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ScienceDirect
Bioelectrochemistry 69 (2006) 201–208
Bioelectrochemistry
www.elsevier.com/locate/bioelechem

A novel method for glucose determination based on electrochemical impedance spectroscopy using glucose oxidase self-assembled biosensor

Reza Karimi Shervedani *, Abdolhamid Hatefi Mehrjardi, Najmehsadat Zamiri

Chemistry Department, University of Isfahan, Isfahan 81746-73441, I.R. Iran

Received 19 October 2005; received in revised form 4 January 2006; accepted 6 January 2006
Available online 23 February 2006

Abstract

A method is developed for quantitative determination of glucose using electrochemical impedance spectroscopy (EIS). The method is based on immobilized glucose oxidase (GOx) on the topside of gold mercaptopropionic acid self-assembled monolayers (Au-MPA-GOx SAMs) electrode and mediation of electron transfer by parabenzoquinone (PBQ). The PBQ is reduced to hydroquinone (H_2Q), which in turn is oxidized at Au electrode in diffusion layer. An increase in the glucose concentration results in an increase in the diffusion current density of the H_2Q oxidation, which corresponds to a decrease in the faradaic charge transfer resistance (R_{ct}) obtained from the EIS measurements. Glucose is quantified from linear variation of the sensor response ($1/R_{ct}$) as a function of glucose concentration in solution. The method is straightforward and nondestructive. The dynamic range for determination of glucose is extended to more than two orders of magnitude. A detection limit of 15.6 μM with a sensitivity of 9.66×10^{-7} $\Omega^{-1}\,mM^{-1}$ is obtained.

Keywords: Electrochemical impedance spectroscopy; Glucose oxidase; Impedimetric biosensor; Mercaptopropionic acid; Self-assembled monolayers

1. Introduction

Electrochemical detection of physiological species, such as glucose, has been the subject of several researches. Great efforts have been devoted to the fabrication and characterization of a large variety of amperometric enzyme biosensors [1–4]. The enzyme dissolved in electrolyte solution or immobilized on a solid electrode serves as a redox centre and reacts selectively with biological species. The reaction product may be used directly or by mediation of a reversible redox couple to determine the species of interest. However, mediated electron transfer is the most efficient process and typically used for biosensors construction [5,6]. Immobilization of enzyme on a solid electrode will decrease the distance between conducting substrate and enzyme redox centre; therefore, the reduced or oxidized mediator will be produced near or inside the diffusion layer, which, in turn, increases the sensitivity and selectivity of the sensor [7,8].

The enzyme may be immobilized in a thin layer at the sensor surface in different ways; as using polymers [9], carbon paste [10], monolayers [11] and multilayer self-assemblies [12]. Among them, immobilization via covalent attachment of enzyme to the functionalized self-assembled monolayers (SAMs) [11–13] is especially useful where miniaturization of the sensor in nanometre scales is required [14]. The functionalized SAMs formed on gold surface are ordered molecular assemblies, which are widely used for the immobilization of proteins and enzymes in biosensors fabrication [15–17].

The biosensors usually contain two basic components connected in series: (i) a biochemical recognition system, which translates information from biochemical domain into a chemical or physical output signal, and (ii) a transducer, which serves to transfer the sensor signal from the output domain of the recognition system to mostly an electrical domain. An electrochemical biosensor is a biosensor with an electrochemical transducer [18]. Most of the biosensor electrochemical transducers are based on potentiometric [19,20] or amperometric detections. The amperometric detections are based on measurement of the current resulting from electrochemical oxidation or reduction of an electroactive species, which is (i) a biocatalytic product (e.g. hydrogen peroxide) or (ii) a redox couple mediating enzyme and the electrode. However, amperometric biosensors have their own inherent limitations, such as

* Corresponding author. Tel.: +98 311 7932715.
E-mail address: rkarimi@sci.ui.ac.ir (R.K. Shervedani).

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doi:10.1016/j.bioelechem.2006.01.003

relatively low output current density, noisy response and gradual deterioration of the enzyme activity that mainly originates from high overvoltage applied to the biosensor. Many attempts have been made to overcome these limitations [5]. For example, composite materials [21], functionalized polymers [22], metal oxides [23] and self-assembled monolayers [7,24] have been investigated. These improvements have been focused on modification of the recognition system. However, it is necessary to seek new electrochemical transducers based on other methods such as electrochemical impedance spectroscopy (EIS) [25].

The EIS is a powerful, nondestructive and informative technique, which is usually used for characterization and study of corrosion phenomena [26], fuel cells and batteries [27], coatings and conductive polymers [28], adsorption behaviour of thin films [29], the SAMs [30,31] and electron transfer kinetics [32]. Recently, the EIS has been used in analytical chemistry to trace modification steps of chemically modified electrodes based on SAMs and to quantify the inorganic [33,34] or biological [35–37] species in solution. The basis of the recognition in these systems has been the blocking of electron transfer kinetic of a redox probe at the SAMs–solution interface by complexation or precipitation reaction connected to analyte, and thus the analyte is recognized indirectly [38,39].

Up to our knowledge, there has been no previous report on glucose biosensors based on faradaic impedance transducers and soluble mediators without any complexation or precipitation biocatalytic reaction at the electrode–solution interface. In this work, glucose oxidase (GOx) is used as an ideal enzyme [40] and immobilized covalently on the topside of the gold mercaptopropionic acid self-assembled monolayers to produce Au-MPA-GOx SAMs. Next, the sensor is used to determine glucose in the presence of parabenzoquinone (PBQ) mediator using EIS. The basis of the recognition system in this work is diffusion of glucose to the sensor. The data are presented and discussed from which a new method is proposed for glucose determination based on the EIS measurements.

2. Experimental

2.1. Chemicals

Glucose oxidase (GOx) (from *Aspergillus niger* 20,000 units/g, EC 1.1.3.4), β-D-Glucose, *N*-hydroxysuccinimide (NHS), parabenzoquinone (PBQ), 3-mercaptopropionic acid (MPA), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and other chemicals were of commercial sources (Merck or Sigma) and used as supplied without further purification except parabenzoquinone that was recrystallized from hot solution of *n*-hexane. All solutions were prepared with double-distilled water. Phosphate buffer solutions (PBS) contained 0.05 M KCl, 0.05 M K_2HPO_4/KH_2PO_4 were used and the pH was adjusted with NaOH or H_3PO_4 dilute solutions. The glucose stock solution was prepared in PBS (pH 7.0) and left at 4 °C overnight to allow the equilibration of the anomers.

2.2. Electrode modification

The polycrystalline gold working electrode (0.0314 cm^2, Azar electrode Co. Urmia, IRAN) was polished using aqueous slurries of alumina (0.3 to 0.05 μm), sonicated in water/chloroform/water for 5 min, and then cleaned electrochemically by cycling the electrode potential between 0.000 and +1.500 V vs. SCE in 0.5 M sulfuric acid until reproducible voltammograms were observed [41]. A roughness factor of 1.8 ± 0.1 was obtained from ratio of the real to geometric surface area of the electrode [42] and attempted to maintain it constant in all experiments [43]. The cyclic voltammograms obtained on the electrode in the presence of reversible marker, $Fe(CN)_6^{3-}$, showed a peak separation that confirms the safety of the system ($\Delta E_p \cong 60$ mV). Immediately before modification, the electrode was thoroughly rinsed with distilled water. Cleaned gold electrode was modified by placing into a 25:75 (v/v) water/ethanol solution containing 20 mM MPA for 12 h to form Au-MPA electrode. The modified electrode was washed with the same ethanolic solution, dried in argon stream, and activated in PBS (pH = 5.5) containing 0.002 M EDC and 0.005 M NHS for 2 h. Then, the electrode was rinsed with the same PBS and immediately placed in PBS (pH = 5.5) containing 500 μg/ml of the GOx enzyme for at least 1.5 h to fabricate Au-MPA-GOx SAMs electrode, washed with PBS, and used for electrochemical measurements.

2.3. Electrochemical measurements

A conventional three-electrode cell, consisting of Au-MPA-GOx modified electrode as working, a saturated calomel electrode (SCE) as reference and a platinum foil with large surface area as auxiliary electrode, was used for electrochemical measurements. The measurements were carried out using Potentiostat/Galvanostat EG&G 273A equipped with EG&G FRA 1025 and interfaced through PCII-GPIB IEEE NI-488.2 card. The EIS, cyclic voltammetry (CV) and chronoamperometry data acquisition were performed using EG&G Powersine™ and EG&G M270® softwares. The electrochemical characterization of Au-MPA SAMs electrode was performed in the presence of 0.5 mM $Fe(CN)_6^{3-}$ redox probe using EIS and CV. Quantitative determination of glucose was performed in the presence of 5 mM PBQ as a mediator by chronoamperometry and EIS methods. All impedance measurements were performed in the frequency range 10 kHz to 100 mHz using a 5 mV alternating voltage superimposed on DC potentials. For the characterization of Au-MPA SAMs electrode, the DC potential was formal potential of the redox couple (i.e. $E^{0\prime}$ of $[Fe(CN)_6]^{3-/4-}$). Quantitative determination of glucose was performed in different DC potentials. Other experimental conditions are described in the respective figures.

The EIS data were approximated using Equivcrt 4.55® software and complex nonlinear least square (CNLS) approximation method [44], from which electron transfer kinetics as charge transfer resistance (R_{ct}), double layer capacitance (C_{dl}) and solution resistance (R_s) were extracted for $Fe(CN)_6^{3-}$ or PBQ. The modified Randles' model in which C_{dl} was replaced