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# Sensitive electrochemical microbial biosensor for *p*-nitrophenylorganophosphates based on electrode modified with cell surface-displayed organophosphorus hydrolase and ordered mesopore carbons

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ABSTRACT

A novel electrochemical microbial biosensor for the rapid monitoring of *p*-nitrophenyl-substituted organophosphates (OPs) compounds based on glass carbon electrode (GCE) modified with both ordered mesopore carbons (OMCs) and cell surface-expressed organophosphorus hydrolase (OPH) (OPH-bacteria/OMCs/GCE) was described in this paper. The genetically engineered *Escherichia coli* strain surface displayed mutant OPH (S5) with improved enzyme activity and favorable stability was constructed using a newly identified N-terminal of ice nucleation protein as an anchoring motif, which can be used directly without further time-consuming enzyme-extraction and purification, thereafter greatly improved the stability of the enzyme. Compared to OPH-bacteria modified GCE (OPH-bacteria/GCE), the OPH-bacteria/OMCs/GCE not only significantly enhanced the current response but also reduced the oxidation overpotential towards oxidizable *p*-nitrophenol (*p*-NP), which was the hydrolysate of *p*-nitrophenyl-substituted OPs. Under the optimized experimental conditions, at +0.84 V (vs. SCE), the current–time curve was performed with varying OPs concentration. The current response was linear with paraoxon concentration within 0.05–25 μM. Similarly, linear range of 0.05–25 μM was found for parathion, and 0.08–30 μM for methyl parathion. The low limits of detection were evaluated to be 9.0 nM for paraoxon, 10 nM for parathion and 15 nM for methyl parathion ($S/N=3$). Thus, a highly specific, sensitive and rapid microbial biosensor was established, which holds great promise for on-site detection of trace *p*-nitrophenyl-substituted OPs.



## 1. Introduction

Organophosphates (OPs) represent one kind of broadly existing acute toxicity species, which are found widely as contaminants (Chough et al., 2002; J and Králové, 2002; Wang et al., 2002; Zhang et al., 2014b). The chemical structures of some common OPs are listed (Supplementary material, Fig. S1). It is estimated that over 1500 kinds of OP compounds have been synthesized during the past 20th century, which are commercially used as pesticides and chemical warfare agents (Brown and Brix, 1998; Eskenazi et al., 1999; Rahimi and Abdollahi, 2007; Schoning et al., 2003). There are stringent restrictive regulations worldwide about OPs especially for *p*-nitrophenyl OPs (e.g. paraoxon, parathion and methyl parathion, etc.), which belong to highest poisonous OPs. For example, paraoxon is used as typical insecticide with a human oral lethal dose of 5 mg/kg (Deo et al., 2005a; Lei et al., 2005; Minton and Murray, 1988; Wang et al., 2003). Parathion would convert into paraoxon through a series of photolysis and metabolic oxidation process (Lei et al., 2007; Lukaszewicz-Hussain, 2010; Mulchandani et al., 2006). So it is highly desirable to ensure that these *p*-nitrophenyl OPs are not present over hazardous levels in food, ground water and soil. Currently, conventional laboratory-based analytical methods for determining *p*-nitrophenyl OPs include primarily gas and liquid chromatography (Pinto et al., 1995), liquid- and thin-layer chromatography (Bravo et al., 2002) and different types of spectroscopy (Mathew et al., 2007; Tang et al., 2014), capillary electrophoresis (Chen and Fung, 2010) and flow injection analysis (Mulchandani et al., 2001; Wang et al., 2003), etc. However, the shortages of these

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analytical technologies such as the time-consuming procedure in sample preparation, the large-scale and expensive instruments and high cost in instrumental maintenance indicated that those technologies were not suitable for on-site test. Accordingly, there are growing demands for field-deployable devices for reliable monitoring of *p*-nitrophenyl OPs.

Organophosphorus hydrolase (EC 3.1.8.1, OPH) can hydrolyze effectively a broad range of organophosphorus esters, which has been widely used as an important component of OPs enzyme biosensors (Ashok Mulchandani, 2011). *p*-nitrophenyl OPs such as parathion methyl parathion and paraoxon can be catalyzed by OPH to form *p*-nitrophenol (*p*-NP), and *p*-NP shows optical absorption at 410 nm approximately. At present, several types of OPH-based biosensors have been introduced, including potentiometric, amperometric or optical devices (Choi et al., 2010; Du et al., 2010; Lee et al., 2010; Tang et al., 2014). Other enzymatic devices based on the inhibition of acetylcholine esterase (AChE) have been widely used for the detection of OPs (Chen et al., 2011; Soltaninejad and Abdollahi, 2009). Generally speaking, although AchE-based biosensors were more sensitive than OPH-based biosensors, they are cumbersome in operating procedures, lots of jamming signals and poor specificity, which only provided the total quantity of a series of toxic inhibitors (Mulchandani et al., 2001).

Microbial surface display could eliminate onerous purification of OPH by fusing it with appropriate anchoring motifs such as outer membrane protein (OmpA) (Karami et al., 2014), ice nucleation protein (INP) (Shimazu et al., 2003) and a-agglutinin (Schofield et al., 2007). However, the OPH activity of surface displayed strains reported earlier is relatively low, which cannot meet the need for sensitive OPH biosensors (Deo et al., 2005b). Therefore, mutated OPH (S5) with improved enzyme activity was displayed on the surface of *E. coli* using INP display system in our laboratory (Tang et al., 2014). The resultant whole cell exhibited excellent OPH activity and stability (Tang et al., 2014), which could provide the ideal bacterial candidate for biosensing of OPs.

In the past decades, electrochemical methods were regarded as high sensitivity, good reproducibility and minimal space and low-cost instrumentation methods, attracting more and more attention for on-site monitoring of *p*-nitrophenyl OPs. Ordered mesopore carbons (OMCs) were popularly used in many aspects due to their outstanding physico-chemical properties (Ndamanisha and Guo, 2012). OMCs with uniform porous channels, large surface area and well-defined pore topology made it the perfect carrier of cell and thus enhance the stability of the enzyme. The oxygen-containing functional groups on the surface of OMCs can combine with the enzyme, and accordingly, which is capable of letting the activity center of enzyme-bacteria exposed to the substrate, and thus retain the enzyme activity (Laothanachareon et al., 2008). Additionally, the edge plane-like defective site on the surface of OMCs can provide a great deal of favorable sites to transmit electrons of electroactive substances (Ndamanisha and Guo, 2012).

In this paper, OMCs were proved to be a satisfactory material for immobilizing cell-surface-expressed OPH in developing novel electrochemical biosensor for *p*-nitrophenyl OPs. The OPH-bacteria with high activity and stability was used directly without further laborous enzyme-extraction and purification, which greatly improved the stability of the enzyme. By detection of the oxidation current of *p*-NP which was the hydrolysis product of *p*-nitrophenyl-substituted OPs, the rapid monitoring of *p*-nitrophenyl OPs was realized. Paraoxon was used as the analyte to systematically study the electrochemical behavior of *p*-nitrophenyl OPs at OPH-bacteria/OMCs/GCE and optimized the experimental conditions. Thus, a highly specific, sensitive and rapid microbial biosensor for trace detection of *p*-nitrophenyl OPs was established. This biosensor would be well-suited for meeting the challenges of on-site determination.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Paraoxon, parathion and methyl parathion were purchased from Sinopharm Chemical Reagent Corporation and used without further purification. The 20 mM stock OP solutions were prepared with methanol and water (1:5) and stored in darkness to avoid photolysis. The experimental solutions should be prepared immediately before use. For safety, these OP compounds should be handled in the fumehood. Direct contact and inhalation should be avoided by taking appropriate security precautions. Nafion (perfluorinated ion-exchange resin, 5 wt% solution in a mixture of lower aliphatic alcohols and water) were purchased from Aldrich Corporation. 0.1 M phosphate buffer saline (PBS, pH 7.4) was served as the supporting electrolyte. All other chemicals were of the highest grade and all the solutions were prepared with ultrapure water. OMCs were synthesized according to the procedure in our published paper, which exhibited large surface area and uniform mesopore channel structure with an average pore diameter of approximately 3.9 nm (Zhang et al., 2013).

### 2.2. Bacterial strains and plasmids, growth of bacteria-displayed OPH

The construction of expression vector pTInaPb-N/Oph has been completed in our previous study (Tang et al., 2014). To obtain the cells surface displayed mutated OPH (S5) for construction of biosensor in the present study, the growth of bacteria was conducted according to the procedures reported earlier (Tang et al., 2014). Briefly, *E. coli* strain BL21 (DE3) was used as the host cell for the expression of recombinant protein INP-OPH. Cells bearing the expressing plasmid pTInaPb-N/OPH were grown in LB media with 50 mg/L kanamycin at 37 °C. Fusion proteins were induced with isopropyl-*β*-D-thiogalactoside (IPTG) at final concentration of 0.1 mM at 25 °C for 8 h. Then cells were harvested and resuspended in 75 mM Tris-HCl buffer with 50 μM $CoCl_2$ (pH 8.0). The procedure for cell fractionation and enzyme activity assay has been described before (Tang et al., 2014).

### 2.3. Electrode fabrication

The bare GCE (3 mm in diameter) was polished successively with 0.3 and 0.05 μm alumina slurry and sonicated for about 3 min. Nafion–OMCs composite was prepared by dispersing 2.0 mg OMCs powder into 1.0 mL Nafion (0.05 wt%) which was diluted from 5 wt% Nafion and sonicated to obtain a homogeneous dispersion. A 10 μL aliquot of this Nafion–OMCs composite was cast onto the cleaned bare GCE. Next, 10 μL of OPH-bacteria aqueous dispersion was added to the modified GCE as to OPH-bacteria/OMCs/GCE and dried overnight at 4 °C. Before use, 4.0 μL of Nafion (0.05 wt%) was syringed onto the surface of modified electrode. The Nafion/bacteria-OPH/GCE was prepared for comparison. The microbial electrode was kept in a refrigerator (at 4 °C) when not in use.

### 2.4. Apparatus and methods

The electrochemical measurements were performed on CHI 660D electrochemical workstation (Chenhua Co., Shanghai, China) with a conventional three-electrode system using bare GCE or modified GCE as working electrode, platinum wire as auxiliary electrode and saturated calomel electrode (SCE) as reference electrode, respectively. All potentials in this paper were recorded versus this reference. PB-10 pH meter (Sartorius AG, Germany) was applied for pH adjustment. All electrochemical measurements