Analytica Chimica Acta 864 (2015) 48–54

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Lateral flow devices for nucleic acid analysis exploiting quantum dots as reporters

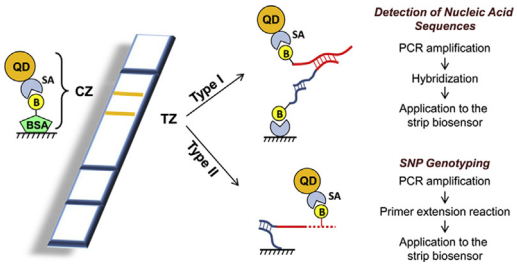
Eleni A. Sapountzi [a], Sotirios S. Tragoulias [a], Despina P. Kalogianni [a], Penelope C. Ioannou [b], Theodore K. Christopoulos [a,c,*]

[a] Department of Chemistry, University of Patras, GR-26504 Patras, Greece
[b] Department of Chemistry, University of Athens, GR-15771 Athens, Greece
[c] Institute of Chemical Engineering and High Temperature Processes, Foundation of Research and Technology Hellas, GR-26504 Patras, Greece

HIGHLIGHTS

- Dipstick tests for DNA hybridization assays and genotyping of single-nucleotide polymorphisms.
- Use of quantum dots as reporters.
- Visual detection without the need for expensive instrumentation.
- Simplicity and low-cost of the assays.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 October 2014
Received in revised form 8 January 2015
Accepted 14 January 2015
Available online 1 February 2015

Keywords:
Lateral flow
Quantum dots
Biosensor
Nanoparticles
Nucleic acids

ABSTRACT

There is a growing interest in the development of biosensors in the form of simple lateral flow devices that enable visual detection of nucleic acid sequences while eliminating several steps required for pipetting, incubation and washing out the excess of reactants. In this work, we present the first dipstick-type nucleic acid biosensors based on quantum dots (QDs) as reporters. The biosensors enable sequence confirmation of the target DNA by hybridization and simple visual detection of the emitted fluorescence under a UV lamp. The 'diagnostic' membrane of the biosensor contains a test zone (TZ) and a control zone (CZ). The CZ always fluoresces in order to confirm the proper function of the biosensor. Fluorescence is emitted from the TZ, only when the specific nucleic acid sequence is present. We have developed two general types of QD-based nucleic acid biosensors, namely, Type I and Type II, in which the TZ consists of either immobilized streptavidin (Type I) or immobilized oligodeoxynucleotides (Type II). The control zone consists of immobilized biotinylated albumin. No purification steps are required prior to the application of the DNA sample on the strip. The QD-based nucleic acid biosensors performed accurately and reproducibly when applied to (a) the visual detection of PCR amplification products and (b) visual genotyping of single nucleotide polymorphisms (SNPs) in human genomic DNA from clinical samples. As low as 1.5 fmol of double-stranded DNA were clearly detected by naked eye and the dynamic range extended to 200 fmol. The %CV were estimated to be 4.3–8.2.

1. Introduction

The detection and quantification of specific nucleic acid sequences are based on the hybridization (molecular recognition) of the target sequence (analyte) with a complementary DNA sequence that is linked, directly or indirectly, to an appropriate

* Corresponding author at: Department of Chemistry, University of Patras, GR-26504 Patras, Greece. Tel.: +30 2610 962951.
E-mail address: tchrist@upatras.gr (T.K. Christopoulos).

http://dx.doi.org/10.1016/j.aca.2015.01.020
0003-2670/

label for signal generation. Presently, there is a growing interest in the development of lateral flow devices for nucleic acid analysis that enable visual detection of the analyte while eliminating several steps required for pipetting, incubation and washing out the excess of reactants.

Fluorometry is the most common technique used in biosensing because of its high sensitivity, simplicity and ability for multiplexing [1,2]. Nanomaterials are gradually replacing the organic fluorophores in the development of novel analytical methods because they exhibit several advantages such as brighter fluorescence, higher photostability and wider Stokes shift [3,4]. Furthermore, nanostructures provide a suitable solid substrate for the construction of biosensors that offer simplicity and higher sensitivity. Nanomaterials such as metallic, silica and carbon nanoparticles and nanotubes, as well as quantum dots (QDs) have been applied to the development of biosensors due to their unique physical, chemical, mechanical, magnetic and optical properties, enhancing the sensitivity and the specificity of detection [5].

QDs are nanometer-scale semiconductor nanocrystals that have been used successfully as reporters in biosensing and bioimaging [6] due to their excellent optical properties and the ability of attaching various molecules to their surface [7–9]. QDs have also been used for the development of immunoassays in a lateral flow strip format. The first electrochemical lateral flow test strip based on QDs was introduced in 2007 [10]. This was an immunosensor strip coupled with a disposable screen-printed electrode for the detection of prostate-specific antigen (PSA) in human serum. CdS–ZnS core–shell QDs, captured on the strip, were determined by stripping voltammetry of the dissolved cadmium. More recently, QD-based fluorometric lateral flow immunoassays, for a variety of analytes, were reported. Both the 'sandwich' type (two-site) and competitive-type immunoassay configurations were exploited.

QD-based competitive-type fluorometric lateral flow immunoassays were developed for the detection of trichloropyridinol (the metabolite of the organophosphate insecticide chlorpyriphos) in rat plasma [11], the detection of mycotoxin ochratoxin A in red wines [12], and the detection of the antibiotic chloramphenicol in milk [13].

Sandwich-type fluorometric lateral flow immunoassays using QDs as reporters were developed for staphylococcal protein A (screening for syphilis) in human blood samples [14], nitrated ceruloplasmin (a biomarker for cardiovascular disease, lung cancer, and stress response to smoking) in spiked human plasma samples [15], alpha fetoprotein (tumor marker for hepatocarcinoma) in human serum samples [16], avian influenza virus in chicken serum samples [17] and anti-hepatitis B virus antibody in human serum [18].

Herein, we describe the first dipstick-type nucleic acid biosensors based on quantum dots. More specifically, this report refers to lateral flow tests exploiting streptavidin-functionalized CdSe–ZnS core–shell QDs as reporters. The biosensors enable sequence confirmation of the target DNA by hybridization and simple visual detection of the emitted fluorescence under a UV lamp. The QD-based nucleic acid biosensors were applied to (a) visual detection of PCR amplification products and (b) visual genotyping of single nucleotide polymorphisms (SNPs) in human genomic DNA. The 'diagnostic' membrane of the biosensor contains a test zone (TZ) and a control zone (CZ). The CZ fluoresces always in order to confirm the proper function of the biosensor. Fluorescence is emitted from the TZ, only when the specific nucleic acid sequence is present. We have developed two general types of QD-based nucleic acid biosensors, namely, Type I and Type II. For Type I biosensors, the test zone contains immobilized streptavidin and the control zone consists of immobilized biotinylated albumin. For Type II biosensors, the TZ contains immobilized oligodeoxynucleotides and the CZ consists of immobilized biotinylated albumin. Moreover, in the Type II sensor, the oligonucleotides were either immobilized directly to the surface of the membrane (Type IIa) or indirectly through the immobilization of an albumin-oligo conjugate (Type IIb). No purification steps were required prior to the application of the DNA sample on the strip either the Type I or the Type II sensors.

2. Materials and methods

2.1. Apparatus and reagents

PCR amplification and allele-specific primer extension reactions were performed in the MJ Research PTC-0150 thermal cycler (Watertown, MA). A digital camera, Konica Minolta DYNAX 5D (Konica Minolta Holdings Inc., Tokyo, Japan) was used for photographing the strips. A UV lamp (Philips HPW 125 W-T, Belgium) was used for the excitation of quantum dots on the strip. The Gel Analyzer software for DNA documentation was purchased from Kodak (New York, NY). The TLC applicator, Linomat 5, and the software WinCats were from Camag (Muttenz, Switzerland).

Quantum dots (585 nm) conjugated with streptavidin (SA) were purchased from Invitrogen (CarlsBad CA). The quantum dots consisted of CdSe as a core surrounded by a shell of ZnS. Terminal deoxynucleotidyl transferase (TdT) was from MBI Fermentas (Vilnius, Lithuania). *Taq* DNA polymerase was obtained from HyTest (Turku, Finland) and New England Biolabs (Beverly, MA). Vent (exo-) DNA polymerase was from New England Biolabs (Beverly, MA). Ultrapure 2′-deoxyribonucleoside 5′-triphosphates (dNTPs) were purchased from Invitrogen (CarlsBad CA). Biotin-11-dUTP was from Applichem (Darmstadt Germany). Immunopore FP nitrocellulose membrane was purchased from Whatman (Florham Park, NJ). The wicking pad, glass-fiber conjugate pad, and absorbent pad were from Schleicher & Schuell (Dassel, Germany). Streptavidin from *Streptomyces avidinii* was purchased from Roche Diagnostics (Mannheim, Germany). EDTA was from Merck (Darmstadt, Germany). Anti-biotin antibody, Sephadex G-25, methanol, sucrose, sodium dodecyl sulfate (SDS) and all other common reagents were from Sigma (St. Louis, MO). Phosphate buffered saline (1 × PBS) consisted of 140 mM NaCl, 2.7 mM KCl, 10 mM NaH_2PO_4, 1.7 mM KH_2PO_4, pH 7.4. Saline sodium citrate (SSC) buffer contained 0.3 M NaCl and 30 mM $Na_3C_6H_5O_7$, pH 7.0. The primers and probes used in this study were synthesized by MWG-Biotech (Ebersberg, Germany) and Invitrogen (Carsbad, CA). The NH_2-dT(30) probe used for the construction of the strip was 5′-NH_2-T_{30}-3′. The universal upstream and downstream primers, used for the amplification of a highly conserved region of the 23S rRNA of *Staphylococcus epidermidis*, were 5′-GCGATTTC(CT)GAA (CT)GGGG(AG)AACCC-3′ and 5′-TTCGCCTTTCCCT CACGGTACT-3′, respectively [19]. The downstream primer was biotinylated at the 5′end. The specific probe for the identification of *S. epidermidis* was 5′-ACGGAGTTACAAAAGAACATGTTAGTTTTT-3′. The upstream and downstream primers, used for the amplification of TLR4 gene were 5′-GTAAAACGACGGCCAGTAGTCCATCGTTTGGTTCTGGGAGA-3′ and 5′-CAGGAAACAGCTATGACGCCATTGAAAGCAACTCTGGTGTG-3′, respectively. The specific primers used in the primer extension reaction for wild type and mutant allele for TLR4 gene were 5′-A_{24}GTGATTTTGGGACAAC-3′ and 5′-A_{24}GTGATTTTGGGACAAT-3′, respectively.

2.2. Construction 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 the required rigidity. The four parts were positioned in such a way that