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Novel oligosaccharides synthesized from sucrose donor and cellobiose acceptor by alternansucrase

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This paper is dedicated to Professor Gérard Descotes on the occasion of his retirement, for his accomplishments in the field of carbohydrate chemistry.

Abstract

Cellobiose was tested as acceptor in the reaction catalyzed by alternansucrase (EC 2.4.1.140) from *Leuconostoc mesenteroides* NRRL B-23192. The oligosaccharides synthesized were compared to those obtained with dextransucrase from *L. mesenteroides* NRRL B-512F. With alternansucrase and dextransucrase, overall oligosaccharide synthesis yield reached 30 and 14%, respectively, showing that alternansucrase is more efficient than dextransucrase for cellobiose glucosylation. Interestingly, alternansucrase produced a series of oligosaccharides from cellobiose. Their structure was determined by mass spectrometry and $\{^{13}C-^{1}H\}$ NMR spectroscopy. Two trisaccharides are first produced: α-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-D-glucopyranose (compound A) and α-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→4)-D-glucopyranose (compound B). Then, compound B can in turn be glucosylated leading to the synthesis of a tetrasaccharide with an additional α-(1→6) linkage at the non-reducing end (compound D). The presence of the α-(1→3) linkage occurred only in the pentasaccharides (compounds C_1 and C_2) formed from tetrasaccharide D. Compounds B, C_1, C_2 and D were never described before. They were produced efficiently only by alternansucrase. Their presence emphasizes the difference existing in the acceptor reaction selectivity of the various glucansucrases.

Keywords: Alternansucrase; *Leuconostoc mesenteroides* NRRL B-1355 and NRRL B-23192; Oligosaccharides; Cellobiose acceptor; α-Glucosyl cellobiose

1. Introduction

Sucrose glucosyltransferases (GTFs), also called glucansucrases, are extracellular enzymes mainly synthesized by *Leuconostoc mesenteroides* and several species of *Streptococcus*.[1] GTFs catalyze the synthesis of an α-glucan polymer from sucrose. Koepsell et al.[2] showed that in the presence of a sugar acceptor molecule, GTFs transfer the glucose coming from sucrose onto the acceptor to produce oligosaccharides. This reaction, named 'acceptor-reaction', competes with α-glucan synthesis. Several authors have classified the acceptor molecules according to their capacity to form oligosaccharides. They

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demonstrated that maltose, isomaltose and α-methyl glucopyranoside are usually the most effective acceptor sugars.[2–5]

GTFs can be classified on the basis of the structure of the glucan they synthesize: dextransucrases (EC 2.4.1.5) synthesize a glucan mainly composed of α-(1→6) linkages into the main chain named dextran whereas mutansucrases (EC 2.4.1.5) produce a polymer with only α-(1→3) linkages in the main chain named mutan. Of particular interest is alternansucrase (EC. 2.4.1.140), the enzyme excreted together with dextransucrase by *L. mesenteroides* NRRL B-1355.[6–8] Indeed, alternansucrase catalyzes the synthesis of an original glucan containing alternating α-(1→6) and α-(1→3) linkages in the main chain. It is named alternan.[7] Various sugars have been tested as acceptors for alternansucrase from *L. mesenteroides* NRRL B-1355.[5,6] Maltose and nigerose were found to be the most efficient acceptors. Transfer of the glucosyl moiety occurred onto the non-reducing end of the acceptor and a series of oligosaccharides containing alternating α-(1→6) and α-(1→3) linkages were obtained demonstrating that the enzyme keeps its specificity during the acceptor reaction. The absence of contiguous α-(1→6) linkages and the presence of α-(1→3) linkages in the main chain, makes alternan and oligoalternans quite resistant to endodextranase hydrolysis. Because of this important property, several authors have suggested that alternan may be valuable as low- or non-digestible (low- or non-caloric) additives or ingredients, e.g., in artificially sweetened food preparations. Alternan could also possibly replace polydextrose, maltodextrins and gum arabic, used as soluble fibers, binders and bulking agents in foods and as components of inks, adhesives, cosmetic creams and ointments.[9]

The acceptor reaction in the presence of cellobiose catalyzed by alternansucrase has never previously been described. This disaccharide was tested as an acceptor for dextransucrase from *L. mesenteroides* NRRL B-512F. Transfer onto the acceptor was very weak and a single reaction product was synthesized with a very low yield. The structure proposed for the cellobiose acceptor reaction product was α-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-D-glucopyranose.[10]

In this work, we describe the reaction, in the presence of sucrose as donor and cellobiose as acceptor, catalyzed by alternansucrase from *L. mesenteroides* NRRL B-23192, a mutant strain of *L. mesenteroides* NRRL B-1355 that mainly produces alternansucrase.[11] The cellobiose acceptor reaction products were purified and their molecular weights and structures were determined by mass spectrometry and $\{^{13}C-^{1}H\}$ NMR spectroscopy.

2. Results

HPLC analysis of cellobiose acceptor reaction products.—The acceptor reaction, in the presence of the cellobiose acceptor, was carried out using a sucrose:cellobiose ratio of one (w/w). As shown in Fig. 1, HPLC chromatograms of acceptor reaction products synthesized with *L. mesenteroides* NRRL B-23192 alternansucrase displayed seven peaks numbered from 1 to 7 on the basis of their retention times. Peaks 1, 2, 3 and 4 also arose on the chromatogram of the products synthesized in the same conditions by dextransucrase from *L. mesenteroides* NRRL B-512F dextransucrase. In order to characterize the oligosaccharides produced with alternansucrase, the products corresponding to each peak were separated by preparative HPLC, individually collected and re-analyzed using analytical HPLC. Analysis of the purified products showed that peaks 1 and 2 corresponded to the α and β enantiomers, respectively, of the same oligosaccharide named A. Peaks 3 and 4 were also due to the α and β forms of another oligosaccharide named B and peaks 6 and 7 corresponded to the α and β enantiomers of oligosaccharide D. Mass spectrometry analysis of the purified oligosaccharides showed that the molecular weight of products A, B, C and D were 504, 504, 828 and 666 g/mol, respectively. Compounds A and B are consequently two trisaccharides, compound C a pentasaccharide and compound D a tetrasaccharide.

Oligosaccharide yield.—Table 1 reports the oligosaccharide yield obtained with both enzymes. *L. mesenteroides* NRRL B-23192 alter-

nansucrase efficiently catalyzed the glucosylation of cellobiose with an overall yield of 30%. Concentrations of A, B, C and D were 10, 7, 2.4 and 11 g/L, respectively. With B-512F dextransucrase, a lower yield, 14.8%, was obtained. The products synthesized were: trisaccharide A (11 g/L), trisaccharide B (1.5 g/L) and oligosaccharide B′ (2.5 g/L). The latter, only produced by dextransucrase, was not further characterized.

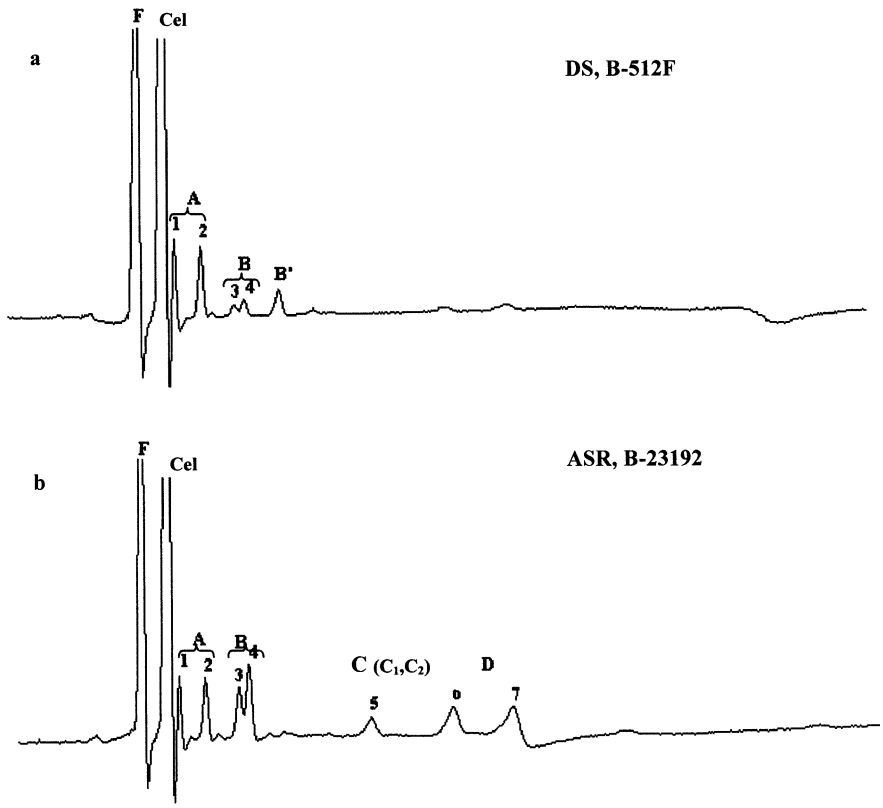

Fig. 1. HPLC analysis of cellobiose acceptor reaction products synthesized by (a) B-512F dextransucrase and (b) B-23192 alternansucrase. Peak identification, F: Fructose, Cel: cellobiose, A: α-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-D-glucopyranose, B: α-D-glucopyranosyl-(1→6)-cellobiose, C_1,C_2: α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-cellobiose or α-D-glucopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→6)]-α-D-glucopyranosyl-(1→6)-cellobiose, D: α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-cellobiose.

Table 1
Concentration and apparent yield of cellobiose acceptor reaction products

Peak	Degree of polymerisation (d.p.)	Concentration (g/L)	Apparent yield (%)
Alternansucrase B-23192			
A	3	10	9.7
B	3	7	6.8
C	5	2.4	2.3
D	4	11	11
Total			30
Dextransucrase B-512F			
A	3	11.5	11
B	3	1.5	1.4
B′	ND	2.5	2.4
Total			14.8

Table 2
^{1}H and ^{13}C NMR data for cellobiose acceptor reaction products A and B

Oligosaccharides	Residue	Chemical shift [a]							Assignment of C-1
		H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	
		C-1	C-2	C-3	C-4	C-5	C-6		
A	α-Glc*p*^{I} [b]	5.27	3.53	3.79	3.54	3.82	3.75	3.71	C-1α
		89.72	76.24	70.25	79.21				
	β-Glc*p*^{I}	4.66	3.28	3.59	3.52	3.44	3.81	3.65	C-1β
		96.5	79.06	73.45	79.41				
	β-Glc*p* (α)^{II}	4.38	3.18	3.37	3.27	3.34	3.77	3.58	C-1,4
		102.92	73.68	71.93					
	β-Glc*p* (β)^{II}	4.36	3.17	3.36	3.26	3.33	3.77	3.56	C-1,4
		102.85	73.70	71.93					
	α-Glc*p* (α)^{III}	4.94	3.40	3.64	3.31	3.82	3.69	3.62	C-1,2
		97.00	71.84	73.30	69.95	71.84			
	α-Glc*p* (β)^{III}	5.20	3.40	3.60	3.31	3.91	3.67	3.63	C-1,2
		98.30	72.06	73.35	69.88	72.24			
B	α-Glc*p*^{I}	5.10	3.46	3.71	3.50	3.82	3.76	3.71	C-1α
		92.80	76.26	72.41	80.63	71.00	61.20		
	β-Glc*p*^{I}	4.53	3.17	3.51	3.48	3.47	3.82	3.67	C-1β
		96.72	74.92	75.40	80.45	75.65	61.30		
	β-Glc*p*^{II}	4.41	3.21	3.40	3.38	3.58	3.81	3.69	C-1,4
		103.71	74.15	76.70	70.68	75.32	67.18		
	α-Glc*p*^{III}	4.84	3.43	3.67	3.29	3.57	3.73	3.63	C-1,6
		99.13	72.50	74.01	70.80	72.93	61.70		

[a] In ppm relative to the signal of internal acetone at 2.1 ppm (^{1}H) in deuterium oxide at 25 °C or at 31.5 ppm (^{13}C) in deuterium oxide at 60 °C.
[b] Glc*p*: glucopyranosyl residue.

NMR structural characterization

Product A. In the proton spectrum of oligosaccharide A, the anomeric region shows the presence of six signals generated by three protons. This indicates a trisaccharide structure in agreement with the mass spectrometry data. The six signals are affected by the mutarotation equilibrium. The signals at 5.27 (H-1 $^{I}\alpha$, 3.5 Hz) and 4.66 ppm (H-1 $^{I}\beta$, 8 Hz) correspond to the reducing unit. The signals at 5.20 (H-1 $^{III}\alpha/\beta$, 3.5 Hz) and 4.94 ppm (H-1 $^{III}\alpha/\alpha$, 3.5 Hz) are also strongly affected by the mutarotation, whereas the last two signals, H-1 $^{II}\alpha/\beta$, 8 Hz at 4.38 and H-1 $^{II}\alpha/\alpha$, 8 Hz at 4.36 ppm, are only slightly affected. By doing 1D-TOCSY experiments on the anomeric protons, with increasing mixing times, we assigned all the proton signals of the three glucosyl residues as shown in Table 2. 2D HMQC experiment allowed the assignment of some carbon-13 signals (Table 2). In the 2D $^{13}C-^{1}H$ HMBC spectrum, inter-residual three-bond connectivities over the glycosidic linkages were observed. Strong cross-peaks between H-4 α of unit I (H-4 $^{I}\alpha$) and C-1 α of unit II (C-1 $^{II}\alpha$,), H-4 $^{I}\beta$ and C-1 $^{II}\beta$, H-1 $^{II}\alpha$ and C-4 $^{I}\alpha$ H-1 $^{II}\beta$ and C-4 $^{I}\beta$ demonstrate that Glc unit II is β-(1→4)-linked to Glc unit I. Strong connectivities between H-2 $^{I}\beta$ and C-1 $^{III}\beta$, H-2 $^{I}\alpha$ and C-1 $^{III}\alpha$, H-1 $^{III}\beta$ and C-2 $^{I}\beta$, H-1 $^{III}\alpha$ and C-2 $^{I}\alpha$ indicate that Glc unit III is α-(1→2)-linked to Glc unit I. The NMR results show that product A is an α-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-D-glucopyranose (Fig. 2).

Product B. The proton spectrum of oligosaccharide B presents four signals generated by three protons. This indicates a trisaccharide structure in agreement with the mass spectrometry data. The signals at 5.10 (H-1 $^{I}\alpha$, 3.5 Hz) and 4.53 ppm (H-1 $^{I}\beta$, 8 Hz) are affected by the mutarotation equilibrium and

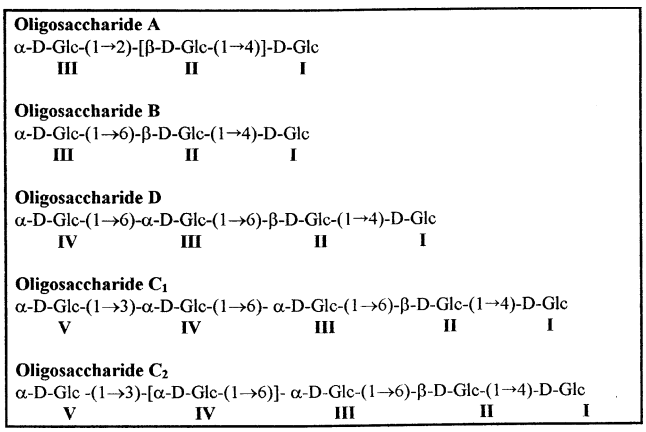

Fig. 2. Structure of cellobiose acceptor reaction products. A: α-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-D-glucopyranose, B: α-D-glucopyranosyl-(1→6)-cellobiose, C_1,C_2: α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-cellobiose or α-D-glucopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→6)]-α-D-glucopyranosyl-(1→6)-cellobiose, D: α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-cellobiose.

correspond to the reducing unit. The two other signals at 4.41 (H-1, 8 Hz) and 4.84 ppm (H-1, 3.5 Hz) correspond to two glucosyl units, which are, respectively β and α linked. By doing 1D-TOCSY experiments on the anomeric protons, we assigned all the proton signals of the three glucosyl residues as shown in Table 2. 2D HMQC experiment allowed the assignment of all the carbon-13 signals (Table 2). By doing 2D $\{^{13}C-^{1}H\}$ HMBC experiment, inter-residual three-bond connectivities over the glycosidic linkages were observed. Strong cross-peaks between H-4 α of unit I (H-4 $^{I}\alpha$) and C-1 α of unit II (C-1 $^{II}\alpha$), H-4 $^{I}\beta$ and C-1 $^{II}\beta$, H-1 $^{II}\beta$, and C-4 $^{I}\alpha$ H-1 $^{II}\beta$ and C-4 $^{I}\beta$ demonstrate that Glc unit II is β-(1→4)-linked to Glc unit I. Strong connectivities between H-6a^{II} and C-1^{III}, H-6b^{II} and C-1^{III}, H-1^{III} and C-6^{II} indicate that Glc unit III is α-(1→6)-linked to Glc unit II. The NMR results show that product B is an α-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→4)-D-glucopyranose (α-D-glucopyranosyl-(1→6)-cellobiose; Fig. 2).

Product C. Mass spectrometry of compound C indicated that it was a pentasaccharide. As shown in Table 3, the proton spectrum agrees with this result and further shows that compound C was a mixture of two pentasaccharides. Indeed, in the anomeric region, eight signals are encountered. Their integration indicated 10 protons corresponding to two pentasaccharides. Signals at 5.1, 4.54, 4.43 and 4.87 ppm are common to both oligosaccharides. The signals at 5.1 ppm (H-1 $^{I}\alpha$, 3.5 Hz) and 4.54 ppm (H-1 $^{I}\beta$, 8 Hz) were affected by mutarotation equilibrium. The signal at 4.43 ppm (H-1 $^{II}\beta$, 8Hz) arose from a β-(1→4)-linked glucosyl unit. As shown in Table 3, the proton signal at 4.87 ppm can be assigned to an α-(1→6)-linked unit substituted on carbon 3. Four additional signals at 4.83, 4.85, 5.23 and 5.21 ppm appeared on the spectrum and were accounted for by four protons. Signals at 5.21 and 5.23 ppm were assigned to an

Table 3
^{1}H and ^{13}C NMR data for cellobiose acceptor reaction products C_1, C_2

Oligosaccharide		Chemical shift [a] in the anomeric region		Assignment of C-1
		H-1	Proton integration	
C	α-Glc*p*^{I} [b]	5.10	2	C-1α
	β-Glc*p*^{I}	4.54	2	C-1β
	β-Glc*p*^{II}	4.43	2	C-1,4
		4.87	2	C-1,6
		4.83	4	C-1,6
		4.85	4	C-1,6
		5.21	4	C-1,3
		5.23	4	C-1,3

[a] In ppm relative to the signal of internal acetone at 2.1 ppm (^{1}H) in deuterium oxide at 25 °C or at 31.5 ppm (^{13}C) in deuterium oxide at 60 °C.

[b] Glc*p*: glucopyranosyl residue.

Table 4
^{1}H and ^{13}C NMR data for cellobiose acceptor reaction product D

Oligosaccharide	Residue	Chemical shift [a]							Assignment of C-1
		H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	
		C-1	C-2	C-3	C-4	C-5	C-6		
D	α-Glc*p*^{I} [b]	5.09	3.46	3.71	3.49	3.82	3.76	3.71	C-1α
		92.80	72.26	72.45	80.66	71	61.20		
	β-Glc*p*^{I}	4.53	3.17	3.50	3.52	3.47	3.82	3.68	C-1β
		96.72	74.92	75.40	80.50	75.64	61.31		
	β-Glc*p*^{II}	4.41	3.21	3.40	3.36	3.59	3.82	3.68	C-1,4
	(β)	103.73	74.10	72.45	70.71	74.20	67.24		
	(α)	103.68							
	α-Glc*p*^{III}	4.85	3.45	3.67	3.37	3.75	3.83	3.66	C-1,6
		99.10	72.45	74.22	70.80	71.35	66.83		
	α-Glc*p*^{IV}	4.83	3.44	3.60	3.30	3.57	3.73	3.63	C-1,6
		98.80	72.52	72.87	70.68	74.20	61.65		

[a] In ppm relative to the signal of internal acetone at 2.1 ppm (^{1}H) in deuterium oxide at 25 °C or at 31.5 ppm (^{13}C) in deuterium oxide at 60 °C.
[b] Glc*p*: glucopyranosyl residue.

α-(1→3)-linked unit (unit V, Fig. 2) and signals at 4.83 and 4.85 ppm were assigned to α-(1→6)-linked residues. From the integration of these signals, it appeared that the peaks at 4.83 and 5.21 ppm can be attributed to one of the two pentasaccharides and those at 4.85 and 5.23 ppm can be attributed to the other. The ratio between the two oligosaccharides is 43/57. Although it would be necessary to separate the two oligosaccharides eluted in peak C to distinguish the individual proton chemical shift assignments, it is clear that two pentasaccharides (C_1, C_2) are formed. Both possess an α-(1→3) linkage and NMR analysis is consistent with the following two possible structures: α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-cellobiose and α-D-glucopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→6)]-α-D-glucopyranosyl-(1→6)-cellobiose (Fig. 2).

Product D. In the proton spectrum of oligosaccharide D we can observe in the anomeric region the presence of five signals due to four protons. The signals at 5.09 (H-1 ^{I}α, 3.5 Hz) and 4.53 ppm (H-1 ^{I}β, 8 Hz) were affected by the mutarotation equilibrium and correspond to the reducing unit. The signal at 4.41 ppm (H-1, 8 Hz) corresponds to a β linked glucosyl unit, whereas the two very close signals at 4.83 and 4.85 ppm correspond to two α linked glucosyl units. By doing 1D-TOCSY experiments on the anomeric protons, we assigned all the proton signals of the four glucosyl residues as shown in Table 2. 2D HMQC experiment allowed the assignment of all the carbon-13 signals (Table 4). In the 2D {$^{13}C-^{1}H$} HMBC spectrum, strong cross-peaks corresponding to inter-residual three-bond connectivities across the glycosidic linkages were observed demonstrating that Glc unit IV is α-(1→6)-linked to Glc unit III, which is itself α-(1→6)-linked to Glc unit II, which is β-(1→4)-linked to Glc unit I. The NMR results therefore show that product D is an α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-cellobiose (Fig. 2).

3. Discussion

The reaction, in the presence of cellobiose acceptor, catalyzed by two glucansucrases, alternansucrase from *L. mesenteroides* NRRL B-23192 and dextransucrase from *L. mesenteroides* NRRLB-512F, were compared. Cellobiose is a disaccharide that had never been tested before as an acceptor for alternansucrase.

Alternansucrase and dextransucrase have only two common cellobiose acceptor reaction products: α-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-D-glucopyranose (compound A) and α-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→4)-D-glucopyranose (compound B). Trisaccharide A results from the transfer of the glucosyl residue coming from sucrose onto the reducing end of cellobiose acceptor through the formation of an α-(1→2)-glucosidic linkage. Its structure agrees with the previously proposed structure for the cellobiose acceptor reaction product obtained in the reaction catalyzed by dextransucrase from *L. mesenteroides* NRRL B-512F.[10] The yields of compound A reached 10 and 11% with alternansucrase or dextransucrase, respectively. In addition, the presence of the α-(1→2) linkage in the molecule prevents its binding to alternansucrase and its glucosylation. However, compound A was considered to be the sole product of the cellobiose acceptor reaction catalysed by dextransucrase from *L. mesenteroides* NRRL B-512F.[10] It was demonstrated here that two other oligosaccharides are synthesized with a much lower yield: α-D-glucopyranosyl-(1→6)-cellobiose (B) and an oligosaccharide of unknown structure (B′).

The most unexpected and interesting result comes from the synthesis of oligosaccharides B, C_1, C_2 and D. Indeed, these novel oligosaccharides were obtained in significant amounts only with alternansucrase. They were synthesized with a reasonable yield (20%) and had never been described before (Fig. 2). They all result from the transfer of the glucose moiety from sucrose onto the non-reducing end of cellobiose. The first product synthesized is trisaccharide B which is in turn glucosylated to produce tetrasaccharide D (α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-cellobiose). The last two products obtained (C_1, C_2) are pentasaccharides. They are not separated by reverse phase chromatography and have a shorter retention time than tetrasaccharide D, showing that they are less hydrophobic. Two structures could be supported by the proton spectrum. Separation of the two products would be necessary to distinguish between them.

Cellobiose, a poor acceptor for dextransucrase from *L. mesenteroi*des NRRL B-512F, was found to be a fairly good acceptor for alternansucrase leading to the synthesis of a series of oligosaccharides which, in turn, can act as acceptors. Therefore, after optimization, the acceptor reaction in the presence of cellobiose (an easily available disaccharide) could be envisaged as a new route for synthesizing novel oligosaccharides. These compounds contain sequences of glucosidic linkages never reported before (α-(1→2), α-(1→3), α-(1→6), β-(1→4)) that may confer to them new and valuable properties for several types of applications.

In addition, from their primary structure homology, dextransucrase and alternansucrase can be classified in the same enzyme family (i.e., family 70 of glucoside hydrolases).[12,13] We have recently shown that very specific segments exist in *L. mesenteroides* NRRL B-1355 alternansucrase when compared to all other glucansucrases.[13] The study presented here further emphasizes the differences in the selectivity of glucansucrases and the need to further investigate the structure–function relationships to address the problem of the selectivity of these enzymes. Indeed, the results obtained with the cellobiose acceptor demonstrate that the topology of the dextransucrase and alternansucrase acceptor binding sites must be significantly different. With alternansucrase, new compounds were synthesized and with a good yield. Two trisaccharides were formed with cellobiose as acceptor showing that either cellobiose binds to the acceptor site in two different ways or that alternansucrase possesses two different acceptor sites. Structure–function studies supported by 3D structures will now be necessary to identify the structural features of these two enzymes involved in their selectivity towards acceptors. By these means, it should be possible to increase the acceptor reaction yield or to achieve better control of the reaction.

4. Experimental

Enzyme production.—Alternansucrase was obtained from *L. mesenteroides* NRRL B-23192, a mutant strain of NRRL B-1355

which produces more alternansucrase.[11] *L. mesenteroides* NRRL B-512F was the source of dextransucrase. Both strains were provided by the NCAUR stock culture collection in Peoria, Il, USA. The cells were stored in 15% glycerol at −20 °C, they were grown at 27 °C on standard medium as previously described.[14] *L. mesenteroides* NRRL B-23192 was grown in a 50 L fermenter for alternansucrase production. *L. mesenteroides* NRRL B-512F dextransucrase was produced in Erlenmeyer flasks on a rotatory shaker at 200 rpm. Cells and other insolubles were recovered by centrifugation. The supernatants containing the soluble alternansucrase and dextransucrase were concentrated by aqueous two-phase partition using polyethyleneglycol (PEG, $M_w = 4000$), taking advantage of the dextranosyl-enzyme complex form in which the enzyme is found.[15] This operation enabled the elimination of the other sucrase activities from the culture supernatant.[16] The precipitate, enriched in insoluble alternansucrase and dextransucrase, was harvested by centrifugation, washed and dissolved in sodium acetate buffer (20 mM, pH 5.4).

Oligosaccharide synthesis and analysis.—Oligosaccharide synthesis was performed at 30 °C in AcONa buffer (20 mM, pH 5.4) supplemented with sucrose (100 g/L), cellobiose as acceptor molecule (100 g/L), $CaCl_2$ (0.05 g/L) and B-23192 alternansucrase or B-512F dextransucrase (0.5 U/mL). The acceptor reactions were carried out until sucrose depletion and stopped by heating for 5 min at 95 °C. The oligosaccharides formed were analyzed by high performance liquid chromatography (HPLC), using a C_{18} column (Prontosyl Eurobond, 5 μm, 250 × 4 mm) and ultrapure water as eluent at a constant flow rate of 0.5 mL/min^{-1}. Oligosaccharide detection was carried out with a differential refractometer. Analyses were carried out at rt during 30 min. Oligosaccharide yield was determined from oligosaccharide concentration measured by HPLC using the following formula: Apparent yield = oligosaccharide concentration/(0.474 × initial sucrose concentration + initial acceptor concentration). Substrate and product concentrations are expressed in g/L.

Oligosaccharide purification.—Cellobiose acceptor reaction products were purified from the 250 mL synthesis (above conditions). After sucrose depletion, the reaction was stopped by heating for 5 min at 95 °C. The medium was centrifuged at 8000 rpm, 4 °C for 30 min in order to eliminate the insoluble polysaccharide. Then, an equal volume of ethanol was added to the supernatant to precipitate the soluble polysaccharide. After 2 h, the precipitate was removed by centrifugation at 8000 rpm, 4 °C for 30 min. Then, the EtOH contained in the supernatant was evaporated off using a Rotavapor and the oligosaccharide mixture freeze-dried. Purification of cellobiose acceptor reaction products was performed by preparative HPLC using a C_{18} column (Ultrasep ES 100, 10 μm, 250 × 4 mm) and ultrapure water as eluent. Oligosaccharide detection was carried out with a differential refractometer. Each peak was collected separately, concentrated and reinjected into an analytical HPLC system. This step was used to check the purity of the compounds and showed that some double peaks corresponded in fact to oligosaccharide anomers (compounds A, B and D).

Structural analyses of oligosaccharides.—Mass spectrometry data of the purified cellobiose acceptor reaction products were obtained by electrospray (MS-ES) measurements. Solvent was ultrapure water at 7 μL/min^{-1} and detection was performed in the positive mode. ^{1}H NMR spectra were recorded on a Varian unit Plus 500 spectrometer equipped with an ultrashim system (operating frequency, 499.836 MHz). Samples were studied as solutions in deuterium oxide (5 mg in 0.75 mL of solvent) at 25 °C in 5 mm o.d. tubes without spinning (internal acetone 2.1 ppm relative to Me_4Si). ^{1}H spectra were recorded using 90 pulses, 3300 Hz spectral width, 12,480 data points, 1.891 s acquisition time, 32 scans were accumulated. 1D-TOCSY experiments were recorded using a soft pulse sequence with an eburp 1-256 shape (186 ms) and (10–80 ms) mixing time. The heteronuclear multiple quantum coherence spectroscopy (HMQC) and heteronuclear multiple bond correlation spectroscopy (HMBC) experiments were obtained by using the Varian

standard pulse sequences. The delay time for the detection of long-range $^{13}C-^{1}H$ couplings was set to 120 ms in HMBC experiments.

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