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Amperometric glucose sensing with polyaniline/poly(acrylic acid) composite film bearing glucose oxidase and catalase based on competitive oxygen consumption reactions

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ABSTRACT

For the purpose of avoiding interference with glucose biosensing caused by coexisting easily oxidizable compounds, the sensing was attempted with an enzyme electrode (PANI/PAA-GOx/Cat) that was fabricated by *co*-immobilizing glucose oxidase (GOx) and catalase (Cat) on polyaniline/poly(acrylic acid) (PANI/PAA) composite films. The glucose sensing was carried out by monitoring the decrease in the O_2 reduction current resulted from competitive consumption of dissolved O_2 caused by electrochemical O_2 reduction and enzymatic glucose oxidation. It was found that the immobilized Cat reproduced O_2 from H_2O_2 accompanying GOx-catalytic glucose oxidation. As a result, the enzyme electrode gave a wide linear range (up to 1.6 ± 0.0 mM glucose) and a high sensitivity (49.3 ± 0.6 μA cm^{-2} mM^{-1}) in the glucose sensing due to increased O_2 concentration in the vicinity of the enzyme electrode. The linear range and sensitivity of the electrode are obviously higher than those of the enzyme electrode without Cat (PANI/PAA-GOx). In contrast, despite the complexity of the electrode reaction, the detection limit of the PANI/PAA-GOx/Cat (26.5 ± 5.2 μM) was almost same as that of the PANI/PAA-GOx. Moreover, high glucose selectivity was achieved by adopting a bioelectrochemical system which functions at a low potential to avoid the interference of coexisting easily oxidizable compounds.

1. Introduction

Glucose sensing is practically important in such fields as medical diagnosis, food analysis and environmental monitoring [1–5]. To detect glucose amperometrically, enzyme electrodes have been fabricated by immobilizing glucose oxidase (GOx) on conductive materials [6–8]. Amperometric glucose sensing can be achieved with a GOx-immobilized electrode through anodic oxidation of H_2O_2 produced by the GOx-catalyzed reaction between glucose and dissolved O_2 [5,9–13]. This sensing method, however, has the problem that it requires a relatively high electrical potential for a practical applications. Therefore, the glucose sensing suffers interference from easily oxidizable compounds such as ascorbic acid, uric acid and acetaminophen that are frequently coexisting with glucose in samples. Although the influence of these compounds can be avoided by incorporating an electron transfer mediator with a lower redox potential, this approach complicates the sensing system and procedure [14,15]. For this reason, the amperometric glucose sensing has been attempted by monitoring a decrease in oxygen concentration at a low electrical potential [16–19].

We have reported recently a novel glucose sensing method combining enzymatic glucose oxidation and cathodic O_2 reduction [20]. This method involves the use of the GOx-immobilized polyaniline/poly(acrylic acid) (PANI/PAA) composite film as an enzyme electrode. The determination of glucose concentration with the enzyme electrode is carried out by monitoring the decrease in the reduction current resulted from competitive consumption of dissolved O_2 caused by electrocatalytic O_2 reduction and enzymatic glucose oxidation. Polyaniline (PANI) has an excellent electrocatalytic activity to reduce dissolved O_2 and is used successfully for the electrochemical O_2 reduction at low potential [21–24]. This property of PANI played a role in suppressing the interference of coexisting easily oxidizable compounds. In this glucose sensing, the concentration of dissolved O_2 is an important factor affecting the sensing performance [20]. However, the concentration of dissolved O_2 in the sample solution is limited unless its concentration is intentionally increased by a certain approach. Thus, effective strategy for increasing the concentration of dissolved O_2 is required to improve the sensing performance.

In this paper, we report an advanced glucose sensing system designed to increase the concentration of dissolved O_2. This system involves an O_2 regenerating reaction, where the enzyme electrode

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bearing both GOx and catalase (Cat) was used as a glucose sensor. Cat is an enzyme that disproportionates H_2O_2 into O_2 and water [25,26]. Therefore, the immobilized Cat regenerates O_2 from H_2O_2 produced by consuming dissolved O_2 and, in this way, increases the O_2 concentration in the vicinity of the enzyme electrode.

2. Materials and methods

2.1. Materials and apparatus

GOx (EC 1.1.3.4, from Aspergillus species, 162 U mg^{-1}) was purchased from Toyobo Co. Cat (EC 1.11.1.6, from bovine liver, 2000–5000 U mg^{-1}) and *N*-cyclohexyl-*N*-(2-morpholinoethyl) carbodiimide metho-*p*-toluenesulfonate (CMC, used as a condensing reagent) were supplied from SIGMA-Aldrich Co. LLC. Aniline and *N*-hydroxysuccinimide (NHS) were obtained from Nacalai Tesque, Inc. Aniline was distilled under reduced pressure and stored under N_2 at −20 °C until use. PAA (molecular weight 250,000) and D-glucose were obtained from Wako Pure Chemical Ind. Other chemicals were of analytical grade and used without further purification. All aqueous solutions were prepared with distilled water passed through a purification system. A glucose solution was allowed to stand for at least 24 h to reach anomeric equilibrium.

A gold electrode (0.5 cm × 2.0 cm) deposited on an alumina plate (1.0 cm × 2.5 cm) was purchased from Sunrise Industrial Co. Ltd., and used as a working electrode. The gold electrodes were cleaned with piranha solution (H_2SO_4:30% H_2O_2 = 3:1) before using them for fabrication of enzyme electrodes. The working area of the gold electrodes was then adjusted to 0.5 cm × 0.5 cm by masking it with a Kapton tape. Electrochemical polymerization and measurements were performed with a conventional three-electrode cell equipped with a potentiostat/galvanostat (μ Autolab Type III, Eco Chemie). A platinum plate and a saturated calomel electrode (SCE) were used as a counter electrode and a reference electrode, respectively.

2.2. Preparation of enzyme-immobilized PANI/PAA composite films

The PANI/PAA composite film was prepared by electrochemical polymerization of aniline in a 0.50 M H_2SO_4 solution containing 0.50 M aniline and 25 mg mL^{-1} PAA as described in a previous publication [27]. The solution was saturated with nitrogen by bubbling via an external source to remove dissolved O_2. The polymerization was conducted by cyclic voltammetry from −0.4 to 0.9 V vs. SCE at a scan rate of 0.05 V s^{-1}. The potential scan was repeated until the amount of passed charge reached to 140 mC. The resulting film was washed with 0.50 M H_2SO_4 and then with distilled water to remove residual reactants.

As illustrated in Scheme 1, the enzyme-immobilized electrodes were fabricated by immobilizing GOx and Cat covalently on the PANI/PAA composite films. The composite films were immersed in a 2 mL aqueous solution containing 100 mg CMC and 15 mg NHS for 20 min to activate the carboxyl groups on the films by esterification with NHS. After washing with distilled water, the composite films were immersed in a 2 mL aqueous solution containing given amounts of GOx and Cat. The enzyme electrodes fabricated thus (PANI/PAA-GOx/Cat_n, where the n means the molar ratio of Cat to GOx) were washed with distilled water and stored in 0.10 M phosphate buffer solution (pH 7.0).

2.3. Electrochemical measurement

The amperometric response of the enzyme electrode to glucose was determined by applying a constant potential of −0.3 V vs. SCE in 20 mL of 0.10 M phosphate buffer solution (pH 7.0) at 25 °C. The solution was continuously stirred with a magnetic bar. After the background current was allowed to be constant, a given amount of glucose solution was added to the solution and the current change was recorded. Measurements were conducted with at least three different samples fabricated under the same conditions. Data points indicate mean values of measured currents with error bars showing standard error.

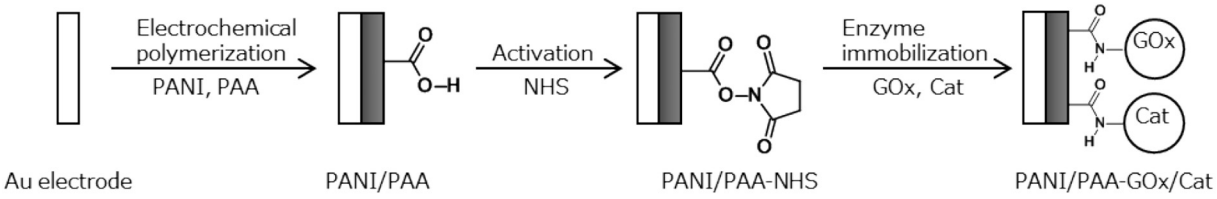
3. Results and discussion

3.1. Glucose sensing with PANI/PAA-GOx/Cat

Fig. 1A shows the principle of the amperometric glucose sensing with PANI/PAA-GOx/Cat. The sensing system involves biocatalytic glucose oxidation with GOx, electrocatalytic reduction of dissolved O_2 on the PANI/PAA composite film and biocatalytic O_2 regeneration with Cat. The amperometric response is observed as the result of competitive consumption of dissolved O_2 caused by GOx-catalyzed glucose oxidation and electrocatalytic O_2 reduction on the composite film. The O_2 regeneration allows the reuse of O_2 that was consumed by glucose oxidation with GOx and, in other words, can supply O_2 in place of the diffusion of it from a bulk solution. In this system, the composite film plays important roles as both the electrocatalyst and the enzyme-supporting material. PANI functions as an electrocatalyst for O_2 reduction. On the other hand, PAA is employed to utilize its carboxyl groups as both doping components and enzyme-binding sites [20,27,28].

Fig. 1B shows the result of amperometric glucose sensing carried out with PANI/PAA-GOx/$Cat_{0.24}$ in an air-saturated phosphate buffer solution (0.10 M, pH 7.0) by successively adding given amounts of 0.10 M glucose solution with a time interval of ca. 100 s. The initial current observed in the absence of glucose corresponds to electrocatalytic reduction of dissolved O_2 on the composite film. The O_2 reduction current was decreased by each addition of glucose and then the current became almost constant within 50 s or less. This current change can be attributed to competition of O_2 consumption between the electrocatalytic O_2 reduction on the composite film and the biocatalytic glucose oxidation with the immobilized GOx (Fig. 1A). Namely, the decrease in the O_2 reduction current reflects the decreased in the concentration of dissolved O_2 in the vicinity of the film caused by the O_2 consumption due to enzymatic oxidation of added glucose. Thus, the amount of added glucose can be related to the decrease in the O_2 reduction current.

Fig. 1C shows the current responses to various concentrations of glucose observed with PANI/PAA-GOx/$Cat_{0.24}$ and PANI/PAA-GOx. PANI/PAA-GOx, which was employed for a comparison, does not have Cat and, therefore, O_2 cannot be regenerated from H_2O_2 accompanying GOx-catalytic glucose oxidation. It can be seen that PANI/PAA-GOx/$Cat_{0.24}$ showed a linear relation between ΔI and glucose concentration up to 1.6 ± 0.0 mM with a sensitivity of 49.3 ± 0.6 μA cm^{-2} mM^{-1}. The linear range and sensitivity of PANI/PAA-GOx/$Cat_{0.24}$ obviously exceeded those of PANI/PAA-GOx (up to 1.3 ± 0.2 mM and 29.4 ± 1.7 μA cm^{-2} mM^{-1}). This can be attributed to the function of co-immobilized Cat to regenerate O_2 from H_2O_2 and to increase the O_2



Scheme 1. Fabrication of enzyme electrodes.