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Short communication

Nanostructured photoelectrochemical biosensor for highly sensitive detection of organophosphorous pesticides

Xinyu Li [a,b], Zhaozhu Zheng [a,b], Xuefeng Liu [c], Shenlong Zhao [a], Shaoqin Liu [a,*]

[a] Key Laboratory of Microsystems and Microstructures Manufacturing, Ministry of Education, Harbin Institute of Technology, Harbin 150080, China
[b] School of Life Science and Technology, Harbin Institute of Technology, Harbin 150080, China
[c] National Center for Nanoscience and Technology, Beijing 100190, China

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ABSTRACT

A sensitive photoelectrochemical (PEC) biosensor for detection of organophosphorus pesticides (OPs) using the nanocomposite of CdSe@ZnS quantum dots (QDs) and graphene deposited on the ITO coated glass electrode as a photoactive electrode is presented. The integration of CdSe@ZnS/graphene nanocomposite with biomolecules acetylcholinesterase (AChE) as a biorecognition element yields a novel biosensing platform. Under visible light irradiation, the AChE–CdSe@ZnS/graphene nanocomposite can generate a stable photocurrent and the photocurrent is found to be inversely dependent on the concentration of OPs. Under the optimal experimental conditions, the photocurrents were proportional to the logarithm of paraoxon and dichlorvos within the concentration range of 10^{-12}–10^{-6} M. The detection limits (LOD) of the proposed biosensor for paraoxon and dichlorvos are as low as 10^{-14} M and 10^{-12} M. The photoelectrochemical biosensor shows good sensitivity, reproducibility, stability, and could be successfully applied to detection of OPs in real fruit samples.

1. Introduction

The analysis of organophosphates pesticides (OPs) residues is an important concern due to their high toxicity, as well as their ability to accumulate, mobility and long-term effects on living organisms (Patel, 2002; Walker and Asher, 2005). Well-established detection methods for OPs such as gas chromatography, high performance liquid chromatography, gas chromatography–mass spectrometry (GC–MS), and liquid chromatography–mass spectrometry (LC–MS), (Chen and Huang, 2006; Leandro et al., 2006; Rotiroti et al., 2005) offer high sensitivity and selectivity for accurate determination of OP pesticides. However, these methods are cost-intensive, time-consuming and often require well trained technicians and experts to run the analysis.

Compared with the conventional methods, photoelectrochemical (PEC) measurement has attracted considerable interests as a newly developed and promising analytical technique for biological assay (Brown et al., 1992; Chen et al., 2010; Cooper et al., 1998; Tokudome et al., 2005). Owing to the complete separation of excitation source (light) and detection signal (photocurrent), PEC measurements exhibit some advantages of both optical methods and electrochemical sensors, such as low background signals, high sensitivity, inherent miniaturization, portability and easy automation. Recently, the PEC sensing platform for OPs has been reported based on photoactive TiO_2 nanoparticles (Li et al., 2011b). However, current PEC sensing systems face at least three challenges: (1) The wide band gap of TiO_2 only allows it to absorb ultraviolet light ($\lambda < 400$ nm). To extend the photoresponse to the visible region, further modification of TiO_2 with organic dyes (Li et al., 2011a; Tu et al., 2010), inorganic semiconductor nanoparticles (Kang et al., 2010; Zhao et al., 2011) and noble metal nanoparticles (Zhu et al., 2009) has to be done. (2) The photocatalytic oxidation of OPs with TiO_2 gives rise to absence of selectivity. Therefore, how to realize the selectivity of a PEC sensor is an urgent problem to be overcome. Actually, coupling photoactive materials with enzyme (Gong et al., 2012) or molecular imprinted polymers (Li et al., 2013; Shi et al., 2011; Wang et al., 2013) is considered to be beneficial for increasing the selectivity of the photocatalyst. (3) The strong oxidizing power of the photogenerated holes upon illumination may cause the deactivation of biomolecules (Wen et al., 2010; Zou et al., 2001).

Owing to narrower band gap and strong absorption in the visible light region (Ellingson et al., 2005), semiconductor nanoparticles or quantum dots (QDs) are important materials for solar-energy conversion. Recently, the integration of QDs with DNA (Freeman et al., 2007; Gill et al., 2005; Willner et al., 2001) or proteins (Katz et al., 2006; Pardo-Yissar et al., 2003; Stoll et al., 2006; Yildiz et al., 2008) has been extensively studied for the

* Corresponding author. Tel.: +86 451 86403493; fax: +86 451 86403483.
E-mail address: shaoqinliu@hit.edu.cn (S. Liu).

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development of PEC sensing platforms (Gill et al., 2008). However, the photocurrent generated by QDs-biomolecule hybrids is quite low due to rapid electron–hole recombination. Many works demonstrate that the photoresponse of QDs can be efficiently improved with the presence of conductive polymers (Granot et al., 2004) or metallic nanoparticles (NPs) (Sheeney-Haj-Ichia et al., 2004; Subramanian et al., 2001) or semiconductor nanoparticles (Nasr et al., 1997; Sant and Kamat, 2002; Zaban et al., 2000), which practically act as electron traps to help separate the photogenerated charges and subsequently enhance interfacial charge transfer. Moreover, very high photoelectrical conversion efficiency was achieved when CdS nanoparticles were attached to carbon nanotube-modified gold electrodes (Sheeney-Haj-Ichia et al., 2005). It has been suggested that the conductive carbon nanotube is capable of providing efficient paths for the transport of conduction-band electrons to the electrode.

Graphene, a sp^2-bonded carbon sheet with a thickness of single atom and a tunable band gap, is a good candidate for the collection and transport of photogenerated charges (Guo et al., 2010; Wang et al., 2011; Zhou et al., 2009). Therefore, the organization of assemblies of graphene and semiconductor QDs on electrode might provide a novel structure for improved PEC functions. Herein, we report the assembly of graphene/CdSe@ZnS hybrid systems on indium-doped tin oxide (ITO) coated glass electrode surfaces. The system reveals efficient PEC functions. The graphene/CdSe@ZnS assemblies are further modified with acetylcholine esterase (AChE). The AChE in assemblies catalyzes the hydrolysis of acetylthiocholine to generate thiocholine, which acts as an electron donor for the photogenerated holes in the valence band of the CdSe@ZnS QDs and thus enhances the photocurrent. However, the enhanced photocurrent will be obviously decreased when the enzyme activity is inhibited by OPs. On the basis of the inhibition effect of OPs on the photocurrent of graphene/CdSe@ZnS/AChE, a rapid and reliable visible light-activated PEC approach is developed for detecting OPs. To our knowledge, this is one of the most sensitive PEC biosensors used for OPs detection till now.

2. Materials and methods

2.1. Reagents and materials

Cadmium oxide (CdO, 98.9%), selenium (99%, powder), sulfur (99.9%, powder), 1-octadecene (ODE, 90%), 2-mercaptoethylamine, mercaptopropionic acid (MPA, 99%), AChE (EC 3.1.1.7 from *Electrophorus electricus*, lyophilized, specific activity 435 U/mg), acetylthiocholine chloride, and poly(ethyleneimine) (PEI, MW 25,000) were purchased from Aldrich. Zinc acetate (99%, powder), oleic acid (80%) and trioctylphosphine (90%) were obtained from Acros Organics, Merck and Fluka, respectively. Graphite powder was obtained from Beijing Chemical Reagents Company. The standard samples of paraoxon, dichlorvos (100 μg mL^{-1} in acetone) were purchased from Beijing (Weiyekechuang Keji Co., Ltd., China). Other chemicals of analytical grade were obtained from commercial sources and used as received. All solutions were prepared with ultrapure water from a Milli-Q water purification system (Billerica, MA). The ITO (about 15 Ω/sq) coated glass was obtained from Leaguer Film Technology (Shenzhen).

2.2. Apparatus

The UV–Vis absorption spectra were measured by using a U4100 UV–Vis–NIR spectrometer (Hitachi, Japan), and the fluorescence spectra were recorded with a Fluoromax-4 fluorescence spectrophotometer (Horiba Jobin Yvon, Japan). If not specifically stated, the samples were excited at 380 nm, and the exciting slit and the emission slit were both 5 nm. The optical properties of solutions and the multilayer films were measured using quartz cuvette with 1 cm path length and a standard solid sample holder, respectively. Electrochemical measurements were performed with a 660 series potentiostat (CH Instruments, Austin, USA) with a conventional three electrode system comprising platinum wire as auxiliary electrode, a standard Ag/AgCl in saturated KCl solution as the reference electrode, and as-prepared graphene/CdSe@ZnS or graphene/CdSe@ZnS/AChE electrode as the working electrode. During the electrochemical measurements, the electroactive surface area of the working electrode was ca. 3.8 cm^2. A 150 W xenon lamp (CROWNTECH, USA) equipped with a filter was used as the irradiation source ($\lambda > 400$ nm).

2.3. Fabrication of the proposed biosensor preparation of graphene/CdSe@ZnS/AChE hybrid modified electrode

An aqueous dispersion of fewer-layered graphene was prepared from pristine graphite according to the modified Hummers method (Li et al., 2008) and the thickness of graphene is approximately 1.51 nm (Fig. S1). MPA-capped CdSe@ZnS QDs with fluorescence peaks at 630 nm were synthesized according to the previous method (Wang et al., 2007). The average particle sizes were estimated to be 5.5 nm (Fig. S2) and the concentration was about 10^{-6} M.

Prior to the preparation of graphene/CdSe@ZnS/AChE electrodes, the bare ITO substrates were cleaned according to a literature procedure (Evenson et al., 1996). Before assembly, the cleaned ITO-coated glass electrodes were functionalized with (3-aminopropyl) trimethoxysilane to yield an amine-functionalized surface. As illustrated in Fig. S3, the $(graphene/PEI/QDs/PEI)_x$ films were alternatively deposited from the prepared graphene solution, 10^{-6} M MPA-capped CdSe@ZnS QDs and 1 mg/mL PEI solution, using an immersion time of 10 min, and then rinsed with pure water and dried under N_2 flow after each layer deposited. AChE enzyme immobilization was achieved by alternatively immersing the obtained $(graphene/PEI/QDs/PEI)_x$ films in 20 mM phosphate buffer (pH 7.0) containing 0.5 mg/mL of AChE and 1 mg/mL PEI (pH 6.0) for 10 min. The resulting graphene/CdSe@ZnS/AChE electrode was stored at −20 °C for use.

2.4. PEC biosensing under visible light illumination

For the measurements of paraoxon and dichlorvos as the model of OPs, the obtained graphene/CdSe@ZnS/AChE electrode was first immersed in phosphate buffer solution (PBS, 0.1 M, pH 7.4) containing different concentrations of standard OPs at 37 °C for 15 min, and then 1.5 mM acetylthiocholine, an enzyme substrate, was added to above solution to study the PEC response. For comparison, the PEC responses were also recorded by immersing the graphene/CdSe@ZnS/AChE electrode in a solution of 1.5 mM acetylthiocholine for 15 min in the absence of OPs. The inhibition of OPs was calculated as follows:

$$\text{Inhibition}(\%) = (I_{without} - I_{with})/I_{without} \times 100$$

where $I_{without}$ and I_{with} were the photocurrent of acetylthiocholine on the graphene/CdSe@ZnS/AChE electrode, in the absence and presence of OP inhibition, respectively.

2.5. Preparation and detection of fruit samples

The procedure for OP determination in real samples was as follows: (1) The fruit sample was first chopped by a steel automatic vegetable fruit chopper machine and extracted with 20 mL PBS solution, then centrifugation at 3000 rpm for 10 min. (2) The