CHEMISTRY LETTERS, pp. 1323 - 1326, 1984.

GLUCOAMYLASE IMMOBILIZED IN POLYSTYRENE FILM

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Glucoamylase was immobilized in a polystyrene film by the method where the enzyme was suspended in benzene containing polystyrene and subsequently the mixture was casted on a glass board to make a film. The activity of the enzyme for soluble starch was found to be maintained through the immobilization.

Utilization of enzymes for industrial production has been desired because of the specific and effective activities of the enzymes for the substrates. A problem for the actualization of this attempt has been a high cost arising from easy denaturation of enzymes and the difficulties in their recovery from the reaction mixture. One of the most powerful methods to overcome this problem is immobilization of enzymes on solid matrices and this has been exclusively used for the industrial purposes.[1,2] However, it has been believed that organic solvents can not be used because their usage causes the fatal enzymic denaturation. The purpose of this study is, therefore, to explore the possibility of the immobilization of glucoamylase in organic solvents.

Glucoamylase[3,4] and reagents used in the present study were purchased from Nakarai Chemicals Co. Ltd. and they were used without further purification. A substrate used was soluble starch and enzymatic activity of both the immobilized (IG) and intact (GA) glucoamylase were determined by measuring the amount of glucose released from the substrate by the hydrolysis with these enzymes. The sugar yield was determined by the Somogyi-Nelson method.[5]

In order to investigate the effect of organic solvents on the denaturation of GA, the enzyme was added to several organic solvents and the suspension was

Table 1. Effect of organic solvents on the activity of GA

Solvent	Power unit / U mg^{-1}
Benzene	717
Ether	685
Toluene	679
Cyclohexane	649
Ethyl acetate	611
Carbontetrachloride	610
Acetone	712
Acetone + H_2O (10%)	695
Acetone + H_2O (20%)	658
None	727

allowed to stand for 12 h at 10 °C. Subsequently residual activity of the enzyme, which was recovered from the suspension by filtration and dried, was measured. As shown in Table 1, benzene was found to be the most desirable for the immobilization among the solvents tested. Although acetone showed preferred behavior, addition of slight amount of water led to the decrease in the residual activity. Therefore, in the present study, benzene solution containing polystyrene (PS) was used for the immobilization. GA was added to the solution and IG was obatined as a film by casting the suspension on the glass board. As shown in Fig.1 by the Lineweaver-Burk plot, thus obtained IG maintained original enzymatic behavior of GA although the constants were changed. The effect of the ratio of GA and PS on the activity of IG was also studied and it was found that when PS concentration was 1%, the preferred weight ratio was ca. 1/10 (GA/PS).

The suitable conditions for the use of IG were explored for pH and temperature. The optimum pH was found to be shifted from 7.0 (GA) to 6.0 (IG) by the immobilization while the optimum temperature was unchanged (50 °C). Under these optimum conditions, it was determined that the activity per mg

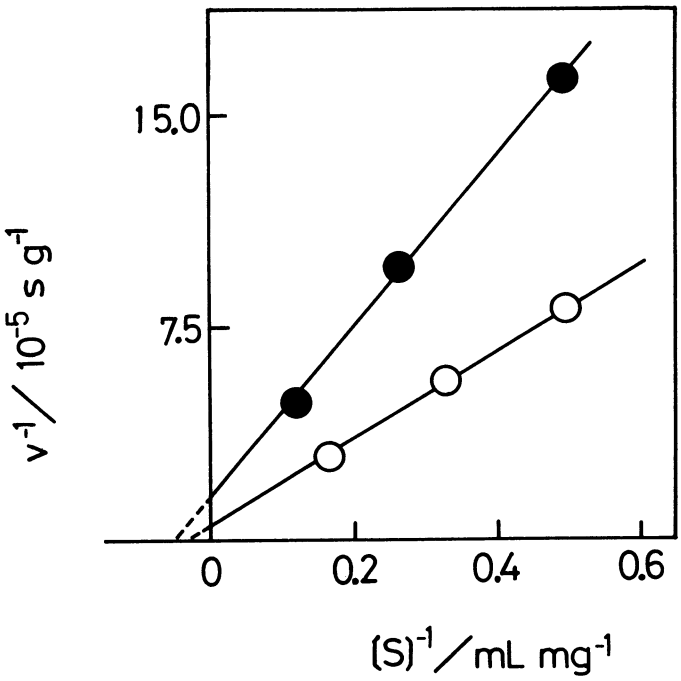

Fig.1. Lineweaver-Burk plot for GA (○) and IG (●). The ratio GA/PS in the preparation was 1/10. The term v is the reaction rate for the substrate concentration of [S].

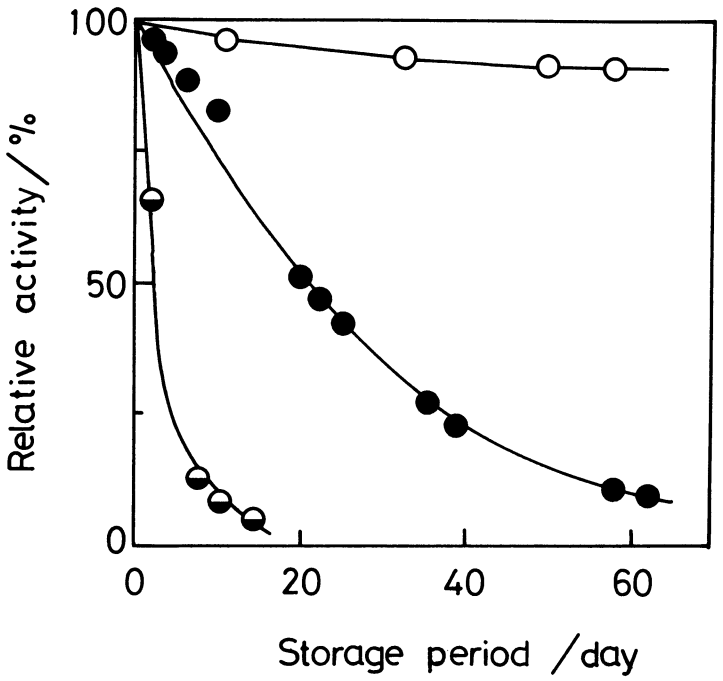

Fig.2. Effect of the storage conditions on the activity of IG. IG was stored dry at 4 °C (○) and in a 1/10 M phosphate buffer (pH 6) at 4 °C (●) and 20 °C (◒).

enzyme in IG was 25% of that of GA. This may mean that a few ten percent of the immobilized enzymes were fixed on the surface of the film while the rest was stuck in the film. Also, the stability of both IG and GA was investigated by measuring under these optimum conditions the residual activities of the enzymes which were allowed to stand at the given pH and temperature for 1 h before the measurement. When the stability against change in one of the factors was studied, the other factor was kept at the optimum value during the storage. As a result, it was found that both the properties were unchanged by the immobilization.

For the practical use of IG, the effect of the storage conditions on the residual activity was studied (see Fig.2). When IG was kept dry at 4 °C, approximately 95% of the original activity was found to remain for 60 d while IG lost its activity readily in solution. Since IG can be dried easily after the preparation, this result implies the advantage of the method used in the present study. Analogous experiments were also carried out for GA and it was found that the rates of the decrease in the enzymic activity of IG were almost equal to those of GA. Also, the enzymic denaturation of IG by the repeated usage was investigated by the successive measurements of the activities under the optimun conditions found here. Each measurement led to the decrease in the activity by ca. 20%.

In summary, we found that the method where GA was immobilized by use of PS benzene solution was effective for the practical purpose of utilizing GA in the industrial purposes.

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(Received May 9, 1984)