Biosensors and Bioelectronics 26 (2011) 2864–2869

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Ultra-sensitive biosensor based on mesocellular silica foam for organophosphorous pesticide detection

Shuo Wu[a,*], Lili Zhang[a], Lin Qi[b], Shengyang Tao[a], Xiaoqin Lan[a], Zhiguang Liu[a], Changgong Meng[a]

[a] *School of Chemistry, Dalian University of Technology, Dalian 116023, PR China*
[b] *School of Materials Science and Engineering, Dalian University of Technology, Dalian 116023, PR China*

ARTICLE INFO

Article history:
Received 16 August 2010
Received in revised form 2 November 2010
Accepted 19 November 2010
Available online 7 December 2010

Keywords:
Mesocellular silica foam
Solid phase extraction
Low limit of detection
Monocrotophos

ABSTRACT

A sensitive amperometric acetylcholinesterase (AChE) biosensor was fabricated based on mesocellular silica foam (MSF), which functioned as both an enzyme immobilization matrix and a solid phase extraction (SPE) material for the preconcentration of target molecules. The hydrophilic interface, the good mechanical/chemical stability, and the suitable pore dimension of MSF provided the entrapped AChE a good environment to well maintain its bioactivity at basic condition. The AChE immobilized in MSF showed improved catalytic ability for the hydrolysis of acetylthiocholine, as evidenced by the increasing of the oxidation current of thiocholine, the enzymatic catalytic hydrolysis production of acetylthiocholine. In addition, the MSF with large surface area showed a modest adsorption capacity for monocrotophos, a model organophosphate used in this study, *via* the hydrogen bond or physical adsorption interaction. The combination of the SPE and the good enzyme immobilization ability in MSF significantly promoted the sensitivity of the biosensor, and the limit of detection has lowered to 0.05 ng/mL. The biosensor exhibited accuracy, good reproducibility, and acceptable stability when used for garlic samples analysis. The strategy may provide a new method to fabricate highly sensitive biosensors for the detection of ultra-trace organophosphorous pesticide infield.

1. Introduction

Organophosphorous pesticides (OPs) are widely used in agriculture for pest control (Wang et al., 2008a; Lee et al., 2010). However, the toxic residuals of OPs in crop, livestock, and poultry products would cause the disfunction of neurotransmitter enzyme, acetylcholinesterase (AChE), and thus harm the human health (Mulchandani et al., 2001). The safety levels fixed by the national and international regulatory agencies depend on the toxicities of OPs, but the maximum concentration of each individual pesticide set by the European Council Directive 98/83/CE should be lower than 0.1 ppb, and the total amount must not exceed 0.5 ppb (Llopis et al., 2009). However, the low concentration of these compounds in food, water and air can hardly be monitored directly using standard analytical instrumentation. Gas chromatography (Noort et al., 2006) and high-performance liquid chromatography (Cappiello et al., 2002) coupled with mass spectroscopy could detect OPs in ppb level. Unfortunately, the tedious sample pretreatments, highly qualified technicians and sophisticated instruments are not suitable for infield detection. Consequently, the development of rapid and sensitive OPs detecting techniques with ultra-low detection limit shows increasing potential for environmental monitoring and food industry.

Amperometric AChE biosensors (Amine et al., 2006; Du et al., 2007; Hildebrandta et al., 2008) present a promising alternative method to the traditional strategies because of their high sensitivity, fast response, and miniature size. Based on the inhibition action of OPs on AChE, the concentration of pesticides could be precisely determined (Amine et al., 2006). The sensitivity and the detection limit of these biosensors depend on enzyme activity (Gong et al., 2009; Sotiropoulou and Chaniotakis, 2005), thus, the immobilization of enzyme on the surface of electrode is a crucial step for the performance of the biosensors. For the protection of enzyme activity, functional nanomaterials such as carbon nanotube (Kandimalla and Ju, 2006; Choi et al., 2009; Chen et al., 2008), gold nanoparticle (Du et al., 2007; Zhao et al., 2009), and mesoporous materials (Shimomura et al., 2009; Sotiropoulou and Chaniotakis, 2005) have been introduced for the construction of enzyme loaded matrix. The integration of advanced materials greatly promoted the sensitivity, stability and detection limit of AChE biosensors. Among them, the mesoporous materials have attracted considerable attention due to their large surface areas, versatile mesostructure, good mechanical/chemical stabilities (Wu et al., 2007; Dai et al., 2004). The pore diameter of mesoporous materials can be easily controlled between in a wide range (2–50 nm) to match the dimension of the immobilized enzyme. The nanopores provide confined space for enzymes

* Corresponding author. Tel.: +86 411 84706293.
E-mail address: wushuo@dlut.edu.cn (S. Wu).

0956-5663/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2010.11.029

to prevent them from unfolding and are therefore beneficial for their activity maintenance (Zhou and Dill, 2001). The good enzyme activity maintenance ability of mesoporous materials has been well established by theoretical simulation (Zhou and Dill, 2001) and a large amount of experimental facts (Vamvakaki and Chaniotakis, 2007; Wu et al., 2007; Dai et al., 2004).

Mesoporous materials have been recently utilized for solid-phase extraction (SPE), which is applied in various fields, including separation science (Pérez-Quintanilla et al., 2007), on-line preconcentration and trace analysis (Trammell et al., 2008). In the past decades, this technique arose for the development of amperometric pesticides biosensors based on the principle of concentrating target pesticide on the surface of electrode, which significantly improve the sensitivity of the biosensors. Large surface area and adsorbing ability are required for a suitable SPE material. Compared with other SPE materials such as ZrO_2 nanoparticle (Du et al., 2008a), ZrO_2/Au nanocomposites film (Wang and Li, 2008), and multiwalled carbon nanotube (Du et al., 2008b), mesoporous material such as three dimensional mesocellular silica foam (MSF) is not only benefit from their large surface area, but pesticides compounds can interact with mesoporous silica with both physical adsorption and hydrogen bond. However, to the best of our knowledge, mesoporous materials are now barely used in the amperometric sensing of electro-inactive compounds for their SPE function. This is a blank area but with great perspective.

Herein, motivated by the super abilities of mesoporous materials, MSF was introduced to the surface of electrode for both enzyme immobilization and SPE of pesticides. An AChE biosensor was fabricated for the detection of a model OP, monocrotophos. The linear range was from 0.05 ppb to 10 ppb with a limit of detection down to 0.05 ppb, which satisfies the demand for ultra-trace pesticide detection.

2. Experimental

2.1. Reagents

Acetylcholinesterase (1000 U/mg) and acetylthiocholine chloride (ATCl) were purchased from Sigma–Aldrich (St Louis, USA) and used as received. Monocrotophos was purchased from Treechem Co. (Shanghai, China). Poly(vinyl alcohol) (PVA) and poly(ethylene oxide)-block-poly-(propylene)-block-poly(ethylene oxide) ($EO_{20}PO_{70}EO_{20}$, Pluronic P123) was purchased from BASF (Germany). 5,5′-Dithiobis(2-nitrobenzoic acid) was purchased from Boao Co. (Shanghai, China). All other reagents were of analytical grade and used without further purification. 0.1 M phosphate buffer solution (PBS) was prepared by mixing the stock solutions of NaH_2PO_4 and Na_2HPO_4. Doubly distilled water was used in all experiments.

2.2. Preparation of AChE loaded MSF suspension

MSF powder was synthesized according to the method reported previously (Schmidt-Winkel et al., 2000). 0.5 mg of the obtained MSF powder was first mixed with 1.0 mL water and stirred for 2 h to obtain the MSF suspension. Then 17 U AChE was added into the suspension and shaken for 20 min to obtain the enzyme entrapped MSF nanocomposite.

2.3. Construction of AChE-MSF-PVA/GCE

Glassy carbon electrodes (3 mm in diameter, CH Instruments, Inc., U.S.A.) were firstly polished to a mirror finish with 0.3 and 0.05 μm alumina slurry followed by thoroughly rinsing with deionized water. After sonicating successively in 1:1 nitric acid, acetone and deionized water, the electrodes were rinsed with deionized water and dried at room temperature. Then 3 μL of the AChE loaded MSF suspension and 3 μL 2% PVA aqueous solution was subsequently cast on the GCE to obtain the AChE-MSF-PVA/GCE, with the total AChE amount of 50 mU on the electrode surface. The PVA membrane could fix the AChE impregnated MSF nanocomposite on electrode surface. The biosensor was stored at 4 °C when not in use. For comparison, AChE-PVA/GCE, MSF-PVA/GCE, and PVA/GCE were fabricated.

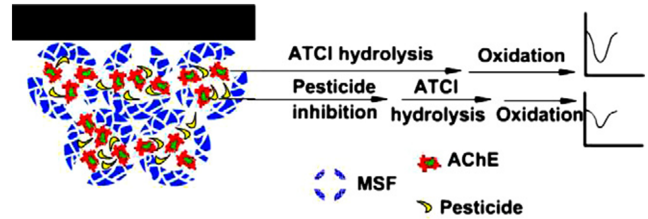

Fig. 1. Schematic diagram for biosensor fabrication and determination of monocrotophos.

2.4. Inhibition measurement using AChE biosensor

A detailed description of the assay process for monocrotophos was illustrated in Fig. 1. For inhibition tests, the original differential pulse voltammetric signal ($I_{P,control}$) was measured in 0.1 M pH 9.0 PBS with 1.5 mM ATCl. Then the electrode was rinsed with water and incubated in an aqueous solution containing the desired concentration of monocrotophos for 10 min. After incubation, the residual signal ($I_{P,exp}$) was recorded at the same condition. The inhibition rate of monocrotophos was calculated as follows:

$$\text{Inhibition}\,(\%) = 100\% \times \frac{I_{P,control} - I_{P,exp}}{I_{P,control}}$$

2.5. Activity and stability assays

500 mg MSF powders were added into 10 mL AChE solution (activity of 20 U), and the mixture was shaken at room temperature in a shaken bath for 2 h for the adsorption of enzyme in the MSF. Then the AChE/MSF composite was centrifuged and washed with deionized water twice. No enzyme was detected in the supernatant according to the Ellman method (Ellman et al., 1961). The composite was dried at 30 °C and stored at −20 °C. The amount of immobilized enzyme in the porous MSF was calculated as 40 mU/mg.

In order to measure the stability of the immobilized enzyme, the AChE/MSF composite was dispersed in pH 9.0 PBS (10 mg/mL). Aliquots were taken every 20 min over a time period of 120 min from the AChE/MSF suspension to analyze its activity using a modified Ellman method (Ellman et al., 1961). For comparison, the stability of the free enzyme in pH 9.0 PBS was also examined.

2.6. Adsorption of monocrotophos

The AChE-MSF-PVA/GCE or the AChE-PVA/GCE was immersed in 1.0 mL 500 ppb monocrotophos solution for 10 min for monocrotophos adsorption. The residual monocrotophos amount after adsorption was calculated by UV adsorption at 265 nm.

2.7. Apparatus

The electrochemical measurements were carried out on a CHI 660c electrochemical working station (CH Instruments Co.). A three-electrode system was employed with AChE-MSF-PVA/GCE as working electrode, a saturated calomel electrode (SCE) as reference