Sensors and Actuators B 191 (2014) 567–571

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Polydopamine nanospheres: A biopolymer-based fluorescent sensing platform for DNA detection

Qian Liu[a], Zonghua Pu[a], Abdullah M. Asiri[b,c], Abdulrahman O. Al-Youbi[b,c], Xuping Sun[a,b,c,*]

[a] Chemical Synthesis and Pollution Control, Key Laboratory of Sichuan Province, School of Chemistry and Chemical Industry, China West Normal University, Nanchong 637002, Sichuan, China
[b] Chemistry Department, Faculty of Science, King Abdulaziz University, Jeddah 21589, Saudi Arabia
[c] Center of Excellence for Advanced Materials Research, King Abdulaziz University, Jeddah 21589, Saudi Arabia

ARTICLE INFO

Article history:
Received 16 July 2013
Received in revised form
30 September 2013
Accepted 13 October 2013
Available online 19 October 2013

Keywords:
Polydopamine nanosphere
Biopolymer
Fluorescence sensing
Nucleic acid
Single-base mutation

ABSTRACT

In this paper, a novel and highly sensitive DNA sensor based on biopolymer is presented. This polydopamine nanospheres-based sensing platform exhibits high selectivity and sensitivity with a detection limit of 5 nM and the sensitivity of this sensing platform increases with increased temperature.

1. Introduction

Simple, fast, cost-effective, and sensitive detection of specific DNA sequences is crucial to the identification of disease-causing pathogens and genetic diseases, as well as other activities. In recent years, we have witnessed the growing importance in developing strategies of DNA detection for various applications in gene expression profiling, clinical disease diagnostics and treatment, fast detection of biological warfare agents, and forensic testing, etc. [1]. Detecting genetic mutations at the molecular level opens up the possibility of performing reliable disease diagnostics in clinical practice even before any symptom of a disease appears. Because single-base mutation can lead to the differences among individuals and it is contributory factor for a number of heritable diseases [2,3], it is of considerable significance to develop DNA diagnostic test capable of single-base mismatch discrimination.

The introduction of simple methods for fluorescent labeling of nucleic acids has enabled fluorescent nucleic acid probes to be used for assays based on fluorescence resonance energy transfer (FRET) or quenching mechanism for DNA detection [4]. Among these probes, Taqman probes, molecular beacons (MBs), and Scorpions are labeled with a fluorescent reporter and a quencher dye at both ends and the fluorescence is only released from the reporter until the two dyes are physically separated after hybridization occurs. However, labeling at both ends with specific dyes suffers from low overall yield and is not cost-effective [5]. It has shown that nanostructures like Au nanoparticles, single-walled carbon nanotubes (SWCNTs), and graphene oxide (GO) can quench fluorescent dyes of different emission frequencies, eliminating the selection issue of fluorophore–quencher pair in such assay [6–9]. More recently, we and other researchers have successfully demonstrated that versatile structures can serve as an effective quencher for fluorescent DNA detection, including carbon nanoparticles [10], nano-C_{60} [11], coordination polymer nanobelts [12] and colloids [13], conjugation polymers [14,15], Ag@conjugation polymer core–shell nanoparticles [16], supramolecular microparticles [17], etc. However, small size gold nanoparticle makes it hard for simultaneous adsorption of multiple DNA probes labeled with different dyes on the same particle surface. For the SWCNT system, an organic solvent like N,N-dimethylformamide (DMF) is used to disperse SWCNT by a period of several hours sonication [8]. As for GO-based system, the preparation of GO using the Hummer's method is time-consuming

* Corresponding author at: Chemical Synthesis and Pollution Control Key Laboratory of Sichuan Province, School of Chemistry and Chemical Industry, China West Normal University, Nanchong 637002, Sichuan, China.
E-mail address: sun.xuping@hotmail.com (X. Sun).

0925-4005/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.snb.2013.10.050

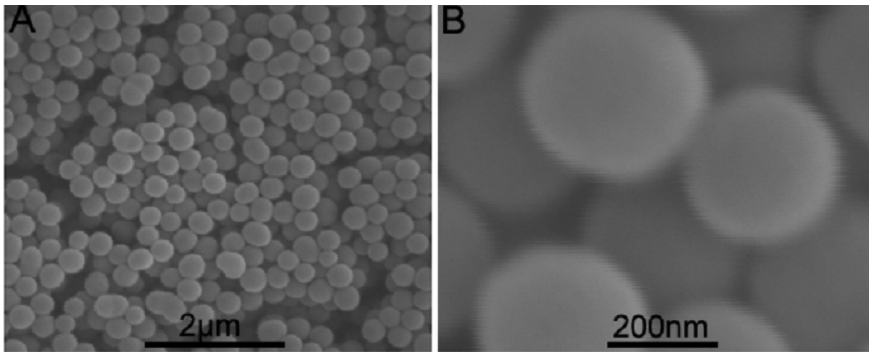

Fig. 1. (A) Low and (B) high magnification SEM images of Pdop-NSs.

and labor-intensive [18]. In addition, all these above quenchers are not bio-related, which may limit their applications. Polydopamine (Pdop), a mussel-inspired adhesive catecholamine, is a biopolymer with excellent biocompatibility and low cytotoxicity, which makes it a versatile platform for bioapplication [19,20]. Herein, we demonstrate the first use of Pdop nanospheres (Pdop-NSs) as a biopolymer-based fluorescent sensing platform for DNA detection. It also suggests that Pdop-NSs can be used as an effective fluorescent sensing platform capable of discrimination of complementary and single-base mismatched target sequences.

2. Experimental

2.1. Reagents and materials

Dopamine (98%, Aladdin Reagent Co. Ltd.), DNA (Shanghai Sangon Biotechnology Co. Ltd.), ammonia aqueous solution (AR, Tianjin Fuyu Ltd.) and absolute ethanol (AR, Tianjin Fuyu Co. Ltd.) were used as received. The water used throughout all experiments was purified through a Millipore system.

Oligonucleotide sequences used are listed below (mismatch underlined):

P_{HIV} (FAM dye-labeled ssDNA):
5′-FAM-AGT CAG TGT GGA AAA TCT CTA GC-3′
T_1 (complementary target to P_{HIV}):
5′-GCT AGA GAT TTT CCA CAC TGA CT-3′
T_2 (single-base mismatched target to P_{HIV}):
5′-GCT AGA GAT TGT CCA CAC TGA CT-3′

2.2. Preparation of Pdop-NSs

Pdop-NSs were synthesized according to reported method [21]. In a typical synthesis, 0.4 mL ammonia aqueous solution, 8 mL absolute ethanol and 18 mL deionized water were mixed together under stirring for 30 min. 0.1 g dopamine was dissolve into 2 mL deionized water, and then poured into the above mixture solution. The self-polymerization reaction of dopamine was allowed to proceed for 30 h.

2.3. Instruments

Scanning electron microscopy (SEM) measurements were made on a XL30 ESEM FEG scanning electron microscope at an accelerating voltage of 20 kV. Fluorescent emission spectra were recorded on a RF-5301PC spectrofluorometer (Shimadzu, Japan). UV–visible spectra were obtained on a UV-5800 spectrophotometer (Shanghai, China). The Fourier transform infrared (FTIR) was recorded on a Bruker Vertex 70 spectrometer.

3. Results and discussion

Fig. 1A shows low magnification SEM image of the products thus prepared, indicating the formation of a large amount of nanospheres. The high magnification SEM image (Fig. 1B) further indicates these Pdop-NSs have smooth surface with diameter in the 200–250 nm range.

We first explored the feasibility of using such Pdop-NSs as a fluorescent sensing platform for nucleic acid detection. FAM-labeled oligonucleotide sequence associated with human immunodeficiency virus (HIV) was used as probe (P_{HIV}). Fig. 2A displays the fluorescence emission spectra of the FAM-ssDNA probe under different conditions. P_{HIV} exhibits strong fluorescence emission without Pdop-NSs (curve a), and the presence of the fluorescein-based dye can account for it. It is obvious that the fluorescence of the free P_{HIV} was hardly influenced by the addition of T_1 in the absence of Pdop-NSs (curve b). However, the presence of Pdop-NSs leads to 68.9% decrease of fluorescence intensity (curve c), indicating that the Pdop-NSs can adsorb ssDNA and quench the fluorescent dye effectively. Upon incubation of the P_{HIV}–Pdop-NSs complex with complementary target T_1 over 30 min, fluorescence shows significant enhancement, leading to about 76% fluorescence recovery (curve d). Fig. 2B shows the fluorescence intensity changes ($F/F_0 - 1$) of the P_{HIV}–Pdop-NSs complex upon addition of different concentrations of T_1 (F_0 and F are fluorescence intensities at 517 nm in the absence and existence of T_1, respectively). It can be seen that increased DNA concentration from 5 to 300 nM results in dramatic increase of fluorescence intensity, suggesting that the Pdop-NSs/DNA assembly approach is effective in probing biomolecular interactions.

The FTIR spectrum of Pdop-NSs (Fig. 3A) shows the characteristic spectral peaks of several functional groups, such as C=C, N—H, and C—N, indicating that Pdop-NS is a π-rich and nitrogen-containing polymer. Thus, Pdop-NSs can strongly and effectively adsorb single-stranded DNA (ssDNA) on its surface via π–π stacking interactions between unpaired DNA bases and Pdop-NSs [22]. Scheme 1 shows a schematic diagram to illustrate the fluorescence-enhanced DNA detection using Pdop-NSs as a sensing platform. The DNA detection is accomplished by the following two steps: (1) Pdop-NS binds dye-labeled ssDNA, leading to fluorescence quenching of the dye due to their very close approximation; (2) the subsequent hybridization of the probe with its target produces double-stranded DNA (dsDNA) which detaches from Pdop-NS due to the absence of unpaired DNA bases and the rigid conformation of dsDNA, leading to fluorescence recovery. The UV–vis absorption spectrum (Fig. 3B) of Pdop-NSs dispersed in Tris–HCl buffer (pH 7.4) in the existence of 5 mM Mg^{2+} exhibits an weak broad absorption peak at 468 nm, suggesting that there have spectra overlap and thus fluorescence resonance energy transfer (FRET) occurs between Pdop-NS and the fluorescent dye FAM [22]. Given the nitrogen-containing nature of Pdop-NS,