Immobilization of Cellulase Using Porous Polymer Matrix

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Synopsis

A new method is discussed for the immobilization of cellulase using porous polymer matrices, which were obtained by radiation polymerization of hydrophilic monomers. In this method, the immobilized enzyme matrix was prepared by enzyme absorbtion in the porous polymer matrix and drying treatment. The enzyme activity of the immobilized enzyme matrix varied with monomer concentration, cooling rate of the monomer solution, and hydrophilicity of the polymer matrix, taking the change of the nature of the porous structure in the polymer matrix. The leakage of the enzymes from the polymer matrix was not observed in the repeated batch enzyme reactions.

INTRODUCTION

Excellent reviews show how extensively the immobilization of enzymes has been studied, and various materials such as organic and inorganic compounds have been used as immobilization carrier.[1-3] The immobilization of enzymes and microbial cells has become an established practice largely due to the ease of separation of the biocatalyst from solution and the subsequent economic advantages of reuse. This has spurred the study of novel enzyme carriers, among them polymeric materials which have been studied due to some rather attractive features, including the variety of materials available. Among polymeric materials, porous soft gels of carrageenan and polyacrylamide etc. have been used for the immobilization of cells rather than enzymes.[4,5] In the immobilization of enzymes using such porous soft gels, enzymes have often been leaked from the gel matrix. We have studied the immobilization of biological substances such as enzymes and cells by radiation polymerization, in which the substances were immobilized under irradiation in the presence of monomers.[6-8]

In this work, we have studied a new immobilization method of cellulase using porous polymer matrix obtained by radiation polymerization of hydrophilic monomers.

MATERIALS AND METHODS

Materials. Cellulase (*Trichoderma viride*, 1×10^4 units/g) was obtained from Yakult Biochemicals Co., Ltd., Japan. 2-Hydroxyethyl acrylate (HEA), 2-hydroxyethyl methacrylate (HEMA), 2-hydroxypropyl methacrylate (HPMA), and methoxytetraethylene–glycol methacrylate (M-4G) were obtained from Shin Nakamura Chemicals Co., Ltd., Japan.

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Preparation of Porous Polymer Matrix. A monomer solution (10–90%) was prepared with monomer and water put into a test tube, and frozen at −78°C. The tube was irradiated with an irradiation dose of 1.0 Mrad by γ-ray from a ^{60}Co source, keeping the temperature of −78°C. After irradiation, the polymer matrix obtained by the polymerization was taken out from the tube at room temperature, and cut to a thin pellet form (0.5–1.0 mm in thickness).

Immobilization of Enzymes. The pellets were put into the vessel containing 1.0% enzyme solution (0.1*M* acetate buffer solution, pH 4.5) and immersed for 24 h at 25°C. After immersion, the pellets were dried in vacuum at 25°C. The amount of the enzymes entrapped in the pellets was determined by measuring the residual enzyme concentration in the enzyme solution with a spectrophotometer at 280 nm, and was 1–10 mg/g.

Enzyme Activity of Immobilized Enzymes. The enzyme reaction with the immobilized enzymes was carried out by the repetition of batch enzyme reaction using 1.0% sodium carboxymethylcellulose solution at 40°C. The enzyme activity (%) remaining in the repeated batch enzyme reaction was obtained from the glucose formation ratio of immobilized enzymes to native enzymes at each batch enzyme reaction (1.0 h). The glucose was measured with a glucose specific reagent, "GOD-PODLK," obtained from Nagase Sangyo Co., Ltd., Tokyo, Japan.[9]

Measurement of Hydrophilicity. The hydrophilicity of polymer matrix was evaluated by measuring the degree of hydration of the polymer. The polymer sample was immersed into distilled water at room temperature for 1 week. Degree of hydration was determined as the ratio of weight of water to the weight of the polymer at swelling equilibrium.

RESULTS AND DISCUSSION

Effect of Drying Treatment of Polymer Matrix on Enzyme Activity

The effect of drying treatment of the polymer matrix absorbing the enzymes on the enzyme activity was studied, in which the polymer matrix was obtained by radiation polymerization of hydrophilic HEA monomer. The relationship between enzyme activity and repeated batch enzyme reaction is shown in Figure 1. The enzyme activity of the immobilized enzyme matrix (the polymer matrix absorbing the enzymes) before drying treatment decreased with the repetition of batch enzyme reaction, but that after dry treatment decreased slightly at the initial stage and became constant. This result shows that the enzymes absorbed in the pores of the polymer matrix become immobilized after dry treatment. The decrease of the enzyme activity of the immobilized enzyme matrix before dry treatment is due to the leakage of the enzymes from the polymer matrix. It was found that this enzyme leakage was stopped by drying treatment of the polymer matrix. The polymer matrix obtained by radiation polymerization of hydrophilic HEA monomer had a porous structure. The formation of the porous structure in the present method results in the melt of ice formed in the polymer matrix by radiation polymerization of the monomer solution at low temperatures.[8] The pores in the porous polymer matrix were used for the trap-

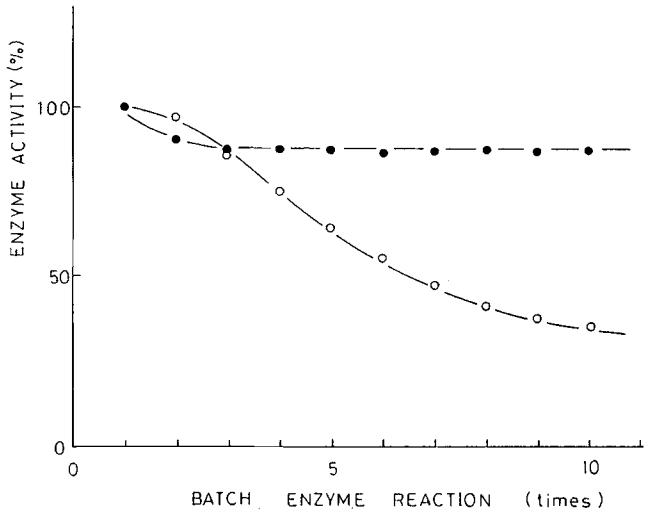

Fig. 1. Variation of enzyme activity with repeated batch enzyme reaction. The polymer matrix was obtained from 30% HEA monomer concentration: (●) after drying treatment; (○) before drying treatment.

ping site of the enzymes as shown in Figure 2. The structure of the pore in the porous polymer matrix was a continuous cylindrical form and the size of the pore was larger than that of the molecular size of the enzyme, so that the enzymes can easily invade the pores. The polymer matrix from hydrophilic HEA monomer was a soft spongelike gel in water, and it changed to a rigid polymer matrix by dry treatment, in which the porous structure disappeared due to shrinking of the polymer matrix. In the present method, the enzymes in the polymer matrix became firmly trapped by shrinking. The state of the trapped enzymes affect the enzyme activity of the immobilized enzyme matrix. Certainly, a slight decrease of the enzyme activity in the dry treated polymer matrix at the initial stage in Figure 1 seems to be leakage of the enzymes, which are loosely trapped near the entrance of the pore and/or temporarily absorbed on the surface of the polymer matrix. However, the decrease of the enzyme activity at the later state of the repeated batch enzyme reaction is not observed as shown in Figure 1, and this indicated that the enzymes are firmly trapped in the pore site of the polymer matrix.

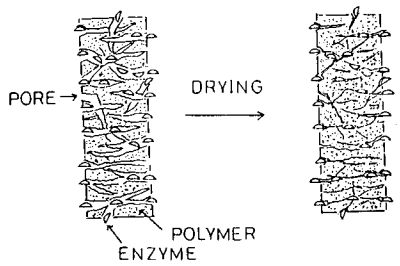

Fig. 2. Proposed state of the enzymes trapped in the porous polymer matrix.

Effect of Monomer Concentration on Pore Size and Enzyme Activity

The pore size in the porous polymer matrix varied with monomer concentration. The relationship between monomer concentration and pore diameter or enzyme activity is shown in Figure 3. The pore size and enzyme activity decreased with increasing monomer concentration. As monomer concentration increased, the number and size of the pore decreased according to the decrease of water concentration, and then the enzyme activity decreased by the decrease of the content of the enzymes trapped in the pores. As can be seen in Figure 3, the apparent size of the pore is very large; therefore, it is elucidated that the porous polymer matrix from low monomer concentrations is able to immobilize large amounts of the enzymes. In fact, the enzyme activity of the polymer matrix from low monomer concentrations appeared to be relatively high. Of course, all the space of the pore is not filled by the enzymes. The enzymes would be trapped in the pores with small sizes rather than large sizes, since the distribution of the pore size is considerably broad. The immobilized enzyme matrix obtained by the dry treatment of the polymer matrix absorbed the enzymes has a slight porous structure, in which the substrate and product are diffused.

The polymer matrix obtained by radiation polymerization of HEA at high temperature above 0°C had a porous structure with discontinuous pores, and it cannot absorb the enzymes even in a swelled state.

Effect of Cooling Rate on Pore Size and Enzyme Activity

The size of the pore in the porous polymer matrix also varied with cooling rate of the tube containing monomer solution in the preparation process. The relationship between cooling rate and average pore diameter or relative enzyme activity is shown in Figure 4, in which the tube was cooled at various cooling rates from room temperatures to −78°C. The size of the pore decreased with increasing cooling rate, and the enzyme activity increased

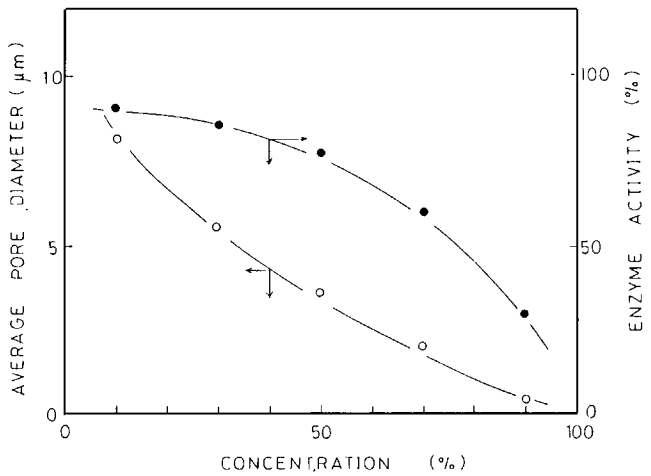

Fig. 3. Relationship between HEA monomer concentration and average pore diameter or enzyme activity: (○) average pore diameter; (●) enzyme activity.

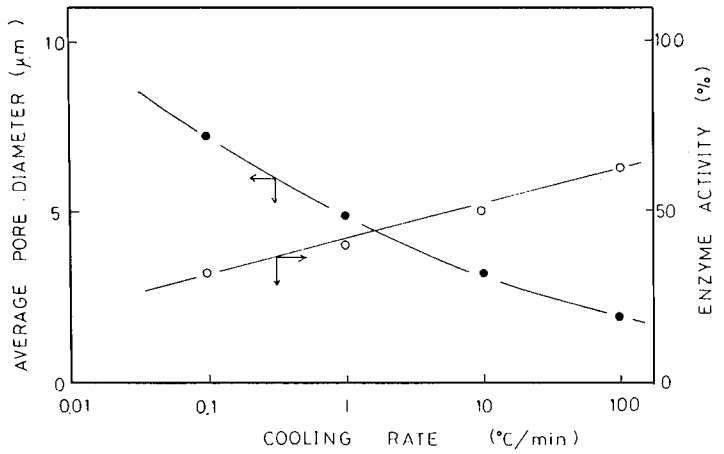

Fig. 4. Relationship between cooling rate and average pore diameter or enzyme activity. The polymer matrix was obtained from 70% HEA monomer concentration: (●) average pore diameter; (○) enzyme activity.

with increasing cooling rate. The variation of the pore size with cooling rate was due to the variation of the size of ice resulting from the regulation of the growth rate of formed ice. The increase of the enzyme activity in large cooling rates appeared to be different from the result in Figure 3. Certainly, as the cooling rate increased, the pore size decreased, but the enzyme activity increased in Figure 4. This result suggests that the increase of cooling rate forms many pores with small sizes, in which the many enzymes are trapped and the enzyme activity is increased. Thus, to obtain the immobilized enzyme matrix having a high enzyme activity, the preparation of the porous polymer matrix which contains a high number of small pores was recommended.

Effect of Hydrophilicity of Polymer Matrix on Enzyme Activity

The porous polymer matrix having various hydrophilicities was prepared by radiation polymerization of various monomers at low temperatures, and the effect of its hydrophilicity on the enzyme activity of the immobilized enzyme matrix was studied. The relationship between degree of hydration and relative enzyme activity is shown in Figure 5. The enzyme activity increased with increasing degree of hydration of the polymer matrix. Since the nature of the pore formed in the polymer matrix hardly changed with the hydrophilicity of the monomer, the increase of the enzyme activity due to increase of the hydrophilicity of the polymer matrix causes the swelling effect of the polymer matrix in water. The polymer matrix having high hydrophilicity is markedly swollen in the enzyme solution, and then the pore size in the polymer matrix of the immobilized enzyme matrix becomes large. Therefore, the enzymes can invade the pore and inner part of the polymer matrix. A high hydrophilic polymer matrix is a very soft gel-like state, and its polymer chains might be interacted with enzyme molecule to entangle both enzymes and polymer chains. This is available for firm trapping of the enzymes in the polymer matrix after dry treatment. Among the

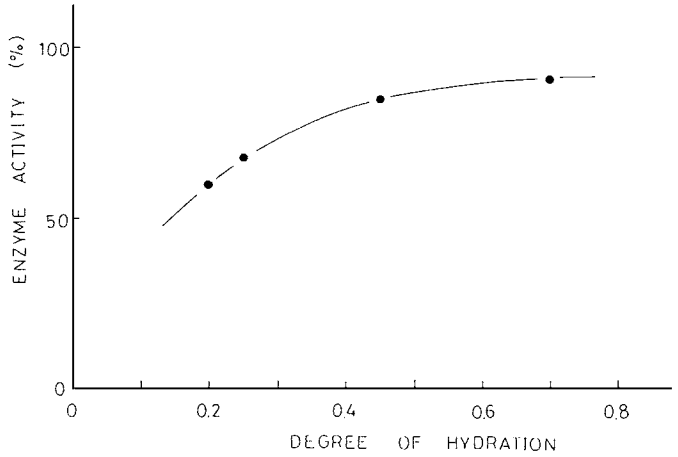

Fig. 5. Relationship between enzyme activity and degree of hydration of the polymer matrix. Monomer concentration 30%.

polymer matrices obtained in this work, the polymer matrix from M-4G monomer gave the most hydrophilic property; the degree of hydration was about 0.7. The immobilized enzyme matrix from this polymer matrix was very soft, but the break of the polymer matrix during repeated batch enzyme reactions was not observed, indicating that the part of the polymer matrix is crosslinked by radiation reaction.

The present immobilization method of enzymes using a porous polymer matrix, which is obtained by radiation polymerization of hydrophilic monomers at low temperatures, can apply to various proteins. In the present method, since immobilized biocatalysts are not irradiated, the immobilization of biocatalysts which are sensitive to irradiation is suitable.

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