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Short communication

Direct electrochemistry and electrocatalysis of myoglobin covalently immobilized in mesopores cellular foams

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ABSTRACT

Myoglobin (Mb) was firstly covalently immobilized into mesopores cellular foams (MCFs) to fabricate the protein electrode (Mb–MCFs/GC electrode) and study the direct electrochemistry of redox protein. The results of UV–vis and FTIR spectra illustrated that the covalently immobilized Mb well retained its native structure and presented good stability. Cyclic voltammetry of Mb–MCFs/GC electrode showed nearly reversible cyclic voltammetric peaks, indicating the direct electron transfer of Mb–Fe^{III}/Fe^{II}. In addition, Mb–MCFs/GC electrode exhibited favorable electrocatalytic reduction to H_2O_2 with high sensitivity, good thermal and long-time stability. Such an avenue, which integrated mesopores cellular foams and redox protein via a simple covalent method, may provide a novel and efficient platform for the fabrication of the third generation biosensor.

1. Introduction

Direct electrochemistry of redox protein has attracted much attention because it not only provides a way to investigate the dynamic and thermodynamic mechanism of the protein reactions but also provides the base to fabricate the third generation biosensors (Deng et al., 2008). However, the realization of the direct electron transfer was not easy for redox protein at bare electrode (Zhang et al., 2007a). It has been proved that choosing suitable matrix and feasible immobilization method for supporting protein could efficiently enhance the direct electron transfer of protein (Zhang et al., 2007b).

So far, polymer, sol–gel, mesoporous silica and inorganic layered materials, etc. have been applied for construction of the third generation biosensor (Zhang and Li, 2004). Among these matrices, mesoporous silica is a promising one because it not only possesses the high specific surface area, good mechanical, chemical and thermal stability, but also provides protective effect for the immobilized biomolecules from disturbance of the environmental variations (Zhang et al., 2007a). In earlier studies, mesoporous silica such as hexagonal mesoporous silica (HMS) with small pore size (4.04 nm) was mainly used (Dai et al., 2004). However, due to the restriction of pore size, only relatively small protein could be effectively immobilized inside the mesoporous channels (Yiu and Wright, 2005). With the development of synthetic technique, more kinds of mesoporous silica have been used in direct electrochemical study, especially those with large pore size, such as SBA-15 (Liu et al., 2007), BMS (Zhang et al., 2007a) and mesopores cellular foams (MCFs) (Li et al., 2009; Zhang et al., 2005, 2007). Among these mesoporous silica, MCFs is one of the most attractive candidates for electrode materials because of its unique structure, such as opened structure and ultra-large mesopores. The large pore size made immobilized protein with high loading and provided low mass-transfer resistance of biosensors. Up to now, physical absorption technique is the main method for construction of mesoporous silicate based protein electrode to study the direct electrochemistry. Although such method is simple, it tends to yield an unstable biosensor because of the inevitable desorption of protein (Yiu and Wright, 2005). In order to overcome the fatal drawback of lack of stability, the method of covalent immobilization of protein into mesoporous silica is preferred (Liu et al., 2008). Though the covalent immobilization method could avoid the leaching of protein from mesoporous matrix, it is also known that the preliminary surface functionalized step of the covalent process often tend to reduce the mesopore opening, thus may further limit the mass-transfer. To overcome such disadvantage, it would be better if covalent method could be used to immobilize protein when mesoporous silica with large mesopores such as MCFs was chose as the matrix. However, to our knowledge, the great potential advantage of mesoporous silica

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with large pore size for covalent immobilization of protein in the field of direct electrochemistry has not yet been studied.

Herein, myoglobin (Mb) was taken as the model of redox protein to study the direct electrochemistry. Mb was covalent linking into MCFs and the immobilized Mb in such matrix could realize reversible direct electron transfer. The resulting electrode displayed linear current responses to H_2O_2 over the concentration range of 3.5–245.0 μM with high sensitivity and long-time stability. In addition, thermal stability of the immobilized Mb was enhanced.

2. Experimental

2.1. Reagents

Myoglobin from equine skeletal muscle (Mb, MW 17,600) was purchased from Sigma Chemical Co. (USA). All other reagents were of the highest grade available used. Deionized double-distilled water was used for making all the solutions (18 MΩ cm^{-1}).

2.2. Synthesis and functionalization of MCFs

MCFs were synthesized by using microemulsion templating in acidic solutions as previously described (Schmidt-Winkel et al., 2000). For post-synthesis functionalization of MCF materials, 1.0 g of calcined MCFs and 5 mL 3-aminopropyltrimethoxysilane were suspended in 30 mL of dry toluene under reflux for 24 h at N_2 atmosphere. The resulting white solid was filtered off, washed with dry toluene and acetone three times and dried under vacuum to obtain the amino-functionalized MCFs (denoted as AFMCFs). To synthesize aldehyde-functionalized MCFs, 0.5 g AFMCFs were added into 20 mL glutaraldehyde solution (2%) under ultrasonic condition for 0.5 h and then stood for 24 h at 4 °C. The resulting solid was then filtered off, washed with deionized water three times and dried under vacuum to obtain the aldehyde-functionalized MCF.

2.3. Preparation of Mb–MCFs composite by covalent binding and Mb/MCFs composite by physical adsorption

10 mg of aldehyde-functionalized MCFs was suspended in 5 mL of Mb solution (2 mg mL^{-1}, pH 6.5) under ultrasonic condition for 10 min and then stood for 24 h at 4 °C. The resulting composite was centrifuged, washed with phosphate buffer solution (PBS) (pH 6.5) three times and dried under room temperature to obtain the MCFs with covalently bonded Mb (denoted as Mb–MCFs). By the similar procedure, 10 mg of calcined MCFs was suspended in 5 mL of Mb solution (2 mg mL^{-1}, pH 6.5) to synthesize the composite of MCFs with absorbed Mb (denoted as Mb/MCFs).

2.4. Preparation of modified electrodes

Mb–MCFs/GC electrode was prepared via a simple casting method. Before the casting, glassy carbon (GC) electrode of 3.0 mm in diameter was polished with 1, 0.3, 0.05 μM alumina powder respectively before each experiment and sonicated in 1:1 nitric acid, acetone and double-distilled water successively. Then, the electrode was allowed to dry at room temperature. 3 mg Mb–MCFs was suspended in 1 mL of PBS (pH 6.5) and 5 μL of this suspension was deposited on the surface of the pretreated GC. It was then left to dry at room temperature. Nafion solution (5%, 10 μL) was then added for encapsulation to obtain the Mb–MCFs/GC electrode (Wang et al., 2009). For comparison, 5 μL of AFMCFs suspension (3 mg mL^{-1}, pH 6.5) was dropped on the surface of GC electrode in the similar way to obtain AFMCFs/GC electrode. These electrodes were then left to dry at 4 °C for overnight.

2.5. Apparatus

Electrochemical measurements were performed using a CHI 630 workstation (CH Instruments Inc., USA). The measurements were based on a three-electrode system with the as-prepared protein electrodes as the working electrodes, a platinum wire as the counter electrode, and a saturated Ag/AgCl electrode as the reference electrode. The buffer solution was purged with highly purified nitrogen for at least 30 min and a nitrogen atmosphere environment was kept during the whole electrochemical measurements.

UV–vis experiments were performed with UV-2100S spectrophotometer (Shimadzu). The FTIR spectra of samples in KBr pellets were recorded on a PerkinElmer instrument. The morphologies of samples were observed utilizing a JEOL 200CX transmission electron microscope (TEM) opened at an accelerating voltage of 50 kV. The pore characters of mesoporous materials were measured by ASAP-2010C adsorption meter.

3. Results and discussion

3.1. Characterization of MCFs and Mb–MCFs composite

As shown in Fig. 1A, MCFs materials possess disordered mesopores with diameters ranging from 25 to 34 nm, which is much larger than the size of Mb. The wall of MCFs was thin and its thickness is estimated to be 2.5–4 nm. Owing to the ultra-large mesopores size and opened pore structures, MCFs could provide sufficient volume for the immobilization of proteins.

The immobilization of Mb into MCFs was monitored by Nitrogen Sorption experiment. Fig. 1B shows the isotherm and pore size distributions curves for amino-functionalized MCFs (curve a) and Mb–MCFs (curve b), respectively. By comparison, the pore volume of Mb–MCFs decreased ca. 37.6% compared to that of

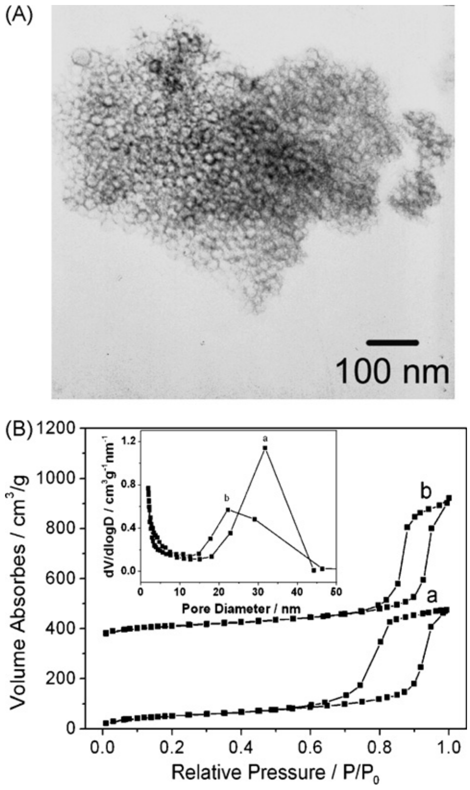

Fig. 1. TEM image for MCFs (A) and N_2 absorption isotherms (B) of AFMCFs (a) and Mb–MCFs (b). Inset of (B) for the pore size distribution of AFMCFs (a) and Mb–MCFs (b).