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Highly-sensitive cholesterol biosensor based on platinum–gold hybrid functionalized ZnO nanorods

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A novel scheme for the fabrication of gold/platinum hybrid functionalized ZnO nanorods (Pt–Au@ZnONRs) and multiwalled carbon nanotubes (MWCNTs) modified electrode is presented and its application for cholesterol biosensor is investigated. Firstly, Pt–Au@ZnONRs was prepared by the method of chemical synthesis. Then, the Pt–Au@ZnONRs suspension was dropped on the MWCNTs modified glass carbon electrode, and followed with cholesterol oxidase (ChOx) immobilization by the adsorbing interaction between the nano-material and ChOx as well as the electrostatic interaction between ZnONRs and ChOx molecules. The combination of MWCNTs and Pt–Au@ZnONRs provided a favorable environment for ChOx and resulted in the enhanced analytical response of the biosensor. The resulted biosensor exhibited a linear response to cholesterol in the wide range of 0.1–759.3 μM with a low detection limit of 0.03 μM and a high sensitivity of 26.8 μA mM^{-1}. The calculated apparent Michaelis constant K_M^{app} was 1.84 mM, indicating a high affinity between ChOx and cholesterol.

1. Introduction

As is known to all, normal concentration of cholesterol in human serum is in the range of 1.3–2.6 mg mL^{-1}, of which ∼30% is sterol and ∼70% is esterified with fatty acids [1]. High cholesterol accumulation in blood serum is strongly correlated with many clinical diseases, such as coronary disease, arteriosclerosis, myocardial infarction, brain thrombosis, lipid metabolism dysfunction, hypertension and so on [2]. Therefore, the determination of cholesterol levels in the blood of human is of great importance in clinical analysis/diagnosis.

In the analytical practice for the cholesterol measurement, high-performance liquid chromatography (HPLC) and gas chromatography (GC) are used frequently, but a very intensive pre-treatment of the samples is needed [3]. As for colorimetry, the method is simple, rapid and capable of being automated, but does require a large amount of serum sample [4]. With regard to spectrophotometry, non-selective and non-sensitive detection as well as the long response time limits its application. As another well-known biochemical detection method for cholesterol, electrochemical technique offers high performance detection as well as simplicity [5]. Because of their low detection limit, rapid response and low cost, amperometric biosensors are more promising than other electrochemical detection. To the amperometric cholesterol biosensors, the enzymatic reactions in the use of cholesterol oxidase (ChOx) as a receptor can be described as follows:

$$\text{Cholesterol} + O_2 \xrightarrow{\text{ChOx}} \text{Cholest} - 4 - \text{en} - 3 - \text{one} + H_2O_2$$

The cholesterol is oxidized by oxygen in the presence of ChOx and H_2O_2 is produced at the same time [6]. The electro-oxidation current of H_2O_2 is detected to determine the concentration of the cholesterol in the sample.

Owing to the unique properties such as good electrical conductivity, strong adsorptive ability and excellent biocompatibility, carbon nanotubes (CNTs) have been widely used in the region of biosensor [7]. Previous studies have indicated that CNTs had fast electron-transfer kinetics and could catalyze the electrochemical reaction of NADH [8], glucose [9,10], neurotransmitters [11], dopamine [12] and so on. Li group reported carbon nanotube modified biosensor for monitoring total cholesterol in blood, and the research stated clearly that CNTs modification not only promoted the electron transfer and almost doubled the sensitivity, but also improved the linearity of the electrode [13]. However, the poor solubility of CNTs in most solvents is one of the limitations in the design of CNT-based biosensing devices. Chitosan (CS), a natural cationic biopolymer, has attracted much interest owing to its interesting properties such as biocompatibility, non-toxicity, low-cost, good film forming ability, high mechanical strength and high

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hydrophilicity [14]. It was usually used as a medium for dispersing multiwalled carbon nanotubes (MWCNTs) in aqueous solution. And the CS-MWCNTs dispersion solutions could keep homogeneous for several months. In the construction of biosensors, the CS-MWCNTs composite is usually combined with other nanoparticles such as Au, Ag, Pt, Cu, TiO_2, SiO_2, ZrO_2 and so on. Recently, some cholesterol biosensors based on CS-MWCNTs have been reported. Tan et al. [15] used sol–gel CS/SiO_2 and MWCNTs composite film to immobilize ChOx for detecting cholesterol and the performance of the biosensor with MWCNTs is superior to the biosensor without MWCNTs. Solanki et al. [16] immobilized cholesterol esterase (ChEt) and ChOx onto SiO_2-CS/MWCNTs bionanocomposite film for the detection of total cholesterol and the linearity of 10–500 mg/dL^{-1} is only two orders. Tsai et al. [6] fabricated amperometric cholesterol biosensor based on CNTs-CS-Pt-ChOx nanobiocomposite. The sensitivity of 44 $mAM^{-1}\,cm^{-2}$ is higher than some other cholesterol biosensors, however, the stability of the prepared cholesterol biosensor was disappointed.

Zinc oxide (ZnO) is an n type semiconductive material with wide energy leap (Eg = 3.37 eV) and large exciton binding ability (60 meV) [17], and has useful electronic and optical properties. It has been demonstrated that both the size and shape or morphology have an influence on the properties of materials. Therefore, numerous attention has been paid to the synthesis of nanostructured ZnO such as nanoparticles [18], nanowires [19], nanobelts [20], nanorods [21], nanotubes [22] and hollow spheres [23] for various applications. ZnO nanorods have attracted considerable interest in the aspect of sensors due to many advantages, including large surface-to-volume ratio, excellent biological compatibility, high electron-transfer rates, non-toxicity and bio-safety. Notably, ZnO with a high isoelectric point (IEP ~9.5) is suitable for the adsorption of low IEP proteins or enzyme. Positively charged ZnO nanorods matrix not only provided a friendly microenvironment for the negatively charged proteins or enzyme to retain its activity but also promoted the direct electron transfer between the enzyme and the electrode to a large extent [24]. Zhang [24] et al. immobilized uricase on ZnO nanorods for a reagentless uric acid biosensor. To improve the properties of ZnO, the combination of ZnO with the metal materials such as Au, Ag, Cu and Pt was reported in the past years. Doping in ZnO with the noble metals [25] offers an effective approach to enhance the properties of ZnO nanostructures, which is crucial for their practical applications especially for the construction of biosensor. Gong [26] et al. prepared a Cu-doped ZnO film by co-sputtering of Cu and ZnO and developed a highly sensitive CO gas sensor. Wang [27] et al. constructed a sensitive SPR biosensor based on ZnO–Au nanocomposites for the determination of rabbit IgG. Wang and Zheng [28] prepared a modified electrode through electrodepositing silver nanoparticles (AgNPs) on a ZnO film, which exhibited wide linear range and low detection limit for the quantitative analysis of H_2O_2. Ahmad [29] et al. synthesized Pt-incorporated fullerene-like ZnO nanospheres by codeposition of $ZnNO_3$ and $H_2PtCl_6{\cdot}6H_2O$ and applied it to develop a highly sensitive amperometric cholesterol biosensor. The metal-ZnO nanostructures built a favorable bridge for electron communication between the electrode and the detecting substrate. To obtain the metal-ZnO nanostructures, the common method is depositing the metal on the surface of ZnO.

The cholesterol biosensors based on MWCNTs [13,30,31], ZnO [32,33], Pt–ZnO nanocomposites [29], Pt-MWCNTs nanocomposites [6,34] have been reported continually, but these biosensors are witnessed by the narrow linear rang, high detection limit or low sensitivity, as well as the unsatisfactory stability. In this paper, Pt–Au@ZnONRs composite was synthesized by the method of multiple-step chemosynthesis and was combined with MWCNTs to construct a cholesterol biosensor. This system provides a new and promising platform for electrochemical devices due to the coupling of advantages of Pt, ZnONRs and MWCNTs. Firstly, ZnO with a high IEP (~9.5) is suitable for the adsorption of the low IEP enzyme (for instance ChOx, IEP = ~4.9), and the large surface-to-volume ratio of MWCNTs and Pt–Au@ZnONRs make for more ChOx immobilized. Secondly, MWCNTs and ZnONRs could retain the enzyme bioactivity and enhance the electron transfer between the active center of the enzyme and the electrode. Thirdly, Pt is a well-known catalyst that has a high catalytic activity for H_2O_2 electro-oxidation, and both MWCNTs and ZnONRs can catalyze the electro-oxidation of H_2O_2, which sharply improve the sensitivity of the biosensor.

2. Experimental details

2.1. Reagents

Cholesterol oxidase (EC 1.1.3.6, 28 $U\,mg^{-1}$), cholesterol, chitosan (CS, MW: 100,000–300,000, deacetylating grade: 70–85%), $H_2PtCl_6{\cdot}6H_2O$ (99.9%), Triton X-100, Nafion (5%) were purchased from Sigma-Aldrich (Shanghai) Trading Co., Ltd. MWCNTs (>95% purity) were obtained from Chengdu Organic Chemicals Co. Ltd. of the Chinese Academy of Science and purified by fluxing in concentrated nitric acid for 7 h prior to use. Tetraethoxysilane (TEOS), 3-aminopropyltriethoxysilane (APTES), trioctylamine, H_2O_2 solution (30%) and 2-propanol (99.9%) were supplied by Chemical Reagent Co., Shanghai, China. Zinc acetate, cobalt (II) acetate, ethanol, ammonia solution, sodium citrate, L-cysteine, ascorbic acid and uric acid were purchased from Chemical Regent Co. Chongqing, China. Nano-Au seed solution was prepared according to the literature [35]. A series of phosphate-buffered solutions (PBS) were prepared by mixing the solutions of 0.05 M KH_2PO_4, 0.05 M Na_2HPO_4 and the supporting electrolyte was NaCl (0.9%). Other chemicals were of analytical-reagent grade without further purification. The cholesterol stock solution was prepared in a 50 mL aqueous solution containing 1 mL of 2-propanol and 1 mL of Triton X-100 in a bath at 60 °C and then diluted with deionized water. Double-distilled water was used throughout this study.

2.2. Apparatus and measurements

The electrochemical experiments were performed at room temperature utilizing an electrochemical workstation (CHI660D) with a three-electrode configuration. A modified glassy carbon electrode (GCE, 4 mm in diameter) was used as the working electrode, with saturated calomel electrode (SCE) as the reference electrode, and platinum as the counter electrode. Transmission electron microscopy (TEM) was performed on a TECNAI 10 (PHILIPS FEI Co., The Netherlands). Atomic force microscopy (AFM) images of the films were achieved by scanning probe microscope (Vecco, USA). X-ray photoelectron spectroscopy (XPS) measurements were carried out with a VG Scientific ESCALAB 250 spectrometer, using Al KR X-ray (1486.6 eV) as the light source. All the electrochemical experiments were carried out at room temperature.

2.3. Cholesterol biosensor fabrication

2.3.1. Preparation of Pt–Au@ZnONRs

Zinc oxide nanorods (ZnONRs) were prepared as following: zinc acetate (0.2335 g) and cobalt acetate (0.01295 g) were mixed in a three-neck flask with trioctylamine (10 mL). The flask was rapidly heated to 300 °C with a condenser. The solids dissolved slowly as the temperature increased, and the color of the solution changed from clear to royal blue. The reaction was continued for 120 min and cooled down to room temperature. The green precipitate was washed several times with ethanol to remove any cobalt precursor, as well as any cobalt metal particles.