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Amperometric glucose-responding property of enzyme electrodes fabricated by covalent immobilization of glucose oxidase on conducting polymer films with macroporous structure

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Abstract

Macroporous conducting polymer films were prepared by the electrochemical copolymerization of 3-methylthiophene and thiophene-3-acetic acid on the ITO-coated glass plates bearing different sizes of polystyrene template particles, and enzyme electrodes were fabricated by covalent immobilization of glucose oxidase on the macroporous copolymer films. It was found that the doping level and conductivity of the copolymer films was significantly affected by the treatment with solvent to remove the polystyrene particles, which was considered to result in deterioration in amperometric glucose-responding property of the enzyme electrodes fabricated with the copolymer films. Three-dimensionally ordered macroporous structure on the copolymer films led to enhancement of amperometric response of the enzyme electrodes, and this effect was attributed to the geometry of the interconnected channel structure formed by the linkage of macropores. It was suggested that the amperometric response of the enzyme electrodes was determined by whether the interconnected channel structure on the copolymer films had long distance regularity and a proper size to allow the enzyme and electron-mediator molecules to penetrate into the interior pores of the copolymer film. In particular, the interconnected channel structure seemed to play an important role in the electron-transfer reaction between the mediator molecules and the surface of electrodes.

Keywords: 3-Methylthiophene; Thiophene-3-acetic acid; Conducting polymer; Glucose oxidase; Enzyme electrode

1. Introduction

During last two decades, the immobilization of enzymes on conducting polymer films has been exploited as an excellent technique to fabricate enzyme electrodes that can be applied to biosensors [1–3] and biofuel cells [4–6]. Generally, the immobilization of enzymes is achieved either by in situ entrapment during the electrochemical polymerization in enzyme-containing solution, or by post immobilization, such as covalent binding and adsorption of enzymes, on conducting polymer

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films [7,8]. No matter what kind of approach is adopted, the morphology of the conducting polymer films should have a great influence on the performance of the enzyme electrodes fabricated with them. It is well known that an increase in surface area or porosity of conducting polymer films will facilitate enzyme immobilization and mass diffusion at solid/liquid interface, and thus improve the performance of the enzyme electrodes.

The preparation of conducting polymer films with ordered macroporous structure has attracted a great interest in recent years owing to the novel three-dimensional structure favorable for their application to enzyme electrodes. Such macroporous structure has been realized for various conducting polymers including polyaniline, polypyrrole, polythiophene and their derivatives, which can be synthesized by chemical [9,10] and electrochemical [11–14] polymerization in the presence of self-assembled template particles. As for the application of such conducting polymer films, an optical-affinity biosensor and a potentiometric biosensor have been fabricated with the highly ordered macroporous film of polythiophene-*co*-(3-thiophenemethanol) [15] and with that of creatinine deiminase doped polypyrrole [16], respectively. Neural prosthetic probe devices were developed by use of macroporous poly(3,4-ethylenedioxythiophene) and polypyrrole films [17]. A nanocomposite of porous polyaniline was applied to fabrication of a nitrite sensor though it was not an example of a biosensor [18]. Glucose oxidase (GOx) and glucose dehydrogenase were immobilized on highly ordered macroporous gold electrodes with precisely controlled pore size and number of pore layers, and the bioelectrocatalytic activity of these enzyme electrodes was investigated in detail [19,20].

Our current interest is to develop enzyme electrodes suitable for biosensors and biofuel cells, in which a large amount of loaded enzyme as well as effective mass diffusion is critically required for achieving high performance of the enzyme electrodes. In the present study, in view of the novel structure and properties of macroporous materials, enzyme electrodes were fabricated by covalent immobilization of GOx on conducting polymer films with macroporous structure. The films were prepared by electrochemical copolymerization of 3-methylthiophene (3MT) and thiophene-3-acetic acid (T3A) on the electrodes bearing polystyrene (PS) particles as the template of the macroporous structure. The PS template was removed by dissolution in tetrahydrofuran (THF) and, in this manner, the macroporous structure was obtained. Fig. 1 shows the structural formula of the copolymer. GOx was immobilized on the surface of the copolymer films through amide linkages by the condensation reaction with COOH groups on the surface. Throughout the present study, the films obtained by the copolymerization of 3MT and T3A with the ratio of 5/1 was employed because they had both considerably high conductivity and a sufficient amount of COOH groups for immobilizing GOx [21]. Amperometric glucose-sensing characteristics of thus fabricated enzyme electrode (GOx-electrode) were compared with those of the one fabricated with the conducting polymer film prepared in the absence of the PS template. The effect of the THF treatment, as well as the size of PS template particles, on the glucose-sensing characteristics of the GOx-electrode was also investigated.

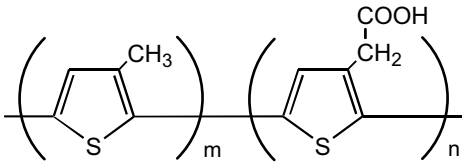

Fig. 1. Structure of 3MT/T3A copolymer.

2. Experimental

2.1. Materials

3MT, tetraethylammonium perchlorate (TEAP) and *p*-benzoquinone (BQ) were purchased from Nacalai Tesque, Inc., and T3A was purchase from Tokyo Kasei Kogyo Co.: all these chemicals were of guaranteed-reagent grade. GOx (EC 1.1.3.4, from *Aspergillus* species) was supplied by Toyobo Co., which had an activity of 165 U/mg. Two kinds of sulfonated PS latex (10 wt% dispersion in H_2O) were obtained from Aldrich Chemical Co., whose average particle diameters were 0.46 and 0.80 μm. 1-Cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluenesulfonate (CMC) from Aldrich Chemical Co. was used as a condensing agent. Other chemicals and solvents were of guaranteed-reagent or analytical grade and used without further purification.

2.2. Apparatus

The electrochemical copolymerization of 3MT and T3A, cyclic voltammetry and measurement of