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Immobilization of Glucoamylase onto Novel Porous Supports Containing Cyclic Carbonate

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Abstract: Glucoamylase was immobilized onto novel porous polymer supports containing cyclic carbonate. The relationship between activity of immobilized glucoamylase and the properties of porous polymer supports was investigated. The operation stability and storage stability of immobilized glucoamylase were studied.

Keywords: Glucoamylase, immobilization, polymer supports, cyclic carbonate.

Usually, before enzyme was immobilized onto support materials, these support materials had been activated through some activators, such as glutaraldehyde. The glucoamylase has been covalently immobilized onto several different support materials through the formation of Schiff base[1-5]. In this work, the glucoamylase was covalently (in the form of σ-bond) immobilized onto the porous polymer supports containing cyclic carbonate without activation. The relationship between the activity of the immobilized glucoamylase and the properties of porous polymer supports was investigated.

The porous polymer supports of vinylene carbonate and 2-hydroxyethyl methacrylate with different diluent agents and different amount of comonomer (2-hydroxyethyl methacrylate) were synthesized, and the porous structure and properties of the polymer supports were tested too[6]. The glucoamylase (16 U/mL) was immobilized onto porous polymer supports in acetic acid-sodium acetate buffer (pH 5.38), followed by mixing at 100 r/min for 16 h at 10°C. The activity of the glucoamylase was determined by measuring the glucose liberated from soluble starch. The amount of glucose produced was determined colorimetrically as described in reference[7], absorbance being determined at 520 nm with a double-beam spectrophotometer (HITACHI Model U-3010), using glucose solution as the standard.

The activity of glucoamylase immobilized onto porous polymer supports with different diluent agents is shown in **Table 1**. When glycerol (87%) was used as the diluent agent, the immobilized glucoamylase showed relatively higher catalytic activity than the other three diluent agents. The difference in the activity was likely caused by the different pore structure of the polymer particle. When glycerol was used as diluent agent, the pore structure was more uniform and the specific surface area was higher. From **Table 1** we can see that the activity of the immobilized glucoamylase increased

with the increasing amount of glycerol.

The activity of the glucoamylase immobilized onto porous polymer supports with different ratios of two kinds of monomers vinylene carbonate (VCA) and 2-hydroxyethyl methacrylate (HEMA) is also shown in **Table 1**. The immobilized glucoamylase showed an optimum when the ratio of the two monomers was 4:3 (mol). The results from **Table 1** indicate that the amount of VCA and HEMA used in experiments can affect on the activity of the immobilized glucoamylase. Of the two monomers, VCA has a functional group cyclic carbonate, it can react with the $-NH_2$ group on the glucoamylase. HEMA is a kind of hydrophilic monomer, and it acts as a comonomer to increase the hydrophility of the porous polymer supports and encourages the immobilization of the glucoamylase onto support materials. So only when the ratio of the two monomers is suitable, the activity of the immobilized glucoamylase can reach the optimum point.

Table 1 Effect of diluent agent and amount ratio of VCA/HEMA(mol) on activity of immobilized glucoamylase

Supports	Diluent agent	Ratio of VCA and HEMA(mol)	Activity of immobilized enzyme (U/g)
S1	Ethanol (100%)	3:2	101.3
S2	Ethylene glycol (100%)	3:2	157.0
S3	Glycerol (17%)	3:2	126.9
S4	Glycerol (35%)	3:2	144.8
S5	Glycerol (87%)	3:2	189.3
S6	Ethanol (100%)	2:1	82.4
S7	Ethanol (100%)	4:3	105.8
S8	Ethanol (100%)	2:3	99.1

In general, the native enzyme only can be used one time, and the residual enzyme can not be separated with products, and then to be deactivated, whereas the immobilized enzyme can be used repeatedly, if its activity does not decrease strongly. We investigated the ability of the immobilized glucoamylase after repeated usage, and the results are shown in **Figure 1**. After repeated usage for 7 times, the activity of the immobilized glucoamylase still maintained about 85% of its original activity, and is no dependent on repeated number.

The native and immobilized glucoamylase were stored at 0-5°C and tested for their activities (**Figure 2**). The activity of the native enzyme decreased to 58% of its initial activity after storage for 23 days, but the immobilized glucoamylase still maintained about 84% of its initial activity after storage for 26 days.

Figure 1 Operating stability of the immobilized glucoamylase

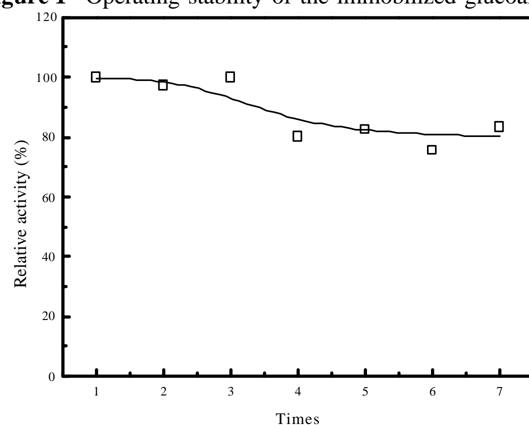
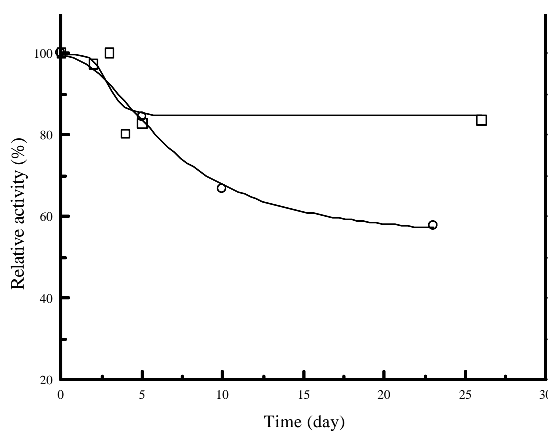


Figure 2 Storage stability of immobilized glucoamylase and native glucoamylase



(□), immobilized glucoamylase; (○), native glucoamylase

In summary, the novel porous polymer supports containing cyclic carbonate can be used to covalently immobilize the glucoamylase. This operation can be carried out under mild conditions without activation. During immobilization, the denaturation of enzyme was slight. After immobilization, the glucoamylase has optimum catalytic activity under some suitable conditions. After storage for 26 days, the immobilized glucoamylase still obtained about 84% of its initial activity, and after repeated usage for 7 times, it still maintained about 85% of its initial activity. In future the novel porous polymer supports may be used to immobilize several enzymes that have free amino groups.

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