ELECTRIC FIELD CONTROL OF ENZYME MEMBRANE ACTIVITY

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Summary

Lipase was immobilized in liquid crystal-collagen membrane. No difference in the optimum pH between the lipase-liquid crystal-collagen membrane and native lipase was observed. However, the immobilized lipase shows flat-pH profiles. The activity of the lipase membrane on a platinum electrode used for cathode increased with increase in terminal voltage and then returned to the initial activity with decrease of terminal voltage.

Considerable worldwide interest has arisen in the use of immobilized enzymes as catalysts in industrial processing and analytical chemistry.[1)]

Activity control of immobilized enzymes also has a potential application in a switch system or a controller for a bioreactor. Photocontrol of enzymes in collagen membrane was reported previously.[2)] It is well known that the molecules of a liquid crystal arrange themselves regularly under an electric field.[3)] Therefore, the diffusion of a material through a liquid crystal membrane can be controlled by an applied electric field. In practice, it is difficult to obtain an enzyme-liquid crystal membrane. Methods for preparation of collagen membrane[4)] and enzyme collagen membrane[5),6)] have been developed by the authors. Enzyme and liquid crystal were entrapped in collagen membrane. In this paper, properties of lipase-liquid crystal-collagen membrane and the electric field control of the lipase-membrane activity are described.

METHODS AND MATERIALS

Lipase from Mucor sp. (8,000 units/g) was obtained from Amano Pharmaceutical Co. 4-cyano-4'-heptylbiphenyl (CHBP) and 4'-Metoxybenzilidene

-4-n-butylaniline (MBBA) were purchased from Fuji Color Co.

The collagen fibril suspension was prepared as described previously.[4] The lipase-MBBA(CHBP)-collagen membranes were prepared as follows: 30 g of collagen fibril suspension (0.8%, pH 4.0) and 120 mg of lipase were mixed sufficiently and then 3 g of MBBA (CHBP) ethanol solution (4%) was added to the mixture. The lipase, liquid crystal and collagen fibril mixture was cast on a Teflon sheet and dried at room temperature. The thickness of the lipase-liquid crystal-membrane was approximately 50 μ.

The lipase-MBBA(CHBP)-collagen membrane-platinum electrode (1 cm x 3 cm) obtained was used as a working electrode. A cell of glass (ϕ 2 cm x 9 cm) with a 28 ml capacity was used for the experiments. A platinum electrode was also used as a counter electrode. The electrode distance was about 1 cm. A potentiostat (Hokuto Denko Co. Ltd. PS-500B) was used to apply a constant voltage across the two electrodes for 20 min. at 37°C.

The lipase activities of various lipase membranes were measured by the method of Fiore and Nord.[7] The activity of lipase or the lipase membrane was measured after 20 min. incubation in a reaction mixture containing 10 ml of olive oil emulsion (75 g of 2% polyvinyl alcohol and 22 g of olive oil) and 10 ml of 33 mM phosphate buffer (pH 7.0).

RESULTS AND DISCUSSION

No leakage of lipase from the liquid crystal-collagen membrane was observed. Figure 1 shows the pH-activity profiles of native lipase and the lipase membranes. The optimum pH of native lipase was 7.0. On the other hand, the lipase-membranes show flat pH profiles. The reason for the flat pH profiles from 4 to 8 may be due to stabilization of the enzyme by the collagen fibril matrix. The relative activity of the lipase membranes was 10%. This low relative activity may be due to a severe diffusion of olive oil emulsion through the membranes. However, the activity of the lipase-liquid crystal-collagen membrane was twice as high as that of the lipase-collagen membrane. As the lipase-liquid crystal-collagen membrane is hydrophobic, olive oil

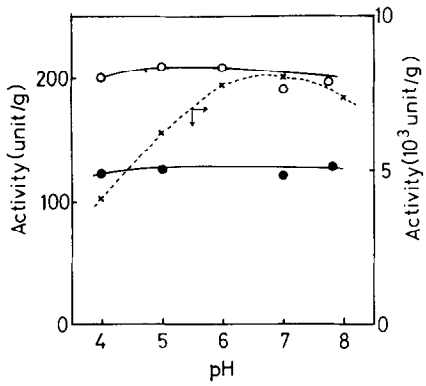

Figure 1. pH-activity profiles of native lipase and the lipase membranes. The activity was measured at 37°C in a 20 ml reaction mixture with 0.5 mg lipase or 7 mg of the lipase-collagen membrane (1:4) or 12 mg of the lipase-MBBA-collagen membrane (1:2:2).
×---× native lipase, ●——● lipase-collagen membrane, o——o lipase-MBBA-collagen membrane.

emulsion may penetrate easily into the membrane.

Figure 2 shows the temperature profiles of native lipase and the lipase membranes. The optimum temperature of native lipase was 37°C. The activity of the lipase membranes could not be determined above 45°C because the collagen membranes shrank at 50°C. No difference in temperature-activity behavious below 40°C were observed between lipase-liquid crystal-collagen membrane and lipase-collagen membrane. However, the activity of lipase-liquid crystal-collagen membrane increased suddenly above 40°C. MBBA used in this experiment is a liquid crystal between 28°C and 42°C. It, however, becomes an isotropic liquid above 42°C. Therefore, the diffusion rate of the substrate may increase with increase in temperature through loosening of the liquid crystal-collagen membrane.

No difference in properties between the lipase-MBBA-collagen membrane and the lipase-CHBP-collagen membrane was observed. Figure 3 shows the effect of voltage on the activity of the lipase membranes. As shown, the activity of the lipase membranes increased with increase in voltage when the lipase membrane-platinum was used for the cathode. On the other hand, no activity

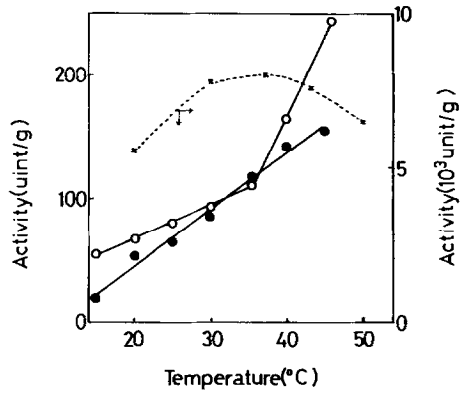

Figure 2. Temperature-profiles of native lipase and the lipase membranes. The activity was measured in a 20 ml reaction mixture with 0.5 mg of lipase or 10 mg of the lipase-collagen membrane (1:4) or 19 mg of the lipase-MBBA-collagen membrane (1:2:2).
×----× native lipase, ●——● lipase-collagen membrane (1:4), o——o lipase-MBBA-collagen membrane (1:2:2).

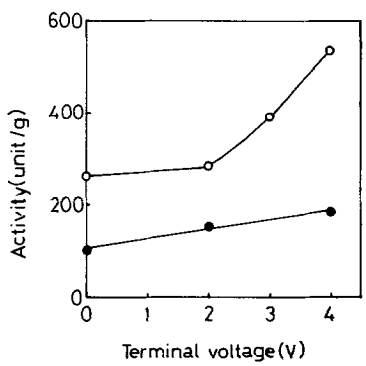

Figure 3. Effect of voltage on the activity of the lipase membranes. The lipase-collagen membrane (1:4, 9 mg) or the lipase-MBBA-collagen membrane (1:2:2, 15 mg)-platinum was used for a cathode. A constant voltage was supplied during activity measurement at 37°C.
●——● lipase-collagen membrane (1:4), o——o lipase-MBBA-collagen membrane (1:2:2).

increase was observed for the lipase membrane when the lipase membrane-platinum was used for the anode. As reported previously, alkali was produced on the cathode during electrolysis.[8] This phenomenon was proved by the determination of pH on the cathode (pH 8-9). As the discharge of free amino groups of collagen fibrils occurs during electrolysis, the collagen membrane

Table 1 Activity Control of the Lipase-MBBA-Collagen Membrane

Exp.No.	Membrane (mg/cm^2)	Activity(unit/g) 3.0 V ⟶ 0.0 V ⟶ 3.0 V		
1	4.57	270	150	230
2	4.40	180	120	160
3	5.00	210	140	240

becomes anionic. Therefore, the swelling of the collagen membrane may occur under electric field so as to promote the diffusion rate of the substrate. Paticulary, in the case of lipase-liquid crystal-collagen membrane, the effect of changes in voltage on lipase activity was remarkable. The reason for the high activity of lipase-liquid crystal membrane may be due to the lubrication effect of the liquid crystal on collagen membrane swollen under electric field.

Table 1 shows the activity control of the lipase-liquid crystal-collagen membrane. Activities of the lipase-liquid crystal-collagen membrane were determined at terminal voltage of 3.0, 0 and 3.0 V. The activity of the lipase membrane at 3.0 V decreased to 55-66 % of initial activity under no voltage and then increased again when voltage was set at 3.0 V. Lipase activity was controlled by the electric field.

Futher activity control experiments could not be performed because the lipase-liquid crystal-collagen membrane was peeled from the surface of the cathode.

The same electric field control of the activity was observed in the case of α-chymotrypsin and urease-liquid crystal-collagen membrane.

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