Talanta 128 (2014) 9–14

Contents lists available at ScienceDirect

Talanta

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

A novel strategy for synthesis of hollow gold nanosphere and its application in electrogenerated chemiluminescence glucose biosensor

Xia Zhong, Ya-Qin Chai *, Ruo Yuan *

Key Laboratory of Luminescence and Real-time Analysis Chemistry, Ministry of Education, College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China

ARTICLE INFO

Article history:
Received 13 December 2013
Received in revised form
21 March 2014
Accepted 29 March 2014
Available online 4 April 2014

Keywords:
Electrogenerated chemiluminescence
Glutaraldehyde
Glucose oxidase
Nanospheres
Glucose

ABSTRACT

Well-distributed hollow gold nanospheres (Au_{shell}@GOD) (20 ± 5 nm) were synthesized using the glucose oxidase (GOD) cross-linked with glutaraldehyde as a template. A glucose biosensor was prepared based on Au_{shell}@GOD nanospheres for catalyzing luminol electrogenerated chemiluminescence (ECL). Firstly, chitosan was modified in a glassy carbon electrode which offered an interface of abundant amino-groups to assemble Au_{shell}@GOD nanospheres. Then, glucose oxidase was adsorbed on the surface of Au_{shell}@GOD nanospheres *via* binding interactions between Au_{shell} and amino groups of GOD to construct a glucose biosensor. The Au_{shell}@GOD nanospheres were investigated with TEM and UV–vis. The ECL behaviors of the biosensor were also investigated. Results showed that, the obtained Au_{shell}@GOD nanospheres exhibited excellent catalytic effect towards the ECL of luminol-H_2O_2 system. The response of the prepared biosensor to glucose was linear with the glucose concentration in the range of 1.0 μM to 4.3 mM (R=0.9923) with a detection limit of 0.3 μM (signal to noise=3). This ECL biosensor exhibited short response time and excellent stability for glucose. At the same time the prepared ECL biosensor showed good reproducibility, sensitivity and selectivity.

1. Introduction

Electrogenerated chemiluminescence (also known as electrochemiluminescence) is the combination electrochemistry and chemiluminescence, which not only possesses the advantages of electrochemical analysis, but also exhibits many characteristics of chemiluminescence analysis [1]. As a new electrochemical detection technology, electrogenerated chemiluminescence (ECL) is of interest to researchers due to its versatility, simplified optical set-up, low background signal and high sensitivity. Electrogenerated chemiluminescence signals are usually obtained from the excited states of a luminophore generated at the electrode surface during the electrochemical reaction [2]. Electrogenerated chemiluminescence technology has been widely applied in the field of biotechnology, food safety and clinical diagnosis [3–5]. In recent years, some enzyme biosensors based on electrogenerated chemiluminescence also have been reported for detecting glucose, alcohol, hypoxanthine, cholesterol, choline, etc. [6–10].

Luminol (2,3-aminophthalhydrazide) has been widely used in constructing electrogenerated chemiluminescence systems because of its low oxidation potential, inexpensive reagent consumption and the high emission yields [11–13]. The electrogenerated chemiluminescence of luminol can be triggered by applying an appropriate positive potential to the working electrode in the presence of hydrogen peroxide (H_2O_2) [1,9]. Some enzymes can produce H_2O_2 during their substrate-specific enzymatic reaction. The intensity of the electrogenerated chemiluminescence signal is directly proportional to the concentration of H_2O_2 which was generated by enzymatical catalysis. Therefore, a sensitive electrogenerated chemiluminescence glucose biosensor could be designed for measurement of glucose by detecting H_2O_2 indirectly [14,15].

Some nanomaterials such as gold nanoparticles (AuNPs) [9,16], platinum nanoparticles [14], palladium nanoparticles [16], graphene [17], TiO_2 nanocrystals [18] and quantum dots [19] have been used in the electrogenerated chemiluminescence system. Among these nanomaterials, gold nanoparticles have excellent catalytic performance to directly enhance the electrogenerated chemiluminescence intensity of luminol-H_2O_2 system [20–22]. So, gold nanoparticles can be used as the immobilization matrix for the design of a novel biosensor with enhanced analytical performance. A lot of hollow capsules nanoparticles have been synthesized and applied [23–26]. Sharma's group [27] has obtained hollow gold nanoparticles by leaching out silver chloride (AgCl) from Au_{shell}@$AgCl_{core}$ nanoparticles with dilute ammonia solution, Jiang's group [28] has synthesized Polystyrene/gold core–shell

* Corresponding authors. Tel.: +86 23 6825 2277; fax: +86 23 6825 4000.
E-mail addresses: yqchai@swu.edu.cn (Y.-Q. Chai), yuanruo@swu.edu.cn (R. Yuan).

http://dx.doi.org/10.1016/j.talanta.2014.03.071
0039-9140/

nanocomposites based on the method of ionic self-assembly and the in *situ* reduction. These preparation process of hollow gold nanoparticles were complicated.

Herein, the aim of this work is to synthesize well-distributed hollow gold nanospheres encapsulating GOD (Au_{shell}@GOD) (about 20 ± 5 nm) in a simple way and develop an ECL biosensor. The prepared hollow Au_{shell}@GOD nanoparticles have large surface area, high conductivity, good biocompatibility, which could effectively increase the loading of GOD and keep the biological activity of GOD. Thus, GOD molecules can exist inside and outside of the Au_{shell}, which could effectively amplify the ECL intensity of luminol-H_2O_2 system. In addition, the Au_{shell} assembling on the electrode can catalyze the electro-oxidization of luminol, and the more strong ECL signal was obtained in neutral aqueous solution. Such fabrication of biosensor exhibited short response time and excellent stability for glucose detecting. At the same time the prepared ECL biosensor showed good reproducibility, sensitivity and selectivity.

2. Experimental

2.1. Reagents and materials

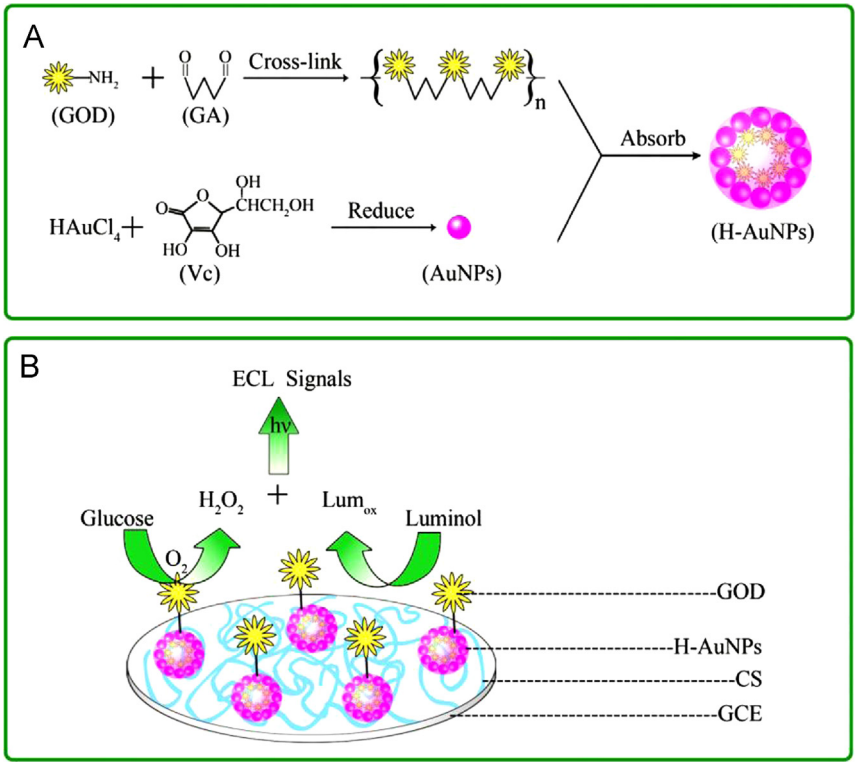
GOD and chitosan (CS) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Gold chloride tetrahydrate ($HAuCl_4 \cdot 4H_2O$) was obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Glutaraldehyde (GA) was purchased from Beijing Chemical Reagent Co. (Beijing, China). L-ascorbic acid (AA) was purchased from Boyi Chemical Reagent Co. (Chongqing, China). Phosphate buffer solutions (PBS) with various pH values were prepared with 0.1 M KH_2PO_4 and 0.1 M Na_2HPO_4. The supporting electrolyte was 0.1 M KCl. All chemicals were analytical grade and were used as received without further purification. Ultrapure water was used throughout all experiments.

2.2. Apparatus

The ECL emission was measured using a model MPI-A electrochemilu-minescence analyzer (Xi'an Remax Electronic Science & Technology Co. Ltd., China) with the voltage of the photomultiplier tube (PMT) set at 600 V in the detection process. Cyclic voltammetry (CV) was performed with a CHI 600D electrochemical work station (Shanghai CH Instruments Co., China). All experiments were performed with a conventional three-electrode system including a bare or modified glassy carbon electrode (GCE, $\Phi = 4$ mm) as working electrode, a platinum wire as auxiliary electrode and a saturated calomel electrode (SCE) as reference electrode for electrochemical experiments, or Ag/AgCl electrode as a reference electrode for ECL experiments. The morphologies of the prepared of Au_{shell}@GOD were tracked by transmission electron microscopy (TEM, H600, Hitachi Instrument, Japan).

2.3. Preparation of hollow gold nanospheres encapsulating GOD (Aushell@GOD)

The hollow gold nanospheres encapsulating GOD were synthesized using GOD cross-linked with glutaraldehyde as a template. Briefly, GOD cross-linked with glutaraldehyde was obtained by mixing 1.0 mL of 2 mg L^{-1} GOD solutions (pH 7.0) with 1.0 mL of 1.0% glutaraldehyde solution. Then, the mixture solution was placed in a refrigerator at 4 °C for 12 h. Subsequently, 0.5 mL of above mixture solution and 100 μL of 1.0% gold chloride tetrahydrate were added in 5.0 mL ultrapure water with stirring. Afterward, 0.01 M ascorbic acid (AA) was injected into the solution with droplet under agitation, and the color of solution changed to claret. In the GOD-glutaraldehyde network structure, $AuCl_4^-$ can be reduced by ascorbic acid to elemental Au that could grow into hollow gold nanospheres (Au_{shell}@GOD) in control. The morphology of Au_{shell}@GOD was measured by TEM. The schematic diagram of the stepwise procedure of the synthesis is illustrated in Scheme 1(A).



Scheme 1. (A) Synthesized process of hollow gold nanospheres (Au_{shell}@GOD); (B) Schematic description of response mechanism.