Journal of Photochemistry and Photobiology A: Chemistry 342 (2017) 15–24

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

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

BSA-AuNCs based enhanced photoelectrochemical biosensors and its potential use in multichannel detections

Shuang Zhao[a,1], Zhengping Li[a,1], Yunxiao Li[a], Jun Yu[a], Guohua Liu[a,b], Ruping Liu[c], Zhao Yue[a,b,*]

[a] Department of Microelectronics, Nankai University, Tianjin, 300350, China
[b] Tianjin Key Laboratory of Optoelectronic Sensor and Sensing Network Technology, Tianjin, 300350, China
[c] Beijing Institute of Graphic Communication, Beijing, 102600, China

ARTICLE INFO

Article history:
Received 22 December 2016
Received in revised form 23 March 2017
Accepted 25 March 2017
Available online 29 March 2017

Keywords:
BSA-AuNCs
GO
LBL
H_2O_2
Dopamine
Photoelectrochemistry
Multichannel
Separation efficiency

ABSTRACT

Herein we present the development of a bovine serum albumin (BSA)-coated fluorescent gold nanocluster (BSA-AuNC)/graphene oxide (GO) hybrid nanocomposite-based photoelectrochemical (PEC) biosensor, which can be used for multichannel detection of both hydrogen peroxide (H_2O_2) and dopamine (DA) within one chip at negative and positive potentials, respectively. BSA-AuNC-based biosensors generally exhibit poor PEC sensing performance owing to their poor separation of photoexcited electron-hole pairs and low electrode coverage. Consequently, improved AuNC/GO hybrid nanocomposites and a layer-by-layer (LBL) method are introduced in this study. The enhancement was investigated by exploring the optimum concentration of GO (0.12 mg mL^{-1}) and LBL number (four layers) for the preparation of the BSA-AuNC materials, revealing that GO plays different enhancement roles in the detection of H_2O_2 and DA. Compared to the results from other gold nanoclusters with different ligands, the photoelectrical enhancement of BSA-AuNCs can be achieved for both negative and positive potentials simultaneously with the same optimum GO concentration and LBL number. Thus, the detection of H_2O_2 and DA was achieved by BSA-AuNC/GO multilayers with enhanced sensing properties and easier fabrication processes. The two enhancement methods acting together led to an improvement in the limits of detection (LODs) from 325 μM to 23 μM (H_2O_2) and from 7.45 μM to 1.5 μM (DA). Thus, our strategy shows great potential for the improvement of PEC sensing structure, and provides a method for multichannel detection.

1. Introduction

Increasing research attention is being dedicated to fluorescent-nanomaterial-based photoelectrochemical (PEC) biosensors for biological analysis [1–4], as they exhibit superior sensing properties and the potential to be developed as light-addressable sensing systems capable of multichannel detection. Quantum dots (QDs) are an important class of photoactive semiconductor nanocrystals and exhibit good PEC properties owing to their unique chemical and physical properties [5–9]. However, most QDs contain heavy metal species such as Cd or Pb, which introduce toxicity issues into their fabrication and use in biological detection [10–12]. Consequently, nanomaterials with lower toxicity, such as doped ZnSe [9], Ag_2S [11] and fluorescent gold nanoclusters (AuNCs) [13–16] have been used as replacements for QDs in fabricating environmentally benign PEC biosensors. AuNCs have many fascinating semiconductor features owing to their discrete energy levels. AuNCs coated with bovine serum albumin (BSA) ligands (BSA-AuNCs) are one of the most promising nanomaterials and have drawn wide attention in recent years. They have many valuable properties, such as good catalytic activity, enhanced photoluminescence, low toxicity, unique charging properties, and ultrafine size, that make them highly promising for application in analysis, sensing, and bioimaging [17]. BSA-AuNCs have been immobilized on gold electrodes (AuEs) with self-assembled materials (SAMs) to form AuNC/SAM/AuE-type devices for PEC detection of hydrogen peroxide (H_2O_2) [18]. Thus, BSA-AuNCs are a suitable substitute for QDs in the fabrication of PEC biosensors.

However, the applications of BSA-AuNC-based PEC biosensors are limited because of their poor PEC performances and low limits

* Corresponding author at: Department of Microelectronics, Nankai University, Tianjin, 300350, China.
E-mail address: yuezhao@nankai.edu.cn (Z. Yue).
[1] Equal contribution to this work.

http://dx.doi.org/10.1016/j.jphotochem.2017.03.034
1010-6030/

of detection (LODs) in practical use. There are two main reasons for these poor sensing performances. First, the charge separation efficiency inside the photoexcited BSA-AuNCs is low because most of the separated electron-hole pairs recombine to release light. Second, the BSA-AuNCs amount of monolayer on gold electrodes is limited, and not all the space on the surface of the gold electrode is efficiently utilized. Thus, inhibiting electron-hole recombination and improving the BSA-AuNC coverage of gold electrode surfaces are primary goals. In this study, both graphene oxide (GO) and layer-by-layer (LBL) methods are introduced to overcome the problems mentioned above and enhance the PEC properties and sensing performances of BSA-AuNC-based biosensors.

GO has been introduced to many nanomaterials in order to improve their PEC properties [19–24]. For example, GO has been used as an electron donor or acceptor in PEC processes to inhibit photoinduced electron-hole recombination and enhance photoelectrical properties [25–28]. Here, GO is used to improve the photoinduced separation of electron-hole pairs in the BSA-AuNCs. Moreover, GO can improve sensing properties by affecting the catalytic properties of BSA-AuNCs [29].

LBL methods have been used to enhance the performances of QD-based PEC biosensors [30,31]. Similarly, BSA-AuNC multilayers fabricated using LBL methods provide larger photocurrents and better sensing properties because LBL deposition of BSA-AuNCs leads to increased surface coverage of gold electrodes.

In this study, both GO and LBL strategies have been used to achieve photocurrent enhancement for BSA-AuNCs at both negative and positive potentials. The negative-potential enhancement improves the sensing performance for H_2O_2 and the positive-potential enhancement improves the detection of dopamine (DA). These two analytes can be detected with one sensing chip simultaneously, which makes the sensing system more practical, time-saving, and more suitable for development as a light-addressable system for multichannel detection of different molecules in the future.

2. Experimental and theoretical methods

2.1. Materials

Hydrogen tetrachloroaurate(III) trihydrate ($HAuCl_4 \cdot 3H_2O$) and KBH_4 were obtained from Aladdin (Shanghai, China). BSA, 0.1 M phosphate buffer solution (PBS, pH 7.4 at 25 °C), toluene, chloroform, and H_2O_2 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Carbon fibers (20 nm) were obtained from XFnano (Nanjing, China). $KMnO_4$, H_2SO_4, $K_2S_2O_8$, and P_2O_5 were obtained from Sinopharm (Shanghai, China). 1,4-Benzenedithiol (BDT) was obtained from TCI (Japan). The chemicals and materials were all analytical grade. 18 MΩ cm ultrapure water was used to prepare all aqueous solutions.

2.2. Synthesis and optical properties of different nanomaterials

BSA-AuNCs were synthesized according to a previously reported method with slight modification [18]. Under vigorous stirring, an aqueous $HAuCl_4$ solution (5 mL, 10 mM, 37 °C) was mixed with a BSA solution (5 mL, 50 mg mL^{-1}, 37 °C). After 2 min, NaOH solution (0.5 mL, 1 M) was added, and the reaction proceeded for another 12 h under stirring at 37 °C. The UV–vis absorption properties of BSA-AuNCs (1.5 μM) were recorded on a Maya2000Pro high-sensitivity spectrometer (Ocean Optics, USA).

$KMnO_4$ and 20 nm carbon fibers were used to synthesize GO [32]. A mixture of H_2SO_4 (5 mL), $K_2S_2O_8$ (0.15 g), and P_2O_5 (0.15 g) were added to 0.2 g carbon fibers at 80 °C and stirred for 5 h. Then, H_2SO_4 (25 mL) was added and the mixture was chilled to 0 °C. Then, 1 g $KMnO_4$ was added slowly, maintaining the temperature below 10 °C. The reaction went to completion overnight. DI water (100 mL) was then added to the reaction mixture while maintaining the temperature below 55 °C in an ice bath. HCL (3.4%) was slowly added to neutralize the mixture. Then, MW 7000 dialysis membranes were used to dialyze the product in DI water. The GO was isolated by centrifugation at 3000 rpm, dried in air, and dispersed to 2 mg mL^{-1} in water.

To obtain BSA-AuNC/GO hybrid nanocomposites, BSA-AuNCs were mixed with treated GO at different ratios and sonicated for 1 h. The electrostatic bonding of the BSA-AuNCs and GO was followed and confirmed by centrifugation of the BSA-AuNC/GO hybrid nanocomposites [17]. The fluorescence spectra of BSA-AuNCs hybridized with different amounts of GO were recorded on a Shimadzu RF-5301 spectrofluorophotometer (Shimadzu, Japan). Transmission electron microscopy (TEM) images of the BSA-AuNC/GO nanocomposites were obtained using a JEM-2100 at 200 kV (JEOL, Japan). Atomic force microscopy (AFM) images of the BSA-AuNC/GO nanocomposites were recorded on a Bruker Dimension Icon (Bruker, USA).

Synthesis and characterization details of AuNCs with glutathione (GSH) and dihydrolipoic acid (DHLA) ligands are given in the Supporting information.

2.3. Fabrication of photoelectrochemical biosensor chips

Gold electrodes (200 nm Au/20 nm Ti/500 um Glass, 7 mm^2) were cleaned and modified with a dithiol. Each gold electrode was cleaned by sequential ultrasonication in ethanol, acetone, and pure water, and then immersed into BDT solution (50 mM BDT in ethanol) for 4 h. Then, the BSA-AuNC or BSA-AuNC/GO nanocomposites were deposited on the BDT-modified electrodes for 5 min, and spinning was used to remove the excess solvent. After being rinsed with water and dried in an N_2 flow, the AuNC/SAM/AuE or AuNC/GO/SAM/AuE chips were fabricated. When the LBL method was applied, the monolayer electrodes were again immersed in 50 mM BDT for another 4 h, followed by incubation in AuNC or AuNC/GO nanocomposite suspension. Again, solvent was removed by spinning, and the surface was dried under N_2. (AuNC/SAM)n/AuE or (AuNC/GO/SAM)n/AuE multilayer chips were obtained by repeating the processes n times. A schematic of the photoelectrochemical sensor fabrication process is shown in Fig. 1.

2.4. Photoelectrochemical measurement system

The PEC sensing measurement system was built based on a CHI 604D potentiostat (CH Instruments, USA) and a lock-in amplifier (SR830, Stanford Research Systems, USA). The electrochemical cell used in this system was lab-fabricated from Teflon. A Ag/AgCl electrode, platinum wire, and the modified gold electrode were used as the reference, counter, and working electrodes, respectively. The light sources used to excite the photosensitive nanomaterials were four light-emitting diodes (LEDs, M365L2: 365 nm/190 mW, M455L3: 455 nm/900 mW, M530L3: 530 nm/350 mW, and MCWHL5: white light/800 mW) and a COP3-A collimation adapter (Thorlabs, USA). The peak wavelength, emitter size, and viewing angle (full angle) of the M365L2 LED were 365 nm, 1 mm × 1 mm, and 120°, respectively. More detailed specifications of the M365L2 LED are shown in the Supporting information. A diagram of the PEC measurement system is shown in Fig. S2 [18].

3. Results and discussion

3.1. Characterization of BSA-AuNCs and BSA-AuNC/GO nanocomposites

Fig. 2(a) shows the UV–vis absorption spectra of BSA-AuNCs and their absorption peak at 520 nm [33]. Furthermore, the