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Cell-based measurement of odorant molecules using surface plasmon resonance

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Abstract

The surface plasmon resonance (SPR) technique has been used for characterizing molecular interactions. Recently, this technique was used for the analysis of interactions between living cells and molecules reactive to cells. In this study, the technique was applied to the cell-based measurement of odorant molecules. HEK-293 cells were used as a heterologous cell system, and ODR-10, the olfactory receptor of the nematode *Caenorhabditis elegans*, as a model olfactory receptor. The ODR-10 was expressed on the cell surface, with the help of the rho-tag import sequence, which was fused at the N-terminus of the ODR-10. Exposure of the cells to 0.1 mM diacetyl, which is an odorant molecule specific to the ODR-10, induced a SPR signal from the HEK-293 cells expressing ODR-10, while no SPR signal was detected from the control HEK-293 cells. The intensity of the induced signal was dependent on the dose of diacetyl. These SPR signals were regarded as a result of the intracellular signaling triggered by the binding of odorant molecules to the olfactory receptors. This method, using a heterologous olfactory cell system and SPR system, can be efficiently used to identify the odorant molecules specific to each olfactory receptor.

Keywords: Surface plasmon resonance; HEK-293 cell; Olfactory receptor; Diacetyl

1. Introduction

Olfactory receptor proteins (ORs), which are the largest G protein coupled receptor family, are characterized by seven transmembrane-spanning proteins [1]. The binding of odorant to the OR changes its relative orientation and triggers the activation of an intracellular signaling cascade via a specific G protein. Subsequently, second messengers, cAMP and IP_3 (inositol triphosphate), are produced by the action of adenylate cyclase and phospholipase C, respectively [2,3]. These second messengers induce the inflow of ions by activating the membrane channels. Several ORs have been expressed in heterologous cell systems, with most of their functions measured by Ca^{2+} imaging assay and QCM (quartz crystal microbalance) [4–10]. However, the Ca^{2+} imaging assay is cost- and time-consuming, and the assay using QCM shows only the interaction between the OR and odorant, without detecting the change in the intracellular molecules resulting from the signal transduction. Therefore, the development of a more efficient assay tool for the identification of odorants specific to hundreds of ORs is required.

The surface plasmon resonance (SPR)-based biosensor has been used to study biomolecular interactions, which it is able to do in real time [11,12]. This technology has made it possible to perform a real-time analysis of the amounts of bound ligand and the rates of association and dissociation of biomolecules with high precision and without the need for labeling [13,14]. It is an optical technique that uses the evanescent wave phenomenon to measure changes in the refractive index very close to a sensor surface. SPR has been used in studying molecular interactions over a wide range of analyte molecular weights and for biological membrane events. Several researches have reported on the characterization of G-protein coupled receptors using SPR [15–18]. SPR technology can also be used as a biosensor together with other technologies [19,20].

Recently, Hide et al. applied the SPR-based biosensing technique to the analysis of cultured living mast cells [21]. The evanescent field on the surface is in the range of several hundreds nm, while the thickness of cell body is several μm. This means that the surface of cells is out of the range of the detectable

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evanescent field. However, they obtained the SPR signals generated from the IgE-sensitized cells stimulated by an antigen, which suggest the SPR signals are derived from the intracellular signaling rather than simple binding kinetics between the ligands and their receptors on the cell surface. Protection of cells from apoptosis is also an important issue for cell-based biosensor on a chip [22,23].

In this study, we applied the SPR technique to analyze the function of an OR in a living heterologous cell system. ODR-10 and diacetyl were used as a model olfactory receptor and model odorant molecules, respectively. The diacetyl is an odorant molecule specific to the ODR-10. When odorants interact with cells expressing an OR, the signal transduction arises from the cAMP or IP_3 pathways. Subsequently, the changes of intracellular components, for example inflow of ions, occur within cells. It is expected that these changes might be detected by SPR. The SPR system was used to detect these intracellular changes resulting from the signal transduction induced by the binding of odorant molecules with the OR on the surface of HEK (human embryonic kidney)-293 cells.

2. Materials and methods

2.1. Apparatus

For a SPR system, SPR*i* (K-MAC, Korea) and sensor chips with bare gold surface were used. The chip (18 mm × 18 mm) was constructed of one body with a prism.

2.2. Reagents

The running buffer for SPR analysis had the following composition: 140 mM NaCl, 5.4 mM KCl, 1 mM $MgCl_2$, 10 mM HEPES [pH 7.4], 5 mM glucose and 1.8 mM $CaCl_2$. The Ca^{2+}-free running buffer for the Ca^{2+} assay had the following composition: 140 mM NaCl, 5.4 mM KCl, 1 mM $MgCl_2$, 10 mM HEPES [pH 7.4], 5 mM glucose and 2 mM EGTA. The PBS (phosphate-buffered saline, pH 7.4) consisted of 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 and 1.8 mM KH_2PO_4. These chemicals and the diacetyl, dimethyl sulfoxide (DMSO), 99% ethanol and poly-D-lysine were purchased from Sigma–Aldrich (USA). Polyclonal anti-mouse Cy2 antibody was purchased from Amersham-Pharmacia Biotech (UK). *Taq* polymerase was purchased from Super-bio (Korea). Restriction enzymes and DNase were purchased from Takara (Japan). The DMEM, fetal bovine serum (FBS), penicillin (100 U/ml), streptomycin (100 μg/ml) and lipofectamine plus reagent were all purchased from GIBCO BRL (USA).

2.3. Cloning of odr-10 and import sequence genes

The *odr-10* gene was obtained by the PCR of *pBluscript SK*$^+$*/odr-10*. PCR amplification was performed using a mixture of *Taq* polymerase, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 mM of each primer and 100 ng plasmid template DNA according to the following protocol: 36 cycles of 94 °C for 1 min, 52 °C for 1 min and 72 °C for 1 min. The forward and reverse primers were 5′-GTTGGAATTCATGTCGGGAGAATTG-3′ and 5′-TAGAAGGCACAGTCGAGG-3′, respectively. The *odr-10* gene sequence was described elsewhere [24]. The *rho-tag* gene was obtained by digesting *pBK-CMV/rho-tag* with *Bam*HI-*Eco*RI, and the resulting 60 bp DNA fragment subcloned into the *pcDNA3* vector (Invitrogen, Netherlands) digested with *Bam*HI-*Eco*RI. The *odr-10* coding sequence was digested with *Eco*RI, and the resulting sequence inserted into *pcDNA3/rho-tag* that had previously been digested with the same restriction enzymes. The *pcDNA3/rho-tag/odr-10* clone was sequenced.

2.4. Culture and transfection of HEK-293 cells

HEK-293 cells were grown in DMEM (Dulbecco's modified eagle medium), supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/ml) and streptomycin (100 μg/ml), in 5% CO_2 atmosphere at 37 °C. Before transfection, the cells were seeded on 6-well and 24-well plates (Nalge Nunc International, USA) and then incubated for one day. Transfection was performed with 1 μg (6-well) and 0.3 μg (24-well) of *odr-10* gene-containing plasmids using lipofectamine plus reagent.

2.5. Immunocytochemistry

HEK-293 cells transfected with the *odr-10* clone were cultivated on 24-well plates for one day, as previously mentioned above. The immunocytochemistry followed a procedure described previously [10].

2.6. RT-PCR

HEK-293 cells were seeded on two 6-well plates and cultivated for 1 day. The cells on one plate were transfected with the *odr-10* clone and cultivated in DMEM supplement with 10% FBS for 1 day. The cells were then harvested and lysed. The total RNA was isolated using PURE*script* (GENTRA Systems, USA), treated with DNase at 37 °C for 1 h, and heat-treated at 80 °C for 10 min. The cDNA of the *odr-10* was synthesized from the total RNA, and then amplified by PCR. The PCR conditions were: 36 cycles of 94 °C for 1 min, 52 °C for 1 min and 72 °C for 1 min. The forward and reverse primers were 5′-GGATCCCGAGCTTGGGTTCG-3′ and 5′-TCACGTCGG AACTTGAGACAA-3′, respectively. The cells on the other plate were also harvested, prepared and followed the same procedure outlined above. The PCR product was analyzed by gel electrophoresis.

2.7. Culture of HEK-293 cells on the gold surface of a sensor chip

The gold surface of SPR analysis chip is not suitable for the culture of cells and therefore, was treated with poly-D-lysine to allow a stable attachment of cells. The gold surface was immersed in 0.1 mg/ml poly-D-lysine solution. After treating for about 2 h at 4 °C, the gold surface was washed five times with ddH_2O, and then immersed in 70% ethanol. This was then sterilized by ultraviolet irradiation for 1 day, and rinsed five times with PBS before use. Cells were seeded on the sterilized gold chip and incubated in DMEM supplemented with 10% FBS.

2.8. Measurement

Cultured HEK-293 cells were maintained for one day, to allow their good adherence to the gold surface, and the *odr-10* clone was transfected into the cells. The sensor chip, with a 50 nm thick gold film, was positioned in a flow-cell unit of the SPR apparatus, with one chamber on each chip according to the manufacturer's instructions. The cells were perfused with running buffer at a flow rate of 50 μl/min. Running buffer (125 μl), with diacetyl, was injected from the side of the inlet at a flow rate of 50 μl/min. The temperature was kept constant at 20.0 ± 0.1 °C during the measurement.

3. Results and discussion

3.1. Expression of olfactory receptor on the surface of HEK-293 cell membrane

To express the ODR-10 protein on the cell surface, a rho-tag import sequence was fused at the N-terminus of the receptor protein [4]. The *rho-tag* gene was inserted into the *Bam*HI/*Eco*RI sites of the *pcDNA3* vector, and the *odr-10* gene cloned into the *Eco*RI site of a *pcDNA3/rho*. The HEK-293 cells were used as a heterologous cell system, as they exhibit high trans-