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Long-term stabilization of enzyme activity by immobilizing enzyme colony in polymaleimidostyrene-modified micelles

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Abstract

Amperometric biosensors fabricated by immobilizing enzyme colony in polymaleimidostyrene-modified micelles exhibited good sensitivity and great long-term stability. To our knowledge, there has not been a general design for various enzyme biosensors. Enzyme micelle membrane which is different from any of other conventional immobilization methods, is an innovative way and will be a well-developed biosensor technology to provide rapid and reliable measurements. The potential of using enzyme micelle membrane to fabricate biosensors will be of great hope.

Key words: enzyme micelle biosensor; long-term stabilization; polymaleimidostyrene; colony

Introduction

The immobilization of urea has mainly focused on the incorporation of the enzyme into polymer films. In our previous work, an L-ascorbic acid (AsA) biosensor has been developed by combining an AGCE or a gold electrode with a thin compact polystyrene (PS) membrane containing enzyme micelles (Wang *et al*., 2007, 2008). The proposed AsA and uric acid sensor exhibited good response with short response time. The enzyme-micelles formed by the reaction of polymaleimidostyrene (PMS) and enzyme could be immobilized into PS membrane which has strong affinity to the polyetheretherketone part of electrode surface. If urease and PMS can form micelles and the micelles can be immobilized into PS membrane, then a novel amperometric biosensor of urea can be expected to realize.

Additionally, we have found that PMS is a convenient immobilization reagent of enzyme in biosensor (Uchiyama *et al*., 2006), because maleimide groups of PMS react with sulphydryl groups of enzyme and have possibility to react with amino groups of enzyme. The activity of PMS-bonded enzyme has long-term stabilization (Tomita *et al*., 2007). However, the PMS-bonded enzyme was adsorbed to porous carbon materials and the response time of the sensors was a few minutes. Therefore, PMS-bonded enzyme was tried to immobilize into thin and compact PS membrane that was directly coated on electrode surface, which procedure was simple and mild.

In this study, a significant advance in the type of enzyme immobilization based on the adsorption to porous carbon sheets toward a simple and disposable type of immobilization into PS membrane was presented. The development was targeting improvement in both sensitivity and stability by design of the immobilization of enzyme. The application of PS membrane as the support of enzyme micelles offers a promising method for biosensors design. The interference from pH and cations was eliminated because of the novel amperometric detect principle that was based on the electrooxidation of carbamic acid. To our knowledge, there is no general design for various enzyme biosensors. Enzyme micelle membrane which is different from any of other conventional immobilization methods, which is an innovative way and will be a well-developed biosensor technology to provide rapid and reliable measurements of food, water pollution and clinical analysis. The potential of using enzyme micelle membrane (EMM) to fabricate biosensors will be of great hope.

1 Experimental

1.1 Reagents

The reagents used in this experiment were reagent grade chemicals. Urease (113 units/mg, from jack beans) and PS was purchased from Wako Pure Chemical Industries Ltd., and it was purified by dialysis and lyophilization. PMS was supplied by Tsukuba Materials Information Laboratory (Japan), and the PMS solution (5.0 mg/mL) was prepared by dissolving it in a chloroform solution. PBS (0.1 mol/L) saturated with oxygen was the support electrolyte changing pH value from 4.8 to 7.0.

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1.2 Apparatus

A computer-controlled electrochemical analyzer (BAS 100B) was used to perform electrochemical measurements. A three-electrode cell with an aminated GCE working electrode covered with polystyrene (PS) membrane containing urease micelles, an Ag/AgCl reference electrode, and a platinum wire counter electrode was employed. A scanning electron microscope (SEM, JSM-5500LV) was employed to observe the surface morphologies (at a magnification of 5000) of PS membranes of the sensors used in this study. All the observation was performed at a voltage of 15 kV.

1.3 Preparation of urease-micelles membrane

The following solutions were mixed: 20 μL of chloroform, 30 μL of PS chloroform solution (10.0 mg/mL), 10 μL of PMS chloroform solution (5.0 mg/mL) and 0.5 mg of urease. The mixture was syringed onto the rotating AGCE surface (200 r/min) by a pipette. The chloroform in the mixture was allowed to be evaporated in 5 min to form a thin compact PS membrane containing urease-micelles. In order to make a good immobilization of enzyme, 10 L of PS solution was syringed onto the dried PS membrane surface and dried again (in 5 min). The resulting enzyme electrode was used immediately or stored in PBS (0.1 mol/L, pH 5.5) in a refrigerator when not in use. All the amperometric measurements were conducted under stable stirring condition.

2 Results and discussion

Long-term stabilization of urease activity by immobilizing enzyme colony in polymaleimidostyrene-modified micelles was obtained. The proposed sensor fabricated using enzyme micelle membrane exhibited good stability. The typical constant (1.3 V) amperometric response curves of urea sensor at pH 5.5 during different storages are shown in Fig. 1. The immobilized urease may maintain its initial activity more than 6 months.

Figure 2 shows the long-term stability of urea sensor. The response increases with the storage time prolongs in the first 3 months. The activity of enzyme kept stable in the next 2 months. By far, the long-term stability of the sensor is still under the way. The increase of the response may be due to both the increase of the hydrophility and the amination of the electrode surface during the measurement. The great long-term stabilization may be due to the immobilization of enzyme colony, because there are free enzymes in the conloies. The major reason why the excellent stabilities of enzyme activities confined in the micelle is that the aggregated enzymes (just like colony) can be immobilized without any physical and chemical treatments, and denature of enzymes like unfolding do not take place for a long time.

Surface morphologies of the PS membranes containing urease-micelles coated on GCE surface was characterized by SEM, Fig. 3 displays the SEM image at a magnification of 5000. It can be seen that the micelles (white parts) are roughly spherical with diameter in the range of 1–3 μm.

PH effect on the response of amperometric current was

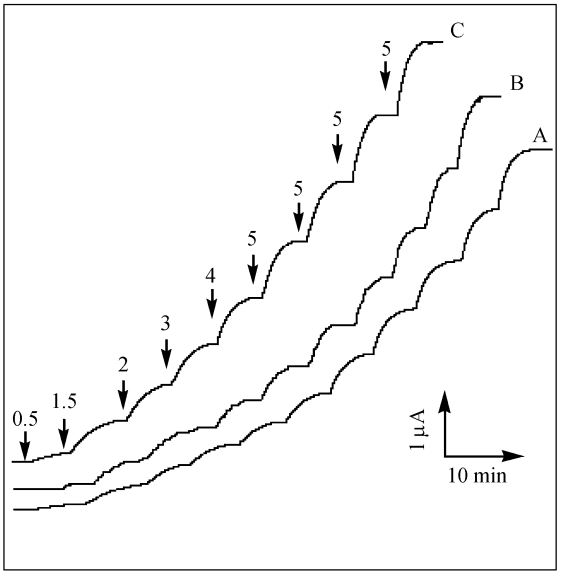

Fig. 1 Typical amperometric response curves of urea sensor. A: 1 d; B: 52 d; C: 140 d.

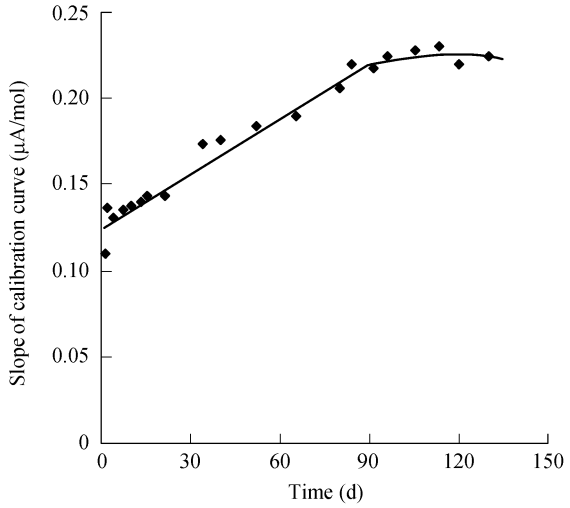

Fig. 2 Long-term stability of the urea sensor.

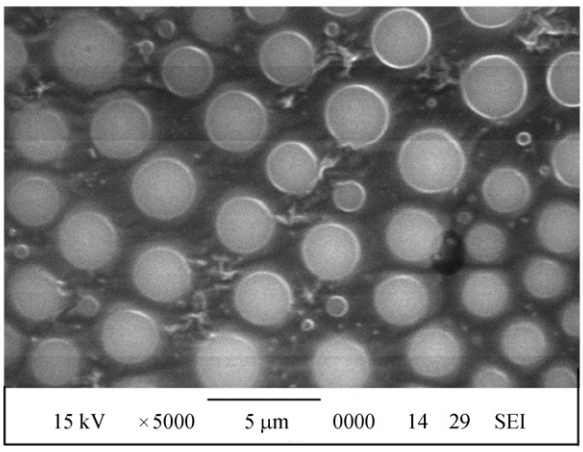

Fig. 3 SEM image of the surface of AGCE coated with PS membrane containing urease-micelles.

studied at freshly prepared sensor. The result is shown in Fig. 4. The sensor exhibited the highest response at pH 5.0. However, the linear response range of urea concentration is short because the amperometric response current started decrease when the concentration of urea was as low as 7 mmol/L. Similar phenomena occurred when the concentration of urea was higher than 26 mmol/L at pH 5.0. In order to obtain broad linear response range, all the detections were carried out at pH 5.5.

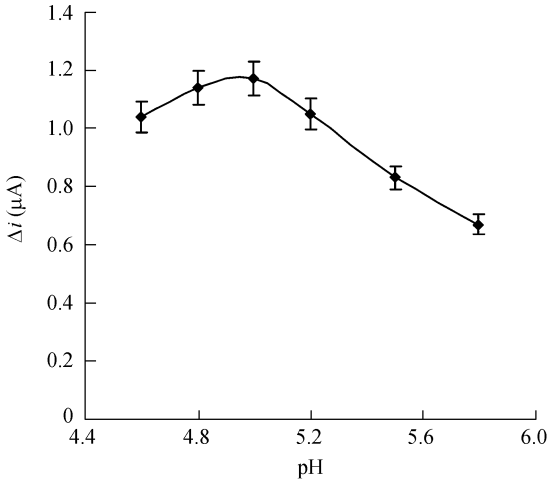

Fig. 4 pH effect on the response current of 5 mmol/L of urea at freshly prepared sensor in phosphate buffer solution (0.1 mol/L).

For principle of detection in this study, a novel principle was used to the amperometric detection of urea. In general, the products of enzymatic reaction of urea are ammonium and carbon dioxide via the hydrolysis of carbamic acid (Fig. 5, process (2)). It has been found that carbamic acid can be oxidized at an applied potential higher than 1.3 V (vs. Ag/AgCl). The finial products are nitrogen, water and carbon dioxide (process (1)). As above mentioned, the amperometric current decreased at a high concentration. The decrease should not be ascribed to the hydrolysis of carbamic acid, because the react rate of process (1) was equal to process (2). The decrease may because the aggregation of nitrogen at the surface of the electrode. The amount of produced nitrogen was large at high concentration of urea. The diffusion of nitrogen is slow compared with the produce of nitrogen in high concentration range of urea. Thus, the produced nitrogen aggregated on the surface of the working electrode results the decrease of the amperometric response current.

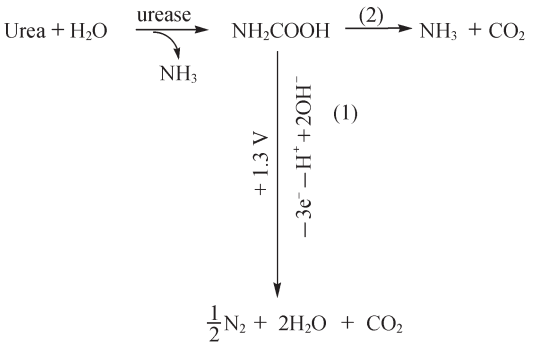

Fig. 5 Principle of amperometric detection of urea using proposed urea sensor.

3 Conclusions

Long-term stability of urease was obtained by the modification using enzyme colony. The immobilized enzyme exhibited good activity after being stored for 5 months. The long-term stability may due to the formation of enzyme micelles which contained enzyme colonies inside its structure and PMS bonding to the outside structure. The increase of the activity during the first 3 months may due to both the increase of the hydrophilicity of the PS membrane and the introduction of nitrogen-containing groups during the detection of urea. The application of enzyme micelles modification method may be a good immobilization method for other enzymes.

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