

Amperometric Glucose Sensor Based on
Electrochemically Polymerized Film of
Hydroxytryptamine

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INTRODUCTION

Much attention has been paid for the preparation of an enzyme electrode simply and quickly. We have been developed that polyion complex membrane is suitable material for immobilizing enzymes [1-3]. However, the membrane can not prepare all the surfaces which form curved ones. In contrast, membranes which polymerized electrochemically can prepare on the surface.

Tyrosine, tryptophane and their derivatives are oxidized electrochemically, and the resultant materials form polymers on the electrode surface [4]. Residue functional groups exist in these polymers, so that enzyme would be immobilized on the polymers by the functional groups. In this paper, we polymerized a tryptophane derivative, 5-hydroxytryptamine electrochemically, and prepared enzyme electrode by immobilizing glucose oxidase (GOD) on the surface by cross-linking agent.

EXPERIMENTAL

Preparation of Enzyme-Immobilized Electrode

A glassy carbon electrode (disk, 3 mm in diameter; Bioanalytical Systems) was immersed into a 0.1 M phosphate buffer (pH 7.0) solution containing 5 mM tryptophane derivative. A potential of +1.0 V vs. Ag/AgCl was applied on the electrode for 30 s. After the polymerization, the electrode was rinsed with buffer solution.

After polymerization, 1 wt% GOD (160 U/mg, Toyobo) solution (20 μ l) and 0.1 wt% glutaraldehyde solution (20 μ l) were successively placed on the polymer, and the electrode was allowed to dry for 4 h. After drying, the electrode was rinsed with buffer and was stored in buffer solution at 4°C before use.

Measurement of Response to Glucose

The enzyme electrode was immersed into a 0.1 M phosphate buffer (pH 7.0) solution (15 ml), and a potential of +1.0 V vs. Ag/AgCl was applied on the base electrode. A certain concentration of glucose solution (150 μ l) was added to the solution, and the oxidation current of hydrogen peroxide produced was measured.

RESULTS AND DISCUSSION

Tryptophane (Trp), 5-hydroxytryptophane (H-Trp), tryptamine (TP) and 5-hydroxytryptamine (H-TP; serotonin) was used for polymerizing electrochemically. Oxidation currents of polymerization using H-Trp and H-TP were larger than those using Trp and TP, so that H-TP was used for following experiments. Melanin-like polymer would be obtained by the polymerization [5]. In the reaction, as the 5 terminal of the indole ring was

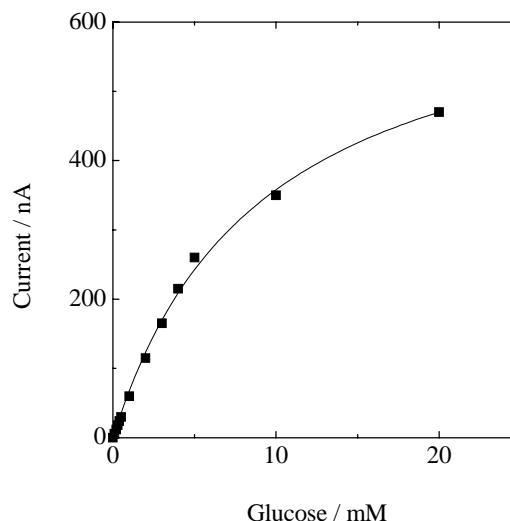


Fig. 1 Calibration curve of glucose.

effective for the oxidation, H-Trp and H-TP would be easily polymerized to melanin-like polymer.

After the polymerization of H-TP, GOD was immobilized on the membrane. The GOD-immobilized electrode was used for the detection of glucose; 6 nA of oxidation current increase was observed when 0.1 mM glucose was added to the 0.1 M phosphate buffer solution (pH 7.0). Addition of other sugars such as fructose, sucrose did not cause the oxidative current increase. Moreover, the response time was ~10 s. The quick response was caused by the thin polymer layer. From the results, it was found that immobilized enzyme retained its activity and produced hydrogen peroxide was oxidized on the base electrode.

The current response was plotted against the glucose concentration (Fig. 1). The current response was linear to the glucose concentration up to 5 mM. The lower detection limit was 0.1 mM glucose (signal-to-noise ratio, S/N = 3). From these data, the electrode would be used as a glucose biosensor.

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