포스터 발표 P-3

Application of Polymer Brush to Enzyme-Multilayered Porous Hollow-Fiber Membrane

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Abstract

Anion-exchange porous hollow-fiber membranes with a thickness of about 1.2 mm and a pore size of about 0.30 μm were used as a supporting matrix to immobilize cycloisomaltooligosaccharide glucanotransferase (CITase). CITase was immobilized to the membrane via anion-exchange adsorption and by subsequent enzymatic cross-linking with transglutaminase, the amount of which ranged from 3 to 110 mg per g of the membrane. The degree of enzyme multilayer binding was equivalent to 0.3 to 9.8. Dextran, as the substrate, was converted into seven- to nine-glucose-membered cycloisomaltooligosaccharides (CI-7, -8, and -9) at a maximum yield of 28% in weight at a space velocity of 10 per hour during the permeation of 2.0 (w/w)% dextran solution across the CITase-immobilized porous hollow-fiber membrane.

Introduction

Enzyme immobilization onto various matrices produces a trade-off between the ease of separation of products from the enzyme and the difficulty of diffusion of substrates to the reaction sites of enzyme in the matrices (*1, 2*). To overcome this dilemma, the use of porous media is suggested as they are powerful matrices for enzyme immobilization: convective transport of the substrate through the pores relieves the resistance.

So far, we have prepared novel porous hollow-fiber membranes to immobilize enzymes in polymer chains grafted onto the pore surface of the membrane by radiation-induced graft polymerization. In addition, multilayering of the enzymes is attained (*3*) because graft chains with ionic groups extend from the pore surface toward the pore interior and provide three-dimensional binding sites for the enzymes (*4-6*). Nakamura et al.(*7*) immobilized aminoacylase in four layers in the polymer brushes, leading to threefold higher activity compared to the enzyme immobilized onto the beads at an identical space velocity. However, the effect of the degree of enzyme multilayering on reaction performance has not been clarified in a quantitative manner.

The objective of this study was twofold: (1) to immobilize cycloisomaltooligosaccharide glucanotransferase (CITase) at various degrees of enzyme multilayering onto the microfiltration membrane, and (2) to evaluate the production of cycloisomaltooligosaccharides using the CITase-immobilized porous membranes. The seven- to nine-glucose-membered cycloisomaltooligosaccharides are promising anticariogenic compounds (*8*). Dextrans with molecular masses ranging from 16 000 to 510 000 were used as substrates.

Experimental

Preparation of Enzyme-Immobilized Porous Hollow-Fiber Membranes. The preparation scheme of CITase-immobilized porous hollow-fiber membranes made of polyethylene (I.D. 1.9 mm, O. D. 3.1 mm, pore diameter 360 nm, porosity 71%) is shown in Figure 1. This scheme consists of the following five steps. (1) Irradiation of electron beam: the original membrane was irradiated with an electron beam in nitrogen atmosphere at ambient temperature. The dose was set at 200 kGy. (2) Grafting of an epoxy-group-containing monomer: the elecron-beam-irradiated membrane was immersed in 10 (v/v)% GMA/methanol solution at 313 K for 12 min. The degree of GMA grafting, as defined below, was 160%:

$$dg\,[\%] = 100\,(W_1 - W_0)/W_0 \tag{1}$$

where W_0 and W_1 are the weights of the original and the GMA-grafted membrane, respectively. (3) Introduction of an anion-exchange group into the graft chain: the GMA-grafted membrane was immersed in 50 (v/v)% aqueous diethylamine solution at 303 K for a prescribed time. The molar conversion of the epoxy group into a diethylamino (DEA) group was determined from the weight gain of the membrane (*3*). The resultant membrane was referred to as a DEA(x) fiber, where x designates the molar conversion. (4) Binding of CITase onto the anion-exchange membrane: 0.44 mg/mL CITase dissolved in 10 mM phosphate buffer (pH 8.0) was forced to permeate from the inside surface of the DEA(x) fiber through the pore rimmed by polymer brush with anion-exchange-groups. The concentration of CITase in the effluent penetrating the outside surface of the DEA(x) fiber was continuously determined from the UV absorbance at 280 nm. The equilibrium binding capacity (EBC), i.e., the amount of CITase bound to the DEA fiber in equilibrium with the feed concentration, was evaluated by integrating the concentration difference between the feed (C_0) and the effluent (C) as follows :

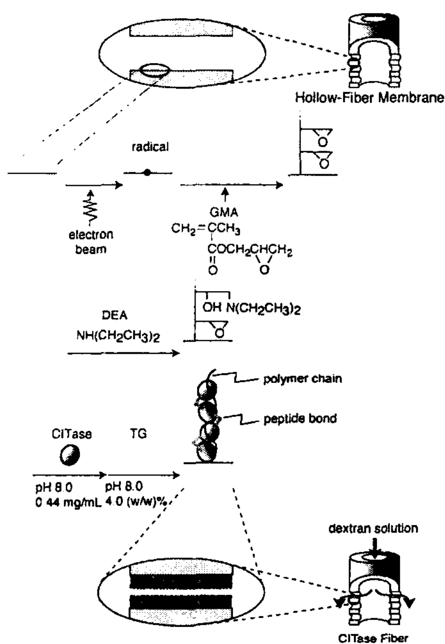


Fig. 1 Preparation scheme of enzyme-multilayered porous hollow-fiber

$$\text{EBC [mg/mL]} = \int_0^{V_e} (C_0 - C)\, dV / W \quad (2)$$

where V, V_e, and W are the effluent volume, the effluent volume for which C reaches C_0, and the weight of the membrane, respectively. The degree of multilayer binding of CITase was calculated by dividing the amount of multilayer-bound CITase by the theoretical monolayer binding capacity. The degree of CITase multilayer binding is defined by dividing the amount of adsorbed CITase by the theoretical monolayer binding capacity. The theoretical monolayer binding capacity was calculated as 11 mg per gram of dry fiber using the following equation:

$$\text{Theoretical monolayer binding capacity} = (M_r\, a_v)/(a\, N_A) \quad (3)$$

where M_r and N_A are the molecular mass of CITase (98 000) and Avogadro's number, respectively. a_v (5.5 m^2/g) and a (7.4 × 10^{-17} m^2) are the specific surface area of the DEA fiber and the area occupied by a CITase molecule, respectively. The calculation method of the theoretical monolayer binding capacity is described in detail in our previous paper (*3*). (5) The bound CITase was cross-linked by immersion of the membrane in 4.0 (w/w)% transglutaminase (TG) solution dissolved in 10 mM phosphate buffer (pH 8.0). Subsequently, the uncrosslinked CITase was eluted by permeating 0.5 M NaCl solution. Cross-linking percentage and the degree of immobilized enzyme multilayering (IEM) were defined as:

$$\text{Crosslinking percentage [\%]} = 100\, [(\text{amt bound}) - (\text{amt eluted})] / (\text{amt bound}) \quad (4)$$

$$\text{Degree of IEM} = (\text{degree of enzyme multilayer binding}) \times (\text{cross-linking percentage}) / 100 \quad (5)$$

where amt bound means the amount of CITase adsorbed and amt eluted, the amount of CITase eluted by permeating 0.5 M NaCl solution. The resultant hollow-fiber membrane was referred to as a CITase(q) fiber, where q is the amount of CITase immobilized.

Production of Cycloisomaltooligosaccharides from Dextran Using CITase-Immobilized Porous Hollow-Fiber Membranes. A 2.0 (w/w)% dextran solution dissolved in 0.1 M acetate buffer (pH 5.5) was permeated from the inside surface of the CITase(q) fiber outward radially at 313 K at a constant permeation rate ranging from 2.0 to 60 mL/h. The space velocity (SV) was defined by dividing the permeation rate by the membrane volume including the lumen part. The effluent penetrating the outside surface was continuously collected using fraction vials. CIs were determined by high-performance liquid chromatography (Hitachi L-7000) with an Amide-80 column (Tosoh Co., Japan). The flow rate of the mobile phase consisting of acetonitrile : water (70 : 30 in volume) was set at 60 mL/h at 308 K.

The yield of CIs and the amount of CIs produced per unit of CITase immobilized were defined as follows:

$$\text{Yield [\%]} = 100\, (\text{total mass of CI-7 to -9})/(\text{mass of dextran fed}) \quad (6)$$

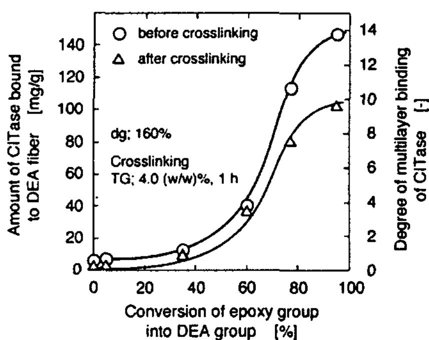


Fig. 2 Amount of ClTase bound to DEA fiber and degree of multilayer binding of ClTase as a function of conversion of epoxy group into DEA group before and after

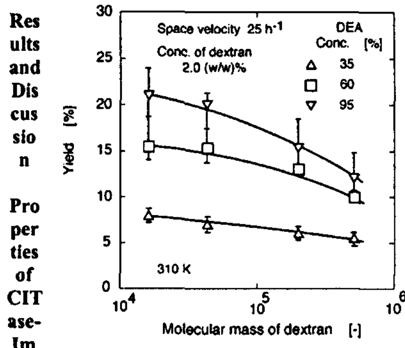
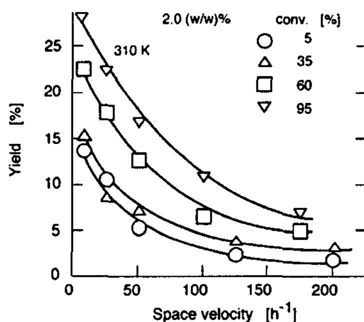


Fig. 3 Effect of molecular mass of dextran on yield of 7- to 9-glucose membered CIs.

Fig. 4 Yield of CIs as a function of space velocity of dextran solution.



Results and Discussion

Properties of ClTase-Immobilized Porous Hollow-Fiber Membranes. The amount of ClTase bound onto the DEA fiber with various molar conversions via electrostatic interaction was evaluated in the permeation mode. EBC of the DEA fiber for ClTase is shown in Figure 2 as a function of molar conversion. The degree of multilayer binding of ClTase, defined by dividing EBC by the theoretical monolayer binding capacity, is also shown in Figure 2. EBC markedly increased at molar conversions higher than 40%, reaching 140 mg per g of the dry DEA fiber at a conversion of 95%. This enzyme multilayering to the polymer brush can be explained by the extension of the graft chain due to electrostatic repulsion.

Subsequently, the enzyme was cross-linked enzymatically with TG. The cross-linking percentage ranged from 70 to 80% for various EBCs. For example, 80% of the enzyme bound onto the DEA(95) fiber was immobilized after the crosslinking treatment, which amounted to the degree of IEM of 9.8. The amount of the immobilized ClTase ranged from 3 to 110 mg per g of the membrane, which was equivalent to the specific activity that ranged from 0.88 to 32 U/mg.

Performance of ClTase-Immobilized Porous Hollow-Fiber Membranes. The effect of molecular mass of dextran on the yield of CIs using the ClTase fiber is shown in Figure 3. The yield of CIs decreased with an increasing molecular mass of dextran. In addition, the higher density of the immobilized ClTase, the more sensitive the yield to the molecular mass of dextran in the feed solution. The dextran with the lower molecular mass more easily accesses the enzymes through the ClTase-immobilized polymer brush.

The yield vs SV of the substrate solution permeating the pores of the ClTase fiber with various amounts of ClTases immobilized is shown in Figure 4. For each ClTase fiber, the yield decreased exponentially with increasing SV. At a constant SV, with an increase in the degree of IEM, the amounts of CIs produced per unit of ClTase immobilized decreased. This is because either diffusion of the dextran into the ClTase-immobilized polymer brushes and intrinsic reaction at the active site of ClTase governs an overall reaction rate.

On an industrial scale, the remaining dextran with a low molecular mass is selectively hydrolyzed into glucose monomer using dextran hydrolase. Subsequently, CIs and glucose monomer can be separated with activated carbon: CIs are selectively adsorbed onto the activated carbon and readily eluted with ethanol.

Conclusions

Anion-exchange groups or positively ionizable groups were introduced into the polymer brush grafted onto a porous hollow-fiber membrane by radiation-induced graft polymerization of an epoxy-group-containing monomer and subsequent addition of diethylamine to the epoxy ring. A solution of cycloisomaltooligosaccharide glucanotransferase (CITase) was forced to permeate through the anion-exchange porous hollow-fiber membranes. With increasing molar conversion of the polymer brush to the diethylamino group, the amount of CITase bound to the graft chain increased up to 110 mg per g of the membrane, which was equivalent to the degree of immobilized enzyme multilayering of 9.8.

After cross-linking the CITase bound to the polymer brushes with transglutaminase to prevent leakage of the enzymes, 2.0 (w/w)% dextran solution was fed to the inside surface of the CITase-immobilized porous hollow-fiber. The higher degree of CITase multilayer binding and the lower molecular mass of dextran resulted in the higher yield of CIs. The yields of seven- to nine-glucose-membered cycloisomaltooligosaccharides at a constant space velocity increased with an increase in the amount of CITase immobilized in the polymer brushes; however, the amounts of CIs per unit enzyme decreased with increasing degree of immobilized enzyme mutilayering. This may be due to the diffusional mass-transfer resistance of dextran through the CITase-immobilized graft chains and the conformational change of CITase induced by multilayering and cross-linking.

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