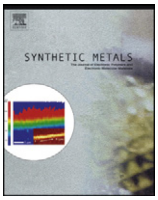
Synthetic Metals 161 (2011) 1861–1868

Contents lists available at ScienceDirect

Synthetic Metals

journal homepage: www.elsevier.com/locate/synmet



Construction of reagentless glucose biosensor based on ferrocene conjugated polypyrrole

Mehmet Şenel

Department of Chemistry, Faculty of Arts and Sciences, Fatih University, B. Cekmece, Istanbul 34500, Turkey

ARTICLE INFO

Article history:
Received 15 April 2011
Received in revised form 14 June 2011
Accepted 19 June 2011
Available online 16 July 2011

Keywords:
Polymer
Ferrocene
Pyrrole
Glucose oxidase
Biosensor

ABSTRACT

Glucose oxidase (GOx) was covalently immobilized onto an electrochemically prepared novel copolymer of ferrocene branched polypyrrole for the construction of an amperometric glucose biosensor. Ferrocene-pyrrole monomers were prepared by condensation reaction between ferrocene ethanol and 3-(1H-pyrrol-1-yl)propanoic acid and ferrocene-pyrrole, 3-(1H-pyrrol-1-yl)propanoic acid, and pyrrole monomers were electrochemically polymerized on the surface of the electrode. GOx was covalently immobilized onto the co-polymer coated electrode surface by using coupling agents to create amine bonds. The prepared copolymers were utilized as conducting films for amperometric glucose sensing after immobilization of GOx. Amperometric response was measured as a function of concentration of glucose at a fixed potential of +0.38 V vs. Ag/AgCl in a phosphate-buffered saline solution (pH 7.0). Results clearly showed that the ferrocene groups on the co-polymer play the role of electron transfer mediator between the redox center of glucose oxidase (GOx) and the surface of the electrode. Also, the effects of pH and temperature, storage, reusability and interference of the amperometric glucose biosensor were investigated.

1. Introduction

The establishment of electron transfer between a redox enzyme and an electrode is an essential prerequisite for the development of biosensors [1] and biofuel cells [2]. To establish an electron transfer, researchers used mediators to access the redox center of an enzyme and then to act as the charge carriers [3,4]. The electro-active compound was chosen as mediator to improve the linear range selectivity, sensitivity, and response time of the bioelectrodes [5]. In view of the development of a molecular device, immobilization of enzymes onto electrode surfaces has been extensively studied. Conducting polymer interfaces are particularly suitable for localizing biomolecules onto micro-sized surfaces [6]. Besides, conducting polymers offer the facility to modulate their electronic properties via molecular interactions [7]. Many researchers have suggested that covalent functionalization of conducting polymers could be achieved by synthesis of functionalized monomers, which are subsequently polymerized [8,9].Ferrocene derivatives are widely used as mediators in the construction of mediated amperometric biosensors. Direct attachment of the ferrocene-based mediators onto polymeric films prevents the mediator from leaching. Many investigators have reported novel redox copolymers where covalent attachment of ferrocene has been attempted such as poly(vinylferrocene-co-hydroxyethyl methacrylate) [10], poly(N-acryloylpyrrolidine-co-vinylferrocene) [11], acryl amide copolymers [12], ferrocene-based polyamides [13], and poly(glycidyl methacrylate-co-vinylferrocene) [14], ferrocene-polythiophene derivative [15]. The determination of glucose is important in the food and fermentation industries and in clinical chemistry, and there have been many reports on this subject. The most frequently employed glucose biosensors are based on glucose oxidase (GOx). In earlier studies, the catalytic conversion of glucose to gluconolactone by GOx in the presence of dioxygen produced hydrogen peroxide which was then detected electrochemically. However, there were sensitivity problems in O_2 concentration and significant interference signals generated by ascorbate, urate, and paracetamol [16]. Later, this problem was overcome by introducing mediators as means of electron transfer instead of using oxygen [6–8]. Recently, due to fundamental interest in electron-transfer reaction between GOx and electrodes and from the perspective of long-term stability of glucose sensors, several redox-active polymers have been prepared and utilized as polymeric mediators [14–22]. Since it has been established that glucose oxidase could be successfully immobilized in polypyrrole derivatives [23–25] and that an electron acceptor to the enzyme, ferrocene, could be easily modified by chemical substitution [26,27], it was hoped that pyrrole-ferrocene conjugates could be synthesized and used in the construction of reagentless mediated biosensors.

E-mail address: msenel@fatih.edu.tr

0379-6779/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.synthmet.2011.06.025

In the present paper, a novel copolymer of N-(3-(1H-pyrrol-1-yl)ethyl)ferrocenecarboxate (Py-Fc) monomer was synthesized and employed as a novel electron-mediating support material for fabricating GOx-immobilized electrodes. For this purpose, the GOx-immobilized electrodes were prepared with the copolymers of various compositions and applied to amperometric sensing of glucose. Their sensing ability was investigated in relation to the effect of the copolymer composition on the conductivity of the film and the immobilization of GOx. It was demonstrated that these redox copolymers function as an enzyme-binding support and a polymeric electron-transfer mediator for enzymatic glucose biosensor.

2. Experimental

2.1. Materials

Glucose oxidase (GOx) (EC 1.1.3.4), 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluenesulfonate (CMC), 1-(2-cyanoethyl)pyrrole (Py-CN), and ferrocene aldehyde were obtained from Aldrich Chemical Co. 3-(Dimethylamino)propyl)ethyl carbodiimide hydrochloride (EDCI), 4-dimethylaminopyridine (DMAP), pyrrole monomer (Py), p-toluene sulfonic acid sodium salt, and glucose were procured from Fluka. Pyrrole monomer was distilled thrice and the *p*-toluene sulfonic acid solution was freshly prepared before use. All other chemicals were of analytical grade and used without further purification.

2.2. Apparatus

Electrochemical polymerizations and measurements were performed using a CHI Model 842B electrochemical analyzer. A small glassy carbon working electrode (2 mm diameter), a platinum wire counter electrode (0.2 mm diameter), an Ag/AgCl-saturated KCl reference electrode, and a conventional three-electrode electrochemical cell were purchased from CH Instruments.

2.3. Synthesis of 1-(2-carboxyethyl)pyrrole (Py-CO_2H)

1-(2-Carboxyethyl)Pyrrole was obtained by hydrolysis of 1-(2-cyanoethyl)pyrrole (Py-CN) according to literature [28]: a mixture of 25 g of Py-CN and 100 ml of 15% potassium hydroxide solution was stirred at 50 °C for 40 h. Then the mixture was cooled to room temperature and acidified by hydrochloric acid. After extraction with ether, the crude product (colorless crystals) was collected on evaporation of ether. The crude product was dissolved in ether and purified by recrystallization from the ether solution. The product was identified as Py-CO_2H by means of FTIR-ATR, ^1H, and ^{13}C NMR spectroscopy (not shown).

2.4. Synthesis of ferrocene alcohol

To a solution of ferrocene aldehyde in tetrahydrofuran (THF) and methanol (1:2, v/v), a suspension of sodium borohydride in THF was gradually added by stirring simultaneously. Once the addition was complete, the reaction was allowed to take place for 1 h at room temperature. After the solvent was removed, the crude product was extracted by ether and recrystallized from *n*-hexane. The solid was then dried at 50 °C under reduced pressure and characterized using FTIR-ATR, ^1H, and ^{13}C NMR spectroscopy (not shown).

2.5. Synthesis of Py-Fc monomer

A solution of EDCI (600 mg, 3.14 mmol) in dichloromethane (4 ml) was added dropwise to a solution of ferrocene alcohol (750 mg, 3.5 mmol), 1-(2-carboxyethyl)pyrrole (500 mg, 3.6 mmol) and DMAP (30 mg, 0.3 mmol) in dichloromethane (6 ml) at −10 °C. After the addition, the reaction mixture was allowed to stir for 20 h at room temperature under argon atmosphere. The reaction mixture was washed thrice with a 1 M sodium hydroxide solution and twice with distilled water. The organic layer was dried using anhydrous magnesium sulfate and the solvent was removed in vacuo. A yellow solid was obtained after crystallization in ethanol.

Table 1
Amounts of GOx immobilized on various copolymer film, apparent Michaelis–Menten constants and maximum current densities for enzyme electrodes.

Abbreviation ($Py_x/PyCO_2H_y/PyFc_z$)[a]	Amount of Immobilized GOx (μg/cm²)	K_m^{app} (mM)	I_{max} (nA)
$Py_{0.6}/PyCO_2H_{0.2}/PyFc_{0.2}$	114	10.15	177
$Py_{0.4}/PyCO_2H_{0.2}/PyFc_{0.4}$	126	4.89	308
$Py_{0.4}/PyCO_2H_{0.4}/PyFc_{0.2}$	132	9.62	223
$Py_{0.2}/PyCO_2H_{0.4}/PyFc_{0.4}$	119	6.78	325
$Py_{0.2}/PyCO_2H_{0.2}/PyFc_{0.6}$	121	4.73	373
$Py_{0.2}/PyCO_2H_{0.6}/PyFc_{0.2}$	146	7.25	260

[a] *x*, *y* and *z* values are mole fractions of monomers in the feed.

2.6. Electrochemical polymerization

Electrochemical polymerization was carried out in a solution of monomers of different concentrations (Py, Py-CO_2H, and Py-Fc, Table 1.) and 1.0 M p-toluene sulfonic acid sodium salt at a fixed voltage of 1.2 V vs. Ag/AgCl. The copolymer films were prepared at an injected charge density of 100 mC/cm² (except for the special requirements). The copolymer Py/Py-CO_2H/Py-Fc was then obtained on the working electrode deposited in the form of a thin film. The composition of the copolymer was determined by means of FTIR-ATR spectroscopy.

2.7. Immobilization of GOx on Py/Py-CO_2H/Py-Fc copolymer film

The copolymer film-coated electrode was immersed in 3.0 ml distilled water containing 25 mg GOx and 120 mg CMC, and allowed to remain for 24 h at 4 °C. The copolymer films treated thus, i.e. GOx-electrodes, were then rinsed with distilled water and stored in 0.1 M phosphate buffer (pH 7.4) at 4 °C.

The amounts of bound protein deposited on the electrodes were determined by the Lowry method [29]. The quantity of covalently bound protein was calculated by subtracting the amounts recovered in the combined washings of the enzyme-electrode from the protein used for immobilization.

2.8. Amperometric measurements

All amperometric measurements were carried out at room temperature in stirred solutions by applying the desired potential and allowing the steady-state current to be reached. Once prepared, the GOx electrodes were immersed in 10 ml of 10 mM PBS solution at pH 7.4 and the amperometric response to the addition of a known amount of glucose solution was recorded. The data shown are the average of three measurements for each electrode.

3. Results and discussion

3.1. Preparation of Py-Fc monomer

Our approach is for the synthesis and biosensor application of the new ferrocene-functionalized polypyrrole derivative. The structure of the new ferrocene-functionalized pyrrole monomer is shown in Fig. 1. The Py-Fc monomer was prepared, at room temperature and under an argon atmosphere, by condensation reaction