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Note

Synthesis of methyl 6-*O*-β-inulotriosyl-α-D-glucopyranoside by intermolecular transglycosylation reaction of cycloinulo-oligosaccharide fructanotransferase

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Abstract

Incubation of cycloinulohexaose and methyl α-D-glucopyranoside in the presence of cycloinulo-oligosaccharide fructanotransferase gave some hetero-oligosaccharides. The main product was a tetrasaccharide whose sugar composition was methyl α-D-glucopyranoside-D-fructose in a ratio 1:3. This oligosaccharide was isolated from the reaction mixture by charcoal-column chromatography and was identified as methyl *O*-β-D-fructofuranosyl-(2 → 1)-*O*-β-D-fructofuranosyl-(2 → 1)-*O*-β-D-fructofuranosyl-(2 → 6)-α-D-glucopyranoside (methyl 6-*O*-β-inulotriosyl-α-D-glucopyranoside), by two-dimensional NMR spectroscopy. © 1997 Elsevier Science Ltd.

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Cycloinulo-oligosaccharide fructanotransferase (CFTase) produces cycloinulo-oligosaccharides from inulin by intramolecular transfructosylation [1] and also catalyzes intermolecular transglycosylation between β-(2 → 1)-fructo-oligosaccharides by disproportionation and coupling reactions [2]. These actions are the same as those of cyclomaltodextrin glucanotransferase (EC 2.4.1.19; CGTase) [3]. CGTase has been used to produce several glucopyranosides because of its wide acceptor specificity during intermolecular transglycosylation [4]. The similarity of the reactions suggests that CFTase may catalyze intermolecular transglycosylation with hydroxylated compounds other than D-fructofuranosyl residues as acceptors. Recently, syntheses of hetero-oligosaccharides with β-fructofuranosidase (EC 3.2.1.26; β-FFase) [5,6] have been reported. β-FFase transfers one fructosyl residue per reaction, but CFTase catalyzes the transfer of one oligofructosyl residue per reaction. In this paper, as a first step to making CFTase more useful, we set out to produce hetero-

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oligosaccharides from cycloinulohexaose and methyl α-D-glucopyranoside using the ability of CFTase to cause intermolecular transglycosylation.

Cycloinulohexaose and methyl α-D-glucopyranoside were incubated with CFTase under the conditions described in the Experimental section, and the products were monitored by HPLC at 5, 24, and 48 h after the reaction started (Fig. 1). By 5 h, five products, designated P_1 to P_5 in order of their elution on HPLC, had appeared in the reaction mixture. Further incubation resulted in increased amounts of P_1, P_2, and P_3 and in the appearance of additional products which accompanied the reduction of cycloinulohexaose. The amounts of P_4 and P_5 did not change. The products of P_1 to P_5 were fractionated directly from the HPLC eluent and their sugar compositions were analyzed. The transfer products were P_1, P_3, and P_5, which seemed to be methyl D-fructosyl-α-D-glucopyranoside, methyl (D-fructosyl)$_3$-α-D-glucopyranoside, and methyl (D-fructosyl)$_6$-α-D-glucopyranoside, respectively (Table 1). On the other hand, P_2 and P_5 were homo-fructo-oligosaccharides, which were inulotriose and inulohexaose, respectively, judging from their retention time. The first step in the formation of these products was probably opening of the fructoside ring in a cycloinulohexaose molecule during the coupling reaction with methyl α-D-glucopyranoside, resulting either in methyl (D-fructosyl)$_6$-α-D-glucopyranoside, or in hydrolysis, with formation of inulohexaose. Both products were immediately used as substrates in further reactions catalyzed by CFTase, resulting in formation of several oligosaccharides with different degrees of polymerization. Previously, we found that CFTase did not cause disproportionation reactions or hydrolyze sucrose or the oligosaccharides of the 1-kestose series with degrees of polymerization up to four, although these saccharides are used as acceptors in the coupling reaction [2]. Therefore, short fructo-oligosaccharides are used only as acceptors by CFTase. When methyl α-D-glucopyranoside was present at a high concentration as an acceptor, short oligosaccharides such as methyl D-fructosyl-α-D-glucopyranoside, methyl (D-fructosyl)$_3$-α-D-glucopyranoside, and inulotriose accumulated in the overall reaction. The hydrolysis by CFTase of cycloinulohexaose was weak, and hardly detectable when an acceptor like sucrose was added (data not shown). In contrast, a considerable amount of inulo-oligosaccharides was produced in this reaction, so perhaps the hydrolyzing activity was accelerated in the presence of such a 'poor' acceptor as methyl α-D-glucopyranoside.

Table 1
Sugar composition of the five products [a] found in the early stage of the reaction

Product	Sugar(s) [b]	Molecular ratio
P_1	MeGlc, Fru	1:1
P_2	Fru	–
P_3	MeGlc, Fru	1:3
P_4	Fru	–
P_5	MeGlc, Fru	1:6

[a] HPLC of the reaction mixture at 24 h shown in Fig. 1 was repeated 5 times, and eluents containing P_1 to P_5 were fractionated each time. The pooled fractions were dried by evaporation, dissolved in 50 μL of distilled water, and purified by further HPLC. The purified samples, after being dried, were dissolved in 50 μL of 10 mM Na-acetate (pH 5.0) containing 10 units of invertase and incubated at 40 °C for 3 h. Then, the sugar compositions were studied by HPLC.
[b] MeGlc, methyl α-D-glucopyranoside; Fru, D-fructose.

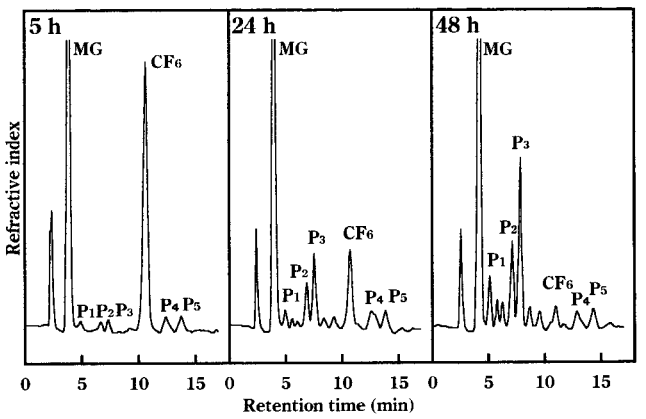


Fig. 1. HPLC patterns of oligosaccharides produced in the reaction mixture. A portion (1 μL) of the reaction mixture was injected into the HPLC apparatus at the designated times after incubation started. MG, methyl α-D-glucopyranoside; CF_6, cycloinulohexaose; P_1 to P_5, products that had appeared by 5 h.

The main product, the tetrasaccharide of methyl (D-fructosyl)$_3$-α-D-glucopyranoside, was purified to give a single peak on HPLC by charcoal-column chromatography. Two-dimensional ^{1}H NMR spectra of the oligosaccharide were measured for identification of where the inulotriosyl moiety was linked to the D-glucopyranosyl residue. In the spectrum of correlation spectroscopy (COSY-45) in Me_2SO-d_6, the signals of OH-protons were assigned by the correlation, $^3J_{H,OH}$, with those of the ring protons of the

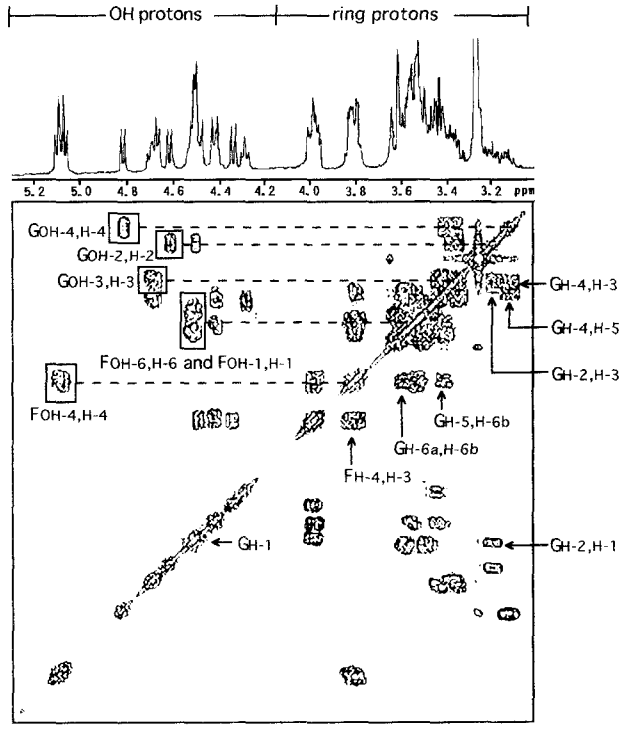
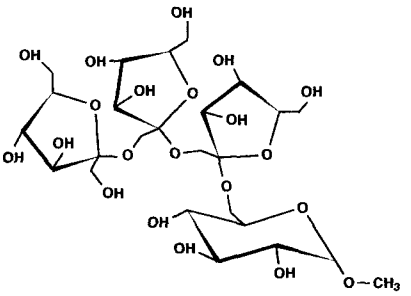


Fig. 2. COSY-45 ^{1}H NMR spectrum of methyl inulotriosyl-α-D-glucopyranoside in Me_2SO-d_6 at 35 °C. G, D-glucopyranosyl residue; F, D-fructofuranosyl residue.

D-glucopyranosyl residue, assigned with reference to a spectrum of COSY-45 measured in D_2O (data not shown). The cross peaks corresponding to $Glc_{OH\text{-}2,H\text{-}2}$, $Glc_{OH\text{-}3,H\text{-}3}$, and $Glc_{OH\text{-}4,H\text{-}4}$ were assigned but no cross peak correlated to $Glc_{H\text{-}6a}$ or $Glc_{H\text{-}6b}$ was found (Fig. 2). That is, there was no $Glc_{OH\text{-}6}$ peak. This result showed that the inulotriosyl moiety was linked to the 6-OH of the D-glucopyranosyl residue; the bond was a β-linkage because of the sensitivity to invertase. The tetrasaccharide was eventually identified as methyl *O*-β-D-fructofuranosyl-(2 → 1)-*O*-β-D-fructofuranosyl-(2 → 1)-*O*-β-D-fructofuranosyl-(2 → 6)-α-D-glucopyranoside, i.e., methyl 6-*O*-β-inulotriosyl-α-D-glucopyranoside (**1**). CFTase catalyzed the transfer of β-(2 → 1)-D-fructo-oligosaccharide moieties to the 6-OH of methyl α-D-glucopyranoside. Recently, the transfer of D-fructosyl residue to methyl α-D-glucopyranoside by β-FFase from an *Arthrobacter sp.* was reported, but only methyl 6-*O*-β-D-fructofuranosyl-α-D-glucopyranoside was produced [5]. β-FFase from *Aspergillus sydowi* catalyzes transfructosylation and produces fructosyl-, inulobiosyl-, and inulotriosyl-trehalose, but methyl α-D-glucopyranoside is not used as an acceptor [6]. The acceptor specificity of CFTase is being examined; CFTase could be useful for the synthesis of new glycosides.



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1. Experimental

Materials.—CFTase was purified from the culture broth of *Bacillus circulans* OKUMZ 31B as described elsewhere [7]. Cycloinulohexaose was prepared as described before [1] from inulin digested with the purified CFTase. Invertase from *Candida utilis* was purchased from Sigma Chemical Co. (St. Louis, MO).

General methods.—Two-dimensional ^{1}H NMR spectra were taken with a Bruker AM360 spectrometer. Trimethylsilane (in Me_2SO-d_6) and 3-(trimethylsilyl)-1-propanesulfonic acid (in D_2O) were used as the internal standards. HPLC was done on a Shimadzu LC-6A instrument with an RID-6A refractive index detector and a column of Capcell Pak NH_2 (4.6∅ × 250 mm, Shiseido Co., Tokyo, Japan). Two elution solvents, 65:35 MeCN–water and 75:25 MeCN–water, were used for oligosaccharide and monosaccharide analysis, respectively, at the flow rate of 1 mL/min. Optical rotation was measured with a Horiba SEPA-200 polarimeter. TLC plates (Whatman HP-KF) were developed with 3:12:4 1-butanol–2-propanol–water. Sugars were detected with naphthoresorcinol–H_2SO_4 reagent [8].

Production and isolation of methyl inulotriosyl-α-D-glucopyranoside.—A reaction mixture containing 10 units of CFTase [7], cycloinulohexaose (0.5 g), methyl α-D-glucopyranoside (2 g), and Tris-citrate (10 mM, pH 7.5, in a total volume of 10 mL) was incubated at 30 °C for 48 h. The oligosaccharide was isolated by charcoal-column chromatography (3.0 × 20 cm, with charcoal for chromatography purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan). The sample was put on the column which was washed beforehand with 1 L of 1:1 EtOH–water and

then with 1 L of distilled water. The column was washed with 1 L of distilled water and then with 1 L of 5:95 EtOH–water. Then the tetrasaccharides was eluted with 1:9 EtOH–water. Oligosaccharides in the fractions were analyzed by TLC. The fractions containing the tetrasaccharide only were combined and evaporated to a syrup at 50 °C under reduced pressure. The syrup was dissolved in MeOH (10 mL) and evaporated to a white powder. The powder was dried under reduced pressure over $CaCl_2$: yield 110 mg, mp 135–140 °C; $[\alpha]_D^{20} + 0.6°$ (*c* 0.72, H_2O).

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References

[1] M. Kawamura, T. Uchiyama, T. Kuramoto, Y. Tamura, and K. Mizutani, *Carbohydr. Res.*, 192 (1989) 83–90.
[2] M. Kawamura and T. Uchiyama, *Biosci. Biotechnol. Biochem.*, 57 (1993) 343.
[3] J.A. DePinto and L.L. Campbell, *Biochemistry*, 7 (1968) 114–120.
[4] S. Kitahata, in the Amylase Research Society of Japan (Ed.), *Enzyme Chemistry and Molecular Biology of Amylases and Related Enzymes*, CRC Press, Boca Raton, FL, 1995, pp 6–17.
[5] K. Fujita, N. Kuwahara, T. Tanimoto, K. Koizumi, M. Iizuka, N. Minamiura, K. Furuichi, and S. Kitahata, *Biosci. Biotechnol. Biochem.*, 58 (1994) 239–243.
[6] M. Muramatsu and T. Nakakuki, *Biosci. Biotechnol. Biochem.*, 59 (1995) 208–212.
[7] M. Kawamura and T. Uchiyama, *Carbohydr. Res.*, 260 (1994) 297–304.
[8] M. Ghebregzabher, S. Rufini, B. Monaldi, and M. Lato, *J. Chromatogr.*, 127 (1976) 133–162.