전도성 고분자를 이용한 포도당 바이오센서의 특성 및 성능

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Conducting Polymer-Based Glucose Biosensor: Characteristics and Performance

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INTRODUCTION

Electrochemically polymerized conducting polymers have been the subject of much recent investigation (Skotheim, 1986). Among the conducting polyheterocyclic films, polypyrrole is of particular interest because the relatively low oxidation potential of the monomer enables films to be grown from aqueous solutions that are compatible with most of biological elements (Asavapiriyanont *et al.*, 1984). The entrapment of enzymes in a polypyrrole matrix during the electropolymerization has been attracting great interest as an immobilization technique.

The electrochemical immobilization has several advantages over conventional methods for the construction of an amperometric biosensor (Fouls & Lowe, 1986). First, one can control the amount and spatial distribution of enzyme in the polymer in a tailored manner, simply by manipulating the preparation parameters such as the charge transferred, as well as monomer and enzyme concentrations in the electrodeposition solution. Secondly, it is also possible to immobilize enzyme on the surface of the electrode by a one-step process regardless of the kinds and shapes of the substrate to be deposited. Finally, the suppression of interference is additionally expected since a conducting

polymer possesses the size exclusion property which is effective in eliminating the interference of electrooxidizable compounds such as ascorbate and urate.

In this work, we constructed a PPy/GOD biosensor as a model system and examined the effects of pyrrole concentration, enzyme concentration, and film thickness on the response of the resulting electrode. The condition of preparing the PPy/GOD biosensor was optimized in terms of the sensitivity for glucose and the suppression of interference.

MATERIALS AND METHODS

Instrumentation

Voltammetric and chronoamperometric measurements were performed with a potentiostat (EG&G Princeton Applied Research, Model 362). Output from the potentiostat was coupled to an IBM-PC compatible computer using a peripheral interface card (PC-LabCard, PCL 812, Advantech Co., Taiwan). All data display and recording were supported by an operating software programmed in C language. The scanning electron microscopy was conducted by using an electron microscope (Philips, 535M). No metal was deposited on the surface of PPy/GOD film.

Preparation of biosensors

All potentials were referred to an Ag/AgCl electrode (BAS, USA). Working electrode was prepared by mounting a platinum rod (dia = 3 mm) in a polyester resin. The electrode surface was mechanically polished with successively finer grades of diamond slurries and alumina slurry down to 0.3 μm (BAS, USA). Electrochemical polymerization was performed in an undivided cell at room temperature using a platinum disk as an auxiliary electrode. The thickness of the film was estimated by assuming that 45 mC/cm^2 of charge yields a film of 0.1 μm thickness. PPy/GOD films were grown potentiostatically at 750 mV in a stagnant and deoxygenated solution of 0.1 M KCl (pH 7.0) containing 0.05 M pyrrole and 0.5 mg/ml glucose oxidase. PPy/HRP films were prepared in the same manner as above except that the polymerization in the presence of HRP were initiated on the top of PPy/GO films which were already formed.

Electrochemical measurement

The polypyrrole-based biosensors were maintained at 700 mV in 0.1 M potassium phosphate buffer (pH 7.0) and overoxidized in order to stabilize the background current. For the amperometric measurement of glucose and other electrooxidizible substances, the steady state anodic current was measured at the potential of 700 mV in the 0.1 M potassium phosphate buffer (pH 7.0) after a spike of corresponding stock solution. The voltammetric experiments were carried out by recording the current-potential traces with linear sweep at the scan rate of 10 mV/sec in stagnant solutions .

RESULTS AND DISCUSSION

To control the thickness of the polymer film and the amount of enzyme that can be immobilized within the film, an investigation of the major parameters that influences the immobilization of glucose oxidase by entrapment in polypyrrole films, prepared by electropolymerization from aqueous solutions containing the enzyme and monomer, was carried out. For the electrochemical variables, which primarily govern mechanically stability of the films, even and adherent films were achieved by polymerization of 0.05 M pyrrole at the potentials ranging from 750 to 850 mV. In the other hand, for the other parameters such as enzyme concentration, electrochemical growth of the films was found to be significantly inhibited by the presence of increasing amount of enzyme in the deposition solution. Scanning electron micrographs revealed that the morphology of the film was drastically changed by the enzyme concentration, indicating that much rougher surface of the film was obtained at lower concentration of enzyme. As an ultimate effect of the change in the film property, the permeability of the resulting film was found to decrease with increasing enzyme concentration in the deposition solution.

The polymerization conditions were examined to maximize the response of the resulting biosensor. It was found that the response was critically dependent on both the enzyme concentration and the film thickness and that there were several suboptimal sets of above parameters, which offer maximum responses. When polymerized at the enzyme concentration of 0.5 mg/ml, the optimum thickness of the film was observed to be 250 mC/cm^2. In the other hand, when deposited at 5.0 mg/ml, the optimum thickness was

about 10 mC/cm^2. Furthermore, the sensitivity of biosensor was enhanced by preparing thicker film at lower enzyme concentration, confirming the dependence of the film permeability on the enzyme concentration.

In order to evaluate the individual effects of the immobilized activity of enzyme and the permeability of the film on the response of biosensor, the response characteristics of biosensor were investigated by an approximate analytical treatment. All experimental data were found to be in a good agreement with the theoretical prediction. Moreover, the derived apparent glucose diffusion coefficient was proven to be inversely proportional to the enzyme concentration during the deposition. Then, the analytical performance of biosensors that were prepared at each suboptimal condition set was compared in terms of the response sensitivity, interference suppression, response time, operational stability, etc. The biosensor, which consisted of thicker film formed at lower enzyme concentration, displayed superior performance to that of thinner one at higher enzyme concentration. Typically, response time was less than 25 sec with the linear range of response up to 10 mM glucose. In addition, the interfering current from ascorbate could be reduced to 3.4 % of that at a bare electrode by virtue of the polymer film. During storage of biosensor at room temperature, the sensitivity in the response remains more than 70% even after 3 weeks.

As an approach to improving the performance against interference, a novel configuration of heterobilayer was developed by overlaying horseradish peroxidase-immobilized film on the top of the underlying glucose oxidase film. *In situ* enzymatic elimination of interference in the proposed system was demonstrated by examining responses either in the absence or presence of interferants in the sample. For glucose concentration higher than 50 mM, the deviation of detection arising from presence of interferants could be reduced less than 7 %.

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