Elaboration of odorant biosensors based on Langmuir-Blodgett technique

Yanxia HOU, Nicole JAFFREZIC-RENAULT, Claude MARTELET and Chaker TLILI
Centre de Génie Électrique de Lyon (CEGELY), Ecole Centrale de Lyon, 69134 Ecully Cedex, France
TEL.: (33)(0)4 72 18 62 43 FAX: (33) (0)4 78 43 37 17 e-mail : Nicole.Jaffrezic@ec-lyon.fr

Aidong ZHANG
College of Chemistry, Central China Normal University, Wuhan 430079, PR China
(Received 13, October 2004 Accepted 31, January 2005)

The advent of electronic noses opened a new kind of analytical approach, which offers an easy, quick, and cheap measurement comparing to conventional analytical approaches. It has potential applications on many different fields, such as assessment of various foodstuffs and beverages, environmental contamination monitoring and medical diagnostics etc. In this study, we used odorant binding protein (OBP) as odor-sensing material for developing such kind of artificial odorant biosensor. And biological films of OBP-1F (rat OBP) were deposited by Langmuir-Blodgett (LB) technique on gold electrode previously functionalised with 1-octadecanethiol (ODT) by self-assembled monolayers (SAMs). It is known that successful immobilization of the Langmuir-Blodgett films depends strongly on the characteristics, particularly stability, of the corresponding Langmuir films at the air/water interface. Hence the characteristics of monolayer of amphiphile octadecylamine (ODA) and the mixed monolayer of ODA/OBP were studied. Electrochemical impedance spectroscopy (EIS) was used to monitor the response of the system to a specific odorant molecule, isoamyl acetate.

Keywords: Odorant-binding protein, Langmuir-Blodgett, Biosensor, Electrochemical impedance spectroscopy

I. INTRODUCTION

Today the human olfactory system is still regarded as the most important analytical instrument for the assurance of odour quality in many industries, such as food and drinks. However, in some other fields, such as environmental contamination and medical diagnostics, it is not practical. Usually conventional analytical approaches, gas chromatography (GC), mass spectrometry (MS) and high pressure liquid performance (HPLC) are used in such domains. Unfortunately, it is well known that they are expensive, complicated and time-consuming. Consequently there is urgent demand for a new kind of analytical approach which can mimic the human sense of smell and provide an easy, quick, and cheap measurement. In the late 1980s and early 1990s research on artificial olfaction has led to significant advances in this field and to the launch of commercial instruments (called ‘electronic nose’) being used in a variety of industries (including food, water and brewing). An electronic nose comprises an array of chemical sensors, where each sensor has only partial specificity to a wide range of odorant molecules, coupled with a suitable pattern recognition system.

In this study, as preliminary try we are dedicated to elaborate a simple artificial olfactory system.
Odor-sensing material, odorant binding protein (OBP), was immobilized on gold electrode by Langmuir-Blodgett technique. Since Langmuir-Blodgett technique has already shown its efficiency for depositing well-defined films of enzymes and antibodies etc. for manufacturing biosensors and it presents numerous advantages such as: rapidity, reproducibility, good control of the amount of the biocomponents and preserving the activities and specific recognition properties of biomolecules. Electrochemical impedance spectroscopy (EIS) was used as transduction technique for monitoring the response of the system to a specific odorant molecule, isoamyl acetate. EIS is a rapidly developing electrochemical technique for the characterization of biomaterial-functionalised electrode surface and has potential application on biosensing, since it represents not only a suitable transduction technique to follow the interfacial interactions of biomolecules, but it also provides a quite powerful method for characterization of the structural features of the sensing interface and for explaining mechanisms of chemical processes occurring at the biofunctionalized electrode/solution interface.

II. METHODS AND MATERIALS

II-A. Materials

Recombinant rat OBP-1F (wt 18.5 KDa) was provided by INRA, Paris. It was produced using the yeast *Pichia pastoris* and purified by reversed phase liquid chromatography as described previously. Purified protein was dialysed extensively against MilliQ H₂O and lyophilized. Isoamyl acetate was obtained from Sigma. Octadecylamine (ODA, purity > 99%) and 1-octadecanethiol (ODT) (purity 98%) were purchased from Fluka and Aldrich Chemical Company, respectively. Solvent chloroform (purity 99.8%) bought from Aldrich Chemical Company was used to dissolve ODA at a concentration of 1 mg/ml and ODT was dissolved at a concentration of 1 mM in ethanol (purity > 99.8%) obtained from Fluka for modification of gold electrode surface. Potassium dihydrogen phosphate and sodium chloride (from Prolabo), and sodium hydroxide from Aldrich were used to prepare 10 mM phosphate buffer solution containing 100 mM NaCl, pH 7.5, which was used for all LB films preparation and impedance measurements. All reagents were of analytical grade (>99%). Water used throughout experiments was purified by an Elga system with a reverse osmosis, deionization, filtration and ultraviolet irradiation systems to have a resistance as high as 18.2 mΩ cm.

II-B. Functionalisation of gold electrode

Gold substrates were fabricated using standard silicon technologies. <100>-oriented, P-type (3-5 Ω cm) silicon wafers were thermally oxidized to grow a 800 nm-thick oxide field. Then, a 30 nm-thick titanium layer and a 300 nm-thick gold top layer were deposited by evaporation under vacuum.

The gold electrodes were previously cleaned with acetone in an ultrasonic bath for 10 min and dried under nitrogen flow, followed by immersion in a 7:3 (v/v) H₂SO₄/H₂O₂ (piranha solution) for 1 min in order to get rid of inorganic and organic contaminants on the electrode surface. They were subsequently rinsed thoroughly in absolute ethanol and finally dried under nitrogen flow.

As soon as the cleaning procedure is terminated, the electrodes were put into a 1 mM ODT solution for 21 h at room temperature in order to make their surface hydrophobic by the formation of SAMs. Excess thiol was removed from the surface by rinsing thoroughly the electrodes with ethanol and finally electrodes were dried under nitrogen flow.

II-C. Langmuir and Langmuir-Blodgett films

Langmuir experiments were performed with a Langmuir trough from NIMA (model 611). The trough was equipped with a chamber preventing external contamination. The subphase temperature was controlled at 10 ± 1 °C by a thermostatic system (Julabo-F25, France). The subphase was constituted of a solution of OBP-1F in PBS at different concentrations or in ultrapure water.

For elaboration of mixed layer of octadecylamine and OBP-1F, the protein solution
in PBS was used as subphase and 22 μL of ODA in chloroform was injected and spread onto interface using a micropipette of 50 μL. After spreading, the monolayers were left for at least 15 min to allow the solvent to evaporate. The apparent molecular area was defined as the ratio of the film area to the number of ODA molecules. Then the compression of the film was initiated at a rate of 5 cm$^2$ min$^{-1}$ until reaching the target surface pressure. Relaxation time of 3 h was required before onset of dipping. While the surface pressure was kept constant and the monolayer was stable, the monolayer was transferred onto hydrophobic gold electrodes by a vertical dipping method at a rate of 3 mm min$^{-1}$. After each upstroke, the electrode was maintained in air for 5 min to dry the films. The transfer ratio (TR) expressed as the ratio of area of film transferred onto the monolayer-coated area on the electrodes was used as a parameter to characterize the LB deposition process. After transfer of LB films functionalized gold electrodes were put into a reaction vessel containing pure undiluted odorant isomyl acetate (10 μL in the chamber) which evaporated freely for 16 h at room temperature. The samples were used for EIS measurements.

For understanding the proper formation of Langmuir and Langmuir-Blodgett films, first we studied the properties of monolayers at the air/water interface. For studying adsorption of protein to amphiphile monolayer at the air/water interface, surface pressure-time curves (referred to as $π$-t) were recorded with opened barrier, namely without compressing force. The characteristics of the monolayer on the surface were studied by measuring the changes in surface pressure upon the monolayer compression at a given temperature, namely surface pressure-molecular area isotherm (referred to as $π$-A). The shape of the isotherm is characteristic of the molecular building up of the film. The stability of the monolayer, which is an important parameter for the stability of the mixed LB films, can be evidenced by molecular area ratio-time evolution isotherms at the target pressure (referred to as $A/A_0$-t) or surface pressure-time evolution at a constant molecular area (referred to as $P_π$-t).

II-D. Electrochemical impedance spectroscopy

Impedance measurements were performed in a conventional electrochemical cell containing a three-electrode system with an Electrochemical Interface SI 1287 and a Frequency Response Analyser 1255B from Solartron. A Pt plate and a saturated calomel electrode (SCE) were used as counter and reference electrode, respectively. Gold electrodes with LB films deposited act as working electrodes with an effective surface of 0.07 cm$^2$. Impedance measurements were done in absence of any redox probe in a PBS at ambient temperature, in a frequency range from 50 mHz to 100 kHz, at a polarization potential of -600 mV/SCE using alternate voltage of 10 mV with a frequency modulation of 10 mV.

III. RESULTS AND DISCUSSIONS

III-A. Adsorption of proteins at the air/water interface

The surface pressure vs time ($π$-t) curves presented in Fig. 1 were measured during adsorption of proteins at the air/water interface at different protein concentrations.

It can be seen that the increase of the surface pressure was dependent on concentration of protein in the subphase and time. The higher concentration of protein produces the higher surface pressure. Such behaviour is in accordance with the kinetics of adsorption, for higher protein concentration, more protein molecules went up towards interface during the same adsorption time.

In addition, from these curves it can be seen that after 250 min the surface pressure continues to increase but very slowly, which means that the adsorption process of protein molecules reaches nearly equilibrium.
molecules, and some protein molecules were inserted into the ODA monolayer.

III-B. Surface pressure-molecular area isotherms

Three different subphases: ultrapure water, PBS at pH 7.5 without and with proteins were used to investigate the characteristics of the corresponding amphiphilic monolayers. The surface pressure – molecular area isotherms of ODA molecules on ultrapure water were considered as a reference. For the mixed film, an apparent molecular area was defined as the ratio of the film area to the number of amphiphile molecules.

It can be seen from Fig. 2 ingredients in the subphase have apparent influence on the characteristics of the amphiphilic monolayers at the air/water interface. Taking the curve of ODA molecules on ultrapure water as reference, the curve obtained with PBS subphase (without protein) is considerably different. We can see from Fig. 2, at low surface pressure, the molecular area of the ODA monolayer with the PBS as subphase was much larger than that of the ODA monolayer in ultrapure water. The difference is possibly attributed to the presence of strongly bound multivalent counter-ions, such as PO₄³⁻ in the PBS subphase, which has some effect on the monolayer.

In the case of PBS subphase with proteins OBP-1F, the apparent molecular area is still greater than without proteins at low surface pressures, it means that interactions exist between proteins and ODA molecules, and some protein molecules were inserted into the ODA monolayer.

Fig. 2. Surface pressure-area isotherms for amphiphile ODA with different subphases: water, phosphate buffer solution (10 mM, pH 7.5) without and with protein OBP-1F (4 mg L⁻¹).

Then with compression, the increase of surface pressure leads to approach of protein and amphiphile molecules until phase transition of the monolayer from liquid to solid occurs, between 30 and 40 mN m⁻¹, where the slope of the π-A curve decreases. In this surface pressure range, protein molecules spread at the air/water interface are gradually expelled from the interface and pushed under the positively charged head of ODA molecules. As a consequence, the surface pressure does not increase significantly. More importantly, a relative high amount of protein is included in the monolayer in the surface pressure range, which is important for the formation of Langmuir-Blodgett films and elaboration of biosensors. For this reason, in the following step we chose 35 mN m⁻¹ as the target pressure for transferring LB films onto gold electrode. And we have studied the stability of the mixed protein/amphiphile monolayer at the air/water interface at this surface pressure.

III-C. Stability of the mixed OBP-1F/ODA monolayer at the air/water interface

The stability of the mixed monolayer is very important because it is related to the transfer efficiency and the quality of deposited LB films. It
can be examined by the evolution of surface pressure with time at a fixed molecular area after the monolayer has been compressed.

In Fig. 3 the stability of the mixed OBP-1F/ODA monolayer at 35 mN m\(^{-1}\) is shown. We can see that during 3 h the monolayer keeps very stable under such condition, which confirms that the surface pressure, 35 mN m\(^{-1}\) can be used as target pressure for transferring LB films.

Fig. 3. Stability of mixed OBP-1F/ODA monolayer at the air/water interface at the surface pressure 35 mN m\(^{-1}\).

III-D. Transfer of Langmuir-Blodgett films

The stable mixed OBP-1F/ODA monolayers were then transferred from air/water interface onto functionalized gold electrodes at the target pressure 35 mN m\(^{-1}\). Since the gold electrodes have been previously functionalised with SAMs of ODT to be hydrophobic, deposition of LB films should occur during the first downstroke of the electrode through air/water interface.

For 4 mg/ml OBP-1F in the subphase, at target pressure of 35 mN m\(^{-1}\), two layers of LB films can be transferred successfully onto ODT functionalised gold electrodes with good reproducibility and quite high transfer ratio (96%, 110%, respectively) as shown in Fig. 4, which corresponds to a Y-type deposition.

After deposition of LB films, scanning electron microscopy (SEM) was used to characterize the films at a submillimeter scale in order to obtain some general topographic informations. As shown in Fig. 5 the structures appear to be heterogeneous, important rearrangements of LB films probably occurring during and/or after the transfer.

Fig. 4. Transfer ratio for two layers of mixed protein OBP-1F/ODA LB films deposition at target pressure 35 mN m\(^{-1}\) (concentration of OBP-1F: 4 mg L\(^{-1}\)).

Fig. 5. SEM image of two layers of mixed OBP-1F/ODA LB films transferred onto gold electrodes at target pressure 35 mN m\(^{-1}\).

III-E. Electrochemical impedance spectroscopy

After mixed LB films deposition on the gold electrode which was previously modified by an ODT SAMs, the sample was mounted as working electrode in the cell. Its Nyquist plot is shown in Fig. 6 curve A. And curve B is the Nyquist plot after exposure to odorant molecules.
Fig. 6. Nyquist ($Z''$ vs. $Z'$) plots measured for gold/thiol/ODA/OBP-1F LB films before (A) and after (B) exposure to odorant molecules. Inset: enlarged Nyquist plot for (B).

Comparing to curve A, we can see diameter of demi-circle for curve B decreases significantly. It means that the electrical resistance decreased. Probably the small odorant molecules can penetrate exterior lipid layer to reach OBP-1F aggregates, which leads to a rearrangement of the aggregates of the LB films. Such molecular reorganization can induce defined interstitial spaces where buffer species can diffuse more easily, which could provoke an electrical shortening between solution and electrode and provides explanation for the strong decrease of the value of the electrical resistance.

IV. CONCLUSION

In this study, we have studied the properties of mixed protein/amphiphile monlayers at the air/water interface. And we found such kind of monolayers are stable and can be transferred efficiently onto functionalised gold electrode, by which we have developed an artificial odorant biosensor. In future work, we plan to elaborate an array of such sensors to detect a wider range of odorant molecules.

ACKNOWLEDGEMENTS

This work was financially supported by Concerted Action NMAC (No 0220306) and the SPOT-NOSED Project (IST-2001-38739).

REFERENCES


Presented at 5th Japan France Seminar on Intelligent Materials and Structures (October 13-15, 2004)