Glucose-Sensing Electrode Based on Carbon Paste Containing Ferrocene and Polyethylene Glycol-Modified Enzyme

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Synopsis. An amperometric glucose-sensing electrode is constructed by the use of a carbon paste containing 1,1′-dimethylferrocene and a polyethylene glycol-modified glucose oxidase. The polymer-modified enzyme is soluble in organic solvents (e. g., hexane) as well as in water, and exhibits a much higher activity than native enzyme does on the surface of a hydrophobic carbon paste eletrode. The higher enzyme activity results in an enhanced electrode response: the current response to glucose on the modified enzyme-based electrode is several times as large as that obtained on a native enzyme-based electrode.

Much effort has been directed to devise an amperometric enzyme electrode for measuring glucose by combining immobilized glucose oxidase (GOD) with an immobilized electroactive species which mediates an electron transfer between the enzyme and the electrode.[1-3] Recently, a novel method for constructing such a mediated glucose-sensing electrode has been developed: a GOD/mediator-containing carbon paste electrode (CPE) has been prepared by doping the carbon paste with the enzyme and a mediator.[4-6] The GOD/mediator-doped CPE is of particular interest, since it can easily be prepared; it also has a close proximity of the enzymatic, mediating and sensing sites, thus providing a rapid current response to glucose. However, hydrophilic GOD molecules are not expected to exhibit high activity in a hydrophobic carbon paste matrix.

Inada et al.[7-9] have reported that enzymes (e.g., catalase and peroxidase) modified with polyethylene glycol (PEG) dissolve in various organic solvents as well as in water. Further, the PEG-modified enzymes have been reported to show catalytic activities, even in organic solvents. Such a PEG-modified enzyme would exhibit a higher activity than the native enzyme in the carbon paste matrix.

We have prepared a glucose-sensing CPE by the use of 1,1′-dimethylferrocene and a PEG-modified GOD. The effect of the modification of GOD on the electrode response is reported in the present paper.

Experimental

GOD (E.C. 1.1.3.4, from *Aspergillus* sp., Grade II) and peroxidase (E.C. 1.11.1.7, from horseradish, Grade I-C) were purchased from Toyobo. α-(4,6-Dichloro-1,3,5-triazin-2-yl)-ω-methoxypoly(oxyethylene) (activated PEG; molecular weight, 5×10^3)[10] was obtained from Sigma, and 1,1′-dimethylferrocene, from Aldrich. The other reagents used were of analytical-reagent grade. Deionized, doubly-distilled water was used throughout.

The GOD, as received (native GOD, 14 mg), and the activated PEG (140 mg) were added into 2 ml of 0.1 mol dm^{-3} borate–KOH buffer (pH 10); this GOD/PEG mixture was then incubated at 37 °C for 2 h, after which the reaction was stopped by adding acetic acid into the solution to make its pH 6. Unattached PEG was removed in an ultrafiltration cell (Millipore) using a PTTK membrane (Millipore; cut off molecular weight, 3×10^4) and 0.05 mol dm^{-3} $(NH_4)HCO_3$ as a dialyzing solution. Finally, a light-yellow-colored powder of PEG-modified GOD (22 mg) was obtained by lyophilization.

The molecular weight of the PEG-modified GOD was estimated by sodium dodecyl sulfate (SDS)–polyacrylamide (4—20%) gel electrophoresis; the molecular marker proteins were myoglobin (1.7×10^4), carbonic anhydrase (3.0×10^4), ovalbumin (4.5×10^4), bovine serum albumin (6.6×10^4), β-galactosidase (1.2×10^5) and myosin (2.0×10^5). The content of FAD (flavin-adenine dinucleotide) in the modified GOD was estimated by measuring the visible absorption of an aqueous solution of the modified enzyme (at 450 nm). The enzyme activities of the modified and native enzymes in solutions and on CPE's were measured by using the *o*-dianisidine/peroxidase-color-producing system.[11]

Two kinds of glucose-sensing CPE's, CPE I and II, were prepared by using the PEG-modified GOD and the native GOD, respectively. The modified or native GOD, 1,1′-dimethylferrocene and carbon paste (BAS CP-O), 10:10:80 by weight, were thoroughly mixed together. A portion of the mixture was placed in a hole (3.2 mm diameter, 4 mm deep) at the end of an electrode body (BAS 11-2010). CPE I or II thus prepared, an Ag/AgCl electrode and a platinum wire served as working, reference and auxiliary electrodes, respectively. The test solution used was a 0.1 mol dm^{-3} potassium acetate buffer solution (20 ml, pH 5, 25 °C). The solution was stirred with a magnetic bar. Oxygen was expelled from the solution by bubbling argon. CPE I and II were stored in the acetate buffer solution at 0 °C when not in use.

Results and Discussion

Figure 1 shows the result of the SDS-polyacrylamide gel electrophoresis. The native GOD gave a single band on the gel at a molecular weight of ca. 7×10^4. This value agrees fairly well with the molecular weight of the subunit in GOD, 7.5×10^4; the native GOD (molecular weight, 1.5×10^5) consists of two identical subunits. On the other hand, the modified GOD gave a broad electrophoretic pattern, indicating that the molecular weight of the subunit increased to be more than 1×10^5 upon attaching activated PEG onto the GOD molecule. The PEG-modified GOD was soluble in organic solvents as well as in aqueous media. For example, the modified enzyme (1 mg) was instantaneously dissolved in hexane (1 ml).

The acetate buffer solution containing the native GOD (5 mg ml^{-1}) and that containing the modified GOD

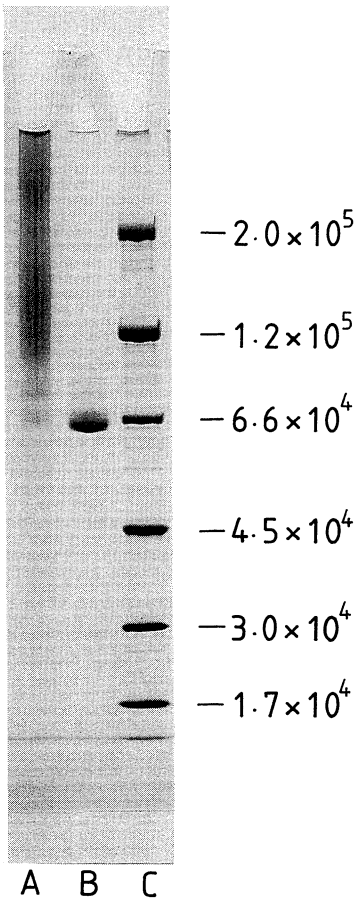

Fig. 1. Electrophoretic patterns of the modified GOD (A), native GOD (B), and marker proteins (C).

(5 mg ml^{-1}) showed optical densities of 0.52 and 0.28, respectively, at 450 nm: the content of FAD in the modified GOD is about a half that in native GOD. When native GOD was incubated in a borate–KOH buffer in the absence of activated PEG, the release of FAD from GOD molecules was not observed. It is therefore indicated that the modified GOD consists of native GOD and the modifier in a weight ratio of ca. 1:1.

The activity of modified GOD in an acetate buffer solution at 25 °C was determined to be 15 U mg^{-1}, whereas that of native GOD under the same conditions was 105 U mg^{-1}. This indicates that the enzyme activity is considerably reduced during the modification process. When the modified GOD was doped into carbon paste, on the other hand, it exhibited a mcuh higher activity than native enzyme. The GOD activities on the surfaces of CPE-I and II were ca. 0.1 and 0.02 U cm^{-2}, respectively. The higher activity of modified GOD in the carbon paste matrix may be attributable to its enhanced effinity for the hydrophobic matrix, which can be proven by the high solubility of the modified enzyme in organic solvents such as hexane.

The increase in the GOD activity in the carbon paste by a modification of the enzyme with PEG resulted in an enhanced response for glucose on CPE I, as compared to

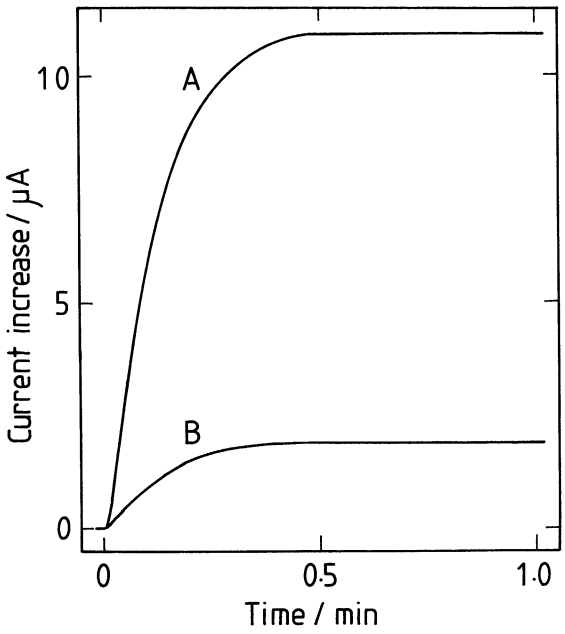

Fig. 2. Response/time curves on CPE I (A) and II (B) for 25 mmol dm^{-3} glucose.

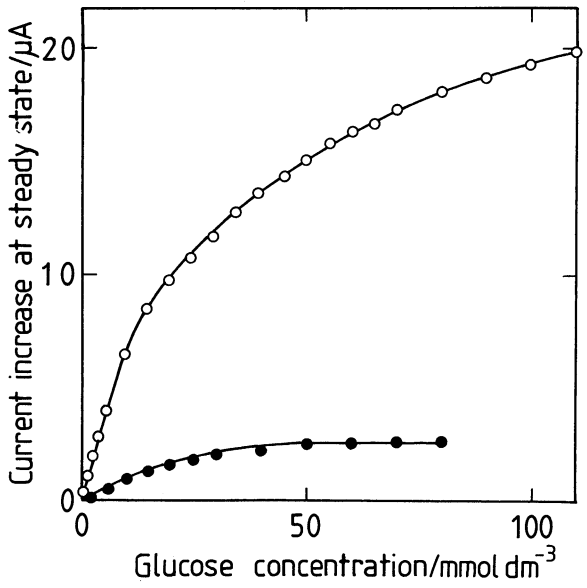

Fig. 3. Relationships between current increase and glucose concentration on CPE I (—○—) and II (—●—).

the case of CPE II. Curves A and B in Fig. 2 show the electrode responses to glucose (25 mmol dm^{-3}) on CPE I and II, respectively. The electrode potential, 0.4 V vs. Ag/AgCl, was sufficient for the oxidation of 1,1′-dimethylferrocene to produce ferricinium ion, and for obtaining ferrocene-mediated current responses to glucose.[4,12] The glucose response on CPE I was about five times as large as that on CPE II.

Figure 3 shows the calibration curves of CPE I and II. CPE I gave a linear current response up to 10 mmol dm^{-3} of glucose, and a siginificant increase in the

response with an increase in the glucose concentration within the range 10—100 mmol dm^{-3}. The detection limit of CPE I was 0.1 mmol dm^{-3} (S/N=5). On the other hand, the detection limit of CPE II was 0.5 mmol dm^{-3} (S/N=5); the electrode response reached a plateau when the glucose concentration was higher than 50 mmol dm^{-3}. These results indicate that the modification of GOD with PEG is effective for widening the dynamic range of the glucose-sensing electrode.

The effect of storage (in the acetate buffer at 0 °C) of CPE I and II was then examined. On each electrode, the response to glucose (25 mmol dm^{-3}) gradually decreased during storage and became ca. 80% of the initial value after 10 d; the long-term stability was, unfortunately, not improved by a modification of enzyme. The decrease in the electrode response was considered to be caused by a leaching of the (modified or native) enzyme out of the carbon paste matrix, since the solution used for storing CPE I or II showed significant GOD activity. Another kind of modification method, to introduce hydrophobic groups onto the surface of GOD molecule, would be required for obtaining enzyme/carbon paste systems with high stability. On the other hand, our preliminary experiments demonstrate that the coating on the surface of CPE I (and that of CPE II) with an anionic polymer (AQ-29D, Eastman)[4] is effective for preventing the enzyme from leaking out of the carbon paste, as well as for diminishing electrochemical interference with such anionic species as ascorbate.

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