Preparation of a Glucose-Sensing Electrode Based on Glucose Oxidase-Attached Polyion Complex Membrane Containing Microperoxidase and Ferrocene

Soichi YABUKI,* Fumio MIZUTANI, and Yoshiki HIRATA

National Institute of Bioscience and Human-Technology (1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan)

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A glucose oxidase-attached polyion complex membrane containing microperoxidase and ferrocene was formed on a carbon electrode. The enzyme electrode gave a reductive current response (100% response time, within 20 s), and steady-state response was proportional to the glucose concentration up to 0.2 mM. Lower detection limit was 1 µM. The current response decreased gradually, owing to the loss of the microperoxidase activity. The enzyme electrode was used for the detection of glucose concentration in beverages. The results obtained agreed well with those obtained by Boehringer F-kit method. The electrode would be useful as a glucose biosensor for real samples.

Key Words: Glucose Biosensor, Glucose Oxidase, Microperoxidase, Polyion Complex Membrane

1 Introduction

Much attention has been paid for the construction of amperometric biosensor with high sensitivity. For the construction of amperometric biosensors, oxidases are often combined with a hydrogen peroxide transducer. Usually a metal or carbon electrode is used for oxidizing hydrogen peroxide as the transducer. For the further improvement in sensitivity, peroxidase-based electrodes have proved to be useful.1–15 Moreover, electrochemical interference current caused by Lascorbate, urate and acetaminophen could be reduced by using the transducer,5–7 because of the applying the negative potential to the base electrode.

Microperoxidase (MP; heme peptide; heme octapeptide, nonapeptide and undecapeptide) are known to have the peroxidase activity. MP has a much smaller molecule (molar mass, ca. 1500-1900 g mol⁻¹) than peroxidase (ca. 40000 g mol⁻¹), which results in a much higher specific activity. Hence, MP was a useful material for constructing a highly sensitive hydrogen peroxide transducer.27–30 In this paper, we prepare glucose oxidase (GOD)-attached peroxidase transducer membrane for applying to the measurement of glucose concentration.

Recently, we have found that enzymes could be easily immobilized into a polyion complex membrane.38–40 The enzyme membrane was prepared by the placing poly-L-lysine, enzyme and poly(4-styrenesulfonate) solutions successively onto an electrode and drying; enzyme molecules were entrapped in a poly-L-lysine/poly(4-styrenesulfonate)-complex. In this paper, we have employed MP and mediator (ferrocene)-immobilized polyion complex membrane as a hydrogen peroxide-transducer, and construct glucose sensor by attaching glucose oxidase onto the polyion complex membrane. The polyion complex membrane is known to restrict the permeation of electrochemical interferents such as L-ascorbate, urate and acetaminophen, because of the permeselectivity based on the solute size with a cut-off molar mass of ca 100 g mol⁻¹.119 Mediators could also be restricted to leak from the membrane. Moreover, the amino residues of poly-L-lysine can be used for attaching enzyme molecules using a cross-linking reagent.

2 Experimental

2.1 Apparatus and reagents

GOD (EC 1.1.3.4, from Aspergillus sp.; 150 U mg⁻¹) was purchased from Toyobo (Osaka). Poly-L-lysine (average MW 100000) and MP (MP-11, degraded from equine heart cytochrome c) were obtained from Sigma Chemical Co. (St. Louis, MO, USA), and poly(sodium 4-styrenesulfonate) (average MW 70000), Aldrich Chemical Co. (Milwaukee, WI, USA). Ferrocene was purchased from Nacalai Tesque (Kyoto), and was re-crystallized before use. All other reagents were of analytical-reagent grade. Double-distilled water was used throughout.

A potentiostat (Model HA150; Hokuto Denko Co., Tokyo) was used in a three-electrode system for electrochemical measurements; the enzyme electrode, an Ag/AgCl electrode (saturated with NaCl; Bioanalytical Systems, IN, USA) and a platinum wire were used as the working, reference and auxiliary electrodes, respectively. The test solution usually used was an air-saturated 0.1 M phosphate buffer (pH 7.0; 15 ml). The solution was stirred with a magnetic bar during the measurements.

2.2 Preparation of glucose oxidase-attached polyion complex membrane containing MP and ferrocene

A glassy carbon electrode (3.2 mm in diameter; Bioanalytical Systems) was used as a base electrode. Polyion complex membrane containing MP and ferrocene was prepared by following the previously reported method.10 After drying the polyion complex membrane containing MP and ferrocene, GOD was attached using
Results and Discussion

After preparing the enzyme electrode, the electrode was immersed into a 0.1 M phosphate buffer (pH 7.0) solution and was applied a potential of −0.2 V vs. Ag/AgCl. Glucose (final concentration; 50 μM) was added to the buffer solution and the reductive current response was observed (Fig. 1). As shown in Fig. 1, the current changed immediately after the addition of glucose, and reached a steady-state current within ~20 s. The current response was not observed when the MP or ferrocene was not immobilized in the membrane. This means that the reductive current response is obtained by the MP-catalyzed reduction of hydrogen peroxide, which is produced through the GOD reaction.

The base current, which was caused by the reduction of oxygen, might be changed by the glucose addition, i.e., the consumption of oxygen by the GOD might influence on the measurements. However, the base current caused by the oxygen reduction was small (~10 nA) in the air-saturated buffer solution, because of the inertness of glassy carbon to the oxygen reduction. The magnitude of the base current corresponds to the magnitude of the response to 25 μM glucose, so that the base current change caused by the oxygen consumption would be small compared with the response current. The change of the base current could be ignored.

The current response was plotted against the glucose concentration (Fig. 2). As the glucose concentration increased, the current response was increased. The current response was linear to the glucose concentration up to 0.2 mM. The lower detection limit was 1 μM (signal-to-noise ratio, S/N = 5). The electrode was useful for highly sensitive determination of glucose.

Magnitude of the response current to the glucose was small to that to the same concentration of hydrogen peroxide. The difference on the responses would be caused by the position of GOD and POD, i.e., GOD was located at the surface of the membrane, the produced hydrogen peroxide would be lost to the bulk solution. To increase the sensitivity of the glucose, several improvements are in progress such that the thinning down membrane and co-immobilization of MP and GOD to bring close to each other.

The long-term stability of the electrode was examined: the current response to 50 μM glucose was measured every day. Figure 3 shows the relationship between the
current responses to glucose and days after the preparation of the electrode. The response was gradually decreased. On the tenth day, though the response magnitude became ca. 60% of the initial value, a linear response was still observed up to 0.2 mM glucose. Thus the electrode could be used at least 10 days. There would be a fear of leaking mediator, ferrocene; ferrocene is immobilized in the membrane so much compared with the MP, and ferrocene is not soluble in the aqueous solution, so that the decrease of the mediator is not influenced on the response. The rather rapid decrease of the response magnitude would be caused by the loss of the MP activity as described previously. Improvement for the long-term stability is now under investigation.

Electrochemical interference from electroactive species such as L-ascorrate and L-cysteine, is a serious problem for applying enzyme electrodes to real samples (e.g., blood and beverage samples). We showed that the current response for the polyion complex system containing MP and ferrocene was not affected so much by the addition of such species, because of the applying much negative potential than those redox potentials to the electrode. The glucose concentrations in beverages were then measured: the results obtained from the proposed electrochemical method were compared to those obtained by using Boehringer F-kit (Boehringer Mannheim, Germany). For the measuring by the proposed method, all the samples were diluted 100 times by distilled water before the measurements, and each 0.15 ml of diluted samples was injected to the test solution (15 ml). Table 1 summarized the results. The results obtained were agreed well with those given by Boehringer F-kit method; the electrode could be used as a glucose biosensor for real samples.

4 Conclusions

A GOD-attached polyion complex membrane containing MP and ferrocene can be prepared easily and quickly. The enzyme electrode gave a quick response (~20 s), and the response was linear to the glucose concentration up to 0.2 mM. Lower detection limit of glucose was quite low (1 µM). Unfortunately the electrode was not so stable, owing to the gradual decrease in the hydrogen peroxide-reducing activity of the MP-ferrocene system. The enzyme electrode was applied to the determination of glucose in beverages. The results obtained were agreed well with those given by Boehringer F-kit method; the electrode could be used as a glucose biosensor for real samples.

References