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Two-phase synthesis of hydrophobic ionic liquid-capped gold nanoparticles and their application for sensing cholesterol

Mingjun Dong, Zhihan Nan, Panpan Liu, Yanjun Zhang, Zhonghua Xue, Xiaoquan Lu, Xiuhui Liu*

Key Laboratory of Bioelectrochemistry & Environmental Analysis of Gansu Province, College of Chemistry & Chemical Engineering, Northwest Normal University, Lanzhou, 730070, China

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ABSTRACT

A novel scheme for fabrication of hydrophobic ionic liquid-capped gold nanoparticles (IL-capped AuNPs) modified electrode is presented and its application potential for cholesterol biosensor is investigated. Highly stable gold nanoparticles were characterized by UV–vis absorption spectroscopy and transmission electron microscopy (TEM). Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) indicated that IL-capped AuNPs nanocomposites showed excellent electrical conductivity. Furthermore, cholesterol oxidase (ChOx) was directly immobilized on the IL-capped AuNPs nanocomposite, and then the direct electrochemistry of ChOx on the modified glass carbon electrode (GCE) was obtained. As a new platform in cholesterol analysis, ChOx-IL-capped AuNPs/GCE exhibited a linear response to cholesterol in the range of 0.1–50 μM with a detection limit of 0.033 μM. Therefore, hydrophobic ionic liquid-capped gold nanoparticles would serve as a good candidate material to construct the related enzyme biosensors.

1. Introduction

A high cholesterol level in human blood will result in some deadly diseases such as arteriosclerosis, hypertension and myocardial infarction, thus the determination of cholesterol is very important in clinical diagnosis [1,2]. A variety of analytical procedures including both chemical and enzymatic methods have been proposed for cholesterol assays [3–6]. Among these, the electrochemical methods based on cholesterol oxidase (ChOx) have made great progress for estimation of cholesterol level in blood and food because of their high sensitivity and fast response time [7–9]. Recently, enzyme-based biosensors researchers focused on the effective immobilization of enzyme on the solid electrode surface and realized the direct electron transfer between ChOx and electrode surfaces [10–12]. But the poor compatibility of the support matrix and the deep embedment of redox active site in the protein may restrict the analytical efficiency of the developed biosensor [13]. In order to solve this problem, many efforts have been made to develop suitable support matrix that provide better environment for loading the enzyme efficiently and maintaining the enzymatic bioactivity.

Gold nanoparticles (AuNPs) have attracted considerable interest in electrochemical biosensor because they can provide higher enzyme loading and retain enzyme bioactivity [14–17]. It was found that the smaller AuNPs could assist the electron transfer generated from the enzyme catalytic redox reaction to the electrode surfaces [18,19]. However, without protection or surface passivation, AuNPs are easy to aggregate owning to their high surface energy, which will greatly reduce the catalytic activity [20,21].

Ionic liquids (ILs) have been proven to be excellent solvents for the immobilization and stabilization of metal nanoparticles due to the much lower driving forces for NP aggregation in ILs [22], thus providing an excellent media for quasi-homogeneous catalysis [23–27]. In addition, some studies about enzymatic catalysis in ILs showed that the activity and stability of the enzymes both increased comparing with those in organic solvents. It was also found that the high hydrophobicity of ILs could be beneficial to enzyme activities and stabilization [28,29]. Recently, our group developed a cholesterol biosensor by immobilizing ChOx on hydrophobic ionic liquid 1-octyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([OMIM][NtF_2]) film which yielded good performance toward the detection of cholesterol, suggesting cholesterol in the solution tends to absorb to the hydrophobic ionic liquid interface [30].

Based on the above consideration, in this work, we first prepared ionic liquid-capped gold nanoparticles by two-phase synthesize as

* Corresponding author. Tel.: +86 0931 7975276; fax: +86 0931 7971323.
E-mail addresses: liuxh@nwnu.edu.cn, liuyr1008@sina.com (X. Liu).

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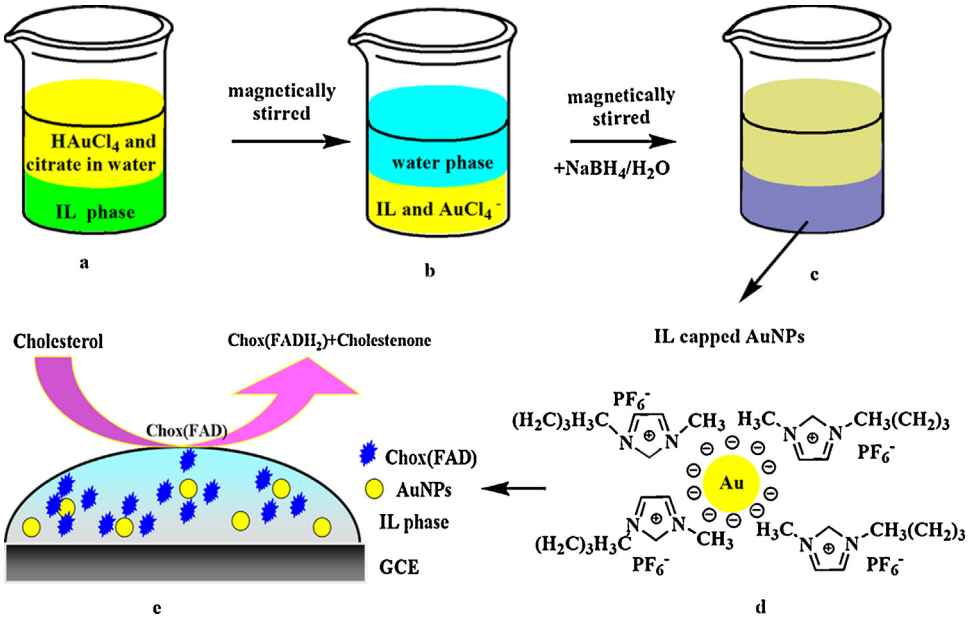

Scheme 1. Schematic illustration reactions of two-phase synthesized of hydrophobic ionic liquid-capped gold nanoparticles and ChOx biosensor fabrication.

shown in Scheme 1, and then fabricated ChOx-IL-capped AuNPs modified electrode to determine cholesterol. Moreover, the direct electrochemistry of ChOx on the modified electrode was observed. By combining the advantages of IL and AuNPs, the ChOx-IL-capped AuNPs/GCE exhibits an excellent response to cholesterol.

2. Experimental

2.1. Reagents

The ionic liquid (IL) of 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]) was produced by Lanzhou Institute of Chemical Physics (Lanzhou, China). Cholesterol oxidase (ChOx, EC 1.1.3.6, 15U/mg) was purchased from Shanghai source leaf biological technology Co. Ltd. (Shanghai, China). Cholesterol was obtained from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). Triton X-100 and chloroauric acid came from Aladdin Chemistry Co. Ltd. (Shanghai, China). Isopropanol and sodium borohydride were acquired from Shanghai Zhongqin Chemistry Co. Ltd. (Shanghai, China). Trisodium citrate was purchased from Xi'an Chemical Reagent Factory (Xi'an, China). Phosphate buffer solution (PBS, pH 7.0) was prepared by mixing suitable amounts of 0.2 M NaH_2PO_4/Na_2HPO_4. Other chemicals were all of analytical grade, and the solutions were prepared by doubly distilled water.

2.2. Apparatus and measurement

Transmission electron microscopy (TEM) micrographs were obtained with a JEOL JSM-6700F field emission transmission electron microscope. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed on a CHI660 C electrochemical workstation (Austin, TX, USA) with conventional three-electrode system. A bare or modified glassy carbon electrode (GCE, 3.0 mm diameter) was served as working electrode. A saturated calomel electrode (SCE) and a platinum electrode were used as reference and auxiliary electrodes, respectively. Electrochemical impedance spectroscopy (EIS) experiments were performed on a multi-channel electrochemical workstation (American Princeton Instruments Corporation). UV–vis spectra measurements were obtained with a UV-1102 spectrometer (Shanghai, China).

2.3. Preparation of ionic liquid-capped gold nanoparticles

As shown in Scheme 1, ionic liquid-capped gold nanoparticles were synthesized by the two-phase system. Firstly, all glassware used in the following procedures was cleaned in a bath of freshly prepared solution of HNO_3/HCl (1:3, v/v), rinsed with twice-distilled water and dried prior to be used. Then 2 mL [BMIM][PF_6] and 2.5 mM $HAuCl_4$ solution were mixed at equal volume into a 10 mL glass vial. 0.0073 g of trisodium citrate was dispersed in a little water and then added them into the mixture solution. After continuously magnetically stirring for 3 min, a freshly prepared aqueous solution of sodium borohydride (0.25 mL 5 mM) was slowly dropwise added into the above solution under magnetic vigorous stirring at room temperature for 20 min. After 10 minutes, the ionic liquid was separated from water at the bottom of the reactor which color changed from yellow to blue, indicating the formation of gold nanoparticles. The supernatant was removed and the colloid was washed by deionized water three times to remove residual trisodium citrate. Finally, the ionic liquid-capped gold nanoparticles were obtained, and its structure was shown in Scheme 1(d) [31].

2.4. Preparation of cholesterol and cholesterol oxidase-ionic liquid-capped gold nanoparticles stock solution

Cholesterol stock solution of 5 mM was prepared in deionized water. Briefly, 1 mL isopropanol and 1 mL Triton X-100 were mixed in a 25 mL volumetric flask, then 0.04835 g of cholesterol added rapidly into the solution and stirred in a bath at 60 °C. Finally, the solution was diluted with distilled water to 25 mL and then it was stored at 4 °C for future use. The stock cholesterol oxidase-ionic liquid-capped gold nanoparticles solution was prepared by mixing 5 mg cholesterol oxidase (ChOx) with 0.2 mL ionic liquid-capped gold nanoparticles stock solution under ultrasonication for 30 min at room temperature until a stable yellow solution was