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Acceptor specificity of cellobiose phosphorylase from *Cellvibrio gilvus*: synthesis of three branched trisaccharides

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Abstract

Cellobiose phosphorylase from *Cellvibrio gilvus* was examined for its acceptor specificity in the synthetic reaction with glucose-1-phosphate, using substrates in which the C-6 substituent of D-Glc had been altered. A range of disaccharides were also tested for acceptor specificity but only those with (1→6)-linkages were successful acceptors. Melibiose, gentiobiose, isomaltose and also the monosaccharide glucuronamide were found to react with cellobiose phosphorylase and glucose-1-phosphate giving β-D-Glc*p*-(1→4)-[α-D-Gal*p*-(1→6)]-D-Glc*p*, β-D-Glc*p*-(1→4)-[β-D-Glc*p*-(1→6)]-D-Glc*p*, β-D-Glc*p*-(1→4)-[α-D-Glc*p*-(1→6)]-D-Glc*p* and β-D-Glc*p*-(1→4)-D-GlcUN*p*, respectively. These products were purified using a range of chromatographic methods and characterised by NMR and FAB-MS. This is the first time cellobiose phosphorylase has been shown to synthesise trisaccharides.

Keywords: β-D-Glucopyranosyl-(1→4)-D-glucuronamide; β-D-Glucopyranosyl-(1→4)-[α-D-galactopyranosyl-(1→6)]-D-glucopyranose; β-D-Glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-D-glucopyranose;β-D-Glucopyranosyl-(1→4)-[α-D-glucopyranosyl-(1→6)]-D-glucopyranose; *Cellvibrio gilvus*; Cellobiose phosphorylase

1. Introduction

Cellobiose phosphorylase (EC 2.4.1.20) has been shown to catalyse the synthesis of a number of Glc-β-(1→4) linked disaccharides from glucose-1-phosphate (G-1-P) and various glycosyl acceptors [1–9]. The reversible phosphorolysis reaction requires the acceptor to maintain the configuration of β-D-Glc*p* at the C-1 and C-4 positions, although the configurations of the C-3 and C-5 positions can also be important [5,6]. Comparison of the data for 6-deoxy and 2-deoxyglucose showed that the role of the hydroxyl group at C-6 is less critical than that of the hydroxyl group at C-2 [6]. Thus 6-deoxy-D-Glc and D-Xyl can both act as successful acceptors and this is confirmed by the synthesis and characterisation of β-D-Glc*p*-(1→4)-6-deoxy-D-Glc*p* [4] and β-D-Glc*p*-(1→4)-D-Xyl*p* [1]. Kinetic data suggests that the hydroxyl group at the C-6 plays a role in enzyme-substrate binding [6].

Our laboratory has been working to fully investigate the acceptor specificity in the synthesis reaction of cellobiose phosphorylase from *Cellvibrio gilvus* with a view to producing novel or useful

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oligosaccharides. In this study we examined the effect that varying the C-6 substituent of D-Glc had on acceptor specificity and confirmed the structures of the oligosaccharides produced using NMR and FAB-MS.

2. Experimental

Chemicals.—Sugars, α-D-Glc-1-phosphate (dipotassium salt), BSA and glucose oxidase were obtained from Sigma Chemical (St. Louis, MO). Glc was removed from isomaltose by treatment with glucose oxidase at pH 5.1 for 24 h at 35 °C followed by elution through a de-ionising ion-exchange column. Ethanol (HPLC grade) was purchased from Nacalai Tesque (Kyoto, Japan). Acetonitrile (HPLC grade) and activated charcoal were obtained from Wako Pure Chemical Industries (Osaka, Japan). SP Sepharose Fast Flow and Q Sepharose Fast Flow were purchased from Pharmacia, Uppsala, Sweden.

General methods.—HPLC was performed using either a TSK Gel-G-oligo-PW column (7.8×300 mm; Tosoh, Tokyo, Japan) or a TSK Gel-Amide-80 column (4.6×250 mm; Tosoh). A Shimadzu HPLC system (Kyoto, Japan) and a GL Sciences RI model 504 detector (Tokyo, Japan) were used. High-pH anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) was carried out using a Dionex DX300 (Dionex, CA) equipped with a Dionex CarboPac PA1 column (4×250 mm) as described previously [5].

DQF-COSY, TOCSY, HMQC, and HMBC NMR experiments were carried out in 99.8% D_2O containing 2-methyl-2-propanol as an internal reference. Chemical shifts were expressed in ppm (δ) downfield from tetramethylsilane. Spectra were obtained at 303 K. All NMR spectra were recorded on a Bruker DRX 600 spectrometer operating at 600.13 MHz (^1H) and 150.15 MHz (^{13}C) using inverse QXI ($^1H/^{13}C$, ^{15}N, ^{31}P) xyz-gradient and QNP (^{13}C, ^{15}N, $^{31}P/^1H$) z-gradient probeheads. Selective HMBC spectra were acquired for *β*-D-Glc*p*-(1→4)-D-GlcUN*p*. Typical parameters used in selective HMBC measurement are described as follows. Shaped pulse: 1 msec pulse width, Gaussian waveform of 512 points. Spectral window of 1446.8×150.9 Hz acquired 2 scans/FID in a 4096×32 matrix and transformed in 2048×64 data points. Digital resolution of f1 dimension: 2.4 Hz. Delay for evolution of long range coupling: 80 ms. Relaxation delay: 2 s. Window function: 90°-shifted squared-sine bell in both dimensions.

Positive ion FAB mass spectra were recorded using a reverse-geometry (BE) double-focusing (JMS SX102A, JEOL, Tokyo) mass spectrometer equipped with a FAB gun producing a 6 keV xenon beam. A resolving power of 3000 was employed with an ion acceleration potential of 10 kV. The spectra were obtained by scanning up to 2000 using a DA 7000 data system. The mass scale was calibrated with Ultramark 1621 (PCR, Gainesville, FL). A matrix of glycerol was used.

Preparation of cellobiose phosphorylase from C. gilvus.—Partially purified cellobiose phosphorylase was obtained by a combination of ammonium sulphate precipitation and hydrophobic interaction column chromatography as described previously [5]. One unit of enzyme activity was defined as the amount of enzyme that produces 1 μmol of D-Glc per min from 10 mM cellobiose and 10 mM P_i at 37 °C.

Oligosaccharide synthesis.—The acceptors (100 mM) were incubated in 50 mM MOPS buffer (pH 7.0), 0.02% BSA, 10 mM G-1-P, and 0.04 U mL^{-1} cellobiose phosphorylase at 37 °C for 24 h. Synthesis was measured by the amount of P_i released from G-1-P using the method of Baginski et al. [10]. For preparative syntheses a solution containing 40 mM sodium phosphate buffer (pH 7.0), 0.02% BSA, 10 mM G-1-P, 0.04 U mL^{-1} cellobiose phosphorylase, and 300 mM acceptor was incubated for 24 h at 37 °C. An exception to this was for the synthesis of the isomaltose trisaccharide in which 50 mM MOPs (pH 7.0) and 100 mM isomaltose were used.

Purification of β-D-*Glc*p-*(1→4)*-D-*GlcUN*p.—The crude reaction mixture (150 mL) was lyophilised and made up to 25 mL with water. It was then applied to a charcoal column (16×500 mm) and eluted with water (20 L, 1 mL min^{-1}) followed by a linear gradient from 0 to 20% aq EtOH (3 L). Fractions (20 mL) were analysed by HPSEC. The product-containing fractions were lyophilised and purified by HPSEC. The product was recovered by lyophilisation (48.5 mg, 9.1% yield based on G-1-P). FAB-MS (positive ion) *m/z*: 140 $[M-GlcO-2H_2O]^+$, 158 $[M-GlcO-H_2O]^+$, 176 $[M-GlcO]^+$, 356 $[M + H]^+$, 378 $[M + Na]^+$.

Purification of β-D-*Glc*p-*(1→4)*-*[*α-D-*Gal*p-*(1→6)]*-D-*Glc*p.—The reaction mixture (30 mL) was applied to a charcoal column (16×500 mm)

and eluted with 1.6% aq EtOH (3 L, 4 mL min^{-1}) followed by a linear gradient from 1.6 to 20% aq EtOH (2 L). The fractions (20 mL) were analysed by HPAEC-PAD, and product-containing fractions were combined and concentrated by rotary evaporation. Further purification was required in order to remove traces of G-1-P and a contaminant present from the melibiose. Therefore the product was purified further using an HPLC TSK-Gel-amide-80 column. Fractions (1 mL) were analysed using HPAEC-PAD, and the product-containing fractions were lyophilised (2.94 mg, 1.9% yield based on G-1-P). FAB-MS (positive ion) *m/z*: 145 $[M-2GlcO-H]^+$, 163 $[Glc]^+$, 325 ($[M-GlcO]^+$, $[M-GalO]^+$), 527 $[M + Na]^+$.

Purification of β-D-*Glc*p-*(1→4)*-[β-D-*Glc*p-*(1→6)*]-D-*Glc*p.—The enzyme reaction mixture (9.75 mL) was applied to a charcoal column (16×500 mm) and eluted using the same conditions as described for the trisaccharide above. The product-containing fractions were combined and concentrated by rotary evaporation and applied to a small charcoal column (5×100 mm). Elution with water (2 mL, 0.4 mL min^{-1}) was followed by 1.6% aq EtOH (28 mL), then a linear gradient from 1.6 to 20% aq EtOH (20 mL), and finally 20% aq EtOH (4 mL). The fractions (2 mL) were analysed by HPAEC-PAD and those containing the product were concentrated (1 mL) and applied to another charcoal column (5×100 mm). The column was washed with 2.5% aq EtOH (30 mL) at 0.2 mL min^{-1} and the product was then eluted using a linear gradient from 2.5 to 20% aq EtOH (30 mL). After lyophilisation of the product-containing fractions, 4.3 mg of *β*-D-Glc*p*-(1→4)-[*β*-D-Glc*p*-(1→6)]-D-Glc*p* was obtained (8.7% yield based on G-1-P). The trisaccharide was 94.3% pure as determined by HPAEC-PAD. FAB-MS (positive ion) *m/z*: 325 $[M-GlcO]^+$, 527 $[M + Na]^+$, 543 $[M + K]^+$.

Purification of β-D-*Glc*p-*(1→4)*-[α-D-*Glc*p-*(1→6)*]-D-*Glc*p.—The crude reaction mixture (10.1 mL) was applied to a charcoal column (16×500 mm) and eluted with 1.6% aq EtOH (2.8 L, 4 mL min^{-1}), and a linear gradient from 1.6 to 20% aq EtOH (3 L). The fractions (20 mL) were analysed by HPAEC-PAD, and the productcontaining fractions were combined and concentrated using rotary evaporation. The sample was then de-ionised with two small columns packed with SP Sepharose Fast Flow (OH^- form, 7×15 mm) and Q Sepharose Fast Flow (H^+ form, 7×15 mm). The product was lyophilised and 4.7 mg of *β*-D-Glc*p*-(1→4)-[α-D-Glc*p*-(1→6)]-D-Glc*p* was obtained (9.1% yield based on G-1-P). The purity was determined to be 96.6% using HPAEC-PAD. FAB-MS (positive ion) *m/z*: 325 $[M-GlcO]^+$, 527 $[M + Na]^+$.

3. Results and discussion

D-Glc derivatives in which the hydroxymethyl group had been modified or substituted were tested for their acceptor ability in the synthetic reaction of cellobiose phosphorylase (Table 1). Cellobiose phosphorylase could utilise D-glucuronamide in the synthetic reaction but was unable to utilise D-glucuronate or D-glucose-6-phosphate. (1→6)-linked Glc disaccharides were also found to have moderate acceptor ability (Table 1). Various (1→1), (1→2), (1→3), and (1→4)-linked disaccharides were also tested but were not found to be acceptors (Table 1).

In order to identify the products formed, the reactions using glucuronamide, melibiose, gentiobiose, and isomaltose were carried out on a preparative scale and the purified products characterised by NMR and FAB-MS. Using TOCSY and DQF-COSY NMR spectra, the ^1H chemical shifts and coupling constants were determined. ^{13}C NMR spectra were assigned using

Table 1
Acceptor specificity in the synthetic reaction of cellobiose phosphorylase

Acceptor		Product concentration[a] (mM)
Common name	Structure	
Monosaccharides		
D-Glucuronamide		0.85
D-Glucuronate		—[b]
D-Glucose-6-phosphate		—
Disaccharides		—
Melibiose	α-D-Gal*p*-(1→6)-D-Glc	1.05
Gentiobiose	β-D-Glc*p*-(1→6)-D-Glc	1.71
Isomaltose	α-D-Glc*p*-(1→6)-D-Glc	1.03
α,α-Trehalose	α-D-Glc*p*-(1↔1)-α-D-Glc	—
Sophorose	β-D-Glc*p*-(1→2)-D-Glc	—
Laminaribiose	β-D-Glc*p*-(1→3)-D-Glc	—
Nigerose	α-D-Glc*p*-(1→3)-D-Glc	—
Maltose	α-D-Glc*p*-(1→4)-D-Glc	—
Xylobiose	β-D-Xyl*p*-(1→4)-D-Xyl	—

[a] Measured by P_i assay.
[b] Activity could not be detected.

Table 2
^{13}C and ^{1}H NMR data for oligosaccharides synthesised by cellobiose phosphorylase

Residues[a]	C-1	C-2	C-3	C-4	C-5	C-6	
	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
	($J_{1,2}$ Hz)	($J_{2,3}$ Hz)	($J_{3,4}$ Hz)	($J_{4,5}$ Hz)	($J_{5,6a}$ Hz)	($J_{5,6b}$ Hz)	($J_{6a,6b}$ Hz)
	β-D-Glc*p*-(1→4)-[α-D-Gal*p*-(1→6)]-D-Glc*p*						
Iα	93.47	72.83	72.93	80.95	70.73	68.14	
	5.211 d [b]	3.587 dd	3.830 dd	3.672 dd	4.111 ddd	3.986 dd	3.854 dd
	(3.8)	(9.8)	(8.8)	(10.1)	(5.3)	(1.9)	(11.9)
Iβ	97.42	75.48	75.92	80.71	75.20	67.98	
	4.660 d	3.289 dd	3.626 dd	3.683 dd	3.765 ddd	3.952 dd	3.897 dd
	(8.0)	(9.3)	(8.8)	(9.7)	(5.5)	(2.0)	(11.8)
IIα	104.20	74.86	77.23	71.15 [c]	77.75	62.88	
	4.496 d	3.317 dd	3.476 dd	3.409 dd	3.456 ddd	3.902 dd	3.723 dd
	(7.9)	(9.3)	(8.9)	(9.7)	(2.2)	(5.7)	(12.4)
IIβ	104.27	74.84	77.23	71.13 [c]	77.74	62.26	
	4.496 d	3.310 dd	3.473 dd	3.410 dd	3.456 ddd	3.902 dd	3.723 dd
	(7.9)	(9.3)	(8.9)	(9.7)	(2.2)	(5.7)	(12.4)
IIIα	100.60	70.11	71.12 [c]	70.84	72.73	62.74	
	4.994 d	3.807 dd	3.861 dd	3.992 m [b]	3.95–3.99 m	3.742 d	3.742 d
	(4.1)	(10.5)	(3.6)	—	—	—	(6.0)
IIIβ	100.49	70.08	71.12 [c]	70.85	72.71	62.78	
	5.001 d	3.816 dd	3.867 dd	3.992 m	3.95–3.99 m	3.742 d	3.742 d
	(4.1)	(10.5)	(3.6)	—	—	—	(6.0)
	β-D-Glc*p*-(1→4)-[β-D-Glc*p*-(1→6)]-D-Gl*p*						
Iα	93.57	72.90	72.95	79.94	70.64	69.24	
	5.208 d	3.569 dd	3.804 dd	3.743 dd	4.066 ddd	4.184 dd	3.986 dd
	(3.8)	(9.7)	(9.0)	(10.0)	(1.9)	(3.9)	(11.2)
Iβ	97.57	75.89	75.51	79.95	75.25	69.33	
	4.656 d	3.284 dd	3.608 dd	3.72–3.75 m	3.72–3.73 m	4.258 dd	3.916 dd
	(8.0)	(9.5)	(8.7)	—	(2.0)	(4.1)	(11.0)
IIα	104.11	74.85	77.19	71.22	77.61	62.32	
	4.554 d [d]	3.309 dd [d]	3.498 dd [d]	3.400 dd	3.465 ddd	3.900 dd	3.722 dd
	(8.0)	(9.4)	(8.7)	(10.2)	(2.2)	(5.7)	(12.4)
IIβ	104.16	74.84	77.19	71.20	77.60	62.30	
	4.553 d [d]	3.301 dd [d]	3.494 dd [d]	3.400 dd	3.465 ddd	3.900 dd	3.722 dd
	(8.0)	(9.4)	(8.7)	(10.2)	(2.2)	(5.7)	(12.4)
IIIα	104.15	74.70	77.41	71.42	77.50	62.43	
	4.492 d	3.298 dd	3.495 dd	3.383 dd	3.450 ddd [e]	3.901 dd [e]	3.715 dd

	(8.0)	(9.4)	(8.7)	(10.2)	(2.2)	(5.7)	(12.4)
IIIβ	104.18	74.70	77.39	71.35	77.54	62.43	
	4.504 d	3.309 dd	3.494 dd	3.382 dd	3.448 ddd [e]	3.898 dd [e]	3.715 dd
	(8.0)	(9.4)	(8.7)	(10.2)	(2.2)	(5.7)	(12.4)
	β-D-Glc*p*-(1→4)-[α-D-Glcp-(1→6)]-D-Glc*p*						
Iα	93.49	72.83	72.95	80.85	70.68	67.99	
	5.212 d	3.587 dd	3.825 dd	3.686 dd	4.104 ddd	4.018 dd	3.819 dd
	(3.8)	(9.8)	(8.9)	(10.0)	(4.9)	(1.9)	(11.7)
Iβ	97.44	75.49	75.93	80.61	75.16	67.83	
	4.665 d	3.289 dd	3.625 dd	3.696 dd	3.754 ddd	3.977 dd	3.871 dd
	(8.0)	(9.5)	(8.6)	(10.0)	(5.1)	(1.9)	(11.7)
IIα	104.19	74.84	77.17	71.19 [f]	77.73	62.29	
	4.504 d	3.313 dd	3.491 dd	3.407 dd	3.467 ddd	3.903 dd	3.723 dd
	(7.9)	(9.4)	(8.9)	(10.0)	(2.2)	(5.8)	(12.4)
IIβ	104.24	74.83	77.17	71.17	77.73	62.27	
	4.504 d	3.306 dd	3.489 dd	3.406 dd	3.476 ddd	3.903 dd	3.723 dd
	(7.9)	(9.4)	(8.9)	(10.0)	(2.2)	(5.9)	(12.4)
IIIα	100.46	73.16	74.69	71.17 [f]	73.67	62.21	
	4.968 d	3.539 dd	3.712 dd	3.431 dd	3.722 ddd	3.845 dd	3.766 dd
	(3.8)	(9.9)	(8.9)	(10.0)	(2.2)	(5.0)	(12.4)
IIIβ	100.35	73.13	74.70	71.17	73.62	62.21	
	4.959 d	3.544 dd	3.716 dd	3.429 dd	3.722 ddd	3.845 dd	3.766 dd
	(3.8)	(9.9)	(8.9)	(10.0)	(2.2)	(5.0)	(12.4)
	β-D-Glc*p*-(1→4)-D-GlcUN*p*						
Iα	93.81	72.49	72.74	81.07	71.03	174.96	
	5.264 d	3.629 dd	3.838 dd	3.792 dd	4.375 d		
	(3.8)	(9.5)	(8.9)	(9.6)			
Iβ	97.65	75.14	75.65	80.78	75.34	174.13	
	4.705 d	3.341 dd	3.649 dd	3.831 dd	4.059 d		
	(8.0)	(9.5)	(8.9)	(9.8)			
IIα	103.64	74.78 [g]	77.12 [h]	71.10 [i]	77.71	62.24 [j]	
	4.458 d	3.301 dd	3.474 dd	3.396 dd	3.450 ddd	3.900 [k] dd	3.718 dd
	(7.9)	(9.4)	(8.9)	(9.7)	(2.2)	(5.7)	(12.4)
IIβ	103.70	74.79 [g]	77.11 [h]	71.13 [i]	77.71	62.26[j]	
	4.462 d	3.293 dd	3.472 dd	3.395 dd	3.450 ddd	3.902 [k] dd	3.718 dd
	(7.9)	(9.4)	(8.9)	(9.7)	(2.2)	(5.7)	(12.4)

[a] Residues I, II, and III refer to the unprimed, primed, and double primed sugar rings, respectively.
[b] d: doublet, m: multiplet.
[c] Marked assignments may be interchangeable.
[d,e] Bracketed assignments could not be unambiguously assigned to the α or β form, but were assigned together on the basis of DQF COSY and TOCSY experiments.
[f–k] Marked assignments may be reversed.

HMQC in combination with HMBC. This approach afforded unambiguous identification of each linkage and residue, and allowed complete ^{1}H and ^{13}C assignment H (Table 2). Complete assignment of the proton signals from the glucose residue of the glucuronamide disaccharide was difficult due to the similar proportions of α and β forms. An attempt to resolve this using HMBC spectra was complicated by the signals from the glucosyl residues in the α and β forms of the disaccharide being in close proximity to each other. Therefore, selective HMBC was used, in which a specific region of the ^{13}C spectrum (f1) can be observed without a folding-back of signals from outside the observed region. This involves selective excitation by a shaped pulse in f1. This afforded a greater resolution in f1 scale, and connectivity between βC-4, βH′-1 and αC-4, αH′-1 could easily be observed (Table 1). The signals from the glucosyl residues in the different forms of the disaccharide were then able to be assigned by using these starting points and tracing the crosspeaks of the DQF-COSY spectrum (Table 2).

The connectivities in the HMBC spectra between the C-4, H′-1 (Fig. 1) and C′-1, H-4 of the glucuronamide disaccharide indicate a (1→4)-linkage, while the coupling constant of H′-1 ($J_{1,2}$ 8.0 Hz, Fig. 1) is indicative of a β linkage. Likewise HMBC crosspeaks indicate both (1→4) and (1→6)-linkages in the three trisaccharides and coupling constants identify the configuration of the linkages.

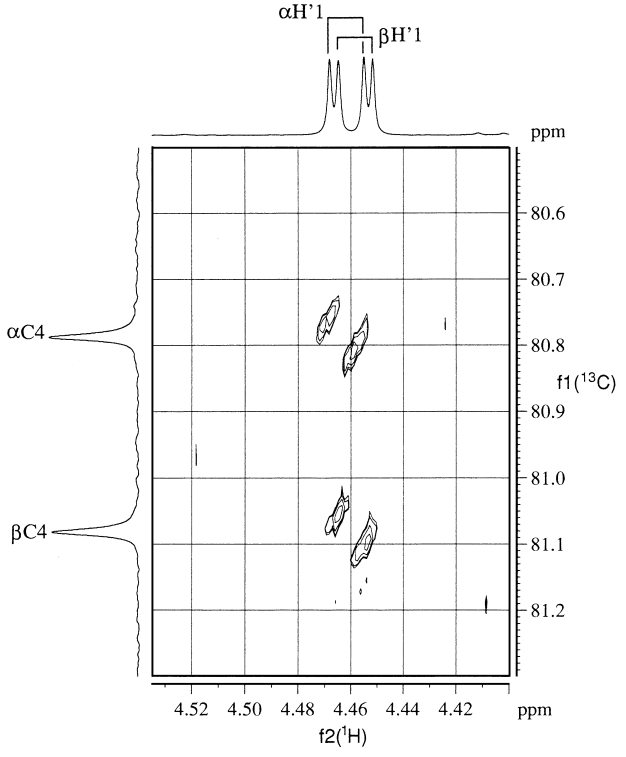

Fig. 1. Expansion of a two dimensional selective HMBC spectrum of β-D-Glc*p*-(1→4)-D-GlcUN*p*. One dimensional ^{13}C (^{1}H fully decoupled) and ^{1}H NMR spectra are shown as projections along the f1 and f2 axes.

The results confirmed that cellobiose phosphorylase had utilised the acceptors, forming the β(1→4)-linked disaccharide and three trisaccharides. To our knowledge this is the first time β-D-Glc*p*-(1→4)-D-GlcUN*p*, β-D-Glc*p*-(1→4)-[α-D-Gal*p*(1→6)]-D-Glc*p*, β-D-Glc*p*-(1→4)-[β-D-Glc*p*(1→6)]-D-Glc*p*, and β-D-Glc*p*-(1→4)-[α-D-Glc*p*(1→6)]-D-Glc*p* have been synthesised. A related product, 4,6-*O*-ethylidene-β-D-Glc*p*-(1→4)-[β-D-Glc*p*-(1→6)]-β-D-Glc*p*-*O*-Me has been chemically synthesised previously in a five step reaction (3.6% overall yield) [11]. The trisaccharide, β-D-Glc*p*-(1→4)-[β-D-Glc*p*(1→6)]-D-Glc*p* is present as part of a *Lactobacillus sake* exopolysaccharide [12] and as part of a cell wall polysaccharide from *Streptococcus faecalis* [13].

This is also the first time that cellobiose phosphorylase has been shown to utilise disaccharides as acceptors to form trisaccharides. Another phosphorolytic enzyme, cellodextrin phosphorylase, has been shown to act on β(1→4)-linked glucose oligomers [14] although so far it has not been reported to occur in *C. gilvus*. In the present study the exclusive formation of a β(1→4) branch on the reducing residue of the (1→6)-linked disaccharides confirms earlier work concerning the specificity of cellobiose phosphorylase. For example, Kitaoka et al. [6] have shown that the acceptor must retain the β-hydroxyl group at the C-1 position; this would explain why cellobiose phosphorylase did not react with the nonreducing residues of the disaccharides. The inability of the (1→2) and (1→3)-linked disaccharides to act as acceptors is also consistent with the failure of cellobiose phosphorylase to utilise acceptors with larger groups at the C-2 (*N*-acetyl-D-glucosamine [6]) and the C-3 (3-*O*-methyl-D-glucose [5]). The utilisation of melibiose, gentiobiose, and isomaltose by cellobiose phosphorylase shows that the enzyme can accept both α and β(1→6)-linked disaccharides. In addition, the similar yields of melibiose and isomaltose prior to isolation indicates that the enzyme may not be specific for the nonreducing residue. It would also be expected that (1→6)-linked oligosaccharides other than those with a reducing glucose residue could act as acceptors providing the reducing residue was an acceptor for cellobiose phosphorylase.

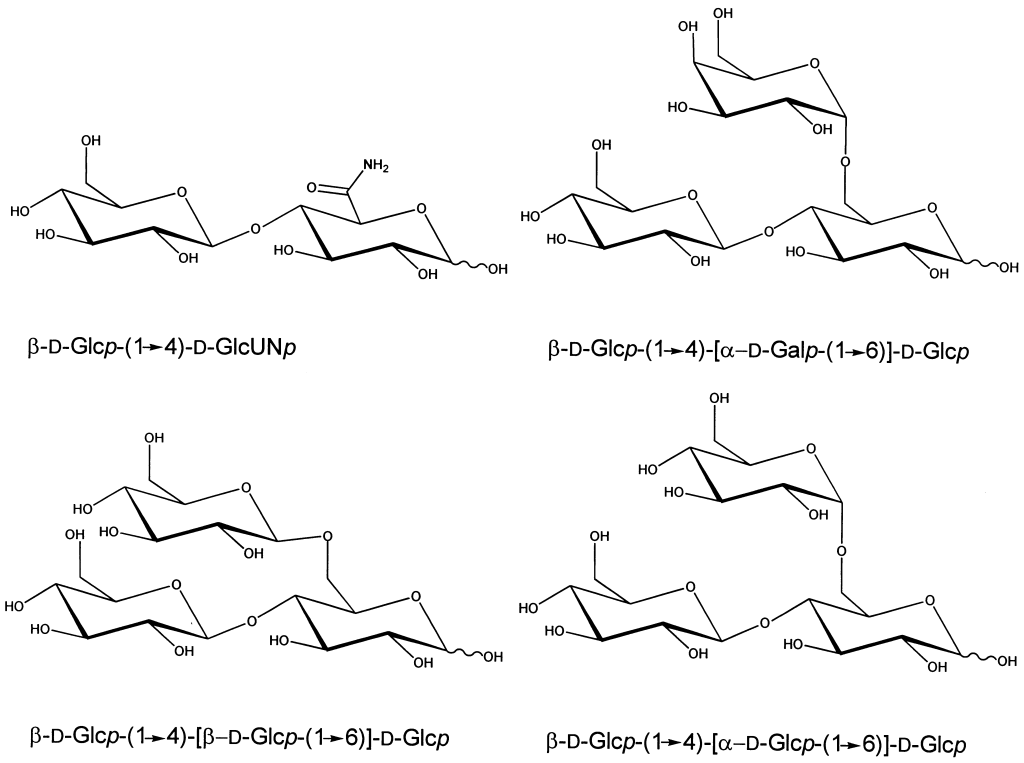

Fig. 2. Structures of oligosaccharides synthesised in the study.

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