Sensors and Actuators B 258 (2018) 88–97

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Effective biosensor based on graphene quantum dots via enzymatic reaction for directly photoluminescence detection of organophosphate pesticide

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ARTICLE INFO

Article history:
Received 9 June 2017
Received in revised form 7 November 2017
Accepted 15 November 2017
Available online 21 November 2017

Keywords:
Graphene quantum dots
Organophosphate
Photoluminescence
Acetylcholinesterase
Choline oxidase

ABSTRACT

In this study, the pesticide sensor by graphene quantum dots (GQDs) and active enzyme, GQDs/enzyme platform, has been developed for monitoring the organophosphate pesticides. In this concept, H_2O_2 generated from the active enzymatic reaction of acetylcholinesterase (AChE) and choline oxidase (CHOx) enables to react with GQDs resulting in a "turn-off" photoluminescence of GQDs. A "turn-on" photoluminescence of GQDs at 467 nm was recovered in the presence of organophosphate. Definitely, the photoluminescence changes of GQDs/AChE/CHOx biosensor reasonably correspond to the amount of pesticide. The detection limit of GQDs/AChE/CHOx biosensor towards dichlorvos was 0.172 ppm (0.778 μM). In this approach, this biosensor offers the promising determination of the organophosphate pesticides and a benefit for easy checking of organophosphate pesticides in food, water and environment with low cost, easy-to-prepare and less toxic to environment.

1. Introduction

Currently, pesticides are widely used in agricultural products to control or kill unwanted insects, weeds, rodents, fungi, bacteria, or other organisms [1]. The Official of Agricultural Economics of Thailand (OAE) reported that Thailand is tending to increase the importation of pesticides every year, about 149,546 tons in 2015 [2]. The widely used pesticides not only can contaminate in fresh fruits and vegetables but also can generate environmental problems including contamination of air, water, and soil. Even small amount of a highly toxic pesticide will cause serious human health problems [3]. Hence, the United States Environmental Agency (EPA) has set a maximum residue limit for organophosphate pesticide such as dichlorvos at 0.01 mg/mL in natural water [1]. Organophosphate pesticides are one type of the pesticide groups. Normally, acetylcholinesterase (AChE) can hydrolyze neurotransmitter acetylcholine to choline and acetate. In the presence of organophosphate compounds, they will covalently bind with an active site of acetylcholinesterase (AChE). This process caused irreversible inhibitor of AChE, leading to the increase of the extremely high level of acetylcholine that causes organ failure and eventual death [4]. Because of high toxicity of pesticides, the sensitive and reliable determination of the organophosphate pesticide residues is of the great importance. Analytical techniques such as GC/MS and LC/MS are usually used to determine the amount of pesticide but these techniques are expensive, time consuming, and require an expertise [5].

In previous reports, the determination of organophosphate pesticides by taking an advantage of inhibition of acetylcholinesterase enzyme were reported with its particular challenges of chemistry for examples of electrochemical method [6–9], fluorescence probe [10–12] and colorimetric detection [13–16]. All of methods are sensitive to detect organophosphate pesticides, but most of the biosensor probes were prepared by a complicated method, highly toxic materials and the use of a large amount of enzymes, leading to high cost. In this work, we attempt to employ an easy and low cost method and a touch on green chemistry in synthesizing the effective photoluminescence GQDs/enzyme biosensor for organophosphate detection.

H_2O_2 is a well-known oxidizing agent that is widely used for determination not only in organophosphates but also in the important compounds in clinical diagnostics such as glucose. In 2010, Song [17] revealed that the graphene oxide (GO) exhibited the peroxidase-like catalytic activity for the oxidation of 3,3,5,5-tetramethylbenzidine (TMB) in the presence of H_2O_2 for determination of glucose. Works by group of Zheng et al. [18] reported better catalytic oxidation of TMB activity from the smaller sized graphene dots (GDs).

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https://doi.org/10.1016/j.snb.2017.11.072
0925-4005/

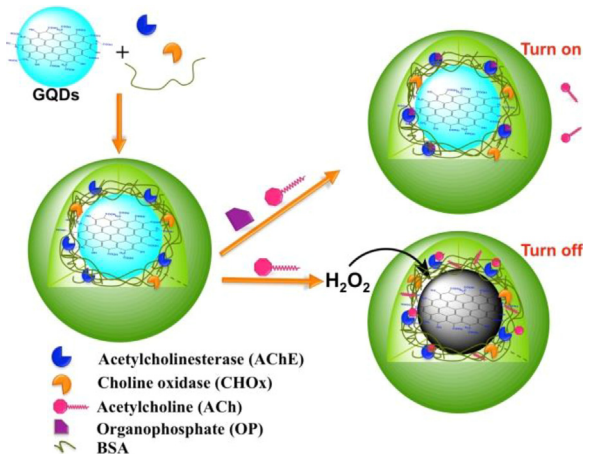

Fig. 1. Proposed GQDs/AChE/CHOx system to determine the organophosphate pesticide.

Graphene quantum dots (GQDs) are the graphene sheets smaller than 10–100 nm [19]. Graphene quantum dots (GQDs) are currently superior in terms of their excellently attractive properties such as their low toxicity and biocompatibility, high photostability against photobleaching and blinking, as well as small particle size resulting in their unique optical and electronic properties including the quantum confinement, tunable band gap, stable fluorescence, high surface area and edge effect [20–23]. The modified size, surfaces and edges of GQDs lead to the change of band gap and the corresponding photoluminescence that takes an advantage for specific detection of target [24]. Apart from the interesting properties of GQDs, for examples of excellent dispersion and good surface grafting, the GQDs, thus, are of intense current interest in various applications such as bioimaging and biosensor for phenolic compound, biothios and glucose molecule [18,24–26]. Taking on a board the ideas of using peroxidase-mimicking catalyst of graphene materials, the GQDs are attractive candidates for this sensing purpose.

This work explored the detection of organophosphate pesticide by using GQDs as a direct photoluminescence probe via enzymatic reaction. We aim to develop the pesticide sensor by GQDs and active enzyme with BSA, GQDs/enzyme platform, for monitoring the organophosphate pesticides. In this concept as shown in Fig. 1, H_2O_2, generated from the active enzymatic reaction of acetylcholinesterase and choline oxidase [15], can react with GQDs, resulting in a "turn-off" fluorescence of GQDs. A "turn-on" fluorescence of GQDs has been recovery in the presence of organophosphate in the system. Definitely, fluorescence changes of GQDs/enzyme biosensor reasonably correspond to the amount of pesticide. In this approach, GQDs/enzyme biosensor is expected to highlight the promising selective determination of the organophosphate pesticides and an attractive material for easy checking of organophosphate pesticides in water with easy-to-prepare and low toxic to environment.

2. Experimental sections

2.1. Materials

Citric acid monohydrous ($C_6H_8O_7{\cdot}H_2O$) was purchased from Merck. Acetylcholinesterase (AChE, 217 units/mg) from *Electrophorus electricus* (electric eel), choline oxidase (CHOx, 14 units/mg) from *Alcaligenes* sp., neurotransmitter acetylcholine (ACh) and bovine serum albumin (BSA) were purchased from Sigma-aldrich. Organophosphate pesticides including dichlorvos, methyl-paraoxon, malathion and parathion were obtained from Sigma-aldrich. Tris–HCl was purchased from Carlo Erba. Milli-Q (MQ) was prepared by ultra pure water system and used throughout the experiment.

2.2. Apparatus

Transmission electron microscopy (TEM) was performed on a JEOL JEM 2010 with a field emission gun operated at 200 kV. The TEM micrographs were used to determine size of the deposited particles by counting approximately 200 particles (ImageJ 1.49 V free software with Java 1.6.0_65(32-bit) obtained from https://imagej.nih.gov/ij/, Wayne Rasband, National Institutes of Health, USA). IR spectrophotometric measurement of dried particle samples was performed on Thermo, Nicolet 6700 FT-IR. Absorption spectra were measured by a Varian Cary 50 UV–vis spectrophotometer. Fluorescence spectra were performed on a Varian spectrofluorometer equipped with a personal computer data processing unit. The light source is Cary Eclipse a pulsed xenon lamp and a detector is a photomultiplier tube. Ultra high performance liquid chromatography coupled with mass spectrometry (UHPLC–MS) used in this experiment was performed on Agilent Technologies Model 1290 (CA, USA) including vacuum degasses, binary pump, agilent jet weaver, autosampler and vacumm oven and Agilent Technologies Model 6490 MS (CA, USA) including a triple quadrupoled mass analyzer, electrospray ionization (ESI) interface and MassHunter software processing. A UHPCL separation was performed on an Eclipse XDB-C18 with 4.6 mm × 50 mm, 1.8 μm from Agilent (USA).

2.3. Preparation of the graphene quantum dots

The preparation of graphene quantum dots (GQDs) was carried out using Dong's method [20]. Firstly, 2 g of citric acid in 5 mL beaker was heated to 200 °C. After 5 min, the solution was turned to orange in 30 min. Then the prepared GQDs liquid was added dropwise into 100 mL of 10 mg/mL^{-1} NaOH solution under vigorous stirring. After that, the solution was adjusted to pH 8.0 by 1 M HCl solution. Then the GQDs were purified by dialysis 2000 Da for 15 h. The GQDs were solidified by freeze-drying, and then they were weighed and characterized by FTIR spectroscopy and TEM techniques.

2.4. Preparation of the proposed biosensors and detection of organophosphate

The GQDs solution was reasonably utilized to determine the organophosphate pesticide (OP) by following the photoluminescence response of GQDs upon the reaction with H_2O_2 generated from the Acetylcholinesterase (AChE) and Choline oxidase (CHOx). Each portion of the proposed biosensors were prepared in the same stock solution. Under 40 experiments, 8 mL of AChE (10 U/mL) and 8 mL of CHOx (1.25 U/mL) in Tris-BSA (1% of BSA in 50 mM pH 8 of Tris buffer) were firstly added into 4 mL of GQDs solution (1×10^{-2} g/mL) and then the solution was added by 16 mL of Tris buffer solution (50 mM pH 8). The solution was stirred for 5 min prior to incubation at 5 °C for 24 h. Stocks of graphene quantum dots immobilized enzymes (GQDs/AChE/CHOx) were divided into 40 vials with 0.9 mL per vial. All vials with GQDs/AChE/CHOx biosensor were kept at 5 °C prior to use. Then the solution of 0.7 mL of MQ-water and 0.2 mL of different concentration of organophosphate pesticide (dissolved in 5% ethanol) was added in the biosensor solution and incubated for 15 min in a water bath at 25 °C. The mixture solution was added by 0.2 mL acetylcholine (10 mg/mL) and incubated by stirring for 30 min. The PL signals were monitored by fluorescence spectroscopy and the measurements were repeated 3 times. The percentage of enzyme inhibition (I%) was plotted against the concentration of organophosphate pesticide to obtain calibration curve. The percentage of enzyme inhibition (I%) was calculated