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Electrochemical formation of polypyrrole-based layer for immunosensor design

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ABSTRACT

This research represents the evaluation of electrochemical formation of conducting polymer polypyrrole-based composite layer, which could be applied in immunosensor design. Polypyrrole (Ppy) layer was formed by mean of potential pulses and bovine leukaemia virus (BLV) protein *gp*51 (*gp*51) was entrapped during this synthesis within formed Ppy layer (*gp*51/Ppy). Some Ppy layer formation aspects were evaluated and mathematical model, which is describing tendencies of *gp*51/Ppy layer formation, was adopted. The interaction of *gp*51/Ppy layer with specific antibodies that are present in the blood serum of BLV infected cattle was evaluated by pulsed amperometric detection.

1. Introduction

The implementation of advanced composite materials, which are suitable for selective interaction with analyte and/or conversion of this interaction into analytical signal, offers new opportunities in sensorics and biosensorics [1]. Unique catalytic [2–4] and affinity [1,5] properties of conducting polymers have been reported in scientific researches. Therefore conducting polymers have been applied in the design of catalytic [6,7] and affinity [1,8] biosensors as: (i) analyte recognizing components [1], (ii) signal transduction systems [1] and/or (iii) immobilization matrices [6].

The capability of conducting polymers to transfer electrical charge is exploited in some sensors [1] and molecular devices. Due to this fact some electrochemically generated conducting polymers could be applied in amperometric enzymatic biosensors [9]. In addition other physicochemical or physical properties (e.g. electrical resistance, electrochemical capacity, optical properties etc.) can be monitored by signal transducer and converted into analytical signal. In such way electrochemically generated conducting polymers could be applied for amplification of analytical signal [10]. Therefore the fabrication of conducting polymer based composite layers is very important for the development of sensors and biosensors [11]. Conducting polymer polypyrrole (Ppy) is often used in the design of electrochemical biosensors. Ppy layers could be formed by several different methods: (i) chemical polymerization initiated by oxidators such as $FeCl_3$ or H_2O_2 [12], (ii) enzymatic polymerization [13] and (iii) electrochemical polymerization [1,23]. Among different methods the electrochemical formation of Ppy-based layers seems very promising due to some advantages that are required for sensor and biosensor design. The most attractive advantages are the possibilities: (i) to dope and to enrich the Ppy layer by selected biological compounds and (ii) to start/stop the polymerization at selected time frame. Therefore, many different electrochemical methods were developed in order to form a wide variety of Ppy layers. These methods are mainly based on potentiostatic [14], galvanostatic [14,15], or potentiodynamic [1,23] techniques. The last mentioned technique seems the most suitable for the formation of stabile conducting polymer layer [1,6] since they allow to increase the concentration of pyrrole monomer at pre-electrode environment during the period when the electrode potential decreases below the potential, which is

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required for the initiation of the polymerization. On the other hand potentiodynamic methods allow significant enrichment of Ppy layer by entrapped biological compound (e.g. enzyme, antibody, single stranded DNA, etc.) [16–20]. In this way the consumption of expensive biomaterials could be reduced. Therefore potentiodynamic methods seem the most attractive for the entrapment of biomaterials, which are used in biosensor design. Potentiodynamic methods based on both potential cycling and potential pulses are mostly applied for the formation of Ppy layers [21], but among these two methods the potential cycling became more popular due to broader availability of this technique in commercial electrochemical devices. However this method chausses significant heterogeneity of formed Ppy layer. These heterogeneities are formed due to potential alteration in wide range. If at certain period of cycle the potential of electrode exceeds +600 mV *vs* Ag/AgCl then the polymerization reaction is initiated [22]; if at certain period of cycle the potential becomes lower than +600 mV *vs* Ag/AgCl then the polymerization is stopped because such potential is too low for the generation of cation-radicals of pyrrole, which are required for the initiation of polymerization reaction. Efficiency of electrochemical polymerization reaction and characteristics of formed Ppy layers depend on setting of parameters such as potential sweep rate and vertex potentials. If at certain period of cycle electrode potential exceed +1200 mV *vs* Ag/AgCl then the electrolysis of water and/or to overoxidation of formed Ppy layer is induced. Therefore more controllable and more reliable is another potentiodynamic method, which is based on rectangular potential pulses with fixed potential values [23]. In this method several discreet potential levels, which are the most suitable for polymerization and for "relaxation" of electrochemical system between polymerization steps, are applied. During the relaxation-step concentrations of pyrrole monomer and materials, which become entrapped within Ppy layer, are restored at pre-electrode environment. Pulsed potential based techniques allow better control of each film formation step and even the application of some additional potential steps, which upon the request could be suitable for some other technological purposes. A range of catalytic biosensors [24], DNA-sensors [25], immunosensors and molecularly imprinted polymer-based affinity sensors [1] were created using pulsed potential based techniques. However the majority of mentioned Ppy-based layers were formed by experimentally determined settings of potential-pulse profile and number of pulses. Therefore additional attempts, which are based on mathematical evaluation of amperometric signals during pulsed amperometric polymerization, are required for better control of Ppy layer formation.

The aims of this study were: (i) to adopt mathematical equation, which is suitable for the evaluation of potential pulses based formation of Ppy layer doped by protein *gp*51 (*gp*51/Ppy) and (ii) to evaluate the interaction of *gp*51/Ppy-based layer with specific antibodies (anti-*gp*51) by pulsed amperometric detection (PAD).

2. Experimental

Chemicals. Basic chemicals including salts (KCl, KH_2PO_4, K_2HPO_4, etc.), which were required for the preparation of buffers were purchased from Sigma–Aldrich (St. Louis, MO, USA). All these chemicals were of analytical or better grade and were used as received from producers unless stated otherwise. Pyrrole of 97% purity was purchased from Sigma–Aldrich (St. Louis, MO, USA) and it was purified additionally by passing through a 5 cm length column filled by Al_2O_3. BLV protein *gp*51 and blood serum containing antibodies against *gp*51 (anti-*gp*51) were obtained from 'Biok' (Kursk, Russia). All solutions were prepared using high purity water with resistivity higher than 18 MΩ cm purified by "Purator-B" Glass Keramic (Berlin, Germany). Oxygen-free solutions were obtained by purging argon gas through the solutions for at least 20 min. All procedures and measurements were carried out at room temperature.

Electrode pretreatment. All solutions were prepared and were used under rigorous exclusion of oxygen by argon. A platinum wire, which was molten in soft glass exposing a disc electrode with a diameter of 1 mm, was applied as a working electrode. Cleaning and pretreatment procedures of the Pt electrode were based on: (i) rinsing with concentrated HNO_3 solution in an ultrasonic bath for 10 min, (ii) rinsing with water, (iii) polishing on a polishing cloth using alumina paste with 3.0, 1.0, and 0.3 μm size grain. After the rinsing with water, then – with 10 M of NaOH, and after this – with 5 M of H_2SO_4 in an ultrasonic bath the electrochemical cleaning/roughening of electrode was applied. This procedure was based on potential cycling in 0.5 M H_2SO_4 between −100 mV and +1200 mV *vs* Ag/AgCl at sweep rate of 100 mV s^{-1}. The potential cycling was performed until the cyclic voltammogram displayed stable features, which are characterizing bare platinum surface. Then the electrodes were deposited in a specially designed low-volume electrochemical cell [26], which allowed to decrease the volume of polymerization solution down to 200 μL. The low-volume electrochemical cell consisted of platinum working electrode (1 mm diameter), platinum auxiliary electrode, and Ag wire, which was covered by AgCl layer and was served as reference electrode. The layer of 'platinum black' was deposited over the platinum working electrode in order to improve the adhesion of the Ppy layer to the electrode surface [23]. In this paragraph mentioned reductive deposition of Pt clusters was performed in oxygen-free 1 mM solution of H_2PtCl_6 containing 0.1 M of KCl by 5 potential cycles in the range between +500 and −400 mV *vs* Ag/AgCl at a sweep rate of 10 mV s^{-1}.

Electrode modification by polypyrrole layers. Ppy layer formation was performed by home-made potentiostat controlled with personal computer. The electrochemical deposition of polypyrrole layer was performed in the same three-electrode miniaturized electrochemical cell, which was briefly described in previous chapter. The solution containing 50 mM of pyrrole, 100 mM of NaCl and 10 mg/mL of *gp*51 was used for the electrochemical formation of Ppy layer doped by *gp*51. The electrochemical formation of *gp*51/Ppy layer was performed by 30 potential pulses with rectangular potential profile, which consisted of two potential levels: (i) 950 mV *vs* Ag/AgCl for 1 s (at this potential the polymerization reaction was initiated) [1,27] and (ii) 0 mV for 10 s (at this potential the *gp*51 and the pyrrole concentrations in the neighbourhood of the electrode was restored) [23].

Electrochemical detection of analytical signal. PAD was applied for the determination of analytical signal, and it was performed using a potentiostat–galvanostat "PGSTAT 30" EcoChemie/Autolab (Utrecht, Netherlands). Conventional, 2.0 mL volume, three-electrode cell consisted of platinum working electrode of 1 mm diameter, which was covered by Ppy layer, platinum auxiliary electrode, and Ag/AgCl in 3.0 M KCl, which was used as reference electrode. All electrochemical measurements were carried out in a Faraday's cage. All PAD experiments prior and after all incubation steps were performed in 0.05 M potassium phosphate buffer, pH 7.0, containing 0.1 M of KCl.

Interaction of gp51/Ppy layer with specific antibody. In order to form a complex between immobilized antigen (*gp*51 protein) and antibody, which is present in the blood serum of BLV infected cattle, the *gp*51/Ppy-modified electrode was incubated in 10 times diluted blood serum containing anti-*gp*51 for 60 min. Some control experiments were performed in order to evaluate the specificity of *gp*51/Ppy-modified electrode. These control experiments were based on the incubation in healthy cattle serum under the same conditions as in the case of BLV infected cattle serum. The serum of healthy cattle contains many different proteins including number