ELECTROCHEMICAL PREPARATION OF UREASE-COLLAGEN MEMBRANE.

I. Karube and S. Suzuki

Research Laboratory of Resources Utilization,
Tokyo Institute of Technology

Meguro-Ku, Tokyo, Japan

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Summary

The electrochemical preparation of urease-collagen membrane has been developed. On the surface of the cathode, an urease-collagen membrane was formed by electrolysis. The urease content of the membrane was almost equal to that of the electrolyte. The relative activity of the urease-collagen membrane was found to be 51 % of that of the native urease. The shape of the pH-activity curve of the membrane was similar to bell-shaped curve obtained for the native urease.

Many methods are now available for crosslinking enzymes to insoluble membrane, such cellulose[1)2) 3)4)], and nylon[5)].

In the case of these methods, some of the enzyme was denatured by cross-linking agent[6)]. The present work describes a new method for entrapping of enzymes to insoluble membrane by electrolysis and some properties of the entrapped enzyme were compared with a soluble one.

EXPERIMENTAL

For a raw material of the membrane, holstein bull calf collagen was used. Collagen fibril suspension was prepared as described perviously[7)]. Urease (from Jack bean 4,000 unit/g) was obtained from Sigma Chemical Co.. As salts prevented electrochemical forming of the membrane[8)], urease solution (50 mg/10 ml) was dialized against a large volume of disstilled water for 24 hrs

to removing salts in the enzyme sample. All subsequent operations were performed below 5°C.

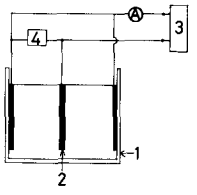

Figure 1. Apparatus for electrochemical preparation of urease-collagen membrane. 1. cell (3cm X 3cm X 11cm). 2. platinum electrode (4cm X 2cm). 3. direct current source. 4. recorder.

Experiments were performed using the apparatus as shown in Figure 1. A cell of acrylic plastic 3 cm long, 3 cm wide and 11 cm deep, fitted with twin platinum anodes to the sides and with a parallel platinum cathode placed centrally to produce an approximately uniform field, was used for experiments. The surface area of all electrodes was 4 X 2 cm^2.

All experiments were carried out at constant current, current density of 2 mA cm^{-2}, for 2 mins without any circulation. 50 ml of 0.35 % collagen fibril suspension contained urease (Collagen : urease = 10 : 1) was employed in each experiments. The electrolyte was cooled in ice to avoid the denaturation of the urease. The wet membrane formed on the cathode was removed from the electrode and washed in a large volume of cooled water. After washing, the membrane immersed in cold acetone(-10°C) and dried up in a vacuum drying apparatus.

The urease activity of the membrane was measured by the method of Van Slyke et al[9]. The amount of the urease entrapped was calculated from cystein content of the collagen membrane.

RESULTS AND DISCUSSION

It became evident from experimental results that the urease

was stable in pH range from 4 to 8 in a short time below 5°C. Therefore, the preparation of the membrane entrapped urease was performed at pH 4.2.

On the surface of the cathode, a milky-white membrane was formed by electrolysis. The dry weight of the membrane was 32 miligram per mAhr after drying at 105°C for 24 hrs. Moreover, the thickness or the weight of the membrane increased linearly with the increase in the current passed. When the current passed was increased, almost all urease in electrolyte were entrapped in the membrane. Furthermore, the urease content of the membrane was almost equal to that of the electrolyte. The relative activity of the urease-collagen membrane was found to be 51 % of that of the native urease. From this results, the urease activity of the membrane was higher than that of the membrane cross-linked enzyme.[1)4)]

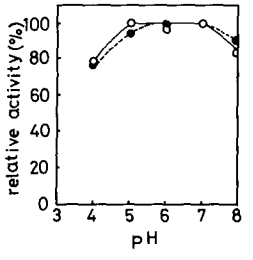

Figure 2. Activities of urease at different pH values between 4 and 8. Activites was measured at 20°C in a 5 ml reaction mixture (pH 7.0, 0.2 M phosphate buffer contained 3 % urea) with 0.5 mg urease or a piece of membrane with equivalent enzymatic activity at pH 7.0. Ordinate : percentage of activity at given pH to activity at optimum pH. Abscissa : pH values. o——o Urease-collagen membrane. ●----● Urease in solution.

Figure 2 shows the activity of urease in solution and in membrane for various pH values after 1 hr incubation at 30°C. The shape of the pH-activity curve of the membrane was similar to bell-shaped curve obtained for the native urease. No signigicant shift of the optimum pH was observed. This resembles the result

that Wittam et al[10] have obtained working with apyrase bound on cellulose matrix. The elution of the urease from the membrane was not detected after 4 hrs incubation at 30°C. This indicate that the urease in the membrane may be entrapped among collagen fibrils network. The activity of the dry membrane was determined after 10 days storage at 20°C. No decrease in the activity of the entrapped urease was noted.

In summarizing, we tend to confirm from these experimental results described above that characteristics of the entrapped urease was unchanged by the electrochemical treatment. So this electrochemical method is useful for preparation of the membrane entrapped enzyme. Moreover, by using this method, many articles entrapped enzyme, such as sack, film, tube and string, are obtained.[11]

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