Influence of Hydration Ability of Monomer on Immobilization of Microbial Cells by Radiation Polymerization

MINORU KUMAKURA and ISAO KAETSU, *Takasaki Radiation Chemistry Research Establishment, Japan Atomic Energy Research Institute, Takasaki, Gunma, Japan*

Synopsis

The influence of the hydration ability of monomer on the immobilization of microbial cells by radiation polymerization was studied using various acrylate and diacrylate monomers. The heats of mixing of the monomers in water were determined, and the hydration abilities of monomer and polymer were evaluated by heat of mixing and water content. The shape of the immobilized cell composites varied with the hydration ability of monomer. The enzymatic activity of the immobilized cell composites varied with the number of ethyleneglycol or methylene units in the monomers. In the immobilization using acrylate monomers, the increase of the hydration ability of the monomer introduced a spongelike composite to the formation of the immobilized cells, and, in diacrylate monomers, the increase of the hydration ability of the monomer introduced a flakelike composite to the formation of the immobilized cells, by which enzymatic activity increased.

INTRODUCTION

Recently, the immobilization of microbial cells has been the subject of increased interest and has been studied by many scientists, resulting in the publication of many reports on the immobilization of microbial cells by entrapping.[1–5] But the relation between the property of the polymer matrix and the enzymatic activity of immobilized cells has not been studied. Little is known about the suitable conditions of the immobilization and the enzymatic properties of the immobilized cells.[5] In the immobilization of microbial cells and enzymes, it is important to study the relation between the hydration ability of monomer and enzymatic activity or shape of immobilized cells because the diffusion of substrate in the polymer matrix (carrier) is affected by the nature of the polymer matrix. In the previous paper, the authors have reported that immobilized cell composites having porous structure were obtained by radiation polymerization of hydroxyethyl methacrylate at low temperatures, and the structure was different from the gel-like matrix obtained by radiation polymerization of acrylamide.[6]

In this paper, the immobilization of microbial cells such as *Streptomyces phaeochromogenes* cells by radiation polymerization at low temperatures was carried out using various hydrophilic and hydrophobic monomers, in which the relation between the hydration ability of the monomer and the enzymatic activity or the shape of the immobilized cell composites was clarified.

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MATERIALS AND METHODS

Microbial Cells and Monomers

Streptomyces phaeochromogenes cells containing active glucose isomerase were obtained from Nagase-Sangyo Co., Ltd. Diethyleneglycol diacrylate (A-2G), triethyleneglycol diacrylate (A-3G), tetraethyleneglycol diacrylate (A-4G), nonaethyleneglycol diacrylate (A-9G), and tetradecaethyleneglycol diacrylate (A-14G) were used as polyethyleneglycol diacrylates (A-*n*G). Hydroxyethyl acrylate (HEA), hydroxybutyl acrylate (HBA), hydroxypentyl acrylate (HPA), and hydroxyhexyl acrylate (HHA) were used as hydroxyalkyl acrylates. These monomers were purchased from Mitsubishi Gas Chemical Co., Ltd., and used without further purification. The purity of these monomers was measured by gas chromatography and was above 99.5%. The impurities were polymerization inhibitor and other monomers which did not affect enzymatic activity of the cells.

Method of Immobilization

A mass of dried cells was suspended in 0.05*M* phosphate buffer solution (0.01*M* $MgSO_4 7H_2O$, pH 7.2) containing the monomer. The total mixture solution was prepared to 2.0 mL. This solution mixture was charged in a glass tube vessel (2.0 cm in length and 0.8 cm in diameter) and rapidly shaken. Immediately after shaking, the vessel was frozen at −78°C. The γ-ray irradiation of 1.0×10^6 R was carried out using Co-60 source of 5×10^5 Ci for 1.0 h at a dose rate of 1.0×10^6 R/h. The irradiation temperature was kept at −78°C by immersing the vessel in a Dewar flask filled with dry ice–methanol.

Heat of Mixing Measurements

The measurement of the heat of mixing of the monomers in water was performed with a closed type calorimeter, which is similar to the calorimeter of Cheeseman and Whitaker.[7] The mixing vessel (10 cm^3) with a magnetic stirring apparatus and sample (water) inlet tube was surrounded by a vacuum jacket, and its temperature was held constant (25°C). The temperature measurement was done with a copper-constantan thermocouple which was fed to a galvanometer. Since the heat of mixing varied with mole fraction, the heats of mixing in various mole fractions of monomer were measured, and the maximum value was obtained.

Measurement of the Enzymatic Activity

The durability of the enzymatic (glucose isomerase) activity of the immobilized cell composites was examined by repeating the batch enzyme reaction (1.0 h at 65°C). The enzymatic activity (%) was obtained from the D-fructose formation ratio in the immobilized and intact cells with each batch enzyme reaction. The enzymatic activity of the cells was evaluated from the initial reaction rate, which was linear during the reaction time of the assay. 1.0% D-glucose solution (0.05*M* phosphate buffer solution containing 0.01*M* $MgSO_4 7H_2O$, pH 7.2) was used as

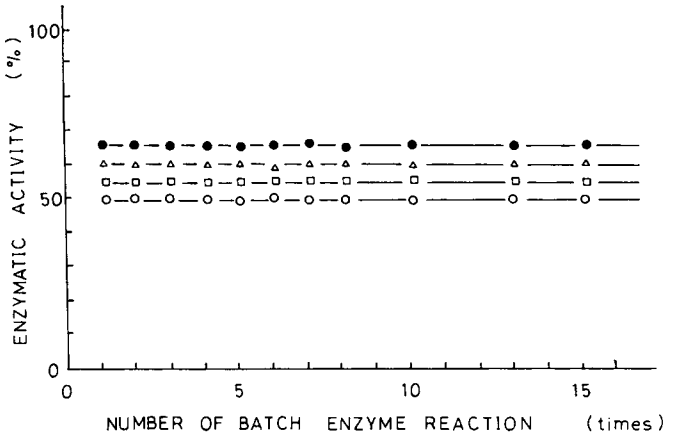

Fig. 1. Variation of the enzymatic activity in A-2G monomer with repeated batch enzyme reaction. Monomer concentration: (●) 15%; (△) 30%; (□) 50%; (○) 70%.

the substrate. The D-fructose formed was determined by the cysteine–carbazole method.[8]

RESULTS AND DISCUSSION

Variation of Enzymatic Activity with Repeated Batch Enzyme Reaction

The enzymatic activity curves in A-2G and A-14G monomer as diacrylate monomers are shown (Figs. 1 and 2) as a function of the number of batch enzyme reaction. The decrease in the enzymatic activity with increasing numbers of batch enzyme reaction in A-2G and A-14G monomer was not observed, indicating that the leakage of the cells from the immobilized cell composites does not occur. The enzymatic activity curves in HEA and HDA monomers as acrylate monomers are shown in Figures 3 and 4, respectively, as a function of the number of batch

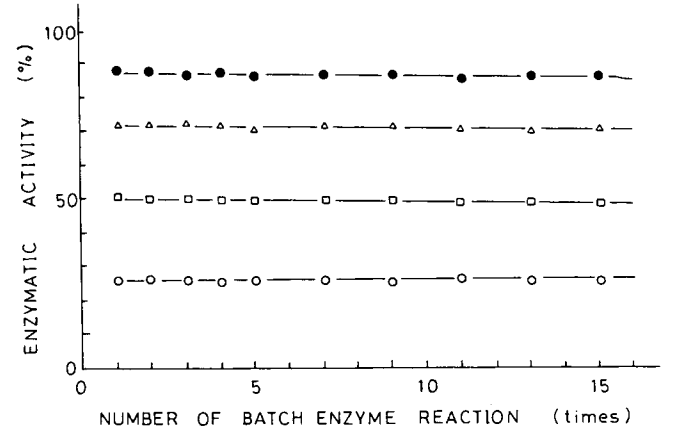

Fig. 2. Variation of the enzymatic activity in A-14G monomer with repeated batch enzyme reaction. Monomer concentration: (●) 15%; (△) 30%; (□) 50%; (○) 70%.

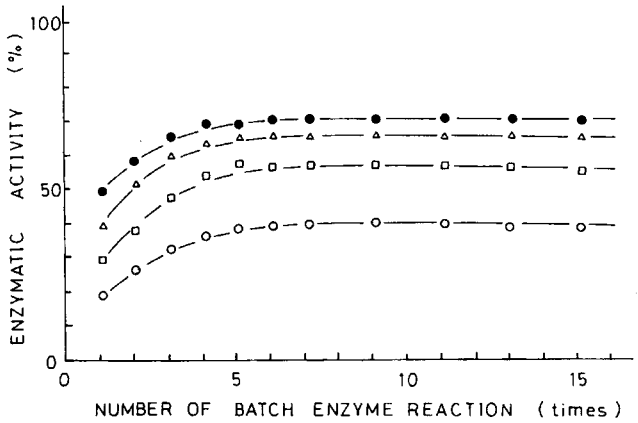

Fig. 3. Variation of the enzymatic activity in HEA monomer with repeated batch enzyme reaction. Monomer concentration: (●) 15%; (△) 30%; (□) 50%; (○) 70%.

enzyme reaction. In the case of HEA monomer, the increase in the enzymatic activity with increasing numbers of batch enzyme reaction at the initial stage was observed to contrast with that in HDA monomer. Such an increase in the enzymatic activity at the initial stage of repeated batch enzyme reaction was observed in hydroxyethyl methacrylate monomer, and it was explained by the swelling effect of the polymer matrix of the immobilized cell composites.[9] The enzymatic activity in HDA monomer was higher than that in HEA monomer, and it did not vary with repeated batch enzyme reaction. The increase phenomena of the enzymatic activity at the initial stage appeared in only HEA monomer among acrylate monomers. Such a phenomena was not observed in the case of diacrylate monomers, though A-14G monomer has considerable high hydration ability, as described in a later section.

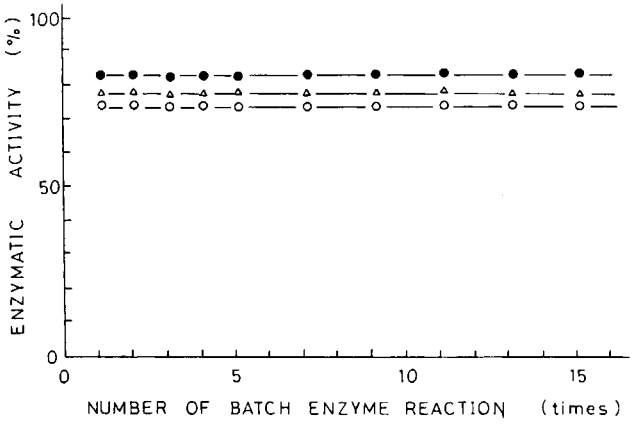

Fig. 4. Variation of the enzymatic activity in HDA monomer with repeated batch enzyme reaction. Monomer concentration: (●) 15%; (△) 30%; (○) 70%.

Relation between Enzymatic Activity and Molecular Structure of Monomer

The relation between the enzymatic activity at the later stage (13 times) of the repeated batch enzyme reaction and the number (n) of ethyleneglycol units

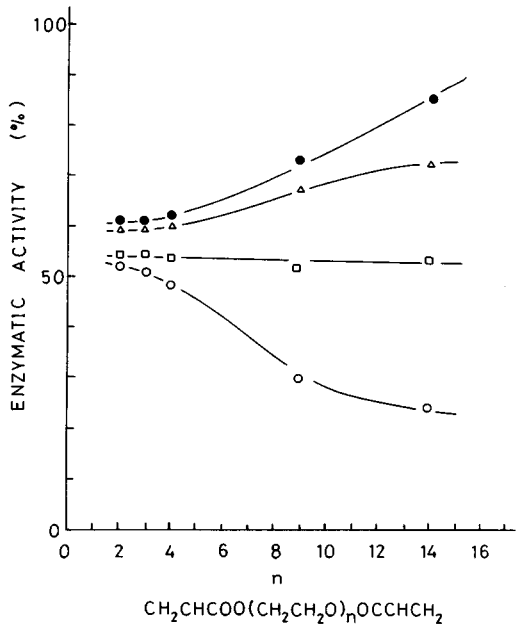

Fig. 5. Relation between the enzymatic activity and the number of ethyleneglycol units in diacrylate monomers. Monomer concentration: (●) 15%; (△) 30%; (□) 50%; (○) 70%.

[—(CH_2CH_2O)—] in diacrylate monomers is shown in Figure 5 as a function of monomer concentration. The enzymatic activity varied considerably with increasing the number of ethyleneglycol units. In the monomers having smaller numbers of ethyleneglycol units, the enzymatic activity did not vary with monomer concentration, but in monomers having larger numbers it varied markedly with monomer concentration.

The relation between the enzymatic activity and the number (n) of methylene units [—(CH_2)—) in acrylate monomers is shown in Figure 6 as a function of monomer concentration. A variation of the enzymatic activity by the number of the methylen units (Fig. 6) was markedly different from that in Figure 5, in which the enzymatic activity in acrylate monomers increased with increasing number of the methylene units. Furthermore, the enzymatic activity in the monomer (n = 6) did not vary by monomer concentration. The variation of the enzymatic activity by the number of the ethyleneglycol or methylene units is related to the hydration ability of monomers and polymers. The heat of mixing of the monomers was examined as described in a later section.

Hydration Ability of Monomer

The present immobilization method by radiation polymerization using glass-forming monomers at low temperatures was carried out in a water–monomer mixture. Therefore, trapping situation of the cells in the process of immobilization should be affected by the hydration ability or hydrophilic property of monomer. The authors have reported that the immobilized cell composites obtained using hydrophilic monomer (hydroxyethyl methacrylate) gave a porous spongelike structure, and while those obtained using hydrophobic monomer (trimethylolpropane triacrylate) gave a fine particle shape without

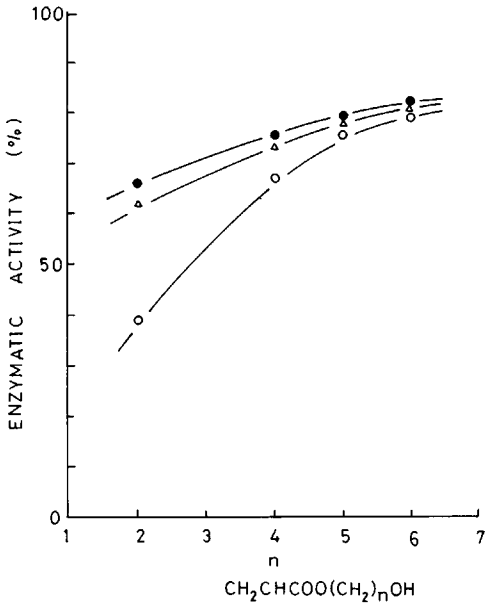

Fig. 6. Relation between the enzymatic activity and the number of methylene units in acrylate monomers. Monomer concentration: (●) 15%; (△) 30%; (○) 70%.

a porous structure.[10] The immobilized cell composites obtained in this work using diacrylate monomers gave a flake shape having a porous structure rather than a particle shape. This difference is due to the hydration ability of the monomer. For example, in the preparation of the immobilized cell composites using diacrylate monomer of $n = 4$, the monomer phase suspended from the mixture containing water and monomer had an incomplete particle shape such as a flakelike shape because the monomer dissolved slightly in the water phase. The polymer matrix of the immobilized cell composites in acrylate monomer, having a smaller number ($n = 2$, HEA), had a soft sponge porous structure and the acrylate monomer, having a larger number ($n = 6$, HDA), had rigid elipse shape of nonporous structure.

The hydration ability of monomer was evaluated by measurement of the heat of mixing ($-\Delta H$) of monomer in water. The relation between the heat of mixing and the number of the ethyleneglycol or methylene units in diacrylate or acrylate monomer is shown in Figure 7. The heat of mixing of acrylate and diacrylate monomers has not been reported though that of some organic solvents has been.[11] The heat of mixing of diacrylate monomers increased markedly with an increase in the number of the ethyleneglycol units, while that of acrylate monomers decreased with an increase in the number of methylene units. From the results (Fig. 7), diacrylate monomers with larger numbers of the ethyleneglycol units had higher hydration ability and the hydration ability of acrylate monomers was relatively low. The heat of mixing generated in the interaction (hydration) of monomer with water corresponded to the hydration ability of monomer with water. The polar group, such as the ether and hydroxyl group, in the monomers is correlated with hydration. The heat of mixing of diacrylate monomers was relatively large; the monomer with $n = 9$ was comparable with that of dioxane

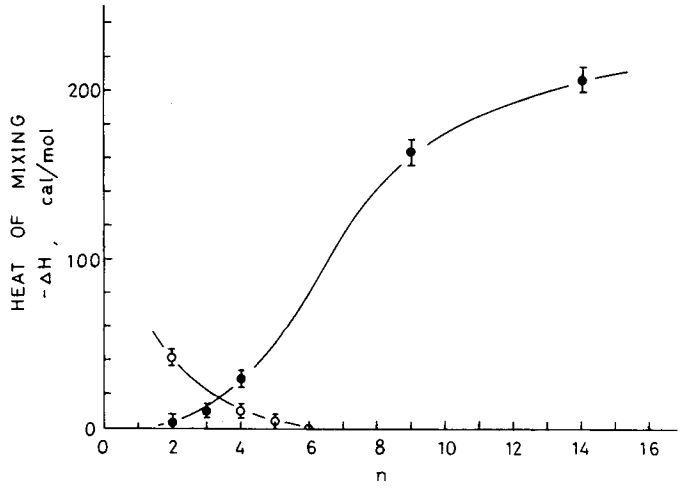

Fig. 7. Relation between the heat of mixing and the number of ethyleneglycol or methylene units in diacrylate or acrylate monomers: (○) acrylate monomer; (●) diacrylate monomer.

(130.5 cal/mol).[11] The heat of mixing of the monomer having $n = 14$ was comparable with that of ethanol. The increase in the heat of mixing with increasing n could be attributed to the increase in the hydration ability owing to the presence of polar ether groups.

Monomers (below $n = 4$) in which the heat of mixing was smaller than 20–30 cal/mol appeared to be hydrophobic monomers, and the immobilized cell composites obtained by radiation polymerization of these monomers in low monomer concentrations were a flake and/or ellipse shape. In these immobilized cell composites, the cells were trapped on their surface parts. Monomers (above $n = 9$) with large heats of mixing were hydrophilic, and the immobilized cell composites obtained using these monomers were a rigid sponge structure in which the cells were trapped on their surface and inner parts. The enzymatic activity of the immobilized cell composites from low monomer concentrations increased with increasing number of ethyleneglycol units (Fig. 5). In the high monomer concentrations, the amount of the cells entrapped in the polymer matrix of the composites increased with increasing number of the ethyleneglycol units (or hydration ability), so that the apparent enzymatic activity decreased. Similar features were observed in the immobilization using acrylate monomers in high monomer concentrations. The enzymatic activity (Fig. 6) in high acrylate monomer concentration (70%) decreased with decreasing number of the methylene units or increasing hydration ability. In the immobilization using acrylate (above $n = 5$) and diacrylate (below $n = 4$) monomers having lower hydration abilities, the enzymatic activity was not affected by monomer concentration (Figs. 5 and 6). These results were reasonable considering that in lower monomer concentrations the cells are trapped on the composite surface of ellipse or flake shape and in higher monomer concentrations some amounts of the cells are entrapped within the inner part of the composite in which the cells cannot react easily with the substrate. In fact, the enzymatic activities of the immobilized cell composites in flake shape from hydrophobic monomers, such as neopentylglycol and 1,3-bytyleneglycol diacrylate, were relatively low and did not

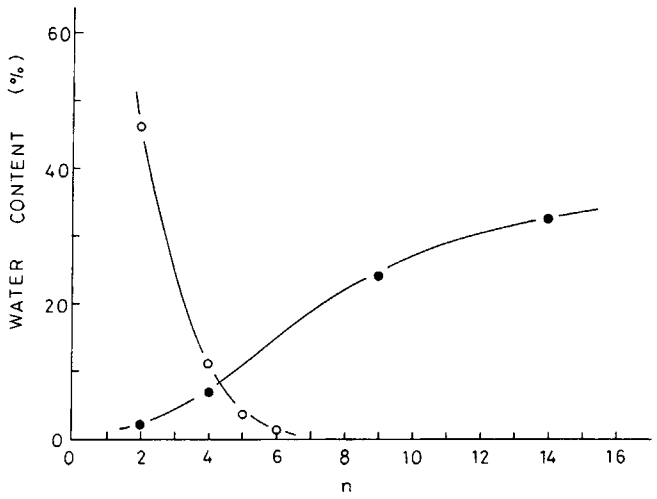

Fig. 8. Relation between the water content and the number of ethyleneglycol or methylene units in diacrylate or acrylate monomers: (○) acrylate monomer; (●) diacrylate monomer.

vary by monomer concentration, as previously reported.[10] In the immobilized cell composites obtained from the monomers having high hydration ability, the cells trapped in the composites can react with the substrate which is diffused through the porous polymer matrix, though, in the composites resulting from high monomer concentrations, the diffusion of the substrate is a rate-determining step in the enzyme reaction. The diffusion rate of the substrate depended on the nature of the polymer matrix of the immobilized cell composites. In high monomer concentrations, the enzymatic activity of the immobilized cell composites with a rigid-sponge porous structure in diacrylate monomer (n = 14) was smaller than that of the immobilized cell composites with a soft-sponge porous structure in acrylate monomer (n = 2). This difference is due to the difference in the diffusion of the substrate which is correlated with the nature of the polymer matrix.

Hydration Ability of Polymer

The hydration ability of the polymer matrix was evaluated by the measurement of the water content of polymer. The relation between water content and the number of the ethyleneglycol or methylene units in diacrylate or acrylate monomer is shown in Figure 8. The water content of acrylate and diacrylate-polymers decreased and increased with increasing n, respectively. The variation of the hydration ability by n in the polymers appeared to be similar to that in the monomers. Of course, the degree of hydration ability decreases by polymerization. In fact, the water content in diacrylate polymers was relatively small due to crosslinking, though the heat of mixing in the monomers was large. The water content in the polymers of hydrophilic acrylate monomer (n = 2) was larger than that in hydrophilic diacrylate monomer (n = 14) due to a crosslinking effect, in which the swollen diacrylate and acrylate polymers were rigid and soft, respectively. The nature of the polymer matrix affects the diffusibility of the substrate in the enzyme reaction with the immobilized cell composites, by which

the enzymatic activity is varied. As described above, the enzymatic activity of the immobilized cell composites from acrylate monomer ($n = 2$) was higher than that from diacrylate monomer ($n = 14$) at high monomer concentrations. Thus, the trapping site of the cells and the shape (and structure) of the immobilized cell composites varied with hydration ability of monomer. Hydration ability of the polymer matrix was correlated with that of monomer.

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