Synthesis of Glucosylxyluloside Using Sucrose Phosphorylase

Hideki TAKAHASHI,* Kozo HARA,* Hitoshi HASHIMOTO,*
Takashi SASAKI** and Hajime TANIGUCHI**,**

* Carbohydrate Research Laboratory, Ensuiko Sugar Refining Co., Ltd.
(13-46, Daikoku-cho, Tsurumi-ku, Yokohama 230, Japan)

** National Food Research Institute
(2-1-2, Kannondai, Tsukuba 305, Japan)

*** Present Address, Iwate Prefecture Brewing and Food Research Institute
(26, Tsushida, Morioka 020, Japan)

Synthesis of glucosylxyluloside (Glc-Xul) by the action of sucrose phosphorylase [EC 2.4.1.7] from *Leuconostoc mesenteroides* was studied. In the transfer reaction using equimolar amount of α -D-glucose-1-phosphate (G-1-P) and D-xylulose, about 25% of D-xylulose was converted into Glc-Xul, whereas only 17% of D-fructose was converted into sucrose when D-fructose was used instead of D-xylulose. In the transfer reaction using equimolar amount of sucrose and D-xylulose, more than 60% of D-xylulose was converted into Glc-Xul. It was found that more than 80% of added D-xylulose was converted into Glc-Xul when the molar ratio of sucrose to D-xylulose was increased to 3.

Sucrose phosphorylase [EC 2.4.1.7] catalyzes two types of transfer reaction. One is a transfer of D-glucose from G-1-P to an acceptor sugar (Eq. (1)). Synthesis of sucrose from G-1-P and D-fructose is the most popular example of this reaction. Another is a transfer of D-glucose from sucrose to an acceptor sugar (Eq. (2)).

This enzyme was found in microorganisms such as *Pseudomonas saccharophila*,¹⁾ *P. putrefaciens*,²⁾ *Clostridium pasteurinum*,³⁾ *Acetobacter xylinum*,⁴⁾ *Pullularia pullulans*,⁵⁾ and *Leuconostoc mesenteroides*.⁶⁾ The enzyme from *P. saccharophila* utilizes D-fructose,¹⁾ L-sorbose,⁷⁾ D-xylulose,⁷⁾ L-ribulose,⁸⁾ L-arabinose⁸⁾ and D-rhamnulose⁹⁾ as an acceptor sugar and produces α -D-glucopyranosyl-(1 $\rightarrow$ 2)- β -D-fructofuranoside (=sucrose), α -D-glucopyranosyl-(1 $\rightarrow$ 2)- α -L-sorbofuranoside (Glc-Sor), α -D-glucopyranosyl-(1 $\rightarrow$ 2)- β -D-xylulofuranoside (glucosylxyluloside = Glc-Xul), α -D-glucopyranosyl-(1 $\rightarrow$ 2)- α -L-ribulofuranoside, α -D-glucopyranosyl-(1 $\rightarrow$ 3)-L-arabinopyranose (Glc-Ara) and α -D-glucopy-

ranosyl-(1 $\rightarrow$ 2)- β -D-rhamnulofuranoside, respectively, in the transfer reaction using G-1-P. The enzyme from *P. saccharophila* was purified and its physicochemical and catalytic properties were extensively studied by Silverstein *et al.*¹⁰⁻¹²⁾

In a hope to develop a useful application of hemicellulose we have studied the preparation of D-xylose from agricultural wastes and its efficient conversion to D-xylulose, and further tried to convert this D-xylulose into Glc-Xul, a sucrose analogue, using sucrose phosphorylase. This compound is expected to show slightly different but important, from the standpoint of application, properties from those of sucrose.

It was reported that *L. mesenteroides* produced higher amount of sucrose phosphorylase per cells than *P. saccharophila* and *P. putrefaciens*.¹³⁾ An efficient method to convert D-xylulose into Glc-Xul using the enzyme preparation obtained from *L. mesenteroides* will be described in this paper.

MATERIALS AND METHODS

Microorganism. Leuconostoc mesenteroides

Acp* = acceptor, Pi** = inorganic phosphate,
Suc*** = sucrose, Fru**** = D-fructose.

ATCC 12291 was purchased from American Type Culture Collection.

Materials. D-Xylose was prepared from D-xylose by the action of xylose isomerase and separated from D-xylose by an ion exchange chromatography. Phosphoglucumutase and glucose-6-phosphate dehydrogenase were purchased from Boehringer Mannheim Yamanouchi Co. Other saccharides and chemicals were purchased from commercial sources.

Preparation of sucrose phosphorylase. The microorganism was cultivated semi-anaerobically according to the method of Vandamme *et al.*¹⁴⁾ Cells were harvested after 15 hr, at early stationary phase, by centrifugation, freeze-dried and stocked at -20°C .

These cells were used as an enzyme source in some experiments. The freeze-dried cells were suspended (20 mg/ml) in 10 mM Tris-maleate buffer (pH 6.7) and sonicated with SONIFIER CELL DISRUPTER 350 (Branson Sonic Power Co.) under cooling condition at 4°C . The resultant suspension was centrifuged and the obtained supernatant was brought to 80% saturation by adding solid ammonium sulfate. After standing overnight at 4°C , the precipitate formed was collected by centrifugation, dissolved in 10 mM Tris-maleate buffer (pH 6.7) and dialyzed overnight against the same buffer. The obtained solution was used as sucrose phosphorylase preparation in most of the experiments.

Assay of enzymatic activity. The enzymatic activity was routinely assayed in a reaction mixture containing 0.1 M sucrose, 0.1 M K_2HPO_4 , 0.1 M KH_2PO_4 , 0.2 M NaHCO_3 , 0.1 M KF (pH 6.7) and a suitable amount of enzyme solution. The reaction mixture was incubated at 37°C for 30 min and boiled for 3 min to inactivate the enzyme, and the amount of G-1-P produced was determined by the phosphoglucumutase-glucose-6-phosphate dehydrogenase method.¹⁵⁾ One unit (U) of sucrose phosphorylase was defined as the amount of enzyme that produced 1 μmol of G-1-P per min under the above conditions.

When the transfer reaction using G-1-P and an acceptor sugar was run, the amount of released inorganic phosphate (Pi) was measured according to the method of Lowry and Lopetz.¹⁶⁾

Synthesis of disaccharides. Except where otherwise stated, the reaction mixture for disaccharide synthesis contains 50 mM of G-1-P or sucrose as a glucosyl donor, 50 mM acceptor sugar and suitable amount of the enzyme in 50 mM Tris-maleate buffer (pH 7.0). The reaction was carried out at 37°C , and aliquots were withdrawn at intervals, boiled for 3 min to inactivate the enzyme, deionized and subjected to high performance liquid chromatography.

High performance liquid chromatography (HPLC). HPLC was carried out with TRI-ROTOR SR-2 (Japan Spectroscopic Co., Ltd.) equipped with an RI detector. MCI GEL CK08EC column ($8 \times 300\text{mm}$, Mitsubishi Kasei Co.) was used at 65°C with the mobile phase of water at a flow rate of 0.6 ml/min for routine analysis. Sample solutions were deionized by adding suitable amount of Amberlite IR-120B and IRA-93, and the resultant supernatants (20 μl) were injected. The amount of each disaccharide was calculated using standard curve of sucrose. The yield of disaccharide formed was defined by the following equation,

$$\text{Yield (\%)} = \frac{\text{amount (mol) of disaccharide produced}}{\text{amount (mol) of added acceptor}} \times 100$$

RESULTS AND DISCUSSION

Preparation of crude sucrose phosphorylase solution. At first the possibility of using dry cells as an enzyme for sucrose synthesis was studied. The cells were incubated with 50 mM each of G-1-P and D-fructose. D-Fructose in the reaction mixture decreased continuously and disappeared almost completely. Sucrose started to accumulate at first, but its accumulation stopped at a concentration of only 7 mM. Instead, large amount of mannitol was produced throughout the incubation. This is probably because of the presence of mannitol dehydrogenase, which converts D-fructose into mannitol.

To avoid the formation of mannitol, the dry cells were then sonicated and the solubilized enzyme preparation was concentrated by 80% saturated ammonium sulfate precipitation. As shown in Fig. 1, this preparation did not form mannitol and, therefore, was used as sucrose

Fig. 1. Sucrose synthesis by the enzyme preparation.

Fifty mM each of G-1-P and D-fructose were incubated with the enzyme preparation (5.5 U/ml) in 50 mM Tris-maleate buffer (pH 7.0) at 37°C. For other details see MATERIALS AND METHODS. ●, Sucrose ; ■, D-Fructose ; ▲, Mannitol.

Table 1. Relative initial rates of disaccharide formation from various acceptor sugars.

Acceptor	Relative initial rate
D-Fructose	100
D-Xylulose	85
L-Sorbose	16
L-Arabinose	14
D-Glucose	0
D-Mannose	0
D-Galactose	0
D-Xylose	0
D-Ribose	0

Fifty mM each of G-1-P and one of the acceptor sugars listed were incubated with 0.2 U/ml of the enzyme preparation in 50 mM Tris-maleate buffer (pH 7.0) at 37°C for 30 min. The amount of released Pi was determined as described in MATERIALS AND METHODS. A reaction mixture containing no acceptor sugar was run as a control. The rate with D-fructose was taken as 100.

phosphorylase in the following experiments.

Acceptor specificity of sucrose phosphorylase. Relative initial rates of Pi release (=disaccharide formation) from G-1-P and one of D-fructose, D-xylulose, L-sorbose and L-arabinose were studied. As summarized in Table 1, the reaction using D-fructose as an acceptor proceeded most rapidly. The reaction using D-xylulose followed, at 85% as fast as that of

sucrose synthesis. Compared with these, the reactions using L-sorbose or L-arabinose as an acceptor proceeded more slowly. D-Glucose, D-mannose, D-galactose, D-xylose and D-ribose did not act at all as an acceptor. These results agree quite well with the data obtained by the partially purified sucrose phosphorylase from *P. saccharophila*, as reviewed by Vandamme *et al.*¹⁷⁾

Synthesis of Glc-Xul by the transfer reaction using G-1-P. Disaccharide synthesis by the transfer reaction using G-1-P and acceptor sugars was studied. The reaction was followed by HPLC. Figure 2 summarizes the results obtained with several acceptors. As is anticipated from Table 1, sucrose synthesis reached an equilibrium most rapidly, after 1 hr of a reaction, with the maximum yield of about 17%. Then, it began to decrease gradually. Glc-Xul was synthesized at the same rate as that of sucrose synthesis, but the synthesis continued even after the sucrose synthesis leveled off, with the maximum yield of 25%. A maximum yield of about 9% was obtained in the case of Glc-Sor synthesis. Synthesis of Glc-Ara lasted longer time and its yield reached 18% after 6 hr and 20% after 8 hr. L-Arabinose is quite unique as an acceptor as it is an aldose and the product formed from it is a reducing disaccharide. Thus D-xylulose was found to be converted into disaccharide with

Fig. 2. Time course of disaccharide formation by the transfer reaction from G-1-P and an acceptor sugar.

Fifty mM each of G-1-P and one of the acceptor sugars were incubated with 8 U/ml of enzyme preparation in 50 mM Tris-maleate buffer (pH 7.0) at 37°C. ○, Sucrose ; ●, Glc-Xul ; ▲, Glc-Sor ; ■, Glc-Ara.

Fig. 3. Time course of disaccharide formation by the transfer reaction from sucrose and an acceptor sugar.

The same conditions as in Fig. 2 were used except that G-1-P was replaced with sucrose. The same symbols were used.

Fig. 4. Effect of molar ratio of sucrose to D-xylulose on the Glc-Xul yield.

Reaction mixtures containing 50 mM D-xylulose and various amounts (50–500 mM) of sucrose were incubated with 5.3 U/ml of enzyme preparation in 50 mM Tris-maleate buffer (pH 7.0) at 37°C for 3 hr. The Glc-Xul yield of each reaction mixture were determined and plotted against the molar ratio.

the highest yield among the tested ketose. However, more than 70% of added D-xylulose remained unutilized even when the synthesis of Glc-Xul reached an equilibrium in this reaction condition.

Synthesis of Glc-Xul by the transfer reaction using sucrose. Disaccharide synthesis by the transfer reaction using sucrose and acceptor sugars was then studied in a hope to obtain higher yield. Figure 3 summarizes the results of three reactions using D-xylulose, L-sorbose or L-arabinose as an acceptor. Clearly synthesis of Glc-Xul proceeded most rapidly among the three disaccharides and more than 60% of added

Fig. 5. Structures of sucrose and Glc-Xul.

D-xylulose (or sucrose) was converted into Glc-Xul within 2 hr under the conditions used. Again, transfer to L-arabinose proceeded slowly and progressively through the tested reaction period.

As D-xylulose is much more expensive than sucrose, the molar ratio of sucrose to D-xylulose in the reaction mixture was changed variously and the yields of Glc-Xul were plotted against the molar ratio. Results are shown in Fig. 4. The yield based on added D-xylulose increased from 63% to 83% on increasing the molar ratio from 1 to 3. However, increasing the molar ratio beyond 3 resulted in no more increase in the yield. As described above, a new method was developed which converts D-xylulose into Glc-Xul with an yield as high as 83%.

Glc-Xul is quite close to sucrose in its structure (Fig. 5); therefore, it is expected to have similar physicochemical and physiological properties to those of sucrose. However, the presence of a small but distinct difference in their structures may give Glc-Xul some different properties from those of sucrose. Extensive characterization of this disaccharide is of considerable interest from standpoints of both basic research and application.

On the basis of the new efficient method described above, a large-scale production of Glc-Xul and its characterization are in progress.

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シュクロースホスホリラーゼを用いた
グルコシルキシロシドの合成

高橋英樹*, 原 耕三*, 橋本 仁*
佐々木 堯**, 谷口 肇**, ***

* 塩水港精糖糖質研究所
(230 横浜市鶴見区大黒町 13-46)

** 農林水産省食品総合研究所
(305 つくば市観音台 2-1-2)

*** 現在の住所: 岩手県醸造食品試験場
(020 盛岡市津志田 26)

Leuconostoc mesenteroides 由来のシュクロースホスホリラーゼ [EC 2.4.1.7] を用い、グルコシルキシロシド (Glc-Xul) の合成を検討した。同濃度の α -D-グルコース-1-リン酸 (G-1-P) と D-キシロースを用いた転移反応において、D-キシロースの約 25% が Glc-Xul に変換された。一方、D-キシロースの代りに D-フラクトースを用いたとき、D-フラクトースの 17% がショ糖に変換された。同濃度のショ糖と D-キシロースを用いた転移反応において、D-キシロースの 60% 以上が Glc-Xul に変換された。さらに、D-キシロースに対するショ糖のモル比を 3 にあげたとき、添加した D-キシロースの 80% 以上が Glc-Xul に変換されることがわかった。