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Short communication

Dipstick-type biosensor for visual detection of DNA with oligonucleotide-decorated colored polystyrene microspheres as reporters

Despina P. Kalogianni[a], Ioannis K. Litos[b], Theodore K. Christopoulos[a,c,*], Penelope C. Ioannou[b]

[a] Department of Chemistry, University of Patras, Patras 26500, Greece
[b] Laboratory of Analytical Chemistry, Department of Chemistry, University of Athens, Greece 15771
[c] Foundation for Research and Technology Hellas, Institute of Chemical Engineering and High-Temperature Chemical Processes (FORTH/ICE-HT), P.O. Box 1414, Patras 26504, Greece

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ABSTRACT

In recent years, there is a continuously growing interest in the development of biosensors for rapid, simple and inexpensive DNA tests suitable for the small laboratory or for on-site testing. Detection is accomplished through electrochemical, optical or gravimetric transduction. We report on the development of disposable dipstick-type DNA biosensors that employ oligonucleotide-decorated colored polystyrene microspheres as reporters and enable visual detection of DNA sequences without the use of instrumentation. The biosensors have been designed to detect DNA molecules that contain both, a biotin moiety and a segment that is complementary to the oligonucleotide attached on the surface of blue or red microspheres. Capture of the hybrids by immobilized streptavidin at the test zone results in the formation of a colored line. The biosensors were applied to: (a) detection of single-stranded DNA, (b) detection of PCR-amplified double-stranded DNA and (c) genotyping of single nucleotide polymorphisms (SNP). The results were compared with sensors based on gold nanoparticle reporters. It is also demonstrated that the microspheres offer the potential for multicolor detection of specific DNA sequences.

1. Introduction

Nucleic acid hybridization assays have evolved significantly to allow a wide spectrum of applications for the routine laboratory or for field testing. In recent years research efforts aim at: (a) High analyte-throughput systems enabling parallel detection of thousands sequences in one or a few samples (e.g., DNA microarrays). (b) High sample-throughput assays based on microtiter wells or spectrally encoded beads that allow analysis of a large number of samples, each for a few target sequences. (c) There is a continuously growing activity on the development of biosensors for rapid, simple and inexpensive DNA tests suitable for the small laboratory or for on-site testing.

The principle of a DNA biosensor entails the immobilization of a DNA probe on the surface of the sensor, hybridization and detection by electrochemical (Lucarelli et al., 2004; Ozsoz et al., 2003), optical (Minunni et al., 2001; Mannelli et al., 2007) or gravimetric transduction (Tombelli et al., 2005).

The types of DNA biosensors listed above require instrumentation and, in most cases, involve several incubation and washing steps. Recent reports from our group and others (Glynou et al., 2003; Matsubara and Kure, 2003; Kalogianni et al., 2006, 2007a,b; Litos et al., 2007; Toubanaki et al., 2008) have described the development of novel disposable biosensors that enable visual detection of DNA without the use of instruments. Moreover, these biosensors are based on a dry-reagent dipstick format that eliminates multiple incubation and washing steps and minimizes the requirements for highly qualified personnel. The transduction principle exploits the characteristic red color of gold nanoparticles (AuNP) that is due to the plasmon resonance peak at 520 nm (Hayat, 1989). Hybridization triggers the accumulation of AuNP at the test zone of the sensor enabling visual detection.

In the present work we report on the development of dipstick-type DNA biosensors that employ oligonucleotide-decorated red and blue colored polystyrene beads as reporters. The performance of the biosensors is evaluated by: (a) the detection of single stranded target DNA, (b) detection of polymerase chain reaction (PCR)-amplified double-stranded DNA and (c) genotyping of single-nucleotide polymorphisms (SNP). In all cases the new sensors are compared with AuNP-based biosensors. In addition, we demonstrate that the bead-based sensors allow dual-color detection of

* Corresponding author at: Department of Chemistry, University of Patras, Patras 26500, Greece. Tel.: +30 2610 996022; fax: +30 2610 997118.
E-mail address: tchrist@upatras.gr (T.K. Christopoulos).

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specific DNA sequences, such as the color-coded identification of bacteria.

2. Materials and methods

The apparatus and reagents are presented in Supplement.

2.1. Coupling of oligonucleotides to microspheres

Microspheres (17 fmol) suspended in 50 μL of 0.1 M MES buffer, pH 4.5, were centrifuged for 3 min at 10,000 × *g* and washed twice with 50 μL of the same solution. The microspheres were resuspended in 100 μL of 0.1 M MES, pH 4.5, sonicated for 5 min and mixed with 100 pmol of 5′ amino-labelled $(dT)_{30}$ oligonucleotide. A 2-μL aliquot of freshly prepared 100 g/L EDC solution, in water, was added and the mixture was incubated at ambient temperature for 30 min. The addition of EDC was repeated with a fresh solution followed by 30-min incubation. The microspheres were pelleted by centrifugation at 10,000 g (3 min) and washed twice with 100 μL of 0.2 mL/L Tween-20. The $(dT)_{30}$-conjugated microspheres were resuspended in 20 μL of tailing reaction mixture (0.2 M potassium cacodylate, 25 mM Tris, pH 7.2, 0.1 mL/L Triton X-100, 2.5 mM $CoCl_2$, 0.5 mM dTTP) and sonicated for 20 s. Terminal deoxynucleotidyl transferase (30 U) was added and the mixture was incubated at 37 °C for 1 h. The microspheres were centrifuged as above and washed once with 100 μL of 0.2 mL/L Tween-20 and once with 100 μL of 1 g/L sodium dodecyl sulphate (SDS). The microspheres were sonicated for 10 s during each washing step. The poly(dT)-conjugated microspheres were finally resuspended in 85 μL of 15% sucrose, 45 mM NaCl, 2.5 g/L SDS and 2.5 mL/L Tween-20 solution.

2.2. Preparation of the dipstick-type DNA biosensor

The dry-reagent strip (4 mm × 70 mm) consisted of an immersion pad, a conjugate pad, a laminated membrane and an absorbent pad assembled on a plastic adhesive backing that provides rigidity. Streptavidin (27 pmol) and poly(dA) (1.2 pmol) were immobilized on the test zone and control zone of the strip, respectively. Blue or red microspheres coupled with poly(dT) strands (1 fmol), were placed on the conjugate pad.

2.3. Visual detection of PCR products by using the DNA biosensor

A 225-bp segment of maize-specific invertase (IVR) gene was amplified from genomic DNA by PCR (Elenis et al., 2007). The product was first hybridized with (dA)-tailed IVR-specific probe. Tailing of the probe was performed as above (Section 2.1) using 0.5 mM dATP and 100 pmol probe. For hybridization, 10 μL of PCR product was mixed with 1 μL of 0.9 M NaCl and 1 pmol probe. The mixture was heated at 95 °C (2 min) and then incubated at 37 °C (5 min). A 5-μL aliquot was applied to the conjugate pad of the sensor. The strip was then immersed into the developing solution containing 40 mL/L glycerol, 10 g/L SDS and 0.5 mL/L Tween-20 in PBS, pH 7.4. SDS and Tween-20 were added in order to avoid the appearance of nonspecific lines at the test zone of the sensor. A colored line (blue or red, depending on the color of the microspheres) at the test zone of the sensor denotes the presence of the amplified product. The control zone is always colored to confirm proper function of the sensor.

2.4. Genotyping of single nucleotide polymorphisms

Genomic DNA was isolated from 200 μL human whole blood using the QiaAmp DNA blood mini-kit. PCR amplification of MBL2 gene was performed as described previously (Litos et al., 2007). PEXT reactions (20 μL) were performed in the thermal cycler in a mixture containing 20 mM Tris–HCl, pH 8.8, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 1 mL/L Triton X-100, 1 mM $MgSO_4$, 0.25 U of Vent (exo-) DNA polymerase, 0.2 pmol amplified DNA, 2 pmol of the appropriate probe (normal or mutant), 2.5 μM each of dATP, dCTP, and dGTP, 1.9 μM dTTP, and 0.62 μM biotin-11-dUTP. The reaction conditions for the SNP at position -550 (c.-619G > C) were: initial denaturation at 95 °C for 5 min, followed by three cycles of denaturation at 95 °C (15 s), primer annealing at 65 °C (10 s) and extension at 72 °C (15 s). For detection of the SNP at position -221 (c.-290G > C), the cycling parameters were as above except that the annealing step was at 55 °C (10 s). PEXT products were subjected to a final denaturation step at 95 °C (5 min) and placed immediately on ice. A 5-μL aliquot of denatured product was applied onto the conjugate pad next to the microspheres. The wicking pad was then dipped into a microcentrifuge tube containing 200 μL of 10 mM phosphate buffer (pH 7.4), 75 mM NaCl, 30 mL/L glycerol, and 10 g/L SDS. The products were detected visually in about 10 min.

2.5. Dual-color biosensor for detection of bacteria

A fragment of the 23S rRNA gene was amplified by PCR using universal primers (Kalogianni et al., 2007b). Specific probes for *Escherichia coli* and *Staphylococcus aureus* were coupled to blue and red microspheres, respectively, as described in Section 2.2. Biotinylated PCR products were denatured at 95 °C (5 min) and a 5-μL aliquot (about 50 fmol) was added to a mixture of the conjugated microspheres (blue and red) (1 fmol of each) in 0.2 M NaCl, 0.1 M Tris, pH 8.0 and 0.8 mL/L Triton X-100. The mixture was incubated for 10 min at 37 °C. The microspheres were pelleted by centrifugation for 3 min at 10,000 g, resuspended in 10 μL of 15% sucrose, 45 mM NaCl, 2.5 g/L SDS, 2.5 mL/L Tween-20 and applied to the sensor. A 2.5-μL aliquot of blue microspheres (0.5 fmol) coupled with $(dT)_{30}$ probe was also applied to the sensor strip. The sensor was then immersed into the developing solution. A blue or red line was formed at the test zone of the sensor indicating the presence of *E. coli* or *S. aureus*, respectively, in the sample. A second line (blue) was formed at the control zone of the strip confirming the proper function of the sensor.

3. Results and discussion

A schematic illustration of the principle of the dipstick-type biosensor for visual detection of DNA by using oligonucleotide-decorated colored polystyrene microspheres as reporters is presented in Fig. 1. Three assay configurations are also presented in Fig. 1: (A) detection of ssDNA, (B) detection of PCR-amplified dsDNA and (C) detection of SNP genotyping products. The biosensor has been designed to detect DNA molecules that contain both a biotin moiety and a segment that is complementary to the oligonucleotide attached on the surface of the microspheres. The sample is placed on the conjugate pad of the sensor, which is then immersed in the developing buffer. The buffer migrates along the strip by capillary action. The DNA is linked, through hybridization, to the microspheres and captured at the test zone through biotin/streptavidin interaction. The proper function of the biosensor is confirmed at the control zone in which immobilized poly(dA) strands capture poly(dT)-decorated microspheres. The size of 300 nm for the microspheres was chosen because it is the optimum for the porosity of the nitrocellulose membrane (5 μm) thereby allowing smooth flow of the microspheres with the developing solution.