Biosensors and Bioelectronics 26 (2011) 2390–2395

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Ultrasensitive quantum dots-based DNA detection and hybridization kinetics analysis with evanescent wave biosensing platform

Feng Long, Shuxu Wu, Miao He, Tiezheng Tong, Hanchang Shi*

Environmental Simulation and Pollution Control State Key Joint Laboratory, Department of Environment Science and Engineering, Tsinghua University, Beijing 100084, China

ARTICLE INFO

Article history:
Received 8 August 2010
Received in revised form 10 October 2010
Accepted 11 October 2010
Available online 16 October 2010

Keywords:
DNA
Quantum dots
Evanescent wave biosensor
Hybridization kinetics

ABSTRACT

Ultrasensitive DNA detection was achieved using a new biosensing platform based on quantum dots (QDs) and total internal reflection fluorescence, which featured an exceptional detection limit of 3.2 amol of bound target DNA. The reusable sensor surface was produced by covalently immobilizing streptavidin onto a self-assembled alkanethiol monolayer of fiber optic probe through a heterobifunctional reagent. Streptavidin served as a versatile binding element for biotinylated single-strand DNA (ssDNA). The ssDNA-coated fiber probe was evaluated as a nucleic acid biosensor through a DNA–DNA hybridization assay for a 30-mer ssDNA, which were the segments of the uidA gene of *Escherichia coli* and labeled by QDs using avidin–biotin interaction. Several negative control tests revealed the absence of significant non-specific binding. It also showed that bound target DNA could easily be eluted from the sensor surface using SDS solution (pH 1.9) without any significant loss of performance after more than 30 assay cycles. A quantitative measurement of DNA binding kinetics was achieved with high accuracy, indicating an association rate of 1.38×10^6 M^{-1} s^{-1} and a dissociation rate of 4.67×10^{-3} s^{-1}. The proposed biosensing platform provides a simple, cheap, fast, and robust solution for many potential applications including clinical diagnosis, pathology, and genetics.

1. Introduction

Highly-sensitive, rapid, and reliable detection of DNA/RNA without expensive instrumentation and extensive sample pretreatment, is in great demand for clinical diagnosis, pathology, and genetics. Among the various detection schemes currently under investigation, optical fiber biosensors based on total internal reflection fluorescence (TIRF) have shown great potential in providing viable solutions to these challenges (Abel et al., 1996; Leung et al., 2007). When optical fiber transmits light on the basis of the principle of total internal reflection, the evanescent wave penetrates essentially into the surrounding cladding of lower refractive index and exponentially decays with distance. The evanescent wave can excite fluorescence primarily from the fluorescent labeled analyte complexes bound to the surface through affinity recognition interactions. The signal changes over time are directly related to the binding kinetics of biomolecular interactions (Golden et al., 1997). Knowledge on hybridization kinetics at a solid-phase interface is critical for the design and optimization of biosensing techniques and the efficient collection of genomic information (Zeng et al., 2003). In addition, quantum dots (QDs) have become powerful tools for biosensing and medical imaging because of their unique optical properties, such as high quantum yield, photostability, narrow emission spectrum, and broad absorption (Michalet et al., 2005; Resch-Genger et al., 2008). Compared with organic dyes, QDs are 20 times brighter and several orders of magnitude more photostable (Chan and Nie, 1998; Sukhanova et al., 2004). The combination of the evanescent wave biosensor and QDs can be used for ultrasensitive DNA detection and analysis of hybridization kinetics at solid interfaces.

This study is the first to propose an all-fiber DNA biosensing platform based on QDs and TIRF. Compared with conventional fiber optic DNA biosensors that need numerous optical components with stringent optical alignment requirements, the proposed DNA biosensor shows considerable promise in obtaining sequence-specific information in a simple, fast, and portable technology. *Escherichia coli* is selected as a model pathogen to validate the detection capabilities of the developed biosensor using QD-DNA as reporter molecules. The interfacial hybridization behaviors of DNA on the sensor surface are determined in real-time, and kinetic parameters are calculated based on the proposed theory.

2. Experimental

2.1. Chemicals and reagents

Ovalbumin (OVA), bovine serum albumin (BSA), 3-mercaptopropyl-trimethoxysilane (MTS), N-(4-maleimidobu-

* Corresponding author. Tel.: +86 10 62773095; fax: +86 10 62771472.
E-mail address: hanchang@mail.tsinghua.edu.cn (H. Shi).

0956-5663/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2010.10.018

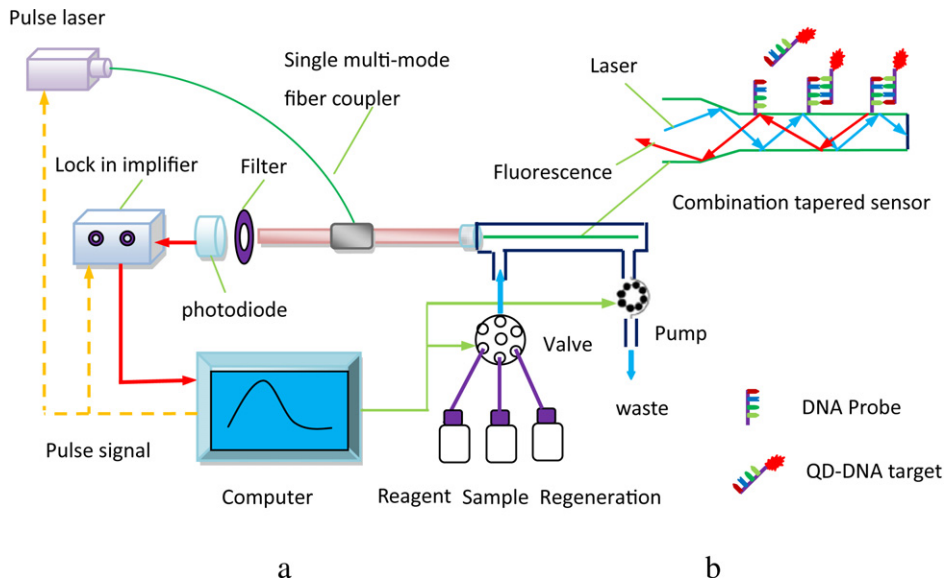

Fig. 1. (a) Schematic diagram of the structure of evanescent wave DNA biosensor and (b) schematic diagram of combination-tapered fiber sensor and mechanical schematic of DNA detection.

tyryloxy) succinimide (GMBS), and 1-ethyl-3-dimethylaminopropyl carbodiimide hydrochloride (EDC) were purchased from Sigma–Aldrich (Germany). The Qdot® streptavidin conjugates (Q10161MP) were obtained from Invitrogen Ltd. (USA). DNA oligonucleotides used were the segments of the uidA gene of *E. coli*, which were supplied by Takara Biotechnology (Dalian) Co. (China). The following oligonucleotide were used: DNA probe, 5′-biotin-TTTTTTTTTTACGCTCACACCGATACCATC-3′; perfectly matched cDNA (PM), 5′-biotin-AGTCTCAGCAGATGGTATCGGTGTGAGCGT-3′; non-complementary DNA (NM), 5′-biotin-AGTCTCAGCAAG-TCTCAGCAAGTC TCAGCA-3′; one-base mismatched cDNA (MM), 5′-biotin-AGTCTCAGCAGATG GTAGCGGTGTGAGCGT-3′, a perfectly matched cDNA labeled by Dylight 680 (Dyl-PM), 5′-Dylight680-AGTCTCAGCAGATGGTATCGGTGTGAGCGT-3′. All oligonucleotides were purified by reverse-phase high performance liquid chromatography (RP-HPLC) to remove failure sequences that interfere in the experiments. A biotin-tethered oligonucleotide stock solution was prepared with 10 mM phosphate buffered solution (PBS, pH 7.4) containing 1 mM EDTA; the stock solution was kept frozen afterwards. The PM, NM, and MM target oligonucleotides were dissolved in TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.4) and kept frozen for storage. All solutions were prepared with ultrapure water from a Millipore Milli-Q system. All other reagents, unless specified, were purchased from the Beijing Chemical Agents (China). All chemicals were of analytical reagent grade.

2.2. Evanescent wave biosensing platform

Fig. 1 presents the schematic of the proposed QDs-based DNA biosensor. Due to the light absorption efficiency of Qdot® nanocrystals used increased dramatically to the blue of the emission (Chan and Nie, 1998; Michalet et al., 2005), the 405-nm pulse diode laser (HuayuanStar Ltd., China) with pigtail acted as the excitation light source. The laser entered from the single multi-mode fiber into the single-multi mode fiber coupler (Beijing Glass Research Institute, China) with a diameter of 600 μm and a numerical aperture of 0.22. Excitation light from the laser was coupled to the fiber-based DNA sensor through a fiber connector. Incident light was propagated along the length of the fiber probe via total internal reflection. The evanescent wave was generated at the surface of the probe and decayed exponentially with distance. It excited the fluorophores labeled in the surface-bound analyte complex. Part of the fluorescence coupled back into the fiber sensor was subsequently filtered by a bandpass filter (FF01-692/40, Semrock, USA), and detected by photodiodes through a digital lock-in amplifier produced in the laboratory. In this system, both the transmission of the excitation light and the collection and transmission of fluorescence were achieved by fiber optics with a single-multi mode fiber optic coupler (Long et al., 2008a). In this way, the optical components required were reduced, and the need for frequent optical alignment became rare. The DNA biosensor is embedded in a flow glass cell (Ø2 × 40 mm, 200 μL) with an inlet and an outlet. The flow cell is attached via Tygon® tubing with an inner diameter of 0.76 mm to a peristaltic pump, which controlled the flow rate and delivered various solutions. Buffer, sample solutions, and regeneration solutions could be exchanged through the six-way valve. Control of the pump and six-way valve, as well as performing data acquisition and processing were automatically performed by a computer. The computer also provided pulse signals of the same frequency for the laser and for the lock-in amplifier.

2.3. Single-strand DNA (ssDNA) labeled with QDs

Biomolecules can be attached either covalently or non-covalently on the surface of QDs. Avidin–biotin cross-linking, one of the most popular methods for conjugating biomolecules on the surface of QD, was used to label DNA with QDs. All biotinylated target DNA were labeled by the QD-streptavidin conjugate as follows: 100 μL of 10 μM biotinylated DNA were added into 2 μM QD-streptavidin conjugate solution and the mixture was stirred gently for 20 min to allow for streptavidin and biotin to bind. Then, to remove any excess unbound biotinylated cDNA, the solution was centrifuged at 2000 rpm in the ultrafiltration unit for at least five buffer exchanges (50 mM borate buffer, pH 8.3). The QD-DNA conjugate solution was stored at 4 °C until use.

2.4. Modification of optical fiber sensor

A combination-tapered fiber optic sensor was prepared as described previously (Long et al., 2008b). The modification method used to immobilize DNA probe onto the sensor surface is shown in Fig. 2. Combination-tapered fiber sensor was initially cleaned with