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ELECTROCHEMICAL PREPARATION OF URICASE-COLLAGEN MEMBRANE

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The electrochemical entrappment of uricase in the collagen fibril network has been developed. The activities of the uricase-collagen membrane prepared at pH 3.8 and at pH 10.4 were 43% and 20% respectively as was compared with that of the native enzyme.

The temperature dependence and the pH dependence of the activity of the membrane were similar to that of the native uricase, which indicated that the properties of uricase in the membrane remained unchanged in the electrochemical entrappment.

The electrochemical preparation of enzyme-collagen membrane has been proposed as one of the effective techniques to immobilize enzymes without loss of activity. Urease[1], amylase[2] and catalase[3] were immobilized in success by this method. Furthermore, the catalase-collagen membrane was applied for an bioelectrochemical sensor specific for hydrogen peroxide[3].

Uricase (E.C.1.7.3.3), which catalyzed the oxidation of uric acid, has been used in monitoring uric acid contained in blood and urine. Immobilization of uricase is expected to offer considerable advantages of stability and continuous use of the enzyme. The electrochemical preparation of uricase-collagen membrane and the properties of the membrane are described in this paper.

The collagen fibril solution was prepared as was described previously[1]. Yeast uricase (from Candita utilis 2.4 I.U./mg) was supplied from Oriental Yeast Co., Ltd. Uricase solution (0.1%) was dialyzed against a dilute NaOH solution (pH 9.2) for two days to exclude the salts which prevented electrochemical forming of the membrane. The enzyme activity decreased to 1.2 I.U./mg during the dialysis. The electrolyte for electrochemical forming of the membrane was prepared by mixing the dialyzed uricase solution with the collagen fibril solution. The apparatus for electrochemical preparation of the membrane is presented previously[1]. The preparation was carried out for both an acidic (pH 3.8) and a basic electrolyte (pH 10.4) at constant current, current density of 4 mA cm^{-2}, for 2 min without any circulation. The membranes formed on the cathode (for an acidic electrolyte) and on the anode (for a basic electrolyte) were washed with water and dried up in a vacuum drying apparatus.

The uricase activity of the membrane was measured by the method of Yamamoto and Nakagiri[4].

The activity of the uricase-collagen membrane prepared at pH 3.8 was 43% as was compared with that of the native uricase. Such a high activity of the uricase-collagen membrane indicates that electrochemical entrappment is effective for uricase, because uricase is very unstable in such an acidic solution. On the other hand, the activity of the uricase-collagen membrane prepared at pH 10.4 was 20%, though uricase is rather stabilized in an alkali solution. The decrease in the activity of the membrane prepared at pH 10.4 may be caused by the de-alkalination around the electrode. In the electrochemical preparation, the electro-osmosis is accompanied and the solution which is removed through the membrane to the anode becomes acidic. This acidic solution may decrease the activity of the membrane. As for the electrochemical preparation at pH 3.8, de-acidification around the cathode is thought to stabilize uricase.

The temperature dependence and the pH dependence of the activity are presented in Figs. 1 and 2 for the uricase-collagen membrane prepared at pH 3.8. These dependence are coincident with those of the native uricase. On the basis of these results, it can be said that the properties of the native enzyme remains unchanged in the electrochemical entrappment.

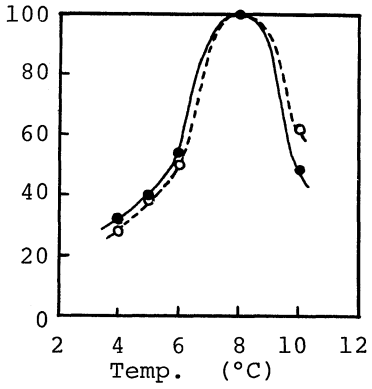

Fig.1 The temperature-activity curve of uricase
●—● uricase-collagen membrane
o--o native uricase

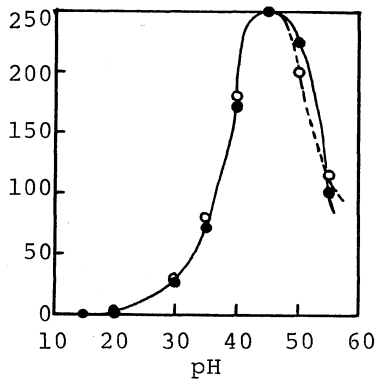

Fig.2 The pH-activity curve of uricase
●—● uricase-collagen membrane
o--o native uricase

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