Talanta 85 (2011) 2154–2158

Contents lists available at ScienceDirect

Talanta

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

Carbon nanospheres enhanced electrochemiluminescence of CdS quantum dots for biosensing of hypoxanthine

Yangyang Zhang, Shengyuan Deng, Jianping Lei, Qiunan Xu, Huangxian Ju*

State Key Laboratory of Analytical Chemistry for Life Science, Department of Chemistry, Nanjing University, Nanjing 210093, PR China

ARTICLE INFO

Article history:
Received 29 March 2011
Received in revised form 13 July 2011
Accepted 17 July 2011
Available online 22 July 2011

Keywords:
Biosensors
Electrochemiluminescence
Quantum dots
Carbon nanospheres
Hypoxanthine

ABSTRACT

This work developed a novel method to greatly enhance the electrochemiluminescence (ECL) of CdS quantum dots (QDs). The ECL amplification was achieved by the assembly of QDs on poly (diallyldimethylammonium chloride)-functionalized carbon nanospheres (PFCNSs), and successfully employed for sensitive ECL biosensing of oxidase substrates. The carbon nanospheres were prepared by a "green" method, and the high loading of QDs on carbon nanospheres led to a 4-times increased ECL intensity with dissolved O_2 as the coreactant. Using xanthine oxidase (XOD) as a model, an ECL biosensor was fabricated by immobilizing the enzyme on the mixing membrane of PFCNSs and QDs. The ECL biosensor showed a fast response to hypoxanthine with a linear concentration range from 2.5×10^{-8} to 1.4×10^{-5} M. The limit of detection was 5 nM at a signal-to-noise ratio of 3. The assay results of hypoxanthine in fish samples were in a good agreement with the reference values by amperometric technique. This facile approach to prepare the PFCNSs/QDs system for ECL biosensing could be of promising application in bioanalysis and electronic device.

1. Introduction

Since the electrogenerated chemiluminescence (ECL) of Si quantum dots (QDs) was reported in 2002 [1], the QDs-based ECL emission has attracted considerable attention. The ECL emission has become a very powerful analytical technique owing to the advantages, such as good stability against photobleaching, simplified optical setup, low cost, and low background noise [2,3]. Multifunctional QDs as ECL signal transduction emitters have been used for construction of versatile biosensing platforms [4]. For example, a QDs-based ECL biosensor for detection of lysozyme has been developed by associating aptamer-lysozyme bioaffinity complexes at an Au electrode [5]. A competitive QDs-based ECL immunoassay coupled with enzymatic amplification has also been proposed for the detection of human IgG [6]. To efficiently enhance the ECL emission, several carbon nanomaterials such as carbon nanotubes (CNTs) [7–11] and graphene [12–15] have been used to load the QDs for improving the sensitivity and the analytical performance of ECL biosensors, especially in the detection of low-abundant proteins. This work used poly (diallyldimethylammonium chloride)-functionalized carbon nanospheres (PFCNSs) to load QDs and develop a high sensitive ECL biosensing method for detection of oxidase substrates.

Carbon-based nanomaterials have extensively been used in improving the ECL emission of QDs due to their unique chemophysical properties and remarkable conductivity. By facilitating CdTe QDs oxidation and trigger $O_2^{\bullet-}$ generation, graphene oxide can produce enhanced ECL emission for selective sensing of glutathione from thiol-containing compounds [13]. Based on the ECL amplification of N-doped carbon nanotubes the CdSe QDs modified electrode shows a five-times stronger cathodic ECL emission, which is more efficient than the three-times stronger ECL emission of CdSe QDs enhanced by CNTs [8]. Compared with CNTs and graphene, carbon nanospheres possess better tunability of particle size and porous nanostructure. The electrical conductivity and chemical stability of CNSs are also acceptable. Thus such nanomaterials have been applied in the electrochemical assays [16,17]. Here CNSs were used for the first time to amplify the ECL emission of QDs, leading to a sensitive ECL biosensor for the detection of oxidase substrates by using hypoxanthine as a model analyte.

Hypoxanthine is an essential metabolite to degrade adenine nucleotide, which is an indicator for the quality control of meat or fish products in food industries. Therefore, it is significant to develop a quick and effective detection method for the determination of hypoxanthine. Various methods have been proposed for the detection of hypoxanthine concentration, such as chromatography [18,19], capillary electrophoresis [20] and electrochemistry [21,22]. In this work, a simple, sensitive and portable ECL method based on CNSs enhanced ECL emission of CdS QDs with dissolved O_2 as a coreactant and consumption of O_2 via xanthine oxidase (XOD)

* Corresponding author. Tel.: +86 25 83593593; fax: +86 25 83593593.
E-mail address: hxju@nju.edu.cn (H. Ju).

0039-9140/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2011.07.059

enzymatic reaction was developed for the detection of hypoxanthine by layer-by-layer assembly of PFCNSs and QDs on a glassy carbon electrode. The PFCNSs/CdS QDs system provided an efficient platform for practical application of ECL biosensing.

2. Experimental

2.1. Materials and reagents

Xanthine oxidase (EC 1.1.3.22, from microbial source, 8.1 U/mg), hypoxanthine (≥99%), mercaptopropionic acid (MPA), chitosan (≥85% deacetylation) and poly (diallyldimethylammonium chloride) (PDDA, MW 200,000–350,000, 20% (w/w) aqueous solution) were purchased from Sigma Chemical Co. (MO, U.S.A.). Cadmium chloride ($CdCl_2{\cdot}2.5H_2O$) was purchased from Alfa Aesar Co., Ltd. (China). Thioacetamide was purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. D-(+)-Glucose, ascorbic acid and uric acid was purchased from Sinopharm Chemical Reagent Co., Ltd. The N_2-saturated solution was obtained by bubbling highly pure N_2 into air-saturated solution for 30 min. Other reagents were of analytical grade and used as received. The ultrapure water (≥18 MΩ, Milli-Q, Millipore) was used throughout the work.

2.2. Apparatus

The electrochemical and ECL measurements were carried out on a MPI-E multifunctional electrochemical and chemiluminescent analytical system (Xi'an Remex Analytical Instrument Co., Ltd.) at room temperature with a conventional three-electrode system. The three-electrode system was consisted of a GCE (5 mm in diameter) as working, a platinum wire as counter and an Ag/AgCl (saturated KCl solution) as reference electrodes. Electrochemical impedance spectroscopic (EIS) measurements were carried out on a PGSTAT30/FRA2 system of Autolab Electrochemical Analyzer (Ecochemie, BV, Netherlands) in 5 mM $[Fe(CN)_6]^{3-/4-}$ with 0.1 M KCl as the supporting electrolyte. After coated with Au film to improve the conductivity, the sample films were examined under a Hitachi S-4800 scanning electron microscope (SEM, Hitachi, Japan). Photoluminescence (PL) spectra were performed on a RF-5301 PC fluorometer (Shimadzu Co., Japan). UV–vis spectra were obtained on a Shimadzu UV-3600 UV–vis-NIR photospectrometer (Shimadzu Co., Japan).

2.3. Preparation of PFCNSs

The CNSs were prepared by a "green" method under hydrothermal conditions according to the method reported by Sun and Li [23]. In brief, 4 g glucose was dissolved in 40 mL water and placed in a 50 mL Teflon-sealed autoclave, which was maintained at 180 °C for 6 h. The resulting black product was isolated and purified by centrifugation, redispersed in alcohol, and oven-dried at 80 °C overnight. The as-prepared CNSs were further treated with 3:1 H_2SO_4/HNO_3 (v/v) to induce carboxylic groups on the surface by stirring for 4 h, followed by filtering and washing repeatedly with water until the pH reached 7. Then, 1 mg/mL of carboxylated CNSs was dispersed in a 0.5% PDDA aqueous solution containing 0.5 M NaCl by stirring for 30 min to give a homogenous brown suspension. Residual PDDA was removed by high-speed centrifugation, and the complex was thrice washed with ultrapure water to obtain PFCNSs.

2.4. Preparation of MPA capped CdS QDs

The synthesis of MPA capped CdS QDs was performed according to a method reported previously [9] with slight modification. Briefly, 86 μL MPA was added in 20 mL of 0.02 M $CdCl_2$ solution followed by adjusting the pH to 10 with 1 M NaOH solution. Then, 20 mL of 0.02 M thioacetamide solution was injected into the solution with extensive sonication in air for 30 min. The resulting solution was refluxed at 80 °C for 10 h to obtain MPA capped CdS QDs. The as-prepared QDs colloid was dialyzed exhaustively against deionized water over one week at room temperature for purification. Finally, the product was condensed by ultrafiltration at 10,000 rpm for 10 min, obtained by decanting the upper phase and kept at 4 °C.

2.5. Fabrication of ECL biosensor

The GCE was polished carefully with 1.0 and 0.05 μm alumina slurry (Beuhler), followed by rinsing thoroughly with deionized water. After successive sonication in acetone and deionized water, the electrode was rinsed with deionized water and allowed to dry under N_2 stream. A 15 μL of 1.0 mg/mL PFCNSs dispersion was spread on the pretreated GCE, which was allowed to dry at room temperature. Then 25 μL of 5 μM CdS QDs solution was covered on the modified layer and dried in air. 10 μL of 3 mg/mL XOD and 10 μL of 0.05% chitosan solutions (both prepared in 0.1 M pH 7.4 PBS) were subsequently dropped onto the CdS QDs/PFCNSs modified electrode to obtain the ECL biosensor. The function of chitosan is to protect the enzyme molecules from leakage. The biosensor was stored in 4 °C refrigerator when not in use.

2.6. Determination of hypoxanthine in real sample

A piece of fish meat was homogenized in 15 mL of ultrapure water for 30 min. The solution was filtered through a filter membrane (0.2 μm pore size). Then ultrapure water was added into the filtrate producing a total volume of 50 mL homogenized sample solution. A mixture containing equal volumes of fish extract and 0.1 M PBS was applied for the freshness analysis. After spiking the standard solutions of hypoxanthine into the samples, the concentrations of hypoxanthine and the recovery of the assay were measured from the decrease of ECL emission.

3. Results and discussion

3.1. Morphology and spectroscopic characterization of CNSs and QDs

The SEM image of carboxylated CNSs showed a relatively narrow size distribution with the average diameter of 200–250 nm (Fig. 1A). The surface of CNSs exhibited a homogeneous spiky and porous structure, which should benefit to the loading of QDs on the CNSs and the diffusion of analytes through interconnected millipores, leading to a sensitive biosensing.

The formation of MPA capped CdS QDs was characterized by UV–vis and PL spectra (Fig. 1B). The UV–vis absorption peak occurred at 391 nm (curve a). According to Peng's empirical equations [24], the size and the concentration of QDs were estimated to be 3.1 nm and 5.8 μM, respectively. The PL spectrum of MPA capped CdS QDs showed a relatively narrow emission with a maximal intensity at 560 nm (curve b). The short PL excited wavelength at 400 nm indicated that the PL emission came from the excited state of QD core [25].

3.2. EIS characterization of modified electrodes

The EIS characterization could demonstrate the modification procedure of the electrode (Fig. 2). The diameter of semicircle in the Nyquist plot at high frequency corresponds to the electron-transfer resistance R_{et}, which can be calculated according to the equivalent circuit (inset of Fig. 2). The bare GCE showed a relatively small R_{et} of 230 Ω (curve a). When PFCNSs were assembled onto GCE,