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Olfactory biosensor for insect semiochemicals analysis by impedance sensing of odorant-binding proteins on interdigitated electrodes

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ABSTRACT

Insects can sensitively and selectively detect thousands of semiochemicals at very low concentrations by their remarkable olfactory systems. As one of the most important olfactory proteins, odorant-binding proteins (OBPs) from insects are the most promising candidates for fabricating biosensors to detect biochemical molecules in the chemical ecology as well as for other biotechnological applications. In this study, we designed an olfactory biosensor by immobilizing OBPs from oriental fruit fly on interdigitated electrodes to detect semiochemicals. After successfully separated and purified, OBPs were immobilized by the special designed polyethylene glycol (PEG), SH–PEG–COOH, to produce a robust sensing membrane. Based on electrochemical sensing, interactions between OBPs and different semiochemicals emitted from host plants of the insect, such as the isoamyl acetate, β-ionone, and benzaldehyde, could be sensitively detected. With related amino acid residues in the hydrophobic cavities distinguished, the interaction forces between semiochemicals and OBPs were analyzed by molecular docking. Integrated biological olfaction proteins of insects, OBPs based biosensors could not only advance the progress in the understanding of chemical communication systems of insects, but also show promising potentials for biosensing applications in many fields.

1. Introduction

Semiochemicals are chemical substances that are emitted by plants, insects and mammals, such as fruity scents, floral odors, and sex or alarm pheromones. They carry messages that are used to communicate between individuals, including pheromone for intraspecific communication and allelochemical for interspecific communication of plans and animals (Sbarbati and Osculati, 2006; Schulz, 2010; Mensah and Moore, 2011; Apps, 2013). Been sensitively and selectively detected by the highly-adapted olfactory systems of animals, semiochemicals play central roles in chemical communications in the natural world by influencing the behaviors of animals, especially insects, which embraced a new research area known as chemical ecology (Pickett et al., 2013; Wehrenfennig et al., 2013). Detecting and monitoring the chemicals that are involved in interactions between living organisms are very important for the understanding of how they can maintain an excellent coordination in complex ecosystems. At the same time, investigating of the molecular mechanisms of olfaction for semiochemicals maybe will offer great benefits for bio-inspired methodologies of artificial olfaction.

Up until now, approximately 3500 animal semiochemicals have been identified, more than 3000 of which are insect semiochemicals, while only a few are mammals semiochemicals (Apps, 2013). Insects are known for their remarkable olfaction ability, which is crucial for them to distinguish, comprehend and estimate the overall situation and even for their survival, reproduction, and social communication (Fan et al., 2011; Forêt and Maleszka, 2006; Lu et al., 2014). Therefore, semiochemicals of plants and animals can be detected by insects at low concentrations over a long distance, even dozens of meters away (Wehrenfennig et al., 2013).

Generally, high-resolution gas chromatography (HRGC), fluorescence probes, electrophysiology and electroantennography (EAG) could be used to sensitively detect semiochemicals. However, there were some drawbacks for practical applications, such as either the advanced instruments used in the experiments were expensive, or the procedures were time consuming and cumbersome due to the labeling of fluorescence probes (Czerny et al.,

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2011; Li et al., 2013; Pickett et al., 2013; Wehrenfennig et al., 2013). Therefore, low-cost and ease of operating approaches with fast response should be developed. Some recent researches indicated that the insect olfaction with broad receptive ranges for chemosensing could provide distinguished responses to a variety of chemicals even if these chemicals do not occur in the animal's natural environment, such as volatile compounds emitted from cancer cells (Strauch et al., 2014). The exceptional olfactory ability had inspired researchers to develop biosensors, which were mainly based on insect behaviors or isolated organs such as antenna (Schroth et al., 1999; Schöning and Poghossian, 2002; Fernández-Grandon et al., 2011), for different analytes detection, like 2,46-trinitrotoluene (TNT) and other explosives (Repasky et al., 2006; Rains et al., 2008), and even for diseases control and diagnosis (Carey and Carlson, 2011; Strauch et al., 2014). Because of the difficulties in interpreting quantifiable behavior responses and the short life time of insect organs (Fernández-Grandon et al., 2011; Wehrenfennig et al., 2013), odorant molecules of natural olfactory systems should be integrated into artificial systems to build specific sensors for semiochemicals detection.

For insects, the prominent selectivity and sensitivity of the olfactory systems are achieved by discriminating filters of the small soluble binding proteins and the odorant receptors (Leal, 2005; Fan et al., 2011; Hansson and Stensmyr, 2011). These soluble binding proteins, which are mainly odorant-binding proteins (OBPs), not only can be expressed and purified easily, but also are highly stable to temperature, pH, solvents and proteases (Wei et al., 2008; Pelosi et al., 2014; Lu et al., 2014). With these excellent properties, biosensors based on mammal OBPs, such as bovine OBP and porcine OBP, had already been studied for detection of ethanol, octenol, and carvone, and obtained high specificity and sensitivity (Hou et al., 2005; Ramoni et al., 2007; Capone et al., 2009; Pietrantonio et al., 2013). For insects, however, only few applications of insect OBPs based biosensors were reported for semiochemical detection. Studies showed that OBPs of honeybee and peptide sequence derived from OBPs of insects (*Drosophila* and *Apis mellifera*) have been used to detect floral odors, alcohols and explosives (Lu et al., 2014, Sankaran et al., 2011; Kuang et al., 2010).

In this paper, an OBP of oriental fruit fly was expressed and purified. Though a self-assembled monolayer, the polyethylene glycol (PEG), the insect OBP was immobilized on the interdigitated electrodes to establish an olfactory biosensor. It could sensitively detect semiochemicals such as asisoamyl acetate, β-ionone, and benzaldehyde that emitted from the fly's host plants by electrochemical impedance. Combined with analysis of molecular docking, the sensors could be used to reflect affinities between insect OBPs and their semiochemicals.

2. Experiment

2.1. Expression and purification of the protein

The expression and purification of the active recombinant BdorOBP2 were cloned from the full-length cDNA of oriental fruit fly, *Bactrocera dorsalis*. Briefly, using reverse transcription-polymerase chain reaction (RT-PCR) and pET-30a (+)/BL21 (DE3) prokaryotic expression system, BdorOBP2 from antenna of oriental fruit flies was cloned and expressed by being transformed into *Escherichia coli* BL21 competent cells. The bacteria were induced by isopropyl β-D-1-thiogalactopyranoside (Merck, Darmstadt, Germany) to initiate the expression of recombinant proteins. After harvesting the bacterial cells, the crude cell extracts, pellet and supernatant were analyzed by 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to check if the recombinant proteins were expressed. Afterwards, the inclusion body of BdorOBP2 was severely precipitated in 1.5 M urea in ddH_2O and finally freeze-dried. The protein was resuspended (67 μg/ml) in phosphate buffered saline (PBS; pH=7.4) and saved under 4 °C for the following biosensor experiments.

2.2. Semiochemicals and reagents

The fruity scents, such as isoamyl acetate, β-ionone, and benzaldehyde, which could specifically bind to BdorOBP2, were used as semiochemicals for biosensing. 4-allylveratrole and butanedione were also tested as the negative control, which were two kinds of widely used odorants in biosensors. Taking 1% methanol as co-solvent, these semiochemicals were prepared with PBS solution for different concentrations: 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M, and 10^{-3} M.

The solvents and reagents, potassium ferricyanide/ferrocyanide ($K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$), PBS, 2-(4-Morpholino) ethanesulfonic acid (MES), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), and α-thio-ω-carboxy poly (ethylene glycol) (MW 2100 Da, COOH–PEG–SH in short) for protein immobilization and impedance detecting were all purchased from Sigma-Aldrich, USA.

2.3. Fabrication of interdigitated electrodes

Interdigitated electrodes, which have uniform electric fields and high electrode coverage, were used to measure impedance changes when semiochemicals binding to BdorOBP2. The fabrication process of the electrode arrays using semiconductor technology had been described in the previous paper (Zhou et al., 2013; Lu et al., 2014). Briefly, A four-inch sterilized Pyrex glass 7740 was chosen as the insulating substrate. After sputtering a layer of Cr

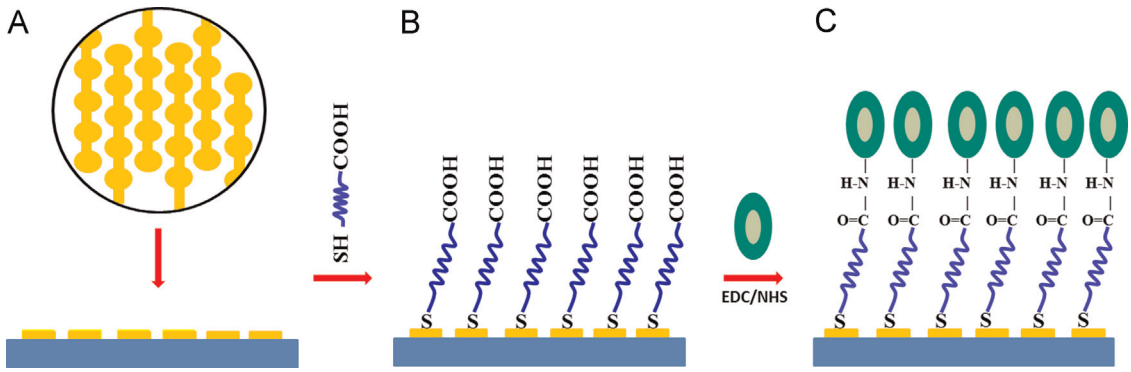

Fig. 1. OBPs immobilized on PEG modified interdigitated electrodes. (A) Sketch map of the interdigitated electrodes, (B) COOH–PEG–SH forms Au–S bonds with gold electrodes, and (C) BdorOBP2 forms covalent amino bonds with PEG-electrodes by EDC/NHS coupling.