Ligation of Nanoparticle Coated DNA Cleaved with Restriction Enzymes

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Received March 27, 2007
Revised Manuscript Received June 14, 2007

Material synthesis at the nanoscale frequently relies on biological molecules for inspiration, selectivity, and specificity. Molecular templates using biomolecules have been used to fabricate structures with specific sizes and shapes.[1−5] Often times, the biological function of the template molecule is utilized to connect nanoscale structures, site-specifically catalyze reactions, or enzymatically modify templated segments.[6−16] The specificity of these biomolecular interactions can often lead to straightforward fabrication methods of materials that are difficult to synthesize by conventional means. One of the simplest and most easily adapted forms of such interactions is that involving the hybridization of single-stranded DNA to its double-helix form. This interaction has been used to drive the formation of complex structures, nanoparticle conjugates, and DNA-based electronics.[17,18] More recently, the introduction of DNA-manipulating enzymes has been investigated.[19−21] We have previously demonstrated the ability of restriction endonucleases, which cut DNA at specific sequences, to fragment λ phage DNA templated with magnetic nanoparticles.[6] DNA ligases, which oppose the function of restriction enzymes by repairing cleaved DNA molecules, have recently been used to generate specific connectivity between DNA-modified nanoparticles.[10]

In this report, we demonstrate how λ phage DNA-based templates coated with $CoFe_2O_4$ nanoparticles can be cleaved by enzymes and subsequently ligated using a T4 ligase. Long DNA molecules have been shown to be an attractive method to generate high-aspect-ratio nanoparticle chains. The λ phage DNA used in this report has a theoretical strecthed length of 16 μm and has been shown to be capable of stretching to lengths longer than 20 μm using a molecular combing technique. The diameter of the structure is defined by the $CoFe_2O_4$ nanoparticles, which at 3.5 nm exceed the ~2 nm diameter of the DNA molecule. This method has several unique advantages to conventional lithography, such as its solution-based processing, inexpensive material and instrument costs, and biochemical reactions available by the template molecule. Recently, we have shown that the BamH1 enzyme retains its function when used to catalyze the cleavage of Fe_2O_3-nanoparticle coated DNA. In this report, we focus on introducing the ligation of such templated fragments to recombine and form one-dimensional structures. These chains of $CoFe_2O_4$ nanoparticles have potential applications in memory storage devices and magnetic field sensors and can provide platforms for fundamental studies on magnetic tunneling junction devices. The restriction enzymes used in this study, BamH1 and EcoR1, generate distinct size fragments due to their different sequence specificity.[22] The fragments generated from these interactions contain sticky-end single-strand overhangs specific to each enzyme and are rejoined during ligation. These two opposing reactions, digestion and ligation, were performed on the templated structures to determine what affect the $CoFe_2O_4$ nanoparticle coating on the DNA would have on the availability of the biomolecule template. Scheme 1 shows an illustration of this process.

The samples were prepared by allowing the λ phage DNA to age in a tris buffered solution of 3.5 nm 2-pyrrolidinone-capped $CoFe_2O_4$ nanoparticles (1 mg/mL) for 1 h at room temperature. From previous studies, we have determined that this will result in the decoration of the DNA with the nanoparticles because of electrostatic interactions.[23] The complexes formed using this method are fairly consistent in particle coverage, though DNA strands with limited particle coating can be found. Following the development of the

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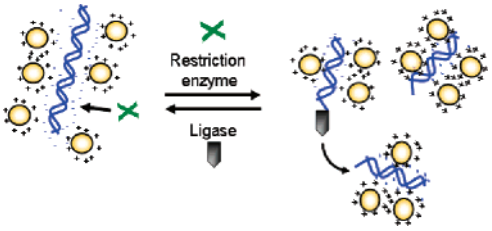
10.1021/cm070850e CCC: $37.00
Published on Web 06/30/2007

Scheme 1



Method used to generate, cut, and repaste $CoFe_2O_4$ nanoparticle-coated DNA. DNA electrostatically combines with positively charged nanoparticles in solution (left). Restriction enzymes are added to site-specifically clip the coated DNA, leaving shorter segments with single stranded overhangs (left to right). T4 ligase is then added to the solution gluing back the cleaved fragments and reforming long nanoparticle-coated DNA strands (right to left).

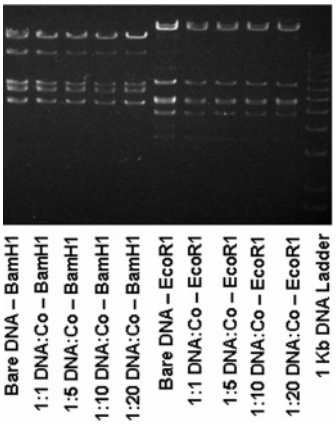


Figure 1. Agarose gel (0.8%) electrophoresis of $CoFe_2O_4$ nanoparticle-coated DNA. Lanes 1−5 show the results of digestion with BamH1; lanes 6−10 show digestion with EcoR1.

templated structures, we introduce either 10 units of BamH1 or EcoR1 into the reaction vessel and allow the reaction to proceed at room temperature for 4 h. After the time expires, the reaction is halted by heating the solution to 65 °C for 15 min to inactivate the enzyme. T4 ligase is then added to the vessel and incubated at room temperature for 30 min. The reaction vessel is then again heated to 65 °C for a period of 10 min to inactivate the ligase enzyme.

The restriction-digested samples were run on a 0.8% agarose gel to determine what affects the $CoFe_2O_4$ nanoparticle presence would have on the reaction. In Figure 1, a gel is shown comparing the activity of the two enzymes in response to increased concentration ratios of nanoparticles to DNA. In lanes 1−5, digests performed with the BamH1 enzyme are shown. The BamH1 enzyme is known to cut λ phage DNA into 5 segments with lengths of 5626, 6527, 6770, 7,233, and 16841 bp. In lane 1, DNA containing no $CoFe_2O_4$ nanoparticles was digested with the BamH1 enzyme. In this lane, the resulting fragments occur at the expected locations and demonstrate high contrast when stained with ethidium bromide. In lanes 2−5, the samples contain DNA that has been coated with $CoFe_2O_4$ nanoparticles at increasing particle concentrations. In these experiments, the DNA:$CoFe_2O_4$ nanoparticle ratios are expressed in terms of the ratio fo micrograms of DNA to micrograms of $CoFe_2O_4$. The BamH1 enzyme appears to produce all of the expected fragments in these samples, though the intensity of the samples appears to decrease with increasing concentrations of $CoFe_2O_4$ nanoparticles. From lanes 6−10, we show that the EcoR1 fragments appear as expected at 3530, 4878, 5804, 7421, and 21226 bp. Similarly, these bands lose staining intensity when high nanoparticle concentrations occur. This may be due to two possible mechanisms. The first being that the nanoparticles surrounding the DNA limit the ability of the enzymes to cleave the strands, thereby resulting in a decrease in the number of fragments formed. The second possibility is that the nanoparticle coating affects intercalation of the ethidium bromide stain, leading to a decreased number of dye molecules captured by the DNA.

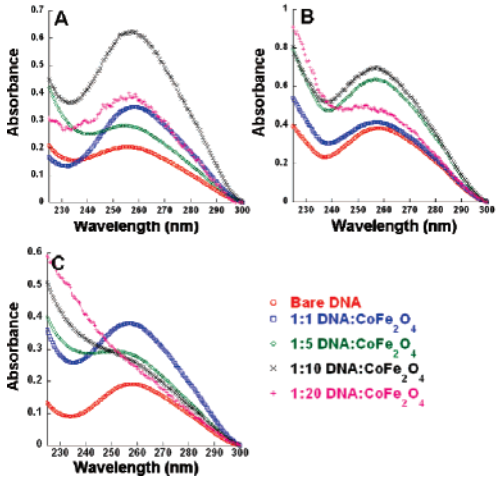


Figure 2. UV−visible spectroscopy of $CoFe_2O_4$-coated DNA templates. (A) Coated strands showing hyperchromicity with increasing nanoparticle concentration. (B) Coated strands digested with EcoR1. (C) Coated strands digested with BamH1.

We performed UV−vis spectroscopy on each sample to better understand which phenomenon occurs. Because the DNA has an intrinsic UV−vis excitation at 260 nm, we could follow the reactions without the use of the ethidium bromide stain. Figure 2A shows the spectra of the samples prior to digestion with either restriction enzyme. The absorbance at 260 nm increases with increasing concentrations of particles from 0.21 with bare DNA to 0.62 when the DNA:$CoFe_2O_4$ nanoparticle concentration ratio is 1:10. At a 1:20 DNA:$CoFe_2O_4$ nanoparticle concentration ratio, the sample is seen to decrease in absorbance. This hyperchromicity trend can be explained by the affect of the nanoparticle environment on the DNA conformation. With increasing particle concentration, the saturation of the DNA with particles begins to occur. The interaction between the particles and DNA induce an altered conformation, in comparison to the samples containing no nanoparticles, leading to higher absorption.

This technique was also used to determine the thoroughness of the restriction digests, panels B and C in Figure 2. When the coated DNA is digested with EcoR1, Figure 2B, the samples containing the greatest concentration of particles show modest increases in absorbance at 260 nm. It is typical for the shorter fragments generated by a restriction digest to have greater absorbance then their longer parent strand. The disparity between samples with nanoparticles versus those without is significant enough in the spectra to imply that the presence of nanoparticles does not impede enzymatic digestion. This can further be seen in Figure 2C, where the coated DNA is digested with BamH1. In the case of the

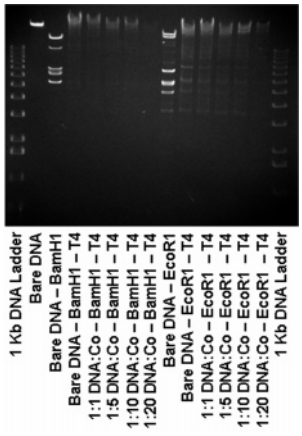


Figure 3. Agarose gel (0.8%) electrophoresis of $CoFe_2O_4$ nanoparticle-coated DNA. Lanes 3−7 show the results of digestion with BamH1 followed by ligation with T4 Ligase. Lanes 9−13 show digestion with EcoR1 followed ligation with T4 Ligase. Bare λ DNA was run in lane 2 as a control. The ligation products in each lane show a band that migrated to approximately the same distance.

BamH1-digested samples, the most significant increase in absorbance occurs in the samples with a 1:1 DNA:$CoFe_2O_4$ nanoparticle concentration ratio, though increases in absorbance are also noted in all nanoparticle-containing samples. These data suggest that the decreased intensity that occurs in staining the gel is from a decreased concentration of stain intercalating into the bases of the coated strands rather than a decrease in enzyme activity. The most compelling mechanisms, on the basis of the experimental data, indicate that the conformation of the DNA strand itself is altered. These spectra are indicative of either an unfolding, unwinding, or twisting of the DNA, causing the alignment of the dipole moments of the bases to become misaligned. In such an alternative conformation the ethidium bromide, which relies on intercalation between bases with specific orientation and geometry, cannot bind as tightly. This explains why there is an increase in the absorbance at 260 nm in the particle concentration spectra, whereas the same samples appear to produce less-intense bands in the electrophoresis gel. It is undetermined as yet as to what causes this conformational change and is currently being investigated using CD spectroscopy.

We performed a ligation of the fragmented coated DNA to further test the inherent biological capabilities of these structures. During ligation, complementary sticky-end fragments are pieced back together to form long DNA strands.[22] To test this, we digested samples of the $CoFe_2O_4$-coated DNA at DNA:$CoFe_2O_4$ ratios (μg:μg) of 1:1, 1:5, 1:10, and 1:20 with either BamH1 or EcoR1. T4 ligase (Promega) and the appropriate buffer were added directly to the samples. After 30 min, the samples were run in a 0.8% agarose gel at 75 V for 4 h (Figure 3). As controls, bare DNA without $CoFe_2O_4$ nanoparticles present was digested and then ligated and run on the same gel. The ligated samples show bands that are equal to or greater than the length of the original λ DNA scaffolds independently of the concentration of nanoparticles present. For both the BamH1-digested samples and the EcoR1-digested samples, the degree of ligation decreases with increasing particle concentration. When the $CoFe_2O_4$ nanoparticle concentration is 20 times that of the DNA, there appears to be little if any ligation and a decrease of templated DNA in either sample. This is most likely due to the increased nanoparticle coating causing a decrease in the ability of the stain to intercalate. This is similar to the decreased gel staining shown in the digestion samples. These results show that the fragments generated by the BamH1 enzyme are more thoroughly ligated than those produced by EcoR1. This occurs in each sample cleaved by EcoR1 regardless of the concentration of nanoparticles, indicating that the conditions necessary for ligation are likely longer than those for the BamH1 fragments. There also appears to be some star activity of the EcoR1 enzyme-digested samples due to buffer compatability with the enzyme. Fragments generated from star activity contain sticky ends that are not complementary to those generated by cleavage at the site-specific site of the enzyme.

These experiments show that despite a nanoparticle coating DNA can be cut and glued back together. Gel electrophoresis experiments demonstrated that even in large ratios of $CoFe_2O_4$ to DNA, BamH1 and EcoR1 could be used to generate fragmented templated DNA. This finding was also confirmed by UV−vis spectroscopy. The UV−vis spectra also indicated that the attachment of nanoparticles alters the conformation of the DNA, resulting in spectra resembling that of single-stranded DNA. Ligation of the digested samples was shown by gel electrophoresis experiments. These proof of concept experiments show another method to "cut and paste" nanoscale scaffolds by utilizing enzymatic tools commonly used in molecular biology. We are currently investigating the use of these methods to generate one-dimensional chains containing multiple types of nanoparticles. The specificity of the biomolecular template can provide an approach to do such with addressability of size-specific chains.

Acknowledgment. We acknowledge the SURI program administered through NASA INAC for summer support of M.V.S.

CM070850E