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Olfactory receptor-based smell nanobiosensors: An overview of theoretical and experimental results

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ABSTRACT

Mammalian olfactory system is the archetype of smell sensor devices. Its complexity resides both in the odorant mechanism of capture by the single olfactory receptor (OR) and in the space organization and codification of the information. The result is a unique profile for each odorant. Our aim is to partially mimick this system, in order to produce a biosensor on nanometric scale. In this paper, we present a microscopic theoretical framework in which the experimental results should be embedded. It consists of the description of the protein at the level of the amino-acid structure in terms of an impedance network able to simulate the electrical characteristics associated with the protein topology.

1. Introduction

The mammalian olfactory system is the most efficient biological sensor we know for odour recognition. In the mammalian nose, tens of millions of neurons express one (or few) [1,2] different odorant receptors (ORs). Each OR is able to react to a few odorants and the simultaneous activation of many neurons, at different level, produces a specific profile (coding) for each specific odour [3]. This is one of the most refined mechanism of pattern recognition and the perspective of using its facilities into a nanodevice, possibly easy to handle, is very attractive. This issue is addressed by a European collaboration which involves eight research groups [4]. The main goal of the project is to construct an array of nanobiosensors whose active part consists of a few specific ORs interfaced with nanoelectrodes. In such a way, various ORs, differently responding to the same odorant compound, should produce a specific odorant profile, like it happens *in vivo*. Actually, *in vivo*, the receptor which captures a specific odorant molecule undergoes a cycle of activation which starts with the protein conformational change (see also, for more information Ref. [5]), then sending the message of successful capture to the olfactory bulb. *In vitro*, only part of this chain of events can be reproduced, cutting the process at different stages, with respect to the kind of hybrid system one should develop [6–9].

In the present approach, the odorant recognition is performed by looking only at the conformational change. As a matter of fact, we are interested to develop a device which should monitor the odorant capture by means of the impedance variation of the receptor, as due to its conformational change. In other words, the entire chain of biochemical reaction is skipped in favor of a direct sensing associated with the conformational change of the protein. In this way we expect a fast response by the device such as it happens in nature. To reach this goal, the active part of the device has to be constituted of a few nanosomes [7] containing specific ORs. Each nanosome is interfaced with nanoelectrodes and the odorant capture is transduced into an electric signal. Specifically, the electrical response is correlated with the conformational change that a single OR undergoes when it captures a specific odorant molecule. An array of nanodevices should be able to produce specific response profiles.

In this work we report a set of experimental results which support the feasibility of the main concept. Experiments were carried out within the Spot-Nosed collaboration [4] and pertain two G protein coupled receptors (GPCRs), the light-receptor bovine rhodopsin and the rat olfactory receptor I7. The comparative analysis of these two different receptors has the main aim to validate the concept of a dependence of the monolayer electrical response on the protein structure and to set up a coherent theoretical framework in which the results can be embedded.

Finally, this short report presents a summary of previous results by the authors and collaborators regarding the possibility to use conformational change as definitive tool for the realization of olfactory receptor-based nanobiosensors for smell.

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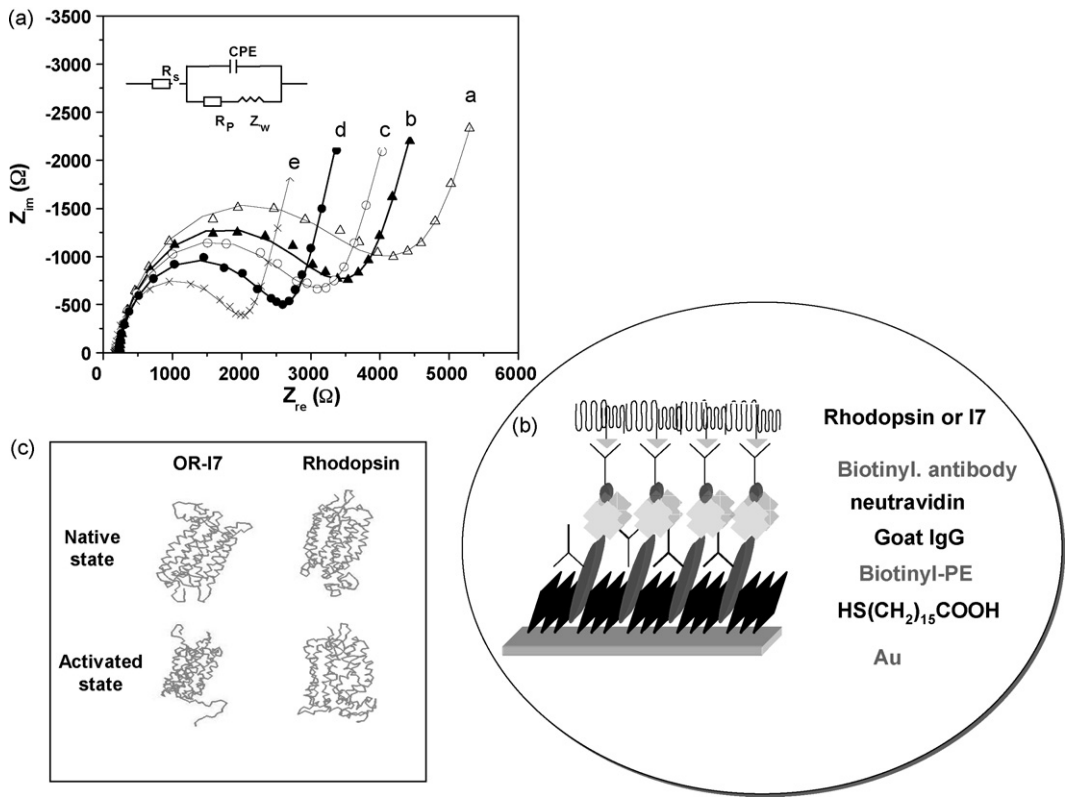

Fig. 1. Electrochemical characteristics of rhodopsin SAM at different levels of assembling with the corresponding circuital analogue (Randles cell) (A). (B) Schematically illustrates the assembling and immobilization process of rhodopsin and I7. In Fig. 1(A): (a) Step I: mixed SAMs modified gold electrode; (b) Step II: blockage with goat IgG; (c) Step III: binding of neutravidin; (d) Step IV: immobilization of biotinylated antibody Biot-Rho-1D4; (e) after injection of 80 ng/ml rhodopsin membrane fraction [8]. (C): Sketch of the backbone of native and activated states of rhodopsin and rat OR-I7, as obtained with MODELLER software (see Section 2.3).

2. Experiment and theory

2.1. Experiment

The debated question on the electrical conductivity of sensing proteins has been partially answered with some experiments, in particular performed on bovine rhodopsin [8]. Electrochemical impedance spectroscopy (EIS) measurements were carried out on self-assembled multilayers (SAMs) of rhodopsin on gold substrate (see Fig. 1(B)), at different stages of multilayer formation [9] (from (a–e) in Fig. 1(A)). The results can be described in terms of Nyquist plots (a), as shown in Fig. 1. Precisely, they can be interpreted by means of a simple electric analogue: the Randles cell.

Different levels of assembling correspond to different Nyquist plots, but only the semicircular part modifies its shapes. This means that in the circuit analogue the Warburg impedance (Z_W) and series resistance (R_s) do not change their value significantly. Indeed, the Warburg and series impedances mainly pertain to the experimental environment. Finally, what really describes the sampling modification is practically only the impedance of the R_P-CPE parallel circuit.

The same procedure was used for testing the selective odorant detection of OR-I7-SAMs [9]. This was tested onto 3 different aldehydes: two specific odorants, octanal (1) and heptanal (2) and one non-specific odorant, helional (3).

The analysis was performed by monitoring the variation of the polarization resistance, say R_P in the Randles cell, at different concentrations of the odorants (Fig. 2). In the circuit analogue the polarization resistance is the element more sensible to the variation of odorant concentration. The net result is a decreasing of R_P for increasing concentrations, with a maximal variation of 12% for octanal, going from a concentration of 10^{-13} M to that of 10^{-4} M. We stress that these results are specific of OR-I7, as confirmed by blank tests and also odorant detection performed with rhodopsin SAMs [9]; in particular the difference between specific and not specific detection is of about a factor of 2 at increasing dose.

The concomitance of these results strongly suggests a correlation between the protein change of conformation (first step of the cycle of activation) and the change of the protein electrical properties.

2.2. Theory

To explore the possible single protein responses, we set up a theoretical framework in which the protein is mapped into a network of impedances. This kind of approach gives us the possibility to connect electrical and topological properties of the receptor [10,11].

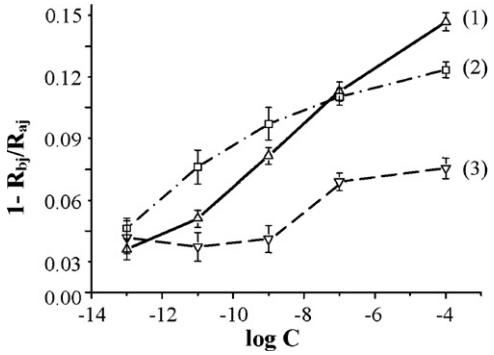

Fig. 2. Rat OR-I7 dose response vs. the log of concentration (expressed in Molarity) of octanal (1), heptanal (2), helional (3). R_{bj} and R_{aj} indicate the polarization resistance before and after the odorant injection, respectively [9].