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Impedance sensing and molecular modeling of an olfactory biosensor based on chemosensory proteins of honeybee

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ABSTRACT

By mimicking biological olfaction, biosensors have been used for the detection of important ligands in complex environments. An olfactory biosensor based on chemosensory proteins (CSPs) was designed by immobilizing honeybee CSPs (Ac-ASP3) on the interdigitated golden electrodes. Its responses to ligands of pheromones and floral odors were recorded by impedance spectroscopy. The relative decrease of charge transfer resistance of the biosensor is proportional to the logarithm of ligand concentration from 10^{-7} M to 10^{-3} M. To explore the molecular recognition processes of the biosensor, the tertiary structure of the protein was modeled and the protein-ligand interactions were investigated by the molecular docking. Our docking results verified the validity of experiments and showed that the specific ligands could form hydrogen bonds with some of the conserved residues, such as Cys 60 and Gln 64 of Ac-ASP3. Furthermore, combining the molecular modeling with impedance detection, the accuracy, specificity and predictability of the ligands binding to the protein could be improved. Thus, CSPs will provide a promising approach for chemical molecular sensing at low concentrations.

1. Introduction

The olfactory system has the ability to discriminate and identify thousands of odorant compounds at very low concentration. The discovery of membrane-bound olfactory receptors (ORs), in both vertebrates and insects, has shown that they can be directly activated by messenger molecules of odorants and pheromone molecules (Buck and Axel, 1991). At the same time, the soluble proteins in high concentration around ORs, which indicate lots of important chemosensory roles, particularly in insects, are mainly classified as odorant-binding proteins (OBPs) and chemosensory proteins (CSPs) (Pelosi et al., 2005; Vieira and Rozas, 2011). Insects can recognize a wide range of chemical signals across animal species and between congener, during which these sensory proteins play a critical role. The chemosensation guide perception of the surrounding environment, detection of predators and location of food and hosts for insects.

The understanding of the biological olfactory system provides valuable insight into odor detection, especially by biosensors. In recent years, many researchers have been trying to develop olfactory biosensors based on olfactory system (Vidic, 2010; Lee and Park, 2010; Glatz and Bailey-Hill, 2011). In those olfactory biosensors studies, ORs have been the very commonly used sensing elements. In the biosensor system, ORs could be activated by odorant molecules, and then the biology interactions are translated into electrical signals just as what happens to the central nervous system (Minic et al., 2005; Lee and Park, 2010; Liu et al., 2010). However, ORs are G protein coupled receptors, which need to stay in their cellular membrane environment to be functional, so that there are inherent difficulties associated with the availability of ORs. OBPs could increase the solubility of volatile hydrophobic odors and make them available to the ligand-binding site of ORs. So OBPs also act as potential sensing materials in olfactory sensors (Ko and Park, 2008; Hou et al., 2005; Sankaran et al., 2010). CSPs represent another protein family that could bind and transport pheromones or other ligands, associated with conformational changes, but they are barely used in olfactory biosensors.

Insect CSPs, which had been identified in several chemosensory as well as non-chemosensory tissues, are deduced to be multifunctional context-dependent proteins involved in diverse cellular processes (Picimbon et al., 2000; Pelosi et al., 2005).

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Besides mediating the solubilization of hydrophobic odorants, CSPs can bind different lipophilic ligands, so that they are believed to be involved in chemical communications, including perception, identification, transport and transduction of semiochemicals from environments (Briand et al., 2002; Ozaki et al., 2008). Moreover, CSPs are stable in a wide range of pH and temperature, which offers an access to large quantity of CSPs expressed in prokaryote for further research (Picone et al., 2001). All of these facts suggest that CSPs possibly provide a new recognizing approach for olfactory biosensor.

Honeybees, which are able to discriminate among a wide range of odors, have been trained to detect specific odors related to explosives, mines and drugs (Repasky et al., 2006; Rains and Tomberlin, 2008). The honey bee CSPs represent the multifunctional insect CSPs family and become attractive models for gaining insights into the organization and function of the olfactory system, as well as the underlying mechanisms of associative olfactory learning (Forêt et al., 2007; Li et al., 2007). This paper focuses on a kind of antennal CSP of Chinese honeybee, *Apis cerana cerana* (Ac-ASP3), with a purpose of detecting its binding properties. Impedance based biosensors are particularly suited for the detection of binding events on the transducer. An impedance biosensor was designed to detect the binding reactions between Ac-ASP3 and the ligands of pheromones and floral odors. On the other hand, to illuminate the ligand binding and explore the essence of biological reaction, the molecular recognition process was simulated by means of molecular docking. And the correlations between protein conformation and electrical impedance properties were discussed.

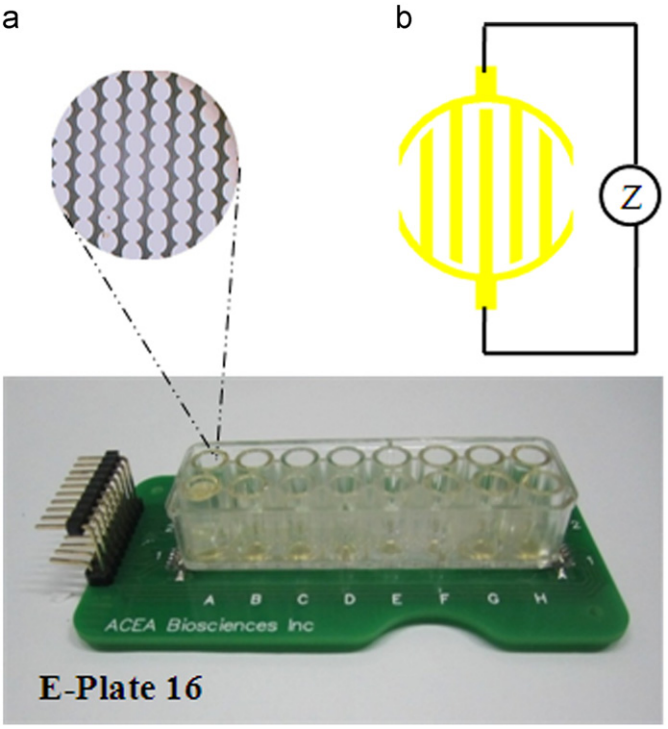

Fig. 1. E-Plate 16 device of the biosensor system. (a) Circle-on-line interdigitated electrodes on the bottom of a well; (b) Sketch map of the impedance measurement of the electrodes.

2. Experiment

2.1. Protein and ligands

The biologically active recombinant Ac-ASP3, whose molecular weight is 18 kD, was cloned from the full-length cDNA of adult worker bees in Chinese honeybee, *Apis cerana cerana* (Li et al., 2007). The protein was prepared in phosphate buffer solution (PBS, pH = 7.4) with a concentration of 130.67 μg/mL.

Ligands of CSPs mostly focus on general volatile odors and pheromones. In this paper, worker bee pheromone (geraniol) and alarm pheromone (isoamyl acetate), with a kind of floral odor (phenyl acetaldehyde) were chosen as tested ligands, additionally, 4-allylveratrole, 3,4-dismethylbenzaldehyde, β-ionone and methy-*p*-hydroxyl benzoate were tested as well. The ligands from 10^{-7} M to 10^{-4} M were diluted with PBS from original concentration of 1 mM in methanol. These reagents were all purchased from Sigma-Aldrich (USA).

2.2. Electrodes processing and electrochemical measurement

The electronic plate (E-Plate 16) with interdigitated electrodes in the bottom of the 16 wells was obtained from ACEA Biosciences Inc. (USA) (shown in Fig. 1). The interdigitated electrodes are circle-on-line electrodes. The diameter of the circle is 90 nm. The width of the line is 30 nm. The distance between two adjacent pairs of electrodes is 80 nm. The electrodes cover approximately 80% of the bottom areas of each well, which allows for maximal sensitivity for the detection of the electrochemical reactions at the electrode interface, with relatively uniform distribution of the electric field while maximizing the coverage area of each well. It has been used successfully for dynamic monitoring of live cells by real-time impedance detection (e.g., Abassi et al., 2004; Atienza et al., 2006). Before protein immobilization, the interdigitated electrodes were cleaned with ultrapure water and dried under nitrogen flow. Nitrocellulose membrane has been used for immobilization of proteins, with its microporous nature of large volume to surface area ratio and high binding capacity to biomolecules, which provides a tremendous number of binding sites per unit area. Several lines of evidence indicate that biomolecules interact with nitrocellulose via a non-covalent, hydrophobic interaction. The nitrocellulose membrane was obtained from Shanghai Haoran Biological Technology Co. (China). Firstly, nitrocellulose dissolved in methanol was added into plate wells in a 5 μL volume which could coat electrodes completely. After about 10 min until the methanol evaporated off, a membrane of nitrocellulose was left on the electrodes. At last, Ac-ASP3 solution was injected into plate wells. A 2-h waiting period was needed to guarantee that the protein molecules were embedded in the nitrocellulose membrane. The residual solution was then drained out with PBS. These operation steps were all conducted at room temperature (18–25 °C).

Electrochemical measurements were performed using the Zahner ZENNIUM electrochemical workstation with the THALES software (Zahner Elektrik, Germany). One pole of the interdigitated electrodes was connected to both the test and sense probes on the electrochemical workstation, and another pole was connected to both the reference and counter probes on the electrochemical workstation (Fig. 1).

The tested frequency was set in the range from 1 Hz to 100 kHz with a 5 mV AC voltage in the impedance measurement program. Ligands at different concentrations were added to the plate wells containing 5 mM K4[Fe(CN)6]/K3[Fe(CN)6] (1:1). At each concentration, electrochemical impedance spectroscopy was recorded for about 40 min. After each measurement, the well was rinsed by PBS for about 15 min. All of electrochemical measurements were performed at room temperature (18–25 °C).

2.3. Molecular modeling

The tertiary structure of Ac-ASP3 was built by I-TASSER server (Zhang, 2008). Based on the amino acid sequence of Ac-ASP3