Journal of Electroanalytical Chemistry 784 (2017) 85–90

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

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

Preparation of photoactive multilayer films with high photocurrent response and detection of thrombin

Weibo Zhang [a], Yaying Shi [a], Shan Zheng [a], Yang Liu [a], Chaobiao Huang [a,b,*]

[a] College of Chemistry and Life Science, Zhejiang Normal University, China
[b] Zhejiang Normal University Xingzhi College, Jinhua, 321004, China

ARTICLE INFO

Article history:
Received 2 August 2016
Received in revised form 26 October 2016
Accepted 30 October 2016
Available online 1 November 2016

Keywords:
Photoelectrochemical
Thrombin
Biosensor
CdS QDs
Pt NPs

ABSTRACT

The CdS quantum dots (QDs) multilayers with high photoelectric conversion efficiency are firstly assembled based on the electrostatic layer-by-layer (LbL) self-assembly between the sequentially adsorbed positively charged Poly(diallyldimethylammonium chloride) (PDDA) and negatively charged CdS quantum dots. The electrodes we fabricated have high photocurrent intensity, which is 8–10 times of the traditional method. Based on CdS QDs multilayers, a sensitive and selective photoelectrochemical (PEC) sensor for thrombin was fabricated by using aptamer (ONS2)-labeled Pt nanoparticles (NPs) as novel energy transfer nanoprobes and thrombin as a 'protective agent'. In the absence of thrombin, ONS2-labeled Pt NPs could be captured on CdS QDs surface by DNA hybridization between the thrombin aptamer (ONS1) and ONS2 and result in photocurrent quenching of sensing system. After the specific binding of thrombin aptamer and thrombin, however, the Pt NPs could not be captured on CdS QDs surface. The thrombin aptasensor displayed a linear range from 10^{-10} mol/L to 10^{-15} mol/L and a detection limit of 10^{-16} mol/L. Fabrication of QDs multilayers with high photoelectric conversion efficiency is of great significance for the future development of PEC biosensor.

1. Introduction

Thrombin is a coagulation protease which generated at sites of vascular injury and does not find in healthy blood [1]. Thrombin plays a pivotal role in hemostasis and blood clotting through selectively cleaving Arg–Gly bonds in fibrinogen to form fibrin and activating platelet. However, over-expression or activity imbalance of thrombin is associated with many diseases such as thrombosis, inflammation, atherosclerosis, and hemophilia [2]. Therefore, it is of great significance for accurately detecting thrombin to diagnosis and treatment of some diseases. Addressing this issue, some thrombin sensors based on small fluorescent organic molecules, proteins, oligonucleotides, or nanoparticles have been reported. For example, Xu et al. reported an energy-transfer based photoelectrochemical protein biosensor, using single stranded DNA (ssDNA) as a distance controller and Au nanoparticles (NPs) functionalized with ssDNA as novel energy transfer nanoprobes [3]. Kong et al. reported a label-free fluorescence thrombin aptasensor, using the SYBR Green I (SGI) dye as the signal probe based on its high specificity for dsDNA [4]. A photoelectrochemical biosensor fabricated based on a Graphene/Quantum-Dot Nanocomposite was proposed for sensitive detection of thrombin. The dual-quenched effect based on the electron transfer of a bipyridinium relay and energy transfer of Au NPs was investigated [5].

Due to its desirable advantages, e.g. simple equipment, cost-effectiveness, high sensitivity and rapidness, the newly developed PEC detection is of special interest for its potential in bioanalysis [6–8]. Its sensing mechanism is that the specific recognition reactions are converted into the corresponding photocurrent change [9]. These conversions usually need the help of photoactive species. QDs as a general photoactive species play an important role in photoelectrochemical sensors. A large number of different quantum dots (such as CdS QDs, PdS QDs, CdSe@ZnS QDs, carbon nitride QDs) are applied in this field [10–13]. Up to now, considerable works have been devoted to PEC study and significant progress has hence been made for DNA [14–16], enzyme [17], and protein [18].

In this work, we report an ultrasensitive protein detection based on the platinum nanoparticles functionalized with nucleic acid strand as novel energy transfer nanoprobes in a properly designed photoelectrochemical system. The integration of ONS2-labeled Pt NPs into the ONS1/CdS QDs/ITO electrode results in a sharp decrease of photocurrent. While, the thrombin could act as a 'protective agent' to prevent ONS1 on the surface of QDs hybridizing with Pt NPs-labeled ONS2. The fabricated aptasensor exhibited high sensitivity and selectivity by photocurrent quenching of CdS quantum dots (QDs) induced by energy transfer between CdS QDs and Pt NPs on the electrode [19].

* Corresponding author.
E-mail address: hcb@zjnu.cn (C. Huang).

http://dx.doi.org/10.1016/j.jelechem.2016.10.061
1572-6657/

2. Experimental

2.1. Chemicals, materials and instrumentation

The oligonucleotide strand (ONS1: 5′-$NH_2(CH_2)_6$-TTTTTTCACTGTG GTTGGTGTGGTTGG and ONS2: 3′-$SH(CH_2)_6$-CCAACCACAGTG) was purchased from Sangon Biotech. Co. Ltd. (Shanghai, China). N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), Poly(diallyldimethylammonium chloride) solution (PDDA; molecular weight = 200,000–350,000, 20%, w/w in water), thioglycolic acid (TGA) were purchased from Aladdin (Shanghai, China). Lyzyme, glucose oxidase (GOD), ACHE and thrombin from human plasma (human α-thrombin, 37.4 kDa, 2582 NIHunits/mg protein) were obtained from Sigma-Aldrich (St. Louis, MO). Tris(hydroxymethyl)aminomethane, Tris(2-carboxyethyl) phosphine hydrochloride(TCEP), Bovine Serum Albumin (BSA) were purchased from shanghai titan scientific Co. Ltd. (Shanghai, China). All chemicals were of analytical grade. Ultrapure water from a NANOpureDiamond (≈18 MΩ, Millipore) source was used throughout the experiments.

2.2. Instrumentation

The 0.24 cm^2 modified ITO slices were used as the working electrode, a Pt wire and a KCl saturated Ag/AgCl was employed as the counter and reference electrode, respectively. A 500 W Xe lamp was used as irradiation source. Photocurrent was measured on an electrochemical workstation (CHI 850d). All the photocurrent measurements were performed at a constant potential of 0 V (versus Ag/AgCl). The TEM images were recorded on a JEM-2100F transmission electron microscope with 200 kV accelerating voltage. The pH values were measured with a model pHS-3C pH meter. The fluorescence spectra were recorded with an RF-5301PC luminescence spectrometer, and the UV–vis absorption spectra were collected with a Perkin Elmer Lambda 950 UV–vis spectrophotometer. Electrochemical impedance spectroscopy (EIS) was recorded by an Autolab PGSTAT302N (Metrohm Autolab B.V., Netherlands).

2.3. Synthesis of visible light excitation TGA-capped CdS QDs

TGA-capped CdS QDs was synthesized according to the previous report with a slight modification [10]. Typically, 50 mL of 10 mmol/L $CdCl_2$ was mixed with 250 μL of TGA, the pH of the solution was adjusted to 11.0 by 1 mol/L NaOH and highly pure N_2 was bubbled throughout the solution for 0.5 h to remove the dissolved O_2. And then 5.5 mL of 0.1 mol/L Na_2S aqueous solution was injected into this almost boiling solution. After that, the mixture was refluxed under N_2 atmosphere for 4 h to obtain TGA-capped water-soluble CdS QDs. Finally, the resulting solution was then diluted with the same volume of water and stored in a refrigerator at 4 °C for further use. Fig. 1B is the UV–vis absorption and fluorescence emission spectra of the prepared CdS QDs.

2.4. Fabrication of electrode

The PDDA/CdS multilayer film was performed by alternately immersing of the cleaned ITO slices into a solution of PDDA (positive) containing certain concentration of NaCl and the TGA — CdS QDs (negative) solution containing different concentration of NaCl for 10 min, respectively. After each dipping step, the films were carefully washed with ultrapure water which is good solvent for the QDs/polyelectrolyte to remove superfluous QDs/polyelectrolytes and to prevent the cross-contamination of solutions. Repeating this process three times leads to a thin film of multilayer CdS QDs. Finally, the electrode was dried for 36 h at 4 °C.

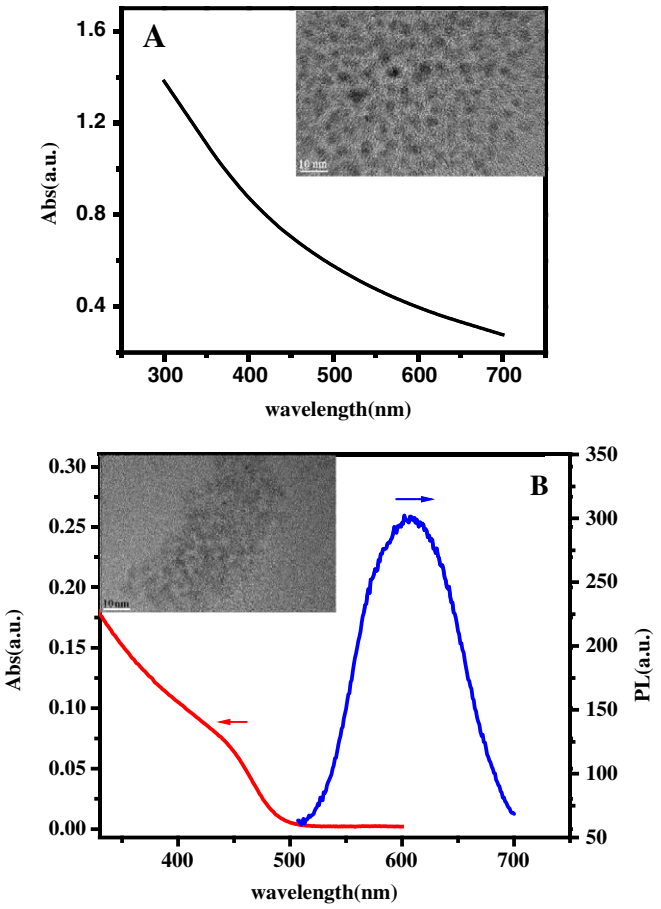

Fig. 1. (A) The UV–vis absorption spectrum of the prepared Pt NPs. (B) The UV–vis absorption spectrum and the PL spectrum of the prepared CdS QDs. Insets in A and B: the TEM images of the Pt NPs and CdS QDs, respectively.

2.5. Synthesis of Pt NPs and Pt NPs labeled DNA

Pt NPs were synthesized according to the previous report [20]. In the typical synthetic process, 10 mL of 38.8 mmol/L citrate sodium was added into 50 mL of 1 mmol/L H_2PtCl_6 heating-reflux solution, followed by boiling the mixture for 30 min and the solution would turn from clear to black.

For preparation of ONS2 modified Pt NPs, 25 μL solution of 10^{-6} mol/L ONS2 was pretreated by 1.7 μL TCEP to cut S—S bond, then the activated ONS2 was added into 2.5 mL colloidal Pt NPs. The mixed solution was kept for 12 h at 4 °C in order to assure the fastness of Pt—S bond. After that, 100 μL BSA solution (8 mg/mL) was added to the mixed solution and kept in the fridge for 1 h to block the nonspecific active binding sites of the Pt NPs. The size of Pt NPs is about 3 ± 1 nm by TEM measurement (Inset in Fig. 1A).

2.6. Immobilization of capture DNA and thrombin detection

Immobilization of ONS1 to the CdS QDs/ITO electrodes was accomplished via the commonly used EDC coupling reactions between –COOH groups on the surface of CdS QDs and the $-NH_2$ groups of ONS1. The CdS QDs/ITO electrodes were immersed in a solution containing 10 mmol/L EDC and 20 mmol/L NHS for 60 min at room temperature. After rinsing, 10 μL of 2 μmol/L ONS1 was spread onto the electrode surface and incubated at 4 °C in a moisture atmosphere overnight. After that, the electrode was rinsed with 10 mmol/L PBS buffer (pH 7.2) to remove the excess ONS1.