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Structural analyses of new tri- and tetrasaccharides produced from disaccharides by transglycosylation of purified *Trichoderma viride* β-glucosidase

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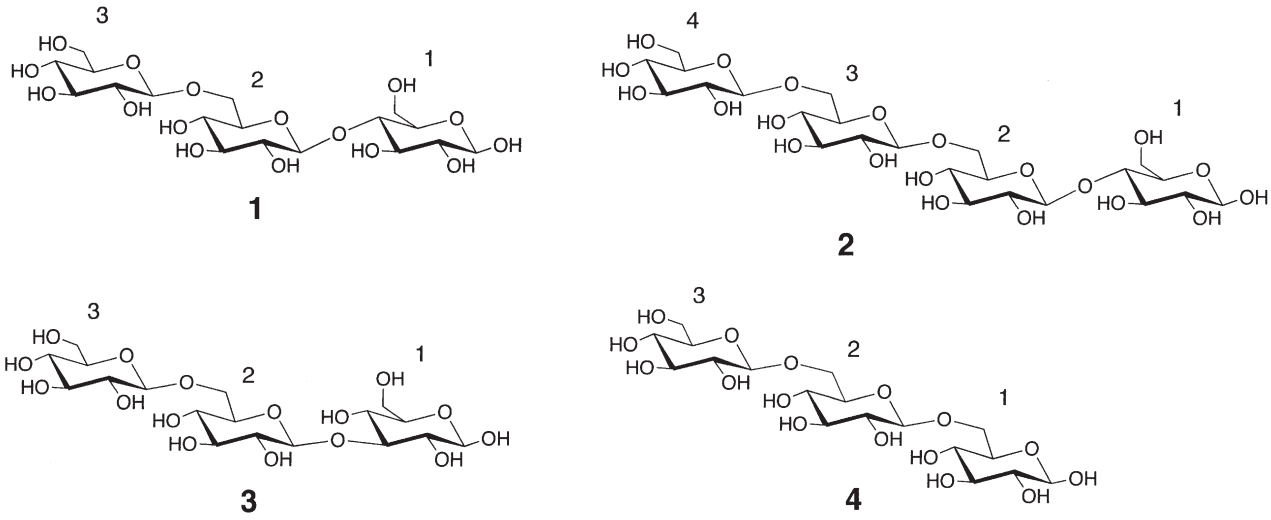
A new β-glucosidase was partially purified from *Trichoderma viride* cellulase. This β-glucosidase catalyzed a transglycosylation reaction of cellobiose to give β-D-Glc-(1→6)-β-D-Glc-(1→4)-D-Glc (1, yield: 18.8%) and β-D-Glc-(1→6)-β-D-Glc-(1→6)-β-D-Glc-(1→4)-D-Glc (2, 3.7%), regioselectively. Furthermore, the enzyme regioselectively converted laminaribiose and gentiobiose into β-D-Glc-(1→6)-β-D-Glc-(1→3)-D-Glc (3, 15.3%) and β-D-Glc-(1→6)-β-D-Glc-(1→6)-D-Glc (4, 20.2%), respectively. The structures (1–4) of the products were determined by ^{1}H and ^{13}C NMR spectroscopies. This high regio- and stereoselectively of the β-glucosidase could be applied for oligosaccharide synthesis.



***Keywords: Trichoderma viride,* β-glucosidase, transglycosylation, two-dimensional NMR spectroscopy, oligosaccharide synthesis**

Introduction

Cellulase is generally characterized by three components [1,2]: endoglucanase [EC 3.2.1.4], exo-glucan cellobiohydrolase [EC 3.2.1.91], and β-glucosidase [EC 3.2.1.21]. Of these enzymes, endoglucanase and exo-glucan cellobiohydrolase act synergistically to hydrolyze crystalline cellulose to form cellooligosaccharides, while β-glucosidase hydrolyzes the cellooligosaccharide to glucose [1,2]. Some β-glucosidases are known to catalyze hydrolysis and transglycosylation [3–7]. Although the physiological significance of the latter reaction is not clear, it is believed to play a important role in the production of cellulase inducers [1]. Thus, clarification of the mechanism of the transglycosylation by β-glucosidase is important to elucidate the biological function of the β-glucosidase in the cellulase-producing microorganisms.

Recently, with increasing interest in oligosaccharide components of glycoproteins and glycolipids, β-glucosi-

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dases have attracted the attention of many researchers because β-glucosidase-catalyzed transglycosylation can be applied to a new method of oligosaccharide synthesis. The transglycosylation by β-glucosidase generally proceeds with substrate specificity and stereoselectivity to the acceptor molecules and has the advantage of using free sugar as both acceptor and donor [3–6]. However, wider use of β-glucosidase has been limited, because most of the β-glucosidases have no regioselectivities for the hydroxyl linkages to the sugar acceptor. Thus, in many cases, the products of the transglycosylation are obtained as mixtures of regio-isomers [5,6]. In order to use β-glucosidase as a catalyst for oligosaccharide synthesis at a practical level, both high regio- and stereoselectivity are necessary for the β-glucosidase.

In this study, we report on the purification of β-glucosidase from *Trichoderma viride (T. viride)* cellulase complex [8–10] and oligosaccharide syntheses from β-glucobioses effected by purified β-glucosidase. We also carried out a structural analysis of the products to elucidate the regioselectivity of the β-glucosidase, which is also described herein.

Materials and methods

Materials

T. viride cellulase ONOZUKA R-10 was purchased from Yakult Pharmaceutical Industry Co. Ltd., Japan. *p*-Nitrophenyl-β-glucopyranoside (*p*NP-Glc), sophorose, laminaribiose, and gentiobiose were purchased from Sigma Chemical Co., USA. The series of cellooligosaccharides from cellobiose through cellopentaose were purchased from Seikagaku Co., Japan.

Analytical methods

Protein concentration was determined spectrophotometrically at 280 nm or by the method of Lowry et al. [11], using bovine serum albumin as standard. Elemental analysis was carried out with a 185 analyzer (Hewlett-Packard Ltd., USA). HPLC was carried out using a LC10-VP apparatus (Shimadzu Co., Japan) with TSK gel amide-80 column (Tosoh, Co., Japan). The ^{1}H and ^{13}C NMR spectra of oligosaccharides were recorded on a Avance 600 spectrometer (Bruker Co., Germany) or a MSL-400 spectrometer (Bruker) at 23 °C. In recording one-dimensional ^{1}H NMR spectra, the water-eliminated Fourier transformation sequence [12] was used. The ^{1}H and ^{13}C chemical shifts were referenced to internal 4,4-dimethyl 4-silapentane sodium sulfonate, 0.015 ppm, and acetone, 31.55 ppm, respectively. Double-quantum filtered ^{1}H, ^{1}H-correlated spectroscopy (DQFCOSY) and total correlation spectroscopy (TOCSY) with a spin-lock time of 60 ms were used to assign ^{1}H resonances. Heteronuclear single quantum coherence (HSQC) and ^{1}H-decoupled multiple-bond heteronuclear multiple quantum coherence (HMBC) were used to assign ^{13}C resonances. Glycosyl-linked position of monosaccharide residue was determined by rotating frame nuclear overhauser and exchange spectroscopy (ROESY) experiment with a spin lock time of 300 ms. All the NMR experiments were performed according to standard pulse sequence. The structural analysis of the oligosaccharides was carried out by observing: the marked down-field shifts of ^{1}H and ^{13}C resonances [13,14] and the type of residual rotating frame nuclear overhauser and exchange connectives from newly bounded glucose residue.

β-Glucosidase activity

50 μL of enzyme solution was incubated with 1 mM *p*NP-Glc (950 μL) in 50 mM acetate buffer, pH 5.0, (buffer *A*) at 40 °C for 10 min. The reaction was stopped by heating the solution at 100 °C for 5 min and then filtering it. The amount of released *p*-nitrophenol in the filtrate was determined by absorbance at 405 nm. One unit of β-glucosidase corresponds to the amount of enzyme that produces 1 μmol of *p*-nitrophenol per min.

Cellobiose hydrolysis

The standard procedure for cellobiose hydrolysis was as follows: 50 μL of enzyme solution (0.1 unit) and 2 mM cellobiose solution (950 μL) dissolved in buffer *A* was incubated at 40 °C for 20 min. The reaction mixture was heated at 100 °C for 5 min and filtered. The amount of glucose in the filtrate was measured by the mutarotase-glucose oxidase method [15].

Purification of β-glucosidase with transglycosylation ability from cellulase

Powder (2 g) of cellulase ONOZUKA R-10 (β-glucosidase activity; 0.051 units/mg-protein) was dissolved in buffer *A*. To this solution was added solid $(NH_4)_2SO_4$ to give 80% saturation. The precipitate formed was collected by centrifugation and desalted by ultrafiltration using a Q0100 filter (Advantec Toyo Co., Japan). The enzyme solution (266 mg protein, 0.121 units/mg-protein) was applied to a HiLoad 26/10 Q Sepharose High Performance column (Pharmacia LKB Biotechnology Co., Sweden) equilibrated with buffer *A* and then eluted with linear gradient of NaCl (0–550 mM) in the buffer *A*. Fractions (25 mL each) were collected, and 50 μL of each fraction was incubated with 500 mM cellobiose (950 μL) in buffer *A* for 12 h at 40 °C. The reaction was stopped at 100 °C for 5 min. The products of the reaction mixture were quantified by HPLC on the basis of peak areas using standard glucose and cellooligosaccharides (degree of polymerization: 2–5). Fractions that could produce trisaccharide (fraction number 18-21 in Figure 1) were collected and concentrated to ca. 3 mL by ultrafiltration. The enzyme solution (45.6 mg-protein, 0.057

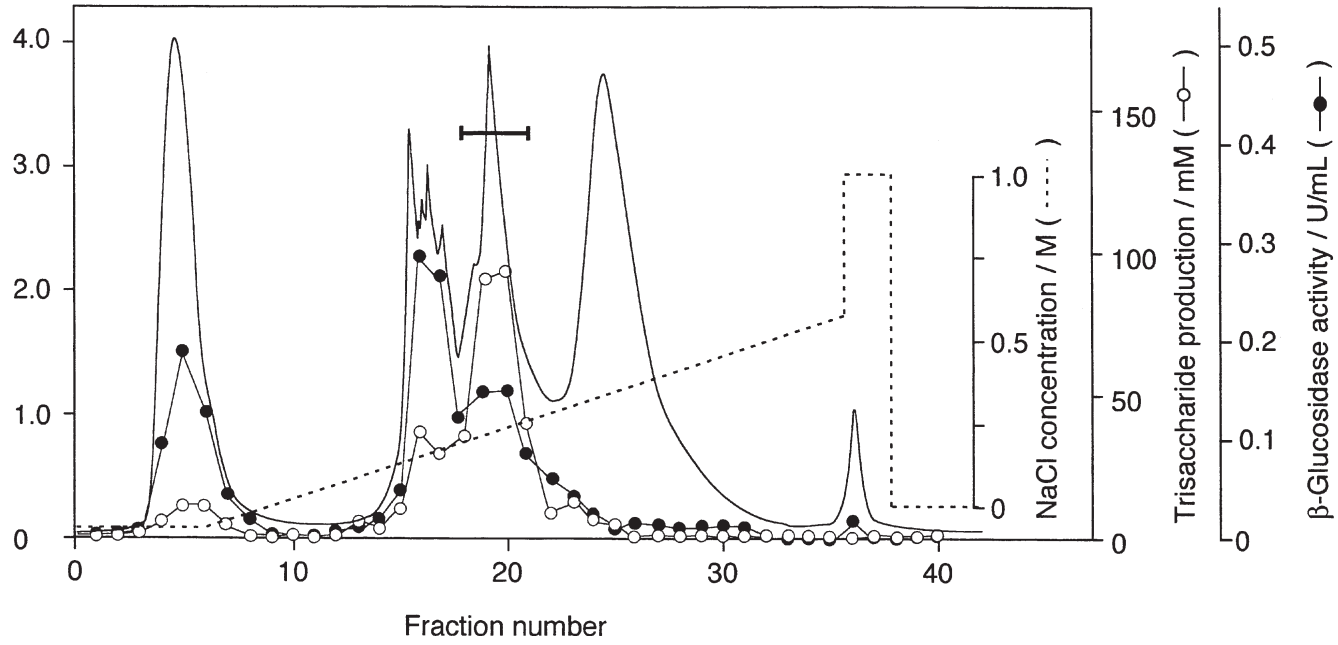

Figure 1. Purification of β-glucosidase by anion-exchange chromatography on HiLoad 26/10 Q Sepharose High Performance. β-glucosidase activity (●) and trisaccharide production (○) were measured. The solid and broken lines represent absorbance at 280 nm and NaCl concentration, respectively.

units/mg-protein) was directly applied to Superdex 75 HR 10/30 column (Pharmacia), eluted with buffer *A* with a flow rate of 1 mL/min. Fractions (1 mL of each) were collected, and the trisaccharide productions of these fractions were estimated according to the procedure described above. Fractions that could produce trisaccharide (fraction number 16-29, data not shown) were collected and lyophilized to give 16.6 mg β-glucosidase (0.141 units/mg-protein). The enzyme was used in the following experiments without further purification.

Measurement of molecular weight

SDS-PAGE was carried out according to Laemmli [16], using 12.5% gel. From the calibration curve obtained by using molecular marker kit (Pharmacia), the molecular weight was estimated as ca. 68.0 kDa.

Effect of pH and temperature on cellobiose hydrolysis

The optimum pH for hydrolysis of cellobiose was measured by the standard procedure using 50 mM acetate buffer (pH 3.0–6.0) or 50 mM phosphate buffer (pH 6.0–8.0) in spite of the presence of buffer *A*. The optimum temperature for the hydrolysis of cellobiose was determined by carrying out the standard procedure at various temperatures between 25 °C and 80 °C. Fifty mM glycine buffer (pH 2.0–3.0), acetate buffer (pH 3.0–6.0), and phosphate buffer (pH 6.0–9.0) were used to measure pH stability. The enzyme solutions at the various pH buffers were kept at 4 °C. After 24 h, the remaining activity toward cellobiose of the enzyme solution was measured according to the standard procedure. The thermal stability was also measured. The enzyme solutions in buffer *A* were incubated at various temperatures between 4 °C and 90 °C for 30 min. The remaining activity toward cellobiose of the incubated enzyme solution was measured according to the standard procedure.

Substrate specificity

Two mM disaccharide solution (950 μL) in buffer *A* was mixed with 50 μL of enzyme solution (0.1 unit) and incubated at 40 °C. An aliquot (100 μL) was withdrawn at appropriate time intervals. The aliquot was heated at 100 °C for 5 min and then filtered off. The amount of glucose in the filtrate was analyzed by the mutarotase-glucose oxidase method [15].

Syntheses of β-D-Glc-(1→6)-β-D-Glc-(1→4)-D-Glc (1) and β-D-Glc-(1→6)-β-D-Glc-(1→6)-β-D-Glc-(1→4)-D-Glc (2) from cellobiose

A solution (1 mL) of buffer *A* containing cellobiose (171 mg) and the purified β-glucosidase (0.141 units) was incubated at 40 °C for 24 h. After the transglycosylation reaction was stopped by heating at 100 °C for 5 min, the mixture was filtered. The filtrate was applied onto a Bio-Gel P-2 column (Bio-Rad Laboratories Inc., USA, 2.5 × 120 cm). The column was eluted with water. The sugar content of the

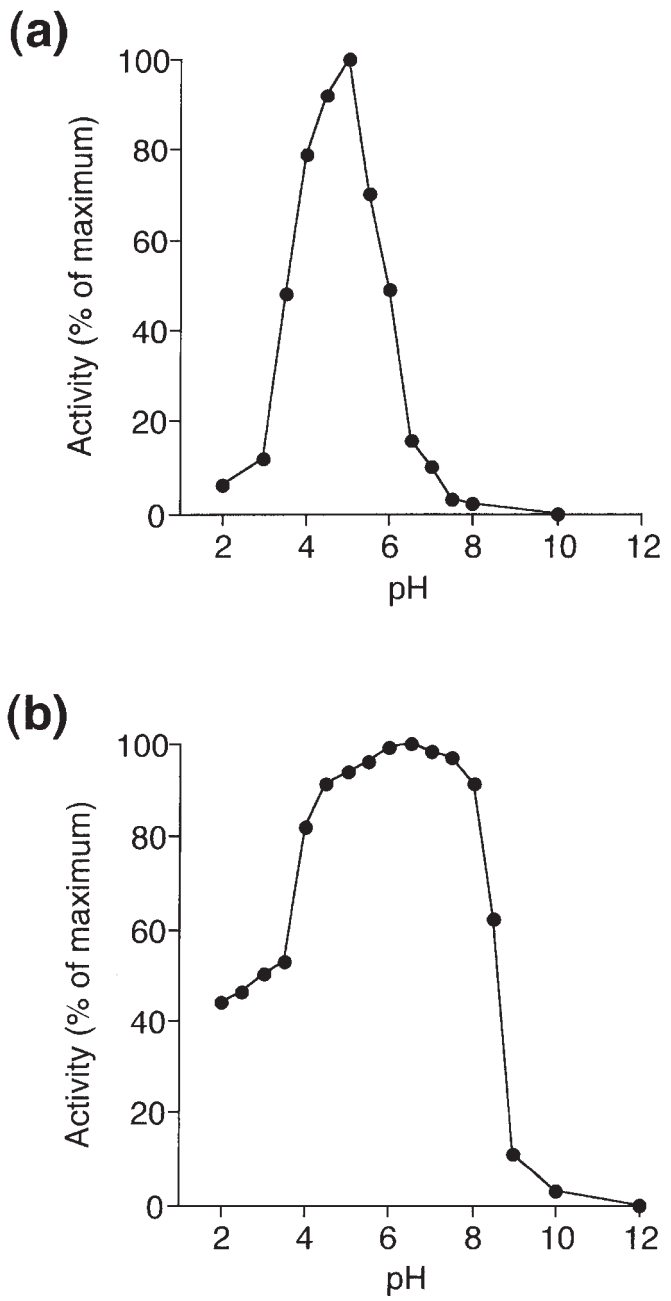

Figure 2. Effect of pH on *T. viride* β-glucosidase. (a) Effect of pH on *T. viride* β-glucosidase. (b) Effect of pH on the stability of *T. viride* β-glucosidase.

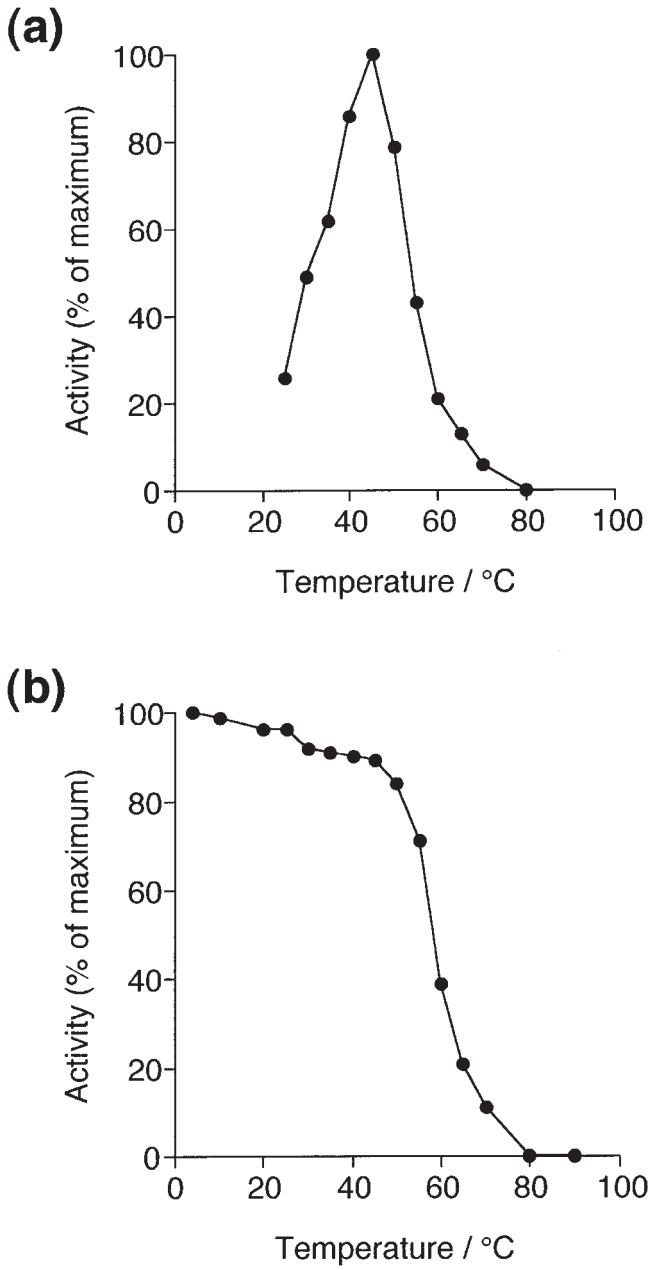

Figure 3. Effect of temperature on *T. viride* β-glucosidase. (a) Optimum temperature for hydrolysis of cellobiose. (b) Thermal stability of *T. viride* β-glucosidase.

fraction was estimated by the Nelson [17]-Somogyi [18] method. Fractions containing trisaccharide and tetrasaccharide were pooled and lyophilized to give **1** (32.2 mg, 18.8% yield) and **2** (6.4 mg, 3.7%), respectively.

Anal. Calc. for $C_{18}H_{32}O_{16}$: C, 42.86; H, 6.39. Found (for **1**): C, 42.73; H, 6.38

Anal. Calc. for $C_{24}H_{42}O_{21}$: C, 43.24; H, 6.35. Found (for **2**): C, 43.30; H, 6.34

Synthesis of β-D-Glc-(1→6)-β-D-Glc-(1→3)-D-Glc (3) from laminaribiose

A solution (1 mL) of buffer *A* containing laminaribiose (171 mg) and the purified β-glucosidase (0.141 units) was incubated at 40 °C for 24 h. After the transglycosylation reaction was stopped by heating at 100 °C for 5 min, the mixture was filtered off. By the purification with Bio-Gel P-2 column chromatography similar to **1** purification, 26.1 mg of **3** was obtained (15.3% yield).

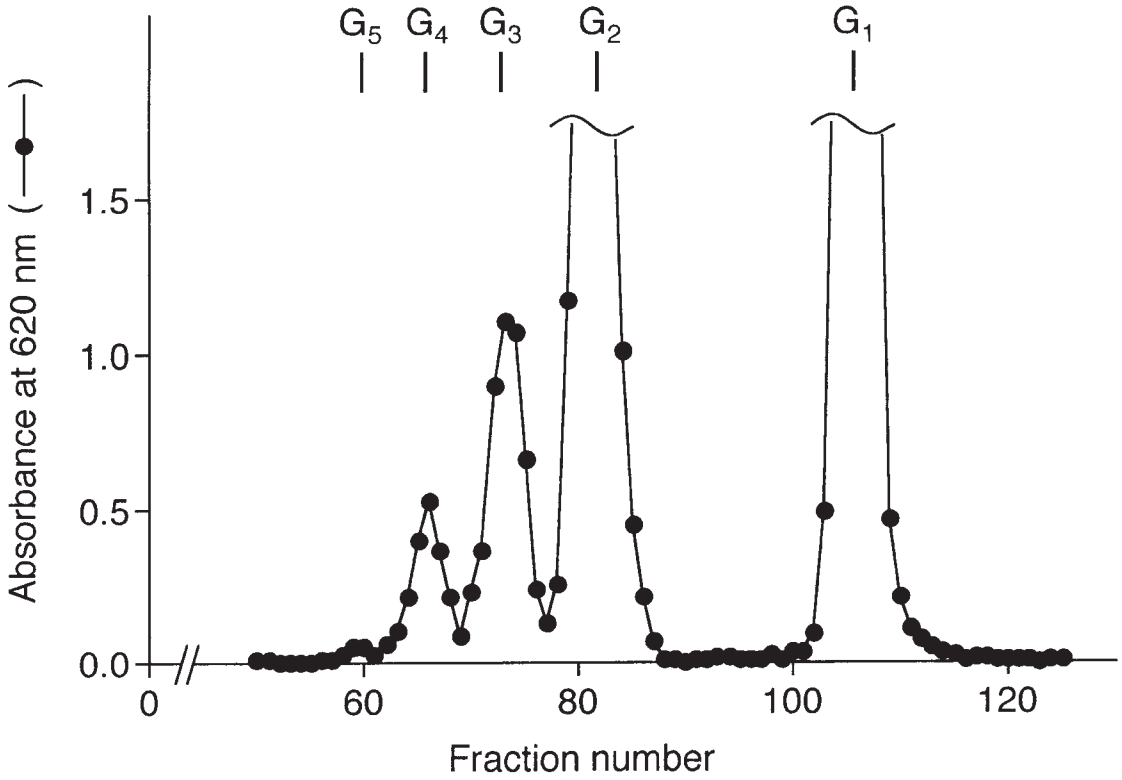

Figure 4. Gel-chromatographic separation of transglycosylation products by the action of β-glucosidase on cellobiose. The positions of G_1, and G_2–G_5 indicated the elution volumes of the standard glucose and the series of cellooligosaccharides from cellobiose through cellopentaose, respectively. 4 mL fractions were collected.

Anal. Calc. for $C_{18}H_{32}O_{16}$: C, 42.86; H, 6.39. Found (for **3**): C, 42.69; H, 6.37

Synthesis of β-D-Glc-(1→6)-β-D-Glc-(1→6)-D-Glc (4) from gentiobiose

A solution (1 mL) of buffer *A* containing gentiobiose (171 mg) and the purified β-glucosidase (0.141 units) was incubated at 40 °C for 24 h. After the transglycosylation reaction was stopped by heating at 100 °C for 5 min, the mixture was filtered off. By the purification with Bio-Gel P-2 column chromatography similar to **1** purification, 34.6 mg of **4** was obtained (20.2% yield).

Anal. Calc. for $C_{18}H_{32}O_{16}$: C, 42.86; H, 6.39. Found (for **4**): C, 42.89; H, 6.34

Results and discussion

Purification of β-glucosidase and its hydrolytic properties

For the purification of β-glucosidase having transglycosylation ability from *T. viride* cellulase complex, HiLoad 26/10 Q Sepharose High Performance column chromatography was performed. The elution of the cellulase after the ammonium sulfate precipitation is shown in Figure 1. Three peaks of β-glucosidase activity associated with the protein peaks were observed. Of these, the β-glucosidase fraction containing transglycosylation ability at the end of the salt gradient was fractionated. After ultrafiltration, the enzyme solution was applied to a Superdex 75 HR 10/30 column. On the gel-filtration chromatogram, one symmetrical peak of β-glucosidase activity associated with the protein peak appeared at the position where the other proteins were not eluted (data not shown). The β-glucosidase fraction had high transglycosylation ability, as described later in this paper.

In the examination of the effect of pH on hydrolysis of cellobiose, as shown in Figure 2, the maximum activity was observed at pH 5.0 in sodium acetate buffer. The purified enzyme was stable in the range pH 4.0–8.0. In the experiment measuring the effect of temperature, as shown in Figure 3, the optimum temperature for cellobiose hydrolysis of the enzyme was observed at 45 °C. The purified enzyme was sensitive to temperature above 50 °C at pH 5.0. The optimum pH and temperature for hydrolysis of cellobiose by purified β-glucosidase corresponded to those by cellulase before the purification (data not shown). In order to investigate the substrate specificity of the purified β-glucosidase in the hydrolysis reaction, the rate of the hydroly-

Table 1. ^{1}H chemical shifts (ppm) of the saccharides (**1–4**) in D_2O at 23 °C

	Chemical shift (ppm)						
Residue[a]	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-6*
β-D-Glc-(1→6)-β-D-Glc-(1→4)-D-Glc (**1**)							
1α	5.226	3.584	3.834	3.620	3.955	3.870	3.968
1β	4.667	3.290	3.641	3.656	3.655	3.815	3.955
2	4.518	3.334	3.522	3.508	3.672	3.882	4.224
3	4.537	3.329	3.517	3.405	3.468	3.736	3.924
β-D-Glc-(1→6)-β-D-Glc-(1→6)-β-D-Glc-(1→4)-D-Glc (**2**)							
1α	5.228	3.587	3.829	3.617	3.954	3.871	3.965
1b	4.667	3.294	3.638	3.647	3.656	3.813	3.957
2	4.520	3.338	3.526	3.509	3.672	3.877	4.227
3	4.520	3.342	3.486	3.484	3.687	3.856	4.230
4	4.555	3.331	3.519	3.405	3.462	3.740	3.928
β-D-Glc-(1→6)-β-D-Glc-(1→3)-D-Glc (**3**)							
1α	5.225	3.621	3.823	3.521	3.871	3.816	3.899
1b	4.664	3.353	3.704	3.509	3.533	3.815	3.911
2	4.713	3.423	3.568	3.513	3.679	3.889	4.226
3	4.549	3.334	3.510	3.406	3.466	3.733	3.926
β-D-Glc-(1→6)-β-D-Glc-(1→6)-D-Glc (**4**)							
1α	5.220	3.531	3.689	3.490	3.878	3.966	4.130
1b	4.666	3.236	3.482	3.462	3.615	3.839	4.198
2	4.516	3.340	3.489	3.486	3.619	3.855	4.223
3	4.516	3.327	3.501	3.396	3.458	3.734	3.919

[a]A number indicates position of glucose residue from reducing-end unit of oligosaccharides.
Underline indicates glycosidically linked position.

sis of the disaccharides was measured. The enzyme hydrolyzes all the disaccharides, and the hydrolysis rates are in this order: β-(1→3)-, β-(1→4)-, β-(1→2)-, and β-(1→6)-linked disaccharide (data not shown). These hydrolytic properties are similar to those of other β-glucosidases [3,6].

Syntheses of oligosaccharides (1–4) by transglycosylation of β-glucobioses using the purified β-glucosidase

We tried to perform the transglycosylation reactions with cellobiose, laminaribiose, gentiobiose, or sophorose as substrates using the purified β-glucosidase. Figure 4 shows the elution profile of transglycosylation reaction products of cellobiose by the purified β-glucosidase from Bio-Gel P-2. Fraction numbers from G_1 through G_5 appeared at the same filtration volume compared with standard glucose and cellooligosaccharides from cellobiose through cellopentaose. The chromatogram showed two remarkable high peaks in the trisaccharide (G_3) and tetrasaccharide (G_4) regions. After the fractions corresponding to the tri- and tetrasaccharide elution were collected and lyophilized, the trisaccharide and tetrasaccharide were identified by ^{1}H and ^{13}C NMR spectroscopy (Tables 1 and 2, respectively) as β-D-Glc-(1→6)-β-D-Glc-(1→4)-D-Glc (**1**) and β-D-Glc-(1→6)-β-D-Glc-(1→6)-β-D-Glc-(1→4)-D-Glc (**2**), respectively. As shown in Fig. 5, there were no signals derived from the other tri- and tetrasaccharides in the ^{1}H NMR analysis. This result indicated that D-glucose residue at the non-reducing end of cellobiose was regioselectively transferred to O-6 of D-glucose residue at non-reducing end of another cellobiose to produce trisaccharide **1.** Furthermore, the β-glucosidase regioselectively catalyzed the transferring β-D-glucose residue of cellobiose to O-6 of the non-reducing terminus of product **1** to synthesize tetrasaccharide **2.** Although the trisaccharide **1** had been reported to be synthesized from cellobiose by *Aspergillus niger* β-glucosidase [3], the tetrasaccharide **2** was synthesized and properly characterized in this experiment for the first time.

A quite similar regioselectivity was observed in the transglycosylation reaction that used laminaribiose or gentiobiose as a substrate instead of cellobiose. Trisaccharides were synthesized from laminaribiose and gentiobiose by the β-glucosidase-catalyzed transglycosylation, although

Table 2. ^{13}C chemical shifts (ppm) of the saccharides (**1–4**) in D_2O at 23 °C

	Chemical shifts (ppm)					
Residue[a]	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
β-D-Glc-(1→6)-β-D-Glc-(1→4)-D-Glc (**1**)						
1α	94.63	73.97	74.34	82.28	72.79	62.79
1b	98.51	76.65	77.48	82.07	77.26	62.93
2	105.49	75.87	78.43	72.28	77.61	71.48
3	105.50	75.94	78.22	72.41	78.69	63.53
β-D-Glc-(1→6)-β-D-Glc-(1→6)-β-D-Glc-(1→4)-D-Glc (**2**)						
1α	94.60	74.00	74.32	82.27	72.81	62.81
1b	98.49	76.69	77.25	82.04	77.51	62.94
2	105.50	75.91	78.46	72.23	77.73	71.43
3	105.70	75.85	78.33	72.45	77.70	71.89
4	105.72	75.91	78.24	72.43	78.71	63.53
β-D-Glc-(1→6)-β-D-Glc-(1→3)-D-Glc (**3**)						
1α	94.68	73.46	85.10	70.35	72.86	62.78
1b	98.51	75.21	87.32	70.42	77.68	62.91
2	105.50	75.53	78.20	71.90	77.79	71.38
3	105.31	75.62	78.12	71.95	78.42	63.42
β-D-Glc-(1→6)-β-D-Glc-(1→6)-D-Glc (**4**)						
1α	94.62	73.26	74.40	71.76	72.76	71.26
1b	98.48	76.65	77.80	71.87	77.94	71.06
2	105.50	75.12	77.86	71.96	77.76	71.42
3	105.51	75.46	78.19	72.30	78.60	63.49

[a]A number indicates position of glucose residue from reducing-end unit of oligosaccharides.
Underline indicates glycosidically linked position.

no formation of tetrasaccharides was observed in HPLC analysis (data not shown). As summarized in Tables 1 and 2, ^{1}H and ^{13}C NMR spectroscopic analysis revealed that laminaribiose and gentiobiose also were regioselectively converted into trisaccharide β-D-Glc-(1→6)-β-D-Glc-(1→3)-D-Glc (**3**) and β-D-Glc-(1→6)-β-D-Glc-(1→6)-D-Glc (**4**), respectively. Productions of no other regioisomers from laminaribiose and gentiobiose were observed by HPLC analysis, respectively. The trisaccharides **3** and **4** were synthesized by enzymatic transglycosylation for the first time. Although structural analyses of the trisaccharides **1, 3,** and **4** had already been performed by ^{13}C NMR [19] and GC-MASS [20,21], complete assignments of all proton and carbon resonances for NMR spectra of the trisaccharides **1, 3,** and **4** were carried out in our experiments for the first time.

On the other hand, when sophorose was used as a substrate for the transglycosylation reaction, trisaccharide was not obtained. In the products from sophorose, small amounts of gentiobiose and cellobiose were detected, which indicated that the β-glucosidase could not recognize sophorose as a glycosyl acceptor in the transglycosylation. From these results of the transglycosylation of four β-glucobioses, we concluded that the purified β-glucosidase have strict specificity for acceptors in transglycosylation reaction, although the enzyme could hydrolyze all the β-glucobioses.

Regarding the mechanism of action with synthetic and natural substrates of glycosidases, *Escherichia coli* β-galactosidase [22–24] has been studied quite extensively. Research has shown that the β-galactosidase has two subsites for binding the lactose (β-D-Gal-(1→4)-D-Glc), one for galactose residue and the other for glucose residue [22–24]. When the β-galactosidase has caused breakage of the glycosidic bond of lactose, glucose has diffused away and the enzyme is in galactosyl form. In the intermolecular transglycosylation of lactose, the enzyme converts lactose into allolactose (β-D-Gal-(1→6)-D-Glc). In the transglycosylation of lactose by the β-galactosidase, Huber et al. [23] suggested that the glucose site of the galactosyl form of the galactosidase becomes highly specific for binding glucose after the glycosidic bond of lactose is broken, and it is best to bind glucose more tightly at the C-6 hydroxyl group, not the other hydroxyl group, because it is in close contact with the active galactose in the galactosyl form of the enzyme.

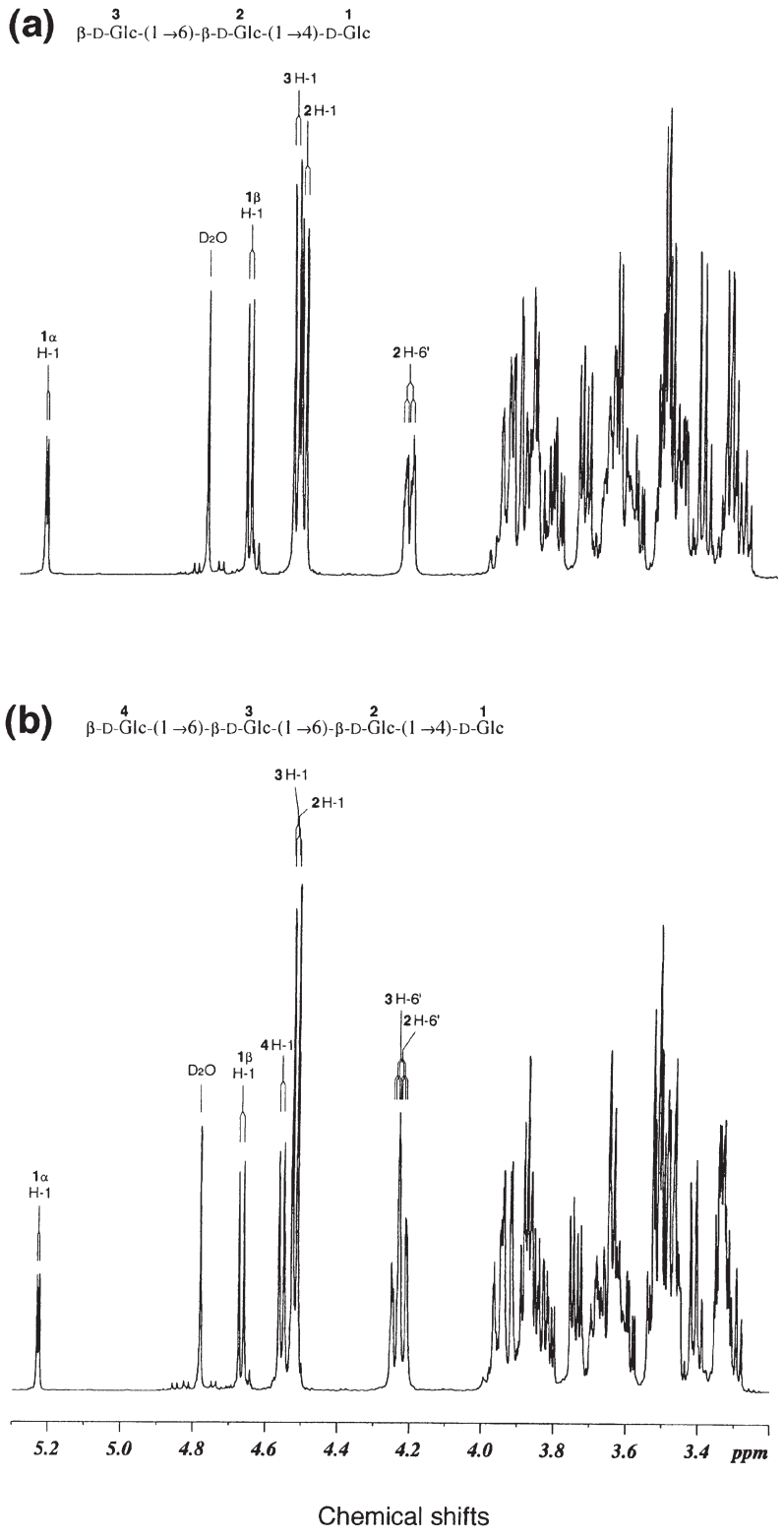

Figure 5. ^{1}H NMR spectra of (a) the trisaccharide **1** and (b) the tetrasaccharide **2.**

Further, Huber and Gaunt [25] suggested that definite specificities for binding of the transglycosylation products were found at the acceptor site (glucose site) of the β-galactosidase. With respect to β-glucosidase-catalyzed transglycosylation, Gopalan et al. [7] suggested that the presence of two binding subsites for acceptor and donor molecules within the catalytic center of the mammalian cytosolic liver β-glucosidase and that the structure of the acceptor-binding subsite have a direct effect on regioselectivity in the transglycosylation. In this experiment, since it is not clear that such sugar binding-sites in the catalytic center of the purified *T. viride* β-glucosidase exist, interpretation of the results is not possible. If the transglycosylation reaction in this experiment proceeds within such acceptor and donor subsites, the acceptor site has no affinity for sophorose and it binds cellobiose, laminaribiose, gentiobiose, or product **1** to bring the C-6 hydroxyl group of the non-reducing end of the acceptor close to the C-1 hydroxyl group of glucose within the donor binding-site in order to produce **1, 2, 3,** or **4,** respectively. To clearly elucidate the regioselectivity of this β-glucosidase, structural analysis of the catalytic region in β-glucosidase is necessary, although such an analysis will be difficult to achieve.

In cellulase-producing microorganisms, cellulose is known to be a common inducer of cellulase formation [1,2]. Since cellulose is insoluble and impermeable to the microorganisms, soluble oligosaccharides such as sophorose [26,27], cellobiose [28,29], and gentiobiose [30] are considered to act as direct inducers of cellulase. Regarding the fungus *T. viride,* sophorose was found to be an inducer of cellulase [26], whereas glucose caused repression of cellulase secretion [28]. The oligosaccharides (**1–4**), though their biological functions and a mechanism for cellulase induction by *T. viride* have not been clear yet, would be expected to play an important role in the metabolism of *T. viride* because of the products synthesized by the transglycosylation of *T. viride* β-glucosidase.

Many researchers have reported that β-glucosidases originating from a wide variety of microorganisms [3,5,8], higher plants [4,6], and animals [7] catalyze transglycosylation reactions. Whereas transglycosylation reactions with these enzymes result in the formation of several isomers, in our experiments, only β-(1→6)-linkages were formed in the transglycosylation products **1–4.** We therefore conclude that the β-glucosidase obtained from *T. viride* cellulase in our experiment has precise regioselectivity as well as stereoselectivity toward the hydroxyl group of the sugar acceptor in the transglycosylation. Thus, this enzyme can be used for the regio- and stereoselective synthesis of oligosaccharides in an aqueous buffer solution.

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