Biosensors and Bioelectronics 68 (2015) 288–294

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Efficient immobilization of acetylcholinesterase onto amino functionalized carbon nanotubes for the fabrication of high sensitive organophosphorus pesticides biosensors

Guangxia Yu [a,b], Weixiang Wu [a,b], Qiang Zhao [a,b], Xiaoyun Wei [a,b], Qing Lu [a,b,*]

[a] Key Laboratory of Environment and Health, Ministry of Education & Ministry of Environmental Protection, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, #13 Hangkong Road, Wuhan, Hubei, 430030, China
[b] State Key Laboratory of Environmental Health (Incubating), School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, #13 Hangkong Road, Wuhan, Hubei, 430030, China

ARTICLE INFO

Article history:
Received 21 October 2014
Received in revised form
15 December 2014
Accepted 2 January 2015
Available online 3 January 2015

Keywords:
Organophosphorus pesticide biosensor
Surface functionalization
Carbon nanotubes
Protein adsorption
Orientation
Paraoxon

ABSTRACT

This work introduced an efficient immobilization of acetylcholinesterase (AChE) onto amino functionalized carbon nanotubes (CNT–NH_2), in order to fabricate high sensitive and practical organophosphorus pesticide (OPs) biosensors. Compared with the pristine, –COOH and –OH decorated CNTs, there were larger amount of enzymes adsorbed on the surface of CNT–NH_2 with a favorable orientation and the best amperometric response was obtained on the AChE/CNT–NH_2/GC electrode. Furthermore, the biosensor modified with CNT–NH_2 showed a high affinity to acetylthiocholine chloride (ATCh) and could catalyze the hydrolysis of ATCh with an apparent Michaelis–Menten constant (K_m) value of 67.4 μM. Using paraoxon as a model compound, wide linear ranges from 0.2 nM to 1 nM and 1 nM to 30 nM, and a low detection limit of 0.08 nM were obtained with satisfactory reproducibility and stability. Moreover, the biosensor had also been successfully employed for the determination of low concentrations of pesticides in real vegetable samples. This method could be extended to other functionalized nano–materials for their application in constructing biosensors.

1. Introduction

Efficient immobilization of the enzyme onto the electrode surface while maintaining its native catalytic activity is the key consideration to develop ultrasensitive enzyme based biosensors. Ideally, it is expected that enzymes can be bound to the electrode surface with a favorable orientation while avoiding conformational changes (Ganesana et al., 2011). The existing immobilization methods include direct physical adsorption (Sotiropoulou et al., 2005), physical entrapment in polymers or sol–gels (Dutta and Puzari, 2014; Zou et al., 2008), cross linking (Lai et al., 2009), covalent attachment (Zhang et al., 2005) and self assembling (Liu and Lin, 2006). However, the enzyme immobilizations with these approaches are hard to control, because as contributions of either hydrophobic interactions, hydrogen bonding or electrostatic attraction, the main driving forces of protein–particle interactions are often not fully enlightened (Meder et al., 2013a). Moreover, these traditional methods usually need complicated steps, leading them to hardly satisfy the requirement of practical applications.

In recent years, surface functionalization, which allows tailoring the surface properties of nano-materials, has been applied to investigate the interaction between protein and particle surface. Numerous researches had demonstrated that specific design of nano–materails by surface functionalization tailored with charge groups (like –COOH, –NH_2, –SO_3H, etc.) might be a tool to control driving forces of proteins adsorption and orientation (Meder et al., 2013a, 2013b). Moreover, certain surface chemistries even feature protein adsorption comparable to antigen–antibody interactions (Hoshino et al., 2010; Monopoli et al., 2012). Meder et al. (2012) had discussed the interaction between the surface of colloidal alumina (Al_2O_3) particles decorated with different functional groups (–NH_2, –COOH, –SO_3H and –PO_3H_2) and three model proteins, bovine serum albumin (BSA), lysozyme (LSZ) and trypsin (TRY). It had been demonstrated that BSA was preferentially adsorbed on the positively charged surfaces of Al_2O_3 and Al_2O_3–NH_2, while LSZ and TRY were adsorbed on negatively charged Al_2O_3–COOH, Al_2O_3–SO_3H and Al_2O_3–PO_3H_2. Furthermore, the regions in

* Corresponding author at: School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, #13 Hangkong Road, Wuhan, Hubei 430030, China. Fax: +86 27 83657765.
E-mail address: qi_weiliao@126.com (Q. Lu).

http://dx.doi.org/10.1016/j.bios.2015.01.005
0956-5663/

protein with dominant positive or negative potential could be the adsorption sites on oppositely charged particle surfaces, indicating that the protein oriented adsorption was influenced by the type of functional groups via electrostatic interactions as driven forces. Gessner et al. (2003) had discussed the influences of different basic and acidic function groups on the surface of polystyrene on the protein adsorption. The studies had shown that proteins with isoelectric point pI > 5.5 preferred to bind acidic functional groups in neutral or subalkalic experiment conditions and vice versa. A conclusion could be drawn that the adsorption should be mainly driven by Coulomb forces. These researches above had provided a prerequisite for the design of functionally graded materials to realize the protein oriented adsorptions, thus we put forward a hypothesis that chemical functionalized nano-materials could also be employed to control the immobilization of enzymes onto the electrode surface without any other cross–linking agent.

Carbon nanotubes (CNTs) have been extensively used for the development of biosensors as electronic bridges and signal amplifiers due to their superior electrical conductivity, high electrochemical catalytic activity, biocompatibility and nontoxicity (Kim et al., 2007). Additionally, CNTs have excellent electrocatalytic activity for thiocholine (Liu et al., 2005), the product enzymatically generated by AChE, which could provide a prerequisite for the construction of AChE based OPs biosensors. As is reported, the isoelectric point of AChE from electric eel is 5.5, thus the net surface charge of AChE is negative in neutral or subalkalic experiment conditions (Choi et al., 2001). Furthermore, the active-site gorge region of AChE had been investigated to be negatively charged (Dvir et al., 2010; Ripoll et al., 1993), thus it could work as an adsorption site bonding to the positively charged nano-materials. Thereby, in this work, we applied the positively charged CNT–NH_2 as a model to demonstrate an efficient immobilization of AChE for the fabrication of high sensitive and practical OPs biosensors.

In this system, amino functionalized CNTs, in addition to serving as electronic bridges and signal amplifiers, could also be a tool to control the efficient immobilization of enzymes. The advantages of this method were as follows: (1) guiding the protein orientation and reducing randomly bounded proteins, therefore improving the sensitivity; (2) improving the affinity between the enzyme and nano-materials, thus promoting the electron transfer mechanism between enzyme and electrode; (3) simplifying the immobilization steps, thus improving the reproducibility and operability. The proposed method could be further applied as a common approach for the design of various biosensors with functionalized materials.

2. Experimental

2.1. Reagents

Acetylthiocholine chloride (ATCh) and acetylcholinesterase (1000 Units/mg, from electric eel) were obtained from Sigma-Aldrich (St. Louis, MO). Multiwalled carbon nanotubes (purity > 95%) were purchased from the Chengdu Organic Chemistry Institute (Chengdu China) and the 3–aminopropyltriethoxysilane (3–APTES) was purchased from Aladdin Industrial Corporation (Shanghai China). The standard sample of paraoxon was obtained from the Future of Shanghai Industrial Co., Ltd. (Shanghai China). Practical vegetable samples were purchased from the local market. All the other reagents were of analytical grade. All aqueous solutions were prepared with doubly distilled water.

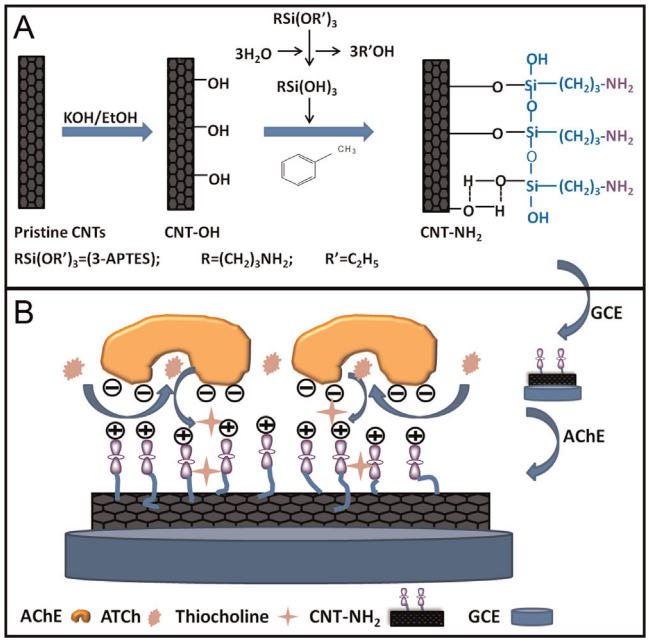

Fig. 1. (A) Schematic representation of amination process of carbon nanotubes; (B) schematic representation of the efficient immobilization of AChE onto the CNT–NH_2 modified electrode.

2.2. Apparatus

Electrochemical measurements were carried out using a conventional three-electrode system. A bare or modified glass carbon electrode was served as working electrode, a standard calomel electrode (SCE) as reference electrode and a platinum wire as counter electrode. Impedance–Time Parameters (current–time) and Differential Pulse Voltammetry (DPV) were performed on a CHI830C electrochemical workstation (Shanghai Chenhua Apparatus, China). Electrochemical impedance spectroscopy (EIS) was worked at a CHI660C electrochemical workstation (Shanghai Chenhua Apparatus, China). Infrared spectrometry (IR) (VERTEX 70, Bruker, Germany) and X-ray photoelectron spectroscopy (XPS, AXIS-ULTRA DLD-600W, SHIMADZU, Japan) were applied to characterize the functionalized CNTs. The gas chromatography (GC) analysis was conducted on an Agilent 7890A (the USA). The amount of adsorbed protein was detected with a UV spectrophotometer (UV–1800, SHIMADZU, Japan).

2.3. Surface functionalization of CNTs

The surface amination of CNTs was carried out by the procedures described by Song et al. (Song et al., 2009). A schematic representation of the amination processes was shown in Fig. 1A. Firstly, 100 mg pristine CNTs were mixed with 100 mL ethyl alcohol (EtOH) and 5 g KOH, and then refluxed for 8 h, followed by the filtration and washing process with ethanol and doubly distilled water to obtain CNT–OH. Secondly, 50 mg CNT–OH was dispersed in toluene via sonication for 30 min and then an excess of 3–APTES solution was added drop by drop to obtain the produced mixture which should be stirred at 80 °C for 8 h. The tubes were then rinsed with toluene and ethanol, dried at 80 °C and finally designated as CNT–NH_2. Besides, CNT–COOH was obtained by acid treatment with H_2SO_4:HNO_3 (1:3) (Tzavalas et al., 2006).

2.4. Preparation of OPs biosensors

Schematic illustration of the enzyme immobilization onto electrode surface was shown in Fig. 1B. Firstly, 5 μL 0.5 mg/mL