Journal of Electroanalytical Chemistry 726 (2014) 112–118

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

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

A new third-generation biosensor for superoxide anion based on dendritic gold nanostructure

Liang Wu, Xiaohua Zhang, Jinhua Chen *

State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, PR China

ARTICLE INFO

Article history:
Received 21 March 2014
Received in revised form 15 May 2014
Accepted 17 May 2014
Available online 27 May 2014

Keywords:
Electrodeposition
Dendritic gold nanostructure
Superoxide anion
Superoxide dismutase
Biosensor

ABSTRACT

In this paper, a facile electrodeposition method without any additive has been successfully adopted to fabricate dendritic gold nanostructure (DenAu) on glassy carbon (GC) electrode and the prepared DenAu/GC electrode has large surface area. A sensitive third-generation superoxide radical ($O_2^{\cdot-}$) biosensor was then constructed by assembled L-cysteine (cys) onto the DenAu/GC electrode to immobilize large amounts of superoxide dismutase (SOD). The direct electron transfer of SOD was successfully realized and a pair of quasi-reversible redox peaks of SOD was observed at the SOD/cys/DenAu/GC electrode in the phosphate buffer solution (25 mM, pH 7.2). Due to the high loading of SOD on the electrode, the resulted biosensor exhibited good analytical performance for $O_2^{\cdot-}$ detection, such as a low detection limit of 2.1 nM, a good stability and reproducibility, especially a wide linear range up to 540 μM.

1. Introduction

Superoxide anion ($O_2^{\cdot-}$), the primary one of the reactive oxygen species (ROS), is generated during various parts in biological systems. It is closely related to the etiology of aging, cancer, and progressive neurodegenerative diseases [1,2]. Therefore, developing the facile and sensitive $O_2^{\cdot-}$ sensors has received increasing attention. Over the past years, many technologies for the detection of $O_2^{\cdot-}$ have been demonstrated, such as chemiluminescence [3], fluorometry [4], and electron spin resonance [5]. Among them, electrochemical methods have been widely used because of their direct, real-time measurements and capability for in vivo detection [6]. It is well known that superoxide dismutase (SOD) is a redox enzyme that has a highly uncommon specificity to $O_2^{\cdot-}$ [7,8]. Due to its ability to efficiently catalyze the dismutation of $O_2^{\cdot-}$ to O_2 and H_2O_2 via a cyclic oxidation-reduction electron-transfer mechanism, SOD offers great potential for the sensitive and selective analysis of $O_2^{\cdot-}$ in electrochemical biosensors [9–12]. The first-generation $O_2^{\cdot-}$ electrochemical biosensors have been constructed based on the detection of H_2O_2 produced from $O_2^{\cdot-}$ dismutation catalyzed by SOD [13]. However, the high potential required for the oxidation of H_2O_2 may cause the simultaneous oxidation of some coexisting electroactive species in biological samples. Meanwhile, the biosensors based on the detection of H_2O_2 seem to be limited in differentiation of H_2O_2 produced by the SOD-catalyzed dismutation of $O_2^{\cdot-}$ from that endogenously produced in the biological systems [14]. The second-generation $O_2^{\cdot-}$ biosensors are based on the electron transfer of SOD realized by redox mediators [15]. However, it is not convenient to add mediators into biosensing systems [16]. Recently, many efforts have been made to the third-generation $O_2^{\cdot-}$ biosensors which is based on the direct electron transfer of SOD without any mediator [17,18].

In the presence of the so-called electron transfer promoters, a reversible redox response of bovine and human Cu/Zn-SOD has been observed on gold electrode [19,20]. Then, some sensitive SOD-based third-generation amperometric biosensors for $O_2^{\cdot-}$ have been developed with good linearity in the nanomolar range and low detection limit [21,22]. For example, Wang et al. prepared $O_2^{\cdot-}$ biosensors based on three kinds of SOD (Cu/Zn-SOD, Fe-SOD, Mn-SOD) modified gold electrodes via 3-mercaptopropionic acid (MPA) with an identical linear range of 13–130 nM min^{-1} [10]. However, from the view of in vivo applications, requirement of wide dynamic linear range, not only in nanomolar concentration but also up to micromolar and even millimolar grade concentration of $O_2^{\cdot-}$, is still a severe challenge. It is well-known that, under normal physiological conditions, $O_2^{\cdot-}$ is in a rather low physiological concentration (10–100 nM). However, in response to traumatic brain injury ischemia-reperfusion, hypoxia, and environmental stresses, the concentration of $O_2^{\cdot-}$ may increase to 0.1 mM [6]. Thus, it is essential to provide quantitative information on $O_2^{\cdot-}$ concentration ranging from the nanomolar to millimolar level for tracking the role that $O_2^{\cdot-}$ plays in physiological and pathological processes.

* Corresponding author. Tel.: +86 731 88821961; fax: +86 731 88821848.
E-mail address: chenjinhua@hnu.edu.cn (J. Chen).

http://dx.doi.org/10.1016/j.jelechem.2014.05.016
1572-6657/

To achieve the sensitive detection of $O_2^{\cdot-}$ with wide linear range, suitable electrode materials with excellent biocompatibility and large surface area for high loading of electroactive SOD should be developed. Due to their unique properties, gold nanostructure has attracted much attention in the past years [23]. Many strategies have been developed to prepare Au nanostructure with special size, shape, and crystal faces [24]. Among them, electrodeposition method has become one of the mostly used approaches because of its convenience, simplification, and easy control. Wang and co-workers prepared a hierarchical and homogeneously dispersed flowerlike gold microstructure by three-step electrodeposition on self-doped polyaniline nanofibers modified glassy carbon (GC) electrode [25]. Choi et al. fabricated a 3-D nanoporous gold thin film on gold substrate through electrodeposition by adding polyethylene glycol (PEG) into the deposition solution [26].

Here, a dendritic gold film was prepared on the GC electrode by a very simple one-step galvanostatic electrodeposition strategy without any additive and used to immobilize large amounts of SOD. Due to the good electronic conductivity, excellent biocompatibility, and the large active surface area of the dendritic gold film, the resulted third-generation electrochemical $O_2^{\cdot-}$ biosensor based on SOD exhibited excellent analytical performance to $O_2^{\cdot-}$, such as the high sensitivity, low detection limit, good stability and reproducibility, especially a wide linear range which is suitable for the determination of the high concentration of $O_2^{\cdot-}$ at some abnormal physiological conditions. And these intrinsic characteristics should sufficiently make the SOD/cys/DenAu/GC electrode very potential for in vivo analysis in future.

2. Experimental

2.1. Reagents and apparatus

Hydrogen tetrachloroaurate (III) ($HAuCl_4$) trihydrate (99.9%) was obtained from Sigma Aldrich and used without any purification. Superoxide dismutase (Cu/Zn-SOD, 2360 U mg^{-1}) was purchased from Bio Basic Inc. and used as received. Phosphate buffer solution (PBS, 25 mM, pH 7.2) was prepared by 7 mM K_2HPO_4, 18 mM KH_2PO_4 and 0.1 M KCl solutions. SOD stock solution of 2 mg mL^{-1} was prepared with PBS and stored at 4 °C. L-Cysteine (cys) was supplied by Bio Basic Inc. and its aqueous solution was freshly prepared and deoxygenated by bubbling pure nitrogen for at least 30 min prior to use. The chemical generation of $O_2^{\cdot-}$ was performed by dissolving KO_2 in DMSO solution. The stock solution of KO_2 was prepared by adding KO_2 to dimethyl sulphoxide (DMSO) which stored together with 4 Å molecular sieves, sonicating the solution for 5 min, and then adding 4 Å molecular sieves to further remove trace H_2O. The concentration of $O_2^{\cdot-}$ was determined by recording the reduction of ferricytochrome c spectrophotometrically using a LabTech UV-2100 spectrometer and using the extinction coefficient (21.1 mM^{-1} cm^{-1}) of ferrocytochrome c at 550 nm [12]. All other chemicals were of analytical grade. Aqueous solutions used throughout were prepared with ultra pure water obtained from Millipore system (>18 MΩ cm).

The morphology of the DenAu/GC electrode was investigated by field emission scanning electron microscopy (FESEM, JSM 6700, Japan). All electrochemical measurements were performed on a CHI 660B Electrochemical Workstation (Chenhua Instrument Company of Shanghai, China). A conventional three-electrode cell was used with a GC electrode (diameter = 3 mm) as the working electrode, a platinum wire as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode. Except the specific statement, the electrochemical measurements were carried out in PBS (25 mM, pH 7.2) at room temperature (25 ± 2 °C). The amperometric experiments were carried out in a gentle stirred, nitrogen-saturated 25 mM PBS at the applied potential.

2.2. Preparation of the DenAu/GC electrode

GC electrode was carefully polished to a mirror-like surface with 0.5 and 0.05 μm alumina slurries, followed by rinsing thoroughly with ultra pure water and dried in nitrogen atmosphere. The electrodeposition of DenAu on the surface of the pretreated GC electrode was conducted at −1.0 mA for different time (300, 500, 700 s) in the solution of 5 mM $HAuCl_4$ and 1 M H_2SO_4 with gentle stirring. The obtained electrode was then taken out of the solution and thoroughly rinsed with the ultra pure water.

2.3. Fabrication of the $O_2^{\cdot-}$ biosensor

By dipping the DenAu/GC electrode into a 1.5 mM deoxygenated cys aqueous solution for 30 min, cys was immobilized on the surface of the DenAu/GC electrode by Au–S bond. Then the obtained cys/DenAu/GC electrode was rinsed with ultrapure water to remove the non-specific adsorbed cys. After that, the cys/DenAu/GC electrode was incubated in 2 mg mL^{-1} SOD solution for 12 h at 4 °C to fabricate the SOD/cys/DenAu/GC electrode. The prepared electrode was rinsed thoroughly with ultrapure water and stored in 25 mM PBS (pH 7.2) at 4 °C while not being used.

3. Results and discussion

3.1. Characterization of the DenAu/GC electrode

Schematic representation of the fabrication procedure of $O_2^{\cdot-}$ biosensor is shown in Fig. 1. The DenAu modified electrode was firstly prepared by a simple electrochemical deposition strategy in a mixture solution of $HAuCl_4$ and H_2SO_4 without any additive and then used as the substrate to form a self-assembled monolayer (SAM) of cys for the immobilization of large amounts of SOD. In order to obtain a porous DenAu nanostructure with huge surface area, the deposition current was set at −1.0 mA where a large amount of hydrogen bubbles were evolved.

Fig. 2A–C shows the morphology of the DenAu with different deposition time of 300 (A), 500 (B) and 700 s (C). With 300 s, the electrode surface was observed to be occupied by nonuniform and scattered irregular nanoparticles (Fig. 2A). This could be attributed to the formed hydrogen gas bubbles which templated the growth of gold nanoparticles. When the deposition time was prolonged to 500 s (Fig. 2B), the simple and unconspicuous dendritic structure could be observed. However, there still were some naked regions on the electrode surface. With 700 s, it could be observed that the surface of the GC electrode was covered by large and symmetrical dendritic Au structure (Fig. 2C). The formation of the DenAu experienced a growth of the nanoparticle to a 3D porous dendritic nanostructure. And the hydrogen bubbles, which were evolved during the whole electrodeposition process, acted as the dynamic template to assist the orientation growth of gold nanocrystal. However, when the deposition time was longer than 700 s, the film of DenAu was too thick and pealed off from the electrode surface. EDS spectrum of the modified electrode with the deposition time of 700 s (Fig. 2D) indicates the electrode is only composed of C and Au elements, which further proves the surface of the DenAu modified electrode is very clean.

Fig. 3 shows the characteristic current–potential (I–E) curves of (a) the bare gold electrode (diameter = 2 mm), and (b–d) the DenAu modified GC electrodes with different deposition time of 300, 500, 700 s. The values of the real surface area (S) of the electrodes could be evaluated from the amounts of charge consumed