Olfactory receptors for a smell sensor: a comparative study of the electrical responses of rat I7 and human 17-40

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Abstract

In this paper, we explore the relevant electrical properties of two olfactory receptors (ORs), one from rat, OR I7, and the other from human, OR 17-40, which are of interest for the realization of smell nanobiosensors. The investigation compares existing experiments, coming from electrochemical impedance spectroscopy, with the theoretical expectations obtained from an impedance network protein analogue, recently developed. The changes in the response due to the sensing action of the proteins are correlated with the conformational change undergone by the single protein. The satisfactory agreement between theory and experiments points to a promising development of a new class of nanobiosensors based on the electrical properties of sensing proteins.

Keywords: smell sensors, olfactory receptors, protein electrical properties

(Some figures in this article are in colour only in the electronic version)

1. Introduction

The research on smell sensors is wide and has long been explored because of the huge number of possible applications in everyday life (food quality assessment, detection of specific molecules produced in some diseases [1], and of drugs and toxic material). In order to make these devices more sensitive, easy to use, versatile and of low cost, recent advances aim at substituting their sensitive part, originally constituted by solid-state gas sensors, with organic/biological material [2]. In particular, olfactory receptors (ORs), extracted from different species, have been recently considered among the best candidates to provide the sensitive action in smell sensors of the new generation [3].

In this perspective, a European collaboration, now called BOND³, was launched in 2009 and collects different kinds of expertise in physics, biology and nanoelectronics. The main goal of the project is to build up an array of nanobiosensors whose active part consists of a few kinds of ORs, selective on specific odorant molecules, interfaced with nanoelectrodes. The ORs, differently activated by the same odorant compound, should produce a specific odorant response [4], as happens in vivo. In particular, in vivo, the OR activation involves modifications of the receptor topology, which, in turn, produces a cascade of events that culminate in the transmission of the capture information to the brain. In vitro, only the initial 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 [4, 5]. Experiments performed with different techniques [5–10] strongly suggest that together with the morphology, this process modifies the protein electrical properties. Therefore, the OR activation could be monitored by means of electrical measurements. In order to describe this mechanism, a theoretical model, able to reproduce the electrical properties of a given protein, as deduced by the aforementioned experiments, has been developed. This model, hereafter called INPA (impedance network protein analogue), describes the protein in terms of an impedance network whose structure reproduces the electrical interaction pathways,

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consistently with the protein topology. In particular, the model offers the possibility of exploring some aspects of the protein morphology and its modifications subsequent to the ligand capture and also to interpret some disputed experimental data within a reliable physical framework.

The aim of this paper is to provide a microscopic interpretation of these experiments and carry out a comparative study of the electrical properties of the rat OR 17 and human OR 17-40. For this purpose, the paper is organized as follows. Section 2 briefly recalls the experimental framework and the theoretical model. Section 3 reports the results together with their physical interpretation. Major conclusions are drawn in section 4.

2. Theory and experiment

ORs are proteins of the G protein-coupled family, GPCR, characterized by their sensing action, i.e. their ability to bind to a selected class of molecules that can both activate (agonist ligands) and inhibit (antagonist ligands) their activity [11]. The GPCR working is strictly related to the activation of the G protein; therefore, when used in vitro, the presence or absence of the G protein could be relevant for the results. A role in the shape of the ligand concentration response also seems to be linked to the presence or absence of odorant-binding proteins (OBPs) [9]. Anyway, the first stage of sensing is the ligand capture, which is associated with a conformational change of the GPCR. Thus, we postulate that this is sufficient to modify the electrical properties of the protein, modification that the presence of the natural environment could only emphasize.

To support our conjecture, here we recall some seminal experiments.

2.1. Experimental framework

The debated question on the electrical conductivity of sensing proteins has been partially answered by some experiments, in particular on two light receptors, bovine rhodopsin and bacteriorhodopsin [5, 8], and on the ORs 17 and 17-40 [6, 7, 9, 10]. The techniques adopted for testing the electrical properties of these proteins are different: electrochemical impedance spectroscopy (EIS), conductive atomic force microscopy (C-AFM) and surface plasmon resonance, to mention only a few of them. The used proteins were taken from sacrificed animals [5], natural microorganisms [8] or expressed in appropriate cells, like yeast [6, 7, 9, 10]. EIS measurements were performed on self-assembled monolayers (SAMs) of the specific protein, rhodopsin [5] or ORs [7, 10]. SAMs were obtained blocking with specific antibodies, like goat IgG, the proteins on a previously functionalized gold substrate. The detailed description of the procedure can be found in [5, 7, 10]. The results can be described in terms of Nyquist plots [5, 7, 10]. Precisely, they can be interpreted by means of a simple electric analogue, the so-called Randles cell. This circuit describes the electrochemical cell by using a RC parallel circuit, with the capacitor mimicking the double-layer capacitance of the electrode/solution interface and the resistance \( R \), the polarization resistance, or the charge transfer resistance, depending on the situation. Finally, in order to describe the diffusion process in the actual solution of an electrochemical cell, the impedance element known as a Warburg element can be added to the RC circuit [5].

In the Randles cell, the polarization resistance is found to be the passive element more sensitive to the variation of odour concentration. Both for rhodopsin [5] and the ORs 17 [7] and 17-40 [10], when activated by the specific ligands, the net result is a decrease in \( R \) with increasing ligand concentration.

2.2. Theoretical framework

As clearly signalled by experiments, the addition of a specific odorant induces a modification of the electrical response in functionalized samples of protein receptors. For these samples, the main modifications in the electrical response are attributed to the single protein conformational change. Therefore, the model we adopt for describing the experimental outcomes describes the protein electrical features as a function of the protein morphology. In doing so, the receptor is represented by a graph of \( N \) nodes where \( N \) is the number of amino acids constituting the protein. Then, two nodes are connected if the distance between the corresponding amino acids is not greater than an assigned value \( R_c \), which defines the interaction radius between neighbouring amino acids. For \( R_c \) varying in the range 3–70 Å, the graph goes from a not connected to a completely connected structure.

The mechanism underlying the specificity of the OR during odour sensing is the protein conformational change. Here, the change in the tertiary structure of the sensing protein, which in turns leads to a geometrical change in the position of the amino acids composing the protein, is taken as the mechanism responsible for the electrical change. In this approach, the resistivity and the dielectric property of the single amino acid are assumed to remain the same in both the native and active states.

The distance \( R_c \) definitely selects the connectivity of the network and is taken here as an adjustable parameter of the model. Accordingly, the choice of its value should be guided by some physical constraints and finally fixed by the comparison of numerical calculations with experiments.

As a further step, the graph is turned into an electrical network by substituting each link between two nodes with an RC parallel circuit whose value depends on the distance between the corresponding amino acids. The RC elementary impedance, which mimics the microscopic charge transfer and the charge polarization, is given explicitly by [2, 12, 13] \[ Z = \frac{l}{A \rho^{-1} + i\varepsilon_0 \omega}, \]
where \( A = \pi(R_c^2 - l^2/4) \) is the cross-sectional area between two spheres of radius \( R_c \), each of them centred on one of the two nodes; \( l \) is the distance between these centres, \( \rho \) is the resistivity, taken to be the same for every amino acid, with the indicative value of \( \rho = 10^{10} \Omega \) m, \( i = \sqrt{-1} \) is the imaginary unit, \( \varepsilon_0 \) is the vacuum permittivity and \( \omega \) is the circular frequency of the applied voltage. The relative dielectric constant of the considered couple of nodes

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By positioning the input and output electrical contacts, respectively, on the first and last node (corresponding to the first and last amino acid of the protein sequential structure), the network is solved within a linear Kirchhoff scheme and its global impedance spectrum is calculated within the standard frequency range 1 mHz–100 kHz.

The choice of the contacts’ position and their point-like size is not mandatory for the model applicability. In previous papers [12, 13], we have made different selections, in order to describe, for example, the C-AFM tip penetration into a bacteriorhodopsin sheet or to take into account the finite size of actual contacts. In both cases, the results have shown a different absolute value of the protein impedance; however, the change of the impedance induced by the conformational change was practically the same. We conclude that, concerning the change of the electrical properties, the choice of the positioning of the contacts is of negligible importance.

3. Results

The change in the protein state, by implying a change in the network structure, leads to a different spectrum of the single protein impedance. Such a variation of the impedance spectrum is taken here as an estimate of the electrical response at a macroscopic level, as resulting from the conformational change of an ensemble of identical proteins. In the following, the investigation of the protein modifications is carried out by means of different tools, such as the analysis of the contact maps, the determination of the protein global resistance and the shape of the impedance spectra.

3.1. Structure and contact map

The global insights into the protein modification, as induced by the ligand capture, are shown by the contact maps reported in figures 1 and 2 for the rat OR I7. In these figures, each point represents a couple of connected amino acids, i.e. a link, in an assigned configuration (native on the left side of the main diagonal and active on the right side of the main diagonal) and for a given value of the cut-off radius. For both values, \( R_C = 6 \text{ Å} \) (figure 1) and \( R_C = 12 \text{ Å} \) (figure 2), we can observe that the main differences between the native and active states are located in the middle region, with an increase in connections for the active state. This is probably due to the protein closing on the binding pocket (there located [14]) subsequent to the ligand capture.

The difference between the number of links in the native and active states of both the ORs under examination is reported in figure 3 for OR I7 and in figure 4 for OR 17-40. In particular, each figure reports the data at two different \( R_C \) values, say 6 and 12 Å. By comparing the rat and human protein, we notice that besides the similarities of the two proteins, a few but significant differences exist. These differences are also present and amplified in the calculated impedance of the receptors where, more than the absolute difference in the number of links, the link position is of relevance for the determination of the impedance [2, 12, 13].

3.2. Resistance and impedance spectrum

The differences between native and active states are also evidenced in the change of the receptor global resistance, \( R \). Concerning this point, figure 5 shows the shape of the relative variation of \( R \) as a function of \( R_C \), for both the proteins. The main result reported in this figure is the larger sensitivity of the rat OR I7 with respect to that of the human OR 17-40. In the former case, it is possible to resolve a maximum difference of about 60%, while in the latter case the maximal resolution is about 20%. The region of maximal sensitivity is the same for both the proteins, corresponding to \( R_C \) in the range 6–14 Å. For the case of OR 17-40, the active state shows the values of \( R \) that are significantly lower than those of OR I7. Furthermore, for \( R_C \) above about 18 Å, calculations evidence for OR 17-40 an inversion of the resistance variation, with the active state becoming less resistive than the native state in two regions of the \( R_C \) values, 18–24 Å and 40–60 Å, respectively.
Figure 3. Difference between the number of links in the native and active states of the rat OR I7 for $R_C = 6$ Å (left side of the diagonal) and $R_C = 12$ Å (right side of the diagonal).

Figure 4. Difference between the number of links in the native and active states of the rat OR I7-40 for $R_C = 6$ Å (left side of the diagonal) and $R_C = 12$ Å (right side of the diagonal).

network, such an inversion is interpreted as a stronger increase of parallel with respect to series connections. Accordingly, the different behaviour shown by the two receptors signals the different peculiarities of the network structure.

The impedance response of single proteins is explored in a wide range of frequencies and the results are given by means of the Nyquist plot. This plot is obtained by drawing the negative imaginary part versus the real part of the global impedance, within a given frequency range (typically from 1 mHz to 100 kHz as in experiments [7, 10]). Figure 6 reports the Nyquist plots with the impedance normalized to the static value of its native state $Z_{\text{nat}}(0)$. The symbols refer to experiments [7], where the crosses refer to no odorant, the empty squares to an odorant concentration of $10^{-9}$ M and the full squares to an odorant concentration of $10^{-4}$ M. The lines refer to theoretical results with the continuous curve referring to the native state configuration with $R_C = 50$ Å, the long dashed line referring to the active state configuration with $R_C = 30$ Å and the dashed line referring to the active state configuration with $R_C = 25$ Å.

Within our model, the Nyquist plots are obtained by using as input data the networks corresponding to the native and active states, at the cut-off radius which, according to figure 5, fits the experimental value of $Z_{\text{nat}}/Z_{\text{nat}}(0)$ at the given odorant concentration. In other words, we conjecture the existence of a correlation between the concentration of odorant and $R_C$, due to the modifications undergone by the protein ensemble in the presence of a given concentration of odorant [15]. The agreement between theory and experiments is found to be satisfactory from both a qualitative and quantitative point of view. We remark that the near ideal semicircle shape of the experimental Nyquist plot is well reproduced, thus confirming that the network impedance model behaves very like a single RC circuit, as expected by the presence of a rather uniform distribution of the time constants associated with the different values of the resistance and capacitance of the network links [12, 13].

Figure 7 reports analogous results for the case OR 17-40, in the native state and in the active state with
recent experiments have made it possible to transduce the sensing action of the protein large to be detectable within the experimental resolution; this measuring the corresponding variations of impedance. In this in principle, to reveal the protein conformational change by the capture cannot be reproduced. Nevertheless, it is possible, especially detection of the conformational change is not a simple task, used by an OR-based nanobiosensor. On the other hand, the first step in the mechanism of odour recognition that should be activated the G protein. As a consequence, a change in the protein conformation (from the native to the active state) which activates the G protein. Consequently, both proteins are able to bind a specific molecule in the tertiary structure as well as similar behaviour in the sensing action. Both proteins are able to bind a specific molecule in the so-called active site, which is placed well inside the protein (among the three, four, six helices) [14]. The capture produces a change in the protein conformation (from the native to the active state) which activates the G protein. As a consequence, a chain of biological events starts, ending with odour recognition by the brain. The detection of the conformational change is the first step in the mechanism of odour recognition that should be used by an OR-based nanobiosensor. On the other hand, the detection of the conformational change is not a simple task, especially in vitro, where the cascade of events subsequent to the capture cannot be reproduced. Nevertheless, it is possible, in principle, to reveal the protein conformational change by measuring the corresponding variations of impedance. In this respect, it is crucial to determine whether this change exists or not and, in the positive case, if such a change is sufficiently large to be detectable within the experimental resolution; this makes it possible to transduce the sensing action of the protein into an electrical signal. Recent experiments [7, 10] have confirmed the possibility of detecting the sensing action of the rat OR I7 and the human OR17-40 due to the capture of a specific ligand. Measurements were performed on self-assembled multilayer (SAM) substrates on which the proteins were anchored. Then the sensitivity to specific and non-specific odorants and also to the dose response was tested. The results are found to be promising for the considered proteins, showing a better sensitivity for the rat OR I7 than for the human OR 17-40 [9]. Furthermore, the experiments were accomplished by an electrochemical impedance spectroscopy (EIS) characterization: the conformational change induces a modification of the impedance value in a wide range of frequencies.

The experiments are microscopically investigated here on the basis of an impedance network approach (INPA) that is found to provide a satisfactory interpretation of available experiments. We notice that the INPA is a single protein model whose results are here extended to those pertaining to a macroscopic sample, by using a rescaling procedure. In particular, we proceed as if all the proteins in the sample work independently; therefore, the net response is simply the sum of the single responses. We are aware that the actual working process is more complex; however, actual knowledge of the OR characteristics, both of the morphological and the electrical aspects, is still widely insufficient to elaborate more sophisticated models. Anyway, at this early stage of investigation we believe that the agreement found between theory and experiments catches the essential features of the correlation between the protein conformational change and the correlated change of its electrical properties. In particular, these results prove the possibility of using proteins as very refined sensors, able to determine, through an electrical signal, the presence and the concentration of the substance to be detected.

4. Discussion and conclusions

We have presented a microscopic interpretation of the electrical properties of two ORs, the rat I7 and the human 17-40. Both ORs pertain to the huge family of seven-helix transmembrane receptors, the so-called G protein-coupled receptors (GPCRs) [16]. Accordingly, they share a similar tertiary structure as well as similar behaviour in the sensing action. Both proteins are able to bind a specific molecule in the so-called active site, which is placed well inside the protein (among the three, four, six helices) [14]. The capture produces a change in the protein conformation (from the native to the active state) which activates the G protein. As a consequence, a chain of biological events starts, ending with odour recognition by the brain. The detection of the conformational change is the first step in the mechanism of odour recognition that should be used by an OR-based nanobiosensor. On the other hand, the detection of the conformational change is not a simple task, especially in vitro, where the cascade of events subsequent to the capture cannot be reproduced. Nevertheless, it is possible, in principle, to reveal the protein conformational change by measuring the corresponding variations of impedance. In this respect, it is crucial to determine whether this change exists or not and, in the positive case, if such a change is sufficiently large to be detectable within the experimental resolution; this makes it possible to transduce the sensing action of the protein into an electrical signal. Recent experiments [7, 10] have confirmed the possibility of detecting the sensing action of the rat OR I7 and the human OR17-40 due to the capture of a specific ligand. Measurements were performed on self-assembled multilayer (SAM) substrates on which the proteins were anchored. Then the sensitivity to specific and non-specific odorants and also to the dose response was tested. The results are found to be promising for the considered proteins, showing a better sensitivity for the rat OR I7 than for the human OR 17-40 [9]. Furthermore, the experiments were accomplished by an electrochemical impedance spectroscopy (EIS) characterization: the conformational change induces a modification of the impedance value in a wide range of frequencies.

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