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Ultrafast colorimetric detection of nucleic acids based on the inhibition of the oxidase activity of cerium oxide nanoparticles

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A label-free colorimetric method to detect nucleic acids, which relies on target DNA induced shielding of the oxidase activity of CeO2 NPs, is developed. With this novel strategy, target nucleic acids can be identified within a few minutes and without the need for post-purification of PCR product.

Methods for detecting nucleic acids are in great demand in the areas of human genetics, clinical chemistry, cytology, and infectiology.1 Common procedures for this purpose involve exponential amplification of target DNA sequences by using the polymerase chain reaction (PCR)2 followed by gel electrophoretic analysis.3 However, the gel-based assays inevitably require tedious experimental procedures, specialized expertise, and long analysis times. An alternative to the use of conventional PCR coupled with gel electrophoresis, real-time PCR is now widely used because it enables immediate detection of target DNA sequences during the amplification process.4 This new method possesses many advantageous features including accurate quantification of target DNA, high sensitivity and specificity, and relatively short processing times.5 Despite some of its distinctive advantages, real-time PCR is limited by the need for special reagents (fluorescent probe or DNA-binding dye) and relatively expensive fluorescence instrumentation, which when combined critically limit applications in point-of-care testing (POCT) and facility limited environments, like those found in developing countries where inferior healthcare facilities exist.6 Thus, the development of simple and practical DNA screening methods that can be utilized in facility limited or POCT environments remains a significant goal.

Colorimetric strategies for DNA sensing are attractive because they enable rapid visual detection without the need for sophisticated instrumentations. In this regard, colorimetric sensing methods employing noble metal nanoparticles (gold and silver) or conjugated polymers (polythiophenes and polydiacetylenes) have been investigated. Although these systems rapidly and sensitively respond to target DNA, they possess several drawbacks including variabilities that depend on experimental environments (e.g., salt concentration, pH, etc) and that might lead to incorrect results. In addition, these types of sensing systems often require laborious pre- and/or post-modification procedures to conjugate the materials with probe oligonucleotides. To overcome these limitations, Li and Rothberg reported a method, which relies on single stranded DNA-induced inhibition of the salt-induced aggregation of gold nanoparticles; however, it still requires post-PCR processing of further denaturation to prepare ssDNA from PCR products and annealing to induce hybridization of probe DNA with ssDNA from PCR products.7

Recently, the unique enzyme-like activities of certain nanoparticles have been utilized as the basis for new colorimetric strategies for DNA detection. For example, the glucose oxidase activity of gold nanoparticles was employed in a novel system to detect DNA hybridization. The approach takes advantage of the remarkably different binding affinities of single (ss) and double stranded (ds) DNA to gold nanoparticles which leads to different levels of inhibition of the oxidase activity of the nanoparticles.8 We also devised a new colorimetric strategy to detect nucleic acids that utilizes target DNA induced inhibition of the peroxidase mimicking activity of magnetic nanoparticles.9 These approaches have opened a new paradigm for the design of simple and rapid methods to detect nucleic acids.

Cerium oxide nanoparticles (CeO2 NPs) have been shown to promote rapid oxidation reactions of various organic substrates that generate colored products without the need for additional oxidizing agents (e.g., H2O2).10 By employing CeO2 NPs, color generation takes place very rapidly within a few min, whereas other enzyme-like nanoparticles usually require several tens of min for color signal development.11,12 In addition, because they are not beset by problems of enzyme instability, CeO2 NPs have been used in place of horseradish peroxidase in more robust and reliable immunoassay systems.13,14 By taking advantage of the meritorious features of the oxidase activity of CeO2 NPs, we have designed a new and convenient label-free colorimetric method to detect nucleic acids. The results of this investigation are described below.

![Scheme 1 Schematic illustration of CeO2 NPs-based, label-free, colorimetric detection of target DNA.](image)

Scheme 1 Schematic illustration of CeO2 NPs-based, label-free, colorimetric detection of target DNA.
The overall strategy involved in devising the new DNA detection method is illustrated in Scheme 1. In the method, a sample containing the target DNA is first subjected to PCR amplification and then mixed with CeO₂ NPs (ca. 25 nm in diameter), followed by addition of 3,3',5,5'-tetramethylbenzidine (TMB), the color producing substrate. It is believed that the amplified target DNA will quickly adsorb on the surface of CeO₂ NPs owing to electrostatic interactions between the negatively charged phosphate backbone and the positively charged surface of the NPs. 14 As a consequence, direct contact of TMB with the NP surface, essential for promotion of the oxidation reaction, would be blocked. Moreover, negatively charged DNA also entraps the positively charged substrate TMB, which would also prevent free access of the substrate to the CeO₂ NPs. Finally, DNA present in the solution should also cause a certain level of aggregation of CeO₂ NPs, which would significantly decrease the availability of CeO₂ NPs surfaces and cause a further reduction of the oxidase activity. 14 These combined effects should cause a significant reduction of the oxidase activity of CeO₂ NPs and a corresponding reduction in the colorimetric signal produced by oxidation of TMB. In contrast, when amplified nucleic acids are not formed because target DNA is not present in the sample, CeO₂ NPs promoted oxidation of TMB should proceed in a normal manner to generate an intense blue colored product. Importantly, the differences in the intensities of colorimetric signals generated in these two cases should be distinguished easily even using the naked eye.

The validity and diagnostic capability of the new strategy was determined by employing the assay system comprised of CeO₂ NPs and varying concentrations of model target DNA from C. trachomatis. The inset is a plot of the absorbance at 652 nm vs. the concentration of target DNA, and the images show a control sample without target DNA and a test sample containing target DNA at a concentration of 120 nM.

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For this purpose, CeO₂ were separated by using centrifugation for 30 s of a solution of CeO₂ NPs and target DNA that is preincubated for 1 min. The NP containing pellet was treated with Eva-green dye, which generates a fluorescence signal by intercalating into dsDNA. 11 Analysis by using confocal microscopy shows that the CeO₂ NPs display a fluorescence signal confirming that adsorption of DNA takes place on the surface of CeO₂ NPs (Fig. S3, ESI†). In contrast, a fluorescence signal was not observed from CeO₂ NPs that were not incubated with the target DNA.

In order to explore the factors involved in DNA induced suppression of the oxidase activity of CeO₂ NPs, we first evaluated adsorption of nucleic acids to the CeO₂ NPs surfaces. For this purpose, CeO₂ NPs were separated by using centrifugation for 30 s of a solution of CeO₂ NPs and target DNA that is preincubated for 1 min. The NP containing pellet was treated with Eva-green dye, which generates a fluorescence signal by intercalating into dsDNA. 11 Analysis by using confocal microscopy shows that the CeO₂ NPs display a fluorescence signal confirming that adsorption of DNA takes place on the surface of CeO₂ NPs (Fig. S3, ESI†). In contrast, a fluorescence signal was not observed from CeO₂ NPs that were not incubated with the target DNA.
remaining in the solution (2, blue), and a test sample containing target DNA was first purged, and a control sample (red) was transformed to 100% vs. the absorbances from tests containing target DNA.

That nucleic acids absorb CeO$_2$ NPs surfaces was also demonstrated by using energy dispersive X-ray spectroscopy analysis (Fig. S4, ESI†). This technique shows that elements associated with both the CeO$_2$ NPs (Ce and O) and DNA (N and P) are present in the NP pellet obtained after removal of the solution containing DNA. In addition, analysis of TEM images probing NP surfaces (Fig. 2a and 2b) and the particle size distributions by measuring dynamic light scattering (DLS) (Fig. S5a, ESI†), show that CeO$_2$ NPs preincubated with nucleic acids, aggregate to a greater extent than those that are not treated with nucleic acids. This presence of nucleic acids on the aggregated CeO$_2$ NPs was also confirmed by the negative value of zeta potential (Fig. S5b, ESI†). This finding indicates that negatively charged nucleic acids induce aggregation of positively charged CeO$_2$ NPs. The combined results demonstrate that nucleic acids adsorb to surfaces and induce aggregation of CeO$_2$ NPs, which contributes to the reduction of catalytic activity of the NPs.

The amount of nucleic acids adsorbed on the CeO$_2$ NPs was estimated by measuring the quantity of nucleic acids remaining in solution after centrifugal removal of the DNA adsorbed CeO$_2$ NPs. As shown in Table S1, greater than 50% of the applied nucleic acids bind on the surface of the CeO$_2$ NPs. To assess the contribution made by unbound nucleic acids remaining in solution to inhibition of catalytic activity of the CeO$_2$ NPs, colorimetric assays to assess oxidase activity were performed before and after removal of the nucleic acid from solutions of DNA and the NPs and then compared to those arising from a control not containing DNA. The results (Fig. 2c) show that a significant reduction in the colorimetric signal occurs even after removal of the nucleic acids in the solution. However, the degree of color intensity reduction is ca. 70% of that of a solution containing nucleic acids in the supernatant. This observation demonstrates that nucleic acids in solution and those absorbed on NP surfaces both contribute to the reduction of oxidase activity of CeO$_2$ NPs.

We propose that electrostatic interactions between positively charged TMB and the negatively charged DNA molecules present in the solution hinder substrate transfer to the CeO$_2$ NPs and causes a reduction in the efficiency of the colorimetric signal producing oxidation reaction. To gain evidence to support the validity of this assumption, the negatively charged substrate, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), was used instead of TMB in the assay system. The results show that PCR products arising from target DNA cause a reduction in the color intensity generated by oxidation of ABTS. In this case however, the extent of reduction is significantly lower than that arising when positively charged substrate TMB is employed (Fig. 2d). Importantly, no significant differences were observed in the degree of colorimetric signal reduction before and after the removal of the unbound nucleic acids remaining in the assay solution (Fig. 2d; 2,3). Importantly, this finding shows that nucleic acids in the solution do not contribute to reduction of the colorimetric signal producing oxidation reaction of ABTS. Thus, it appears that nucleic acids in the solution do not interact with and cause aggregation of negatively charged ABTS whereas, as we proposed, their interaction with the positively charged TMB inhibits oxidation by CeO$_2$ NPs.

Finally, the new assay method was applied to the direct analysis of PCR products arising from unpurified genomic DNA. As can be seen by viewing the graph in Fig. 3, an obvious reduction of colorimetric signal takes place even when unpurified PCR products (600 bp) at 100 nM are assayed using the CeO$_2$ NPs system. Although the extent of signal reduction (ca. 50%) is lower than when purified PCR product is used (ca. 70%), the reduced color intensity associated with unpurified PCR products is still easily distinguished from a negative control using the naked eye.

In conclusion, a new, simple and ultrafast colorimetric method for the detection of nucleic acids has been developed. The assay relies on target DNA induced inhibition of the oxidase activity of CeO$_2$ NPs. By using the new technique, DNA samples can be analyzed by naked-eye detection within a few minutes without the need for post-purification of PCR product, chemical modifications of either the CeO$_2$ NPs or DNA, and tedious salt treatment steps, all of which are generally required in gold nanoparticle-based DNA detection systems. The clinical utility of the simple CeO$_2$ NPs-based colorimetric detection strategy was successfully demonstrated by its use in diagnosing C. trachomatis using a human urine sample. As a consequence of its many advantageous features, the new assay procedure should find wide use in POC applications. Furthermore, the strategy employed to design the new DNA sensor has the potential for being used to create assay systems to detect single nucleotide polymorphisms coupled with allele-specific PCR and biomolecules that are rationally designed to inhibit the oxidase activity of CeO$_2$ NPs.

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Notes and references


