A novel amperometric biosensor based on gold nanoparticles-mesoporous silica composite for biosensing glucose

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We report a novel bienzyme biosensor based on the assembly of the glucose oxidase (GOD) and horseradish peroxidase (HRP) onto the gold nanoparticles encapsulated mesoporous silica SBA-15 composite (AuNPs-SBA-15). Electrochemical behavior of the bienzyme bioconjugates biosensor is studied by cyclic voltammetry and electrochemical impedance spectroscopy. The results indicate that the presence of mesoporous AuNPs-SBA-15 greatly enhanced the protein loadings, accelerated interfacial electron transfer of HRP and the electroconducting surface, resulting in the realization of direct electrochemistry of HRP. Owing to the electrocatalytic effect of AuNPs-SBA-15 composite, the biosensor exhibits a sensitive response to H₂O₂ generated from enzymatic reactions. Thus the bienzyme biosensor could be used for the detection of glucose without the addition of any mediator. The detection limit of glucose was 0.5 µM with a linear range from 1 to 48 µM.

1 Introduction

The accurate, rapid quantitative determination of glucose is of great importance in clinical chemistry, biochemistry and food analysis[1]. In the last decades, the electrochemical enzymatic glucose sensors have attracted more attention due to their high specificity[2]. Glucose oxidase (GOD) can catalyze the oxidation of glucose to hydrogen peroxide (H₂O₂) and gluconolactone in the presence of dissolved oxygen. However, the major challenge for the development of new biosensors for the determination of glucose is the elimination of interferences, which hindered the sensitivity of the devices. These interferences mainly come from the presence of more easily oxidizable compounds present in biological fluids than the liberated H₂O₂ produced by the enzymatic reaction. Recently, co-immobilization of horseradish peroxidase (HRP) with a H₂O₂ producing oxidase gives a biosensor with selectivity towards the substrate of the oxidase. For example, Kulys et al.[3] designed the first oxidase/peroxidase bienzyme electrode with a film containing HRP and GOD to determine glucose by measuring the amount of H₂O₂ produced through the peroxidase. Zambonin et al. [4] fabricated a glucose bienzyme sensor based on HRP and GOD immobilized in an electropolymerized film. Narayanan et al. [5] developed a bienzyme nanobiocomposite biosensor using functionalized carbon nanotubes for the detection of H₂O₂ and glucose. The coupling of several enzymes through substrate or co-substrate regeneration among these multienzymatic systems is quite promising for amplifying the electrochemical responses in biosensing
applications.

On the other hand, SBA-15, as a type of mesoporous silica, has a large internal surface, tunable uniform pore size, chemical and thermal stability. It has attracted considerable attention in biosensor platforms for the detection of hydrogen peroxide and glucose. Recent studies have demonstrated that SBA-15 is able to improve the direct electron transfer reaction of some biomolecules, which is attributed to its mesoporous structure. Additionally, gold nanoparticles with appealing electrical properties and biocompatibility have already been used in sensor fabrication, which have been found to be able to accelerate electron exchange between electrode and protein. Therefore, immobilization of enzyme on gold nanoparticles encapsulated mesoporous SBA-15 will show certain advantages over those glucose biosensors in which enzymes are directly immobilized on bare electrode surfaces.

In this paper, the gold nanoparticles were encapsulated into the channels of mesoporous silica SBA-15 to fabricate a AuNP-SBA-15 hybrid material. This composite was further used for the immobilization of biocatalysts to develop a mediator-free amperometric glucose biosensor. One of the enzymes, GOD converts glucose to gluconic acid with H$_2$O$_2$, which is the substrate for the second enzyme, HRP. HRP is in direct electronic communication with the electrode via AuNPs-SBA-15 thus bringing about the electrocatalytic reduction of H$_2$O$_2$, which can be further measured by amperometric method. The biosensor showed high performance characteristics with a broad detection range and a long-term stability of H$_2$O$_2$ and glucose, which implied that the proposed method provided an excellent platform for sensitive electrochemical sensing and biosensing.

2 Experimental

2.1 Chemicals

GOD (activity 250 units/mg), HRP (250 units/mg) and poly(ethylene-oxide)-poly(propylene oxide)-poly (ethyleneoxide) block copolymer EO$_{20}$PO$_{70}$EO$_{20}$ (P123) were purchased from Sigma Co. Hydrogen peroxide (30 w/v), HAuCl$_4$·4H$_2$O and Tetraethyl orthosilicate (TEOS) were from Shanghai Chemical Reagent Co. (Shanghai, China). (3-Aminopropyl)triethoxysilane (APTES) was obtained from Nanjing Reagent Factory (Nanjing, China). Phosphate buffer solutions (PBS, 0.1 M) with various pH values were prepared by mixing stock standard solutions of Na$_2$HPO$_4$ and NaH$_2$PO$_4$ and adjusting the pH with H$_3$PO$_4$ or NaOH. All other chemicals were of analytical grade and used without further purification.

2.2 Synthesis of SBA-15 and AuNPs-SBA-15 nanostructures

The mesoporous SBA-15 was synthesized according to the method described by Zhao et al. The synthesis of the AuNPs-SBA-15 nanocomposite was as follows: First, the temple-free SBA-15 material was rehydrated by refluxing with water for 6 h, followed by heating at 200°C overnight. After cooling to room temperature, the sample was immersed in an ethanol solution of the APTES and refluxed at 70°C for 6 h. The functionalized SBA-15 was filtered, washed with a large amount of ethanol and dried. Subsequently, 0.4 g of functionalized SBA-15 was mixed with 6 mL of an aqueous 1 wt% HAuCl$_4$ solution at pH 2, followed by stirring for 30 min. After reaction, the mixture was filtered, washed with water, and then dried in air. The resulting yellowish solid was further reduced by the addition of 5 mL of 0.1 M KBH$_4$ aqueous solution and stirred for 30 min. The AuNPs-SBA-15 was collected by centrifugation and further washed with water.

2.3 Preparation of TEOS stock sol-gel solution

A homogeneous stock sol-gel solution was prepared by mixing 600 µL of ethanol, 50 µL of TEOS, 10 µL of 5 mM NaOH, and 60 µL of H$_2$O in a small test tube at room temperature. After being sonicated, the sol-gel was formed and stored at 4°C. The sol-gel solution was prepared freshly before each electrode modification experiment.

2.4 Construction of the biocatalyst bioconjugate biosensor

The biocatalyst bioconjugate biosensor was fabricated as follows: First, 5 mg of AuNPs-SBA-15 composite was dispersed in 1 mL of pH 7.0 PBS containing 5 mg of HRP and 5 mg of GOD, and shaken at room temperature overnight. Subsequently, 5 µL of this suspension was dropped onto the glassy carbon electrode (GCE, 3 mm in diameter) and dried in a silica gel desiccator. After 2 h, 5 µL of stock silica sol-gel solution was pipetted to cover the bioconjugate-modified GCE. Finally, the electrode was left to dry at 4°C overnight. The modified