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Amperometric determination of H_2O_2 at nano-TiO_2/DNA/thionin nanocomposite modified electrode

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ABSTRACT

We report electrochemical preparation and characterization of a new biosensor made of nanostructured titanium dioxide (nano-TiO_2) particles and deoxyribonucleic acid (DNA). Thionin (TN) redox mediator was electrochemically deposited onto DNA/nano-TiO_2 modified glassy carbon electrode (GCE). The X-ray diffraction analysis, atomic force microscope (AFM) and scanning electron microscope (SEM) were used for surface analysis of TN/DNA/nano-TiO_2 film. In neutral buffer solution, TN/DNA/nano-TiO_2/GCE biosensor exhibited excellent electrocatalytic activity towards the reduction of hydrogen peroxide (H_2O_2) and oxygen (O_2). The biosensor shows excellent analytical performance for amperometric determination of H_2O_2, at reduced overpotential (−0.2 V). The detection limit and liner calibration range were found to be 0.05 mM (S/N = 3) and 0.05–22.3 mM, respectively. In addition, determination of H_2O_2 in real samples was carried out using the new biosensor with satisfactory results. The TN/DNA/nano-TiO_2/GCE showed stable and reproducible analytical performance towards the reduction of H_2O_2. This biosensor can be used as an amperometric biosensor for the determination of H_2O_2 in real samples.

1. Introduction

Metal oxides are emerging as important materials because of their versatile properties such as high-temperature superconductivity, ferroelectricity, ferromagnetism, piezoelectricity and semiconductivity. Recently, nanostructured TiO_2 particles preparation and their applications in photovoltaic studies, photocatalysis and environmental studies (water and air purification) have attracted much attention among the researchers [1–6]. The emerging sensor technology based on nanoparticles (NPs) and nanocomposites with chemical and biological molecules is much beneficial for direct and real applications. For example, the TiO_2–oligonucleotide nanocomposites not only retain the intrinsic photocatalytic capacity of TiO_2 and the bioactivity of the oligonucleotide DNA, but also possess the chemically and biologically unique new property of a light-inducible nucleic acid endonuclease, which could became a new tool for gene therapy [7]. Recent advances in hybrid nanotechnology involving nucleic acids are predominantly linked with sequence-specific nucleic acid interactions, and are oriented towards cellular imaging or DNA microarray development [8–11].

Recently, the anti-17β-estradiol antibody was immobilized on polyelectrolyte polyacrylic acid-modified TiO_2 nanoparticles for water treatment [12], sonogel carbon electrode modified with nanostructured TiO_2 for catechol detection [13], a nano-TiO_2 film/nafion modified on a glassy carbon electrode (GCE) used for investigation of dopamine [14], nano-TiO_2 and hemoglobin were co-modified on pyrolytic graphite electrode to study the photovoltaic effect of TiO_2 nanoparticles [15,16] and carbon electrode modified with TiO_2/metal nanoparticles for the detection of trinitrotoluene [17] have been reported.

The DNA has been used for the immobilization of protein molecules [18–22]. DNA can enhance electron transfer between electrode and heme proteins in myoglobin–DNA films [23]. Hemoglobin on DNA/poly-2,6-pyridinediamine modified Au electrode for detection of H_2O_2 [24], DNA/poly(*p*-aminobenzensulfonic acid) bi-layer modified GCE for detection of dopamine and uric acid [25], electrochemical behavior of neomycin at DNA-modified gold electrodes are investigated [26]. An electrochemically deposited thin film of DNA is suitable platform for fabrication of biosensors [27–29].

In the present work, we report a novel nanocomposite biosensor for the detection of H_2O_2 based on thionin incorporated bi-layer of DNA/nano-TiO_2 film modified electrode. X-ray diffraction pattern, atomic force microscope (AFM) and scanning electron microscope (SEM) have been employed for surface characterizations of TN/DNA/nano-TiO_2 films modified electrode. Cyclic

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voltammetry and amperometry have been used for investigation of electrochemical properties of nanocomposite modified electrode. Using amperometry, linear range and detection limit of H_2O_2 were explored. In addition, we report electrocatalytic reduction of oxygen (O_2) using TN/DNA/nano-TiO_2 coated electrode.

2. Experimental

2.1. Materials and apparatus

Deoxyribonucleic acid sodium salt (17.5 A_{260} units/mg), thionin dye (dye content >95%) and titanium oxosulfate $TiOSO_4$ were purchased from Sigma–Aldrich (St. Louis, MO, USA). H_2O_2 (30%, w/w), potassium ferrocyanide, sulfuric acid (H_2SO_4, assay 95%), nitric acid (HNO_3, assay 60%) and sodium hydroxide (purity 93%) were purchased from Wako pure chemicals (Osaka, Japan). Potassium nitrate, sodium acetate and sodium dihydrogen phosphate were received from E-Merck (Darmstadt, Germany) and other chemicals were of analytical grade and used without further purification. Double-distilled water was used in all experiments. Diluted H_2O_2 standard solutions were freshly prepared directly prior to use. The commercial antiseptic and contact lenses cleaning H_2O_2 solutions were purchased from a local drug store in Taipei.

Electrochemical measurements were performed by a CHI750A Electrochemical Work Station (CH Instrument Inc., USA). Glassy carbon electrode from BAS (West Lafayette, USA) and indium tin oxide-coated glass (ITO) electrodes were purchased from Merck Display Technologies, Ltd. (Darmstadt, Germany). ITO thickness and resistance were 30 ± 10 nm and 80 Ω, respectively. Size of the glass: 300 mm × 350 mm × 0.7 mm. ITO or GCE are used as working electrodes. ITO substrates were cleaned by using detergent, diluted nitric acid and then finally rinsed with distilled water. Platinum wire is used as the counter electrode. All the cell potentials were measured with respect to an Ag/AgCl [KCl (sat)] reference electrode. Amperometry measurements for H_2O_2 were performed on a Bi-potentiostat Model CHI750A (TX, USA) having an analytical rotator model AFMSRK with MSRX speed control (PINE Instruments, USA). Hitachi scientific instruments (London, UK) model S-3000H scanning electron microscope was used for surface image measurements. The AFM images were recorded with a multimode scanning probe microscope system operated in tapping mode using Being Nano-Instruments CSPM-4000, Ben Yuan Ltd. (Beijing, China). Electrochemical impedance measurements were performed using impedance measurement unit, IM6ex ZAHNER, Messsysteme (Kroanch, Germany). All experiments were carried out at room temperature.

2.2. Electrochemical synthesis of TiO_2

Electrochemical synthesis of TiO_2 nanoparticles were carried out onto ITO electrode from the bath solution containing 0.02 M $TiOSO_4$, 0.03 M H_2O_2, 0.05 M HNO_3 and 0.05–0.25 M KNO_3 (pH 1.4). The deposition was performed at room temperature (25 ± 2 °C) under potentiostatic conditions (−2.0 V vs. Ag/AgCl). This led to the formation of a white colored gel film on the electrode surface. Each deposition has been conducted for 30 min. For the preparation of multiple TiO_2 layers electrosynthesis was repeated three or four times, with drying steps at 150 °C in between, after which the final annealing step takes place at 400 °C for 1 h to obtain crystalline TiO_2 film. The substrates were weighed prior to coating and after annealing to determine the amount of deposited TiO_2. Nearly 20% mass reduction was observed after heat-treatment at 400 °C for 1 h, due to water elimination from the film [30]. Thereafter, crystalline TiO_2 particles were scratched from the ITO surface and collected in 10 mL brown colored vial and later used for modification of GCE.

Cathodic electro-deposition of TiO_2 film from $TiOSO_4 + H_2O_2 + HNO_3 + KNO_3$ (pH 1.4) solutions involves the indirect deposition of a gel of hydrous titanium oxo-hydrides (Eq. (3)), resulting from the reaction of titanium peroxo-sulfate Eq. (2) with hydroxide ions produced by nitrate electrochemical reduction [30–32].

$$NO_3^- + H_2O + 2e^- \rightarrow NO_2^- + 2OH^- \quad (1)$$

$$TiOSO_4 + H_2O_2 \rightarrow Ti(O_2)SO_4 + H_2O \quad (2)$$

$$Ti(O_2)SO_4 + 2OH^- + (x+1)H_2O \rightarrow TiO(OH)_2{\cdot}xH_2O_2 + SO_4^{2-} \quad (3)$$

Annealing of the gel at 400°C for an hour, results in the formation of crystalline TiO_2.

$$TiO(OH)_2{\cdot}xH_2O \rightarrow TiO_2 + (x+1)H_2O. \quad (4)$$

2.3. Preparation of modified electrodes

2.5 mg of synthesized TiO_2 NPs was added into 5 mL double-distilled water and then ultrasonicated for 10 min to create a suspension with a concentration of 0.5 mg mL^{-1}. After being diluted five times, the mixture of 10 μL TiO_2 NPs suspension was spread evenly onto the surface of the well cleaned GCE which was dried for 6 h in the absence of light. Finally, the modified electrode was thoroughly rinsed with double-distilled water. The deposition of DNA layer was carried out under constant potential of +1.5 V for 30 min in 0.1 mg mL^{-1} DNA solution [25,33]. This electrode was described as DNA/nano-TiO_2/GCE. For comparison, the DNA deposition was made on a bare GCE to prepare a DNA modified GCE, denoted as DNA/GCE.

Consequently, DNA/nano-TiO_2/GCE electrode was cycled in 0.1 M phosphate buffer solution (PBS) containing 1×10^{-5} M thionin (between −0.45 and 0.2 V) for 20 cycles. Afterwards, the electrode was thoroughly rinsed with double-distilled water and then dried at 4 °C for an hour in the absences of light. When not in use, the electrode was stored in aqueous solution of 0.1 M PBS (pH 7.0) at 4 °C. It was named as TN/DNA/nano-TiO_2/GCE and then used for further studies. For comparison, TN/nano-TiO_2/GCE, TN/DNA/GCE and TN/GCE coated electrodes were prepared and used for further investigation.

3. Results and discussions

3.1. Electrochemical deposition of TN

Fig. 1 shows the consecutive cyclic voltammograms (CVs) of TN adsorption onto DNA/nano-TiO_2 bi-layer modified electrode in pH 7.0 PBS containing 1×10^{-5} M TN. The CVs of the electrochemical deposition of TN film onto a DNA/nano-TiO_2/GCE was characterized by the TN redox couple in the scanning potential region between 0.0 and −0.4 V. The continuous increase in anodic and cathodic peak currents of the TN redox couple indicated the surface deposition of TN molecules (Fig. 1). After the 20th cycle, TN adsorption reached saturation. According to the literature reports, DNA is negatively charged [24–26] which could be firmly attached onto biocompatible nano-TiO_2 layer [7]. The irreversible doping of TN occurs onto DNA/nano-TiO_2 layer due to the interaction between negatively charged DNA and the positively charged TN from its solution (pK_a 7.8) [34]. In this experiment, during the electrochemical deposition process, TN