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Studies of Syntheses and Permeabilities of Special Polymer Membranes

61. New Method for Enzyme Immobilization by a Polyion Complex Membrane

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Summary

A new method of enzyme immobilization by a polyion complex was proposed. An enzyme immobilizing membrane was prepared by ultrafiltrating a mixture consisted of quarternized chitosan, sodium polyacrylate and invertase in an aqueous NaBr solution. The permeation and hydrolysis characteristics of aqueous sucrose solution through the invertase immobilizing membrane was studied under some conditions. A hydrolysis rate of sucrose by the invertase immobilizing membrane corresponded to the Michaelis-Menten type reaction.

Introduction

Enzymes can be immobilized by various techniques such as a covalent binding, adsorption, gel entrapment onto carriers and microcapsulation. When enzymes are immobilized, in general, their activities are lowered compared with their native state. However, there are some merits in the enzyme immobilization such as the easiness to separate enzyme and product, the possibility of continuous operation, the effective use of enzyme etc. The present report focuses on a new method of invertase immobilization by polyion complex membrane, which were prepared by applying the ultrafiltration process to a mixture containing quarternized chitosan, sodium polyacrylate and invertase in aqueous NaBr solutions, and on hydrolytic profiles of aqueous sucrose solutions through the invertase immobilizing membranes in the ultrafiltration.

Experimental Part

Materials: Chitosan (Kyowa Yusi Ind.) was a free amino groups of 74.9 mol%. Quarternized chitosan (q-chitosan) with an ion exchange capacity of 3.6 mmol/g was synthesized by stirring a mixture of chitosan (58.7 mmol) and methyl iodide (211 mmol) in a mixed solvent (1 ℓ) consisting of water (69 vol%) and methanol (40 vol%) at 45 °C for 24 h. Sodium polyacrylate

(PAANa) (Toa Gosei Chem. Ind.) with an ion exchange capacity of 9.1 mmol/g was employed as a polyion. Invertase (Wako Pure Chem. Ltd.) containing 1.0 mg in 1.0 ml of aqueous glycerol solution (50 vol%) was from yeast. All reagents used were pure grade from commercial sources. Microporous poly(propylene) film 3501 (Polyplastics Co. Ltd.) with a maximum pore diameter of 0.4x0.04 μm was used as a support of the enzyme immobilizing polyion complex membrane.

Preparation of membrane: PAANa (0.4 g) and q-chitosan (1.0 g) were separately dissolved in an aqueous NaBr solution (20 wt%). Equal volumes of these two aqueous solutions were mixed. The casting solution was prepared by diluting the mixed solution with aqueous NaBr solution (20 wt%) to 0.6 wt% in total polymer concentration.

A sketch for preparation of the enzyme immobilizing polyion complex membrane is shown in Figure 1. The casting solution (2 g) was poured onto the microporous poly(propylene) film mounted on the porous support in the ultrafiltration cell, and ultrafiltrated at 0 ^{o}C and 2 kg/cm^{2} under nitrogen gas. First polyion complex layer was formed on the poly(propylene) film. The mixed solution containing the invertase solution in 2 g of the above casting solution was put on the first polyion complex layer and second layer, in which enzyme was immobilized, was formed by ultrafiltrating at 0^{o}C and 2 kg/cm^{2}. Finally, third polyion complex, the same as the first layer, was laminated on the second layer containing invertase. The three layer immobilized polyion complex membranes were washed by permeating phosphate buffer solution (pH 5.3) at

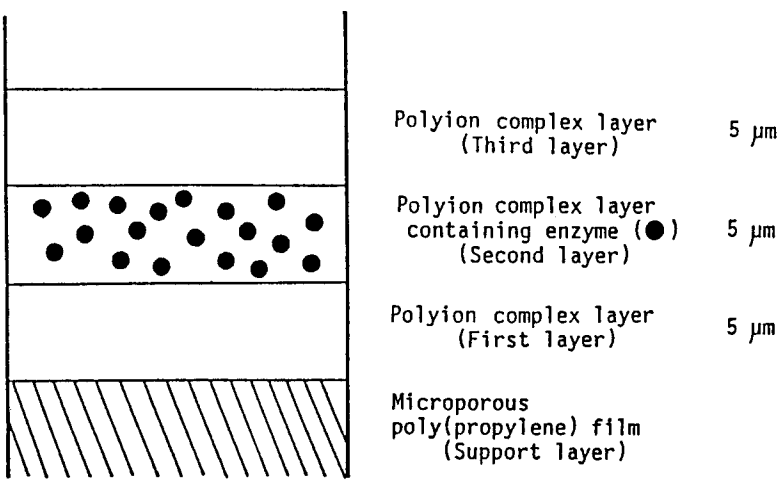

Figure 1. Sketch of the enzyme immobilization polyion complex composite membrane.

50 °C and 3 kg/cm^2 to remove NaBr completely.

Apparatus and measurements: The ultrafiltration cell used was presented in earlier papers[1,2]. The effective membrane area was 11.3 cm^2. The permeation rate was determined by weighing the permeated liquid through the invertase immobilizing composite membrane. Concentrations of sucrose, glucose and fructose were measured by high performance liquid chromatography.

Results and Discussion

Effect of initial sucrose concentration on the amount of decomposed sucrose (DA) in the permeated liquid through the invertase immobilizing polyion complex membrane with time is shown in Figure 2. The amount of decomposed sucrose was quantitative and did not change with the permeation time. In this figure open square plots were the results for the permeation of aqueous sucrose solution in pH 5.3 buffer solution after the invertase immobilizing polyion complex membrane was washed by permeating the buffer solution of pH 7.0. In this case the degree of decomposition of sucrose decreased to about 20 %. This result suggests that an activity of invertase is predominantly controlled by pH of washing and feed solutions.

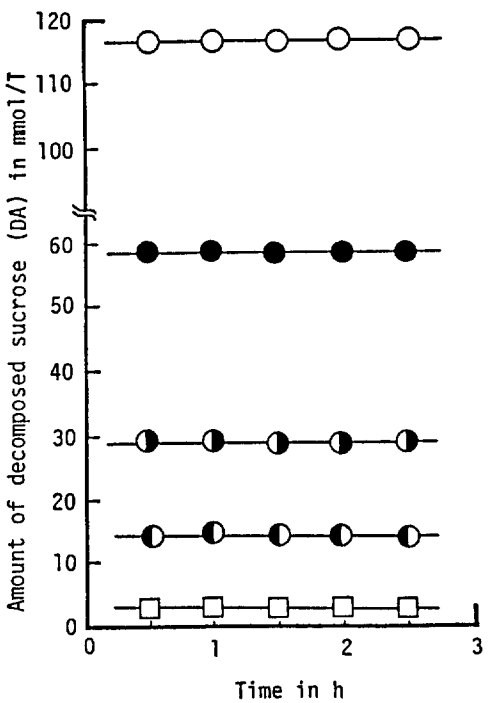

Figure 2. Effect of the initial sucrose concentration on the decomposed amount of sucrose through the invertase immobilizing polyion complex membrane with time. Initial sucrose concentration in buffer solution at pH 5.3: (◐) 14.6 mmol/l, (◑) 29.7 mmol/l, (●) 59.9 mmol/l, (○) 117.9 mmol/l. (□): aqueous sucrose solution (15.0 mmol/l) in the buffer solution at pH 5.3 was subjected to the permeation of the immobilized membrane after washing with the buffer solution at pH 7.0. Operating conditions: 50 °C, 3 kg/cm^2.

The decomposition rate of sucrose which in contact with the immobilized enzyme is defined as the amount of sucrose decomposed per time.

$$V = DA/Tr \qquad (1)$$

where V (mol/l s) is the decomposition rate of sucrose, DA (mol/l) is the decomposed amount of sucrose and Tr (s) is the residence time of the sucrose molecules in the invertase immobilizing membrane as defined by Eq. (2):

$$Tr = AL/PR \qquad (2)$$

where A (cm^2) is the effective membrane area, L (cm) is the effective membrane thickness, PR (cm^3/s) is the permeation rate. The effective membrane thickness is that of the second layer containing invertase ($5x10^{-4}$ cm). The relation between the decomposition rate of sucrose and the initial sucrose concentration is shown in Figure 3. As can be seen in Figure 3, the decomposition rate depends on the substrate concentration as its low concentration range but is not dependent on it in its high concentration range. This profile suggests the rate equation on a Michaelis-Menten type reaction.

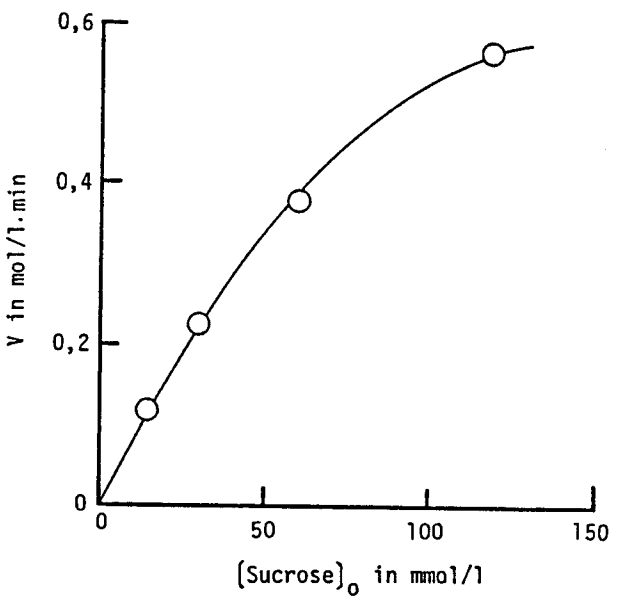

Figure 3. Effect of the initial sucrose concentration on the decomposition rate of sucrose by the invertase immobilizing polyion complex membrane at 50 ^{0}C and 3 kg/cm^2.

Figure 4 is a Lineweaver-Burk plot of the reaction. A good linear relationship is obtained. This result suggests that the decomposition of sucrose through the invertase immobilizing polyion complex membrane can be interpreted as a normal enzymatic reaction.

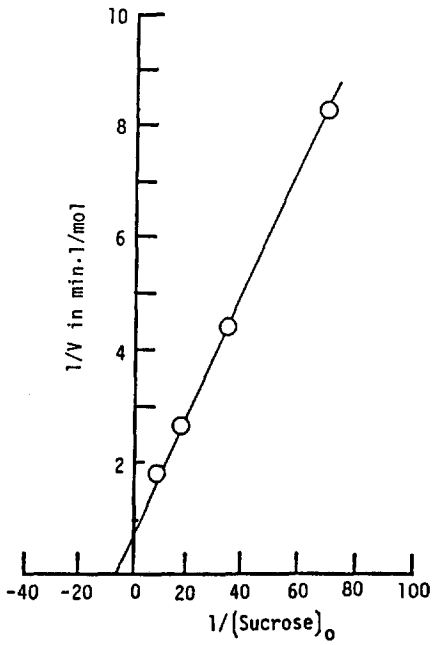

Figure 4. Lineweaver-Burk plot for the decomposition of sucrose by the invertase immobilizing polyion-complex membrane at 50 ^{0}C and 3 kg/cm^2.

The kinetic data obtained from the intercepts of axis in Figure 4 are summarized in Table 1, comparing with those of native invertase. The value of maximum decomposition rate, V_{max}, of sucrose for the invertase immobilizing membrane is greater than that for the native enzyme. This is mainly caused by the fact that the existence amount of the enzyme per unit volume is higher in the enzyme immobilizing membrane. The reciprocal of Michaelis constant, $1/K_m$, which is evaluated as the affinity constant between the substrate and enzyme molecules, is slightly larger in the system of enzyme immobilizing membrane. Polymer matrix constructet the polyion complex membrane becomes a barrier for an approach of substrate molecule to the immobilized enzyme molecule. But when the substrate molecule is once contacted the immobilized enzyme molecule, these molecules interact in the limited space in the polymer matrix.

Table 1. Kinetic data for hydrolysis of sucrose by native invertase and invertase immobilizing membrane

Enzyme	K_m (M)x10	V_{max} (MS^{-1})	$1/K_m$ (M^{-1})
Native [a)]	3.27	1.10×10^{-4}	3.06
Immobilized [b)]	1.56	2.29×10^{-2}	6.41

a) Invertase (20 μl) was used in sucrose aqueous solution (50 ml). Reaction conditions were at pH 5.3 and 50 °C for 3 h.

b) Invertase (20 μl) was immobilized in the polyion complex membrane. Permeation conditions were at pH 5.3, 50 °C, and 3 kg/cm^2 for 3h.

Consequently, the affinity constant becomes higher in the enzyme immobilizing membrane.

If some enzymes can be immobilized by the method of this report, we can easily prepare a laminate type composite enzyme immobilizing membrane.

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