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Effect of the Polymer Matrix on the Immobilization of Lipase by Radiation Polymerization

Minoru Kumakura and Isao Kaetsu

Takasaki Radiation Chemistry Research Establishment,
Japan Atomic Energy Research Institute, Takasaki, Gunma, Japan

SUMMARY

Effect of the hydrophobicity of the polymer matrix on the immobilization of lipase by radiation polymerization was studied. The enzymatic activity of the immobilized enzyme composites was affected by hydrophobicity, and optimum hydrophobicity existed for the enzyme reaction at the surface of the immobilized enzyme composites. Furthermore, the enzymatic activity was affected by monomer concentration. In the immobilization using polyethyleneglycole diacrylate monomers, the enzymatic activity increased with increasing number of ethyleneglycole unit in the monomer molecule.

INTRODUCTION

Immobilization of lipase has been studied by various methods (KOSUGI and SUZUKI 1973; BROCKMAN et al. 1973; KILARA et al. 1977; LIEBERMAN and OLLIS 1975). However, attempts relating to the use of various polymer supports have not been made systematicaly. The adsorption of lipase on hydrophobic surfaces has been studied by some workers(SORDA et al. 1957; LEDFORD and ALAUPOVIC 1975; KOSUGI and SUZUKI 1976). Purification of lipase appling adsorption property of lipase on hydrophobic surfaces has been studied(LEDFORD and ALAUPOVIC 1975). In the immobilization of lipase, a hydrophobic property of the polymer matrix is seemed to be regarded as important factor. The authors have studied the immobilization of microbial cells by radiation polymerization of various glass-forming monomers at low temperatures (KUMAKURA et al. 1979). In this work, the immobilization of lipase by radiation polymerization has been studied using various hydrophobic and hydrophilic monomers to evaluate the effect of property of the polymer matrix.

MATERIALS

Lipase was obtained from Sigma Chemical Company. Hydroxyethyl methacrylate(HEMA), hydroxyethyl acrylate(HEA), trimethyrolpropane trimethacrylate(TMPT), and polyethyleneglycole diacrylate(A-nG; n is number of ethyleneglycole unit $(-(CH_2CH_2O)-)$ were used as glass-forming monomers, which

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were obtained from Mitsubishi Gas Chemical Co., Ltd.

IMMOBILIZATION METHOD

The enzyme dissolved in 0.01 M phosphate buffer solution (pH 7.5) was mixed with monomer. This mixture solution was charged in a glass tube and quickly shaken. Immediately after shaking, the tube was frozen at -78°C. The gamma-irradiation(total dose; 1.0 MR) was carried out at a dose rate of 1.0 MR/hr for 1 hr at -78°C. After irradiation, the immobilized enzyme composites obtained using hydrophilic monomers were cut to pellet form at room temperature.

ENZYMATIC ACTIVITY OF THE IMMOBILIZED COMPOSITES

Enzymatic(lipase) activity(%) remaining after repeated batch enzyme reaction of the immobilized enzyme composites was examined by hydrolysis of glycerol triacetate, and obtained from the acid formation ratio in immobilized and native enzyme with each batch enzyme reaction at 35°C for 30 min. The amount of the acid formed by the enzyme reaction was measured by titration method with 0.05 N NaOH.

RESULTS AND DISCUSSION

The enzymatic activity of the immobilized enzyme composites obtained with hydrophilic HEMA monomer was examined as a function of monomer concentration as shown in Fig.1. The enzymatic activity had a maximum at monomer concentrations of 50 - 60% and decreased with increasing or decreasing monomer concentration. The decrease of the enzymatic activity at lower and higher monomer concentrations was

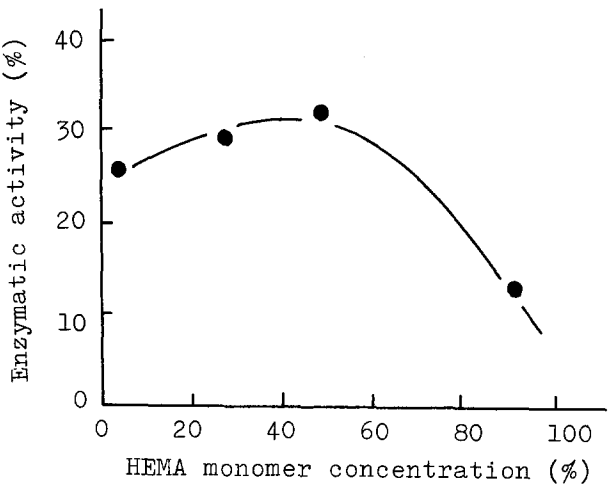

Figure 1. Relation between HEMA monomer concentration and the enzymatic activity

caused by a leakage of the enzyme from the polymer matrix and the decrease of the quantity of the enzyme trapped on the surface of the polymer matrix. The polymer matrix of the immobilized enzyme composites obtained in high monomer concentrations had a porous structure of small porosity; for example, the porosity in 80% HEMA monomer concentration was about 15%, so that considerable amount of the enzyme was entrapped into the polymer matrix. The immobilized enzyme composites obtained in HEMA monomer concentrations of 50 - 60% had a suitable porous structure for the trapping of the enzyme on the surface of the polymer matrix, so that the substrate hydrolyzed easily. The reaction of the immobilized enzyme composites with insoluble substrate should occur on the surface of the polymer matrix, in which the contact action of the substrate with the polymer matrix would be affected by the property and structure of the polymer matrix. The immobilized enzyme composites were obtained with various hydrophilic and hydrophobic monomers, and the relation between the enzymatic activity and water content of their polymers is shown in Fig.2. The enzymatic activity decreased with increasing water content and had a slight maximum, indicating that the enzyme reaction with the enzyme immobilized is affected by hydrophobicity of the polymer matrix. Furthermore, the result suggested that optimum

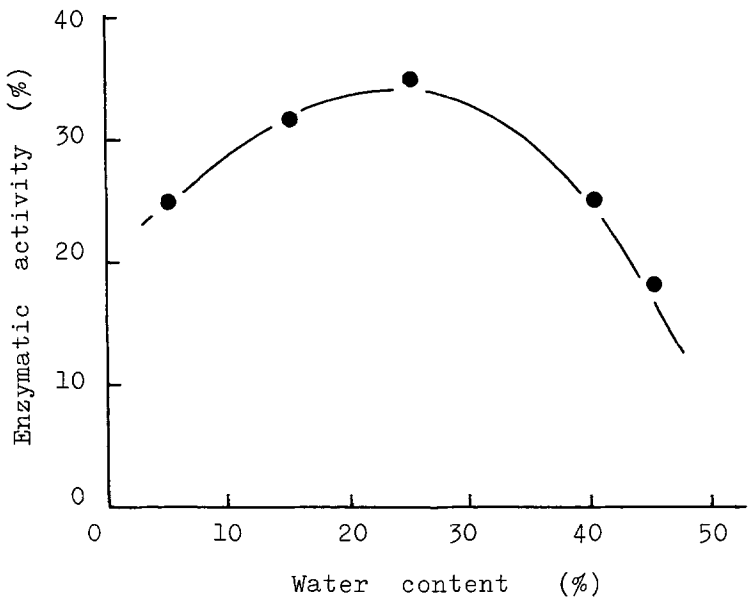

Figure 2. Relation between water content of the polymer matrix and the enzymatic activity.

Monomer concentration : 50%

hydrophobicity exists for the enzyme reaction of the enzyme at the surface of the polymer matrix. This hydrophobicity should be depended on the property of the substrate and enzyme. The immobilized enzyme composites obtained with hydrophobic TMPT monomer gave a particle shape on which the enzyme is trapped on the surface of the partcle. Such a immobilized enzyme particle is suitable for the enzyme reaction with insoluble substrate. On the other hand, hydrophilic immobilized enzyme composites obtained with HEA monomer gave a gel-like porous structure, in which the enzyme might be entrapped into the polymer matrix. Previously reported(KUMAKURA et al. 1979), such a very hydrophilic porous structure was important for a mild immobilization of microbial cells in which the diffusion of the substrate into the polymer matrix was easy. Also, in immobilization of the enzyme such as glucoamyrase having the substrate of small molecular weight, the enzymatic activity of the immobilized enzyme composites obtained with HEA monomer was relatively high(YOSHIDA et al. 1980). This was different markedly from the result in the immobilization of lipase.
Lipase was immobilized with A-nG monomers having various number(n) of ethyleneglycole unit. The relation between n and the enzymatic activity is shown in Fig.3. The enzymatic

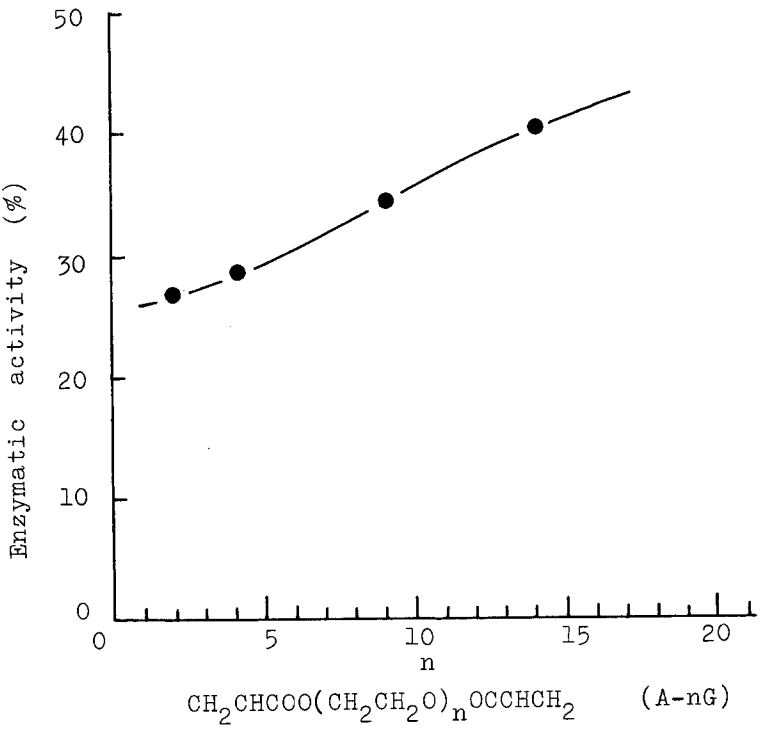

Figure 3. Relation between the number of ethyleneglycole unit in A-nG monomers and the enzymatic activity.
Monomer concentration : 50%

activity increased with increasing number of ethyleneglycole unit. The shape of the immobilized enzyme composites obtained with A-nG monomers which are bi-functional monomers having a cross-linking property, is a flake form. The particle size of the flake decreased with increasing number of ethyleneglycole unit. The increase of the enzymatic activity with increasing number of ethyleneglycole unit would be caused by the partcle size and hydrophobicity of the polymer matrix. The hydrophobicity of the polymer matrix increased with decreasing number of ethyleneglycole unit, in which the water content of A-14G monomer was comparable with that of HEMA monomer.

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