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One-Step Electrodeposition of Gold-Graphene Nanocomposite for Construction of Cholesterol Biosensor

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Abstract

The determination of cholesterol is increasingly important for the fact that high serum cholesterol level was related with many diseases. In this work, a new cholesterol biosensor was developed based on the gold-graphene nanocomposite material prepared by one-step electrodeposition. The nanomaterial formed on a glassy carbon electrode was proved by scanning electron microscope and the electrochemical behavior of the modified electrode as well as the cyclic voltametry and electrochemical impedance spectroscopy of the ferricyanide/ferrocyanide couple. After the immobilizing of cholesterol oxidase cross-linked with chitosan, a simple cholesterol biosensor was prepared. The biosensor responded linearly to cholesterol in the concentration range of 0.25 to 5.0 mM with a detection limit of 50 μM. The sensor has the advantages of easy preparation, short response time and good selectivity. Thus, it could be used to determine the free cholesterol in serum.

Keywords: Electrodeposition; Nanocomposite; Biosensor

Introduction

Graphene (GE), a new advent of carbon material with single layer of carbon atoms in a two-dimensional lattice, dominated the carbon nanomaterials for its advantages of fast electron transportation and low raw material prices. The integration of GE with noble metal nanoparticles had shown the promising applications in electrochemical biosensors [1-5]. In the work of Aravind et al. [3], the substantial effect of Au nanoparticles (AuNPs) dispersed over graphene nanoplatelets was proved. Electrodeposition is a convenient technique for preparing both GE and AuNPs. And it is suitable for the fast modification of nanomaterials on a bare electrode with good adherence and optional film structure control. Hu et al. [6] have provided a general route for fabrication of graphene-based noble metal nanomaterials composite. The graphene sheets suspension was cast onto the surface of the pretreated GC electrode, the AuNPs were electrodeposited on the GE/GCE electrode after the evaporating of solvent. Xia et al. [7] have fabricated AuNPs/GE modified electrode by dipping bare GCE into graphite oxide suspension and chloroauric acid solution and electrodepositing under different potential alternately for ten times.

Cholesterol, as an important and indispensable substance of animal tissue cells, the determination of high level cholesterol is of practical significance [8,9]. The electrochemical analysis of cholesterol is usually developed by fabricating electrochemical biosensor based on cholesterol oxidase (Chox). Most of them are based on measuring the variation of the anodic or cathodic current during the oxidation or reduction of H_2O_2 produced from the oxidation of cholesterol by dissolved oxygen in the presence of Chox [10-12].

In this work, we investigated the one-step electrodeposition of Au-GE nanocomposite from a mixture of GO and Au(III) on the surface of a bare glassy carbon electrode (GCE) to use the unique electrocatalytic properties of gold and graphene (Au-GE) composite for the reduction of H_2O_2. Then, the preparation of a cholesterol biosensor was developed by the incorporation of Chox with the aid of chitosan (CS) to this modified electrode. The determination of free cholesterol in human serum was also performed.

Results and Discussion

Characterization of Chox-CS/Au-GE/GCE

Nyquist plots of bare GCE, Au-GE/GCE and Chox-CS/Au-GE/GCE in 0.1 M KCl solution containing 5 mM Fe(CN)63−/4− were recorded to characterize the electrode modification. It was observed that the diameter of the semicircle (Ret) of the bare GCE was about 180 Ω. It was reduced to 150 Ω after the Au-GE composite was deposited on the electrode surface, showing that the presence of gold nanoparticles and GO on the electrode surface could obviously improve the electron transfer rate with the interface resistance decreased. The Ret of Chox-CS/Au-GE/GCE increased significantly, which is about 400 Ω, demonstrating that Chox has been successfully immobilized to the electrode surface.

The cyclic voltammogram of the prepared Au-GE/GCE in pH 7.4 PBS solution was also recorded. The anodic peak at +0.99 V and the cathodic peak at +0.32 V correspond to the eletrochemical oxidation of Au(0) and the eletrochemical reduction of gold oxide [13]. Thus, the generation of Au(0) on the surface of the electrode could be concluded. From SEM image of Au-GE/GCE, AuNPs were well separated on the thin layer graphene sheets.

Performance of Chox-CS/Au-GE/GCE

To evaluate the performance of the Chox-CS/Au-GE/GCE as a cholesterol biosensor, the amperometric response for successive addition of cholesterol at −0.35 V was recorded in 0.1 PBS (pH 7.4). It could be seen that the biosensor exhibits a rapid response to the addition of cholesterol. When an aliquot of cholesterol is added into 5 mL 0.1 M PBS at pH 7.4, the reductive current rises obviously to reach a stable value. The linear range is from 0.25 to 5.0 mM with the estimated detection limit of 50 μM. The current response of the Chox-

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CS/Au-GE/GCE electrode reached steady state within 4 s. The sensor has faster response compared to the electrochemical sensors based on some other nanomaterials such as $NiFe_2O_4$/CuO/FeO-CS [14], carbon nanotubes/gold nanoparticles [15] and Pt–Pd bimetallic nanoparticle decorated graphene [1]. The short response time of the bioelectrode is attributed to the rapid electron communication characteristic of the Au-GE composite.

The effect of the presence of ascorbic acid, uric acid, glucose, which may interfere in the determination of cholesterol in real samples, was investigated using the Chox-CS/Au-GE/GCE. The current was decreased obviously when the cholesterol was added, demonstrating the excellent response of the biosensor toward the target substrate. However, there was no obvious response when glucose, uric acid or ascorbic acid was added. These phenomena indicate that the sensor has good selectivity.

The application of the enzyme biosensor developed in this work to the analysis of real samples was tested. The analysis was evaluated using human serum spiked with cholesterol at three different concentration levels. The RSD and recovery showed that the biosensor has great potential for the analysis of cholesterol levels in real clinical samples.

The storage stability of the biosensor was examined within a week. The current response of the biosensor to 1.0 mM cholesterol remained almost unchanged when the fabricated biosensor was stored in 4 °C in a refrigerator. This showed that the bioactivity of the immobilized Chox could be maintained by the biocompatible Au-GE nanocomposite and CS at least one week.

Conclusion

In this study, a new cholesterol biosensor was fabricated based on the Au-GE nanocomposite. The nanocomposite was prepared by the simple one-step electrodeposition which is more efficient than the chemical reduction or alternate electrodeposition. The biosensor showed potential application in the serum cholesterol determination with the feature of short response time.

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