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A novel mesoporous silica nanosphere matrix for the immobilization of proteins and their applications as electrochemical biosensor

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ABSTRACT

A mesoporous silica nanoshpere (MSN) was proposed to modify glassy carbon electrode (GCE) for the immobilization of protein. Using glucose oxidase (GOD) as a model, direct electrochemistry of protein and biosensing at the MSN modified GCE was studied for the first time. The MNS had large surface area and offered a favorable microenvironment for facilitating the direct electron transfer between enzyme and electrode surface. Scanning electron microscopy, transmission electron microscopy, UV–vis spectroscopy and cyclic voltammetry were used to examine the interaction between GOD and the MSN matrix. The results demonstrated that the immobilized enzyme on the MSN retained its native structure and bioactivity. In addition, the electrochemical reaction showed a surface controlled, reversible two-proton and two-electron transfer process with the apparent electron transfer rate constant of 3.96 s^{-1}. The MNS-based glucose biosensor exhibited the two linear ranges of 0.04–2.0 mM and 2.0–4.8 mM, a high sensitivity of 14.5 mA M^{-1} cm^{-2} and a low detection limit of 0.02 mM at signal-to-noise of 3. The proposed biosensor showed excellent selectivity, good reproducibility, acceptable stability and could be successfully applied in the reagentless detection of glucose in real samples at −0.45 V. The work displayed that mesoporous silica nanosphere provided a promising approach for immobilizing proteins and fabrication of excellent biosensors.

1. Introduction

Since the discovery of MCM-41-type ordered mesoporous silica by Mobil corporation scientists in 1992, a large amount of research has been conducted on its controlled syntheses and applications [1]. Due to good biocompatibility [2,3], stable mesoporous structures, large surface areas, and tunable pore sizes and volumes, MCM-type mesoporous silica materials have made them ideal candidate for hosting molecules of various sizes, shapes and functionalities [4]. In particular, mesoporous silica nanoparticles have attracted great attention in many research fields over the past few years [5]. Great endeavors have been made for the use of mesoporous silica nanoparticles in controlled drug/gene release and as delivery carriers [6–11], biosensors [12,13], biomarkers [14,15] and enzyme supporters [3,16,17]. As a protein immobilizing matrix, mesoporous materials can incorporate proteins through physical or chemical action with good adsorption due to its large specific surface area [16]. The incorporation of mesoporous materials into redox enzymes could provide an active biomaterial [18]. Lin's group for the first time synthesized a novel mesoporous silica nanosphere (MSN), which was further used to construct a controlled-release delivery system for pharmaceutical drug molecules and neurotransmitters [4]. To the best of our knowledge, MSN has been not used for immobilization of proteins for applications in electrochemical biosensing.

The detection of blood glucose levels is of great importance for the diagnosis and therapy of diabetics. Glucose oxidase (GOD) as an ideal mode enzyme has been extensively used in fabrication of electrochemical glucose biosensors due to its catalytic ability to oxidize glucose [19–22]. However, an ultimate goal of glucose sensing is to develop the third generation biosensor based on the direct electron transfer (DET) between the cofactor FAD of GOD and the electrode surface without the mediator [23]. Unfortunately, the redox center in biomolecules is usually seated deeply in cavity of the enzyme molecules, which makes it hard to realize DET of enzyme to the electrode [24]. Thus, many novel nanomaterials, such as gold nanoparticles [25,26] carbon nanotubes [27–30], graphene [31,32], titanium oxide [33] and tin disulfide [34] have been explored to immobilize GOD for accelerating DET of redox enzyme on the surface of electrode.

In this work, MSN was synthesized according to previous method with some modifications. By immobilizing GOD on MNS modified electrode, a novel electrochemical biosensor was constructed. The MNS materials have an average particle size of 115 nm and an average pore diameter of 2.3 nm. The large surface area, uniform porous structure and favorable microenvironment

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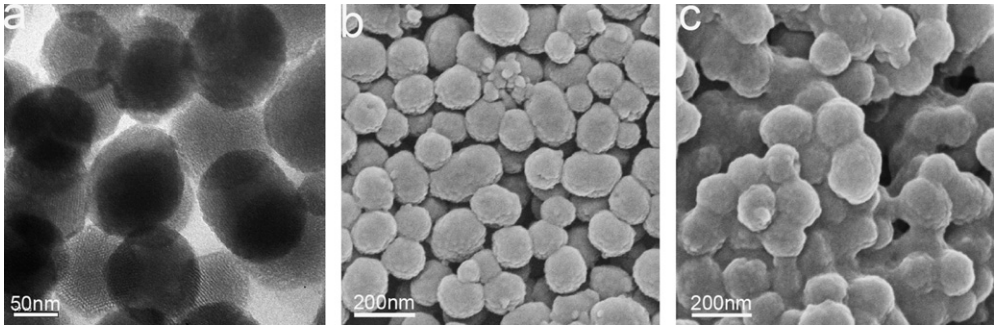

Fig. 1. (a) TEM image of MSN, (b) SEM images of MSN and (c) GOD–MSN.

facilitate the direct electron transfer between enzyme and electrode. A pair of obvious and well-defined redox peaks of GOD could be observed at the GOD–MSN/Nafion modified electrode. The resulting glucose biosensor showed high sensitivity, low detection limit, excellent selectivity and good reproducibility. Thus the mesoporous silica nanosphere provided a promising platform for immobilizing proteins for electrochemical biosensing.

2. Experimental

2.1. Reagents and materials

GOD (EC 1.1.3.4, 108 U mg^{-1}, from *Aspergillus niger*) was purchased from Amresco. D-(+)-Glucose and Nafion were purchased from Sigma-Aldrich. n-Cetyl-trimethylammonium bromide (CTAB) and tetramethoxysilane (TMOS) were supplied by Sinopharm Chemical Reagent Co., Ltd. A stock solution of D-glucose was prepared and allowed to mutarotate at room temperature for 24 h before use. Phosphate buffer solution (PBS) was 0.1 M Na_2HPO_4 and NaH_2PO_4 and its pH was adjusted with H_3PO_4 or NaOH solutions. All other chemicals and reagents are of analytical grade and were prepared using distilled water.

2.2. Apparatus

Electrochemical measurements were carried out on a CHI 852C electrochemical workstation (Co., CHI, USA). All experiments were performed with a three-electrode system using a glassy carbon electrode (GCE, Φ=3 mm) as the working electrode, a platinum wire as the auxiliary electrode and a saturated calomel electrode (SCE) as reference electrode. The cyclic voltammetry experiments were carried out at a scan rate of 100 mV s^{-1} in an electrochemical cell filled with 5.0 mL PBS. All pH measurements were made with S-25 digital pH-meter with glass combination electrode.

Transmission electron microscopy (TEM) images were obtained with a Philips Tecnai-12 transmission electron microscope (Holland) at an acceleration voltage of 100 kV. Scanning electron microscopy (SEM) images were obtained with a Hitachi S-4800 scanning electron microscope (Japan) at an acceleration voltage of 15 kV. UV–vis spectra were recorded using UV-2550 spectrophotometer (Shimadzu Co., Japan).

2.3. Synthesis of MCM-41-type mesoporous silica nanosphere

MCM-41-type mesoporous silica nanosphere was synthesized according to previous method with some modifications [4]. 0.2 g of CTAB was firstly dissolved in 96 mL of distilled water. 0.7 mL of NaOH (aqueous, 2.0 M) was added to CTAB solution, and then the solution temperature was adjusted to 80 °C. 1.0 mL of TMOS was added dropwise to the solution, and the mixture was allowed to stir for 2 h at 80 °C. Subsequently, the resulting white precipitate was isolated by filtration. After the silica particles were calcined at 550 °C for 5 h to remove the template, the mesoporous silica nanosphere was finally obtained.

2.4. Preparation of the GOD–MSN/Nafion/GCE

The GCE was firstly polished successively with 0.03 and 0.05 μm alumina slurry (Buhler) followed by rinsing thoroughly with distilled water, and finally sonicated in 1:1 nitric acid, acetone and distilled water and dried in air. 3.0 mg of MSN was dispersed in 1.0 mL of distilled water under ultrasonic stirring, and then 0.5 mL of the MSN suspension was mixed with equivalent volume of 8.0 mg mL^{-1} of GOD to form GOD–MSN mixed solution. Subsequently, 6.0 μL of the mixed solution was dropped on the surface of the pretreated GCE and dried in a desiccator. Finally, 5.0 μL of 0.5% Nafion solution was dropped on the surface of GOD–MSN/GCE to form GOD–MSN/Nafion modified electrode. The modified electrode was rinsed throughout with distilled water to wash away the loosely adsorbed enzyme molecules. The modified electrode was then stored in pH 7.0 PBS at 4 °C in a refrigerator before use.

3. Results and discussion

3.1. Characterizations of MSN, GOD and GOD–MSN

The TEM and SEM images of the synthesized mesoporous silica nanosphere shown in Fig. 1(a and b), indicate that MSN materials are composed of well-dispersed spherical nanoparticles with average diameter of 115 nm. The pore characterization of MSN was investigated by the N_2 isothermal adsorption experiments. The specific surface area, pore volume and average pore diameter were calculated to be 645.3 $m^2 g^{-1}$, 0.43 $cm^3 g^{-1}$ and 2.7 nm using the BET and BJH method, respectively. As can be seen from the TEM image, ordered mesopores and parallel channels appear. The uniform porous structure is advantageous to obtain a high enzyme loading. When GOD was immobilized in the MSN matrix, SEM image of the formed GOD–MSN (Fig. 1c) displayed uniform and homogeneous morphology.

The UV–vis absorption spectra of the MSN, native GOD and GOD–MSN were shown in Fig. 2. For the single MNS, no obvious peak was observed in the wavelength range of 200–700 nm, while the spectrum of GOD showed three characteristic absorption peaks. The absorption peak at 276 nm was ascribed to the characteristic of polypeptide chains, and the two weak peaks at 381 and 458 nm were attributed to the oxidized form of flavin group in protein structure [35]. The position and shape of absorption peaks of the GOD–MSN are almost the same as those