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Piezoelectric olfactory receptor biosensor prepared by aptamer-assisted immobilization

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ABSTRACT

Inspired by the very high sensitivity and specificity of biological olfactory system, engineers pay much attention to biomimetic olfactory biosensors due to their excellent performance and great commercial prospects. In this study we presented an olfactory receptor (OR)-based piezoelectric biosensor with high sensitivity and specificity for the odorant detection, in which aptamers were employed for the specific and effective olfactory receptor immobilization to improve the overall performance of biosensors. An olfactory receptor of *C. elegances*, ODR-10, was expressed heterologously in HEK-293 cells with a hexahistidine (His_6) tag on the N terminus. His_6-tagged ODR-10 was extracted from the plasma membrane of transfected HEK-293 cells and used as the sensing element of biosensors. Anti-His_6 aptamers were immobilized covalently onto the gold surface of piezoelectric quartz crystal microbalance (QCM) electrode to capture the His_6-tagged ODR-10 specifically, as well as achieve the purification of ODR-10 simultaneously. The immobilization procedures were characterized by electrochemical methods and scanning probe microscopy (SPM). Additionally, physical adsorption method was used as a control method to illustrate the influences of immobilization methods on the performances of OR-based biosensors. The results demonstrated that the piezoelectric biosensor prepared by aptamer-assisted immobilization method showed high specificity and improved sensitivity. The detection limit was as low as 1.5 ppm (v/v). Additionally, the aptamer-assisted receptor immobilization method has great potential to become a universal protein immobilization technique in biosensing, and be applied into the development of OR-based biosensor array for the simultaneous detection of several odorants. This aptamer-assisted olfactory receptor biosensor has great application prospects in many fields, such as food safety, environmental monitoring, and disease diagnosis.

1. Introduction

The nature's olfactory system has an excellent capacity in recognizing and discriminating various odorants. Since the concept of "bio-electronic nose" was proposed in 1998 by Göpel [1], mimicking nature's olfactory system has become a new trend of biosensors for odorant detection. In the biological olfactory system, olfactory receptors (ORs) act as the sensitive elements of olfactory sensory neurons (OSNs), which play a great role in odorant specific recognition and discrimination [2]. ORs belong to the G protein-coupled receptor (GPCR) family. The binding of odorants and ORs will trigger the intracellular signal transduction cascades and induce the depolarization of OSNs. The unique functions of ORs interacting with the specific odorant molecules make them ideal candidates as sensitive elements of biomimetic gas sensors toward odorant detection [3]. Glatz and Bailey-Hill have made a comprehensive comment on mimicking nature's nose, and discussed various olfactory biosensors as well as their applications [4]. Various transduction techniques have been employed in the research of OR-based biosensors, including field effect transistor (FET) [5,6], quartz crystal microbalance (QCM) [7–9], surface acoustic wave (SAW) device [10,11], light addressable potentiometric sensor (LAPS) [12,13], surface plasmon resonance (SPR) [14], bioluminescence resonance energy transfer (BRET) [15], and intracellular calcium responses [16]. Almost all the odorant-induced information can be utilized for olfactory biosensing. However, one major issue in this field is how to achieve functional immobilization of defined ORs with high efficiency on the solid surface of secondary transducers.

The antibody-directed specific immobilization of ORs is more specific and efficient than that of physical adsorption method,

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but the procedure is relative complex and the antibody is much expensive [17]. Recent years, aptamers have been widely viewed as promising complementary molecules to antibodies, which are single-stranded oligonucleic acid molecules [18]. Compared with antibodies, aptamers have a lot of advantages, such as more resistant, being more easily modified with functional groups or tags, and less expensive. They have been used to replace antibodies in many applications, such as enzyme-linked immunosorbent assay (ELISA) [19], Western blot [20], biosensing applications [21], and protein micro-arrays [22]. Concerning the unique advantages of aptamers, this study explore the possibility of using aptamers to achieve the high specific and effective functional immobilization of ORs onto the surface of secondary transducers.

We present an OR-based piezoelectric biosensor prepared by the aptamer-assisted immobilization method. Quartz crystal microbalance (QCM) is a commonly used mass sensitive piezoelectric transducer [9], whose oscillation frequency changes with the mass loading on the crystal. The interactions between ORs and odorants can be detected by monitoring the oscillation frequency changes of QCM. A well-characterized olfactory receptor of *C. elegances*, ODR-10, is employed as a model of ORs. Diacetyl is the only volatile ligand to activate ODR-10 [23], and can induce the intracellular calcium elevation of HEK-293 cells expressing ODR-10 in a dose-dependent way [16]. In the present study, ODR-10 was expressed on the plasma membrane of HEK-293 cells with a His_6-tag on its N-terminus. The surface of gold electrodes of QCM was modified by specific anti-His_6 aptamers via Au—S bonds, which could only capture His_6-tagged ODR-10 specifically through anti-His_6 aptamers. Purification of ODR-10 was also achieved simultaneously due to the specific interactions between aptamers and His_6-tags. The immobilization procedures of ODR-10 were characterized by electrochemical methods and scanning probe microscopy (SPM). The specificity and sensitivity of this OR-based piezoelectric biosensor were tested. In addition, physical adsorption method was used as a control method to illustrate the influences of immobilization methods on the performances of OR-based biosensors.

2. Materials and methods

2.1. Preparation of functional olfactory receptors

Here an olfactory receptor of *C. elegances*, ODR-10, was employed as a model of ORs, which were produced by cellular heterologous expression techniques. The full-length *cDNA* of *odr-10* was amplified by polymerase chain reaction (PCR) from *pEGFP-N1/rho-tag/odr-10* [24]. A pair of specific primers were designed: the forward primer 5′-AGAGGTACCGATGCACCACCACCACCACCA CATGAACGGGACCGAGGGCCCAA-3′, containing the *KpnI* restriction enzyme cutting site (the underlined part) and His_6 sequence (the dotted part); and the reverse primer 5′-TGTGGATCCTCATT-ACTACGTCGGAACTTGAGACAAATTGG-3′, containing the *BamHI* enzyme cutting site (the underlined part). PCR was performed in a 50 μL reaction volume using 1.0 μL of the plasmid template *pEGFP-N1/rho-tag/odr-10*, 1 μL of the forward primer, 1 μL of the reverse primer, 4 μL of 10 mM dNTP mixture, 0.5 μL of STAR™ polymerase, 10 μL of 5× PCR buffer (containing Mg^{2+}), and 32.5 μL of deionized water. The reaction cycle conditions were 30 cycles of 94 °C denaturation for 30 s, 60 °C annealing for 1 min, and 72 °C extension for 1 min. The obtained *His_6-tag/rho-tag/odr-10* sequence was subcloned into the *KpnI* and *BamHI* sites of the expression vector *pcDNA 3.1*(+). The *rho-tag* import sequence was used to facilitate the localization of ODR-10 on the cell plasma membrane. *His_6-tag* can indicate the expression level of ODR-10 and assist the functional specific immobilization of ORs on the sensor surface. The expression vector *pcDNA 3.1*(+)/*His_6-tag/rho-tag/odr-10* was confirmed by restriction enzyme mapping and DNA sequencing.

Then the plasmid *pcDNA 3.1*(+)/*His_6-tag/rho-tag/odr-10* was transfected into the heterologous HEK-293 cells to express ODR-10 on the plasma membrane. HEK-293 cells were cultured in DMEM (Dulbecco's Modified Eagle's Medium) (Gibco, UK) supplemented with 10% fetal bovine serum, penicillin (100 U/mL) and streptomycin (100 μg/mL), at 37 °C in the 5% CO_2 incubator. HEK-293 cells were seeded and cultured in 6-well plates. When being 80% confluent, cells were transfected with 4 μg of the expression plasmid and 10 μL of Lipofectamine™ 2000 (Invitrogen) for each well according to the Lipofectamine™ 2000 instructions. After 48 h, transfected cells were collected for subsequent assays.

Reverse transcription polymerase chain reaction (RT-PCR) was conducted to indicate the expression of ODR-10 at mRNA level. Total RNA was collected from transfected cells by TRIZOL and analyzed with PrimeScript® 1st Strand cDNA Synthesis Kit (Takara). The cDNAs were used as templates for PCR amplification. Forward primer: 5′-AGAGGTACCGATGCACCACCACCACCACCACATGAACGG GACCGAGGGCCCAA-3′, and reverse primer: 5′-TGTGGATCCTC-ATTACTACGTCGGAACTTGAGACAAATTGG-3′. The reaction products were resolved by electrophoresis. The expected length of the target product was 1120 bp.

Western blot was conducted to verify the expression of His_6-tagged ODR-10 at the protein level. Transfected cells were collected and lysed. The protein supernatants were loaded on a SDS-PAGE gel and transferred onto a PVDF membrane. Then the membrane was incubated with the primary antibody anti-His_6 mouse IgG, followed by incubation with the secondary antibody DyLight 680 conjugated goat anti-mouse IgG. The bands were visualized with the Odyssey infrared imaging system. The expected size of target protein was about 45 kDa.

The membrane protein extraction kit (Sangon Biotech, China) was used to extract the functional His_6-tagged ODR-10 from the plasma membrane of transfected HEK-293 cells. About 5×10^8 cells were collected from 6-well plates and washed with ice cold cell wash buffer for three times. After resuspended in 1 mL extraction buffer (containing 1 μL protease inhibitor and 1 μL DTT), cells were sonicated with a sonicator for 3–4 times and centrifuged at 14,000 rpm for 10 min at 4 °C. The supernatant was transferred to a new vial and centrifuged at 13,000 rpm for 5 min at room temperature. The pellet was the total cellular membrane protein, containing proteins from both plasma membrane and cellular organelle membrane. By further centrifuging, 10 μL of the plasma membrane proteins were harvested. This solution was the mixture of total plasma membrane proteins, including His_6-tagged ODR-10, and stored at −70 °C for the development of biosensors.

2.2. Aptamer-assisted olfactory receptor immobilization and characterization

The aptamer-assisted immobilization method was proposed to immobilize the His_6-tagged ODR-10 efficiently and specifically. Its schematic was shown in the inset of Fig. 1. Before immobilization, the gold electrode surface of QCM was cleaned thoroughly to remove inorganic and organic contaminants, and then incubated with a mixture solution for 21 h to form a self assembly monolayer (SAM) through Au—S bonds, which contained 5 μM of thiol-modified anti-His_6 aptamer molecules (5′-HS-$(CH_2)_6$-GCTATGGGTGGTCTGGTTGGGATTGGCCCCGGGAGCTGGC-3′) [25] and 50 μM of blocker molecules 11-mercaptoundecanoic acid (11-MUA) (Sigma, USA). 11-MUA was used to block free sites of the electrode surface and provide suitable steric spaces for the aptamers. Thereafter the gold electrode was rinsed with ethanol thoroughly and dried under the nitrogen flow. 10 μL of the plasma membrane extraction protein solution, containing the His_6-tagged