materials

Communication

Long-Term Stability of a Cellulose-Based Glucose Oxidase Membrane

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Abstract: A cellulose-based glucose oxidase membrane was prepared on a glassy carbon (GC) electrode. The current response of the electrode to glucose was measured by applying a potential of 1.0 V *vs.* Ag/AgCl on the base GC and was proportional to the concentration of glucose up to 1 mM. The long-term stability of the electrode was examined by measuring the daily glucose response. Over four months, the response magnitude was maintained and then gradually decreased. After 11 months, though the response magnitude decreased to 50% of the initial value, the linear response range did not change. Therefore, the electrode could be used as a glucose biosensor even after 11 months of use. The entrapment of the enzyme in the cellulose matrix promoted the stability of the enzyme, as revealed by data on the enzyme activity after the enzyme electrode was immersed in urea. Therefore, the cellulose matrix may be used to improve the performance of biosensors, bioreactors and bio-fuel cells.

1. Introduction

Numerous studies have focused on improving the stability of enzyme membranes for use in biosensors and bioreactors [1], because increasing the long-term stability of enzyme membranes [2,3] may extend the lifetime of biosensors and bioreactors.

To increase the long-term stability, various factors that decrease the stability should be eliminated. Such factors may include [4]: (1) the lifetime of the immobilized enzymes and membrane materials; (2) strength of immobilization or entrapment and (3) fouling and contamination of the samples. Typically, removing more than one factor should be addressed to increase the stability of the enzyme membrane.

Many studies have been conducted regarding the solid immobilization of enzyme membranes and it has been reported that covalent bonding or cross-linking between the base material and enzyme facilitates strong immobilization [5–8]. However, the required reagents may alter the enzyme. To avoid this issue, enzyme entrapment has been employed for the immobilization [9–16]. Furthermore, our group has developed enzyme entrapment methods, which avoid enzyme activity loss including the polyion complex membrane [3,14,15] and cellulose-based membrane [4,16].

For cellulose-based enzyme membranes, excellent long-term stability was achieved as the response magnitude did not change up to four months after preparation [16]. Furthermore, cellulose was stable against chemical and biological contamination and the lifetime of the enzymes in the membranes was extended. In this study, the electrode responses during long-term usage were examined.
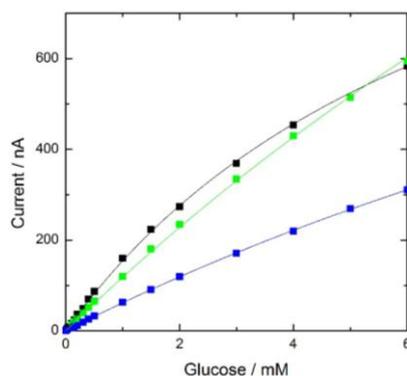
2. Results and Discussion

2.1. Current Response to Glucose on Cellulose-Based Glucose Oxidase Membrane Electrode

As described in the experimental section, glucose oxidase (GOD) was immobilized in the cellulose membrane on the glassy carbon (GC) electrode. The current response of the electrode to glucose was measured. The characteristics of the electrode were similar to those described in a previous report [16] as the response time was *ca*. 10 s. Because the response was not observed when the electrode without GOD was utilized, the increase in the oxidation current was caused by the oxidation of hydrogen peroxide produced by the enzymatic reaction.

The response current was plotted against glucose concentration (black line in Figure 1) and was proportional to the glucose concentration, up to 1 mM. The lower detection limit was 10 μM glucose, which was similar to that in a previous report [16]. Thus, the electrode can be used as a sensitive glucose biosensor and the preparation of the cellulose-based GOD membrane is easy and inexpensive.

Figure 1. Calibration curves of glucose based on cellulose-based glucose oxidase membrane electrode. The black curve was obtained on the 2nd day after preparation. The green and the blue curves were obtained on 117th day and 329th day, respectively.
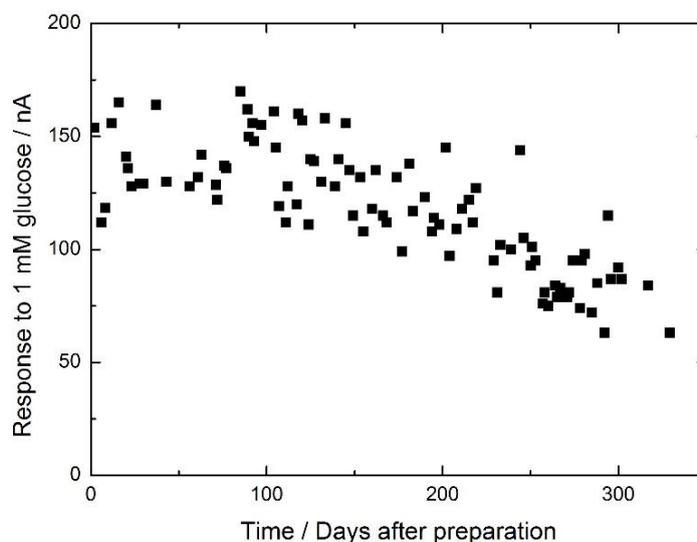
2.2. Long-Term Stability of Cellulose-Based GOD Membrane

The long-term stability of the enzyme membrane was examined by measuring the current response of the electrode to 1 mM glucose daily for 11 months (Figure 2). During the first four months after preparation, the magnitude of the current response was almost the same as the initial value. The calibration curve of the electrode on the 117th day after preparation (green plot in Figure 1) was the same as the initial curve (black plot in Figure 1). After four months, the current gradually decreased and eventually reached *ca*. 50% of the initial value after 11 months. However, the calibration curve on the 329th day after preparation (blue plot in Figure 1) had a similar shape to the initial curve, but a different magnitude, meaning the proportional response range against the glucose concentration had not changed. The magnitude of the response to 1 mM glucose on the 2nd, 117th and 329th day were determined to be 161 $\pm$ 10, 119 $\pm$ 6 and 63 $\pm$ 5 nA (number of the measurements, *n* = 5), respectively, *i.e.*, the magnitude of the error was within several percent of the signal. Therefore, the electrode can be used as a biosensor for 11 months.

The long-term stability is likely related to the characteristics of the cellulose matrix, enzyme capture, and stabilizer effects. The cellulose membrane could be penetrated by small molecules such as L-ascorbate and acetoaminophene [16]. On the other hand, the enzyme entrapped in the cellulose membrane remained within the membrane for four months, likely because of the structure of the cellulose matrix. The enzyme electrode could be used as a biosensor even after 11 months of electrode use because of the capture and stabilization of the enzyme. Though the measurement error was less than 10%, the fluctuation of the stability data was larger, likely because of the lack of temperature control during the measurements.

Figure 2. Long-term stability of the cellulose-based glucose oxidase (GOD) membrane. The daily response of the electrode to 1 mM glucose is shown.
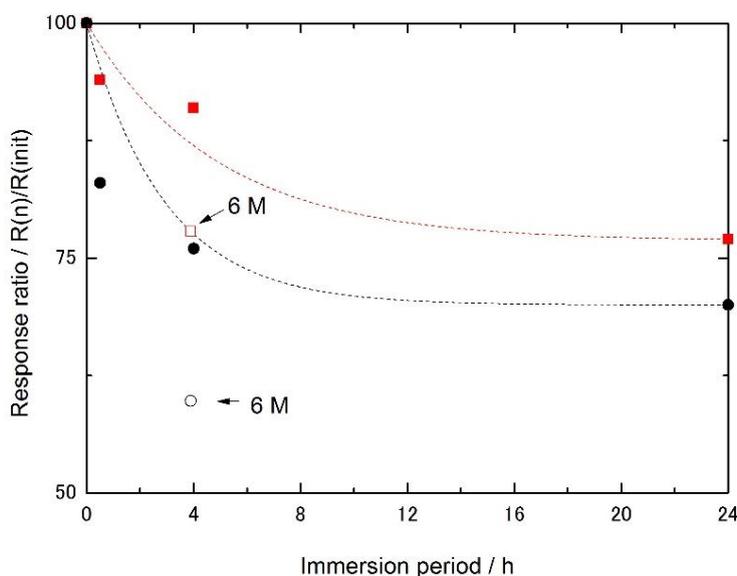
2.3. Effect of a Denaturant on Electrode Response

The effect of urea, a denaturant, on the electrode response to glucose was measured by immersing the enzyme electrode in a 3 M solution of urea. The ratio of the current response before and after the immersion is plotted in Figure 3. The response of the cellulose-based enzyme electrode gradually decreased with the immersion time (red squares in Figure 3). The response of the polyion complex membrane containing the enzyme was also measured (black circles in Figure 3). Open marks represent the results obtained when the electrodes were immersed in a 6 M solution of urea. Notably, neither membrane was altered after immersion in urea. A comparison of these two matrices revealed that the enzyme in the cellulose matrix retained a higher activity than the enzyme in the polyion complex membrane matrix. Cellulose appeared to stabilize the entrapped enzyme and thus enhance its activity.

Based on the permeation characteristics of the two membranes, urea could easily penetrate the membranes [14,16]. Nevertheless, the immobilized enzymes were not denatured to the same degree, which may be related to the different characteristics of the matrix materials. In particular, cellulose is a hydrophilic material, whereas the polyion complex is hydrophobic [3,17]. The environment was altered by the type of matrix materials and thus the surroundings of the enzyme were also altered. As a result, the cellulose matrix was more suitable to increase the immobilized enzyme lifetime. A more detailed investigation is now in progress.

Figure 3. Response ratio to 1 mM glucose before and after immersion of the electrode in 3 M urea. The red squares represent cellulose-based GOD membranes. The black circles represent polyion complex membranes containing GOD. The open marks represent immersion in 6 M urea.

3. Experimental Section

3.1. Materials

GOD (203 U·mg^{-1}; EC 1.1.3.4; obtained from *Aspergillus niger*), cellulose solution (5 wt%) in ionic liquid (1-ethyl-3-methylimidazolium acetate), poly-L-lysine (average MW 100,000), and

polystyrene sulfonate (average MW 70,000) were obtained from Aldrich (St. Louis, MO, USA) and were used as received. All other reagents were of analytical reagent grade.

A GC electrode (disk, 3 mm in diameter) was purchased from Bioanalytical Systems (West Lafayette, IN, USA).

3.2. Preparation of Cellulose-based Enzyme Membrane

A modified version of a reported procedure [16] was carried out. Briefly, GOD was dissolved in water at 5 wt%, and the GOD solution (20 μL) was dropped on a GC electrode. The electrode was allowed to dry for 2 h. Then, 20 μL of cellulose solution in an ionic liquid was spread on the electrode for coating. The electrode remained stationary for 1 min to allow the formation of uniform layer, and then the electrode was immersed in water for 5 min to remove the ionic liquid. After drying for 4 h, the electrode was used for the experiments.

3.3. Measurement of Current Response to Glucose

The electrode was immersed in a 0.1 M citrate buffer solution (pH 5.5, 15 mL), and a potential of 1.0 V *vs.* Ag/AgCl was applied to the base electrode to measure the oxidation current of hydrogen peroxide produced by the enzymatic reaction. The current response to glucose was then measured by the addition of a solution of glucose to the buffer. After the measurement, the electrode was stored in a refrigerator at 4 °C.

The long-term stability of the electrode was evaluated based on the daily current response to 1 mM glucose.

The effect of urea on the electrode response was also measured. The current responses of 1 mM glucose were measured before and after the immersion of the electrode into a 0.1 M citrate buffer solution (pH 5.5) containing urea.

All measurements were performed at room temperature (23 ± 2 °C).

3.4. Preparation of GOD-Entrapped Polyion Complex Membrane

A GOD-entrapped polyion complex membrane was prepared by the successively dropping polystyrene sulfonate (20 μL, 20 mM monomeric unit), GOD (20 μL, 5 wt%), poly-L-lysine (20 μL, 20 mM monomeric unit) solutions on the GC electrode. After drying for 4 h, the electrode was used for subsequent experiments.

4. Conclusions

A cellulose-based GOD membrane was prepared on a GC electrode. The current response of the electrode to glucose was measured by applying a potential of 1.0 V *vs.* Ag/AgCl on the base GC. The current response to glucose was proportional to glucose concentration up to 1 mM. The long-term stability of the electrode was examined by measuring the daily glucose response. During the first four months, the response magnitude did not change, and after this period, the response gradually decreased. After 11 months, although the response magnitude decreased to 50% of the initial value, the linear

response range had not changed. Thus, the electrode could be used as a glucose biosensor even after being used for 11 months.

The long-term stability may be due to the enzyme entrapment by the cellulose matrix. The entrapment in the cellulose matrix promotes the stability of the enzyme, as confirmed by data regarding enzyme activity after the enzyme electrode was immersed in urea. Thus, the cellulose matrix might improve the performance of biosensors, bioreactors, and bio-fuel cells.

Conflicts of Interest

The authors declare no conflict of interest.

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