J. Appl. Glycosci., **55**, 1–3 (2008)
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Note

Synthesis and Structure Analysis of Glucosylpsicose Produced by Cyclomaltodextrin Glucanotransferase

(Received April 19, 2007; Accepted September 3, 2007)

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Abstract: Glucosylpsicose was synthesized by transglycosylation with α-cyclodextrin and D-psicose using cyclomaltodextrin glucanotransferase (EC 2.4.1.19; CGTase). The structure of glucosylpsicose was analyzed by HPLC to determine sugar composition and molecular mass, by methylation analysis using GC-MS and by α- and β-glucosidase treatments to determine linkage, and ^{1}H- and ^{13}C-NMR spectrometries to obtain the anomeric configuration of the glycosidic linkage. By chemical analysis, it was found that the structure of glucosylpsicose is 1-*o*-α-D-glucopyranosyl-D-psicose.

Key words: D-psicose, cyclodextrin, cyclomaltodextrin glucanotransferase

Recently, it has become apparent that D-psicose (D-psi) causes plant growth regulation effects and induction of defence gene transcription,[1)] and provides no energy for growth in rats.[2)] It might be expected that transglycosylation products with D-psi would have better functionality than the rare sugar itself, because oligosaccharides function as prebiotics, cosmetics and therapeutic materials.[3)] Previously, we designed some probable structures of xylosylpsicoses, which were synthesized by transglycosylation with arabinoxylan and D-psi using endo-1,4-β-D-xylanase (EC 3.2.1.8) from *Aspergillus sojae*.[4)]

Here, we describe the preparation of glucosylpsicose (GP) produced by cyclomaltodextrin glucanotransferase (EC 2.4.1.19, CGTase). This enzyme is widely used in the synthesis of various glycosides. Evidence for the structure of the disaccharide is provided.

D-Psi was supplied by the Rare Sugar Research Center, Kagawa University. Cyclodextrin (α, β and γ) and maltooligosaccharides (maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose and maltoheptaose) were obtained from Hayashibara Biochemical Laboratories (Okayama). Dextrins Pinedex #100, Pinedex #4 and Pinedex #1 were purchased from Matsutani Chemical Industry Co., Ltd. (Hyogo), and dextrin for chemicals (DEX) was obtained from Wako Pure Chemical Industries (Osaka). CGTase from *Bacillus stearothermophilus* was purchased from Seikagaku Corporation (Tokyo). α-Glucosidase from rice, β-glucosidase from almonds, β-amylase from barley and amyloglucosidase from *Rhizopus* sp. were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). Other chemicals were obtained from Wako Pure Chemical Industries.

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Abbreviations: Glc*p*, glucopyranose; Psi*p*, psicopyranose; Psi*f*, psicofuranose.

Transglycosylation products were analyzed with a Shimadzu HPLC System using a Shodex KS-802 column (Showa Denko, Tokyo; 0.8×30 cm). Unless otherwise specified, the transglycosylation reaction mixture containing 10 μL of 25% (w/w) α-cyclodextrin (α-CD), 10 μL of 25% (w/w) D-psi in 50 mM sodium acetate buffer (pH 5.5) and 15 U of CGTase was incubated at 40°C for 1 h. The concentration of transglycosylation product was determined using isolated GP as a standard.

Figure 1 shows a chromatogram of the reaction products in the reaction mixture. With D-psi, a few new peaks—together with some peaks of hydrolyzates of α-CD—were detected, since CGTase catalyzed not only transglycosylation (intermolecular and intramolecular), but also hydrolysis of CD.[5)] From the result, we confirmed that GP was a major transglycosylation product under the reaction conditions.

The glucosyl donor specificity of the enzyme for producing GP is shown in Fig. 2. The enzyme acted on various glucosyl donors, *e.g.,* maltooligosaccharides, CD and dextrins, to produce GP; glucose did not act as a substrate. The quantity of GP was increased with the increasing degree of polymerization (DP) of maltooligosaccharides. Among them, CD was a good glucosyl donor for the production of GP under the conditions used.

For isolation of GP, a reaction mixture containing 10 g of α-CD, 10 g of D-psi and 5000 U of CGTase in 15 mL of 20 mM sodium acetate buffer (pH 5.5) was incubated at 40°C for 24 h. This reaction mixture (10 mL) was placed in a Toyopearl HW-40 S (Tosoh Co., Ltd., Tokyo) column (5.0×95 cm) and eluted with distilled water. The fractions containing GP were concentrated, and the solution was separated using an AG 50 W (Ca^{2+}) column (2.6×95 cm) with distilled water as the eluent.

The purity and DP of the GP were analyzed using a Hypercarb column (Thermo Fisher Scientific Inc., Waltham, MA, USA; 0.46×10 cm) and a TSK gel G-oligo

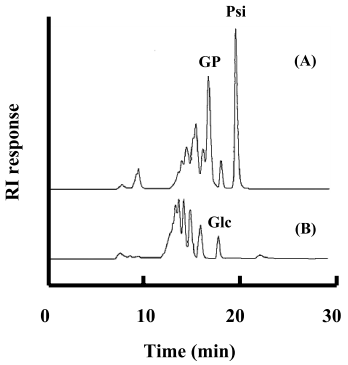

Fig. 1. Chromatogram of reaction products by CGTase from a mixture of α-CD with or without D-psicose.

(A), with D-psicose; (B), without D-psicose; Psi, psicose; GP, glucosylpsicose; Glc, glucose.

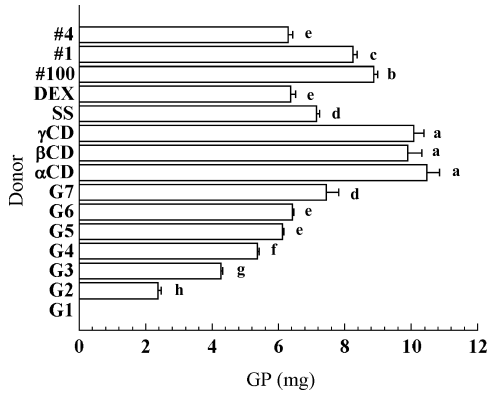

Fig. 2. Glycosyl donor specificity for GP production.

G1, glucose; G2, maltose; G3, maltotriose; G4, maltotetraose; G5, maltopentaose; G6, maltohexaose; G7, maltoheptaose; αCD, α-cyclodextrin; βCD, β-cyclodextrin; γ CD, γ-cyclodextrin; SS, soluble starch; DEX, dextrin; #100, pinedex #100; #1, pinedex #1; #4, pinedex #4. The TG reactions with various glucosyl donors were carried out in a mixture containing 75 U of CGTase, 50 mg of D-psicose and 50 mg of various glucosyl donors in 0.25 g of 0.05 M sodium acetate buffer pH 5.5 at 40°C for 48 h. Each value is presented as mean±SE of 5 samples. Values not sharing a common letter are significantly different by Student's *t*-test at $p < 0.05$.

PW column (Tosoh), which was calibrated with standard glucose oligomers (DP 1−20; Seikagaku Corporation). The sugar composition of GP was analyzed according to the method of Pazur[6] using a Shodex KS-802 column with D-glucose and D-psi as standards.

From the purification steps, *ca.* 1.6 g of GP was obtained from 10 g of D-psi in the reaction mixture; the yield of GP was 8.4% according to the following equation: transglycosylation product (mol)/ acceptor (mol)× 100. The isolated GP showed a single peak not only on the Shodex KS-802 column, but also on the Hypercarb column. Moreover, the DP of isolated GP was estimated to be two by a TSK gel G-oligo PW column. The sugar was hydrolyzed with 0.2 N HCl at 100°C for 3 h. The hydrolyzate was separated into glucose and psicose in a molar ratio of 1:1 by HPLC (data not shown).

Next, two mg of GP was methylated by the method of Hakomori.[7] After extraction with chloroform, a methylated sample was hydrolyzed with 1 M trifluoroacetic acid solution at 70°C for 30 min, and the methylated monosaccharides were reduced with 1% $NaBH_4$ solution and then acetylated with acetic anhydride at 100°C for 3 h. The

Table 1. Results of methylation analysis of glucosylpsicose.

Peaks	Retension time	Methylated sugar	Linkage site	Rate of composition*
P1	26.18	2,3,4,6-tetra-*o*-Methylglucitol	Glc 1→	1.00
P2	26.33	2,3,4,6-tetra-*o*-Methylallitol	→1 Psi	1.06

*The rate of composition of each methylated sugar was caluculated on the basis of the number of carbons from each peak as 2,3,4,6-tri-*o*-methylglucitol; 1.00.

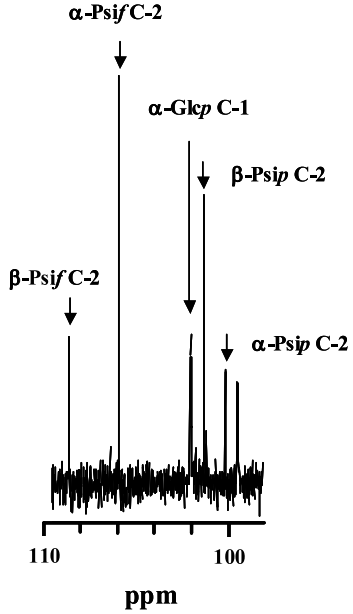

Fig. 3. ^{13}C-NMR spectrum of GP.

The ^{13}C-NMR spectrum was recorded at 25°C. Chemical shift is shown in ppm downfield from an internal sodium 3-(trimethylsilyl)-2,2,3,3-d_4-propionate.

partial methylated alditol acetates were analyzed with a coupled system of gas chromatograph-mass spectrometry (GC-MS), namely, the Hewlett Packard HP5890 series II with an SPB-5 capillary column (Sigma-Aldrich Japan K. K., Tokyo; 25 m×0.25 mm internal diameter, I.D.) coupled to JEOL Auto-Mass system II (JEOL, Tokyo). The temperature profile for GC was as follows: hold initially for 1 min at 60°C, wash and then increase to 280°C at a rate of 8°C/min. The MS conditions were as follows: electron impact mode (70 eV) and a scanning rate of 1 s/scan.

Two major peaks (P1 and P2) were detected by GC under the described conditions; the peaks were identified as 2,3,4,6-tetra-*o*-methylglucitol and 2,3,4,6-tetra-*o*-methylallitol respectively, on the basis of the reported data[8] in a molar ratio of 1:1.06 (Table 1). These results indicated that the transglycosylation product was linked between the C-1 positions of each sugar.

The ^{1}H- and ^{13}C-NMR spectra were recorded with a JEOL NM-SCM 40 spectrometer; GP (40 mg) was dissolved in 0.6 mL of deuterium oxide (D_2O) at 25°C with sodium 3-(trimethylsilyl)-2,2,3,3-d_4-propionate as the internal reference at 399.7 MHz for ^{1}H-NMR analysis and 100.4 MHz for ^{13}C-NMR analyses.

The signal at 5.1 ppm signals in the ^{1}H-NMR spectrum (data not shown) and the signal at 102 ppm signal in the ^{13}C-NMR spectrum were assigned to the anomeric proton

and carbon of the C-1 of α-Glc*p*, respectively. Furthermore, the ^{13}C-NMR signals at 100.2 and 101.3 ppm were assigned to the α- and β-anomer of the C-2 of Psi*p*, and those at 105.9 and 108.7 ppm were assigned to the α- and β-anomer of the C-2 of Psi*f* respectively, on the basis of the reported data[9,10] (Fig. 3). These data indicated that the D-psi residue of GP was presented as mixtures of four tautomers, i.e. α- and β-Psi*p*, and α- and β- Psi*f* in the solution.

Additionally, GP (0.5 mg) was incubated with β-glucosidase, amyloglucosidase, β-amylase or α-glucosidase (0.25 U each) in 200 μL of 50 mM sodium acetate buffer pH 5.5 for 18 h at 40°C. Whereas no hydrolysis of GP under these conditions occurred with β-glucosidase, amyloglucosidase or β-amylase, α-glucosidase caused hydrolysis of GP. From the chemical analysis, the structure of GP was identified as 1-*o*-α-D-glucopyranosyl-D-psicose.

Nakajima has already reported GP is produced from ρ-nitrophenyl α-D-glucoside and D-psi by α-glucosyltransferase from *Protaminobactor rubrum*.[11] Additionally, Morimoto *et al.* synthesized an α-D-glucopyranose-(1→2)-β-D-psicofuranose using sucrose phosphorylase from *Leuconostoc measenteroides* with α-D-glucosyl-1-phosphate and D-psi.[12] However, details of the structure of GP, which was produced by CGTase, are reported here for the first time. For industrial application of GP, the transglycosylation reaction with CGTase may be a useful method for mass production of GP, since the reaction uses a readily available α-glucosyl complex, *e.g.*, soluble starch, maltooligosaccharides and dextrin as a glucosyl donor.

In acceptor specificity of CGTase, Kitahata *et al.* reported the structure is an effective acceptor, which has the same configuration of the free C-2, C-3 and C-4 hydroxy groups as D-glucopyranose, and the transglycosylation product forms an α-1,4-glucosidic linkage.[13] However, the linkage site of GP was the C-1 position of each sugar. Therefore, the mechanism of transglycosylation of glucosyl residue to D-psi was not based on the above mentioned factors, but it might be similar to that of ascorbic acid.[14] The acceptor specificity of CGTase for tautomers of D-psi and the efficient production of GP, and the elicitor effect on plants will be reported in due course.

This work was supported in part by a grant from the Technical Development Association Project (Sugar-Bio-Cluster) of the Kagawa Prefectural Government, Japan. We wish to thank Mrs. Noriko Kagawa, Food Research Institute of Kagawa Industrial Technology Center, for much helpful collaboration.

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シクロマルトデキストリン グルカノトランスフェラーゼを用いた グルコシルプシコースの合成と構造解析

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シクロマルトデキストリングルカノトランスフェラーゼ (EC 2.4.1.19; CGTase) を用いて，α-シクロデキストリンとD-プシコースからグルコシルプシコース (GP) を合成した。GPの構造はHPLCによって糖組成と分子量，GC-MSを用いて結合部位を分析し，α-グルコシダーゼおよびβ-グルコシダーゼ処理，並びに^{1}H-および^{13}C-NMR分析によってグリコシド結合のアノマー構造を得た．化学分析の結果からGPの構造は，1-*o*-α-D-グルコピラノシル-D-プシコースであることが明らかになった．