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Electrochemical biosensor based on glucose oxidase encapsulated within enzymatically synthesized poly(1,10-phenanthroline-5,6-dione)

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ABSTRACT

This study is focused on the investigation of electrocatalytic effect of glucose oxidase (GOx) immobilized on the graphite rod (GR) electrode. The enzyme modified electrode was prepared by encapsulation of immobilized GOx within enzymatically formed poly(1,10-phenanthroline-5,6-dione) (pPD) film. The electrochemical responses of such enzymatic electrode (pPD/GOx/GR) *vs.* different glucose concentrations were examined chronoamperometrically in acetate-phosphate buffer solution (A-PBS), pH 6.0, under aerobic or anaerobic conditions. Amperometric signals of the pPD/GOx/GR electrode exhibited well-defined hyperbolic dependence upon glucose concentration. Amperometric signals at 100 mM of glucose were 41.17 and 32.27 μA under aerobic and anaerobic conditions, respectively. Amperometric signals of the pPD/GOx/GR electrode decreased by 6% within seven days. The pPD/GOx/GR electrode showed excellent selectivity in the presence of dopamine and uric acid. Furthermore it had a good reproducibility and repeatability with standard deviation of 9.4% and 8.0%, respectively.

1. Introduction

Diabetes is a rapidly growing problem, currently affecting many millions of people. It is expected that this number will increase two folds within next 20 years. Although there is no cure for diabetes, patients can reduce disease associated complications through the tight control of blood glucose levels [1–4]. For this reason the research and development of sensors for the management of diabetes is an important research area. Even though progress in this field is rapid, the ultimate goal of achieving long-term, accurate and continuous glucose monitoring directly in patient body has been still a challenge [5–8]. However, several instrumental methods have been reported for the determination of glucose [9–13]. Among them, electrochemical techniques have been found to be the most promising because of their high sensitivity, stability, reproducibility, low cost and easy maintenance [14,15]. On the other hand, in some cases, electrochemical techniques based on the electron transfer between a redox enzyme and an electrode are challenging because of the deeply imbedded redox center in the protein shell of enzyme. Hence in order to provide an efficient electron transfer between redox enzymes and electrodes, the modification of electrode surfaces with suitable redox mediators has been applied as a very common approach [16–18]. For this purpose some polymers are suitable as the immobilization matrix because of their simple preparation procedure, biocompatibility, chemical inertness, mechanical stability and limited permeability, which extends linear detection range. On the other hand the most exploited feature of glucose oxidase (GOx) in enzyme based glucose sensors is their ability to form hydrogen peroxide during catalytic reaction. Hence formed hydrogen peroxide initiates synthesis of polymer, which then forms a shell over the enzyme. The formed polymeric shell significantly reduces the diffusion rate of glucose and formed products. Some studies related to enzymatic polymerization have been performed by different monomers such

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as pyrrole, aniline, thiophene and some others [19–24]. However just few reports have been published related with application of either immobilized or polymerized phenanthroline derivatives in sensor design [17,25,26].

The 1,10-phenanthroline-5,6-dione, one of phenanthroline derivative, is a versatile ligand due to its two diiminic nitrogen atoms suitable for the binding of metal ions, and the *o*-quinoid moiety involved in redox reactions [27,28]. Although, several studies have been carried out in order to clarify the spectroscopic properties of phenanthroline derivative–metal complexes and their applicability in electrochemical sensors [26,29–31], due to our best knowledge, there are no scientific reports on application of polymerized 1,10-phenanthroline-5,6-dione in sensor design. From the point of this view, in order to evaluate advantages of such kind of ligand, the aim of this study was to design the electrode based on immobilized GOx and enzymatically synthesized poly(1,10-phenanthroline-5,6-dione).

2. Experimental

2.1. Chemicals and equipments

All chemicals were of analytical-grade and were used as received. The GOx from *Aspergillus niger* (E.C. 1.1.3.4.) with catalytic-activity of 295 U/mg and glutaraldehyde were purchased from AppliChem GmbH (Darmstadt, Germany). The D-(+)-glucose was obtained from Carl Roth GmbH&Co (Karlsruhe, Germany). 1,10-phenanthroline-5,6-dione (PD) was purchased from Sigma–Aldrich (Berlin, Germany).

1.0 M solution of glucose was prepared in distilled water at least 24 h before use to allow glucose to mutarotate and to reach the equilibrium between α- and β-forms. 10 mg/mL solution of GOx was prepared in the acetate/phosphate buffer solution, which is the mixture of 0.05 M sodium acetate and 0.05 M sodium phosphate buffer with 0.1 M KCl (A-PBS), pH 6.0. The PD solution was prepared in acetonitrile.

The amperometric measurements were performed in an electrochemical three-electrode cell placed inside of a Faraday-cage at an ambient temperature (25 °C) using an Autolab PGSTAT 30 Potentiostat/Galvanostat operated by the GPES software Eco Chemie (Utrecht, Netherlands). The graphite rod (GR) electrode (3 mm diameter × 30 mm length, 99.999%) purchased from Sigma–Aldrich (Berlin, Germany) was used as a working electrode. Ag/AgCl in saturated KCl (Ag/AgCl/KCl_{sat}) and Pt wire were used as the reference and the auxiliary electrodes, respectively.

The bare and modified electrode surfaces were characterized by Hitachi SU-70 field emission scanning electron microscope (FE-SEM). Just before the characterization electrodes were prepared freshly, thoroughly washed with distilled water, dried at room temperature and then used for characterization.

2.2. Electrode preparation

In order to avoid contamination of oxidation products and to obtain a clean electrode surface, the surface of the GR electrode was hand-polished with fine emery paper, washed with ethanol and distilled water, and dried at room temperature before further treatments with enzyme and enzymatic polymerization.

2.3. Immobilization of GOx on the GR electrode surface

In order to prepare GOx-modified (GOx/GR) electrode, 3.0 μL solution of GOx (1 mg/mL) was dropped on the electrode surface three times. Each next drop was added after drying of previously added drop at room temperature. At the end of this treatment GOx/GR electrode was stored for 20 h over a 5% solution of glutaraldehyde at +4 °C in a closed vessel as previously described [14,15,17]. After cross-linking the GOx/GR electrode was thoroughly washed with distilled water in order to remove non-cross-linked enzyme.

2.4. Enzymatic polymerization of 1,10-phenanthroline-5,6-dione on the GOx/GR electrode

GOx/GR electrode surface was used as the substrate suitable for the enzymatic polymerization of 1,10-phenanthroline-5,6-dione. For this aim, GOx/GR electrode was immersed into A-PBS, pH 6.0, containing 25 mM of glucose and 5 mM of PD and it was stored at +4 °C, for 24 h. By this treatment, enzymatically poly(1,10-phenanthroline-5,6-dione) modified electrode (pPD/GOx/GR) was prepared. Prior to electrochemical measurements the pPD/GOx/GR electrode was thoroughly washed with distilled water (Scheme 1).

2.5. Electrochemical measurements

Dependence of amperometric signal on different glucose concentrations of the pPD/GOx/GR electrode was investigated at +600 mV (*vs.* Ag/AgCl/KCl_{sat}) and signals are presented as plots indicating changes in current *vs.* different glucose concentrations. In order to compare the effect of polymerization to the electron transfer between enzyme and electrode, the GOx/GR electrode was evaluated at similar conditions.

The experiments were performed under aerobic and anaerobic conditions in order to determine the effect of the oxygen to the electron transfer ability of mediator. For the measurements under anaerobic conditions, all solutions were purged with argon of 99.999% purity for 30 min before electrochemical measurements. Argon atmosphere was maintained over the solution during the electrochemical experiments.

The sensitivity, selectivity, repeatability, reproducibility and stability of the pPD/GOx/GR electrode were investigated. In order to perform selectivity measurements, the electrochemical response of the pPD/GOx/GR electrode was evaluated in the presence of dopamine and uric acid. The repeatability and reproducibility of the pPD/GOx/GR electrode were evaluated at 100 mM of glucose for the same electrode and for the three similarly fabricated electrodes, respectively. The stability of the pPD/GOx/GR electrodes was examined within 7 and 14 days by storing the electrodes at +4 °C in the closed vessel over the A-PBS, pH 6.0, between the measurements.

3. Results and discussion

The GOx is a flavine adenine dinucleotide-dependent enzyme that catalyzes oxidation of β-D-glucose by molecular oxygen to hydrogen peroxide and D-glucono-1,5-lactone (Eq. (1)), which subsequently hydrolyzes it to β-D-gluconic acid (Eq. (2)). It was thought that the hydrogen peroxide formed by the interaction between GOx and glucose could be used as initiator of PD polymerization (Eq. (3)):

$$\text{Glucose} + O_2 \xrightarrow{\text{GOx}} \text{Gluconolactone} + H_2O_2 \quad (1)$$

$$\text{Gluconolactone} + H_2O \rightarrow \text{Gluconic acid} \quad (2)$$

$$n\text{PD} \rightarrow (\text{PD})_n \quad (3)$$

Recently, enzymatic polymerization has been presented as an alternative approach for enzyme entrapment within polymeric films [16,19,21,22]. It is simple as one-step and environmentally