EFFECT OF THE HYDROPHILICITY OF THE POLYMER MATRIX ON IMMOBILIZED α-CHYMOTRYPSIN

Minoru KUMAKURA and Isso KAETSU

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

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α-Chymotrypsin was immobilized by radiation polymerization at low temperatures and the effect of the hydrophilicity of the polymer matrix on the enzyme activity and thermal stability was studied. The activity and thermal stability of immobilized chymotrypsin increased with the increasing hydrophilicity of the polymer matrix or monomer. The thermal stability was affected by the form and pore size of the polymer matrix; chymotrypsin immobilized on a soft-gel polymer matrix exhibited an enhanced thermal stability.

Recently, various procedures using immobilized enzymes have been described and some immobilized enzymes have received application even in industrial practice. The different methods of immobilization of enzymes on various supports have been reviewed recently[1-4]. Each enzyme and each application will probably require a preliminary investigation of the most suitable support and immobilization technique to be carried out. In preceding experiments we studied the immobilization of biological material by radiation polymerization at low temperatures where immobilized microbial cells retain a high activity of their enzymes[5,6]. In this work, α-chymotrypsin was immobilized by radiation polymerization of various monomers and the effect of the hydrophilicity of the polymer matrix on the properties of the immobilized enzyme was examined. Immobilized proteinases are often more stable than the native ones because they are protected against autolysis[7-15]; they can therefore be used in various processes.

EXPERIMENTAL

Materials: α-Chymotropsin was obtained from Sigma Chemical Co. 2-Hydroxyethyl methacrylate (HEMA), 2-hydroxyethyl acrylate (HEA), 1,3-butyleneglycoldimethacrylate (BG), neopentyl glycol dimethacrylate (NPG), and tetradecaethyleneglycol dimethacrylate (14G) were purchased from Shin Nakamura Chemical Co., Ltd. Hammarstein casein and trichloroacetic acid were from Merck and Kanto Chemical Co., Ltd., respectively.

Preparation of immobilized enzyme: The monomer–enzyme solution (1·0 ml), containing the enzyme (1·0 mg/ml), $CaCl_2$ (2 mmol l^{-1}), and monomer in 0·1 mol l^{-1} phosphate buffer (pH 7·4) in a glass tube 20 cm long and 0·8 cm in diameter, was shaken vigorously. Immediately after

the shaking, the content of the tube was cooled down to −78°C and irradiated with 1·0 Mrad of γ-rays from a ^{60}Co source. After the irradiation, the polymerized matrix with the immobilized enzyme was cut to thin (0·5 mm) pellets.

Degree of hydration: The hydrophilicity of polymers was evaluated by measurement of the degree of hydration. The degree of hydration of the polymer was determined as the ratio of the weight of water to the weight of the polymer at swelling equilibrium at 25°w in water.

Enzyme assays: The assays of the immobilized enzyme were carried out in the batchwise arrangement. The assay, (reaction time 30 min), using 0·5% Hammarstein casein as a substrate, at 25°C, was repeated several times with the same batch of immobilized enzyme. After each assay, 10% trichloroacetic acid was added to the reaction mixture and undigested casein was filtered off. The hydrolysis of casein was determined by measuring the absorbance of the filtrate at 280 nm. The relative activity of the immobilized enzyme in each assay was determined from the quantity of digested casein and expressed in relation to the quantity of casein digested by the native enzyme.

Thermal stability of the immobilized enzyme was determined by measuring the residual activity after thermal treatment in buffer solution at various temperatures for 30 min.

RESULTS AND DISCUSSION

The activity of chymotrypsin immobilized by radiation polymerization of various monomers was examined as a function of the number of repeated assays carried out with the same enzyme batch (Fig. 1). With the increasing number of the batch assays the activity of the enzyme immobilized on matrices prepared from hydrophilic HEA and 14G increased gradually and became constant, whereas on matrices from hydrophobic NPG and BG it decreased first and then became constant; hence the enzyme activity in the hydrophilic monomers is obviously higher than that in the hydrophobic monomers. The enzyme immobilized by irradiation of the solution of hydrophilic monomers formed a bulk on a porous polymer matrix, the enzyme being trapped on its surface. On the other hand, the enzyme immobilized by irradiation of solutions of hydrophilic monomers formed particles (size 100−200 μm in diameter) with the enzyme on their surface. Thus, the method described here can yield the immobilized enzymes in various forms according to the properties of the monomer used to start with. The immobilization method using hydrophilic monomers gives rise to a bulk of the enzyme on a porous polymer matrix as a result of the melting of ice originally formed by irradiating and freezing the homogeneous monomer − enzyme solution. The pore size of the porous structure varied with the monomer concentration and freezing rate. The formation of the immobilized enzyme particles from solutions of the hydrophobic monomers by radiation polymerization was a result of the dispersion of monomer particles yet without a dispersing agent. The surface area of the porous polymer matrix with the immobilized enzyme, prepared from the hydrophilic monomers, is larger than that of the matrix prepared from the hydrophobic monomers. The activity of the enzyme immobilized on supports prepared from the hydrophilic monomers was higher than on the supports from the

hydrophobic monomers, as seen in Fig. 1. The degree of hydration of the polymer, both NPG and BG, was comparable (about 0·05). By contrast, the degree of hydration of the polymer prepared from HEA and 14G is much higher (0·45 and 0·25, respectively). The polymer matrix prepared from HEA was a soft, gel-like sponge whereas that prepared from 14G was a rigid-sponge structure because 14G is a bifunctional monomer yielding a cross-linked polymer. This difference in the nature of the polymer matrix is a result of the difference between a monofunctional and a bifunctional monomer and was also reflected by variations in the enzyme activity (Fig. 1): the soft polymer matrix can swell in water, and does not resist the diffusion of substrate and product, unlike the rigid polymer matrix.

The activity of the enzyme immobilized from solutions of hydrophobic monomers varied with the monomer concentration as shown in Fig. 2; a 30–50% monomer concentration appeared to be optimal for the activity of the enzyme. The low enzyme activity observed at low and high monomer concentration was obviously due to leakage and entrapping of the enzyme in the polymer matrix, respectively, With the increasing monomer concentration the porosity of the polymer matrix decreases and the enzyme is being entrapped in the polymer matrix; hence the enzyme reactivity is restricted by resistance to diffusion of substrate and product. As shown in Fig. 2, the enzyme activity in HEA was higher than that in HEMA, considering the difference in the hydrophilicity of the polymers; the degree of hydration of the HEMA polymer was 0·26. The activity of the immobilized enzyme was therefore considerably dependent on the hydrophilicity of the polymer matrix.

The thermal stability of the immobilized enzyme varied markedly with the condi-

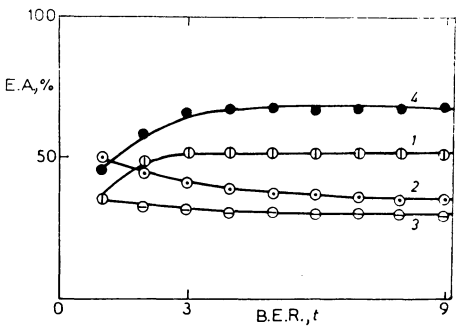

FIG. 1

Activity of the immobilized enzyme as function of the nature of the monomer; Monomer concentration: 50%. Monomer: *1* 14G, *2* NPG, *3* BG, *4* HEA

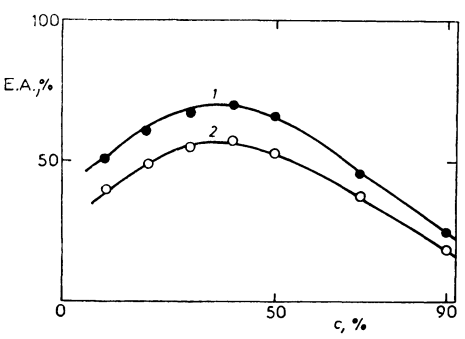

FIG. 2

Effect of monomer concentration on the enzyme activity. Monomer: *1* HEA, *2* HEMA

tions of immobilization such as the concentration of monomer, as shown in Fig. 3. The enzyme was markedly stabilized by immobilization and the thermal stability of the enzyme immobilized from HEA solutions increased with the increasing HEA concentration. The porosity of the polymer matrix prepared from HEA decreased with the increasing monomer concentration; the porosity and pore size at 90% monomer concentration were 4–5% and 0·5–1 μm (diameter), respectively. The enzyme is firmly attached on the surface of the polymer matrix though a part of it is probably entraped inside the matrix. In the matrix, the enzyme molecules are isolated from each other and autolysis obviously does not take place. Moreover, excess thermal motion of the enzyme molecules at high temperatures is limited by the folding of the polymer chains in the matrix. As a matter of fact, the residual enzyme activity of the native enzyme at 60–70°C was almost zero, whereas the activity of the immobilized enzyme was still high. Since the inactivation of the enzyme at 60–70°C is mainly due to thermal denaturation rather than autolysis, the immobilization of the enzyme from solutions of hydrophilic monofunctional monomers seems to enhance the thermal stability of the enzyme. On the other hand, the thermal stability of the enzyme immobilized from the solution of bifunctional 14G, which is a slightly hydrophilic monomer only, was low and comparable with that of the native enzyme, as shown in Fig. 4. The enzyme on the polymer matrix prepared from bifunctional monomers was immobilized on a rigid structure; a part of the enzyme molecules was very firmly trapped on its surface and hence this exposed part became

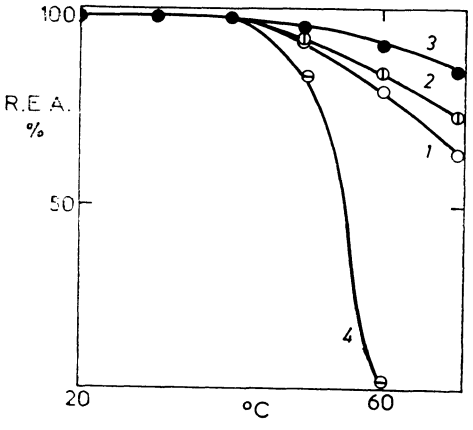

FIG. 3

Thermal stability of the enzyme immobilized from HEA solutions. *1* 20% HEA, *2* 50% HEA, *3* 90% HEA, *4* native enzyme

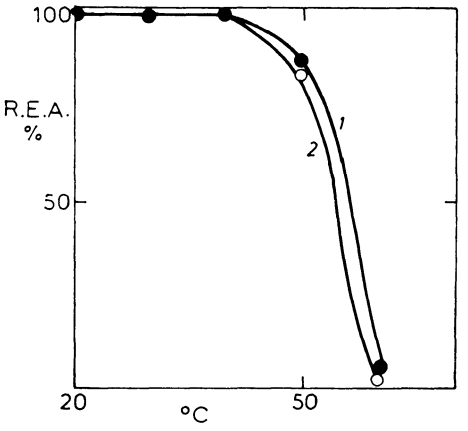

FIG. 4

Thermal stability of the enzyme immobilized from 14G *1* 50% 14 G, *2* native enzyme

denaturated by heating. Moreover, the porous structure of the polymer matrix prepared from bifunctional monomers is different from that prepared from the monofunctional monomers; the porosity of the structure prepared from bifunctional monomers is larger than the porosity of the matrices prepared from monofunctional monomers because of the differences in the hydrophilicity of both monomers. These differences in the porous structure probably affect the thermal stability, since the thermal stability of the immobilized enzyme varies with the concentration of the monofunctional monomer (such as HEA) from which the polymer matrix had been prepared.

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