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DNA-Based Magnetic Nanoparticle Assembly Acts as a Magnetic Relaxation Nanoswitch Allowing Screening of DNA-Cleaving Agents

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The creation of nanostructures capable of sensing DNA interactions and screening DNA cleaving agents has been a major goal in bioorganic and medicinal chemistry. Systems that utilize the exquisite molecular recognition capability of biomolecules such as DNA to guide the assembly of nanoparticles have previously been reported. For example, DNA-labeled gold[1] and CdSe nanoparticles (quantum dots)[2] possessing unique colorimetric and fluorescent properties have been used as biosensors of DNA hybridization. Recently, we developed magnetic nanoparticles conjugated to synthetic oligonucleotides and found that when the monodisperse nanoparticles hybridize to a target they assemble into highly stable nanoassemblies (clusters),[3] with a concomitant decrease in the spin–spin relaxation time (T2) of adjacent water protons. These magnetic nanoparticles have been referred to as magnetic relaxation switches (MRS).

Here we report the design of a pair of MRS, denoted P1 and P2, which can be used to detect DNA cleaving and methylating enzymes. The oligonucleotide sequences were chosen so that P1 and P2 would self-hybridize with the formation of a MRS nanoassembly that exhibits a more pronounced effect on T2. We hypothesized that restriction of endonucleases (by cleaving the double stranded oligonucleotide linking P1 and P2) would cause the nanoassembly to switch to a dispersed state and produce an increase in T2. To test this hypothesis we prepared a pair of MRS (P1 and P2) that self-assemble to form a BamHI recognition site (Figure 1a).[4] P1 (10 μg Fe/mL) had a T2 of 61.6 $\pm$ 0.3 ms, while P2 (10 μg Fe/mL) had a T2 of 60.4 $\pm$ 0.5 ms. Meanwhile, the T2 of an equimolar mixture of P1 and P2 (total 10 μg Fe/mL) had a T2 of 32.3 $\pm$ 0.6 ms ($p < 0.0001$), due to self-hybridization of the particles and formation of nanoassemblies. Incubation with BamHI[5] resulted in an increase in T2 back to baseline levels (59.4 $\pm$ 0.4 ms). T2 changes were specifically inhibited by the addition of a synthetic complementary oligonucleotide and other endonucleases did not cause an increase in T2. Atomic force microscopy revealed that P1/P2 consisted of stable nanoassemblies with average sizes raging from 300 to 400 nm (Figure 1b). After 1 h of incubation with BamHI, the nanoassemblies were no longer present and monodisperse nanoparticles (50–60 nm) were observed instead (Figure 1c).

To further prove that changes in T2 were in fact due to the BamHI cleavage of double stranded oligonucleotide holding the MRS nanoassembly together, we subjected the oligonucleotide on the nanoassemblies to electrophoresis after treatment with endonucleases. The oligonucleotide was released from the nanoparticles with use of DTT and electrophoresed as shown in Figure 2.[6] The oligonucleotide from both the untreated and Eco R1-treated nanoassemblies moved as a single band, while BamH1 treatment yielded two faster moving bands. These results indicate that the MRS nanoassemblies served as a substrate for BamHI and suggest that they could serve as a substrate for other DNA modifying enzymes.

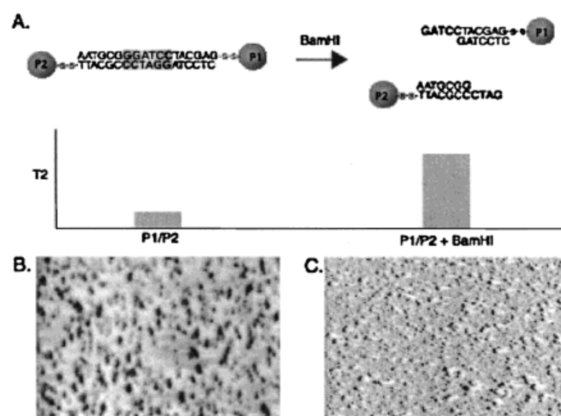

Figure 1. (A) Diagram of MRS nanoassembly (P1/P2). Sequence specific cleavage by BamHI results in separation of the particles in the nanoassembly with a corresponding increase in T2 relaxation times. For clarity, only two nanoparticles are shown. Phase contrast AFM images of MRS nanoassembly (P1/P2) before (B) and after (C) BamHI treatment. Regions of high contrast (dark spots) were identified as either a nanoparticles assembly or free particle. Bar = 500 nm.

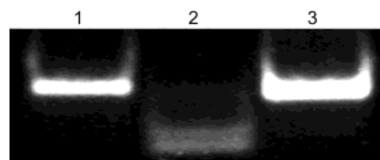

Figure 2. Nondenaturing gel electrophoresis of the released oligonucleotide from (lane 1) P1/P2, (lane2) BamHI-treated P1/P2, and (lane 3) EcoRI-treated P1/P2.

DNA methyltransferases cause changes in chromatin structure by methylating adenine and cytosine and play a key role in regulating gene expression.[7] To examine whether the MRS nanoassemblies P1/P2 could serve as substrate for DNA methylase, we treated the nanoassemblies with dam methylase,[8] which methylates the adenine residue within the sequence GATC, see Figure 1A. The methylated nanoassemblies P1/P2 were then treated with DpnI, a restriction endonuclease that specifically cleaves the methylated sequence GATC. The effect of treating methylated and nonmethylated MRS nanoassemblies with DpnI is shown in Figure 3. DpnI caused an increase in T2 from 48 $\pm$ 0.5 ms to 112 $\pm$ 2.4 ms with the methylated nanoassembly after 1 h of incubation, while no change in T2 was observed with the nonmethylated nanoassembly. These results indicate that the DpnI cleaved nanoassemblies held

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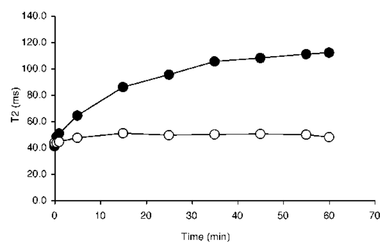

Figure 3. Time-course of water T2 relaxation times of methylated MRS nanoassembly (P1/P2) treated with DpnI (●). Nonmethylated MRS nanoassembly treated with DpnI is shown as a control (○).

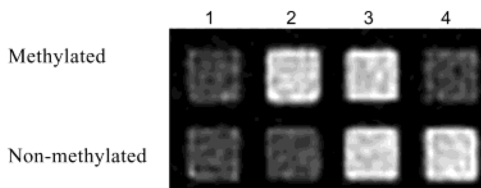

Figure 4. T2-weighted MR image (1.5 T, spin−echo sequence: TR = 3000 ms, TE = 80 ms) of methylated and nonmethylated magnetic nanoassemblies incubated with various restriction endonucleases in a portion of a 384-well plate: lane 1, nontreated (control); lane 2, DpnI-treated; lane 3, BamHI-treated; lane 4, MboI-treated.

together by methylated oligonucleotide and not those joined by nonmethylated oligonucleotides.

Finally, we investigated whether the activity of endonucleases on MRS nanoassemblies could be detected by parallel magnetic resonance imaging (MRI) of entire 384 well plates rather than sequential analysis with benchtop NMR relaxometers. This imaging approach has previously been shown to be capable of processing tens of thousands of samples within a few hours of imaging.[9] Methylated and nonmethylated MRS nanoassemblies (3 μg/mL) were incubated with DpnI, BamHI, or MboI endonucleases in a 384 well plate (Figure 4). The MR signal intensity for control of methylated and nonmethylated nanoassemblies was identical, indicating methylation did not alter T2 (lane 1). Methylated nanoassemblies treated with DpnI showed a higher signal intensity (higher T2) than nonmethylated nanoassemblies (lane 2). The opposite effect was obtained when methylated and nonmethylated nanoassemblies were treated with MboI (lane 4). These findings correlate well with the specificity of these endonucleases; methylation of adenine within GATC enhances cleavage by DpnI while it inhibits cleavage by MboI.[10] Finally the same effect (higher signal intensity) was observed with both methylated and nonmethylated MRS nanoassemblies treated with BamHI (lane 3), which is known to cleave both methylated and nonmethylated sequences.[10]

In conclusion, MRS nanoassemblies can act as biosensors for the recognition and real-time monitoring of protein based or small molecule DNA cleaving agents. Most DNA-cleavage assays use radioactive probes and electrophoresis and are difficult to automate in high-throughput applications. Recently, methods have been developed where molecular beacons are used to monitor DNA cleavage as a function of increase in fluorescence of the sample.[11] Since the described method correlates DNA cleavage with the magnetic properties of the solution (changes in water relaxivity) and does not involve the use of optical methods, experiments can be carried out in turbid media. In addition, complex molecules containing multiple fluorochromes that can interfere in optical assays due to absorption or high background fluorescence could be screened for possible DNA binding. This method could also be adapted to other types of interaction such as protein−DNA interactions. Furthermore, conventional NMR spectrometers could be adapted to measure water T2 relaxation times and recent developments in high-throughput NMR[12] will further expand throughput of the magnetic nanoassembly based assay. These qualities will make the developed system ideal for high-throughput drug discovery screening of complex mixtures and biological samples for DNA-cleaving activity.

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(4) Two self-complementary 3′-alkanethiololigonucleotides (AAT-GCG-GGATCC-TAC-GAG-$(CH_2)_3$-SH and CTC−CTA-GGATC−CGC-ATT-$(CH_2)_3$-SH) were conjugated to the nanoparticles as described in ref 3. The resulting conjugates (MRS), denoted P1 (AAT-GCG-GGATCC-TAC-GAG-$(CH_2)_3$-S-S-CLIO) and P2 (CTC-CTA-GGATC-CGC-ATT-$(CH_2)_3$-S-S-CLIO), have an average of 3 oligonucleotides per particles. Formation of MRS-nanoassemblies upon mixing P1 and P2 was determined by atomic force microscopy (AFM) (Dimension 3100, Digital Instruments). Images were recorded by using tapping mode and a surface area of 5×5 μm^2.

(5) The restriction endonuclease digestion was performed at 37 °C with 0.4 U/μL of BamHI, (New England BioLabs) in 500 μL of 10 mM Tris HCl, 10 mM $MgCl_2$, and 50 mM NaCl pH 7.4 containing MRS nanoassembly P1/P2 (10 μg of Fe/mL). The water relaxation of the solution was measured at either time intervals or after 1-h incubation and compared to control samples (with no enzyme) using a 0.47 T NMR relaxometer (Bruker NMR Minispec, Billerica, MA).

(6) MRS nanoassembly P1/P2 (1 μg of Fe) was digested with BamHI or EcoRI (O.4 U/μL) for 1 h. The samples were then incubated with 4 mM DTT for 2 h to cleave the oligonucleotide from the nanoparticles and run by nondenaturing polyacrylamide (10%) gel electrophoresis. SYBR Gold acid stain was used to visualize the DNA.

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(8) Methylation of the MRS nanoassembly P1/P2 (10 μg of Fe/mL) was performed at 37 °C for 90 min with 0.16 U/μL of dam methylase (New England BioLabs) in 500 μL of 10 mM Tris HCl, 10 mM $MgCl_2$, 50 mM NaCl, 0.08 mM *S*-adenosylmethionine pH 7.4. The water relaxivity (T2) of both the methylation and control reaction was recorded before and after addition of restriction endonuclease DpnI, MboI, or BamHI (0.4 U/μL).

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