Colloids and Surfaces A 529 (2017) 113–118

Contents lists available at ScienceDirect

Colloids and Surfaces A

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

Research Paper

Enhanced direct electron transfer of glucose oxidase based on gold nanoprism and its application in biosensing

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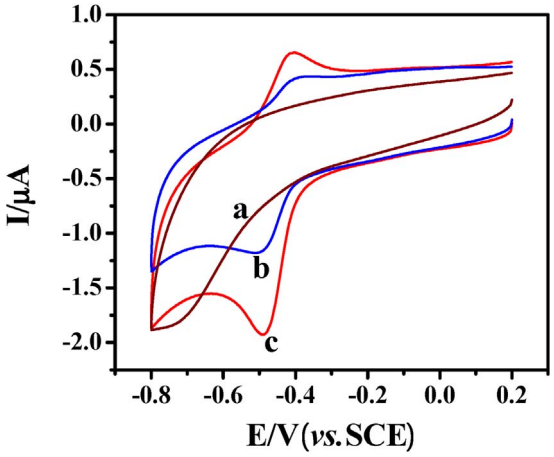
GRAPHICAL ABSTRACT

A pair of distinct and well-defined redox peaks is observed at the GOD–AuNP–chitosan/GCE (curve c) with the formal potential of −0.460 V (vs. SCE) and the peak to peak separation was 52 mV.



ARTICLE INFO

Keywords:
Enhanced electron transfer
Gold nanoprism
Glucose oxidase
Biosensing
Glucose

ABSTRACT

Gold nanoprism (AuNP) was used for immobilization of glucose oxidase (GOD), and the direct electrochemistry of GOD–AuNP–chitosan modified GCE and glucose biosensing were studied. Transmission electron microscopy, UV–vis spectroscopy and electrochemical impendence spectroscopy were employed to confirm the morphology and film modification changes of the prepared biosensor. Results showed that the AuNP can provide a favorable and biocompatible microenvironment for facilitating the direct electron transfer between proteins and electrode surface. It was found that the special structure of gold nanoprism exhibited enhanced performances in direct electron transfer of GOD and glucose sensing. The adsorbed GOD displayed an apparent electron transfer rate constant (k_s) of 24.92 s^{-1}. The constructed biosensor exhibited a good response to glucose with linear range from 0.05 to 1.2 mM (R^2 = 0.9975), low detection limit of 0.01 mM and high sensitivity of 11.83 μA mM^{-1} cm^{-2}. The proposed biosensor offers an alternative method for the determination of glucose in real samples and has potential applications in the fabrication of other biosensors with redox proteins.

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http://dx.doi.org/10.1016/j.colsurfa.2017.05.049
Received 2 March 2017; Received in revised form 5 May 2017; Accepted 20 May 2017
Available online 25 May 2017

1. Introduction

Studies on direct electrochemistry of redox protein/enzyme have attracted increasing interest, which provide a desirable model for designing electrochemical biosensors [1,2], biofuel cell [3] and bioreactor devices [4] and studying mechanisms of proteins and enzymes in biological systems [5]. However, it is difficult for redox protein to exchange electrons directly with bare solid electrodes, because its redox center is deeply immersed in the insulated protein shells, which makes it hard to realize direct electron transfer (DET) of between GOD and the bare electrode [6,7]. Thus, some efforts have been devoted to retain the biological activity and promote DET behaviors of GOD via selected matrix [8,9].

Many types of nanomaterials have been applied in bioelectrochemical analysis due to their large specific surface area and excellent biocompatibility [10], such as gold nanoparticles [11], graphene [12], carbon nanotube (CNT) [13] and so on. Among them, gold nanomaterials has been extensively exploited as biosensors due to it can provide a suitable microenvironment to immobilize enzyme and facilitate the electron transfer between the immobilized enzyme and electrode surface [14]. Compared to other biocompatible materials, chitosan has good biocompatible, biodegradable, non-toxic and excellent film forming ability [15]. So it was commonly used to disperse nanostructured materials and immobilize proteins for constructing biosensor [16].

Glucose oxidase (GOD) is an ideal mode enzyme for studying the electron transfer properties and has been widely applied to monitor glucose owing to its high specificity toward glucose [17–21]. Nanomaterial could facilitate the electron transfer between the immobilized enzyme and electrode surface. The electron transfer rate is related to structure of nanomaterial [22]. In this work, gold nanoprisms (AuNP) was synthesized successfully, and dispersed in chitosan solution and then mixed with GOD. The as-prepared GOD–AuNP–chitosan composite was modified on the surface of glassy carbon electrode (GCE) to construct a novel electrochemical biosensor. UV–vis displayed that the AuNP–chitosan provided a favorable microenvironment for GOD to retain its original structural confirmation and bioactivity. The constructed electrode showed excellent direct electrochemical behavior and was successfully applied in glucose detection, indicating that gold nanoprisms can provide a promising alternative material for immobilizing biomolecules.

2. Experimental

2.1. Materials

Glucose oxidase (E.C. 1.1.3.4, 182 U/mg, Type X-S from *Aspergillus niger*), Chitosan (> 90% deacetylation) was got from Shanghai Yuanju Biotechnology Co., Ltd. Cetyltrimethlyammonium bromide, hydrogen tetrachloroaurate trihydrate ($HAuCl_4{\cdot}3H_2O$, 99.9%), sodium borohydride ($NaBH_4$, 99.995%), sodium hydroxide (NaOH, 99.998%), and sodium iodide (99%) were obtained from Aldrich. All other chemicals were of analytical grade and used without further purification. All aqueous solutions were prepared with ultrapure water from a Milli-Q Plus system (Millipore).

2.2. Apparatus

All of the electrochemical experiments were performed with a CHI660D electrochemical workstation (Shanghai Chenhua Instrument Co. Ltd., China) using a three-electrode system, where a standard saturated calomel electrode (SCE) served as the reference electrode, a platinum wire electrode as the auxiliary electrode, and the modified electrode GCE (3 mm in diameter) as the working electrode. Scanning electron micrographs (SEM) were carried out on a JSM–6390A (JEOL, Japan) scanning electron microscope using an accelerating voltage of 20.0 kV. Transmission electron micrographs (TEM) were carried out by E.M. 912 Ω energy filtering TEM (120 kV). UV–vis adsorption spectra were investigated by Cary 50 Scan UV–vis spectrophotometer (Varian, Australia). The dynamic light scattering (DLS) experiments were performed on a particle size analyzer, model Zetasizer 1000HS (Malvern instruments, UK). Fourier transform infrared spectroscopy (FTIR) was recorded with TENSIR 27 (Bruker, German).

2.3. Preparation of gold nanoprism and modified electrodes

Gold nanoprism was simply synthesized according to previous method [23]. Briefly, 1 mL of 0.1 M $NaBH_4$ was added to the mixture of 1 mL of 0.01 M $HAuCl_4$, 1 mL of 0.01 M sodium citrate and 36 mL of water while stirring vigorously. The resulting mixture was aged for 4 h in order to allow the hydrolysis of unreacted $NaBH_4$. Then, the gold nanoparticle seeds were prepared. The gold nanoprism was obtained by three-step growth of seeds. 1 mL of seeds solution was added to solution containing 0.25 mL of 10 mM $HAuCl_4$, 0.05 mL of 100 mM NaOH, 0.05 mL of 100 mM ascorbic acid, and the mixed solution was gently shaken. Then, 1 mL of mixed solution was added to 9 mL of CTAB prepared solution containing 50 μM NaI under shaking. The resulting solution was added to solution including 2.5 mL of 10 mM $HAuCl_4$, 0.50 mL of 100 mM NaOH, 0.50 mL of 100 mM ascorbic acid, and 90 mL of 0.05 M CTAB prepared solution. Finally, the gold nanoprism was obtained when the color of solution changed from clear to deep magenta-purple.

Prior to the modification, the GCE was polished successively with 0.3 and 0.05 mm alumina slurry to obtained mirror like surface, and rinsed with doubly distilled water, followed by sonication in 1:1 ethanol and deionized distilled water. Then, the GCE was allowed to dry in a stream of nitrogen. 200 μL gold nanoprism solution and 1.0 mL 0.5 wt% chitosan solution (containing 2.0 mg mL^{-1} GOD) were mixed to form GOD–AuNP–chitosan mixed solution. Subsequently, 6 μL of the mixture was dropped on the pretreated GCE surface. Then, the modified electrodes were dried at 4 °C in a refrigerator.

3. Results and discussion

3.1. Characterization of the AuNP and GOD–AuNP–chitosan

The morphology and microstructure of the as-synthesized AuNP were first investigated by SEM in Fig. 1A. Meanwhile, these nanoparticles were further characterized by transmission electron microscope (TEM), are shown in Fig. 1B and C, which exhibits an uniform prism morphology with about 150 nm side length. The size distribution can be estimated by dynamic light scattering (DLS) studies. The spectrum shows the presence of two distinct peaks (Fig. 1D). The peaks at 13 nm and 155 nm are attributed to thickness and side length of the AuNP structure, respectively [24]. These measurements agreed with TEM results.

The UV–vis absorption spectra of the AuNP–chitosan, native GOD and GOD–AuNP–chitosan are shown in Fig. 2. From curve a, the surface plasmon resonance due to gold could be seen at about 526 nm [23]. The UV–visible spectrum of GOD (curve b) exhibited two well-defined absorption peaks at 380 and 455 nm which are attributed to the oxidized form of the flavin groups present in GOD [25]. The two peaks for GOD in GOD–AuNP–chitosan composite (curve c) are almost the same as those for native GOD, indicating that the original structural confirmation and native structure of GOD has not been altered during the immobilization process.

Fig. 2B shows FTIR spectra of the AuNP–chitosan, native GOD and GOD–AuNP–chitosan. The adsorption peak of chitosan at 1058 cm^{-1} (curve a) is attributed to frame symmetric and asymmetric flexible vibrations, and peak at 1411 cm^{-1} is assigned to CH_2 bending vibration [26]. The two characteristic peaks of native GOD are displayed at 1640 and 1544 cm^{-1}, which are ascribed to amide I and II bands of GOD