Preparation of D-Psicose from D-Fructose by Immobilized D-Tagatose 3-Epimerase

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D-Tagatose 3-epimerase (D-TE) from Pseudomonas sp. ST-24 immobilized on Chitopearl beads of BCW 2503 was used in the preparation of D-psicose from D-fructose. When D-fructose solution (10%) was passed through a column packed with immobilized D-TE about 20% of the D-fructose was converted to D-psicose. This column was continuously used for 10 d at 45°C and about 90 g of D-psicose were obtained from D-fructose (500 g). Through the coupling with immobilized D-TE and D-xylose isomerase, D-psicose could be prepared directly from D-glucose.

[Key words: D-tagatose 3-epimerase, Pseudomonas sp. ST-24, D-xylose isomerase, D-psicose preparation, D-fructose, D-glucose]

A bacterium, Pseudomonas sp. ST-24, was isolated during a study of the preparation of rare keto-sugars from galactitol (1). During the course of a recent investigation, it was found that the cell-free extract of the bacterium, as well as the washed cell suspensions, could actively epimerize between D-tagatose and D-sorbose (1-3). Furthermore, we discovered a new epimerase in the crude extract of strain ST-24 (2) and named it D-tagatose 3-epimerase (D-TE) (3). We used this enzyme for the preparation of rare keto-sugars, D-sorbose (4) and D-psicose, from the inexpensive sugars D-tagatose and D-fructose, respectively. In this note, a simple method for the preparation of D-psicose from D-fructose by immobilized D-TE is described.

The bacterium, Pseudomonas sp. ST-24, was cultivated for 60 h at 30°C with shaking in medium containing 0.26% (NH₄)₂SO₄, 0.24% KH₂PO₄, 0.56% K₂HPO₄, 0.01% MgSO₄·7H₂O, 0.05% yeast extract, 1.0% D-glucose, and 0.05% D-tagatose (3). The cells were harvested by centrifugation and then ruptured. The enzyme was partially purified (4) by MnCl₂ treatment and polyethylene glycol fractionation (10-40%), each performed twice. This enzyme preparation was used for immobilization.

D-TE was immobilized by the following method. D-TE solution (partially purified) was added to Chitopearl beads of BCW 2503 (Fuji Spinning Co., Tokyo) which had been equilibrated with 50mM Tris-HCl buffer, pH 7.5 (buffer A), and shaken for 2 h. The immobilized enzyme thus prepared was washed with buffer A. The enzyme activity of native and immobilized D-TE was measured as described previously (4). One unit of D-TE activity was defined as the amount of enzyme which produces 1 pmol of D-sorbose from D-tagatose per minute at 30°C.

The equilibrium ratio of D-fructose to D-psicose using the immobilized D-TE was determined. The reaction was carried out at 30°C with shaking in a suspension (1.0 ml) containing 100 mM D-fructose in 25 mM Tris-HCl buffer, pH 7.5 and immobilized D-TE (0.2 units). After 48 h incubation, about 20% of the D-fructose was converted to D-psicose (80% of the D-fructose remained). Moreover, D-psicose could be prepared from D-glucose by coupling with immobilized D-TE and D-xylose isomerase (Sweetzyme T; from Novo Industry, Denmark). At equilibrium state, about 10% of the D-glucose was converted to D-psicose (Figs. 1 and 2).

The continuous production of D-psicose from D-fructose was performed using the column packed with immobilized D-TE. D-TE (200 units) was immobilized on 50 ml (38.5 g, wet weight) of Chitopearl beads BCW 2503, previously sterilized in an autoclave. The immobilization was performed at 45°C with shaking at 120 rpm for 10 d. After immobilization, the column was washed with buffer A, and D-psicose was eluted with buffer A. The column was continuously used for 10 d at 45°C and about 90 g of D-psicose were obtained from D-fructose (500 g).

[FIG. 1. Conversion of D-glucose to D-psicose using D-tagatose 3-epimerase and D-xylose isomerase.]

[FIG. 2. Preparation of D-psicose from D-glucose by immobilized D-TE and D-xylose isomerase.]

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Epimerization of D-fructose (10%) in a column packed with immobilized D-TE at 45°C.

The substrate solution (10% D-fructose in buffer A) was passed through the column at a flow rate of 500 ml/d. Product in the eluted reaction mixture was detected by HPLC (Fig. 3). After continuous reaction for 10 d, the conversion ratio had decreased by about 10%, and about 20% of the D-fructose supplied was converted to D-psicose (90 g).

After the reaction, 50 ml of the reaction mixture were concentrated by vacuum evaporation at 35°C. The sample was treated with activated charcoal for 1 d at room temperature, centrifuged at 12,000 rpm for 30 min, and finally filtered to remove the charcoal. The filtrate was deionized by passing through Diaion SK1B (H+ form) and Amberlite IRA-411 (CO32− form) ion-exchange resin columns. After evaporation under a vacuum at 35°C, the concentrate was applied to a column (2.5 × 97.0 cm) of Dowex 50W-X2 (Ca2+ form). Figure 4 shows the elution profile of the product, D-psicose. The fractions containing the product were pooled and evaporated to a syrup under vacuum. The syrup solidified on being kept in a desiccator in the cold and with addition of a very small amount of authentic D-psicose crystal.

The product was characterized by analysis of the infrared spectrum and optical rotation. The infrared spectrum was measured on an infrared spectrophotometer (Nihonbunko, model A-302) using KBr tablets. The retention time on HPLC analysis using a Hitachi column GL-C611 (60°C, with 0.01 mM NaOH at a flow rate of 1.0 ml/min) and the infrared spectrum (Fig. 5) of the isolated crystal coincided with those of authentic D-psicose. The specific optical rotation of the product was observed to be +4.1 (20°C, in H2O), while that of authentic D-psicose has been reported to be +3.1 in the literature (5). Based on these results, the product formed from D-fructose was identified as D-psicose.

Methods for the production of D-psicose which utilize bases have been reported (5-8). Doner (9) and Beveridge et al. (10) investigated the optimum conditions for the base-catalyzed isomerization of D-fructose to D-psicose. However, the yield of D-psicose in this isomerization was low (8%) (10), and some by-products appeared (9). We have reported that Alcaligenes sp. 701B could produce D-psicose from D-talitol, D-tagatose, or galactitol (11). In this study, we have shown that it is possible to directly prepare large amounts of D-psicose from inexpensive sugars such as D-fructose or D-glucose. D-Psicose has already been used as a substrate for the production of allitol (12) and other rare sugars. Thus, we believe that the method for the mass preparation of D-psicose from D-fructose described in this paper is a feasible and simple way to prepare these rare sugars.

REFERENCES


