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Development of label-free impedimetric platform based on new conductive polyaniline polymer and three-dimensional interdigitated electrode array for biosensor applications

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ABSTRACT

Novel label-free impedimetric platform based on a three-dimensional interdigitated electrode array (3D-IDEA) sensor and new conductive polymer as a transducer for oxidoreductases is introduced. This platform is cost-effective, simple to construct and miniaturize. Monomer of conductive polymer N-(N',N'-diethyldithiocarbamoylethylamidoethyl) aniline (AnD) was deposited onto 3D-IDEA by chemical polymerisation. It was found that the polymer film resistance depends on the redox-potential of the solution. For the first time polyAnD was used as enzyme immobilisation matrix. Pyrroloquinolinequinone (PQQ) dependent alcohol and glucose dehydrogenases were immobilized on 3D-IDEA covered with polyAnD by two different methods. 3D-IDEA sensors with enzymes, which were immobilised by physisorption on polyAnD layer, showed specific response in the presence of 1 μM of the corresponding substrates. Obtained results revealed that PQQ dependent dehydrogenases can re-oxidize on polyAnD via direct electron transfer (DET) from enzyme active site to the polymer surface. This process can be monitored by methods of electrochemical impedance spectroscopy (EIS) and chronoamperometry. Presented study shows that EIS method gives a useful tool for research of re-oxidation process and interaction of electroactive enzymes with conducting materials giving information required to construct and develop analytical devices.

1. Introduction

Nowadays there are numerous fields such as medicine, microbiology, food and environmental protection where ultrasensitive and fast sensors for determination of various analytes are required. The sensors based on biocatalysts and electrochemical detection gained wide attention from researchers due to their unique sensitivity, cost-effectiveness, short-time analysis, capability of measurements in dispersed and nontransparent solutions. Moreover these devices are easy to miniaturize.

There are different electrochemical methods, which are employed to construct biosensors: amperometry, voltammetry, potentiometry, coulometry, conductometry and electrochemical impedance spectroscopy (EIS) [1]. EIS is a powerful analytical tool that is employed successfully in chemical and biochemical analysis [2–5], as well as to study biocatalysis on the surface of electrodes [6–8]. EIS is widely applied in physical and biological sciences especially in the field of biosensor research [9]. EIS can provide information on the changes of biocatalyst structure or electrode matrix structure [10,11]. Moreover, in comparison with other electrochemical methods, EIS setup does not require a reference electrode and analysis can be performed without high potential treatment of the electrode surface, that can destroy thin and

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sensitive bioactive layer of the sensor [12]. Impedimetric biosensors are employed for quantitative and qualitative immunoanalysis [10], determination of nucleoacids, their mutations [13] and different redox-active compounds [14], e.g. H_2O_2. However, the possibility of impedance measurements often requires the presence of redox-active species in test solution, e.g. usually ferricyanide/ferrocyanide couple is used. In this case Faradaic charge transfer resistance is measured, which may be affected by interactions of a target biomolecule with a probe-functionalized sensor surface [15]. To avoid addition of redox-agents and to be able to perform the measurements immediately on the area close to the electrode surface the capacitive biosensors based on interdigitated electrode arrays (IDEA) were introduced [16]. In this case biochemical reactions at the sensor surface are registered as capacitance changes in non-Faradaic measurements. Planar microband electrodes of IDEA, between which the impedance is measured, are very closely situated, so that in this case changes in electrical properties of the interdigital space may affect the sensor impedance [15]. Several years ago the concept of a three-dimensional interdigitated electrode arrays (3D-IDEA) with electrode fingers separated by an insulating barrier was proposed [9,17]. The specific design of this sensor structure allows for enhancement of its sensitivity for biochemical reactions taking place at the sensor surface [18]. The principles and applications of 3D-IDEA sensors were recently discussed in [15].

Another opportunity to avoid the addition of redox-compounds or special labels for analytes into the test solution is an application of electrochemical systems based on redox-active enzymes – oxidoreductases, since some of them can possess direct electron transfer (DET) from an enzyme active site to the electrode surface. The effective direct re-oxidation of enzyme onto electrode surface ensures the highest selectivity of analysis, independence of oxygen concentration in the media, analysis at lower potential and simplifies the sensor construction [19]. Due to this, DET became a phenomenon, which is extensively investigated during last several decades. Oxidoreductases are the class of enzymes [20] that catalyze selective oxidation or reduction of many organic compounds and this class is of interest for various industrial applications. The redox-active moiety of most of the oxidoreductases is deeply buried within the enzyme globule [21]. Such spatial isolation protects enzyme active site from surrounding and provides effective kinetic barrier for DET. Thus, due to unique three-dimensional structure only certain types of oxidoreductases may participate in DET and only on specific electrode materials [22].

Broad applicability of 3D-IDEA sensors to study interactions of biomolecules [18] can be useful to apply this sensor type for investigation of oxidoreductases. A synergism of 3D-IDEA and unique selectivity of oxidoreductases can be promising to design label-free ultrasensitive new impedimetric biosensor, based on determination of the changes only in the electrical properties of immobilised enzyme layer and enzyme immobilisation matrix. In order to construct such biosensor platform several requirements must be fulfilled: 1) the enzyme must possess DET; 2) the enzyme immobilization matrix must provide for enzyme adhesion, stabilization and orientation; 3) the immobilization matrix must have reasonable conductivity [23–26].

To address the first issue one can consider pyroloquinolinquinone (PQQ) dependent oxidoreductases which represent a unique group of enzymes that can act as electrocatalysts, facilitating the electron transfer between different conductive electrode materials and their substrate molecule with no mediator involved in the process [27–29]. It is important to note that PQQ dependent enzymes do not react with molecular oxygen and, consequently, they are attractive for construction of the third generation biosensors [19] based on the DET between enzyme and electrodes. Previous investigations showed that PQQ dependent enzymes exchange electrons with gold, silver, pyrolytic graphite, several organic polymers, glassy carbon and carbon paste electrodes [30–35]. In addition, the efficiency of the DET is highly dependent on the intrinsic properties of the electrode material.

In order to address the second and the third issues one can apply various conducting polymers which are very attractive materials as their properties may be tuned chemically in a desired way to assist with electron transfer [36,37].

Conducting polymers, especially polyanilines, have a great potential in electronics and optics [38,39] and especially in the field of chemical sensors and biosensors [3,37,40,41]. Due to their peculiar electrochemical and optical properties these materials can be used as components of different transducers in potentiometric, amperometric and impedimetric/conductimetric sensors [3]. They are frequently employed as sensitive elements of "chemiresistors" for gas sensing [42]. These polymers were found to change their electronic conductivity in response to changes in pH or redox-potential of the solution in contact with the polymer [43,44] and were suggested to be applied as chemical or redox-sensors [45]. Biosensors with conducting polymers are most commonly used in amperometric detection [3], though conductimetric sensors with different enzymes have also been reported [46–48]. Recently new *N*-substituted aniline derivatives with interesting features were reported in the literature [36,49]. These monomers contain two essential parts: aniline and methacrylamide or dithiocarbamate. Recently one of such monomers *N*-(*N*',*N*'-diethyl dithiocarbamoyl ethyl amido ethyl)aniline (AnD) [36] is introduced. The aniline part of this substance can be polymerized in both chemical and electrochemical way and conductivity of the resulting polymer is similar to that of aniline. An attractive feature of polyAnD molecule is that each monomer unit has a dithiocarbamate ester moiety, and it can be used for further surface modification. UV grafting can be applied for grafting of other polymers over polyAnD via iniferter activation. Moreover, it was shown, that polyAnD can be used as molecular imprinted polymer for amperometric determination of certain organic compounds [50]. These features seem useful and are more advantageous in comparison with other conducting polymers for construction and directional modification of electrode matrix for design of bio-compatible enzyme immobilisation matrix with high surface area.

In this study we introduce a novel label-free impedimetric platform based on 3D-IDEA sensor and new conductive polyaniline polymer polyAnD as a transducer for oxidoreductases. The three different PQQ dependent dehydrogenases were applied to test suitability of the proposed impedimetric platform for studying of electron transfer processes between enzymes and immobilization matrix and for biosensing purposes as well.

2. Experimental

2.1. Enzymes and chemicals

Membrane bound PQQ dependent alcohol dehydrogenase (mADH) from *Gluconobacter sp.* 33, E.C. 1.1.5.5, was isolated and purified by the method reported in [51]. The activity of the enzyme solution in 5 mM Tris buffer (pH 7.5) with 1 mM $CaCl_2$, 0.02% Triton-X-100 and 0.5% sucrose was 200 U/ml. Ethanol was used as default substrate for the mADH.

Soluble PQQ dependent alcohol dehydrogenase (sADH) from *Pseudomonas putida* HK5, E.C. 1.1.9.1, was isolated and purified by the method reported in [52]. The activity of the enzyme solution in 5 mM Tris buffer (pH 8.5) with 5 mM $CaCl_2$ was 130 U/ml. 1,2-propandiol was used as the default substrate for the sADH.

Soluble PQQ dependent glucose dehydrogenase (GDH) from *Acinetobacter calcoaceticus*, E.C. 1.1.5.2, was isolated and purified by