Journal of Electroanalytical Chemistry 781 (2016) 377–382

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

journal homepage: www.elsevier.com/locate/jelechem

Photoelectrochemical biosensor for acetylcholinesterase activity study based on metal oxide semiconductor nanocomposites

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ARTICLE INFO

Article history:
Received 28 April 2016
Received in revised form 27 June 2016
Accepted 5 July 2016
Available online 6 July 2016

Keywords:
Photoelectrochemical biosensor
TiO_2 nanotubes
Nanocomposite
Acetylcholinesterase
Cadmium ion

ABSTRACT

Acetylcholinesterase (AChE) is a well-known serine hydrolase and its dysfunction could disturb cholinergic neurotransmission which is related to the pathogenesis of neurodegenerative disorders. In this report, a photoelectrochemical biosensor based on metal oxide semiconductor nanocomposite was fabricated to investigate the effect of cadmium ion (Cd^{2+}) on the activity of AChE. The photoelectrochemical nanocomposite was prepared by anodic oxidation of titanium (Ti) foil to form titanium dioxide (TiO_2) nanotubes (TNs) array followed by cathodic deposition of zinc oxide nanorods (ZnONRs) onto the TNs. AChE was immobilized on the obtained nanocomposites and the biosensor showed enhanced photoelectrochemical response under visible light irradiation. The experimental results showed that Cd^{2+} exhibited interesting dose-dependent and time-dependent effects on AChE activity. Specifically, high concentration of Cd^{2+} inhibited while low level of Cd^{2+} could stimulate the activity of AChE. These findings are of great significance for the study of enzyme activity influenced by metal ions and related pathogenesis investigation of neurodegenerative disorders.

1. Introduction

Photoelectrochemical biosensing is a burgeoning analytical technique endowed with high sensitivity because of the effective separation of excitation source from the detection signal of which potential or current is employed [1–3]. For this new technique, the most important basis is choosing the photoelectric materials with high photoelectric conversion property. Up to now, inorganic semiconductors, organic photoelectrical materials, and some biomolecules with photoelectrochemical activity have been used as photoelectric materials in biosensor development [4–6]. Titanium dioxide (TiO_2) nanomaterials have attracted much attention owing to its good photoelectric conversion efficiency, high specific surface area, abundant resource and nontoxicity. However, the band gap of 3.2 eV confines the excitation light in the UV region. Many modification methods, such as dye sensitization, metal and nonmetal atoms doping, and semiconductor coupling have been applied to improve the photoresponse of TiO_2 upon the visible light irradiation [7–9]. For example, the semiconductor coupling method increases the photoelectric response and stability of TiO_2 by suppressing the charge recombination [10]. Therefore, semiconductor coupling materials are promising for biosensor development with good photoelectrochemical performance.

Acetylcholinesterase (AChE) is a well-known serine hydrolase playing a critical role in acetylcholine (ACh)-mediated neurotransmission. The abnormal change of AChE activity such as inhibition of AChE could lead to accumulation of ACh in the synaptic cleft and result in disturbed neurotransmission, which is related to the pathogenesis of neurodegenerative disease [11–13]. Cadmium (Cd) is a particularly hazardous element because of its easy uptake by plants and high tendency to accumulate in food chain crops. Exposure to Cd via occupational exposure, diet, or tobacco is hazardous to human health characterized by kidney dysfunction and bone damage [14]. Besides that, it was found that Cd may have direct effect on the central nervous system related to Parkinson's disease (PD) and Alzheimer's disease (AD). For instance, Bungo Okuda's group reported a representative case of PD after acute Cd poisoning in clinic treatment [15]. Some animal experiments have also confirmed that the pathological change of substantial nigra in rat caused by Cd is similar to the clinical pathological change of PD [16–18]. In recent years, the researches about the effect of various metal ions on AChE activity have also drawn much interests [19,20]. Although conventional colorimetric methods are frequently used for the determination of AChE activity [21,22], photoelectrochemical biosensing by the introduction of semiconductor nanomaterials into biological research offers a new option for probing enzyme activity [23,24].

In this work, we prepared metal oxide semiconductor nanocomposites marked as ZnONRs/TNs which were composed of zinc oxide nanorods (ZnONRs) and titanium dioxide nanotube (TNs) arrays. Then, we used this nanocomposite to fabricate a photoelectrochemical biosensor

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http://dx.doi.org/10.1016/j.jelechem.2016.07.007
1572-6657/

AChE/ZnONRs/TNs by immobilizing AChE on the nanocomposite substrate. ZnONRs/TNs exhibited higher photoelectrochemical activity than pure TNs and the prepared biosensor also showed high affinity to acetylthiocholine with a low value of Michaelis-Menten constant (K_m) (K_m = 0.138 mM). The effects of various concentration of Cd^{2+} on AChE activity were studied using the photoelectrochemical biosensor. Experimental results demonstrated that Cd^{2+} displayed dose-dependent and time-dependent effects on AChE activity.

2. Experimental section

2.1. Chemicals and materials

AChE (type V-S, from electric eel, 1000 U/mg), acetylthiocholine (ATCh), and pieces of Ti foil (dimensions: 15 mm × 4 mm × 0.5 mm, >99% purity) were purchased from Sigma-Aldrich (St. Louis, MO, USA). $(NH_4)_2SO_4$, NH_4F, $Zn(NO_3)_2$, $H_2C_2O_4$ and chitosan (CS) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All other chemicals were of analytical grade. 0.1 M phosphate buffer solution (PBS) was prepared by using Na_2HPO_4 and KH_2PO_4. All solutions were prepared using Milli-Q (Millipore, Bedford, MA, USA) A-10 gradient (18 M Ω·cm) deionized water.

2.2. Apparatus

Electrochemical experiments were done at room temperature with a CHI 832 electrochemical system (CH Instruments, Austin, TX, USA). Scanning electron microscopic (SEM) images were recorded by a Hitachi S4800 (Hitachi, Japan). The energy dispersive X-ray spectroscopy was measured and recorded by EDX Genesis fitted to the Hitachi FE-SEM S4800. For verifying the formation of crystal structure of the samples such as TNs and ZnONRs/TNs, the X-ray diffraction (XRD) analysis was done by a Rigaku Model D/max2550 (XRD, Model D/max 2550 V, Rigaku Co., Japan) using a diffractometer with monochromatized Cu KR radiation (λ = 1.5418 Å), performed over angular ranges of 2θ from 20° to 90°, scanned at a speed of 0.01°/s and steps of 0.01°. The equipment was operated at 35 kV and 25 mA. Reflectance spectra was measured and recorded in the range from 200 to 800 nm by a Hitachi U-3900 spectrometer (Hitachi, Japan) with an integrating sphere and with $BaSO_4$ as a reference.

2.3. Preparation of biosensors

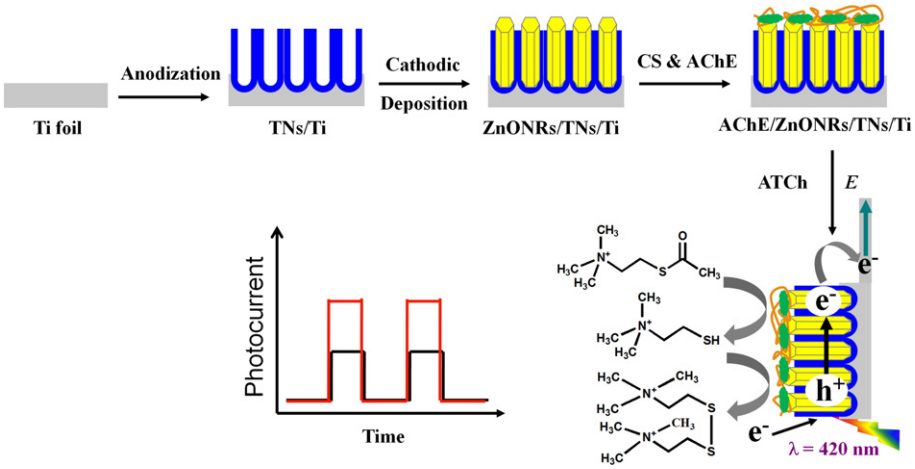
After being mechanically polished with abrasive papers of different fineness in sequence, Ti foils were rinsed in an ultrasonic bath of cold distilled water, acetone, and isopropyl alcohol for 10 min, respectively. Subsequently, these cleaned Ti foils were treated with the mixed solution (HF: HNO_3: H_2O = 1:4:5 in volume) for 1 min. Then Ti foils were again rinsed with acetone and deionized water for 10 min successively and dried under nitrogen (N_2) at room temperature. The highly ordered TiO_2 nanotube (TNs) array electrodes were prepared by a similar electrochemical anodic oxidation technique reported previously [25]. Briefly, the anodization process was carried out at room temperature using a direct current power supply (HuGuang Electrical Appliance Co., Shanghai) in a cylindrical electrochemical reactor (the radius is 30 mm and height is about 70 mm), with the pretreated Ti foil serving as the anode and carbon rod serving as the cathode. The electrolyte was 1 M $(NH_4)_2SO_4$ with the addition of small amounts of NH_4F (0.1 M) and oxalic acid ($H_2C_2O_4$, 1/12 M). A potential of 20 V and anodization time of 90 min was used in this study. Anodized substrates were immediately rinsed with deionized water and dried with a N_2 stream. Then, these substrates were annealed at 500 °C for 1 h to convert the amorphous phase to the anatase crystalline phase; both the heating and cooling rates were kept at 2.5 °C·min^{-1}.

For the preparation of ZnONRs/TNs, direct cathodic deposition of ZnONRs on TNs substrate was performed as follows. The TNs served as working electrode in a conventional three-electrode system, and a standard Ag/AgCl electrode and a platinum electrode served as the reference and counter electrodes, respectively. The electrolyte was prepared by dissolving 1 mM $Zn(NO_3)_2$ in deionized water. The cathodic deposition was performed at −1.0 V at 65 °C for 30 min. The obtained electrodes were annealed at 450 °C for 1 h; both the heating and cooling rates were kept at 2.5 °C·min^{-1}.

For the preparation of AChE/ZnONRs/TNs biosensor, enzyme solution (10 μL) containing 1.0 mg·mL^{-1} AChE and 5.0 mg·mL^{-1} chitosan was dropped on one side of the obtained ZnONRs/TNs substrates and allowed to dry in air at room temperature. The obtained biosensor was denoted as AChE/ZnONRs/TNs. For comparison, AChE/TNs biosensor was prepared using the same procedures except the loading of ZnONRs by cathodic deposition. Both the modified electrodes were stored at 4 °C in a refrigerator before use.

2.4. Photoelectrochemical determination

All the photoelectrochemical measurements were performed using a three-electrode system comprising the Ti foil as working electrode, a platinum wire as auxiliary electrode and saturated calomel electrode (SCE) as reference electrode. The photoelectrochemical responses were measured at a constant potential of 0.7 V with the illuminated monochromatic light (λ ≥ 420 nm) on and off in a 0.001 M PBS (pH = 7.0) containing ATCh. The PBS solution was deaerated by highly pure N_2 for 20 min before photoelectrochemical experiments and then



Scheme 1. Schematic illustration for the preparation of the AChE/ZnONRs/TNs biosensor and the photoelectrochemical response of ATCh.