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BIOPHYSICS LETTER

A new concept of olfactory biosensor based on interdigitated microelectrodes and immobilized yeasts expressing the human receptor OR17-40

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Abstract This work shows the feasibility of an olfactory biosensor based on the immobilization of *Saccharomyces cerevisiae* yeast cells genetically modified to express the human olfactory receptor OR17-40 onto interdigitated microconductometric electrodes. This olfactory biosensor has been applied to the detection of its specific odorant (helional) with a high sensitivity (threshold 10^{-14} M). In contrast, no significant response was observed using a non-specific odorant (heptanal), which suggests a good selectivity. Thus, this work may represent a first step towards a new kind of bioelectronic noses based on whole yeast cells and allowing a real time monitoring of olfactory receptor activation.

Keywords Olfactory biosensor · Conductometric electrodes · Odorant · Human olfactory receptor

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Abbreviations

OR	Olfactory receptor
PBS	Sodium phosphate buffer saline
SSTR2	Somatostatin receptor 2
DMSO	Dimethylsulfoxide

Introduction

The mammalian olfactory system can identify and discriminate a great number of odorants (Buck and Axel 1991; Firestein 2001). Detection of these different odorants results from the association of the odorant molecules with olfactory receptors (ORs), carried by olfactory sensory neurons. The discovery and characterization (Buck and Axel 1991) of the great multigene family encoding ORs opened the way for both fundamental and biotechnological investigations. The expression of many olfactory receptor genes was carried out in different heterologous systems (Mombaerts 2004), in particular yeast (Minic et al. 2005a, b). Progress in the field of olfaction makes attractive the idea to develop an artificial odor-sensing system (bioelectronic nose). These systems may have many potential applications in different fields: medicine, environment, food or cosmetic industries, etc.

Recently, scientific studies describing systems for odorants detection using different bio-receptors have been published. Some of them are based on the direct immobilization of fractions of the olfactory mucosa (Wu 1999), or from the membrane fraction of cells expressing an olfactory receptor (Gomila et al. 2006; Hou et al. 2007; Sung et al. 2006; Vidic et al. 2006). We reported the selective growth of whole yeast cells made to express olfactory receptors, in response to their odorant ligand stimulation (Pajot-Augy et al. 2003), and their bioluminescent response due to

activation of a luciferase reporter (Minic et al. 2005a). However, up to now, no olfactory biosensor based on direct electrical measurements on whole genetically modified cells has been described.

In order to develop this whole-cell olfactory biosensor, we immobilized modified *Saccharomyces cerevisiae* yeast cells [described in Minic et al. (2005a)] expressing the human olfactory receptor OR17-40 on interdigitated thin film microelectrodes. The purpose was to detect the conductometric changes of yeasts heterologously expressing OR1740, due to ionic exchanges resulting from the recognition of the odorant ligand molecule by the olfactory receptor.

Experimental

Materials

The OR17-40 olfactory receptor, first human olfactory receptor characterized (Wetzel et al. 1999), was expressed heterologously in the yeast *S. cerevisiae* as previously reported (Minic et al. 2005a). For yeast culture: adenine, glucose and galactose were purchased from Sigma; yeast nitrogen base (YNB) from Difco; adenine, synthetic dropout CSM media without HIS, LEU, TRP, URA (CSM-X) from QBiogen; lactic acid from Fisher. The odorant helional was a generous gift from Givaudan-Roure, courtesy of Boris Schilling (CH-Dubendorf); heptanal (purity 95%) was purchased from Aldrich. Dimethylsulfoxide (DMSO) (purity ≥99.9%), and poly(lysine) were bought from Sigma.

Preparation of culture media

Different culture media were prepared as follows, medium A: 6.7 g YNB + 0.74 g CSM-X in 950 mL ultrapure water; glucose medium: medium A + 40 mg/L adenine + 2% glucose; lactate medium: medium A + 40 mg/L adenine + 3% lactate; galactose medium: medium A + 40 mg/L adenine + 2% galactose.

Yeast culture

Saccharomyces cerevisiae yeast strains based on MC18, kindly provided by Pr. Ian Connerton, U. Nottingham, UK, were cultured as follows: (1) growth in 2 mL glucose medium for 24 h at 30°C under 200 rpm agitation to reach an optical density (OD) at 600 nm between 1 and 3; (2) centrifugation and washing: cultures were centrifuged for 5 min at 2,500×*g* then two times washed with sterilized ultrapure water to remove glucose; (3) incubation at 30°C for 4–6 h in 2 mL lactose medium to completely eliminate glucose; (4) centrifugation: at 2,500×*g* for 5 min; (5) induction of receptors expression [human olfactory receptor 17-40, or rat somatostatin receptor SSTR2 (Price et al. 1995)] in 5 mL galactose medium at 15°C for 60 h.

Sensor design

The conductometric transducer is composed of two identical pairs of gold interdigitated electrodes (thickness 0.5 mm, dimensions 5 × 30 mm) fabricated by vacuum deposition on a ceramic substrate (sintered aluminum oxide) at the Institute of Semiconductors Physics, Kiev, Ukraine (Fig. 1a). An intermediate layer of chromium (0.1 µm thick) was used for better gold adhesion. Each finger of the electrode was 20 µm wide and 1 mm long, with 20 µm spacing between fingers of the electrodes in the pair. To delimitate the sensitive area of the transducer, the central part of the chip was covered with epoxy resin.

Yeast immobilization

Saccharomyces cerevisiae yeasts were immobilized on the interdigitated gold microelectrodes coated with poly(lysine): one drop (0.7 µL) of 0.1% polylysine solution was deposited on the two pairs of electrodes (reference and working electrode). The transducer was then dried 15 min at room temperature. After drying, one drop (0.7 µL) of yeast suspension was deposited on the surface of each pair of electrodes (*S. cerevisiae* expressing rat SSTR2 receptor on the reference electrode and *S. cerevisiae* expressing human OR17-40 on the working electrode). Finally, electrodes were dried for 20 min at room temperature. In Fig. 1b yeasts deposited on electrode surface were visualized under the optical microscope. The spherical form of yeasts is clearly visible (showed in Fig. 1b with red circles).

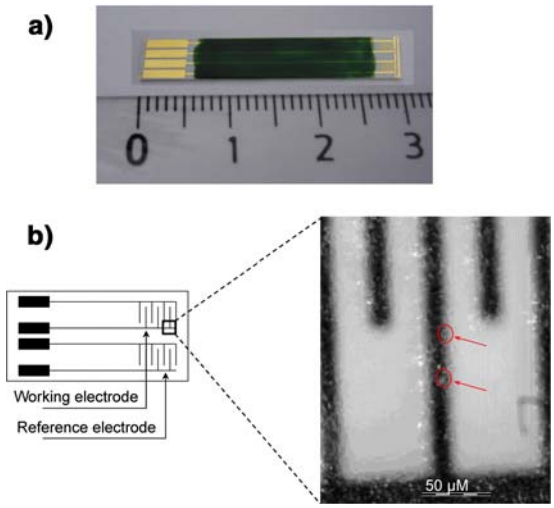

Fig. 1 **a** Gold conductometric transducer. **b** Schematic representation and microscopic view of interdigitated microelectrodes with deposited yeast cells

Preparation of odorant solutions

Stock solutions of odorants (10^{-1} M) were freshly prepared before experiments by dilution of pure odorant in DMSO. A dilution of 10^{-4} M was then directly prepared in ultrapure water, and further dilutions were obtained by successive 1:10 dilutions in ultrapure water. To take into account a possible solvent effect in biosensor response, control solutions at the various dilutions were prepared, replacing the odorant by ultrapure water.

Measurements

Measurements were performed in 5 mL of 150 mM NaCl, pH 7.4, 10 mM sodium phosphate buffer saline (PBS) at room temperature under magnetic stirring. A sinusoidal wave of 100 KHz frequency with small-amplitude alternating voltage (10 mV peak-to-peak about 0 V) was applied by a generator. These conditions were used to reduce faradaic processes, double-layer charging and concentration polarization at the microelectrode surface. After stabilization of output signal, 100-fold concentrated odorant solutions were added into the measurement cell to reach the final odorant concentration. The differential output signal (between reference electrode and working electrode) was recorded using Stanford Research Systems Lock-in amplifier SR510. Thus, the output of the lock-in amplifier is directly proportional to the cell conductance and the responses of the biosensor were recorded as a function of final odorant concentration on the yeast cells.

Results and discussion

An initial study (Wetzel et al. 1999) had shown that helional is the preferential ligand of human OR1740. After immobilization of the yeast expressing OR17-40 on the working electrode and the yeast expressing SSTR2 on the reference electrode, measurements were performed by adding different concentrations of helional in the measurement cell already containing PBS. The kinetics of biosensor response after odorant addition is presented in Fig. 2a. The curve shows a fast increase of the signal value followed by a slightly slower decrease. The fast biosensor response and its rapid disappearance suggest the absence of steric hindrance, due to both the biological sensing element being simply adsorbed on the polylysine surface, and the small size of the odorant molecules. The shape of this curve is similar to that observed for transient intracellular calcium concentration increase recorded in human embryonic kidney cells (HEK-293) expressing human OR17-40, or the rat OR I7 upon stimulation by their respective odorant ligands (Ko and Park 2005; Levasseur et al. 2003). Thus, the formation/dissociation of the ligand–receptor complex significantly modifies the conductance on the surface of the electrode. In fact, once the ORs bind the ligand odorant molecule, a cascade of events is initiated in the yeast. The activated olfactory receptor interacts with the $G_{\alpha olf}$ subunit of the trimeric G protein hooked in the membrane, causing it to stimulate the downstream elements of the yeast mating signal transduction pathway (Pajot-Augy et al. 2003). This possibly modifies the conductivity of the cell through the opening of some ion channels, which could explain the conductance variation phenomenon. In contrast, as presented in Fig. 2a, only a small signal variation was obtained with the control. This effect is most likely due to the solvent non-specific adsorption/interaction on yeast membrane.

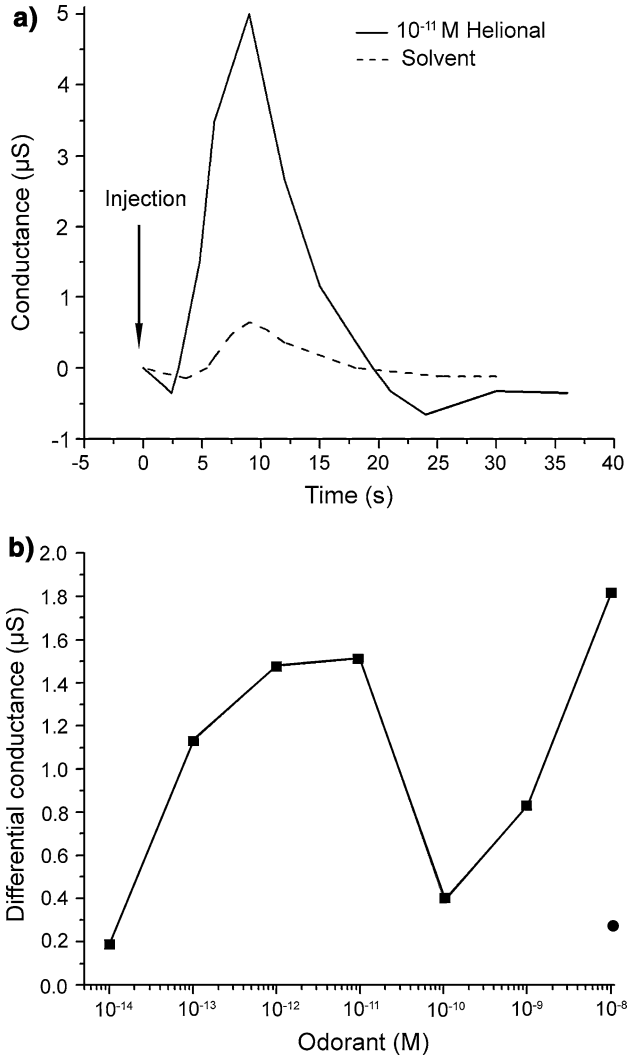

Fig. 2 **a** Kinetics of yeast OR17-40 biosensor response upon 10^{-11} M helional or control addition. **b** Dose–response curve for helional detection (*square dots*), plotted as differential conductance between responses to odorant and solvent. The round dot at 10^{-8} M refers to differential conductance for heptanal detection

Figure 2b reports conductance variation versus helional concentration. We observed a threshold for biosensor

response at 10^{-14} M with a peak at 10^{-12} to 10^{-11} M, then an important signal decrease at 10^{-10} M, and a major increase of response for concentrations up to 10^{-8} M. This dose–response curve is different in comparison to "classical" sigmoid ones, with signal saturation at high ligand concentration typically observed for G protein coupled receptors (Erlenbach et al. 2001; Price et al. 1995), or other proteins (Hou et al. 2005; Marrakchi et al. 2006). However, this phenomenon was already reported with olfactory receptors, including OR1740 (Gomila et al. 2006; Levasseur et al. 2003; Minic et al. 2005a; Vidic et al. 2006). Thus, it seems that the olfactory biosensor developed can indeed detect the biological phenomenon of odorant ligand recognition and binding by its receptor.

The selectivity of the biosensor was checked at the concentration giving the maximum helional response (10^{-8} M), by comparing this signal to that upon a non-specific odorant stimulation (heptanal). As can be seen in Fig. 2b, only a negligible signal variation was obtained in the biosensor response to heptanal in comparison to helional. This result suggests that our conductometric biosensor based on immobilized *S. cerevisiae* yeasts expressing OR17-40 specifically recognizes the odorant ligand of this receptor.

In addition to specificity and selectivity, we were interested in the biosensor response repeatability. The conductance variation was followed after successive additions of helional (10^{-8} M). A signal decrease of about 26% was observed between two successive helional additions in a row, but the decrease was only 6% after a 20 min lapse-time in PBS between two additions. We conclude that a lag phase of more than 20 min between measurements is needed for the system to recover its response ability, time for the olfactory receptor relaxation after its change of conformation upon the odorant ligand binding (Vidic et al. 2006).

Conclusion

The conductometric biosensor based on immobilized *S. cerevisiae* yeasts expressing the human OR 17-40 showed interesting potentialities in odorant detection. The dose–response curve obtained suggests that the biosensor elaborated preserves the natural receptor characteristics of odorant recognition. This original olfactory sensing system may constitute an initial step towards the use of conductometric interdigitated microelectrodes as transducers for the development of biosensors based on genetically modified cells. As a perspective, combining arrays of miniaturized microelectrodes coated with different yeast cells expressing a variety of ORs could lead to operational artificial bioelectric noses to detect a panel of various odorants.

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