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Hydrogen peroxide biosensor based on microperoxidase-11 immobilized in a silica cavity array electrode

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ABSTRACT

Hydrogen peroxide biosensor based on the silica cavity array modified indium-doped tin oxide (ITO) electrode was constructed. An array of silica microcavities was fabricated by electrodeposition using the assembled polystyrene particles as template. Due to the resistance gradient of the silica cavity structure, the silica cavity exhibits a confinement effect on the electrochemical reactions, making the electrode function as an array of "soft" microelectrodes. The covalently immobilized microperoxidase-11(MP-11) inside these SiO_2 cavities can keep its physiological activities, the electron transfer between the MP-11 and electrode was investigated through electrochemical method. The cyclic voltammetric curve shows a quasi-reversible electrochemical redox behavior with a pair of well-defined redox peaks, the cathodic and anodic peaks are located at -0.26 and -0.15 V. Furthermore, the modified electrode exhibits high electrocatalytic activity toward the reduction of hydrogen peroxide and also shows good analytical performance for the amperometric detection of H_2O_2 with a linear range from 2×10^{-6} to 6×10^{-4} M. The good reproducibility and long-term stability of this novel electrode not only offer an opportunity for the detection of H_2O_2 in low concentration, but also provide a platform to construct various biosensors based on many other enzymes.

1. Introduction

The electrical contact between redox proteins and the electrode has attracted much interest, not only in fundamental studies about charge transfer properties of the redox proteins but also for further development of sensitive biosensors and bioelectronics devices [1–6]. Direct electron transfer (DET) is fundamental in fabricating a biosensor with high sensitivity and fast response. To achieve this, the redox protein must be immobilized on the electrode surface. Directly immobilizing a redox enzyme on the surface of a metal electrode or a metal nanoparticles modified (in particular gold nanoparticles) electrode are widely believed to be the most effective ways for enhancing the electron transfer (ET) between the enzyme and electrodes [7]. For example, microperoxidase-11(MP-11), as a hydrolytic digestion product of cytochrome *c*, consists of eleven amino acids with a covalently linked Fe III-protoporphyrin IX heme site [8]. Owing to the small size and relatively unshielded heme group, MP-11 could be the best candidate to serve as the core of a biosensor. However, MP-11 has been shown, in previous studies, to be electrochemically inactive at the bare gold electrode [9]. Consequently, over the past two decades, great efforts have been devoted towards the development of immobilization procedures to promote DET and bioelectrocatalytic activity. The binding of redox enzymes to the conducting nanostructures is accomplished through several methods, such as chemisorption via thiol groups, electrostatic adsorption by modifiers, or coupling by bifunctional reagents to surface functionalities [10–14] Lötzbeyer and Schuhmann pioneered the immobilization of MP-11 onto the gold surface [15,16]. They demonstrated that direct and fast electron transfer could occur when MP-11 was covalently immobilized on the cystamine modified gold electrode, which was also confirmed by Jiang et al. using 3-mercaptopropionic acid to bind MP-11 [17]. In these papers, the self-assembled monolayers (SAMs) technique was utilized to immobilize enzymes via a covalent link to sulphur-containing compounds chemisorpted on a conductive substrate. With the usage of these "flexible" mediators (owing to the easiness of preparation and the possibility of changing the alkyl chain length and the functional terminated group) [18], small molecules (usually O_2, H_2O_2) in the electrolyte solution can diffuse in and react with the active centre of the enzyme immobilized on the organosuphur molecule and then react with the electrode surface; thus, ET between the enzyme and the electrode is achieved [19]. In addition, previous researches have revealed that the distances between electrodes can be greatly reduced when electrochemical reactions occur in a very small volume, resulting in the overlap or coupling of two diffusion layers in the electrodes; therefore, high signal intensities and optimal signal-to-noise ratios can be

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achieved under ambient analyte conditions [20–22]. Thus, it is reasonable to expect that if MP-11 is placed into a micro or nanoreactors to fabricate a kind of microelectrode (at least microcathode), we might obtain a novel biosensor with fast response and high sensitivity.

Microreactors provide a microenvironment for chemical reactions to occur inside it and acts as a microdevice for material and energy conversion [23], which gives us an approach that enables a chemical reaction to be studied on a molecular level. There are several methods for fabricating micro or nanodevices, such as lithographic methods, nano-imprinting and scanning probe microscope (SPM) writing techniques [24,25]. However, the relatively high cost of these facilities is beyond the purchasing power of most laboratories. Compared to lithographic methods and SPM writing processes, template-based methods are time-saving approaches with low equipment cost for preparing surface nanostructures [26]. A general method for fabricating bowl-shaped nanovoid architectures was introduced by Velev et al. [27,28], and has been well-developed by the same group and many other groups [24,29–34]. With highly ordered monodispersed nanoparticles (usually silica and PS particles) as a template, functional materials can be deposited into the interspace of the template particles. Cavities that are opened at both the top and bottom are obtained after the template is removed, with the same shape and size as the template. To a certain extent, microelectrodes can be fabricated on the condition that the micro or nanovoids were arranged on a conductive substance; the nanobowls may then be used as ultrasmall containers for holding fluids at nanoscale volumes. The species in the solution are provided direct access, through the open pore and exposed bottom, to the underlying surface.

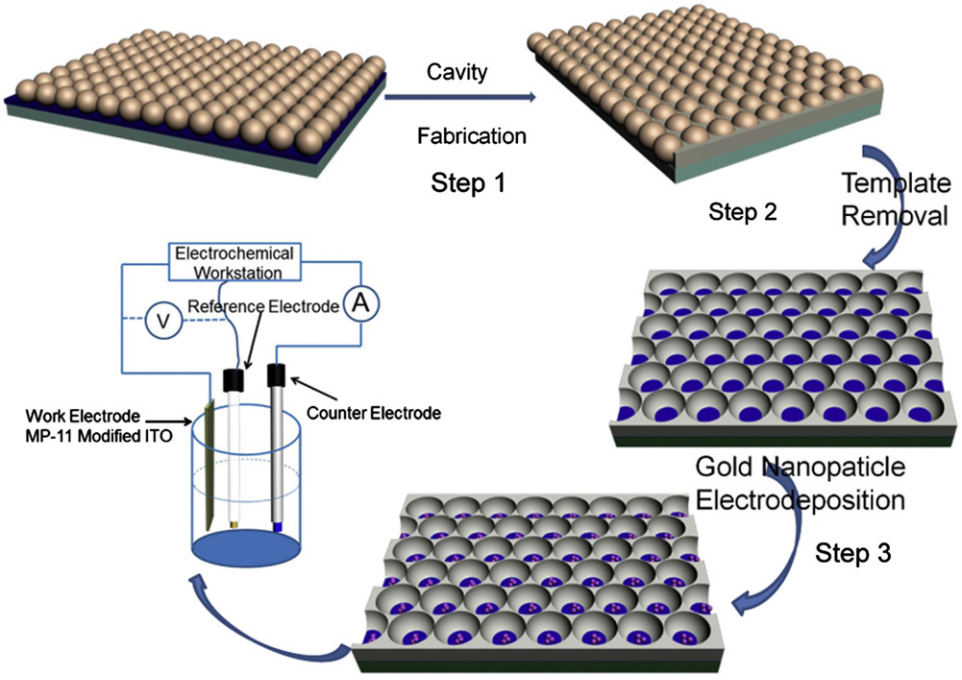
In this paper, we report a novel hydrogen peroxide biosensor based on a silica cavity array electrode. A two dimension array of hemispherical silica cavities, opened at top and bottom, was fabricated on the surface of ITO electrodes using 2 μm diameter polystyrene (PS) as the template, and followed by the electrochemical deposition of gold nanoparticles (GNPs) at the bottom of the cavities. A monolayer of 4-mercaptobenzoic acid (MBA) was then attached to the GNPs through S–Au bond, and acted as a mediator to covalently immobilize MP-11. Scanning electron micrographs (SEM), electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) were employed to characterize and delineate the electrochemical performance of this novel electrode. It was found that the SiO_2 cavity provided a favorable microenvironment for MP-11 to achieve its direct electron transfer and to maintain its electrocatalytic activities. The electrocatalytic performance of the modified electrode to hydrogen peroxide was also investigated.

2. Experimental section

2.1. Materials and apparatus

Microperoxidase-11 was purchased from Sigma-Aldrich, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), *N*-hydroxysucinimide (NHS), 4-mercaptobenzoic acid and polyvinylpyridine (PVP), tetramethoxysilane (TMOS) were purchased from J&K (Japan). A fresh solution of H_2O_2 was prepared before being used. The supporting electrolyte was 0.1 M KH_2PO_4/K_2HPO_4 phosphate buffers solution (pH 6.0–8.0). Polystyrene (PS) particles were synthesized by a dispersion polymerization of styrene in ethanol–water mixtures according to literature [35]. Ultrapure water used in washings and all buffer solutions preparations was produced in Millipore-*Q* system.

The SEM were observed on a Hitachi S4700 (Japan) field emission scanning electron microscope. Cyclic voltammograms (CV) and electrochemical impedance spectroscopy (EIS) were recorded on a CHI 660D electrochemical station (Shanghai Chen-Hua Instruments CO LTD., China). A bare or modified ITO electrode was employed as the working electrode. In order to maintain the constant surface area of the working electrode, the surface of the electrode was sheltered by a sticker, only a circular portion of 0.5 cm diameter was exposed to the solution. A platinum and an Ag|AgCl (saturated KCl) electrode were used as the auxiliary and reference electrodes, respectively. All electrochemical measurements were conducted in argon-purged (at least 30 min and kept under an argon atmosphere during whole course) solution and at room temperature.



Scheme 1. Schematic illustration of the procedures for fabricating the MP-11 modified electrode and the conventional three-electrode system..