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Fabrication of enzyme electrodes with a polythiophene derivative and application of them to a glucose fuel cell

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ABSTRACT

Two kinds of enzyme electrodes were fabricated by covalent immobilization of glucose oxidase (GOx) and bilirubin oxidase (BOx) on the films of a thiophene derivative having carboxyl groups as binding sites on their surfaces. The electrode bearing GOx (GOx/Copolymer electrode) and that bearing BOx (BOx/Copolymer electrode) were applied to a glucose fuel cell as an anode and a cathode, respectively. The open circuit voltage of 0.61 V was achieved by use of the BOx/Copolymer as the cathode, whereas the voltage became 0.41 V when a Pt black (PtB) electrode was used instead. The short circuit currents of 0.54 and 0.84 mA cm^{-2} were obtained by use of the BOx/Copolymer and PtB cathodes, respectively. The biofuel cell equipped with the GOx/Copolymer anode and the BOx/Copolymer cathode gave the maximum power density of 0.15 mW cm^{-2} at the cell voltage of 0.35 V, which was twice as large as that generated with the PtB cathode.

1. Introduction

Biofuel cells are novel devices in which biocatalysts such as enzymes and microorganisms are used for conversion of chemical energy to electrical energy [1–5]. As is the case with a traditional fuel cell, the biofuel cell is composed of anodic and cathodic compartments separated by a polymer electrolyte membrane. However, it is a marked difference between the biofuel cell and the traditional one that biological substances catalyze the electrochemical reaction in the former instead of precious metals in the latter. As for fuels, abundant organic substances such as ethanol [6,7] or glucose [8–10] can take part in the oxidation process on the anode of the biofuel cell. Among them, glucose is a notable biofuel that can be obtained in quantity by bacterial decomposition of cellulose in waste materials [11]. On the other hand, in the same way as the traditional fuel cell, molecular oxygen can be incorporated into the reduction process on the cathode of the biofuel cell.

The authors have been studying a biofuel cell system equipped with an enzyme electrode as an anode for converting chemical energy of glucose to electric energy [12]. In this system, glucose oxidase (GOx) immobilized on the anode oxidizes glucose to gluconolactone to become a reduced form and then is oxidized by an electron-transferring mediator. As a result, the mediator becomes a reduced form and supplies electrons for cathodic reduction of oxygen. The enzyme electrode has been fabricated with a conducting polymer film prepared by electrochemical copolymerization of 3-methylthiophene (3MT) and thiophene-3-acetic acid (T3A). This film (3MT/T3A copolymer film) is useful as an electrode material because of its high conductivity (10^{-3} S cm^{-1}) and large surface area [13,14].

It is well known that conjugated polymers, which are synthesized from such monomers as aniline, pyrrole and thiophene, have electron conductivity in spite of being organic substances [15–19]. These polymers have attracted attention because a variety of functional groups used as enzyme-binding sites can be introduced into them by selecting appropriate monomers. Thus the conducting polymers are considered as important components of enzyme electrodes in both aspects of electron transfer and enzyme immobilization [20–25]. From these viewpoints, the 3MT/T3A copolymer film is a promising candidate for the electrode material because it has not only high conductivity but also carboxyl groups as shown in Fig. 1, which can immobilize enzyme molecules covalently through amide linkages.

In a biofuel cell using enzyme electrodes as both an anode and a cathode, crossover reaction can be negligible due to the highly substrate-selective nature of enzymes and, therefore, it is unnecessary to separate anodic and cathodic compartments by a polymer electrolyte membrane. This affords flexibility for the construction of the biofuel cell and leads to a reduction of cost. In addition, the biofuel cell needs no precious metals such as platinum. Laccase and bilirubin oxidase (BOx) act as biocatalysts for cathodic reaction [26–29]. These enzymes can catalyze the four-electron reduction of dioxygen to water. If electrodes are modified by immobilization of such enzymes, they can be used as cathodes of biofuel cells.

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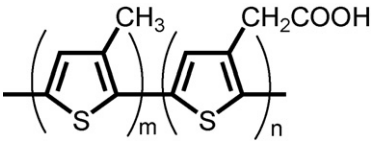


Fig. 1. Molecular structure of 3MT/T3A copolymer.

Furthermore, BOx shows a high activity at pH 7, which is favorable for a practical application. In the present work, BOx as well as GOx was immobilized covalently on the 3MT/T3A copolymer film by the condensation reaction with the carboxyl groups on the surface of the copolymer film. This paper deals with a novel glucose fuel cell system constructed by use of the GOx-immobilized and BOx-immobilized copolymer films as an anode and a cathode, respectively. The performance of the glucose fuel cell was investigated and compared with that of the cell with a Pt black (PtB) cathode known as a general O_2 reduction electrode.

2. Experimental

2.1. Materials and apparatus

GOx (EC 1.1.3.4, from *Aspergillus* species) was supplied by Toyobo Co., which had an activity of 154 U mg^{-1}. BOx (EC 1.3.3.5, 28 units mg^{-1}) from *Myrothecium verrucaria* was purchased from Sigma Chem. Co. 3MT and tetraethylammonium perchlorate were obtained from Nacalai Tesque, Inc. T3A was purchased from Tokyo Kasei Kogyo Co. D-Glucose and hydrogen hexahydrate palatinate (IV) hexahydrate were obtained from Wako Pure Chemical Ind. *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD) from Sigma Chem. Co. and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) from Aldrich Chemical Co. were used as electron transfer mediators. Lead (II) acetate trihydrate, 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho-*p*-toluenesulfonate (CMC), which was used as a condensing agent for enzyme immobilization, and Nafion 112 (perfluorinated membrane with a thickness of 0.002 in.) were supplied by Aldrich Chemical Co. Other chemicals and solvents were of guaranteed-reagent or analytical grade and used without further purification. All aqueous solutions were prepared with distilled water passed through a purification system.

Electrochemical experiments were carried out with a potentiostat/galvanostat (Hokuto Denko Corp. HA-150G), a bipolar coulomb/amperehour meter (Hokuto Denko Corp. HF-203D) and an arbitrary function generator (Hokuto Denko Corp. HB-105A) in a conventional three-electrode cell. A Pt-plate and a saturated calomel electrode (SCE) were used as a counter electrode and a reference electrode, respectively.

2.2. Preparation of electrodes

Enzyme electrodes were fabricated with 3MT/T3A copolymer films as illustrated in Scheme 1. The copolymer film was formed on a Au electrode (0.5 cm × 0.5 cm) by electrochemical polymerization at an applied potential of +2.2 V vs. SCE in an acetonitrile solution containing 0.45 mol L^{-1} 3MT, 0.05 mol L^{-1} T3A and 0.10 mol L^{-1} tetraethylammonium perchlorate. Prior to the polymerization, for removal of dissolved oxygen, the solution was saturated with nitrogen by bubbling via an external source. The polymerization was continued until a charge of 0.80 C was passed. The copolymer film obtained thus was rinsed with distilled water to remove residual monomers.

GOx and BOx were immobilized covalently on the 3MT/T3A copolymer films through amide linkages. The copolymer film was immersed into distilled water containing each enzyme (0.5 mg mL^{-1}) and CMC (24 mg mL^{-1}) for 1 h at room temperature. The enzyme-immobilized films prepared thus (GOx/Copolymer and BOx/Copolymer electrodes) were rinsed with distilled water and stored in a phosphate buffer solution (0.10 mol L^{-1}, pH 7.0) at 4 °C.

A PtB electrode was prepared with a Pt plate in the following manner [30–32]: in advance the Pt plate was washed by sonicating in acetone for 15 min and in distilled water for 15 min and then dried. PtB was formed on the Pt plate by electrodeposition at −1.0 V vs. SCE in an aqueous solution containing 10 mg mL^{-1} hydrogen hexahydrate palatinate (IV) hexahydrate and 0.5 mg mL^{-1} lead (II) acetate trihydrate for 10 min. Then the PtB was activated by 10 sweep segments of cyclic voltammetry from −0.5 to 0.5 V vs. SCE at a scan rate of 50 mV s^{-1} in a 0.10 mol L^{-1} H_2SO_4 solution and rinsed with distilled water. The PtB electrode obtained thus was stored in distilled water at room temperature.

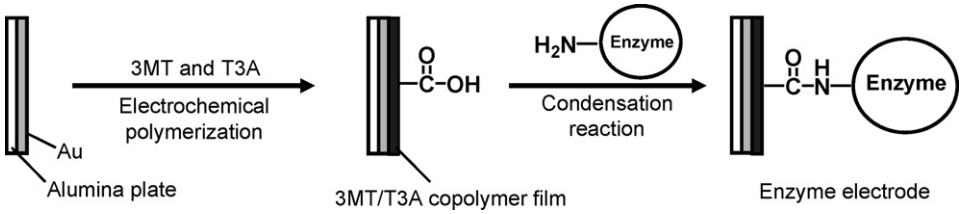
2.3. Assay of immobilized enzyme

The amount of enzyme immobilized on 3MT/T3A copolymer film was determined by the analysis with Folin–Ciocalteu phenol reagent after alkaline copper treatment according to the method of Lowry [33], in which colorimetry was carried out at 750 nm with a Shimadzu UV-3100 PC spectrometer.

2.4. Electrochemical measurements

The electrochemical properties of the enzyme electrodes and the PtB electrode were examined by acquiring polarization curves with the three-electrode cell. The measurements were carried out at a scan rate of 5 mV s^{-1}. The GOx/Copolymer electrode was examined in a phosphate buffer solution (0.10 mol L^{-1}, pH 7.0) containing 0.10 mol L^{-1} glucose and 1.0 mmol L^{-1} TMPD. The PtB and BOx/Copolymer electrodes were examined in a phosphate buffer (0.10 mol L^{-1}, pH 7.0) saturated with dissolved oxygen and, in the case of the BOx/Copolymer electrode, 1.0 mmol L^{-1} ABTS was added to the buffer solution.

A glucose fuel cell was constructed with the enzyme electrodes as illustrated in Fig. 2. In the present study, TMPD and ABTS were used as electron-transferring mediators in a dissolved state and, therefore, the cell was separated into anodic and cathodic compartments with a polyelectrolyte membrane (Nafion 112). Both the compartments were of 20 mm diameter and 45 mm depth. The GOx/Copolymer electrode was used as the anode, and the



Scheme 1. Preparation of the enzyme electrode with the 3MT/T3A copolymer film.