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Enhancement of odorant detection sensitivity by the expression of odorant-binding protein

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Abstract

Odorant-binding proteins are low molecular weight, soluble proteins that are secreted by glands of the nasal cavity. Their function is known to be the transport of hydrophobic odorants. This feature is important to artificial olfactory biosensors, which operate in the aqueous phase. In this study, one of rat odorant-binding proteins, OBP3, was inserted into a mammalian expression vector pcDNA3, expressed, and secreted from human embryonic kidney-293 (HEK-293) cells. The his_6 tag and signal peptide of the prelysozyme (plys) were fused with OBP3 for the detection and secretion of the proteins, respectively. The secretion level of OBP3 was maximal at 3 h of incubation time. The secreted OBP3 increased the solubility of a hydrophobic odorant, octanal, which is the specific odorant of rat olfactory receptor I7. The secreted OBP3 also bound to olfactory receptor I7. These interactions consequently increased the cellular signal intensity stimulated by the odorant in the cells expressing olfactory receptor I7. Our findings indicate that odorant-binding protein can be effectively used to increase the sensitivity of olfactory receptor-based biosensors.

Keywords: Odorant-binding protein; Olfactory receptor I7; HEK-293; Olfactory receptor-based olfactory biosensor

1. Introduction

The first step in smell perception is the activation of G-protein-coupled receptors, which are designated as olfactory receptors (ORs), by odorant molecules. This occurs at the cilia of olfactory neural cells in aqueous nasal mucus (Breer et al., 1994). An odorant is defined as any molecule capable of stimulating the olfactory neurons (Pelosi, 1994). An odorant molecule should be dissolved in aqueous mucus phase in order to stimulate the olfactory neurons. However, odorant molecules are mostly hydrophobic, and are only slightly dissolved in aqueous phase. Odorant-binding proteins (OBPs), which are abundant in mucus, are considered to play a role in dissolving hydrophobic odorants into an aqueous compartment of the mucus phase (Bignetti et al., 1985; Pevsner et al., 1985; Pelosi, 1994, 1996). The OBPs are small, water-soluble, extracellular proteins found in the aqueous mucus in the olfactory epithelium. These proteins belong to the lipocalins, which are a large family of carrier proteins that are generally involved in the transport of hydrophobic molecules in aqueous media (Flower, 1994; Pevsner et al., 1988). The molecular mass of OBPs are approximately 20,000 Da. These proteins reversibly bind to odorants with dissociation constants in a micromolar range (Briand et al., 2000; Paolini et al., 1999).

Since a bovine OBP was first discovered for its binding ability, several OBPs have been identified in a variety of vertebrates including rat, pig, mouse, and rabbit (Pelosi et al., 1982; Pevsner et al., 1988; Dal Monte et al., 1991; Dear et al., 1991a,b; Pes et al., 1992, 1998; Pes and Pelosi, 1995). OBPs are synthesized by glands of olfactory epithelium and secreted into olfactory mucus (Ohno et al., 1996; Utsumi et al., 1999).

The functions of olfactory receptors expressed in heterologous cells have been investigated, and biosensors that utilize these olfactory receptors have been developed (Zhang et al., 1997; Ko and Park, 2005, 2006; C.-S. Lee et al., 2006; J.Y. Lee et al., 2006; Sung et al., 2006). These biosensors were developed based on the interaction between olfactory receptors and odorant molecules. OBPs are expected to enhance the sensitivity in these molecular interactions, especially when odorant molecules are highly hydrophobic. In a recent decade, OBPs were expressed in *E. coli* and purified in order to identify their functions (Löbel

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et al., 1998, 2001; Briand et al., 2000). However, OBPs have not been expressed in animal cells, and the functions of OBPs on hydrophobic odorants and the potential interactions between OBPs and ORs have not been clearly identified.

In this study, human embryonic kidney-293 (HEK-293) cells were transiently and stably transfected with an OBP gene that was genetically manipulated for the secretion of an OBP using a signal sequence of the prelysozyme. Application of the OBP to an artificial olfactory biosensor system was investigated in order to increase its sensitivity, which is among the important issues that must be considered in relation to these biosensors, together with other issues such as effective secondary transducer systems (Choi et al., 2004; Kang et al., 2006; C.-S. Lee et al., 2006; J.Y. Lee et al., 2006; Park et al., 2006) and apoptosis on a cell-based biochip (Kim and Park, 2003).

2. Materials and methods

2.1. Reagents

Tris, dimethyl sulfoxide (DMSO), glycerol, urea, ethylenediaminetetraacetic acid (EDTA), *N*-lauroylsarcosine, β-mercaptoethanol, bromophenolblue, sodium dodecyl sulfate (SDS), $C_4H_4KNaO_6{\cdot}4H_2O$, NaOH, $CuSO_4{\cdot}5H_2O$, NaCl, KCl, $MgCl_2$, $CaCl_2$, glucose, HEPES, acetone, protease inhibitor cocktail, and octanal were all purchased from Sigma–Aldrich (USA). Skim milk was purchased from DIFCO (USA). The ECL (enhanced chemiluminescence) system, anti-his antibody, polyclonal anti-mouse Cy2 antibody, and HRP-linked mouse IgG were purchased from Amersham-Pharmacia Biotech (UK). Fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), penicillin (100 U/ml), streptomycin (100 μg/ml), G418, and Lipofectamine plus reagent were all purchased from GIBCO BRL (USA). Restriction enzymes were purchased from Takara (Japan). *Taq* polymerase was purchased from Super-bio (Republic of Korea). pcDNA3, pVgRXR, pIND vector, and ponasterone A were purchased from Invitrogen (Netherlands).

2.2. Procedures

2.2.1. Cloning of the OBP gene

A signal peptide DNA of the prelysozyme, which is designed to contain two restriction enzyme sites of KpnI and EcoRV, was synthesized and hybridized (Genotech Co., Korea). This DNA fragment was digested with KpnI–EcoRV and cloned into a pcDNA3 vector digested with the same restriction enzymes.

A rat odorant-binding protein3 gene (OBP3) containing the *his$_6$* gene was amplified from pQE31-OBP3 by PCR, using the following primers: OBP3-N (EcoRV); 5′-ATGAGGATATCTCACCATCACCAT-3′, OBP3-C (XhoI); 5′-ATCAACTCGAGTCCAAGCTCAG-3′. PCR was performed with a mixture of *Taq* polymerase, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 mM of each primer, and 100 ng templates according to the following protocol: 36 cycles at 94 °C for 1 min, 52 °C for 40 s, and 72 °C for 40 s. The PCR product was double-digested with EcoRV–XhoI and inserted into the pcDNA3 vector containing signal peptide DNA of the prelysozyme that had previously been digested with the same restriction enzymes. pcDNA3-plys-his$_6$-OBP3 was sequenced to identify the correct clone.

2.2.2. Cloning of the olfactory receptor I7 gene

The rat olfactory receptor *I7* gene was amplified by PCR with pVL-I7 as a template using the following primers: I7-N (EcoRI); 5′-GAATTCATGGAGC GAAGGAAC-3′, I7-C (EcoRV); 5′-TCTGATATCGACCTAACCAATT-3′. The rho-tag import sequence was obtained by digesting pBK-CMV containing *rho-tag* DNA with BamHI–EcoRI, and the resulting 60 bp fragment was subcloned into a pIND vector (Invitrogen) that had previously been digested with BamHI–EcoRI. The pIND-rho clone was identified by sequencing. Finally, the *I7* gene obtained by PCR as described above was digested with EcoRI–EcoRV and inserted into these vectors, which were previously digested with the same restriction enzymes.

2.2.3. Transfection of olfactory receptor I7 genes into HEK-293 cells

Human embryonic kidney-293 (HEK-293) cells were grown in DMEM supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 μg/ml) in 5% CO_2 at 37 °C. Transfection was performed as described in our previous work (Ko and Park, 2006).

2.2.4. Construction of HEK-293 cells stably expressing OBP3

HEK-293 cells were grown on six-well plates and transfected with 2 μg of pcDNA3-plys-his$_6$-OBP3 using Lipofectamine plus reagent. HEK-293 cells stably expressing OBP3 were obtained and selected by treatment with G418 (500 μg/ml) for 2–3 weeks.

2.2.5. RT-PCR for identifying OBP3 mRNA

HEK-293 cells were grown on six-well plates and transfected with 2 μg of pcDNA3-plys-his$_6$-OBP3 using Lipofectamine plus reagent. The control HEK-293 cells and the HEK-293 cells that were transiently transfected with OBP3 were cultivated for a day, harvested, and lysed. The total RNAs were isolated using PUREscript (GENETRA system, USA). The cDNA of OBP3 was synthesized from the total RNAs and amplified by the following PCR protocol: 36 cycles at 94 °C for 1 min, 52 °C for 1 min, and 72 °C for 1 min.

2.2.6. Protein preparation

HEK-293 cells were cultivated in two T-25 flasks. HEK-293 cells in one flask were transfected with 4 μg of pcDNA3-plys-his$_6$-OBP3, and cells in another flask were used as a control. After incubation for 6 h, the medium was exchanged with fresh medium containing serum, and cells were further cultivated for 24 h. The medium was then exchanged with the standard solution (140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5 mM glucose, 10 mM HEPES, pH 7.4). After