Biosensors and Bioelectronics 65 (2015) 176–182

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Ultrasensitive detection of lead ion sensor based on gold nanodendrites modified electrode and electrochemiluminescent quenching of quantum dots by electrocatalytic silver/zinc oxide coupled structures

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ARTICLE INFO

Article history:
Received 13 June 2014
Received in revised form
8 October 2014
Accepted 9 October 2014
Available online 19 October 2014

Keywords:
Electrochemiluminescence
Gold nanodendrites
Ag/ZnO coupled structures
Lead ion
Oligonucleotide

ABSTRACT

A signal-off electrochemiluminescence (ECL) DNA sensor based on gold nanodendrites (Au NDs) modified indium tin oxide (ITO) electrode for the detection of lead ion (Pb^{2+}) was developed. Well-defined Au NDs were prepared on ITO electrode using low-potential synthesis, assisted by ethylenediamine. Based on Pb^{2+}-specific deoxyribozyme, the silver/zinc oxide (Ag/ZnO) with coupled structure, prepared by one-pot method, was close to the surface of the electrode to catalyze the reduction of part of H_2O_2, the coreactant for cathodic ECL emission, leading to a decrease of ECL intensity. In addition, taking advantage of the larger surface area to capture a large amount of capture probe as well as excellent conductivity of Au NDs, the sensor could detect Pb^{2+} quantitatively in a wider range, and performed excellent selectivity. Furthermore, such simple and sensitive DNA sensor was successfully applied for the detection of Pb^{2+} in lake water and human serum samples, respectively.

1. Introduction

Toxic metal ions always act as severe environmental pollutants and pose serious risk to human health (Lin et al., 2011). Lead ion (Pb^{2+}), as one of them, widely distributed in ambient air, water, soil, and even food, and it is one of the most hazardous metal pollutants (Li et al., 2009). Therefore, rapid and sensitive detection of Pb^{2+} is of great significance for environmental protection as well as disease prevention and treatment. Nowadays, many analytical methods for detection of Pb^{2+} have been developed, such as fluorescence, colorimetric method, atomic absorption spectroscopy, inductively coupled plasma mass spectrometry, and electrochemical method. (Li et al., 2010; Wang et al., 2008; Ghaedi et al., 2009; Ataro et al., 2008; Shen et al., 2008). However, the practical applications suffered from poor selectivity or weak sensitivity. In order to avoid the limitations above, nucleic acid-based Pb^{2+} sensors have recently attracted considerable attention as optimized solution for detection of Pb^{2+} (Xiang et al., 2009), among which, deoxyribozyme (DNAzyme) biosensors for Pb^{2+} have attracted more attention (Shen et al., 2008). In this work, electrochemiluminescence (ECL) was employed to sensitively detect Pb^{2+} based on Pb^{2+}- specific DNAzyme due to its inherent features, such as low cost, rapid response, wide linear range and high sensitivity (Liu and Ju, 2008).

The design and synthesis of nano-structures on electrode surface have been recently studied to improve sensitivity of biosensor by increasing electroactive surface area for analysis (Guo and Wang, 2007). The incorporation of nano-structures, including nanoparticles, nanorods, nanotubes and nanodendrites, onto electrode surface has been increasingly considered for diverse analytical applications. Among them, gold nanodendrites (Au NDs) have drawn great attention due to its availability of large surface area within a structure. Up to now, a couple of electrodeposit strategies have been proposed on synthesis of Au NDs (Feng et al., 2012; Huan et al., 2011) due to its ease of control, highly pure and uniform deposits, and simple operation, which can make the dendritic structures directly deposited on electrode surface (Feng et al., 2012; Li et al., 2011). To improve the sensitivity of the Pb^{2+} sensor, a facile and template-free method was adopted for electrodeposition of "clean" Au NDs with the assistance of ethylenediamine (EDA) for initial branching, which was used for immobilization of more cadmium sulfide quantum dots (CdS QDs).

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http://dx.doi.org/10.1016/j.bios.2014.10.022
0956-5663/

As well known, semiconductor QDs have been widely used in bioanalysis (Dong et al., 2010) due to their controllable size and emission wavelength, high photoluminescence (PL) yield, and good chemical stability (Yin and Alivisatos, 2005). The electrochemically reduced or oxidized semiconductor QDs can also react with the coreactants to generate ECL in the vicinity of the electrode (Ding et al., 2002). As a coreactant used in the ECL system of semiconductor QDs in aqueous solution, hydrogen peroxide (H_2O_2) can enhance ECL signals of semiconductor NCs greatly (Jiang and Ju, 2007). Recently, consumption of ECL coreactant in an enzymatic reaction, one kind of mechanism of ECL emission of QDs, has attracted considerable concern (Jie et al., 2008; Jie et al., 2010), which leads to target-concentration-dependent decrease of ECL emission. However, nonenzymatic assays that employ metal oxides and their composites are attracting increasing attention due to its quite low cost, easy operation, fine stability even at high temperature and little need of maintenance (Palanisamy et al., 2012; Zhao et al., 2009). In particular, zinc oxide (ZnO) is an imperative wide band gap semiconductor material and it has great potential applications in H_2O_2 sensing (Liu et al., 2009; Zhu et al., 2007). Up to now, great efforts in the modern catalysis field have been devoted to the design and fabrication of ZnO with high active facets exposed for enhancing catalytic properties (Gu et al., 2013). Among them, a facile one-pot solvothermal method for synthesis of Ag/ZnO coupled structures have been reported, which displayed excellent electrocatalytic response for the detection of H_2O_2 (Zhang et al., 2012). Herein, a sensor for detection of Pb^{2+} was fabricated based on the quenching ECL of CdS QDs, in which H_2O_2 was reduced by Ag/ZnO.

In this work, a sensor for the detection of Pb^{2+} was constructed by immobilizing CdS QDs and capture probe on Au NDs modified indium tin oxide (ITO) electrode. With Pb^{2+}-induced activation of DNAzyme, the Ag/ZnO coupled structures were close to the surface of the electrode to catalyze the reduction of part of H_2O_2, the coreactant for cathodic ECL emission, leading to a decrease of ECL intensity. The experimental results indicated that the DNA sensor not only exhibited excellent analytical performance, but also provided a promising potential in clinical diagnosis, especially in point-of-care testing.

2. Materials and methods

2.1. Reagents

All oligonucleotides were synthesized and purified from Shanghai Linc-Bio Science Co. Ltd. (Shanghai, China). 6-Mercapto-1-hexanol (MCH) was purchased from Nanoport. Co. Ltd. (Shenzhen, China). Poly(diallyldimethylammonium chloride) (PDDA), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide (EDC), N-hydroxysuccinimide (NHS, 98%), mercaptopropionic acid (MPA), thioacetamide, and gold chloride ($HAuCl_4$) were obtained from Alfa Aesar China Ltd. Silver nitrate, cadmium chloride ($CdCl_2 \cdot 2.5H_2O$), ammonia solution, ethylene glycol (EG), and EDA were obtained from Sigma (St. Louis, MO, USA). Zinc acetate dehydrate ($Zn(CH_3COO)_2 \cdot 2H_2O$), and polyvinylpyrrolidone (PVP) were purchased from Nektar (Huntsville, AL). All chemicals and solvents used were analytical grade available and were used as received. The sequences of oligonucleotides are presented with the following sequences:

(S_1): 5′- SH-$(CH_2)_6$-TTTCATCTCTTCTCCGAGCCGGTCGAAATAGTGA-GT-$(CH_2)_7$-NH_2-3′;
(S_2): 5′- ACTCACTATArGGAAGAGATG-3′;

The ultra-pure water was obtained from a Lichun water purification system (≥ 18 MΩ cm, Jinan, China) and used throughout. The buffers involved in this work are as follows: DNA immobilization buffer, 10 mM Tris–HCl and 0.1 M NaCl (pH 7.4); hybridization buffer, 10 mM phosphate buffered saline (PBS, pH 7.4) with 0.25 mM NaCl; washing buffer, 10 mM PBS, and 0.1 mM NaCl (pH 7.4). Buffer for ECL is 10 mM Tris–HCl buffer (pH 7.4) containing 0.1 M KCl, and H_2O_2 (1.0 mM) was used as the coreactant.

2.2. Apparatus

The ECL measurements were conducted on a flow injection luminescence analyzer (IFFM-E, Xi'an Remex Electronic Instrument High-Tech Ltd., Xi'an, China) with the voltage of the photomultiplier tube (PMT) set at 800 V. Cyclic voltammetric measurements (CVs) were performed with a CHI 760D electrochemical workstation (Shanghai CH Instruments, China). Transmission electron microscopy (TEM) images of Ag/ZnO were obtained from a Hitachi H-800 microscope (Japan). Electrochemical impedance spectroscopy (EIS) was carried out on an IM6x electrochemical station (Zahner, Germany). Scanning electron microscope (SEM) images were obtained on a QUANTA FEG 250 thermal field emission SEM (FEI Co., USA). Energy dispersive spectrometer (EDS) was obtained on Oxford X-MAX50 EDX (Oxford, Britain). Ultraviolet visible (UV–vis) was recorded on a UV-3101 spectrophotometer (Shimadzu, Japan). The PL characterization was achieved on a LS-55 spectrofluorometer (P.E. USA). All experiments were carried out with a conventional three-electrode system with the modified ITO electrode as working electrode, a platinum counter electrode and an Ag/AgCl (sat. KCl) as reference electrode.

2.3. Preparation of Au NDs

Prior to the preparation of Au NDs, the washed ITO glass electrodes (length 4 cm and width 1 cm) were immersed into 1 M NaOH solution (pH 11.3). Then, the substrates were rinsed thoroughly with water and dried under a stream of N_2. The Au NDs were prepared according to the reported method (Feng et al., 2012). A typical experiment was performed under the deposition potential of 0.0 V for 600 s in 0.5 M H_2SO_4 containing 2.5 mM $HAuCl_4$ and 150 mM EDA. After electrodeposition, the electrodes were washed with water and dried by nitrogen.

2.4. Synthesize of the CdS QDs

The water-soluble CdS QDs were prepared using MPA as stabilizing agent according to a method similar to that reported previously (Han et al., 2011). Briefly, 86 μL of MPA was added to 20 mL of 20 mM $CdCl_2$ solution. After adjusting the pH to 10, 20 mL of 20 mM thioacetamide aqueous solution was added with extensive stirring for 30 min. After refluxing at 80 °C for 10 h, the formed CdS colloid was dialyzed exhaustively against water overnight at room temperature to obtain CdS QDs solution. The product was condensed by ultrafiltration at 10,000 rpm for 10 min, and the upper phase was decanted and kept at 4 °C. UV–vis and PL spectra were used to characterize the formation of CdS QDs (Fig. S1).

2.5. Preparation of Ag/ZnO coupled structures

The Ag/ZnO coupled structures were synthesized by the reported method (Zhang et al., 2012). Typically, 0.4390 g of $Zn(CH_3COO)_2 \cdot 2H_2O$, 0.2000 g of PVP, and 12 mL of 0.2 M ammonia solution were mixed in a beaker under stirring. Then 4 mL of EG and 2 mL of 0.1 M $AgNO_3$ solution were dropwise added into the solution above under stirring. After being stirred for 30 min, the