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Surfactant bilayers for the direct electrochemical detection of affinity interactions

Mikhail Yu. Vagin [a,*], Arkady A. Karyakin [a], Tibor Hianik [b]

[a] *Faculty of Chemistry, M.V. Lomonosov Moscow State University, 119899, Moscow, Russia*
[b] *Faculty of Mathematics, Physics and Computer Science, Comenius University, Mlynska Dolina F1, 842 48 Bratislava, Slovak Republic*

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Abstract

Simple methods of preparing the direct affinity sensors are proposed. Due to the self-consistent introduction of a hydrocarbon chain bound with oligonucleotide pentadecathymidylate (dT_{15}) into the hydrophobic region of surfactant bilayer or the adsorption of antibodies on the bilayer surface, the immobilizations of oligonucleotide or antibodies were carried out correspondingly. The responses were detected by impedance spectroscopy. Whereas the specific DNA-coupling caused the decrease of real part of impedance, the antibody–antigen interaction caused the increase of real part. The obtained results give an opportunity for the development of impedimetric affinity sensors for clinical analysis or for the detection of various environmental pollutants.

Keywords: DNA sensor; Surfactants; Affinity interactions; Impedance spectroscopy

1. Introduction

In modern medicine, there is a problem of express specific recognition of different bioactive compounds and determination of specific human, viral and bacterial DNA sequences in diagnosis [1] and a search for adequate therapy. It is promising to use for these aims biosensors based on the detection of affinity interactions by some powerful electrochemical methods such as electrochemical impedance spectroscopy [2,3].

The bilayers composed of surfactant molecules mimic the cell membrane structure [4] and are good barriers for ions [5]. They have been already used for the direct detection of affinity interactions [6]. Bilayer lipid membranes (BLM) have a biocompatibility and similar physical properties with natural cell membranes [7]. However, the wide use of BLMs is restricted by low stability [8]. The membranes of uncharged surfactant Brij-52 self-assembled onto the gold electrode are much more stable [9]. In the present work, we propose to use bilayers of Brij-52 on gold electrodes as transducers for the detection of affinity interactions. The new affinity sensors possess a high stability. The level of signal/noise ratio in proposed systems is higher than in previously reported biosensors [7,10].

2. Experimental

All substances, procedures and experimental set up were used as in Ref. [9]. The horseradish peroxidase and antibodies against it were from Immunotech (Moscow, Russia) and used in glycerin protective buffer solution with a 10^{-4} M concentration and were stored in -20 °C. All electrochemical methods and equipment were used as in Ref. [9].

The synthesis of the dT_{15} in the 5-hexadecylphosphoryl-pentadecathymidylate ($C_{16}pdT_{15}$) as well as complementary (dA_{15}) and noncomplementary $dAT_8C_6G_7$ oligonucleotides was performed by phosphoramidite method as described elsewhere [11]. DNA-probe ($C_{16}pdT_{15}$), dA_{15} and $dAT_8C_6G_7$ were used in double distilled water solution with 10^{-6} M concentration.

The electrodes for affinity sensors were prepared as follows. Five microliters of Brij-52 solution in pure ethanol (concentration 10 mM) was syringed onto the top of the gold disk electrode, and the solvent was allowed to evaporate. Then the electrode was put in the phosphate buffer solution. The bilayer membrane was self-assembled over-

* Corresponding author. Fax: +7-95-9395417.
E-mail address: MVY@enzyme.chem.msu.ru (M.Y. Vagin).

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night. Afterward, the electrode with membrane was put into the 10^{-8} M buffer solution of $C_{16}pdT_{15}$ or 10^{-6} M buffer solution of antibodies against horseradish peroxidase and also left overnight.

For the response detection, the electrodes were placed into an electrochemical cell in buffer solution and the impedance spectra of the background were recorded. Then the different oligonucleotides or horseradish peroxidase solutions were added into the cell with electrode with immobilized DNA-probes or antibodies correspondingly. The series of impedance spectra were recorded again.

3. Results and discussion

In the present work, we proposed to use the possibility of self-consistent introduction of a hydrocarbon chain bound with oligonucleotide into the hydrophobic region of a Brij-52 bilayer. The adsorption on the Brij-52 bilayer was accepted during the incubation. The complementary oligonucleotide coupling with bound one and the antigen–antibody binding on the transducer surface should provide the changes of membrane interfacial properties and therefore the response generation.

The background spectra of electrodes with Brij-52 bilayer with immobilized particles (Figs. 1 and 2) and the spectra detected after addition of complementary oligonucleotide dA_{15} or antigen are shown in Figs. 1 and 2, respectively. It can be seen that the addition of detected particles leads to the changes of spectrum. An addition of

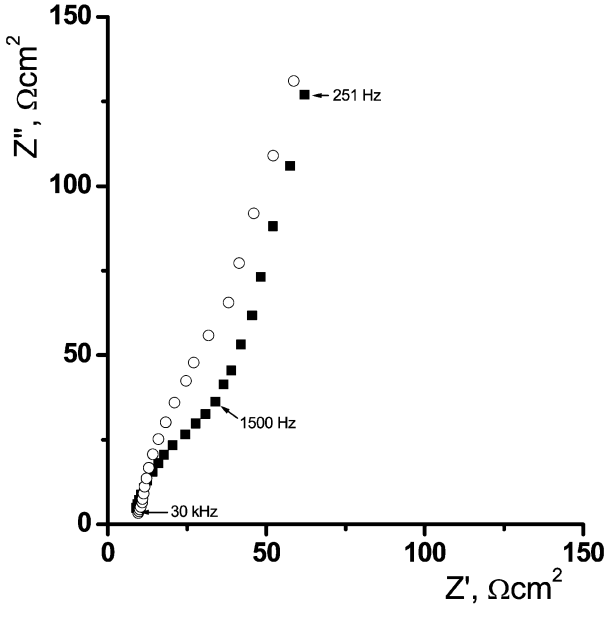

Fig. 1. The background impedance spectrum for the electrode with immobilized oligonucleotides T_{15} (■) and spectrum detected in presence of complement oligonucleotide A_{15} (10^{-8} M) (○). 0.1 M NaCl, 0.05 M KH_2PO_4, pH 7.4; 0.1 V Ag/AgCl/0.1 M KCl.

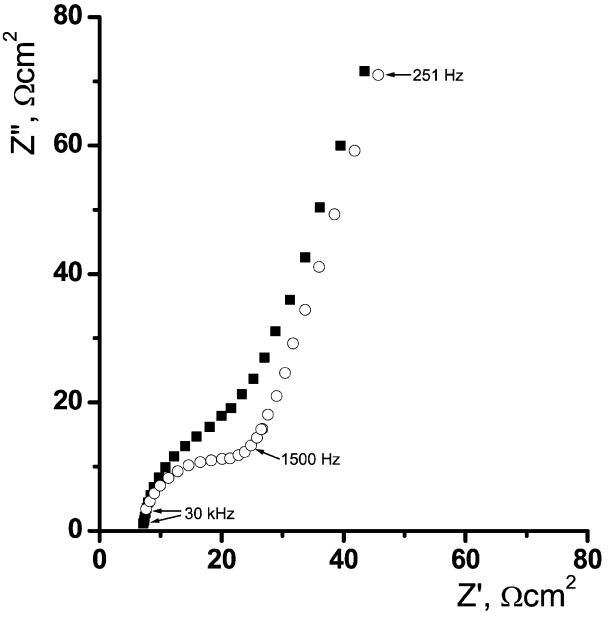

Fig. 2. The background spectrum for the electrode with immobilized antibodies against horseradish peroxidase (■) and spectrum detected in the presence of antigen (peroxidase 3×10^{-5} M) (○). 0.1 M NaCl, 0.05 M KH_2PO_4, pH 7.4; 0.1 V Ag/AgCl/0.1 M KCl.

dA_{15} causes the decrease of real part whereas antigen presence mainly leads to the increase of real part of impedance. In both cases, the imaginary part of the impedance did not change drastically. Therefore, the complement coupling leads to the decrease of membrane resis-3tance probably caused by local violations of inner bilayer order due to the hydrocarbon chains of modified oligonucleotides. At the same time, the large antigen binding ($M_{peroxidase} = 40{,}000$) increases the thickness of the interfacial layer on the electrode surface and further shields ions permeation. This effect increases the real part of the impedance. The background spectra for the Brij-52 bilayer system with immobilized DNA-probes were the same to the spectra measured in the presence of noncomplementary oligonucleotide $dAT_8C_6G_7$. Also the spectra for pure surfactant layer on the electrode in absence and in presence of antigen were identical. Thus, the responses are obviously caused by specific DNA coupling or antigen–antibody interactions.

To develop the biosensor based on the affinity interactions detection it is not necessary to measure whole impedance spectrum in wide frequency range of applied potential. The most significant changes were observed in the real part of impedance in the frequency range 1–10 kHz (Figs. 1 and 2). The highest responses were observed on the frequency of 2 kHz for the DNA-sensor and 3 kHz for immunosensor. The changes of the response were approximately 40% and 50% from background level correspondingly, which is higher than in earlier reported systems [7,10].

4. Conclusion

In the present work, the simple methods of preparing direct affinity sensors were proposed. Due to the immobilization of the oligonucleotides or antibodies on the transducer surface we could detect the specific binding of complement oligonucleotides or antigen–antibody interaction correspondingly. The different nature of response manifestation was shown. The changes of the response were approximately 40% and 50% from background level correspondingly, which is higher than in reported systems [7,10].

The obtained results give an opportunity for the development of impedimetric affinity sensors for the direct detection of specific DNA sequences or of different antigens.

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