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Immobilization of glucosyltransferase from *Aureobasidium*

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SUMMARY

Glucosyltransferase from *Aureobasidium*, which produces panose and isomaltose from maltose, was immobilized by alginate gel or DEAE-cellulose at high efficiency (71 and 41% respectively). Alkylamine porous silica was less efficient as a support. The enzymatic profiles of immobilized enzymes were almost identical to the native one except that their stabilities to extreme pH, metal ions and inhibitors were improved. Both immobilization procedures successfully produced high amounts of panose, 125 mg ml^{-1} (alginate gel) or 141 mg ml^{-1} (DEAE-cellulose), from 300 mg ml^{-1} of maltose.

INTRODUCTION

In previous papers, we reported the production, purification and properties of glucosyltransferase (EC 2.4.1.24) from *Aureobasidium* [3–5] which produces panose and isomaltose from maltose. We have investigated the immobilization of glucosyltransferase to construct a bioreactor system for industrial production of isomalto-oligosaccharides such as panose. While the immobilization of neopullulanase for the production of panose by the hydrolyzing reaction was reported [8], there is little information about the immobilization of glucosyltransferase.

In the present paper, we examined three methods, namely alginate gel (entrapment), DEAE-cellulose (ionic bond) and porous silica (covalent bond) for the immobilization of glucosyltransferase from *Aureobasidium* sp. ATCC 20524 and describe the immobilization efficiencies and the enzymatic properties of the preparations.

MATERIALS AND METHODS

Cultivation and preparation of enzyme

Aureobasidium sp. ATCC 20524 was cultivated for glucosyltransferase production in liquid culture (maltose 2.5%, yeast extract 1.5%, K_2HPO_4 0.75%, $MgSO_4 \cdot 7H_2O$ 0.05%, pH 7) at 30 °C for 2 days under the same conditions as described previously [3]. Glucosyltransferase was solubilized by Kitalase (endo-β-1,3-glucanase; Wako Pure Chemical Industries Ltd, Osaka, Japan) and purified by fractionations using ammonium sulfate and S-Sepharose Fast Flow, DEAE-Cellulofine and Sephadex G-200 chromatography before immobilization as described in a previous paper [3].

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Preparation of immobilized enzyme

Immobilization of the enzyme (5.1 μkat mg^{-1} protein) using alginate gel, DEAE-cellulose and porous silica was carried out as follows:

Alginate gel. The immobilization was carried out by dropping an aqueous solution (10 ml) containing Na-alginate (2–10% w/v; Wako) and the enzyme (0.33–1.7 mkat) into a gently stirred 5% (w/v) $CaCl_2$ solution (50 ml), and then curing the resultant gel (approx 4 mmϕ) in the solution with stirring for 1 h as described previously [7]. The excess enzyme was then washed off with water and the preparation was used for further experiments.

DEAE-cellulose. DEAE-cellulose (0.1–1 g) [1] was treated in 0.5–5 ml of 0.5 M NaOH and then washed with water before utilization for immobilization of enzyme. Enzyme solution (1.7–8.3 mkat) and the prepared support (0.1–1 g) was mixed in a total volume of 3–30 ml and the mixture was stirred at room temperature for 1 h and the excess enzyme was then washed off with water as described previously [6]. The preparation was used for further experiments.

Porous silica. The alkylamine porous silica [2] (0.3–1 g) was activated by addition of 2.5% (v/v) glutaraldehyde (3–10 ml) with stirring for 1 h, and excess glutaraldehyde was washed off with water. Enzyme solution (0.17–8.3 mkat) and the activated support (0.1–1 g) were mixed in a total volume of 5–50 ml and stirred at room temperature for 2 h and the excess enzyme was then washed off with water as described previously [2]. The preparation was used for further experiments.

Enzyme activity assay

The appropriate amount of immobilized enzyme (approx 17 nkat) was employed in the reaction mixture [2,6,7]. The enzyme was assayed using 30% (w/v) maltose as substrate in a total volume of 1 ml of 75 mM McIlvain buffer, pH 5. The

reaction was carried out at 65 °C for 10 min and stopped by boiling for 10 min as described previously [3].

Glucose released in the reaction mixture was measured using glucose oxidase (Glucose test B; Wako). Panose and other products were measured by HPLC with μ-Bondaspare NH_2 (3.9 × 15 mm, Waters, Tokyo, Japan) as described previously [3]. One katal of enzyme activity is defined as the quantity of enzyme responsible for the transfer of 1 mol of glucose per second.

The values given in Results and Discussion are the means from at least three experiments.

RESULTS AND DISCUSSION

Immobilization of enzyme

The effect of alginate concentration on enzyme immobilization is shown in Fig. 1. While a wide range of alginate concentration, 2–10% (w/v), gave high immobilization efficiency, more than 56%, the optimum concentration was 4% (w/v).

The effect of enzyme concentration on the efficiency of immobilization using alginate gel and DEAE-cellulose is shown in Figs 2 and 3, respectively. There was a tendency to increase the amount of immobilized enzyme with the increase of enzyme concentration, and the maximum efficiency of immobilization, 71% (alginate gel) and 41% (DEAE-cellulose), was found with 0.17 and 5 mkat g^{-1} support of added enzyme, respectively. While the immobilization efficiency of alginate gel was higher than that of DEAE-cellulose, the amount of immobilized enzyme per g support of DEAE-cellulose (2.1 mkat g^{-1}) at the maximum efficiency was approximately 18-fold of that of alginate gel (0.12 mkat g^{-1}). Immobilization using DEAE-cellulose was completed within 1 h after addition of enzyme to the support (Fig. 4).

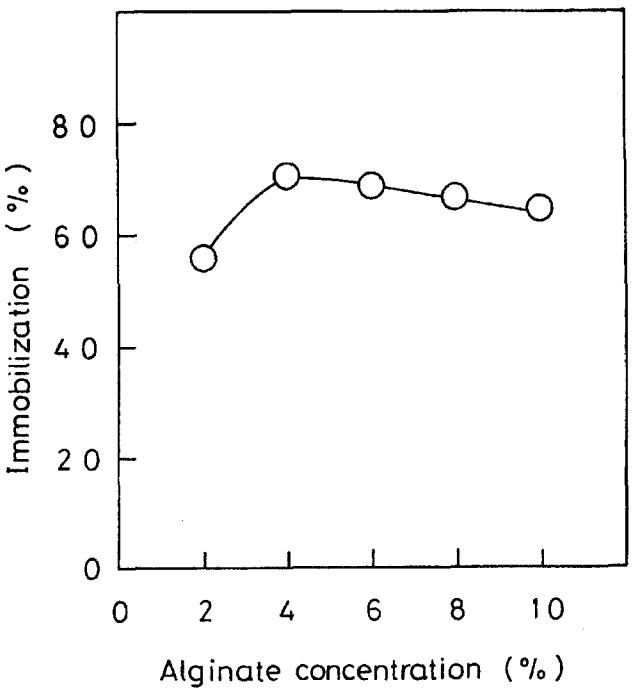

Fig. 1. Effect of alginate concentration on enzyme immobilization using 0.17 mkat g^{-1} support of enzyme.

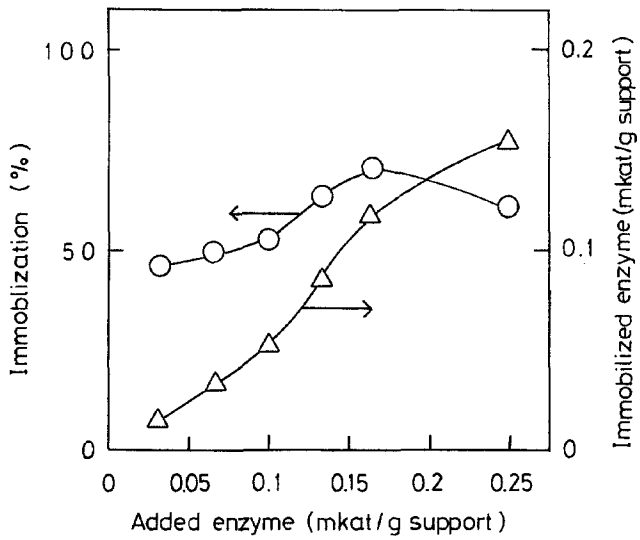

Fig. 2. Effect of the amount of added enzyme on immobilization by alginate gel. Symbols: immobilization, ○—○, immobilized enzyme, △—△.

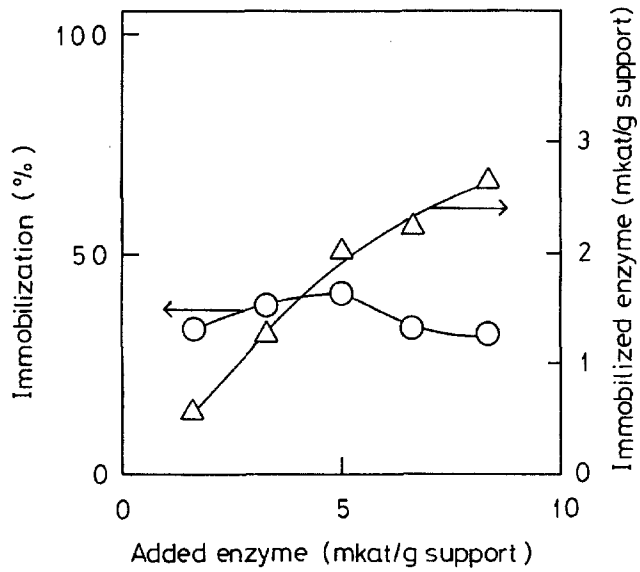

Fig. 3. Effect of the amount of added enzyme on immobilization by DEAE-cellulose. Symbols: immobilization, ○—○, immobilized enzyme, △—△.

While immobilization of the enzyme using alkylamine porous silica at various conditions (pore size, amount of amino groups on the support and the amount of added enzyme) was carried out as described previously [2], the efficiency was lower than 6%. So alginate gel and DEAE-cellulose were selected as the support for further experiments.

Properties of the immobilized enzyme

The effect of reaction pH on the activity of immobilized and native enzymes is shown in Fig. 5. The optimum pH of the enzyme, 5 [3], was not changed after immobilization by

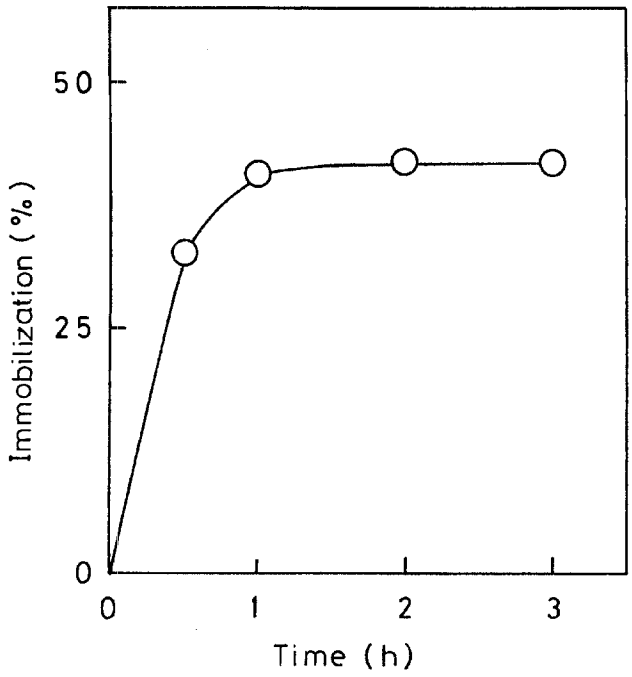

Fig. 4. Time course of enzyme immobilization by DEAE-cellulose.

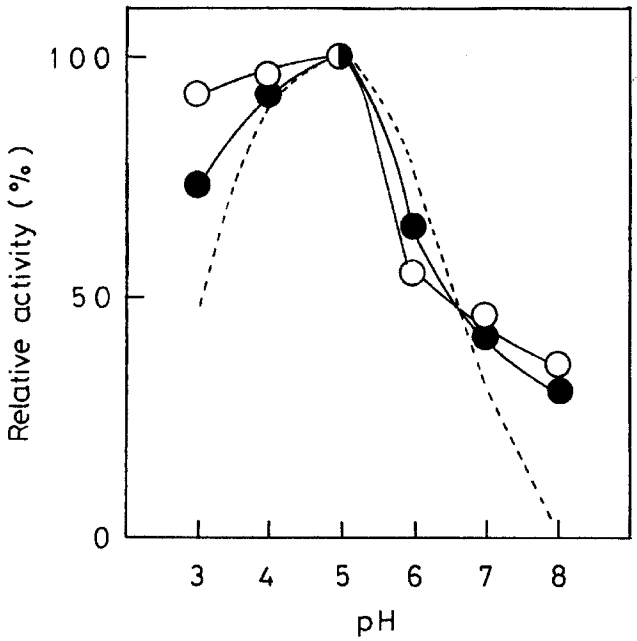

Fig. 5. Effect of pH on the activity of immobilized and native enzymes. Symbols: immobilized enzyme, alginate gel ○—○, DEAE-cellulose ●—●; native enzyme, -------.

either method. The stabilities of immobilized and native enzymes at various pHs are shown in Fig. 6. Both immobilization procedures resulted in enzyme which was stable within the range of pH 4–6 and which retained more than 96% of its maximum activity after 3 h. The enzyme released from support was not detected at acidic and alkaline pHs. Stability of the native enzyme at pH 3 and 8 [3] was increased after immobilization.

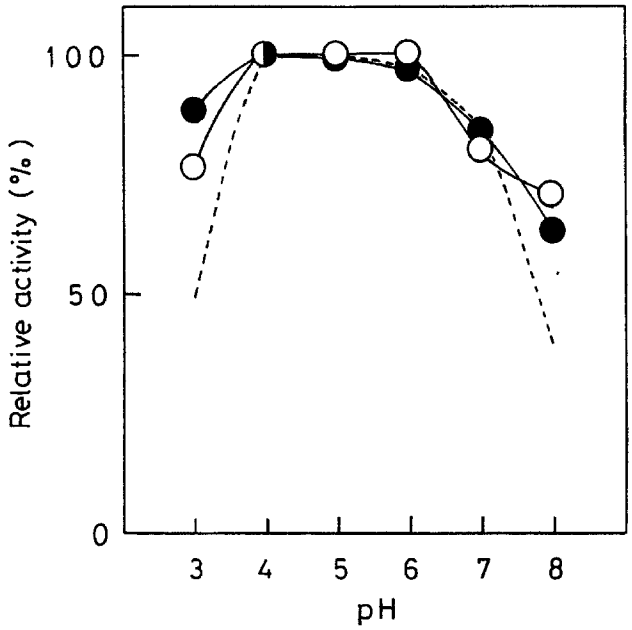

Fig. 6. Effect of pH on the stabilities of immobilized and native enzymes. Symbols: immobilized enzyme, alginate gel ○—○, DEAE-cellulose ●—●; native enzyme, -------. The activities were measured after 3 h incubation at each pH.

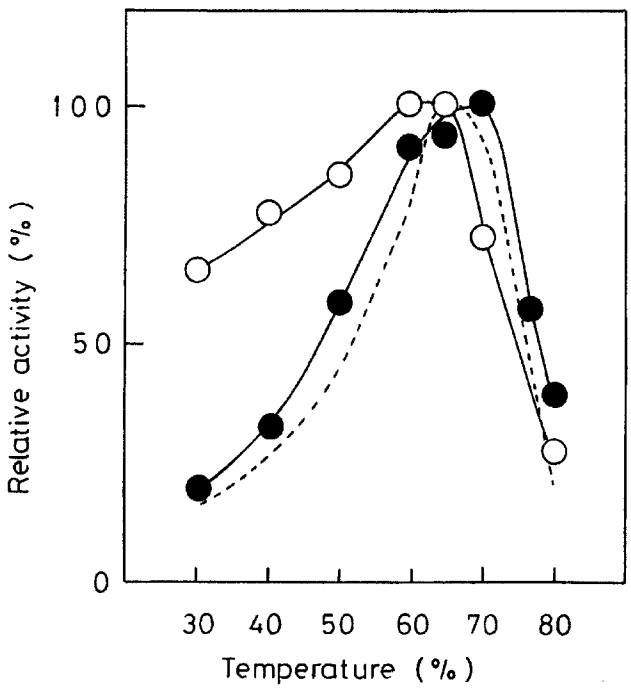

Fig. 7. Effect of temperature on the activities of immobilized and native enzymes. Symbols: immobilized enzyme, alginate gel ○—○, DEAE-cellulose ●—●; native enzyme, -------.

The effect of reaction temperature on the activity of immobilized and native enzymes is shown in Fig. 7. The optimum tempeature of both immobilized enzymes, 65–70 °C, was almost identical to that of the native enzyme [3].

The stability of immobilized and native enzymes at various

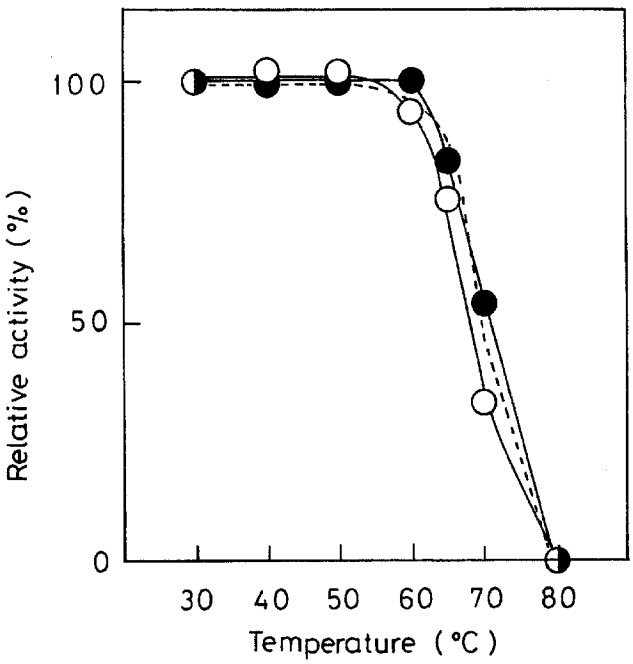

Fig. 8. Effect of temperature on the stabilities of immobilized and native enzymes. Symbols: immobilized enzyme, alginate gel ○—○, DEAE-cellulose ●—●; native enzyme, -------. The activities were measured after 15 min incubation at each temperature.

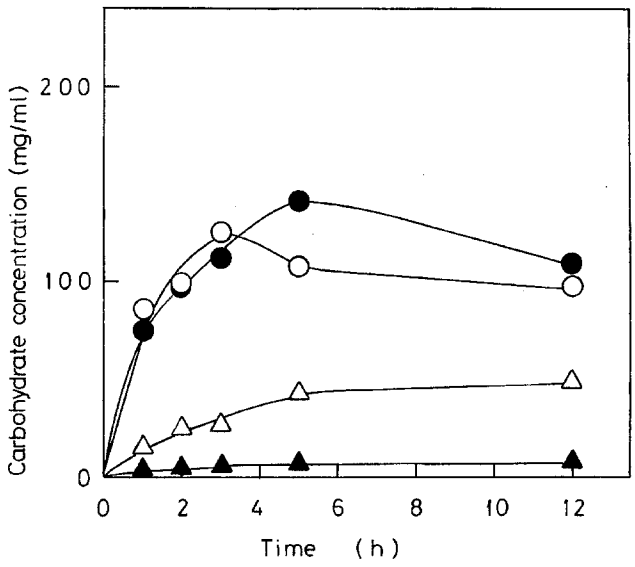

Fig. 9. Time course of product formation by immobilized enzymes at 55 °C. Approx 83 nkat of enzyme were employed in the reaction mixture. Symbols: alginate gel, panose ○—○, isomaltose △—△; DEAE-cellulose, panose ●—●, isomaltose ▲—▲.

TABLE 1

Effect of metals and organic inhibitors on the activity of immobilized and native enzymes

Compound (1 mM)	Relative activity (%)		
	Native	Immobilized	
		Alginate gel	DEAE-cellulose
None	100	100	100
$HgCl_2$	0	63	0
$AgNo_3$	1	96	2
$AlCl_3$	1	119	102
$NiSO_4$	21	78	104
$CuSO_4$	23	53	108
$BaCl_2$	64	106	91
$CoCl_2$	72	101	109
$ZnSO_4$	83	95	98
Nitrilotriacetic acid	17	102	103
Monoiodoacetic acid	44	112	102
Sodium arsenate	44	88	89
Sodium fluoride	52	95	102
Sodium citrate	89	78	109
p-Chloromercuricbenzoic acid	90	99	90

temperatures is shown in Fig. 8. The immobilized enzymes retained more than 93% (alginate gel) or 99% (DEAE-cellulose) activity at 60 °C, but were inactivated at 80 °C after 15 min as was the native enzyme [3].

Time course of enzymatic reaction

In Fig. 9, the time course of enzymatic reaction by both immobilized enzymes is shown. The efficiency of panose production to initial maltose concentration reached 42% after 3 h (alginate gel) and 47% after 5 h (DEAE-cellulose) which was the same as the level, 46%, of native enzyme [3].

Effect of metal ions and inhibitors on the enzymatic activity

The effect of metal ions and other enzyme inhibitors on the activity of immobilized and native enzymes is shown in Table 1. Inhibition of both immobilized enzyme preparations by metal ions and inhibitors was less than that of the native enzyme. As the inhibition of metal ions, especially Hg^{2+} and Ag^{+}, to alginate gel-immobilized enzyme was less than to the native enzyme, chelation of metal ions with hydroxyl groups of alginate is suggested. Other metal ions and inhibitors tested did not inhibit native or immobilized enzyme.

Glucosyltransferase from *Aureobasidium*, which is considered to be useful for the production of isomalto-oligosaccharides by a glucosyl-transferring reaction, was successfully immobilized at high efficiency by alginate gel or DEAE-cellulose. The result was superior stability to extreme pH, metal ions and inhibitors.

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