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Direct electron transfer and electrocatalysis of microperoxidase immobilized on nanohybrid film

Ying Liu, Mingkui Wang, Feng Zhao, Zhihui Guo, Hongjun Chen, Shaojun Dong *

State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Changchun, Jilin 130022, Graduate School of the Chinese Academy of Sciences, Beijing 100039, China

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Abstract

The nanohybrid film (NHF) consisted of multi-wall carbon nanotubes (MWTNs) with attached gold nanoparticles was used to immobilize protein. The NHF provides a favorable microenvironment for microperoxidase (MP-11) to perform direct electron transfer (DET) at glassy carbon electrode. The NHF and MP-11 attached covalently were confirmed by transmission electron microscopy, atomic force microscopy and X-ray photoelectron spectroscopy. MP-11 immobilized on NHF surface exhibits a surface-controlled quasi-reversible cyclic voltammetric behavior. The electrochemical parameters such as apparent heterogeneous electron rate constant (k), formal potential ($E^{0'}$) and the influence of pH on $E^{0'}$ in the process were estimated by cyclic voltammetry. The MP-11 immobilized on the NHF retains its bioelectrocatalytic activity to the reduction of H_2O_2. The apparent Michaelis–Menten constant (K_m) and stability of the H_2O_2 response were investigated. Moreover, it can catalyze O_2 through four-electron to water in pH 7.0 buffer solution. The kinetic constants for O_2 reduction (k_s, αn_α) were obtained. The NHF to immobilize protein with DET shows potential applicability of nanofabrication of biosensors and biofuel cells.

Keywords: Microperoxidase-11; Carbon nanotubes; Gold nanoparticles; Electron transfer; Electrocatalysis; Nanohybrid film

1. Introduction

The direct electron transfer (DET) reaction of proteins has been attracted more and more interests. It can be applied to the study of physiological electron transfer processes and enzyme-catalyzed reaction in biological systems. And so further development of biosensors, biofuel cells and other bioelectrocatalytic reactors can be provided. Carbon nanotubes (CNTs) with electrocatalytic properties and small size can facilitate electron transfer between enzymes and other biomolecules and electrode [1–3]. The versatile biosensors with improved performances have been developed by immobilizing biomolecules on CNTs [4–12]. The single-wall carbon nanotubes (SWNTs) and multi-wall carbon nanotubes (MWNTs) are the two types of CNTs [13]. MWNTs have good communication with redox proteins and fast electron transfer kinetics [14]. They make redox active center of proteins be close to the surface of proteins [1,15–18]. However, It is difficult to perform the adhering of biomolecules on CNTs owing to CNTs hydrophobic properties. Indeed, proteins have been realized DET [1] in solution or immobilizing on the functionalized CNTs [10] at the modified electrode by CNTs. To our knowledge, only few biomolecules, as streptavidin [10] and DNA [19,20], were observed to adsorb on MWNTs via hydrophobic and nonspecific interaction.

The metal nanoparticles (MNPs) show the properties binding with a wide range of biomolecules and chemical

* Corresponding author. Tel.: +86 431 5262101; fax: +86 431 5689711.
E-mail address: dongsj@ns.ciac.jl.cn (S. Dong).

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ligands. Proteins immobilized on the surface of MNPs with well-remained bioactivity have been extensively studied [21,22]. The metal colloidal film was often used as an interface of redox-active monolayer [23–26]. Gold nanoparticles (GNPs) can provide an environment more similar to the native environment of proteins than that of other MNPs. They help the proteins adopt a favorable orientation as they are binding with the electrode surface and may develop conducting channels between enzyme and electrode surface [27–30], thus improving the electron transfer rate between the enzymes and electrode. Recently, our groups have demonstrated that GNPs can provide the capability for DET of proteins [31–34]. Microperoxidase-11 (MP-11), is the heme-containing polypeptide of cytochrome c and its DET and electrocatalysis have been studied previously [35,36]. MP-11 monolayer on Au electrode and the function of reduction H_2O_2 were reported by Katz and Willner [37]. They also developed the superstructures with three-dimensional and multi-layer-arrays consisting of GNPs and MP-11 [38]. But owing to the poor film-forming of GNPs, it is necessary to provide a matrix to produce GNPs film.

Here, we use MWNTs as the matrix to attach GNPs, which form a novel nanohybrid film (NHF). The DET and bioelectrocatalytic reduction to hydrogen peroxide and oxygen of MP-11 immobilized on the NHF surface were obtained.

2. Materials and methods

2.1. Materials

MP-11, cysteamine (2-mercaptoethanethiol), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbo-diimide hydrochloride (EDC) were purchased from Sigma and used as received. 4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid sodium salt (Hepes, pH 7.5) was obtained from Aldrich and used without further purification. MWNTs (95%, 20–50 nm) purchased from Shenzhen Nanotech. Port. Co., Ltd. (Shenzhen, China) were purified with HNO_3. A fresh solution of H_2O_2 was prepared daily. All other chemicals were of analytical grade. 0.1 M phosphate buffer solutions (PBS, pH 7.0), which were made from Na_2HPO_4 and NaH_2PO_4, were always employed as supporting electrolyte except in the pH-dependent experiments. Pure water was used throughout, which was obtained using a Millipore-Q water purification apparatus with resistivity over 18 MΩ cm.

2.2. Preparation of GNPs

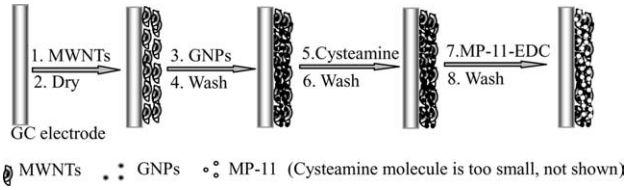
All glassware used in the following procedures were cleaned in a bath of freshly prepared 3:1 HCl:HNO_3 (aqua regia) and rinsed thoroughly in water prior to use. Au colloid (3–4 nm) was prepared according to the literature [39], and confirmed by transmission electron microscopy (TEM) (data not shown here).

2.3. Preparation of MP-11/GNPs/MWNTs/glassy carbon (GC) electrode

One milligram of MWNTs was dispersed with the aid of ultrasonic agitation in 10 mL of dimethylformamide (DMF) to give a 0.1 mg mL^{-1} black suspension. The GC electrode (3 mm in diameter) was polished with 1.0, 0.3 and 0.05 μm alumina slurry sequentially and then washed ultrasonically in water and ethanol for a few minutes, respectively. Firstly, the cleaned GC electrode was coated by casting 15 μL of MWNTs suspension and dried under an infrared lamp. Secondly, the MWNTs/GC electrode was immersed in Au colloidal solution for 10 h at 4 °C. The modified GC was thoroughly rinsed with water to remove weakly adhered GNPs on MWNTs surface. Thirdly, the modified electrode was immersed in 20 mM of cysteamine in ethanol for 3 h at 4 °C. Again, the electrode was rinsed exhaustively with plenty of water to remove physically adsorbed cysteamine. Finally, about 0.3 mM of MP-11 was dissolved in 0.01 M Hepes buffer with 10 mM EDC. The cysteamine-modified electrode was immersed into this solution for 3 h at 4 °C. Then MP-11 was immobilized onto the surface of GNPs/MWNTs/GC electrode. The fabrication of the MP-11/GNPs/MWNTs/GC electrode is sketched in Scheme 1.

2.4. Measurements

Cyclic voltammetry (CV) and amperometry measurements were carried out at a CHI 660B electrochemical workstation (CH Instruments, USA) with a conventional three-electrode cell. The modified electrodes were as the working electrode. Coiled platinum wire and Ag/AgCl (saturated KCl) electrode were used as the counter electrode and the reference electrode, respectively. The PBS were purged with high-purity nitrogen for at least 30 min prior to experiments and a nitrogen environment was then kept over the solution in the cell. All experiments were performed at room temperature.



Scheme 1. Illustrations of preparation procedures of MP-11/GNPs/MWNTs/GC electrode.