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Direct electrochemistry of horseradish peroxidase on TiO_2 nanotube arrays via seeded-growth synthesis

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ABSTRACT

Horseradish peroxidase (HRP) was successfully immobilized on vertically oriented TiO_2 nanotube arrays (NTAs), which was prepared by a seeded-growth mechanism. The nanotubular structure of TiO_2 was characterized by scanning electron microscope (SEM). After encapsulated HRP on TiO_2 nanotube arrays, the direct electron transfer of HRP was observed. Owing to the redox reaction of electroactive center of HRP, the HRP/TiO_2 NTAs modified electrode exhibited a pair of quasi-reversible peaks with the peak-to-peak separation of 70 mV and the formal potential of −0.122 V (vs. SCE) in 0.2 mol L^{-1} phosphate buffer solution (PBS, pH 7.0). The number of transference electron was 0.84 and the direct electron transfer (ET) constant (k_s) was 3.82 s^{-1}. The HRP/TiO_2 NTAs modified electrode displayed an excellent electrocatalytic performance for H_2O_2 and the formal Michaelis–Menten constant (K_m^{app}) was 1.9 mmol L^{-1}. The response currents had a good linear relation with the concentration of H_2O_2 from 5.0×10^{-7} mol L^{-1} to 1.0×10^{-5} mol L^{-1} and 5.0×10^{-5} mol L^{-1} to 1.0×10^{-3} mol L^{-1}, respectively.

1. Introduction

Tubular inorganic nanostructures have attracted a great deal of attention in both scientific and technological studies (Iijima and Ichihashi, 1993; Hueso and Mathur, 2004). They offer great potential for use in fields such as electronics, optics, advanced catalysis, energy storage/conversion, molecular filtration, and tissue engineering (Wang, 2000; Yang and Sheng, 2000). Furthermore, their tubular structures have prompted research into the physical and chemical properties of molecules confined in their inner nanospaces and eventually may lead to the mimicking of biological channels (Jung et al., 2002). Over the past several years specific TiO_2 nanostructures have become a focus of considerable interest due to the favorable physical, optical, and electrical properties (Hagfeldt and Gratzel, 2000; Varghese et al., 2003; Bavykin et al., 2006). TiO_2 nanotubes, in particular, nanotube arrays (NTAs) continue to be rigorously investigated due to the unique advantages, such as the increase in effective internal surface area without a concomitant decrease in geometric and structural order, the ability to influence the absorption and propagation of light by precisely designing and controlling the geometrical parameters of the architecture and the attractive electron percolation pathways for vectorial charge transfer between interfaces (Prakasam et al., 2007; Paulose et al., 2006; Yoriya et al., 2007). Owing to the specific characteristics, TiO_2 NTAs have demonstrated their utility in a variety of applications including room-temperature hydrogen gas sensing (Varghese et al., 2003), generation of hydrogen by water photoelectrolysis (Mor et al., 2005), photocatalyst (Albu et al., 2007), and dye-sensitized solar cells (Kang et al., 2007). In contrast to random nanoparticle systems, all of the applications indicate that the nanotube-array architecture offers excellent photocatalytic ability, dramatically improved charge transport properties and attractive electron percolation capability.

The open mesoporous morphology of TiO_2 NTAs, the high specific surface area, and the presence of micropores for reactant molecules facilitate transport of reagents to the active sites during the catalytic reaction. In addition, their chemical stability, nontoxicity, and excellent charge transfer properties make TiO_2 NTAs become a potential ideal material for biomacromolecule immobilization and biosensor fabrication. For example, a bioelectrocatalytic system based on glucose oxidase functionalized TiO_2 NTAs by anodic oxidation of Ti foil was developed for H_2O_2 and glucose measurement (Xie et al., 2007). Random titanate ($H_2Ti_3O_7$) nanotubes (TNT) system was proven to be an efficient platform for the immobilization of myoglobin (Mb) and the direct electrochemistry of Mb on TNT film was obtained due to the aqueous-like

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environment provided by the hydrophilic TNT surfaces (Liu et al., 2005). A H_2O_2 sensor was designed by coadsorption of HRP with thionine on TiO_2 NTAs by anodizing Ti sheets in a dilute HF solution (Liu and Chen, 2005). However, the direct electron transfer of HRP was not observed at ordered TiO_2 NTAs/Ti electrode, which was contrary to the free dispersed TNT modified electrode. It might ascribe to the obstructive action of barrier layer formed between Ti substrate and TiO_2 NTAs during the anodization process.

In order to make sure the barrier layer is the restricted factor for the direct electron transfer of HRP or not, we seek for new methods to prepare TiO_2 NTAs. Except for anodic oxidation (Prakasam et al., 2007; Paulose et al., 2006; Yoriya et al., 2007), hydrothermal synthesis (Bavykin et al., 2004; Chen et al., 2004) and template synthesis (Hoyer, 1996; Li et al., 2005) have also been used to prepare nanometer-sized TiO_2 tubules. Recently, Tian and coworkers developed a one-step, templateless method to directly prepare TiO_2-based nanotubes arrays (Tian et al., 2003). In this synthesis, the nanotubes were prepared by seeded with TiO_2 nanoparticles and barrier layer could not be formed during the process of TiO_2 NTAs anchored on Ti substrate. Therefore, the seeded-growth mechanism was used to prepare TiO_2 NTAs without barrier layer and biomarcomolecule, HRP, was successfully encapsulated into the NTAs for bioelectrochemical study. We found that the bioactivity of HRP was well retained and the direct electron transfer was also observed at the TiO_2 NTAs electrode for the first time. It suggests that the method for NTAs preparation is a crucial step for the direct electrochemistry of biomolecules. Moreover, the direct bioelectrochemistry of HRP immobilized on TiO_2 NTAs provided a new strategy for the third generation biosensor fabrication.

2. Experimental

2.1. Chemicals and apparatus

Horseradish peroxidase (HRP, 298 U/mg, 44,000) and Ti foil (>99.99%, 1.0 cm × 1.0 cm × 0.1 cm) were purchased from Sigma. P25 (TiO_2 powder) was provided by Degussa. Other chemicals were of analytical grade and used without further purification. Phosphate buffer solution (PBS, 0.2 mol L^{-1}) with various pH values was prepared by mixing calculated $Na_2HPO_4{\cdot}12H_2O$ and KH_2PO_4. All solutions were made up with twice-distilled water.

Scanning electron microscope (SEM) images were obtained on a SEM (S-4800, HITACHI, Japan). Electrochemical measurements were performed at a CHI660c electrochemical workstation (Shanghai Chenghua Instruments Company, China). The electrochemical cell was a three-electrode system where the TiO_2 NTAs modified electrode was used as the working electrode, gold wire electrode as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode.

2.2. Preparation of TiO_2 NTAs

TiO_2 NTAs were prepared according to Tian et al. (2003) in a Teflon reactor (Jiangsu Zhenghong Plastics Company, China). In a typical preparation, 1.0 g of P25 was dispersed in 20 mL twice-distilled water ultrasonically. After 5 min ultrasonic, the TiO_2 suspension was centrifuged for 1 min at 1000 rpm to remove coarse particles and aggregates. By dipping Ti foil into the suspension, the TiO_2 nanoparticles were successfully coated onto the surface of Ti. Thereafter, the Ti foil with nanoparticles was reacted with 10 mL of 10 mol L^{-1} NaOH solution in a sealed Teflon reactor at 150 °C for 6 h. After that, the Ti foil with newly formed film was removed from the reactor, washed with twice-distilled water and dried in air.

2.3. HRP immobilization

HRP was deposited onto TiO_2 NTAs electrode by soaking electrode in the enzyme solution of 1000 U/mL for 12 h (pH 7.0). During this period, HRP was adsorbed onto TiO_2 NTAs because the former was positively charged (isoelectric point is 8.9) (Zhou et al., 2002) and the latter was negatively charged as a result of the fact that the pH was over the isoelectric point (pH ~ 5.9) (Liu et al., 2007). After encapsulation, electrode was washed with twice-distilled water and kept in pH 7.0 PBS at 4 °C.

2.4. Electrochemical measurements

All experimental solutions were de-aerated by bubbling pure nitrogen for 20 min, and a nitrogen atmosphere was kept over the solution during measurements. All electrochemical measurements were performed at room temperature (23 ± 2 °C).

3. Results and discussion

3.1. Characterization of TiO_2 NTAs

Scanning electron microscope is used to characterize formation of TiO_2 nanotubes. After dipped Ti foil into the TiO_2 suspension, the TiO_2 nanoparticles is successfully coated on the surface of Ti substrate (Fig. 1a) and the diameters of nanoparticles are about 30–50 nm. These nanoparticles are used as the nucleation sites to grow extended nanostructures. After reaction with NaOH in sealed Teflon reactor at 150 °C for 3 h, the SEM image is shown in Fig. 1b. At this stage, nanoporous titania surfaces are obtained due to the growth of TiO_2 from the nucleation sites. Fig. 1c is an illustrative FESEM top-surface image of a nanotube arrays sample, obtained by reaction with NaOH at 150 °C for 6 h. The diameters of TiO_2 nanotube range from 30 nm to 150 nm. Fig. 1d displays the cross-sectional image of NTAs, the vertical alignment of the nanotubes is clearly observed and the inner diameter is fairly uniform. It indicates that TiO_2 NTAs based on seeded-growth mechanism can be well prepared by controlling the reaction time.

3.2. Direct electron transfer of HRP at TiO_2 NTAs electrode

After modification procedure, the HRP/TiO_2 NTAs electrode is characterized by cyclic voltammetry. Fig. 2A shows the cyclic voltammograms of TiO_2 NTAs (a) and HRP/TiO_2 NTAs (b) electrode in pH 7.0 PBS at 100 mV s^{-1}. As for TiO_2 NTAs electrode, there is no peak observed at the electrochemical window. However, a couple of redox peaks appear for HRP/TiO_2 NTAs electrode with cathodic peak at −157 mV and the corresponding anodic peak at −87 mV, which results from the direct electron transfer between the HRP and the underlying electrode. The peak-to-peak separation of 70 mV and the cathodic current (I_{pc}) almost equal to the anodic peak current (I_{pa}) suggest that HRP immobilized on TiO_2 NTAs undergoes a quasi-reversible electrochemical reaction. The electrochemical results indicate that HRP has been successfully immobilized on the TiO_2 NTAs and its electrochemical activity is well retained. Integration of CV reduction peak of HRP gives the amount of reduction charge (Q) passed through the electrode, and the surface concentration of electroactive HRP (Γ^*) is about 2.5×10^{-11} mol cm^{-2}, which is obtained according to the Q–Γ^* relationship (Murray, 1986). The total amount of adsorbed HRP (Γ) is about 2.1×10^{-10} mol cm^{-2}, indicating electroactive HRP on the TiO_2 NTAs is about 12% of the total proteins deposited on the electrodes. Liu and coworkers have demonstrated that the direct electron transfer of Mb immobilized on TNT is due to the hydroxyl group, the surface charge, and the tubular morphology of TNT (Liu et al., 2005). However, no redox