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Electrochemical study of human olfactory receptor OR 17–40 stimulation by odorants in solution

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Abstract

The human olfactory receptor OR 17–40 co-expressed with α-subunit of G_{olf} protein in yeast was attempted as a bio recognition part of impedimetric biosensor. The receptor in its natural membrane environment was anchored to a gold-coated glass substrate modified with thiol-based multilayer. Step-by-step building-up of the OR 17–40 based biofilm was monitored using surface plasmon resonance technique. Stimulation of the OR 17–40 with its cognate odorant helional in phosphate-buffered saline was probed by means of electrochemical impedance spectroscopy under various conditions. Activation of OR 17–40 in the presence of GTP-γ-S at 4 °C was found to improve the sensitivity of the developed label-free biosensor, probably via the enhancement of the specific biochemical signal.

Keywords: Impedance spectroscopy; SPR; Bioelectronic nose; Human olfactory receptor; Helional

1. Introduction

Simultaneous detection of different odorants in liquid and gaseous mixtures is extremely important for quality control in food, beverage and fragrance industries. Nowadays, the instruments capable of a broad-band routine analysis of complex aroma are mainly represented by mass spectrometers, or gas chromatographs combined to mass spectrometers (GC/MS). However, a spectrum of odor peaks obtained by means of GC/MS does not actually provide sufficient information about aroma quality itself which, however, directly arises from the human odor impressions [1]. The arrays of semiselective sensors coupled to the pattern-recognition systems and called *artificial (electronic) noses* [1–3] present another kind of analytical instrumentation that mimics natural olfactory system operation. As sensitive elements of electronic noses, conductive organic polymers [4,5], porphyrins [6], calixarenes [7–9], can be used.

In the last years, the curiosity of scientists to achieve a deeper understanding of the functional peculiarities of olfactory system of vertebrates has stimulated the development of a *bioelectronic nose* [1,10]—multisensor device whose biorecognition part consists in natural G protein coupled olfactory receptors. Many recent reports concern the elaboration of individual sensors based on the ORs expressed either in olfactory sensory neurons [10] or in heterologous cells [11–14] and coupled to various transducers like quartz-crystal microbalance [13,14], light-addressable potentiometric sensor [10], substrates for the SPR (surface plasmon resonance) [11] and the EIS (electrochemical impedance spectrometry) [12] techniques.

Some authors consider the human olfactory receptors to be narrowly selective for the individual odorants in contrast to the rodent ORs which are able to respond to a large repertoire of ligands [15]. However, the sensitivity of human nose does not

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arise from the values of binding constants of odorant-receptor coupling itself but rather from the signal processing in the higher brain structures [2], and therefore a choice of appropriate data processing model is an extremely important step in the bioelectronic nose elaboration.

Artificial neural networks are one of the most widely used pattern-recognition models [16,17] dealing with complex non-linear dose-dependence relations and therefore can be very suitable for the bioelectronic noses. However, dealing with time-consuming complex training of such networks requires very high stability of ORs coupled to the transducers and a satisfactory low level of stochastic responses as well.

Previously, our group has studied the sensitivity of heterologously expressed rat OR I7 [12] and human OR 17–40 [11] towards a set of different odorants, by means of the EIS and the SPR technique, respectively. In this work, for the first time, a stimulation of SPR chip-coupled OR 17–40 by two ligands, preferential (helional) and unrelated (heptanal), was probed by means of nonfaradaic EIS. In order to improve a resolution of such an impedimetric label-free sensor via the enhancement of the biochemical signal, ligand-receptor interactions were also investigated in the presence of the guanosine triphosphate (GTP-γ-S).

2. Materials and methods

2.1. Biomaterials and chemicals

Gα_{olf} protein and human OR 17–40 tagged with cmyc sequence on its N-terminus were co-expressed in yeast *Saccharomyces cerevisiae* (strain MC18) [18] and the membrane fraction was prepared as described in [11]. Stock suspension of membrane fraction with protein content 2.85 mg/ml was aliquoted and frozen at −80 °C. Monoclonal anti-cmyc antibody (Ab) was obtained from Roche Molecular Biochemical and biotinylated by means of DSB-X™ Biotin Protein Labeling Kit (Molecular Probes, Leiden, Netherlands). Stock solution of Ab (2.55 mg/ml) was divided into aliquots and stored at −20 °C.

16-Mercaptohexadecanoic acid (90% purity) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-biotinyl sodium salt (biotinyl-PEA) were purchased, respectively, from Aldrich and Avanti Polar Lipids. GTP-γ-S (guanosine-5′-O-(3-thiotriphosphate); MW 563, 93% purity), bovine serum albumin (BSA; 98% purity) and goat IgG were obtained from Sigma, neutravidin—from Pierce. Ethanol (99.8%), HCl (37%), HNO_3 (65%), H_2O_2 (30%) and NH_4OH (25%) were purchased from Fluka and used as received.

Dimethyl sulfoxide (DMSO) for odorant dilution and heptanal were obtained from Sigma. Helional was a kind gift from Givaudan-Roure (Switzerland).

As a working buffer, a phosphate-buffered saline (PBS) was used with the following composition: 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 3 mM KCl, 150 mM NaCl, pH 7.0 [19]. All reagents for PBS preparation were of analytical grade. All aqueous solutions were prepared with ultrapure water from Milli-Q system.

2.2. SPR technique and gold-coated substrates

Surface plasmon resonance spectrometer “Biosuplar 3” (www.micro-systems.de) was developed at the Institute of Semiconductor Physics of the National Academy of Sciences of Ukraine (Kyiv). This optoelectronic device based on the phenomenon of the surface plasmon resonance in the Kretchmann’s optical configuration was controlled by a computer via self-developed software. Gold film (45 nm) deposited through Cr adhesion layer (1–1.5 nm) onto a glass chip represented the sensor surface. An incident beam of p-polarized light from a semiconductor laser diode ($\lambda = 650$ nm) excited an oscillation of electronic plasma (i.e. surface plasmon) in this metallic film. A special prism capable of rotation on a computer-defined angle provided optimal conditions for the plasmon excitation. A plasmon resonance itself was registered as a drastic decrease of reflected light intensity.

In the present study, the SPR spectrometer flow cell (V~20 μl) was connected to a Gilson Minipuls 3 pump.

2.3. Pretreatment of sensor surface

Before work, substrates were cleaned by a mixture “aqua regia” ($H_2O + HCl + HNO_3$, 16 + 3 + 1, v/v) during 1–1.5 min and then—with a basic mixture ($H_2O + H_2O_2 + NH_4OH$, 5 + 1 + 1, v/v) during 15 s. Thoroughly rinsed with water, every chip was then immersed into ethanol for several seconds.

2.4. Self-assembly of the mixed layer onto gold

To obtain mixed self-assembled monolayer (SAM) on gold surface, 1 mM mercaptohexadecanoic acid and 0.1 mM biotinyl-PEA dissolved in ethanol were incubated with freshly cleaned chip during 21 h [20]. Mercaptohexadecanoic acid is fixed onto Au via the chemisorption of SH-groups, as to biotinyl-PEA, it can be inserted between long-chain thiols due to the numerous hydrophobic interactions. Such mixed SAM provides a good basis for the further anchoring of biomolecules to the chip surface. To elute unfixed molecules, chip was rinsed with ethanol, and dried under a nitrogen flow.

2.5. Blocking step and formation of the upper supporting layers

In order to saturate all non-specific adsorption sites on SAM surface, heterogeneous layer was treated by 1 mM solution either of IgG or BSA in PBS in the flow cell: 0.3 ml of blocking molecules solution was run on the chip at the flow rate (FR) 0.04 ml/min.

Neutravidin (0.5 μM, 0.3 ml solution in PBS) and biotinylated anti-cmyc Ab (0.5 μM, 0.3 ml solution in PBS) were subsequently run on the blocked SAM at the FR 0.04 ml/min. Before the formation of any upper molecular layer, the previous one was rinsed with PBS during 5–15 min.

2.6. Preparation and immobilization of OR 17–40

Stock suspension of OR 17–40 in its natural membrane fraction was thawed and resuspended in PBS on ice up to the