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Immobilization of β-fructofuranosidase from *Aureobasidium* on DEAE-cellulose

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Key words: Immobilization efficiency; Continuous reaction; Fructo-oligosaccharide; Fructosyl-transferring enzyme

SUMMARY

β-Fructofuranosidase, which produces fructo-oligosaccharides (1-kestose and nystose) from sucrose, was purified from *Aureobasidium* and immobilized on DEAE-cellulose at especially high efficiency (95%). The enzymatic profiles of the immobilized enzyme were almost identical to those of the native form except that the stability was slightly improved. The immobilized enzyme was stable during long-term continuous reaction for up to 360 h.

INTRODUCTION

We have investigated the immobilization of β-fructofuranosidases (EC 3.2.1.26), which produce fructo-oligosaccharides (1-kestose and nystose) from sucrose [6], to construct a bioreactor system for industrial production of fructo-oligosaccharides. Previously, we immobilized the enzyme on alkylamine porous glass [3,4] and silica [2,5] covalently by glutaraldehyde and described the immobilization efficiency, properties of the preparation and operational conditions for continuous production of fructo-oligosaccharides. Recently, we found that the enzyme could be immobilized on DEAE-cellulose, which has been used for the industrial preparation of immobilized glucose isomerase [1], at especially high efficiency. Usually, ion exchangers such as DEAE-cellulose are recyclable and are also easy to use for immobilization of enzymes. While a previous work described the utilization of DEAE-cellulose as a support of β-fructofuranosidase for determination of the effect of glucose isomerase on production of fructo-oligosaccharides [7], there is no published report concerning the immobilization efficiency and continuous reaction. We describe them together with the properties of the preparation in the present paper.

MATERIALS AND METHODS

Cultivation and preparation of enzyme

Aureobasidium sp. ATCC 20524 was cultivated for β-fructofuranosidase production in liquid culture (sucrose 20%, yeast extract 2%, $NaNO_3$ 1%, K_2HPO_4 0.75%, $MgSO_4 \cdot 7H_2O$ 0.1%, pH 6.5–7) under the same conditions as described previously [6]. β-Fructofuranosidase was solubilized by kitalase (β-1, 3-glucanase) and partially purified by fractionations involving ethanol, calcium acetate and ammonium sulfate as described in a previous paper [6].

Preparation of immobilized enzyme

DEAE-cellulose (0.1–1 g) [1] was treated in 0.5–5 ml of 0.5 M NaOH and then washed with water following 0.75 M McIlvain (citric acid/Na_2HPO_4) buffer, pH 5.5, before utilization for immobilization of the enzyme. Enzyme solution (ca. 10–500 U) was added to the prepared support and the mixture was stirred at room temperature for 1 h. Excess enzyme was then washed off with water. The preparation was used for further experiments.

Enzyme activity assay

The immobilized enzyme (ca. 50 mg) was employed in the reaction mixture. The enzyme was assayed using 30% (w/v) sucrose as substrate in a total volume of 1 ml of 75 mM McIlvain buffer, pH 5.5. The reaction was carried out at 50 °C for 20 min and stopped by boiling for 10 min.

Glucose released in the reaction mixture was measured by the glucose oxidase method (Glucose test B; Wako, Japan). Kestose and nystose were measured by HPLC with a μ-Bondaspare NH_2 column (3.9 × 15 mm, Waters, Tokyo, Japan) under conditions as described previously [6]. One unit of enzyme was defined as the quantity of enzyme responsible for the transfer of 1 μmol of fructose in 1 min.

Continuous reaction of immobilized enzyme

The immobilized enzyme (ca. 1 g support, ca. 80 U) was packed in a glass column (6 mm i.d.) with a bed volume of ca. 1.7 cm^3. The enzyme column was operated continuously at 30 °C using 30% (w/v) sucrose as substrate in 75 mM McIlvain buffer (pH 5.5) at a flow rate of 3.5 ml h^{-1}.

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RESULTS AND DISCUSSION

Immobilization of enzyme

The effect of enzyme concentration on the efficiency of immobilization is shown in Fig. 1(A). While there was a tendency to increase the amount of immobilized enzyme as enzyme concentration increased, the maximum efficiency of immobilization, 95%, was found with 100 U of added enzyme, per gram of support. The value is higher than that for the immobilization using inorganic support (50.3%) [2]. Immobilization was complete within 1 h after addition of enzyme to the support (Fig. 1(B)).

Enzymatic profiles of the immobilized enzyme

The effect of pH on the activity and stability of immobilized and native enzyme is shown in Fig. 2. The optimum reaction pH of immobilized enzyme, 5–5.5, was not changed after immobilization. The immobilized enzyme was stable within the wide range of pH 3–9 and retained more than 90% of its original activity after 3 h. The enzyme released from support was not detected at acidic and alkaline pHs. The pH stability of the enzyme was relatively improved after immobilization.

The effect of temperature on the activity and stability of immobilized and native enzyme is shown in Fig. 3. The optimum temperature of immobilized enzyme, 55 °C, was almost identical to that of the native enzyme (50–55 °C). The immobilized enzyme was stable at 50 °C but inactivated at 70 °C after 15 min. At 55 °C, the immobilized enzyme still exhibited more than 63% of its original activity.

The effect of metal ions and *p*-chloromercuribenzoate (PCMB) on the activity of immobilized and native enzymes is shown in Table 1. Inhibition of the immobilized enzyme by metal ions and PCMB was less than that of the native enzyme. Other metal ions and inhibitors tested did not inhibit either enzyme.

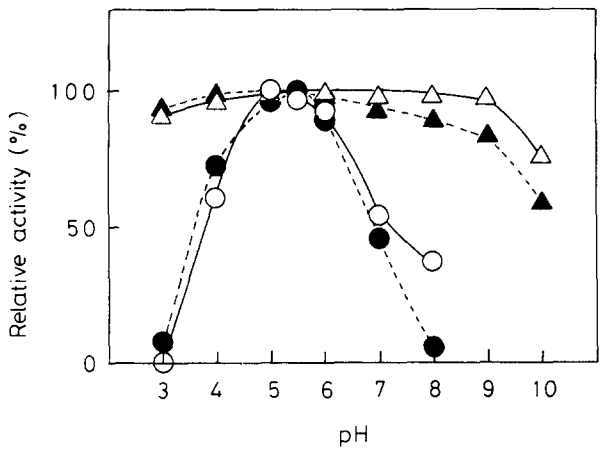

Fig. 2. The effect of pH on the activity and stability of immobilized and native enzyme. Symbols: immobilized enzyme, activity -○-, stability -△-; native enzyme, activity --●--, stability --▲--. The activities were measured after 3 h incubation at each pH (pH 3–8, McIlvain buffer; pH 8–10, Michaelis buffer) to determine stabilities of the enzyme.

Continuous reaction of immobilized enzyme

Continuous production of fructo-oligosaccharides was carried out under the conditions described in Materials and Methods, and the result is shown in Fig. 4. The immobilized enzyme was stable during long-term continuous reaction and the effluent concentration of fructo-oligosaccharides (1-kestose + nystose) remained in the range of 105–127 mg ml^{-1} for up to 360 h.

While the enzymatic profiles were not so improved after immobilization using DEAE-cellulose as support, the immobilization efficiency, stability during long-term continuous operation and brevity of the immobilization were excellent. We conclude this method to be advantageous for

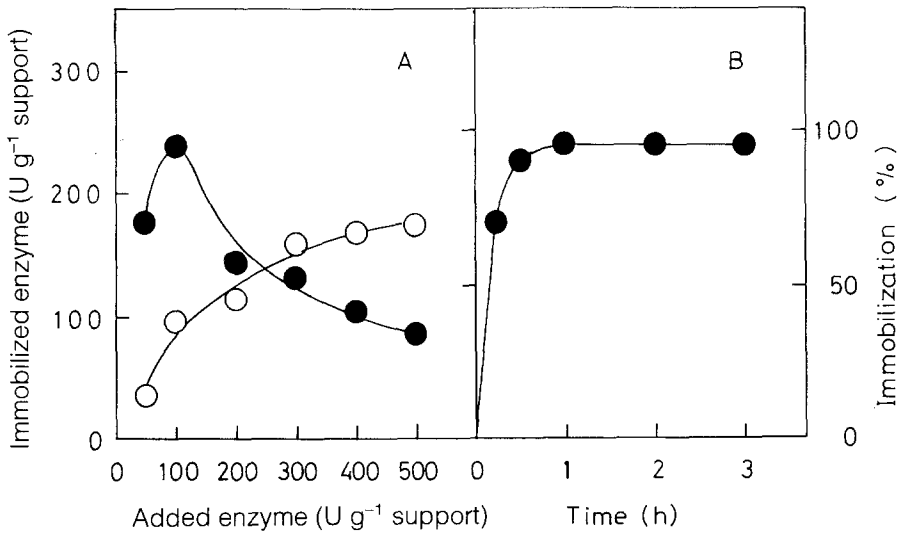

Fig. 1. The effect of the amount of added enzyme on immobilization on DEAE-cellulose. Symbols: immobilized enzyme, ○; immobilization, ●.

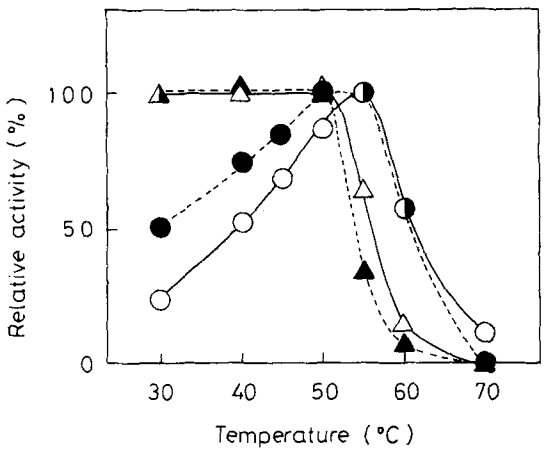

Fig. 3. The effect of temperature on the activity and stability of immobilized and native enzyme. Symbols: immobilized enzyme, activity -○-, stability -△-; native enzyme, activity --●--, stability --▲--. The activities were measured after 15 min incubation at each temperature to determine stabilities.

TABLE 1

Effects of metal ions and PCMB on the activities of immobilized and native enzyme

Metal ion (1 mM)	Relative activity (%)	
	Native	Immobilized
$HgCl_2$	0	2
$CuSO_4$	1	5
$Pb(OAc)_2$	11	21
$ZnSO_4$	41	51
$AgNO_3$	31	68
PCMB	80	92
Control (no addition)	100	100

the immobilization of β-fructofuranosidase from *Aureobasidium*.

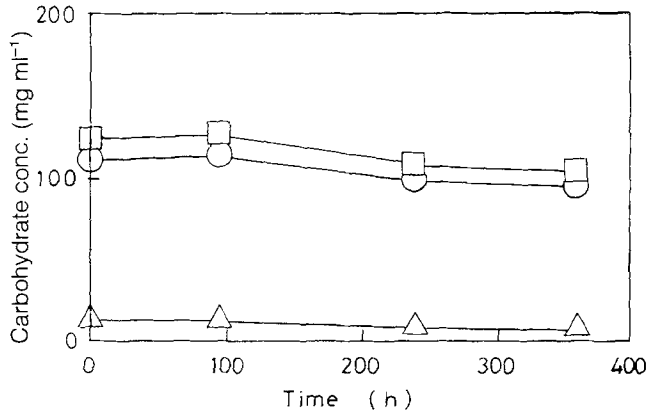

Fig. 4. Continuous reaction by a column packed with the immobilized enzyme. Symbols: 1-kestose, ○; nystose, △; total fructo-oligosaccharides, □.

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