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Dual signal amplification coupling dual inhibition effect for fabricating photoelectrochemical chlorpyrifos biosensor

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ABSTRACT

Photoelectrochemical (PEC) detection is an attractive analytical tool as it allows for an elegant and sensitive assay. However, designing a novel detection strategy to achieve an excellent PEC analytical performance is still highly challenging. Herein we design a novel photoelectrochemical (PEC) chlorpyrifos biosensor based on dual signal amplification strategy coupling dual inhibition effect. Dual signal amplification strategy was achieved by coupling the graphene quantum dots sensitized $CuFe_2O_4$ magnetic nanocrystal clusters (GQDs–$CuFe_2O_4$ MNCs) with the amplification of enzymolysis products. In this biosensing architecture, the GQDs–$CuFe_2O_4$ MNCs prepared by electrostatic adsorption showed nearly 4-fold and 30-fold enhancement photocurrent compared with the pure $CuFe_2O_4$ MNCs and GQDs, respectively. And the contact angle measurement demonstrated that the GQDs–$CuFe_2O_4$ MNCs exhibited good biocompatibility. Based on all these above advantages, the GQDs–$CuFe_2O_4$ MNCs were immobilized on the magnetic electrode surface by a fast and simple magnetism-assisted assembly, and the acetylcholinesterase (AChE) was further coated on the surface of the as-prepared multifunctional electrode. Due to thiocholine (enzymolysis products) acts as a sacrificial electron donor to scavenge the holes, compared with the GQDs–$CuFe_2O_4$ MNCs modified electrode in the acetylthiocholine chloride solution, the photocurrent of the resulting AChE-based biosensor was further significantly enhanced. Based on the dual inhibition of AChE activity by chlorpyrifos and the formation of Cu-chlorpyrifos complex hindered the electron transfer of $CuFe_2O_4$ MNCs toward the electrode surface, the proposed AChE-based biosensor can be applied to the quantification of chlorpyrifos with a linear range from 0.001 $\mu g\,mL^{-1}$ to 1 $\mu g\,mL^{-1}$ and a detection limit of 0.3 $ng\,mL^{-1}$ (S/N = 3). This novel dual signal amplification strategy opens up a new avenue for achieving high sensitivity in the field of PEC biosensing.

1. Introduction

Photoelectrochemical (PEC) sensors have aroused extensive interests in the fields of biology, medicine and environment monitoring [1–3], due to their advantages of simple operation, excellent accuracy, and low-cost [4–8]. Nowadays, to achieve an excellent PEC analytical performance, one major challenge is to amplify the detectable signal during the measurement. To realize this issue, various signal amplification strategies have been exploited. The strategies can be divided into two categories depending on the components of a PEC sensing system, a proper PEC transducer and an electron/hole donor. One of the signal amplification strategies is exploiting innovative PEC transducer and designing appropriate sensitized structures. Another is to get a more efficient electron donor which is produced by biocatalysis reaction chain. In a first aspect, the various PEC transducers, especially semiconductors-based transducers (such as TiO_2 [9–11], CdS [10,12,13], CdSe [14,15], BiOX [16,17], gold nanoclusters [18] and so on) have been investigated widely in PEC sensors due to their excellent PEC properties. Thus, searching for innovative nanomaterials with excellent PEC properties is obviously desirable to improve the performance of the PEC biosensors.

$CuFe_2O_4$ nanomaterial, as multifunctional magnetic material, has been widely applied in lithium-ion storage and photocatalysis degradation due to its electrical properties, low cost, high abundance and environmental friendliness [19,20]. Partic-

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ularly, $CuFe_2O_4$ possesses unique optical and narrow band gap (1.4 eV) [21,22], which is a promising candidate of the visible-light-responsive photocatalysts. However, the inherent poor conductivity [23,24] and the recombination of photo-generated electron-hole pairs [25] limited its potential application in the electrochemical field, especially PEC field. As an alternative strategy, appropriate doping with carbonaceous materials can improve the performances of $CuFe_2O_4$ nanomaterials, effectively. According to the previous reports, graphene quantum dots (GQDs) could be used as an enhanced material for improving the performance of pure nanomaterials by GQDs sensitization [26,27]. Thus, as a signal amplification strategy, the photoelectrical performance of $CuFe_2O_4$ could be improved by GQDs sensitization.

Besides exploiting new photoactive materials and designing appropriate sensitized structures, selecting an efficient electron donor is also crucial to signal amplification. Up to now, to amplify the signals, almost all recent works have resorted to the use of various enzymes as biocatalysts to produce the electron donor. Such as ascorbic acid (AA) is in situ generated by alkaline phosphatase hydrolyzing the substrate 2-phospho-L-ascorbic acid [10,12,28], and thiocholine is produced from the hydrolysis of acetylthiocholine catalyzed by acetylcholinesterase (AChE). Among kinds of electron donor species, AA and thiocholine have demonstrated themselves as an ideal candidate for signal amplification in PEC detection.

Chlorpyrifos, one of the most frequently used organophosphate pesticides, controls a broad spectrum of insects of economically important crops [29–31]. However, the use of this compound could elicit the inhibition of AChE and lead to the disruption of nerve function [31,32]. Thus, it is of extremely significance for the detection of chlorpyrifos in environmental samples and agricultural products. Herein, we developed a novel PEC biosensor based on dual signal amplification strategy by coupling the GQDs sensitized $CuFe_2O_4$ magnetic nanocrystal clusters ($CuFe_2O_4$ MNCs) with the amplification of enzymolysis products for the detection of chlorpyrifos. GQDs can effectively improve the PEC property of $CuFe_2O_4$ MNCs, and the PEC signal was further amplified by the enzymolysis products. The inhibition of AChE activity by chlorpyrifos prevented the production of thiocholine, and the formation of Cu-chlorpyrifos complex hindered the electron transfer of $CuFe_2O_4$ MNCs towards the electrode surface. The inhibitions above resulted in the obvious decrease of photocurrents. Based on the dual inhibitions, a proposed AChE-based biosensor can be applied to the detection of chlorpyrifos. This PEC biosensor showed good performance in chlorpyrifos monitoring with a rapid response and low detection limit.

2. Experimental

2.1. Reagents

Acetylcholinesterase (AChE, Type C3389, 500 U mg^{-1} from electric eel), acetylthiocholine chloride (ATCl) and carbaryl were purchased from Sigma–Aldrich (USA). Chlorpyrifos, carbaryl, parathion-methy and acetamiprid were purchased from Aladdin Chemistry Co. Ltd. Pentachlorophenol (PCP) and ethoprophos were purchased from J&K Scientific Ltd. Pyrolyzing citric acid (CA), $CuCl_2$, $FeCl_3{\cdot}6H_2O$, NaAc, polyethylene glycol 20 000 and ethylene glycol were purchased from Sinopharm Chemical Reagent Co., Ltd. PBS (0.1 M, pH 7.0) was used as the supporting electrolyte, which was prepared by mixing stock standard solutions of NaH_2PO_4 and Na_2HPO_4, and adjusted the pH with 0.1 M H_3PO_4 or NaOH solution. Other reagents were of analytical grade and used as received without further purification, and all solutions were prepared with twice-distilled water.

2.2. Apparatus

Zeta potentials were measured on a Malvern ZEN2600 Zetasizer Nano Z. Transmission electron microscopy (TEM) image was taken with a JEOL 2100 transmission electron microscopy (JEOL, Japan) operated at 200 kV. X-ray photoemission spectroscopy (XPS) was recorded on a VG MultiLab 2000 system with a monochromatic Mg-Kα source operated at 20 kV. X-ray diffraction (XRD) analysis was conducted on a Bruker D8 diffractometer with high-intensity Cu Kα (λ = 1.54 Å). UV–vis absorption spectra were measured by UV–2450 spectrophotometer (Shimadzu, Japan). The magnetization curves were obtained at room temperature on an LDJ 9600 (LDJ Electronics, Troy, MI) vibrating sample magnetometer (VSM), and the static water contact angles were measured with a commercial instrument (CAM 200, KSV Instruments Ltd., Helsinki, Finland).

All the electrochemical and PEC measurements were conducted using CHI660B electrochemical analyzer (Chen Hua Instruments, Shanghai, China) and recorded by a conventional three-electrode system, where a magnetic glassy carbon electrode (mGCE, 10 mm in diameter and 65 mm in length) with an inserted glassy carbon (3 mm in diameter and 2 mm in depth) worked as working electrode. The mGCE was purchased from Tianjin Incole Union Technology Co., Ltd. (Tianjin China), Ag/AgCl (saturated KCl solution) as reference electrode and platinum wire as counter electrode, respectively. The PEC measurement was performed in 0.1 M PBS at 0 V, and a 250 W Xe lamp (Beijing Trusttech Co. Ltd.) was used as the visible light source with an intensity (passing through a 400 nm UV-cut filter) of 100 mW cm^{-2}. Electrochemical impedance spectroscopy (EIS) was performed in a 0.1 M KCl solution containing 5 mM $Fe(CN)_6^{3-/4-}$ with a frequency range from 0.01 Hz to 10 kHz at 0.23 V, and the amplitude of the applied sine wave potential in each case was 5 mV.

2.3. Preparation of GQDs–$CuFe_2O_4$ MNCs

The GQDs were prepared by directly CA according to the previous literature [33]. The $CuFe_2O_4$ MNCs were prepared using a hydrothermal method with some modifications [34]. Briefly, $CuCl_2$ (0.34 g, 2.5 mmol) and $FeCl_3{\cdot}6H_2O$ (1.35 g, 5 mmol) were dissolved in ethylene glycol (40 mL) to form a clear solution, followed by the addition of NaAc (3.6 g) and polyethylene glycol 20 000 (1.0 g). The mixture was stirred vigorously for 30 min and then sealed in a teflonlined stainless-steel autoclave. The autoclave was heated to and maintained at 200 °C for 8 h and allowed to cool to room temperature. The black products were washed several times with ethanol and dried at 60 °C for 6 h.

The GQDs–$CuFe_2O_4$ MNCs were achieved as follows: 100 mg $CuFe_2O_4$ was dispersed into 50 mL above GQDs water solution (2 mg/mL). The final mixture was stirred for 30 min. At last, the precipitate was under magnetic separation and dried in vacuum at 45 °C for 24 h to obtain the GQDs–$CuFe_2O_4$ MNCs.

2.4. Fabrication of the modified electrodes

Prior to each modification, mGCE was first polished with polishing clothes and 1.0, 0.3, and 0.05 μm alumina slurry. After successive sonication in ethanol and water, the electrode was rinsed with water and allowed to dry at room temperature. 8 mg of the as-prepared GQDs–$CuFe_2O_4$ MNCs was dispersed in 200 mL water. After that, the pretreated mGCE was immersed shallowly in 3 mL of the above suspension nearly all the hybrid was assembled on the electrode surface in 5 min by magnetism without any fixing agent (labeled as GQDs–$CuFe_2O_4$ MNCs/mGCE). For comparison, pristine $CuFe_2O_4$ MNCs modified mGCE was also prepared in the same way (labeled as $CuFe_2O_4$ MNCs/mGCE). The GQDs of the same concentration were dropped on the surface of mGCE (labeled