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Analytical Methods

Selenium containing conducting polymer based pyranose oxidase biosensor for glucose detection

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ABSTRACT

A novel amperometric pyranose oxidase (PyOx) biosensor based on a selenium containing conducting polymer has been developed for the glucose detection. For this purpose, a conducting polymer; poly(4,7-bis(thieno[3,2-b]thiophen-2-yl)benzo[c][1,2,5] selenadiazole) (poly(BSeTT)) was synthesized via electropolymerisation on gold electrode to examine its matrix property for glucose detection. For this purpose, PyOx was used as the model enzyme and immobilised via physical adsorption technique. Amperometric detection of consumed oxygen was monitored at −0.7 V vs Ag reference electrode in a phosphate buffer (50 mM, pH 7.0). K_M^{app}, I_{max}, LOD and sensitivity were calculated as 0.229 mM, 42.37 nA, 3.3×10^{-4} nM and 6.4 nA/mM cm^2, respectively. Scanning electron microscopy (SEM), Electrochemical Impedance Spectroscopy (EIS) and cyclic voltammetry (CV) techniques were used to monitor changes in surface morphologies and to run electrochemical characterisations. Finally, the constructed biosensor was applied for the determination of glucose in beverages successfully.

1. Introduction

The development of conducting polymer based biosensors has rapidly increasing in the fields of biological analysis, health care and food processing industries for the detection of various analytes (Cesarino, Moraes, Lanza, & Machado, 2012; Gerard, Chaubey, & Malhotra, 2002; Singh, Chaubey, & Malhotra, 2004). Sensor performance depends largely on the surface properties, interaction between the enzyme molecule and electrode surface and protection of three dimensional structure of enzyme molecule. Therefore, conducting polymers were emerged as one of the most fascinating transducers due to their simple preparation (Chaubey & Malhotra, 2002; Malhotra & Chaubey, 2003; Soylemez, Kanik, Nurioglu, Akpinar, & Toppare, 2013). Conducting polymer based biosensors bring simple, accurate, reliable and low-cost determination of various analytes and act as a very effective analytical tool in the food quality control, selectivity and high sensitivity (Gvozdenovic et al., 2011; Kesik et al., 2013; Ramanavicius, Ramanaviciene, & Malinauskas, 2006). Furthermore, they function as a three dimensional matrix for biomolecule deposition. Their charge transfer ability serves as excellent matrices for biomolecules providing enzyme mimic environment (Tuncagil, Ozdemir, Odaci Demirkol, Timur, & Toppare, 2011; Türkarslan, Kayahan, & Toppare, 2009).

The flavoenzyme pyranose 2-oxidase (PyOx, glucose 2-oxidase, pyranose: oxygen 2-oxidoreductase, EC 1.1.3.10) is a type of oxido-reductase enzyme. PyOx catalyses C-2/C-3 oxidation of numerous sugars to their corresponding dicarbonyl derivatives (aldos-2-uloses or glycosid-3-uloses), coupled to the reduction of FAD (Halada, Leitner, Sedmera, Halt Rich, & Volca, 2003; Marešová, Palyzová, & Kyslík, 2007). Exhibiting a high affinity to its corresponding 2-keto sugars, with accompanying generation of hydrogen peroxide, PyOx was used in various biotechnological applications in carbohydrate chemistry. The working principle of the biosensor is based on the following:

$$\text{d-glucose} + O_2 \rightarrow \text{2-dehydro-d-glucose} + H_2O_2$$

Glucose oxidase (GOx) is a type of oxido-reductase enzyme that catalyses the oxidation of glucose to hydrogen peroxide and D-glucono-δ-lactone. GOx shows high resistance to adverse microenvironment conditions such as denaturing agents and acidic medium. (Uzun et al., 2013). Although PyOx and GOx have a similar mechanism, PyOx has several important advantages. The particular

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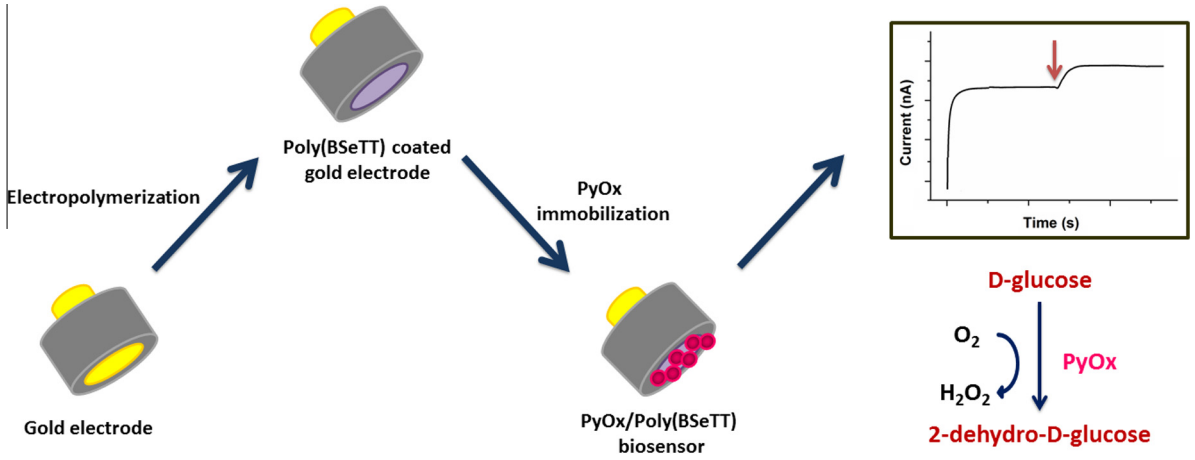

Scheme 1. The typical representation of construction of the proposed biosensor.

advantages in biosensor construction are as follows: its excellent stability and high affinity for D-glucose (km ~1 mM), ability to oxidise efficiently various sugars provide a better alternative than GOx. Moreover, its sensitivity is twice compared to GOx (Odaci, Telefoncu, & Timur, 2008). In the fabrication of biosensors, determination of suitable matrix and immobilisation strategies are the most significant factors. Surface properties of the biosensor also exhibit crucial importance for the successful immobilisation. A suitable matrix should be biocompatible, non-toxic to biorecognition element and durable. In this work, immobilisation of PyOx was performed via physical adsorption within the conducting polymer of 4,7-bis(thieno[3,2-b]thiophen-2-yl)benzo[c][1,2,5]selenadiazole (poly(BSeTT)). Due to the presence of aromatic units in the polymer backbone, the immobilisation was achieved with the help of π–π stacking interactions of the polymer and enzyme molecules. These strong interactions stabilize tertiary structure of proteins effectively (Uzun et al., 2013). For this purpose, BSeTT was synthesized and electrochemical polymerisation of the monomer was performed on gold electrode. After preparation of the gold electrodes, PyOx was immobilised onto the Selenium (Se) containing polymer using glutaraldehyde as the cross linking agent for the construction of glucose biosensor. Due to the biocompatible properties of Se moiety, a robust, high sensitive and long life biosensor can be achieved easily. Due to the unique redox property of Selenium, Se-containing polymers led researchers to explore many opportunities in various applications. Moreover, it was reported that Se shows a protective role against oxidative stress (Valencia-Rodriguez et al., 2012). Hence, Se containing conducting polymers help developing efficient, rapid and high accuracy glucose biosensors. A preparation of proposed biosensor was depicted in Scheme 1. Optimisation and characterisation studies were performed to achieve the best results in fabrication of the biosensor. The application of biosensor was tested via determining glucose in beverages.

2. Material and methods

2.1. Materials

Pyranose oxidase (PyOx; pyranose: oxygen 2-oxidoreductase, E.C.1.1.3.10, from *Coriolus* sp. (10.8 U/mg solid), glucose, $NaClO_4$, $LiClO_4$ were purchased from Sigma–Aldrich and used with no further purification. Dichloromethane (DCM), acetonitrile (ACN) were purchased from Merck (Darmstadt, Germany). For enzyme immobilisation, a phosphate buffer solution (pH 7.0) consisting of 0.025 M Na_2HPO_4 (Fisher Scientific Company) and 0.025 M NaH_2PO_4 (Fisher Scientific Company) was used. As the substrate, a glucose solution was prepared by dissolving 0.18 g of glucose in 10 mL pH 7.0 buffer solution. All chemicals were of analytical reagent grade.

2.2. Apparatus

For amperometric measurements, a Palmsens potentiostat (Palm Instruments, Houten, The Netherlands) was used. Electropolymerisation was performed with Voltalab 50 potentiostat. All electrochemical measurements were performed in a three-electrode cell consisting of gold electrode (BaSi (AUE) 1.6 mm diameter) as the working electrode. A platinum wire as the counter electrode, and a Ag wire as the pseudo reference electrode were employed. Amperometric measurements were performed in a three-electrode system. In amperometric analyses, the data were given as the average of three measurements and standard derivations were recorded as ±SD. All measurements were performed at ambient conditions (25 °C). For surface investigation of the biosensor, scanning electron microscope (SEM) (JEOL JSM-6400 model) was used. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker Spectrospin Avance DPX-400 Spectrometer. Chemical shifts were given in ppm downfield from tetramethylsilane. HRMS study was done with a Waters SYNAPT MS system. Electrochemical Impedance Spectroscopy (EIS) was performed with a GAMRY Reference 600 (GAMRY Instruments Inc., Pennsylvania, USA).

2.3. Synthesis of the monomer BSeTT

Synthesis and characterisation of the monomer, BSeTT was carried out according to a previously described method (Toksabay, Hacıoğlu, Unlu, Cırpan, & Toppare, 2014).

After bromination of benzothiadiazole, the product, 4,7-dibromo-2,1,3-benzothiadiazole, was reduced to achieve 3,6-dibromobenzene-1,2-diamine. To obtain 4,7-dibromobenzo[c][1,2,5]selenadiazole, the diamine product was reacted with selenium dioxide. Subsequently, the product was coupled with stannylated thienothiophene using Stille coupling reaction. Then, the product was subjected to column chromatography to afford dark purple solid to have the monomer 4,7-di(thieno[3,2-b]thiophen-2-yl)benzo[c][1,2,5]selenadiazole (BseTT). Scheme S1 shows synthetic route of the BSeTT.

2.4. Biosensor preparation

Before the experiments, the gold electrode surface was initially polished with alumina powder and was conditioned in 0.5 M H_2SO_4 solution by cycling the potential between 0 and +1.5 V until