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Biocatalytic materials having a porous structure

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Biocatalytic materials having a porous structure have been prepared by radiation polymerization at low temperatures using hydrophilic 2-hydroxyethyl methacrylate monomer and α-glucosidase. The biocatalytic (enzyme) activity of the materials was examined by enzyme reaction. The enzyme activity was affected by the pore size and surface area, and the optimum pore diameter giving a high enzyme activity was 20 to 40 μm. The enzyme activity was also affected by irradiation temperature and addition concentration of cross-linking monomer. The heat and pH stability of the materials were higher than those of the native enzyme.

1. Introduction

Since biological catalysts such as enzymes are soluble in water and generally employed in batch reactions they are used but once, they are destroyed in further processing or they cannot be recovered from the reaction economically. Immobilization technology offers the opportunity to re-use the biological catalysts and thus reduce the consumption of life and the need for additional resources to produce that life. Thus, the technique of immobilization of enzymes by various methods have been developed to circumvent many difficulties associated with the use of soluble enzymes [1–4]. The enormous achievement made in the past few years in the field of immobilized enzymes has been largely due to the development of suitable carriers for the enzymes. The enzymes have been usually immobilized by a chemical binding or entrapping method, in which enzymes were trapped on the surface or in the inner part of the carrier. To obtain immobilized enzymes with high activity it is necessary to prepare the carrier (material) having a large surface area.

In this work, biocatalytic polymer materials having a porous structure have been prepared by radiation polymerization at low temperatures and its effect was studied.

2. Experimental procedure

2.1. Monomer and biocatalyst

2-Hydroxyethyl methacrylate (HEMA) and diethyleneglycol dimethacrylate (2G) were used as monomers, and obtained from Shin Nakamura Chemical Industry Co, Ltd. α-Glucosidase was used as the enzyme (biocatalyst), and obtained from Tokyo Kassei Industry Co, Ltd.

2.2. Preparation of biocatalytic materials

The solution mixture containing the biocatalyst and monomer dissolved in 0.05 M phosphate buffer solution, pH 7.0, was put into a glass tube (0.8 cm in diameter and 20 cm in length) and rapidly shaken with an irradiation dose of 1×10^6 rad at an irradiation dose rate of 1×10^6 rad h^{-1} by γ-ray from a ^{60}Co source. The irradiation temperature was maintained by immersing the tube in a Dewar vessel filled with a dry ice–methanol mixture. After irradiation, the biocatalytic materials obtained by the polymerization were cut into a pellet form.

2.3. Enzyme reaction

The enzyme reactions with the biocatalytic materials were carried out by repeating a batch enzyme reaction, in which the temperature and time of the reaction were 35° C and 1.0 h. Maltose

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(2.0%) dissolved in the phosphate buffer solution was used as a substrate throughout this work. The concentration of glucose formed in each batch enzyme reaction was measured with a glucose-specific reagent, God-Podik, obtained from Nagase Sangyo Co, Ltd. The enzyme activity (%) of the immoblized enzyme materials remaining in the batch enzyme reaction was obtained from the glucose formation ratio of the immobilized and native enzymes for each batch enzyme reaction.

2.4. Measurement of pH and heat stability

pH stability of the immobilized enzyme materials was determined by measuring the remaining enzyme activity after treatment in buffer solutions of different pH at room temperature for 1.0 h. Heat stability of the immobilized enzyme materials was measured as the residual enzyme activity after heat treatments at various temperatures for 1.0 h.

3. Results and discussion

3.1. Effect of material size

Materials with various pore sizes were prepared and the enzyme activity was examined by repetition of batch enzyme reactions. The relationship between enzyme activity and number of batch enzyme reaction is shown in Fig. 1. The enzyme activities of the materials with small pores decreased slightly at first and became constant at the later stage of repeated batch enzyme reactions. The enzyme activities of the materials with large pores initially increased slightly and after that became constant. The enzyme activities of the materials with small pores at the later stage were higher than those with large pores indicating that the enzyme activity is affected by the surface area of the material. It is thought that the enzyme reactions with the enzymes entrapped in the polymer matrix of the materials with large pores are restricted by the diffusion resistance of the substrate through the polymer matrix having a porous structure. Since the practical use of immobilized enzymes is usually carried out using reactors of column or vessel type, the materials with small pores prepared by the present method are favourable. As can be seen in Fig. 1, the enzyme activities of the materials at the later stage were constant. This indicated that the enzymes in the materials are firmly trapped in the polymer matrix and so do not leak from the porous polymer matrix.

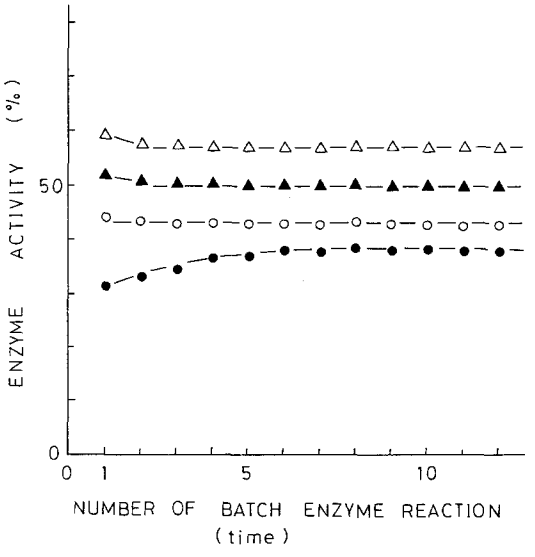

Figure 1 Effect of the size of the material. Monomer concentration: 30% HEMA: irradiation temperature: −24° C; size of material: • 1.6 cm diameter × 2.5 cm; ○ 1.6 cm diameter × 1.5 cm; ▲ 1.6 cm diameter × 0.8 cm; and △ 1.6 cm diameter × 0.5 cm.

3.2. Relationship between pore size and enzyme activity

The porous structure in the materials prepared by various monomer concentrations was examined, and the relationship between average pore diameter and enzyme activity at the later stage is shown in Fig. 2. As pore size increases, the enzyme

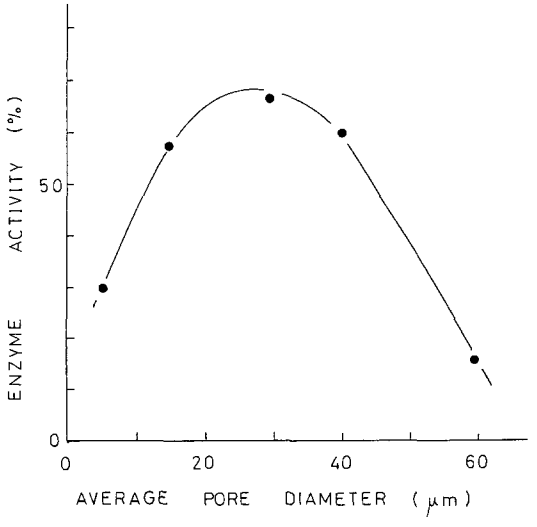

Figure 2 Relationship between pore size and enzyme activity. Size of material: 1.6 cm diameter × 0.3 cm.

activity increased at first, reached a maximum, and then decreased. Thus, the optimum pore size for enzyme activity appeared to be 20 to 40 μm in diameter, which corresponds to 30 to 50% monomer concentration. As previously reported, the materials prepared by radiation polymerization of hydrophilic monomers at low temperatures had a porous structure, in which the pore size was mainly dependent on monomer concentration. The key point of the present immobilization technique was utilization of the porous structure. This was achieved by radiation polymerization at low temperatures under the presence of monomer, water and enzyme. The enzymes in the materials would be trapped on the surface of the porous polymer matrix, because enzymes and small ice particles are separated from the super-cooled monomer phase at low temperatures (−78° C) and so enzymes are localized between ice particle and monomer phase in the preparation process. Such enzymes localized on the monomer phase are firmly immobilized by the polymerization of the monomer. The enzymes entrapped in the polymer matrix with small pore sizes seem to be affected by the diffusion resistance of the substrate on the enzyme reaction. In fact, the enzyme activity in smaller pore diameters was low as seen in Fig. 2. The low enzyme activity in large pore diameters was due to the leakage of the enzymes from the polymer matrix. The mechanical strength of the materials prepared from lower monomer concentrations, below 10%, is small and the enzymes are leaked from the large pores of the polymer matrix during repeated enzyme reactions. Thus, the porous polymer matrix with pore diameter of 20 to 40 μm appeared to be suitable materials.

3.3. Effect of irradiation temperature

The relationship between irradiation temperature and enzyme activity is shown in Fig. 3. The enzyme activity decreased markedly with rising irradiation temperature. This decrease of the enzyme activity was mainly due to the deactivation of the enzymes by irradiation, since it is known that enzymes are deactivated by irradiation at room temperature. It was found that enzymes were not deactivated by irradiation at low temperature (−78° C). Thus, the radiation polymerization at low temperatures using glass-forming monomers was suitable for the immobilization of biocatalysts.

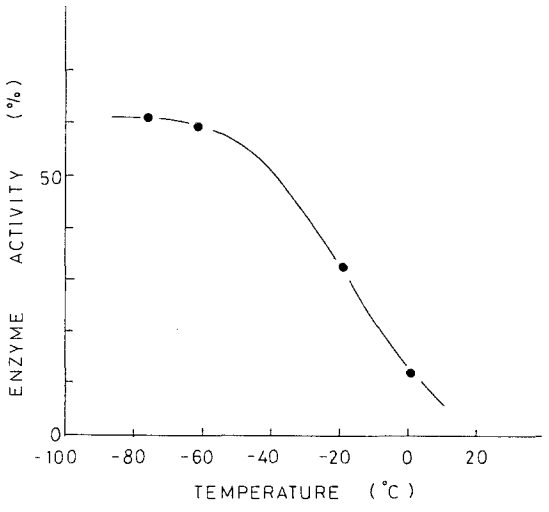

Figure 3 Effect of irradiation temperature. Monomer concentration: 30% HEMA; size of material: 1.6 cm diameter × 0.5 cm.

3.4. Effect of monomer cross-linking on the enzyme

The effect of a cross-linking monomer, such as 2G, on the enzyme activity was studied (Fig. 4). The enzyme activity decreased with increasing concentration of 2G. The polymer matrix was cross-linked by the addition of a biofunctional monomer and the enzymes become entrapped within the rigid cross-linked polymer matrix. The effect of the addition of the cross-linking monomer might appear in the immobilization at low monomer concentrations.

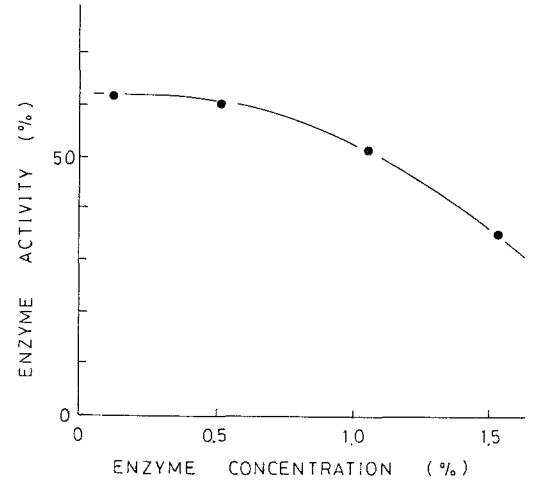

Figure 4 Effect of cross-linking monomer (2G) concentration. Irradiation temperature: −78° C; monomer concentration: 30% (HEMA and 2G); size of material: 1.6 cm diameter × 0.5 cm.

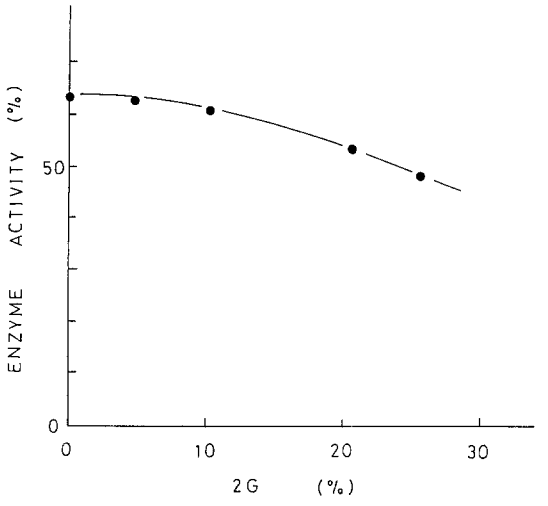

Figure 5 Effect of enzyme concentration. Irradiation temperature: −78° C; monomer concentration: 30% HEMA; size of material: 1.6 cm diameter × 0.5 cm.

Fig. 5 shows the relationship between enzyme concentration and enzyme activity. The enzyme activity was almost constant at enzyme concentrations below about 0.3% but it decreased with increasing enzyme concentration. This decrease of the enzyme activity at high enzyme concentrations would be due to the leakage of the enzymes. Thus, the critical enzyme concentration in 30% monomer concentration for which enzymes are not leaked appeared to be about 0.3%. This value, which is enzyme content in the wetted materials, corresponded to the enzyme content of about 0.99% in the dried materials at a monomer concentration of 30%. It is proposed that this critical enzyme concentration is increased by an increase in monomer concentration since the leakage of the enzyme is related to the pore size in the porous structure.

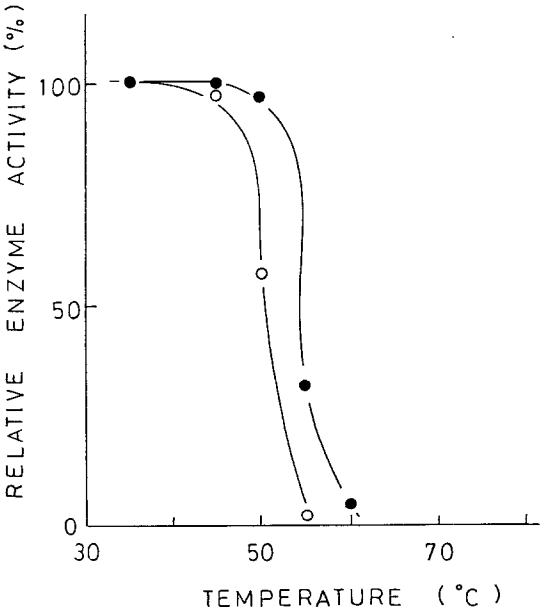

Figure 6 Heat stability curve. Monomer concentration: 30% HEMA; irradiation temperature: −78° C; • immobilized enzyme materials (1.6 cm diameter × 0.5 cm); and ○ native enzymes.

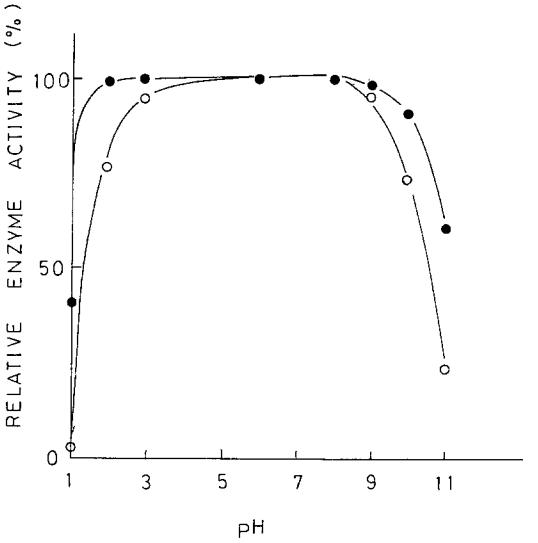

Figure 7 pH stability curve. Monomer concentration: 430% HEMA, irradiation temperature: −78° C; • immobilized enzyme material (1.6 cm diameter × 0.5 cm); and ○ native enzymes.

3.5. Heat and pH stability

The heat and pH stability of the materials was examined as shown in Figs. 6 and 7. The heat stability curve in the native enzymes was shifted to the high temperature side by immobilization as can be seen in Fig. 6. This result indicated that the heat stability of the materials was higher than that of the native enzymes. The pH stability of the enzymes was also increased by immobilization as seen in Fig. 7. The increase in the heat stability by immobilization results in the protection effect of the polymer matrix for the enzymes. For such a protection of enzymes, the porous polymer matrix prepared might be effective.

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