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Ferrocene-Mediated Enzyme Electrode for Glucose with the Use of Conducting Polymer Support

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Synopsis. An amperometric glucose-sensing electrode has been constructed by immobilizing glucose oxidase and fine particles of ferrocene in a photo-crosslinked poly(vinyl alcohol) layer. The magnitude of the response of the ferrocene-mediated electrode to glucose (0.5—50 mmol dm^{-3}) increases when polypyrrole, an electrically conducting polymer, is incorporated into the poly(vinyl alcohol) support.

Much effort has been directed at devising an amperometric electrode for glucose by combining immobilized glucose oxidase (GOD) with immobilized electroactive molecules that mediate the electron transfer between the enzyme and an electrode:[1-4)]

$$\text{glucose} + \text{GOD/FAD} \rightarrow \text{gluconolactone} + \text{GOD/FADH}_2$$
$$\text{GOD/FADH}_2 + 2\text{M}^+ \rightarrow \text{GOD/FAD} + 2\text{M} + 2\text{H}^+$$
$$2\text{M} \rightarrow 2\text{M}^+ + 2e \text{ (at the electrode)}$$

In this scheme, GOD/FAD and GOD/FADH$_2$ represent the oxidized and reduced forms of flavin adenine dinucleotide within GOD, respectively, and M$^+$/M, the mediator which is assumed to be a one-electron couple. One of the most convenient ways for preparing such a mediator-based glucose electrode is the use of a membrane-type polymer containing an immobilized GOD/mediator-couple. However, the rate of charge transport through the nonconducting layer is much lower than desired,[5)] which seriously limits the application of the layer system.

The use of an electrically conducting polymer as the support seems to be a suitable approach, since the conducting support is expected to enhance the effectiveness of the mediator by providing a larger microscopic electrode surface area and a larger percentage of active centers than the case of nonconducting support being used.[6-9)] We report here on the construction and preliminary testing of a ferrocene (bis(cyclopentadienyl)iron, abbreviated FcH)-mediated glucose electrode. Two kinds of polymer supports, an insulator and a conductor, were employed for the construction of the glucose electrode with the use of the GOD/FcH-couple; the current response to the substrate was examined in connection with the electrical conductivity of the support.

Experimental

A type-I layer, a poly(vinyl alcohol) (PVA)-based one, was prepared from a mixture of GOD (Sigma Chemical Co., Type X), FcH (Aldrich Chemical Co., ground to a powder with a diameter less than 1 μm), and a photo-crosslinkable PVA (generous gift from Dr. K. Ichimura of this Institute, 8 wt. % aqueous solution, pH 7), 1 : 3 : 30 by weight, according to a procedure similar to that described previously.[10-12)] In the type-I layer, GOD was physically entrapped in the crosslinked PVA matrix and FcH was embedded in the matrix. The thickness of the prepared type-I layer was about 20 μm. A type-II layer, with a matrix comprising a polymer mixture of crosslinked PVA and polypyrrole (PPy), was prepared by incorporating PPy into the type-I layer. The incorporation of PPy was performed through the oxidative polymerization of pyrrole with K$_2$S$_2$O$_8$ as follows. The type-I layer was placed so as to be in contact with a monomer-containing solution from one side and an oxidant-containing solution from the other side (for 30 min at 25 °C):

> aqueous solution containing 0.1 mol dm^{-3} pyrrole, 0.05 mol dm^{-3} KClO$_4$, and 0.1 mol dm^{-3} phosphate buffer (pH 7)/type I layer/aqueous solution containing 0.1 mol dm^{-3} K$_2$S$_2$O$_8$, 0.05 mol dm^{-3} KClO$_4$, and 0.1 mol dm^{-3} phosphate buffer (pH 7)

The type-II layer, thus prepared, was thoroughly washed with 0.1 mol dm^{-3} phosphate buffer (pH 7).

The type-I or -II layer was separately placed on a platinum disk electrode (diameter, 5 mm) and covered with a polyester mesh (diameter, 20 mm). The mesh was held in place with rubber rings so that the layer was directly in contact with the electrode surface. The test solution used was a phosphate buffer (0.1 mol dm^{-3}, pH 7, 30 °C), being saturated with argon, unless otherwise stated. A saturated calomel electrode (SCE) and a platinum wire were used as reference and auxiliary electrodes, respectively. All potentials given in the present paper were measured against an SCE. The layer-attached electrodes were stored in a phosphate buffer (0.1 mol dm^{-3}, pH 7) at 4 °C when not in use.

The GOD activities in the layers were measured by using the 4-aminoantipyrine/phenol/peroxidase color-generating system[13)] at a glucose concentration of 0.1 mol dm^{-3}. The diffusion coefficients of glucose in the layers, D's, were determined by applying a method similar to that described elsewhere.[12)] The electrical conductivities of the layers were measured by using a two-point probe method, after a thorough washing of the samples with distilled water and drying under a vacuum below 10^{-1} Pa.

Results and Discussion

The parameters measured for the type-I and -II layers are given in Table 1. As shown in Table 1, the incorporation of PPy into the PVA matrix brought about a slight decrease in the GOD activity and a little decrease in the D-value, but a remarkable increase in the electric conductivity, as expected. However, the

Table 1. Parameters Obtained for the Type I and II Layers

Type of layer	GOD activity in the layer	Diffusion coefficient of glucose, D	Electrical conductivity
	U cm^{-3}	cm^2 s^{-1}	s cm^{-1}
I	135	4×10^{-7}	$<10^{-8}$
II	125	1×10^{-7}	2×10^{-4}

conductivity of the type-II layer, 2×10^{-4} S cm^{-1}, was still lower than that of a PPy-containing polymer mixture obtained under appropriate conditions.[8,9,14–16] Further examinations for obtaining the PPy/PVA mixture with a higher conductivity are in progress.

Nevertheless, the increase in the conductivity of the support was effective for enhancing the electron transferability between the mediator and the electrode. Figure 1 shows cyclic voltammograms on the layer-attached electrodes for the immobilized redox system of ferricinium ion (FcH^+) and FcH. The electrode coated with the type-II layer exhibited much larger current peaks than that with the type-I layer.

It is expected that the enhanced electron transferability of the FcH^+/FcH system in the type-II layer results in an increased current response to glucose, compared with the case of the type-I layer. Figure 2 shows the response/time curves. The electrode potential, 0.25 V, was sufficient for the oxidation of FcH to form FcH^+ (Fig. 1). The electrode with the type-II layer gave a larger current response, as expected, than that with the type-I layer.

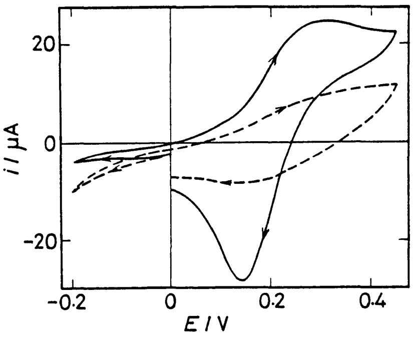

Fig. 1. Cyclic voltammograms on the electrode covered with the type-I layer (----) and with the type-II (——) layer. Potential sweep rate, 50 mV s^{-1}; solution, argon-saturated phosphate buffer (not containing glucose).

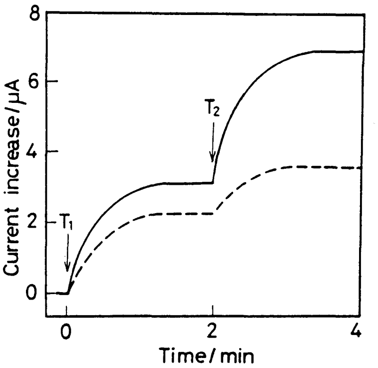

Fig. 2. Response/time curves of the electrodes using type-I (----) and type-II (——) layers to glucose. Electrode potential, 0.25 V; rotating rate of the layer-attached electrode, 20 Hz. T_1 and T_2 are the time points when glucose is added into the argon-saturated buffer to be 5 mmol dm^{-3} and 20 mmol dm^{-3}, respectively.

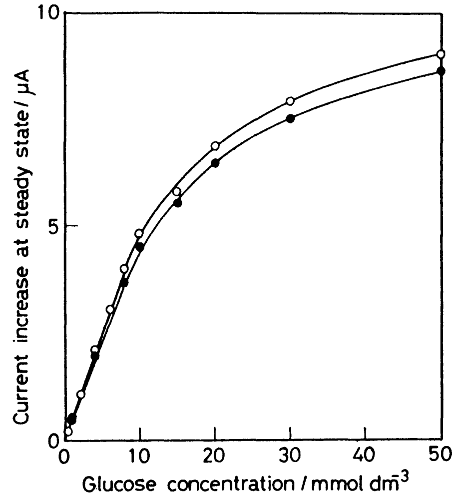

Fig. 3. Calibration curves for glucose of the electrode with the type-II layer in argon (—○—) and air (—●—) saturated buffers. Electrode potential, 0.25 V; rotating rate of the electrode, 20 Hz.

A few additional characteristics of the electrode with the type-II layer, as a glucose-sensing one, were then examined. Figure 3 shows calibration curves of the electrode obtained in argon-saturated and air-saturated solutions. The introduction of air into the test solution brought about a decrease in the current response by ca. 5% at all the glucose concentrations examined (0.5—50 mmol dm^{-3}). The decrease in the response is considered to be caused by interference with oxygen in the air: oxygen reacts with $GOD/FADH_2$ to form GOD/FAD and hydrogen peroxide, and the electrode potential was not sufficiently positive to reoxidize the hydrogen peroxide produced. However, in each solution, the electrode gave a linear current response in the range of 0.5—8 mmol dm^{-3} glucose and a discernible increase in the response with an increase in the glucose concentration above 8 mmol dm^{-3}. These results indicated that the electrode could be used for a determination of glucose in the high concentration range (e.g., blood glucose concentration range), even in an air-saturated buffer. Subsequent experiments were, therefore, performed in air-saturated buffers. The relative standard deviations were ca. 1.5 and 2.5% for 10 successive measurements of 4 and 15 mmol dm^{-3} of glucose, respectively. The long-term stability of the electrode was examined as follows: the determination of 4 mmol dm^{-3} glucose was carried out 10 times a day, every day for one month. The average value of the measurement on each day did not virtually change during this period.

Recently, another method involving the use of PPy for the construction of glucose-sensing electrodes has been reported: GOD was entrapped in a PPy layer on an electrode by the electrolytic polymerization of pyrrole in the presence of the enzyme.[17–19] The procedure for the preparation of the glucose electrode seems to be of particular interest due to its simplicity. However, the resulting GOD/PPy electrode, unfortunately, showed a rather poor stability.[17] The current response of the electrode to glucose diminished within a few days owing to the leaching of GOD out of the PPy matrix and to the chemical and/or electrochemical decomposition of PPy during the measurement of hydrogen peroxide, the product of the GOD-catalyzed reaction in the absence of the non-physiological mediator such as FcH^+/FcH couple. On the other hand, the present electrode was essentially free from these problems, which resulted in the higher stability of the electrode, although the procedure for its construction was more complicated. The approach to obtain a glucose electrode having a high performance by a simpler process is in progress, with the use of the conducting support system.

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