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Potentiometric study of quinohemoprotein alcohol dehydrogenase immobilized on the carbon rod electrode

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Abstract

Enzymes, which exhibit redox properties and are able to directly exchange electrons with conducting materials, are currently of special interest in the fields of biosensorics and bioelectronics. The detection of new electronic properties makes them even more attractive for these growing fields. Quinohemoprotein alcohol dehydrogenase (QH-ADH) from *Gluconobacter* sp. 33 was demonstrated as 'nano-sized electrical power generator' able to separate the electrical charges and generate a measurable electrical potential. This phenomenon was investigated potentiometrically in electrochemical system where QH-ADH was applied as the catalyst oxidizing ethanol thereby converting the energy of this chemical reaction to an electrical potential. A basic immobilization technique based on cross-linking with glutaraldehyde was applied for the immobilization of QH-ADH onto a carbon rod. The maximal open circuit potential generated by QH-ADH immobilized on carbon rod electrode was −115 mV versus an inactivated QH-ADH-modified electrode (Inactiv-ADH/carbon). If 10 mM of redox mediator $K_3[Fe(CN)_6]$ was added to the solution the potential rose to −190 mV versus Inactiv-ADH/carbon. The influence of concentration of Na acetate buffer, pH 6.0, on registered potential was approximately at the same level as the influence of KCl concentration (influence of ionic strength). This result implies that local pH changes do not play a significant role in the development of QH-ADH-modified carbon electrode potential. The potentiometric signal was more stable than amperometric signal based on the same QH-ADH-modified carbon electrode. The ability to directly generate electric potential opens new opportunities for the application of QH-ADH and other direct electron transfer exhibiting enzymes in the design of new potentiometric sensors, biofuel cells and self-powering bioelectronic devices.

Keywords: Bioelectronics; PQQ; Alcohol dehydrogenase; Potentiometry; Biosensor; Bionanotechnology; Direct electron transfer

1. Introduction

Biosensors are combining the basic principles of microelectronics, chemistry, biotechnology and even nanotechnology, they attract considerable attention in fields such as medicine, environmental monitoring, pharmaceutics and food quality control. The biosensor consists of a bio-specific sensing element that responds to a given property of the substance being sensed. The sensing element is usually in contact with/or integrated within a suitable transducer. The transducer then detects the interaction of bio-specific element with the analyte [1]. Here significant attention is paid to the electrochemical sensors. The class of catalytic biosensors is one of the most important classes of the family of electrochemical biosensors. Enzymes are biological components most commonly used in catalytic biosensors, while electrochemical transduction is the most popular method, often employing potentiometric or amperometric techniques.

In potentiometric devices the analytical information is obtained by converting the biorecognition process into a potential signal, whereas the amperometric types are based on monitoring the current associated with oxidation or reduction of an electroactive species involved in the recognition pro-

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cess. Different types of enzymes were applied in the design of catalytic–electrochemical sensors, but it is evident that the most promising approach for the development of electrochemical biosensors is to establish direct electrical communication between the biomolecules and the electrode surface [2–8]. Here conducting polymers [3], redox polymers [4–7] and soluble [7–9] or covalently attached to enzyme redox mediators [10] were applied as electron transfer shuttles that enable direct amperometric signal detection. Further methodology of amperometric biosensor development involves the application of redox enzymes for the targeted oxidation/reduction of analytes at the electrode supports and the generation of the electrical signal output. Towards this goal, it is essential to tailor integrated enzyme electrodes that exhibit 'electrical contact' with the conductive supports [11]. Different classes of oxidative enzymes (e.g., oxidases, dehydrogenases) require the application of different molecular tools to establish this electrochemical communication [12,13]. Electron transfer mediators shuttling electrons between the enzyme active centers and electrodes are usually necessary for the efficient electron transfer from the flavin adenine dinucleotide (FAD)-dependent oxidases (e.g., glucose oxidase) [14]. $NAD(P)^+$-dependent dehydrogenases require soluble $NAD(P)^+$ cofactor and an electrode catalytically active for the oxidation of NAD(P)H and regeneration of $NAD(P)^+$ to establish electrical contact with the electrode [15]. But the majority of enzymes mentioned are unable to transfer electrons without additional electron shuttles [16,17]. The study of direct and reversible electron transfer is of great interest for the development of direct (mediator free) biosensors [18,19]. The first direct electron transfer between protein and electrodes was observed in late 70's for cytochrome *c* [20,21] and laccase adsorbed on graphite [22]. Since then, a great number of papers have been published concerning the problem of direct electron transfer between redox enzymes and conducting surfaces [23–26]. Redox enzymes containing heme-*c* are the most promising enzymes in this context [27–29]. Such an enzyme is the heme-*c* containing pyrroloquinoline quinine (PQQ)-dependent alcohol dehydrogenase (QH-ADH) from *Gluconobacter* sp. 33. QH-ADH was reported to demonstrate direct electron transfer between the enzyme and conducting polymer polypyrrole [3] and carbon [28,29]. This unique property of QH-ADH was employed in the design of amperometric biosensors [30]. To establish an optimal signal all QH-ADH-based amperometric sensors were operating at constant potential ranging from +200 to +400 mV versus Ag/AgCl [3,29,30]. However, the ability to generate electrochemical potential by well-established direct electron transfer chain of QH-ADH has never been investigated as potential transduction principle. Moreover, the traditional potentiometric biosensors are based on enzymes affixed on the top of a pH or ion-selective electrode which is quantifying the change in potential as a function of the change in pH or in particular ion concentration [31]. The absolute majority of potentiometric sensors are operating according to this scheme. The advantages of potentiometric biosensors are simplicity and low cost of both production process and measuring electronic instruments. Moreover, the most successful schemes that are able to generate sufficient electrical current, might be adopted for the design of biofuel cells [32] and for powering nano-sized bioelectronic devices operating at physiological conditions [33].

The aims of the current study were: (i) to design a new type of potentiometric sensor based not on pH or ion concentration changes as usual potentiometric system is functioning but on QH-ADH-generated electrochemical potential based on the ability to separate charges and transfer electrons via established intrinsic electron transfer chain towards carbon surface; (ii) to investigate the general principles of potentiometric signal generation in QH-ADH-modified carbon electrodes.

2. Experimental section

2.1. Chemicals

Alcohol dehydrogenase from *Gluconobacter* sp. 33 (E.C. 1.1.99.8) was isolated and purified according to previously reported protocol [34] by Dr. I. Bachmatova and Dr. L. Marcinkeviciene, Institute of Biochemistry (Vilnius, Lithuania). The enzyme had an activity of 171 U/ml and 7.6 mg/ml concentration of proteins. Carbon rod electrodes 99.999% purity, low density, 3 mm in diameter were obtained from Sigma–Aldrich (St. Louis, USA). An aliquot of thermally inactivated QH-ADH was prepared if separated portion of enzyme was stored at 55 °C for 6 h. Enzymatic activity of alcohol dehydrogenase after thermal inactivation was undetectable.

2.2. Electrode preparation

Enzyme-modified graphite electrodes were prepared as follows: first, rods of spectroscopic graphite were cut, and polished on fine emery paper, followed by rinsing the electrode surface with water and drying at room temperature before coating with enzyme. Then 3 μl of enzyme solution were equally distributed on the electrode surface and electrodes were stored for 20 h over the 5% solution of glutaraldehyde at 4 °C in the closed vessel as it was previously described [35]. Over each electrode 0.0228 mg/ml of protein with initial QH-ADH activity of 0.513 U was distributed. For the experiments with reduced QH-ADH activity electrodes containing mixtures 4:1, 2:1, 1:1, 1:2, 1:4, 1:8 and 1:16 of active QH-ADH and inactivated QH-ADH were prepared and immobilized according to the protocol previously described in this chapter.

2.3. Electrochemical measurements

All electrochemical measurements were performed by potentiostat-galvanostat PGSTAT 30 with GPES3 v3,2