Note

A Transxylosylation Product, α -D-Xylosylisoprimeverose, Synthesized by *Bacillus* α -D-Xylosidase from Isoprimeverose

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We have described the purification and some properties of an α -D-xylosidase from *Bacillus* sp. No. 693-1.^{1,2)} In our previous study, it was observed that the α -D-xylosidase, like most other β -D-xylosidases,^{3~5)} had transfer activity at a high substrate concentration (5%). This paper describes the transxylosylation activity of *Bacillus* α -D-xylosidase using *O*- α -D-xylopyranosyl-(1 $\rightarrow$ 6)-D-glucopyranose (isoprimeverose) as a substrate, and the formation and isolation as well as identification of the main transfer product.

A reaction mixture which contained 5% of the substrate (isoprimeverose, α -1,3-xylobiose prepared in our laboratory,²⁾ or methyl α -D-xyloside from Sigma Co., Ltd.), the purified *Bacillus* α -D-xylosidase²⁾ (0.1 units/ml reaction mixture), and 0.02% of NaN_3 in 10 mM McIlvaine buffer (pH 7.5) was incubated at 45°C. Samples of the reaction mixture were taken at intervals for thin-layer chromatography (TLC). As shown in Fig. 1, the enzyme had transfer activity on all the three substrates tested, isoprimeverose (Fig. 1A), α -1,3-xylobiose (Fig. 1B), and methyl α -D-xyloside (Fig. 1C). It can be seen from Fig. 1A that several transfer products with different degrees of polymerization were formed at the beginning of the reaction (1 hr). When it was incubated longer, the transfer products with higher degrees of polymerization disappeared and three main products, which include two hydrolysis products (xylose and glucose) and one transfer product (P-I), accumulated. Although more P-I accumulated after 1 hr of incubation, a reaction period of 2 hr was selected for easier separation of P-I from other transfer products on a charcoal column described below.

Isoprimeverose (0.5 g) and the enzyme (1.0 unit) were incubated under the conditions mentioned above. After thermal inactivation of the enzyme, the incubation mixture was chromatographed on a charcoal column. The sugars were successively washed with 400 ml of distilled water (to remove monosaccharides) and 600 ml of 8% ethanol (to remove isoprimeverose), and then eluted with a linear 8~35% ethanol gradient. As shown in Fig. 2, the

Fig. 1. Thin-layer Chromatograms showing the Course of Transfer Reaction.

The reaction was done as described in the text and TLC was done with solvent system of 1-propanol-water (85:15, v/v) (Fig. 1A) and acetone-water-chloroform-methanol (8:0.5:1:1, v/v) (Fig. 1B, 1C). S₁, authentic cello-oligosaccharides (C₁~C₄); S₂, authentic xylo-oligosaccharides (X₁~X₅); C₁, glucose; C₂, cellobiose; C₃, cellotriose; C₄, cellotetraose; X₁, xylose; X₂, xylobiose; X₃, xylotriose; X₄, xylotetraose; X₅, xylopentaose.

A: Isoprimeverose. B: α -1,3-Xylobiose. C: Methyl α -xyloside.

Fig. 2. Charcoal Column Chromatogram of Transfer Reaction Mixture.

The reaction mixture described in the text was adsorbed on a charcoal column (2.1 × 38 cm). The column was washed with distilled water and 8% ethanol, and then eluted with an ethanol linear gradient (8 ~ 35%). The size of each fraction was 10 ml and the flow rate was 25 ml/hr. TLC was done as described in Fig. 1.

S₁, xylose and glucose; S₂, isoprimeverose; Xyl, xylose; Glc, glucose; IP, isoprimeverose; P-I, the main transfer product; P₁, T-C-P₁; P₂, T-C-P₂; P₃, T-C-P₃.

effluents were pooled to form three parts. TLC analysis showed that T-C-P₁ contained only xylose and glucose, while T-C-P₂ was mainly isoprimeverose. T-C-P₃ consisted of a transfer product, P-I. T-C-P₃ fractions (Nos. 122 ~ 160 in Fig. 2) were pooled, concentrated, spotted on a filter paper (2.2 cm i.d.), and put into the bottom of a column (2.2 × 50 cm) packed with dry Silica gel 60 (Merck Co., Ltd.). The carbohydrates were eluted with a solvent system (1-propanol-water = 85:15, v/v) by the ascending method. The flow rate was 15 ml/hr and 5 ml fractions were collected. The eluates were analyzed by TLC and HPLC. Purified P-I (49 mg) was homogeneous on TLC (Fig. 3) and HPLC (data not shown).

Methylation and methanolysis analyses²⁾ were done to identify its glycosyl composition and position of glycosidic linkage. Authentic β -1,4 and β -1,3-xylobiose (prepared in our laboratory^{6,7)}), and *O*- α -D-glucuronopyranosyl-(1 $\rightarrow$ 2)-*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 4)-D-xylopyranose (GlcUAX₂, a gift from Dr. I. Kusakabe) were analyzed simultaneously. Measurement of total carbohydrate content and specific rotation as well as TLC, HPLC, and GLC analyses were done as described in our previous paper.²⁾ The configuration of the glycosidic linkages of P-I was

identified by measuring its specific rotation and also by enzymatic hydrolyses. The reaction mixture was composed of 5 mg of P-I and 0.2 unit of the *Bacillus* α -D-xylosidase or 0.1 unit of the partially purified *A. niger* β -D-xylosidase²⁾ in 1.0 ml of 10 mM McIlvaine buffer (pH 7.5 for the α -D-xylosidase, or pH 4.0 for the β -D-xylosidase) and 0.02% NaN₃. It was incubated at 45°C for α -D-xylosidase hydrolysis, or 55°C for β -D-xylosidase hydrolysis. The hydrolyzates (200 μ l each) were removed at intervals for TLC (Fig. 3).

As shown in Fig. 3, the purified transfer product P-I was located between cellobiose and cellotriose as well as between xylotriose and xylotetraose. Its R_{xy1} value was 0.52 on TLC. It showed $[\alpha]_D^{25} + 143.5^\circ$ ($c = 0.29$, in water). After methylation followed by methanolysis analysis, it gave only xylose and glucose derivatives, which were 1,2,3,4-tetra-*O*-Me-D-Xylp, 1,2,3-tri-*O*-Me-Xylp, and 1,2,3,4-tetra-*O*-Me-Glcp in a molar ratio of 1:1:1 (α - and β -anomers) on GLC. From these results, two alternative structures of *O*-D-xylopyranosyl-(1 $\rightarrow$ 4)-*O*-D-xylopyranosyl-(1 $\rightarrow$ 6)-D-glucopyranose and *O*-D-xylopyranosyl-(1 $\rightarrow$ 6)-*O*-D-glucopyranosyl-(1 $\rightarrow$ 4)-D-xylopyranose can be considered. It was found that the fungal β -D-xylosidase

Fig. 3. Thin-layer Chromatogram of Enzymatic Hydrolyzates of the Transfer Product (P-I).

The reactions were done as described in the text. TLC analysis was done with a solvent system of 1-propanol–water (85:15, v/v).

S₁, authentic cello-oligosaccharides (C₁ ~ C₄); S₂, xylooligosaccharides (X₁ ~ X₅); C₁, glucose; C₂, cellobiose; C₃, cellotriose; C₄, cellotetraose; X₁, xylose; X₂, xylobiose; X₃, xylotriose; X₄, xylo-tetraose; X₅, xylopentaose.

A: Hydrolysis by *Bacillus* α -D-xylosidase. B: Hydrolysis by *A. niger* β -D-xylosidase.

showed no hydrolytic activity on P-I even after 12 hr of incubation (Fig. 3B) while P-I was completely hydrolyzed to xylose and glucose within 15 min by the bacterial α -D-xylosidase (Fig. 3A). It has been clarified that the α -D-xylosidase can hydrolyze only α -D-xylosidic linkages (including α -1,4-linkage), but not α -D-glucosidic linkages.^{1,2)} Moreover, the $[\alpha]_D$ value of P-I was positive, +143.5°. Therefore, the structure of P-I was proposed to be *O*- α -D-xylopyranosyl-(1 $\rightarrow$ 4)-*O*- α -D-xylopyranosyl-(1 $\rightarrow$ 6)-D-glucopyranose. The other possible structure of P-I was excluded since this sugar was easily hydrolyzed by the α -D-xylosidase. It is interesting that among the three kinds of α -D-linked xylobioses,²⁾ the α -1,4 linkage was the most easily hydrolyzed by *Bacillus* α -D-xylosidase, but this linkage mode was mainly formed by transxylosylation of the same enzyme.

The results obtained from this work indicate that such an α -D-xylosidase could be used not only for the structural analysis of saccharides containing α -D-xylosidic linkages, but also for the enzymatic processing of oligosaccharides with α -D-xylosidic linkages.

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