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Electrochemical Response of β-Galactosidase- and Glucose Oxidase-containing Microcapsule-immobilized Electrode

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A polyamide microcapsule containing bi-enzyme, β-galactosidase (β-Gal) and glucose oxidase (GOD), was prepared by interfacial polymerization. The average diameter of the microcapsule was evaluated to be 5 μm. The bi-enzyme-containing microcapsule was immobilized with tetrathiafulvalene on a carbon electrode surface and was characterized by cyclic voltammetry. The oxidation current increased with the increase of the lactose concentration. It was concluded that β-Gal and GOD retained their enzymatic activity in the microcapsule and reacted with lactose, and tetrathiafulvalene penetrated the polyamide membrane.

There has been a considerable recent interest in the development of an enzyme containing microcapsule-immobilized electrode.[1–4] The enzyme containing microcapsule-immobilized electrode has several advantages as follows: (1) improvement of the enzyme stability, (2) immobilization of high amounts of enzymes, and (3) protection against interfering substances.[1,5]

For example, Rochefort et al. prepared laccase containing microcapsule-immobilized electrode, and used it for a biofuel cell cathode.[5] We also prepared a glucose oxidase (GOD) and tetrathiafulvalene (TTF) containing microcapsule, and fabricated a full screen-printed enzyme containing microcapsule-immobilized electrode.[6] An enzyme ink was prepared by mixing the microcapsule and a commercial carbon ink. The GOD in carbon ink retained catalytic activity by microencapsulation. Recently, a bi-enzyme and nicotinamide adenine dinucleotide (NADH) containing liposome-immobilized electrode was also reported.[7] Production of microcapsules is simple and inexpensive in comparison with that of liposomes. However, to our best knowledge, there are no reports of a bi-enzyme containing microcapsule-immobilized electrode being fabricated. In the present study, we newly prepared bi-enzyme, β-galactosidase (β-Gal), and GOD containing microcapsule-immobilized electrode (BMIE) and characterized it by electrochemical measurements.

The bi-enzyme-containing microcapsules were prepared by interfacial polymerization.[6] An aqueous solution containing 0.125 M hexamethylenediamine (HMDA), 0.375 M diethylenetriamine (DETA), sodium carbonate β-Gal, and GOD was prepared. The aqueous solution was mixed with cyclohexane–chloroform (volume ratio: 4:1) solution containing 2 wt% Span 80, and emulsified using a homogenizer (Ika Works, T18 Basic S2) at 10000 rpm. Then, cyclohexane–chloroform containing 0.55 M terephthaloyl dichloride was added to the emulsified solution, and agitated for 30 min using a chemical stirrer at 800 rpm. The bi-enzyme containing microcapsule-suspended solution was centrifuged and rinsed five times with ultrapure water. The prepared bi-enzyme-containing microcapsules were immobilized on the surface of a homemade screen-printed carbon electrode. A 5 μL of aliquot of the TTF solution and a 10 μL of the microcapsule suspension were casted on the carbon electrode surface followed by casting 10 μL of the nitrocellulose solution. Then, the electrode was dried at room temperature for 12 h. Electrochemical characterization of the BMIE was performed by cyclic voltammetry by using a potentiostat ALS/CH Instruments Model 1206A in a three-electrode system. A satulated KCL/Ag/AgCl electrode and Pt wire were used as reference and counter electrodes, respectively. The CV were performed in a 67 mM phosphate buffer solution at 27 °C from −0.2 to 0.4 V without agitating the solution. The scan rate was 5 mV s^{-1}.

A polyamide microcapsule is a small sphere with a porous membrane around it. The polyamide microcapsule has high stability to chemical agents and high mechanical strength. A fast interfacial polymerization, which is the key to an efficient entrapment of the enzymes into the core of the microcapsules, can be achieved in the case of the polyamide microcapsules. In the previous study, GOD in the microcapsule selectively reacts with a particular low-weight-molecular substance such as glucose, which can penetrate the porous membrane.[6] In addition, it is reported that the polymerization reaction does not particularly inhibit the activities of the enzyme in the microcapsule.[1,6] For these reasons mentioned above, microencapsulation of β-Gal and GOD was performed by using polyamide microcapsule with interfacial polymerization. The polyamide capsule wall is formed by polymerization between the hydrophilic monomer (DETA and HMDA) and the hydrophobic monomer (TDC) at the oil/water interface of a microdroplet. Hence, the capsule diameter predominantly depends on the size of the microdroplets. Figure 1 shows the optical micrograph of the microcapsules. As you can see, the spherical microcapsules were prepared. The diameter of the microcapsule distributed from 2 to 13 μm. The average diameter of microcapsules calculated from the micrograph was 5 μm.

Figure 2 shows the reaction scheme of the bi-enzyme, β-galactosidase (β-Gal), and glucose oxidase (GOD) containing microcapsule-immobilized electrode (BMIE).[8,9] β-Gal catalyzes

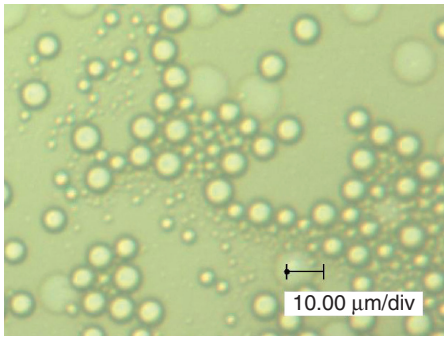

Figure 1. Optical micrograph of the microcapsules.

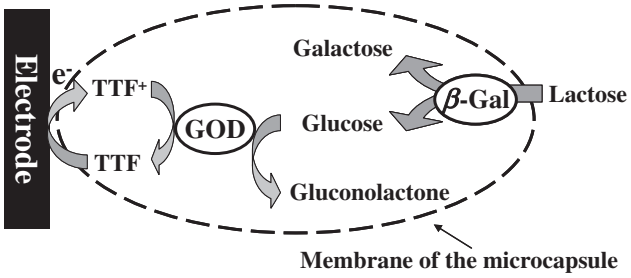

Figure 2. Reaction scheme of the microcapsule-immobilized electrode.

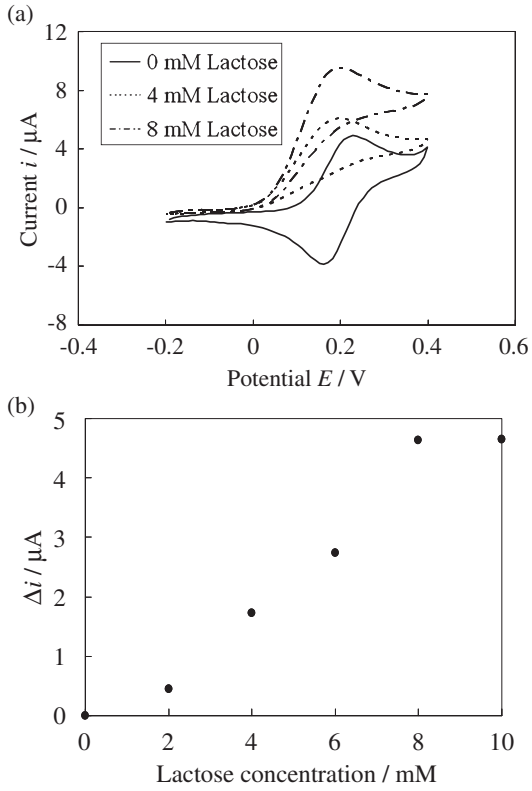

Figure 3. Cyclic voltammograms of the BMIE (a) and calibration curve for lactose (b).

the hydrolysis of the disaccharide such as lactose. Lactose diffuses to the inside of microcapsule and is decomposed to D-galactose and D-glucose. The D-glucose is oxidized to gluconolactone in the presence of GOD. GOD changes to the reduced form. The reduced GOD reacts with the mediator, TTF^+. TTF^+ is reduced to TTF. TTF is diffused and oxidized on the carbon electrode surface. As a result, the current increases when the lactose presents in a solution.

To evaluate the activities of the encapsulated β-Gal and GOD, cyclic voltammetry was performed. Figure 3a shows the cyclic voltammograms of the BMIE. In the absence of the lactose, the BMIE in cyclic voltammetry yielded a pair of oxidation and reduction waves of TTF. In the presence of lactose, the oxidation current started to flow at about 0 V and drastically increased at higher potential than 0.05 V in the anodic scan. These results indicate that the BMIE is active to detect the dissolved lactose which is catalyzed by the entrapped β-Gal and GOD. In addition,

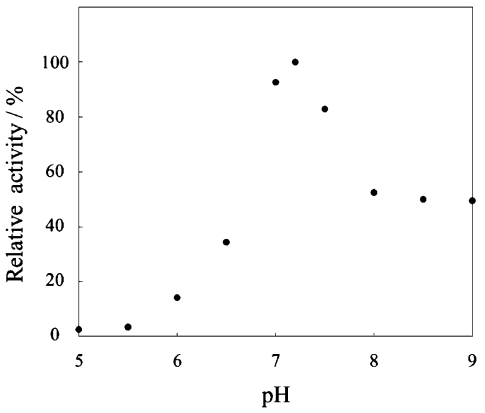

Figure 4. Effect of pH on the response of bi-enzyme containing microcapsule-immobilized electrode (BMIE). The maximum response was set as 100%.

the TTF molecules were found to penetrate the polyamide membrane and to function as a mediator which promotes the electron transfer between the active site of GOD and carbon electrode surface. The relation between the values of peak current of the cyclic voltammograms and lactose concentration were plotted for preparation of a calibration curve. Figure 3b shows the calibration curve of the BMIE for lactose. The oxidation current increased with the increase of the lactose concentration up to 8 mM lactose. We investigated the repeatability of the BMIE. At least two repeated uses of the sensor, the reproducibility was obtained within ±5% error.

The pH of the buffer in the microcapsule influences the sensor response greatly. The effect of pH on the response of the BMIE was studied from pH 5.0 to 9.0 using phosphate buffer. Figure 4 shows the pH dependence of the BMIE evaluated by cyclic voltammogram. The optimal pH for the BMIE was found to be about 7.1. This result is in good agreement with that of the non-microcapsule lactose biosensor reported previously.[9]

In summary, the BMIE is found to respond to lactose. Lactose is the characteristic carbohydrate of dairy products such as milk. Lactose concentration is a basic marker for the evaluation of milk quality.[10] In addition, lactose is also a basic parameter in wastewater control. Therefore, the quantification of lactose is an important topic in many areas. The BMIE has potential for the application to lactose biosensors. In future work, a full screen-printed lactose biosensor will be fabricated by using the present bi-enzyme containing microcapsule and its stability investigated.

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