001. In addition, the T2-weighted MR image demonstrates signal intensity modulation by light stimulus. The mean signal intensity (arbitrary units) for the sample kept in the dark is 249 ± 5 as compared to 211 ± 3 for the light activated sample, a decrease of 15%, as measured by *Image J* software (National Institutes of Health).

In conclusion, this preliminary work reports a "smart" T2 agent that is reversibly activated by a visible light stimulus. We demonstrate that the isomerization of the SP/MC motif, when tethered to an iron oxide nanoparticle, incites significant changes in T2 relaxation time with concurrent size changes consistent with our proposed mechanism. Previously reported contrast agents are activatable in only one direction, meaning they turn "on" or "off" only. Weissleder and colleagues[10] report an activatable T2 agent that is activated with aggregation and is capable of a one-time reverse of aggregation, but the agent is not truly reversible as the reversal is afforded by irrevocable cleavage of DNA. Our probe offers the benefit of being activatable and reversible; therefore, it may have utility for continuous, dynamic monitoring of biological activity. In addition, our preceding work has demonstrated nanoparticle uptake by cells and our capability to endow these probes with a targeting ability through surface modification.[15,16,19]

We have previously reported gadolinium based MRI contrast agents containing spiropyran groups and found that those agents were responsive to oxidation/reduction; therefore, we are currently investigating the redox sensitivity of the T2 agents reported herein. In addition, due to their light sensitivity these T2 agents have exciting potential to act as noninvasive, MRI gene reporters for the luciferase expression system. Firefly luciferin, upon contact with luciferase, emits ~550 nm light which overlaps extensively with the absorbance of CLADIO-NH-SP and can induce isomerization of the molecular switches. Figure 1b shows the overlap of our probe's absorbance (blue) with the emission of firefly luciferin (orange) as measured in solution studies. Typically, luciferase gene reporter systems are of most use in semitransparent or thin specimens, due to the limited depth of penetration of light. An MRI probe activatable by the luciferase/luciferin system would provide a method to use the luciferase system for examination of gene expression in deep tissues.

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Supporting Information Available: Experimental procedures and characterization of CLADIO-NH-SP. This material is available free of charge via the Internet at http://pubs.acs.org.

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