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IMMOBILIZATION OF ENZYME TO GRAFT-POLYMER ON GLASS SURFACE

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The immobilized glucoamylase (from Rhizopus niveus) was prepared by amidination reaction to partially imidoesterized polyacrylonitrile which had been grafted onto flaky glass surface preirradiated by electron-accelerator.

Several numbers of investigations have been reported so far regarding the immobilization of enzyme onto inorganic carriers.[1-3] Inorganic carriers are very useful for its dimensional stability and for its inertness to the enzyme activity. There has not been studied on the immobilization of enzyme onto polymers grafted onto inorganic carriers surface. In the enzymatic reaction, it can be expected that the immobilized enzyme is less structurally shielded than when it is entrapped within a polymer matrix. The preparation of polymers grafted onto alumino silicate glass by preirradiation method have been previously reported.[4-6] The benefit of this method is that we can feasible graft polymers onto silicate carriers such as glass and control the molecular weight or the number of grafted chain. The present paper is concerned with the preparation and activities of the immobilized glucoamylase by amidination reaction to imidoesterized polyacrylonitrile which had been grafted preliminary onto flaky glass surface.

Preparation of graft-polymer.

2.5 g of flaky Pyrex glass from Owence Corning Corporation was treated with $K_2Cr_2O_7$-H_2SO_4 solution for 3 days as previously reported,[4] and was dried after washing with distilled water. The flaky glass was irradiated by Hitachi Van de Graff type electron beam accelerator (1.5 MeV & 10 μAmp) with 10 Mrad total dosage at room temperature. It was sorked into acrylonitrile monomer at room temperature for 2 days. Then, the polymer-glass conjugate was washed with N,N-dimethylformamide in order to remove acrylonitrile monomer and homopolymer, and dried in vacuo. The yield of grafted polymer was shown in Table 1.

Preparation of immobilized glucoamylase.

Graft-polymer onto glass surface was partially imidoesterized by dry hydrogen gas in methanol at 0°C for 3 hours,[7,8] and was washed with excess methanol. Then, 1.5 g of activated polymer-glass conjugate was suspended with 62.5 mg enzyme (16.9 units/mg) in 25 ml of water. The immobilization reaction was carried out under stirring at 30°C for 1 hour, and the product was washed twice with distilled water or buffer under stirring at 30°C for 1 hour.

Assay of protein content.

500 mg of the immobilized enzyme was hydrolyzed with 6N-HCl at 110°C in a sealed tube for 48 hours according to the procedure of Crestfield, Moore and Stein.[9] The amino acid in the suspension was assayed by means of paper chromatography using ninhydrin.[10] The protein content was shown in Table 1.

The reusabilities of the immobilized enzymes were compared with those of the simply adsorbed ones on the glass surface by means of the repeated test of hydrolysis of 1% w/v soluble starch under rigorous stirring at a 400 rpm and at 40°C. The evaluation on their activities was defined with the amount of produced glucose by the method of Somogyi-Nelson.[11,12] It was observed that the remained activities of the immobilized enzymes were 45% or 34% of the initial ones after 10 times of repeated test as illustrated in Fig. 1.

As for the leakage of the enzyme from the carrier, the activities of the simply adsorbed ones decreased clearly by the repeated washing. It was also recognized that the decreased activities of the immobilized enzymes were almost due to the leakage of the enzyme because decreasing enzymatic activities were observed in the filtrates of each wash. Therefore, the activity yield of the immobilized ones was determined from the activity after 10 times washings, and shown in Table 1. The activity yields were found to be 28.6% or 31.9% comparing with the activity of the equivalent weight of native enzyme.

Table 1.

Enzyme conditions of washing	Adsorbed distilled water (●)	Immobilized distilled water (○)	Immobilized McIlvaine (◉) buffer pH 8.3
initial activity (μg/ml.min.g)	47	100	77.4
remained activity (10 times) (μg/ml.min.g)	0	34	34.8
protein content (μg/g)	—	848	780
graft-polymer content (mg/g)	—	13.4	13.4
activity yield (%)	—	28.6	31.9

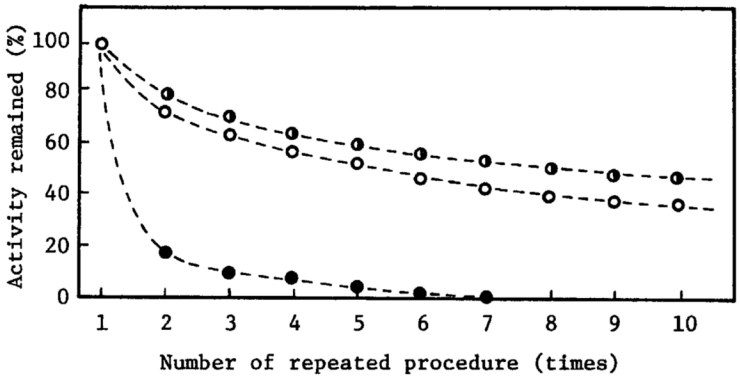

Fig. 1. Durability tests of the immobilized enzyme and adsorbed ones.

These results indicate that the enzyme was immobilized by the amidination reaction, while repeated washing was necessary to remove the adsorbed activity.

In order to obtain higher yield of graft-polymer and protein content, the highly Al_2O_3 contaning silicate[4] is expected to be more reliable carriers.

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