CONTINUOUS PRODUCTION OF 1-KESTOSE BY β-FRUCTOFURANOSIDASE IMMOBILIZED ON SHIRASU POROUS GLASS

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SUMMARY 1-Kestose was produced continuously and selectively from 40 % (w/v) sucrose solution at fast flow rate by a column packed with an immobilized β-fructofuranosidase on shirasu porous glass.

INTRODUCTION

Inorganic supports are easier to use in continuous systems because of their stability to microbial attack and to changes in pH and solvent conditions (Weetall, 1969). Porous glass has been used as support for the immobilization of α-amylase (Ramesh and Singh, 1981), glucoamylase (Goaxing et al., 1982) etc.. We also immobilized β-fructofuranosidase covalently onto shirasu porous glass (SPG) which was produced from shirasu (volcanic ashes) (Nakashima and Kuroki, 1981) and observed its enzymatic profiles (Hayashi, Ito et al., 1991).

In the present paper, we describe the continuous selective production of 1-kestose by β-fructofuranosidase from Aureobasidium (Hayashi, Nonoguchi et al.) immobilized onto SPG. 1-Kestose (O-β-D-fructofuranosyl-(2→1)-β-D-fructofuranosyl α-D-glucopyranoside) is a fructooligosaccharide which has become important because of its favorable functional properties for food (Yamashita et al., 1984; Hidaka et al., 1987). The immobilization of β-fructofuranosidase on DEAE-cellulose has been reported (Kida et al., 1988). There is, however, no report about the continuous production of 1-kestose by the immobilized β-fructofuranosidase on SPG.
MATERIALS AND METHODS

Cultivation and preparation of enzyme

_Aureobasidium_ sp. ATCC 20524 was cultivated for β-fructofuranosidase production and the enzyme was solubilised from the harvested cells by Kitalase as described previously (Hayashi _et al._, 1989, 1990).

The enzyme was partly purified before the immobilization by ethanol, (CH₃COO)₂Ca, (NH₄)₂SO₄ and DEAE-Sephadex A-25 (Hayashi, Ito _et al._, 1991). The enzyme solution was concentrated with a membrane filter (Amicon UM 10) and then used for immobilization.

Preparation of immobilized enzyme

Preparation of support and immobilization of enzyme

were carried out by the procedures of Weetall (1977) with a little modification. Silanization of SPG (64.1 nm, Fuji-Devison Chemical Ltd.) and activation by glutaraldehyde were carried out as described previously (Hayashi, Ito _et al._, 1991). Enzyme solution (ca. 600 U) was added to ca. 1 g of the activated support and stirred at room temperature for 2 h. Then the excess enzyme was washed off with water. The preparation was used for further experiments.

Enzyme activity assay

The activity of immobilized enzyme was assayed using 30 % (w/v) sucrose as substrate in 75 mM McIlvain buffer (pH5.5) as described previously (Hayashi, Ito _et al._, 1991). 1-Kestose and other products were measured with HPLC fitted with μ-Bondapack CH (3.9 x 30 mm) column under the following conditions: temperature, 27°C; mobile phase, acetonitrile/water; 77:23, v/v; flow rate, 1 cm² min⁻¹; and RI detector. One unit of the enzyme was defined as the quantity of enzyme responsible for the transfer of 1 μmol of fructose in 1 min.

Continuous operation of the column packed with the immobilized enzyme

The immobilized enzyme (ca. 0.7 g support, 250 U) was packed in a glass column (6 mm ID) with a bed volume of ca. 0.6 cm³. The enzyme column was continuously operated at the flow rate stated in RESULTS AND DISCUSSION using 40 % (w/v) sucrose in 75 mM McIlvain buffer (pH 5.5) as substrate.

RESULTS AND DISCUSSION

Effect of flow rate of sucrose solution on the