Immobilization of Catalase by Polyion Complex

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SYNOPSIS

Catalase was immobilized in a polyion complex from poly(sodium 2-acrylamide-2-methyl-1-propanesulfonate) and poly[2-(trimethylammonioethyl) methacrylate chloride]. Polyion complex formation between the two polyions was found to proceed stoichiometrically by the turbidimetric titration. Immobilization of catalase was achieved by conducting the complexation in the presence of the enzyme, which was established to be incorporated in the polyion complex networks. The fixed catalase showed higher thermal stability than the original enzyme. Furthermore, the stability and the activity of the fixed catalase were improved by annexing inert components such as albumin, histone, or lecithin at the time of polyion complex formation.

INTRODUCTION

Immobilization of the enzyme is of much use due to its wide applications to medical, chemical, analytical, and industrial uses.[1-4] This paper reports an immobilization of the enzyme by the polyion complex and the effect of some inert components on the properties of the fixed enzyme. The polyion complex is prepared by stoichiometrical mixing of polyanion and polycation, and the polyion complex usually forms an insoluble polymer network.[5,6] An enzyme is expected to be included in the networks, if the complexation is carried out in the presence of the enzyme.

In the present work, poly(sodium 2-acrylamide-2-methylpropane sulfate), P(NaAMPS), was used as a polyanion, and poly[2-(trimethylammonioethyl)methacrylate chloride], P(QDM), as a polycation. Catalase was chosen as an enzyme because catalase has a high activity for the decomposition of hydrogen peroxide,[7,8] and the measurement of activity can easily be conducted by iodometry. The complexation of catalase by P(NaAMPS)/P(QDM) and the effect of addition of some inert components on the immobilized catalase are investigated.

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EXPERIMENTAL

Materials

Sodium 2-acrylamide-2-methylpropane sulfonate (NaAMPS) and quaternized dimethylaminoethyl methacrylate (QDM) were kindly donated by Nitto Chemical Co., Ltd., and were dried under vacuum. Catalase (from bovine liver) and histone (type II from calf thymus) were purchased from Sigma Co., and albumin (from bovine serum) and lecithin (from beans) were purchased from Wako Chemical Co.

Polymerization of NaAMPS and QDM

P(NaAMPS) and P(QDM) were synthesized by radical polymerization using AIBN as an initiator. NaAMPS or QDM (1.0 mol/L), AIBN (5.0×10^{-3} mol/L), and methanol were sealed in a glass ampoule under vacuum, and allowed to polymerize at 60°C for 4 h. The polymerization was quenched by *p*-benzoquinone (2.5×10^{-5}). The product was precipitated by THF, and purified by the reprecipitation from methanol solution by THF.

Turbidimetric Titration

The methanol solutions of 0.03 wt % P(NaAMPS) and P(QDM) were mixed at 25°C. After 1 min of

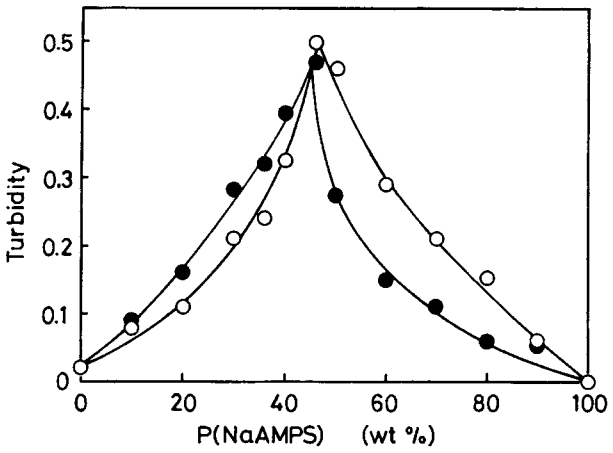

Figure 1 Turbidity of P(NaAMPS) and P(QDM) mixture at 380 nm, 25°C: (○) Methanol solution of P(NaAMPS) was added to the methanol solution of P(QDM); (●) reverse of (○).

stirring, the absorbance at 380 nm was measured to determine the turbidity (τ):

$$\tau = \ln(I_0/I)$$

Immobilization of Catalase

The 1.0 wt % P(QDM) solution containing catalase in the presence or absence of protein or lecithin was mixed with 1.0 wt % P(NaAMPS) solution, and the resultant precipitate was collected by centrifugation (3000 rpm, 30 min). The immobilized catalase was dried under vacuum. All solutions were prepared using phosphate buffer (pH 7.0, 1.0 mol/L) as solvent.

Measurement of Catalase Activity

Activity of catalase was expressed by the amount of decomposed hydrogen peroxide in a minute per 1 g immobilized catalase.[8,9] The immobilized catalase (about 0.1 g) and the phosphate buffer were put into a test tube, and the tube was incubated at 25°C for 30 min. The phosphate buffer was removed by decantation, and 3.0 mL of 0.025 mol/L hydrogen peroxide solution was put into the test tube and allowed to react at 25°C. After 10 min, 1.0 mL of 1.0 mol/L sulfuric acid solution was put into the test tube to terminate the reaction, and the residual hydrogen peroxide was determined by iodometry.[10] Ten percent KI was added to the test tube (excess of KI for the residual hydrogen peroxide) and a drop of 1% ammonium molybdate into the test tube and stored under dark for 5 min. Free iodine was titrated with 0.01 mol/L sodium thiosulfate.

In case of the measurement of the thermal stability, the immobilized catalase and phosphate buffer were heat-treated at a prescribed temperature for 5 min. In the pH sensitivity study of the catalase activity, pH of the hydrogen peroxide solution was changed by dilute HCl or dilute NaOH aqueous solutions.

RESULTS AND DISCUSSION

Polyion Complex Formation

The turbidimetric titration curves for P(NaAMPS) and P(QDM) are shown in Figure 1. The peak position was not changed by the mixing order of both reagents, i.e., the titration curve for the addition of P(NaAMPS) to P(QDM) and that for the addition of P(QDM) to P(NaAMPS) had the peaks at the same weight fraction of P(NaAMPS). At the peak position, polyanion and polycation were estimated to react according to the stoichiometry, i.e., the equivalent amounts of anions and cations were reacted. Thus, for the immobilization of catalase, stoichiometrical amounts of the polyanion and the polycation were employed in the present experiments.

Immobilization of Catalase

Results of the immobilization of catalase are shown in Table I. The conversion, which means the yield of the insoluble fraction from the reaction mixture of polycation, polyanion, and catalase, is independent of the catalase concentration in feed. It seems that the catalase did not interfere with the polyion complex formation, and thus the catalase is esti-

Table I Immobilization of Catalase by Polyion Complex[a]

Sample No.	Catalase in Feed 10^{-12} mol	Conversion (%)	Immobilization Ratio (%)	Relative Activity (%)
C1	8	65.3	11.5	53.0
C2	16	70.3	14.3	38.4
C3	24	67.9	20.8	30.5
C4	48	71.5	31.1	20.4
C5	75	70.8	54.4	11.8
C6	120	71.0	60.3	5.5

[a] Catalase solution (1.0 mL) was mixed with 2.0 wt % P (QDM) aq. solution (10 mL) and 2.0 wt % P(NaAMPS) aq. solution (10 mL) was added at 25°C.

Table II Immobilization of Catalase by Polyion Complex Prepared in the Presence of Protein or Lecithin

Sample No.	Additive (μg/mL)	Conversion (%)	Immobilization Ratio (%)	Activity (10^{-5} mol g^{-1} min^{-1})
CC1	None —	70.8	10.7	1.80
CC2	Albumin 19.2	63.6	0	2.12
CC3	Histone 16.8	51.8	4.3	3.21
CC4	Lecithin 22.7	53.6	8.6	3.32

[a] Catalase solution (1.0 mL, catalase = 34.4 pmol) was mixed with 1.0 wt % P (QDM) aq. solution (3.0 mL), 1.0 wt % additive solution (1.0 mL), and then 1.0 wt % P(NaAMPS) aq. solution (3.0 mL) at 25°C.

mated to be physically included in the polyion complex networks. The immobilization ratio in Table I means a ratio of the immobilized catalase to that in the feed. The ratio decreases with the increase of the catalase concentration in feed, but the relative activity became higher for the higher catalase concentration. In that case, the relative activity means a ratio of the observed activity of the immobilized catalase to the calculated activity, which was evaluated from immobilization ratio. The product of the immobilization ratio and the relative activity is constant for all catalase concentration studied here, i.e., the activity of the immobilized catalase appears to be proportional to the catalase concentration.

Effect of Additives

The effect of additives (albumin,[8] histone, or lecithin) to the activity of the immobilized catalase is shown in Table II. The conversion depended on a kind of additive, i.e., there might be an interaction between the additive and the polyion complex. Although the immobilization ratio was lower when the additive was used, the activity of the immobilized catalase became higher than in the absence. Additives did not decompose hydrogen peroxide and did not affect the activity of the catalase solution. Additives may change the structure or geometry of immobilized catalase in the polyion complex networks, and the change may have enhanced the activity of the catalase.

The thermal stability of the immobilized catalase is shown in Figure 2. The catalase solution lost its activity by heating at 50°C, but the immobilized catalase maintained its activity even at 50°C. The immobilized catalase coexisting with albumin or histone remained active even at 70°C. The additives were found to improve the thermal stability of the immobilized catalase.

The pH sensitivity of the immobilized catalase is shown in Figure 3. Optimum pH for the immobilized catalase is 7.0. This optimum pH value is the same

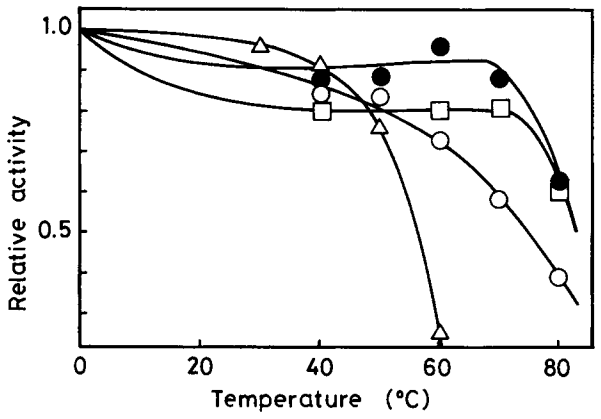

Figure 2 The effect of temperature on the activity of immobilized catalases: (○) immobilized catalase; (●) immobilized catalase containing histone (type II); (□) immobilized catalase containing albumin; (△) catalase solution.

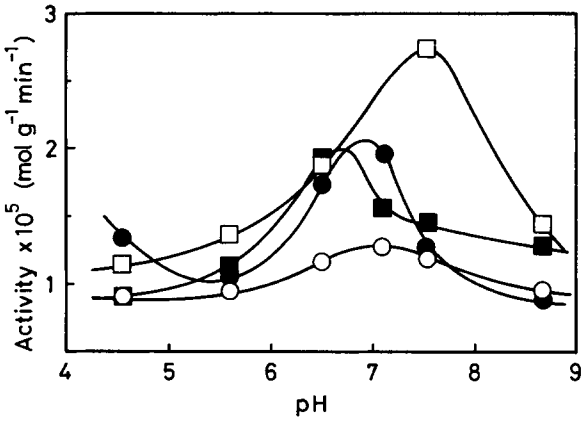

Figure 3 pH sensitivity of immobilized catalases: (○) immobilized catalase; (●) immobilized catalase containing histone (type II); (□) immobilized catalase containing albumin; (■) immobilized catalase containing lecithin.

as that for the catalase solution. The immobilized catalase showed the higher activity when it was with the additive at the neutral area. The optimum pH value of the fixed catalase with an additive shifted to basic side in the case of albumin, to acidic in the case of histone and lecithin. Because albumin is an acid protein, the optimum pH shifted to the basic side in order to neutralize the inner part of polyion complex.[11] The reverse shift of pH sensitivity by histone is interpreted by same explanation as albumin, since histone is a basic protein.

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