Preparation and Properties of Immobilized Glucoamylase on a Magnetically Anisotropic Carrier Comprising a Ferromagnetic Powder Coated by Albumin

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Glucoamylase was immobilized by glutaraldehyde combined with albumin coated on the surface of a powder of $SrFe_2O_4$ as the carrier. This carrier obtained a magnetically anisotropic property under exposure to a static magnetic field. The carrier was able to oscillate and rotate in an alternating magnetic field. The rate of hydrolysis of starch to glucose increased in the alternating magnetic field. This is because the transport of molecules may be facilitated through the interface of the carrier surface and solution by oscillation.

There have been many papers concerning immobilized enzymes on a ferromagnetic powder used as a carrier.[1–5] The magnetic carrier has the advantage of being a collecting carrier by magnetic attraction; aggregation of the magnetic carrier due to a cohesive property gives rise to a lowering of the apparent activity of an enzyme. A group of authors has reported on a study regarding magnetically anisotropic gel beads (MA gel beads), which include a ferromagnetic powder exhibiting a strong coercive force.[6–8] Since the MA gel beads had the property of being able to oscillate in an external alternating magnetic field (AMF), the diffusion layer on the surface between the MA gel beads and the solution could be thinner, owing to this oscillation. In turn, the transport of molecules through the surface could be facilitated. However, these MA gel beads were supposed not to be very useful for enzyme reactions concerning high molecular substrates, since the transport of a high molecular substrate inside gel beads might be difficult. It was, consequently, expected that the enzyme was immobilized on the surface of the carrier, which exhibited a magnetically anisotropic property (defined as MA carrier).

The purpose of this study was to immobilize enzymes (glucoamylase) on the surfaces of MA carriers, and to examine the property of immobilized enzymes as well as the effect of an external AMF on the rate of hydrolysis of starch to glucose.

Experimental

Materials. The Sr-ferrite ($SrFe_2O_4$) used exhibited the following properties: saturation magnetization of 64 emu g^{-1}, a coercive force of 3.2×10^3 Oe (1 Oe=1000/4 π A m^{-1}), and plates 0.2—10 μm long. The glucoamylase used was obtained from Sigma Chemical Co. (Product No. A-7255, crude, from Rhizopus mold). Egg-albumin, soluble starch and other chemicals used were either of guaranteed grade or the best commercially available.

Preparation of Immobilized Glucoamylase. Into 2 g of distilled water, 0.5 g of egg-albumin was added in order to be dissolved; then, 1 g of $SrFe_2O_4$ was added and stirred well to make a suspension. This mixture was poured into 200 cm^3 of hot water with stirring in order to coagulate albumin on the surface of $SrFe_2O_4$. A powder of $SrFe_2O_4$, which exhibited a magnetically isotropic property, was coated by albumin and collected by an Sm–Co magnet and then washed with distilled water. The magnetically anisotropic property of $SrFe_2O_4$ coated by albumin resulted from a collecting process by the Sm–Co magnet. The MA carrier was mixed and reacted with 5 cm^3 of 50% glutaraldehyde at 30 °C during a 12 h period in an AMF (100 Oe, 50 Hz), and then washed with distilled water. The powder was mixed and reacted with 5 cm^3 of a glucoamylase solution containing the requisite concentration over a 1 h period in the same AMF, and then washed with distilled water. We then obtained immobilized glucoamylase comprising the MA carrier, the size of which was 0.1—0.3 mm. A schematic diagram is given in Fig. 1.

Properties of Immobilized Enzymes. The activities of immobilized enzymes were surveyed under various conditions. Ten—300 mg of crude enzyme was immobilized on the surfaces of MA carriers prepared by the above-mentioned process

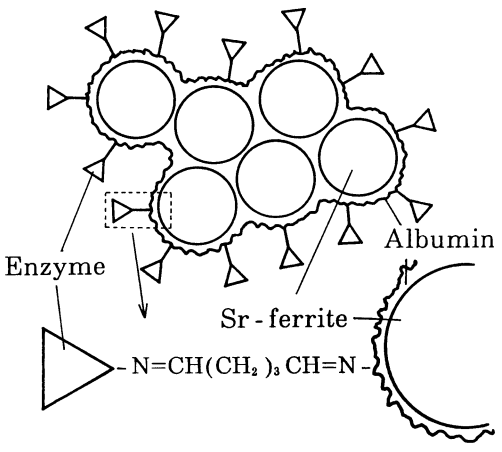

Fig. 1. Schematic diagram of the immobilized enzyme on the MA carrier.

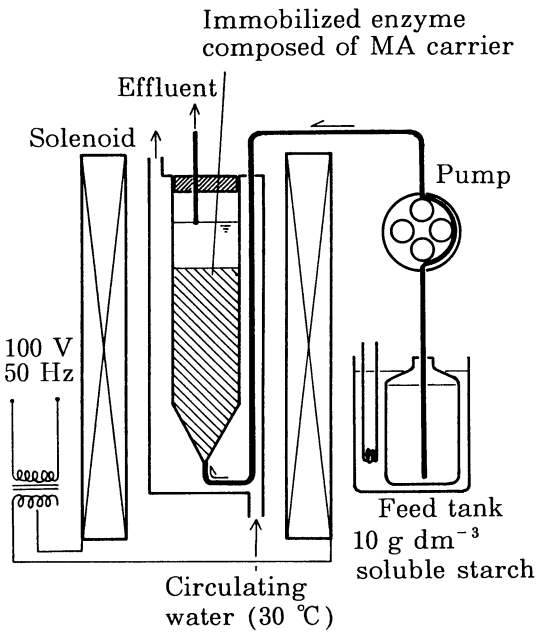

Fig. 2. Equipment for the continuous hydrolysis of 10 g dm^{-3} soluble starch in the AMF.

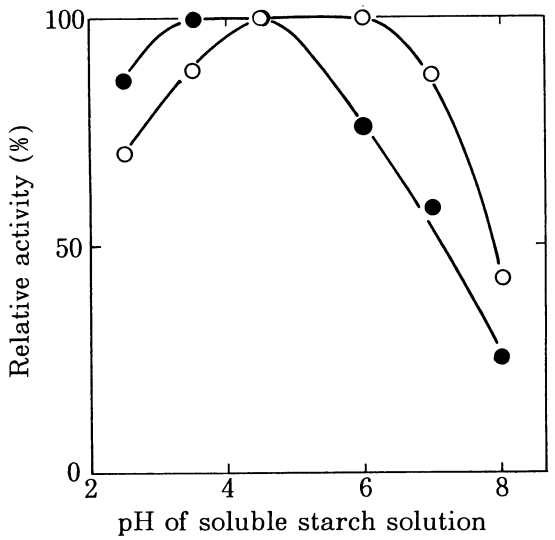

Fig. 3. pH profile of immobilized (○) and native (●) glucoamylase. The MA carrier immobilizing 10 mg of crude enzyme was examined in order to determine the relative activity. At 100% relative activity, both the activities of the native and immobilized enzyme were equal to 9.0 and 3.3 unit mg^{-1} of the enzyme, respectively.

with 1 g of $SrFe_2O_4$, 0.5 g of albumin and 2 g of water (approximately 3 g of wet MA carrier). Fifty cm^3 of 45 g dm^{-3} soluble starch were hydrolyzed by this immobilized enzyme under a pH of 2—8 in a 100 ml-jacketed beaker with a stirrer rotating at 80 rpm. One gram of a substrate was converted to 4.6 mmol of glucose by the catalysis of glucoamylase. Consequently, the hydrolysis (%) was defined as the ratio of the glucose concentration to 46 mmol dm^{-3} of glucose when 10 g dm^{-3} of soluble starch was used as a substrate solution. The following buffer solutions were used: a mixture of glutamate and dipotassium hydrogenphosphate at pH 2.5; that of acetic acid and potassium acetate in the range between pH 3.5—6.0; and that of potassium dihydrogenphosphate and dipotassium hydrogenphosphate in the range between pH 7.0—8.0. All of the buffer solutions were prepared as 0.1 mol dm^{-3} of the ionic strength. The glucose obtained by hydrolysis was determined by the Hanes' method with 0.1—0.5 cm^3 of solution pipetted out at every requisite time. The enzyme activity was determined from the rate of glucose formation. One enzyme unit is defined as the amount of enzyme that liberates 1 μmol of glucose min^{-1}.

The Bioreactor in an Alternating Magnetic Field. The enzyme reaction was carried out using a column bioreactor, as shown in Fig. 2. The column comprised a jacketed glass-tube (1 cm in inside diameter). The column was placed at the center of axis of the solenoid (5 cm in inside diameter, 15 cm long). The immobilized enzyme prepared with 2 g of $SrFe_2O_4$, 1 g of albumin and 4 g of water (as mentioned in above section) was filled in the column, the height of which became 9 cm. The temperature of the column was kept at 30 °C by circulating water. Soluble starch was used as a substrate. Ten g dm^{-3} soluble starch solution was flowed upward in the column at 45 cm h^{-1}.

Results and Discussion

pH Profile. The pH profiles of both immobilized and native enzymes are given in Fig. 3. The optimum pH of the immobilized enzyme was higher than that of the native one. Many carboxyl groups remained on the surface of the carrier, since glutaraldehyde reacted with the amino groups belonging to the enzyme or albumin. This would cause a lowering of the local pH on the carrier. The pH of the soluble starch solution, itself, in this work was 5—6, which was optimum for the immobilized enzyme. Thus, a buffer solution was not

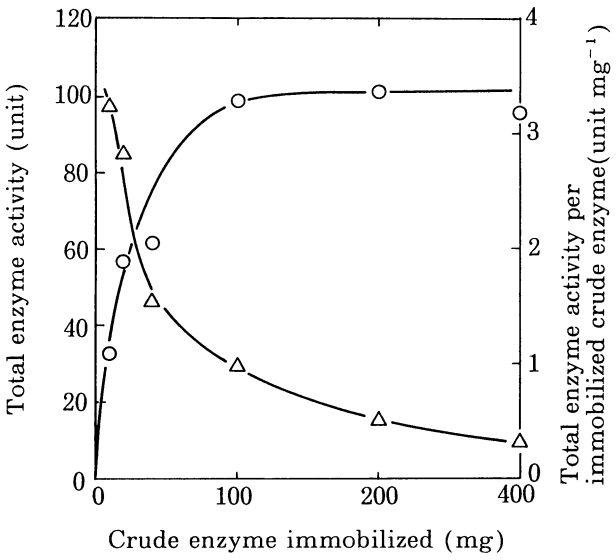

Fig. 4. Relationship between the amount of crude enzyme immobilized and its enzyme activity. ○: total enzyme activity, △: total enzyme activity per crude enzyme immobilized.

necessary for the pH control of the starch solution in a long-term experiment.

The Effect of the Amount of Enzyme Immobilized on the Enzyme Activity. The relationship between the amount of crude enzyme immobilized and the total enzyme activity is shown in Fig. 4. The total enzyme activity increased upon increasing the amount of crude glucoamylase over the range of 0—100 mg, and reached a plateau at more than 100 mg. Also, the enzyme activity per immobilized crude enzyme is shown in Fig. 4. The less was the amount of enzyme immobilized, the greater was the total enzyme activity. As compared with the same amount of native enzyme, the activity of which is 9 unit mg^{-1} under the condition of pH 4.5 and 30 °C, the immobilized crude enzyme gave 36% of activity (41% by applying an AMF of 200 Oe) in the case that 10 mg of the crude enzyme was immobilized. This is better than the other results, in which the recovered yield of activity against the total amount of glucoamylase or protease, which catalyzes the substrate of high molecular compound, was reported to be 2—20%.[9-12]

Magnetic Effect on Fluidization of the Carrier. The enzyme reaction was carried out under the exposure of the AMF in a column (Fig. 2). The MA carrier was observed to oscillate and rotate as it was synchronized with the frequency of the AMF, and fluidization occurred in the column. The glucose concentration in the effluent could be increased by applying the AMF accordingly. Consequently, the apparent activity of the immobilized enzyme (C_{AMF}/C_0) increased as shown in Fig. 5. This suggested that the movement of the carrier might facilitate molecular transfer on the surface of the carrier. Fluidization might prevent clogging and channelling in the column; it provided the advantage that the flow rate of the substrate solution was freely controllable, since fluidization occurred regardless of the flow rate in the column. The relationship between the AMF and the relative activity of the enzyme is shown in Fig. 6. The relative activity is defined as 1 in the absence of the AMF. The relative activity increased up to about 100 Oe of magnetic strength, reaching the limit at more than 100 Oe at a frequency of 50 Hz. Partial fluidization occurred at a lower intensity of the AMF. The MA carrier fluidized completely at an intensity of about 100 Oe. The fluidized bed expanded with a stronger movement of the MA carrier at higher intensities than 100 Oe. Judging from the result given in Fig. 4, a sufficient magnetic effect was obtained to transport molecules by complete fluidization at about 100 Oe.

Long-Term Experiment in an Alternating Magnetic Field. A long-term experiment was carried out under exposure of the AMF (100 Oe, 50 Hz). The results are

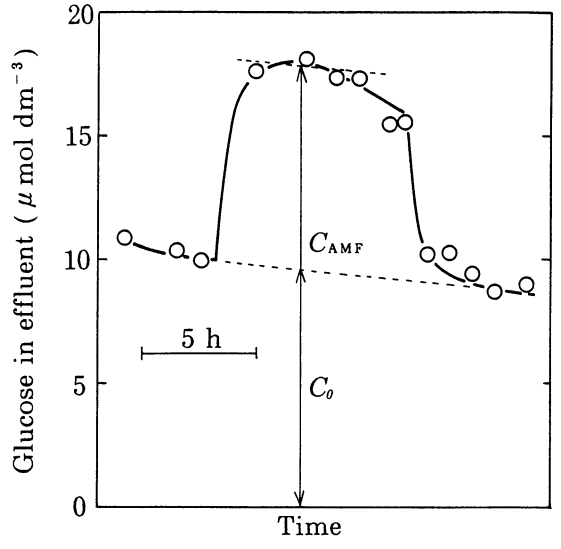

Fig. 5. Concentration change of glucose in the effluent when the AMF (100 Oe, 50 Hz) was on-and-off. The increase in the apparent activity was defined as C_{AMF}/C_0.

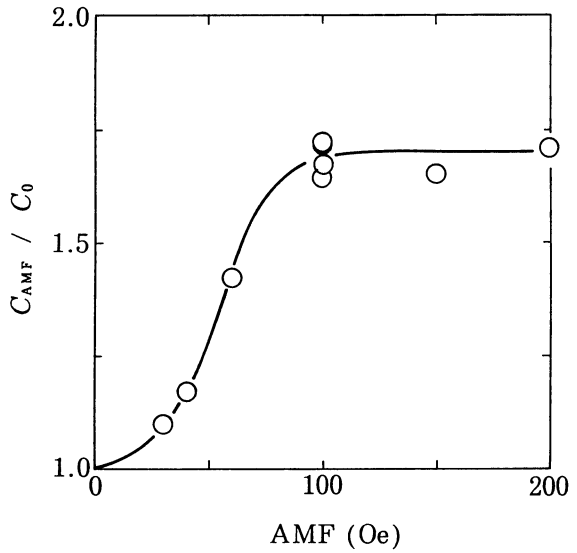

Fig. 6. Relationship between the AMF and C_{AMF}/C_0.

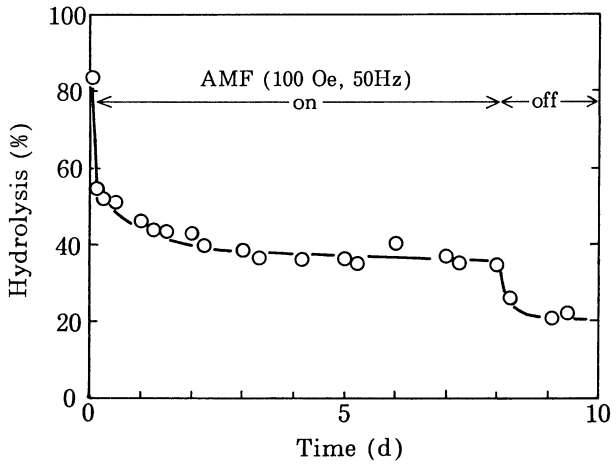

Fig. 7. Consecutive hydrolysis from soluble starch to glucose in the AMF.

given in Fig. 7. The enzyme activity was found to remain almost constant after 3—8 d of reaction time, despite the rate of hydrolysis, which decreased in the beginning. The enzyme activity decreased to about 1/2 after turning the AMF off on 8 d. In other words, the magnetic field created an increase in the apparent activity of the immobilized enzyme. Clogging in the column was prevented by fluidization of the carrier in the AMF during the reaction.

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