Chapter 49
Odorant Binding Proteins as Sensing Layers for Novel Gas Biosensors: An Impedance Spectroscopy Characterization

S. Capone, C. De Pascali, L. Francioso, P. Siciliano, K. C. Persaud and A. M. Pisanelli

Abstract In this work, an ab-initio study of the electrical response to odorants of a self-assembled monolayer of a pig OBP immobilized onto a miniaturized Si-substrate equipped with gold interdigitated electrodes (IDE), was started. Electrical Impedance Spectroscopy (EIS) was used as electrical characterization technique and a dedicated experimental set-up was arranged in order to carry out EIS measurements in controlled environment. The EIS data was fitted by using a fitting software based on Levenberg–Marquardt (LEVM) algorithm to determine the equivalent circuit of the system.

49.1 Introduction

In animal olfactory systems, odour sensing is fulfilled in a primary step by Odorant Binding Proteins (OBPs) and Olfactory Receptors Proteins (ORPs). OBPs are abundant small soluble extracellular proteins secreted in the nasal mucus of many animal species and in the sensillar lymph of chemosensory sensilla of insects. Vertebrate OBPs belong to the lipocalin superfamily. The members of this superfamily have poor sequence similarity but all of them show a conserved folding pattern, that is an 8-stranded β-barrel flanked by an α-helix at the C-terminal end of the protein chain. The β-barrel defines a central apolar cavity, called calyx, whose

S. Capone (✉) · C. De pascali · L. Francioso · P. Siciliano
Institute for Microelectronics and Microsystems, CNR-IMM,
via Monteroni, 7310 Lecce, Italy
e-mail: simona.capone@le.imm.cnr.it

K. C. Persaud · A. M. Pisanelli
School of Chemical Engineering and Analytical Science (SCEAS),
University of Manchester, Manchester, UK

G. Neri et al. (eds.), *Sensors and Microsystems*,
Lecture Notes in Electrical Engineering 91, DOI: 10.1007/978-94-007-1324-6_49,

role is to bind and transport hydrophobic molecules [1]. The exact role of OBPs in vertebrates is not yet well defined, but in insects the role is very clear. The OBPs bind molecules that are transported to the receptors. Here, a OBP releases in a reversible way the bound odorant molecule to the Olfactory Receptors Proteins (ORPs), that are heptahelical membrane proteins coupling via G-proteins on to intracellular transduction cascades. Extensive research in the last two decades have extended the range of OBPs' putative function to a wider range of different functions, however, their precise physiological role still remains elusive [2, 3].

Great expectations in gas sensing rise from a novel biomimetic approach that throws out the innovative idea to use native as well as engineered non-natural biological molecules, as OBPs or ORPs, as possible active elements in gas sensors of new generation. A major driving force to study OBPs and ORPs is the idea that such biological elements are optimized for specific olfactory tasks by evolution and natural selection. The main natural feature of such proteins, that make them attractive for biomimetic sensing platforms, is that the binding of the odorant molecule may induce a small conformational change of the protein. Indeed, the β-barrel of an OBP encloses a quite stable ligand-binding site composed of both an internal cavity and an external loop scaffold. It is the diversity of cavity and scaffold that gives rise to a variety of different binding modes each capable of accomodating ligands of different size, shape and chemical character. Such binding events might, by itself, cause a change in the electrical properties of the protein. The possible connections between binding events and electrical properties changes might build the novel detection principle of biohybrid sensors of new generation. In this context, the electrical characterization of OBPs and ORPs in controlled environment is of extreme importance both from a fundamental and applied point of view.

Recent electrochemical impedance studies of specific ORPs stimulation by odorants in solution performed in conventional electrochemical cell with a three electrodes system have been reported [4–6].

In this work, for the first time, we transfer a self-assembled monolayer of a porcine Odorant Binding protein (pOBP) onto a miniaturized Si-substrate equipped with gold interdigitated electrodes (IDE) and we characterized it by Electrical Impedance Spectroscopy (EIS) in air both in absence and in presence of odorant molecules (as methanol), in order to measure an expected odorant-dependant signal change. EIS is a powerful method for the analysis of the complex electrical impedance of a system, and it is particularly suited to the detection of binding events on the transducer surface [7].

49.2 Experimental and Methods

49.2.1 Biomaterials and Chemicals

Porcine odorant binding protein (pOBP) is a monomer of 157 amino acid residues, purified in abundance from pig nasal mucosa [10]. Recombinant Pig Odorant Binding Protein (modified in the 2 position with a Cysteine Residue, so that the

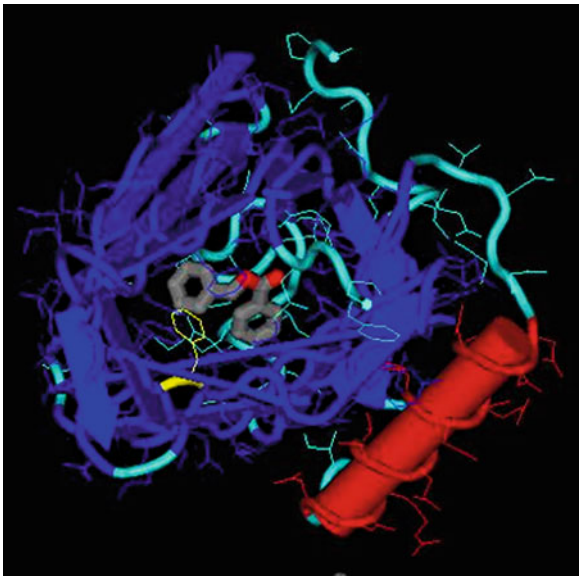


Fig. 49.1 Structure of the Porcine OBP (courtesy Dr. P. Pelosi, Un. of Pisa)

protein could be directly immobilised on a gold electrode surface, courtesy Paolo Pelosi, University of Pisa) present in pig saliva and belonging to the protein subclass of chemical communication was considered in this work. Its typical structure is shown in Fig. 49.1. The Pig OBP was suspended in a working 10 mM phosphate buffer solution pH 7.4.

49.2.2 Preparation of Au IDE Trasduction Platform and Pig OBP Immobilization

Miniaturized silicon substrates (1.5 × 1.5 mm sized) equipped with gold interdigitated electrodes were prepared using standard photolithography technologies. Starting from a 3″ Si wafer with a 500 nm-thick SiO_2 layer, a 20 nm-thick titanium layer and a 200 nm-thick gold top layer were deposited by evaporation under vacuum. Eleven pairs of fingers spaced 10 μm were prepared in a central area of 440 × 440 μm. The single die was cut and bonded onto a standard TO-39 socket for electronic components.

A 1 μl aliquot of Pig OBP was placed on a Si substrate functionalized by Au IDEs, previously cleaned with acetone and isopropilic alcohol, washed in distilled water and dried in nitrogen. After drying the Pig OBP in air, an self-assembly of the protein formed. Due to the cysteine substitution of the Pig OBP easy immobilization onto the Au microelectrodes is achieved Fig. 49.2.

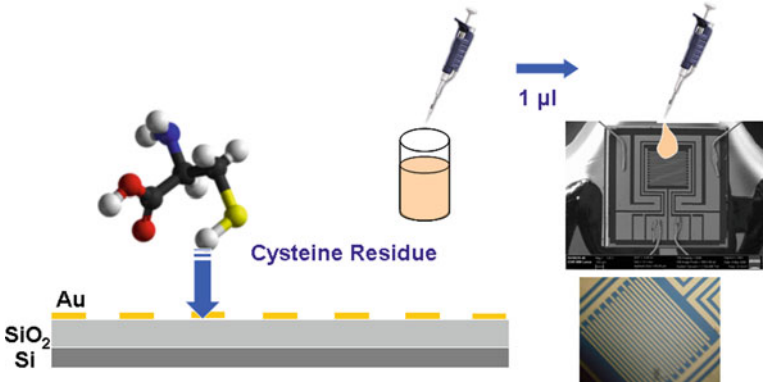


Fig. 49. 2 Deposition and immobilization of OBP onto IDE transducer

49.2.3 Electrical Impedance Spectroscopy Set-up

After immobilization of the Pig OBP layer onto Au IDE transduction microplatform, the sample bonded onto TO-39 socket was introduced into a suitable brass test chamber for Electrical Impedance Spectroscopy (EIS) measurements. An LCR-meter (mod. HP 4284A) was used for the experiments carried out in a frequency range of 20–1 MHz with a sinusoidal voltage input at a polarization potential of 0 V with a frequency modulation of 20 mV. The sample was connected to LCR-meter in 4-terminals configuration (HiPotential-LowPotential, HiCurrent-LowCurrent) by 1 m long BNC cables. The EIS test chamber was connected to a gas-mixing bench in order to evaluate the AC characterization of the Pig OBP in air in controlled conditions (gas flow, relative humidity, odorant concentration in air). The system allows to get different concentrations of gas and/or volatiles by suitable dilution of the related analyte flow in reference air flow. A scheme of the Electrical Impedance Spectroscopy set-up for Pig OBP electrical characterization is reported in Fig. 49.3.

49.3 Results-EIS Analysis

Impedance measurements on biological system are of fundamental importance to understand charge transport as well as charge exchange, surface phenomena and binding events, and diffusion processes. Impedance measurements are made by applying a small alternating voltage V of known angular frequency ω (=2πf, f frequency) and small amplitude V_0 to a system and measuring the amplitude I_0 and phase shift ϕ of the concomitant electrical current that develops across it. Impedance is usually represented by a phasor, i.e. a vector Z of magnitude $Z_0 = |Z|$

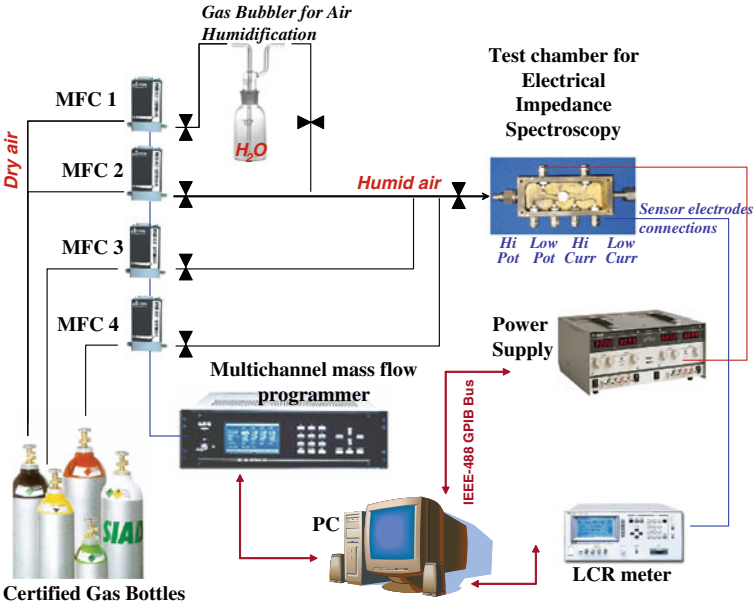


Fig. 49.3 Electrical Impedance Spectroscopy set-up for Pig OBP electrical characterization

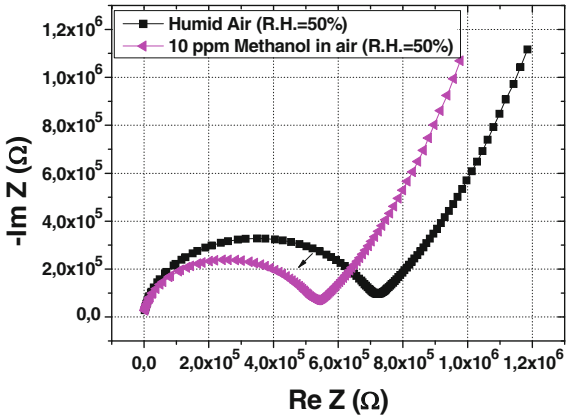
and phase ϕ = arg|Z|. In cartesian coordinates, impedance becomes a complex number:

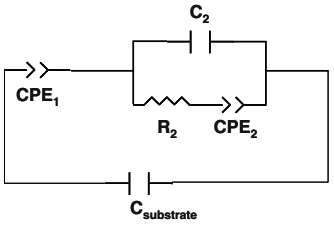
$$\begin{aligned} Z(\omega) &= Z_0 \cdot \exp(j\omega) \\ &= Z_0 \cdot (\cos\phi + j\sin\phi) \\ &= R + jX \end{aligned} \tag{49.1}$$

The Real (ReZ) and Imaginary (ImZ) parts of Z describe the resistance (R) and reactance (X) respectively and can be represented by an appropriate electrical circuit in series. Therefore, the results of an impedance measurement can be illustrated in two different ways: using a Bode plot which plots log|Z| and Φ as a function of log(f), or using a Nyquist plot which plots (-ImZ) versus ReZ.

The analysis of a impedance spectra is often complicated and ardous, since it consists in building an equivalent circuit model of the measured system and in determining the parameter values of all the elements of the equivalent circuit, by non-linear fitting of EIS data.

In this work different samples of the considered Pig OBP were immobilized onto the Au interdigitated microlectrodes and analysed by EIS. After first drying in ambient air, each Pig OBP sample was put into the test chamber under a constant flow of humid air and the impedance spectra acquired stepwise in discrete intervals in order to study the evolution of the system up to a steady state indispensable to measure the EIS spectrum.





		Air RH=50%	10 ppm Methanol in air R.H.=50%
$C_{substrate}$		9.60 pF	7.36 pF
CPE_1	Y_0	$0.11 \cdot 10^{-5}$ F(rad/s)$^{1-\alpha}$	$0.13 \cdot 10^{-5}$ F(rad/s)$^{1-\alpha}$
	α	0.01	0.03
C_2		9.04 nF	0.64 nF
R_2		1.02 MΩ	0.10 MΩ
CPE_2	Y_0	$0.71 \cdot 10^{-8}$ F(rad/s)$^{1-\alpha}$	$0.25 \cdot 10^{-7}$ F(rad/s)$^{1-\alpha}$
	α	0.80	0.70

Fig. 49.4 Nyquist plots for a Pig OBP under exposure in humid air at RH = 50% and under 10 ppm ethanol in humid air at RH = 50%; equivalent circuit and table with fitted circuital parameters

In the following we report the results related to a Pig OBP sample for which a steady state in humid air at RH = 50% was achieved. We exposed the sample to methanol in low concentration (10 ppm in humid air RH = 50%) by measuring the impedance curves both in reference humid air (RH = 50%) and in presence of methanol vapours (Fig. 49.4). As can be observed the impedimetric curve under exposure to methanol is smaller as compared to the impedimetric curve in humid air. This effect can be attributed to occurred binding events between the Pig OBP sample and the analytes molecules, that cause probable conformational changes and/or molecular rearrangement of the proteins. Experiments tests and data analysis are in course in order to explain such gas sensing phenomena.

The experimental impedance spectra were best fitted with the equivalent circuit shown in Fig. 49.4 by making use of a Complex NonLinear Least Squares (CNLS) method based on a Levenberg–Marquardt (LEVM) algorithm [8] In particular, a LEVM software developed by Macdonald [9] was used for fitting.

Both the structure as well as the elements of the equivalent circuit are correlated to different physichemical properties of the biological system under investigation. Once a good fit with the combination of a representative circuital model and suitable starting fitting parameter values is achieved, the next step is to explain the

obtained fit results in terms of the real components of the system and the phenomena associated with the biological element. Such studies are in progress.

49.4 Conclusions

An ab initio study of electrical impedance spectroscopy (EIS) of a Pig Odorant Binding Protein exposed to air and target odorant species was started. Only a few studies regarding EIS on Odorant Binding Proteins (OBPs) and Odorant Receptors Proteins (ORPs) are reported in the literature and these study the protein only in solution. This study is, hence, particularly interesting since: (a) it is a fundamental study of the electrical properties of Odorant Binding Proteins outside their living ambient, useful to understand the preservation of their functionality, (b) it is an explorative study to estimate the feasibility of a novel chemosensor based on a OBP or ORP.

Moreover, a equivalent circuit model was developed and a very good fit for the impedance spectra were obtained. However, a lot of efforts have to be devoted both to the definition of a experimental measurement procedure and to understand the sensing mechanisms by which the odorant molecule interacts with the OBP layer by using the circuit model obtained by EIS characterization.

Acknowledgment We are grateful to GOSPEL (FP6-IST 507610) for financing this initial study, and to Prof. Paolo Pelosi, University of Pisa for the Pig Odorant Binding Proteins.

References

1. Flower DR, North ACT, Sansom CE (2000) The lipocalin protein family: structural and sequence overview. Biochim Biophys Acta 1482:9–24
2. Breer H (2003) Olfactory receptors: molecular basis for recognition and discrimination of odors. Anal Bioanal Chem 377:427–433
3. Pelosi P (2001) The role of perireceptor events in vertebrate olfaction. Cell Mol Life Sci 58:503–509
4. Hou Y, Jaffrezic-Renault N, Martelet C, Tlili C, Zhang A, Pernollet J-C, Briand L, Gomila G, Errachid A, Samitier J, Salvagnac L, Torbiro B, Temple-Boyer P (2005) Study of Langmuir and Langmuir-Blodgett films of odorant-binding protein/amphiphile for odorant biosensors. Langmuir 21(5):4058–4065
5. Hou Y, Jaffrezic-Renault N, Martelet C, Zhang A, Minic-Vidic J, Gorojankina T, Persuy M-A, Pajot-Augy E, Salesse R, Akimov V, Reggiani L, Pennetta C, Alfinito E, Ruiz O, Gomila G, Samitier J, Errachid A (2007) A novel detection strategy for odorant molecules based on controlled bioengineering of rat olfactory receptor I7. Biosens Bioelectron 22:1550–1555
6. Benilova IV, Minic Vidic J, Pajot-Augy E, Soldatkin AP, Martelet C, Jaffrezic-Renault N (2008) Electrochemical study of human olfactory receptor OR 17–40 stimulation by odorants in solution. Mater Sci Eng C 28:633–639
7. Lisdat F, Schäfer D (2008) The use of electrochemical impedance spectroscopy for biosensing. Anal Bioanal Chem 391:1555–1567

8. Marquardt DW (1963) An algorithm for least-squares estimation of nonlinear parameters. SIAM J Appl Math 11:431
9. Macdonald JR (1963) CNLS (Complex nonlinear least squares): immittance, inversion, and simulation fitting programs—LEVM Manual. SIAM J Appl Math 11:431 (Version 8.08, Issue data: February, 2007D.W. Marquardt.)
10. Vincent F, Spinelli S, Ramoni R, Grolli S, Pelosi P, Cambillaud C, Tegoni M (2000) Complexes of porcine odorant binding protein with odorant molecules belonging to different chemical classes. J Mol Biol 300:127–139