Bio-Bar-Code-Based DNA Detection with PCR-like Sensitivity

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Since the polymerase chain reaction (PCR), primer-mediated enzymatic DNA amplification method, was introduced in 1985, PCR has significantly impacted the biological and medical communities.[1-4] PCR revolutionized medical diagnostic systems that rely on detecting and quantifying DNA targets of interest. However, PCR is often criticized for its complex, expensive, time-consuming, and labor-intensive procedure and narrow target DNA quantification range after PCR amplification.[5-7] Many DNA detection assays have been developed using radioactive labels, molecular fluorophores, chemiluminescence schemes, electrochemical tags, and most recently nanostructure-based labels.[8-17] Although some nanostructure-based methods are approaching PCR sensitivity, none thus far have achieved the 5–10 copy sensitivity level offered by PCR. If one could develop a methodology that allows for PCR-like signal amplification and eliminates the drawbacks of PCR, it would be extremely beneficial.

Herein, we report a PCR-less target DNA amplification method that relies on novel two-component oligonucleotide-modified gold nanoparticles (NPs) and single-component oligonucleotide-modified magnetic microparticles (MMPs), and subsequent detection of amplified target DNA in the form of bar-code DNA using a chip-based detection method. Recently, we showed that one can detect prostate specific antigen (PSA) at low attomolar (aM = 10^{-18} M) levels with the bio-bar-code amplification (BCA) approach.[18,19] BCA utilizes oligonucleotide-modified NPs for signal amplification and MMPs for easy and clean separation from unreacted elements. It would be a significant advance to be able to use the BCA to detect DNA targets (DNA-BCA) in addition to protein targets (protein-BCA). In this report, we show that this is possible in a format that offers 500 zeptomolar (zM = 10^{-21} M) sensitivity (~10 copies in the entire 30 μL sample).

For the proof-of-concept experiment reported herein, an oligonucleotide sequence associated with the anthrax lethal factor (5′ GGATTATTGTTAAAT- - -ATTGATAAGGAT 3′) was chosen as an initial target. This sequence is important for bioterrorism and biowarfare applications, and it has been well studied in the literature (Figure 1).[10,14-15] For DNA detection via the BCA approach, two types of probes have been prepared (Figure 1A). The first is an iron oxide MMP, which has a magnetic iron oxide core with an amine-modified silane coating. These particles were functionalized with alkanethiol-capped oligonucleotides that are complementary to one 12-mer portion (5′ ATTGATAAGGAT 3′) of a target sequence using a sulfosuccinimidyl 4-*N*-maleimidomethyl cyclohexane-1-carboxylate (sulfo-SMCC) linker.[20] The second probe is a gold NP (30 nm) modified with two types of oligonucleotides, one that is complementary to the target sequence of interest (5′ GGATTATTGTTAAAT 3′) and different from the region recognized by the MMP, and the other which is complementary to a bar-code sequence that is a unique identification tag for the target sequence. The NPs are loaded with the target binding and bar-code binding DNA by treating citrate-stabilized particles with a
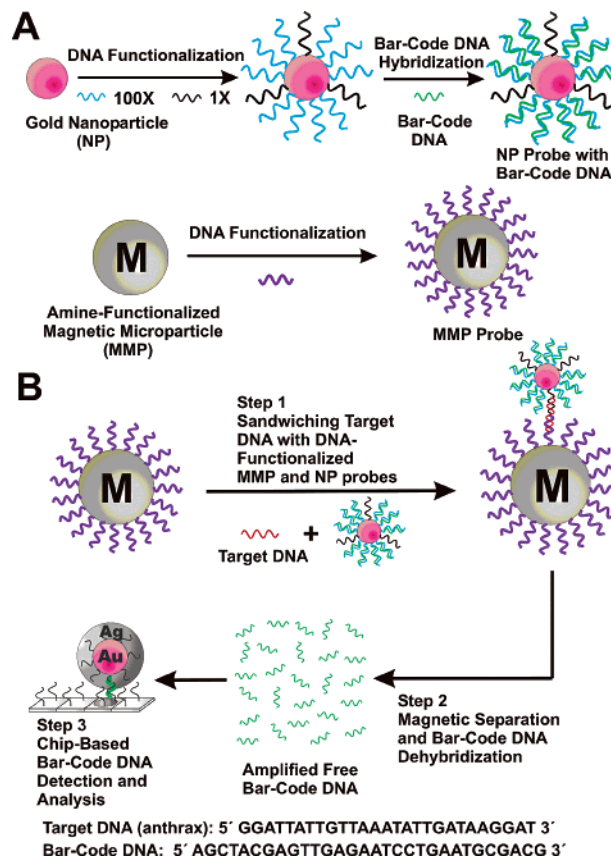

Figure 1. The DNA-BCA assay. (A) Nanoparticle and magnetic microparticle probe preparation. (B) Nanoparticle-based PCR-less DNA amplification scheme.

solution containing the two alkyl-thiol capped oligonucleotides at a 100:1 ratio (see Supporting Information). This ratio provides one route for target amplification because the bar-code DNA rather than the target DNA is identified in the BCA approach. Using literature methods,[21] we determined the average total number of oligonucleotide strands to be ~360 and the ratio of bar-code binding DNA to target binding DNA to be ~70:1.

In a typical DNA-BCA detection experiment, two control DNA sequences [1 μL of 10 pM control DNA 1 (5′ CTATTATAAT-AAAATATTTATATAGCA 3′) and 1 μL of 10 pM control DNA 2 (5′ GAATTATAGTTAACTATAGC TAAGGAT 3′)] were added to each 30 μL test sample. Prior to use, the NPs (5 mL) were loaded with bar-code DNA by hybridization [30 nm probes were at 300 pM concentration; 120 μL of bar-code DNA was introduced at 57 μM concentration to effect hybridization; centrifugation of the particles followed by washing with 0.1 M PBS buffer (0.1 M NaCl in 10 mM phosphate-buffered solution at pH 7.4) and subsequent resuspension resulted in activated NP probes].

The assay was initiated by adding 50 μL of the MMP probes (0.5 mg/mL) to a solution containing target DNA in single-stranded

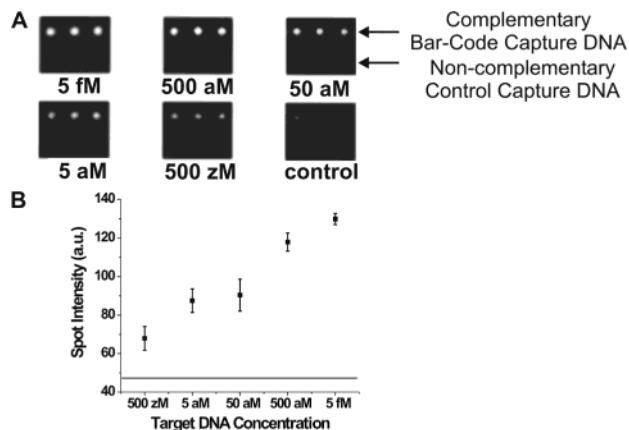10.1021/ja049384+ CCC: $27.50 © 2004 American Chemical Society

Figure 2. Amplified anthrax bar-code DNA detection with the Verigene ID system. (A) Anthrax bar-code DNA detection with 30 nm NP probes. (B) Quantitative data of spot intensities with 30 nm NP probes (Adobe Photoshop, Adobe Systems, Inc., San Jose, CA). The horizontal line represents control signal intensity (47 ± 2).

Figure 3. Single base mismatch experiment.

form (Figure 1B). The system was allowed to stand at room temperature for 10 min. The NP probes (50 μL, 250 pM) were then added to the solution and given 90 min to hybridize. After hybridization, the MMPs with target-linked NPs along with unreacted MMPs are pulled to the wall of the reaction vessel by a magnetic field (BioMag multi-6 microcentrifuge tube separator, Polysciences, Inc., Warrington, PA). Unreacted solution components (DNA and NPs) were washed away with PBS buffer. This washing step was repeated several times to effect removal of NPs that were not specifically bound to the MMPs through hybridization. Finally, the magnetic field was removed, 50 μL of NANOpure water (Barnstead International, Dubuque, IA) was added to the reaction vessel, and the system was heated to 55 °C for 3 min to ensure the release of the bar-code DNA. Reintroduction of the magnetic field removed all of the MMPs from solution, leaving bar-code DNA for detection.

To analyze the results of the assay, the scanometric method was used.[14] The scanometric method is a chip-based DNA detection method that relies on oligonucleotide-modified gold NP probes (5′ TCTCAACTCGTAGCT-A$_{10}$-SH 3′-Au) and NP-promoted reduction of silver(I) for signal amplification. The maleimide-modified glass chips were spotted with 5′ capture DNA strands (5′ SH-A$_{10}$-CGTCGCATTCAGGAT 3′) using a DNA microarrayer (spot diameter is 175 μm, and the distance between two spots is 375 μm; GMS 417 Arrayer, Genetic MicroSystems, Woburn, MA). The nonspot area was then passivated by immersing the chip into a solution containing an A$_{10}$ alkylythiol-capped oligonucleotide sequence (10 μM of 5′ SH-AAAAAAAAAA 3′) overnight. After NP probes mixed with bar-code DNA solution were added to a bar-code capture DNA-modified chip, the spots on a chip were labeled with NP probes and bar-code DNA strands. The spotted chip was then exposed to silver enhancement solution (Ted Pella, Redding, CA) for further signal enhancement. The developed spots were then read with a Verigene ID (identification) system (Nanosphere, Incorporated, Northbrook, IL), which measures the scattered light from the developed spots and provides a permanent record for the assay.

Each test was carried out three times on the same chip. The spot intensities show that one can differentiate a solution containing 500 zM concentration of the target DNA from the control with only noncomplementary DNA strands (Figure 2). The 30 μL sample used to do this experiment had approximately 10 DNA strands in the entire sample, showing the BCA approach provides a sensitivity that is comparable to that of PCR-based techniques.[5−7] Moreover, the DNA-BCA assay shows a wide detection range with a linear relationship between target concentration and spot intensity over a
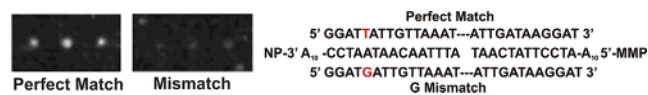
4 orders of magnitude concentration range (Figure 2B). Spot intensity saturates above 5 fM target concentration.

Finally, the BCA approach allows one to differentiate single base mismatches, even under relatively high sensitivity conditions, Figure 3. To test the selectivity of this system, the assay was carried out in two solutions, each containing either 500 aM concentration of target or oligonucleotide with a single base mismatch (in red, Figure 3). To effect stringency, the assay solution temperature was raised to 45 °C and held there for 3 min prior to magnetic separation (step 2 in Figure 1B). Scanometric detection showed a positive signal for the solution containing target and a negative for the one with mismatch.

In conclusion, a novel approach to DNA detection has been developed that provides high selectivity with a sensitivity that is comparable to many PCR-based approaches without the need for enzymatic amplification. Because the DNA-BCA approach is a pseudo-homogeneous system with both MMP and NP in solution, large concentrations of the probes can be used to very efficiently bind DNA target, thereby reducing the time required for high sensitivity detection experiments. Indeed, an advantage of the DNA-BCA approach over conventional microarray sandwich assays is that the entire assay can be carried out in 3−4 h, regardless of target concentration. The system has an excellent dynamic range and is ideally set up for multiplexing.

Supporting Information Available: Detailed nanoparticle and magnetic particle probe preparation. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA049384+