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Glucosylation of α-butyl- and α-octyl-D-glucopyranosides by dextransucrase and alternansucrase from *Leuconostoc mesenteroides*

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Abstract

For the first time, glucosylation of α-butyl- and α-octylglucopyranoside was achieved using dextransucrase (DS) of various specificities, and alternansucrase (AS) from *Leuconostoc mesenteroides*. All the glucansucrases (GS) tested used α-butylglucopyranoside as acceptor; in particular, DS produced α-D-glucopyranosyl-(1→6)-*O*-butyl-α-D-glucopyranoside and α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-*O*-butyl-α-D-glucopyranoside. In contrast, α-octylglucopyranoside was glucosylated only by AS which was shown to be the most efficient catalyst. The conversion rates, obtained with this enzyme at sucrose to acceptor molar ratio of 2:1 reached 81 and 61% for α-butylglucopyranoside and α-octylglucopyranoside, respectively. Analyses obtained from liquid chromatography coupled with mass spectrometry revealed that different series of α-alkylpolyglucopyranosides regioisomers of increasing polymerization degree can be formed depending on the specificity of the catalyst.

Keywords: Alkylpolyglucopyranosides; Dextransucrase; Alternansucrase; *Leuconostoc mesenteroides*; Acceptor reaction

1. Introduction

Alkylpolyglucosides (APG) belong to the class of non-ionic surfactants. They can be obtained via a Fischer glucosylation based on a condensation between fatty alcohols and carbohydrates.[1–5] This class of surfactants has retained the attention for the last 20 years, especially because their synthesis involves renewable raw materials and can be carried out on a large industrial scale.[5] In addition, they are non-toxic,[1,5–7] and have a good biodegradability thanks to the hemiacetal linkage between the two natural moieties, readily hydrolysed.[5]

APG consist of a (fatty) alkyl chain coupled to several glucosyl units: they can be characterized either by the average degree of polymerization (DP), which is the number of glucosyl units per alkyl chain, or by the hydrophilic/lipophilic balance (HLB) corresponding to the relative weight of the two moieties. Currently, industrially available APG have an average DP comprised between 1.3 and 1.6.[1] These two characteristics are important for the surfactant properties,[8–10] which vary according to the DP or HLB values, and thus for the potential applications in detergent, cosmetic, pharmaceutical, and food fields.[7,11]

The modification of DP (or HLB) of these compounds is consequently of great interest, because it gives access to new properties and applications. But so far, HLB can only be altered by the choice of the alkyl chain length used for the APG synthesis. Indeed, glucosidic moiety modification via chemical way would imply numerous and tedious protection/deprotection steps. A good alternative is thus to consider the enzymatic glucosylation of α-alkylglucosides (α-AG) using glucansucrases (GS), which is more specific.

GS are extra cellular transglucosidases mainly produced by *Leuconostoc mesenteroides* and *Streptococcus* species.[12,13] Sucrose, a largely industrially available and cheap carbohydrate, is used as a glucosyl donor. In its presence, GS catalyse the synthesis of high molecular

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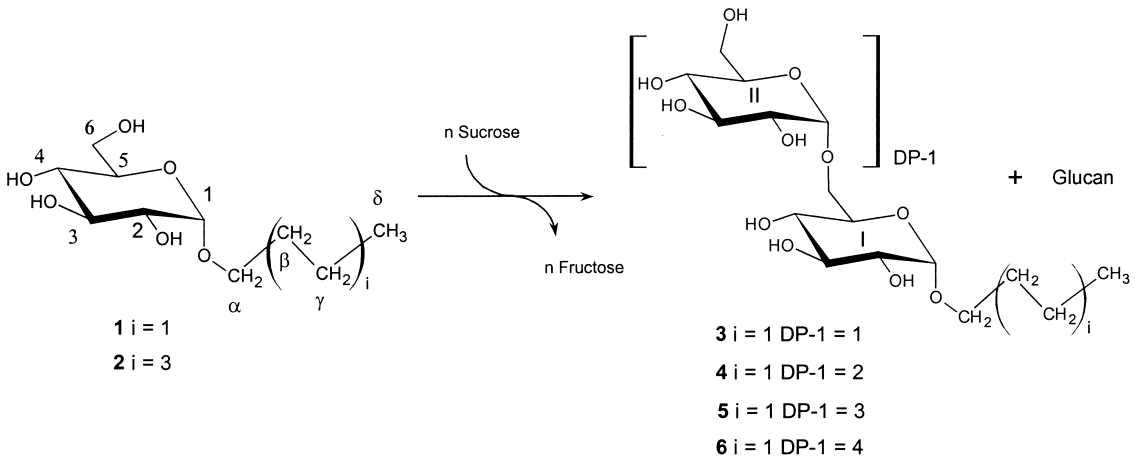

Fig. 1. Scheme of the glucosylation reaction of α-BG **1** as acceptor, using DS from *L. mesenteroides* NRRL B-512F as catalyst, in the presence of sucrose as glucosyl residue donor.

weight α-glucan polymers according to the equation: *n* sucrose → glucan + *n* fructose.[14] This reaction occurs by a glucosyl transfer from sucrose to a growing polyglucose chain. In the meantime, fructose is released in the medium.

Owing to the classification of GS based on the sequence similarities,[15] GS mostly belong to the family 70 of glucoside hydrolases.[16] They can also be classified according to the structure of the glucan formed, and in particular the nature and the frequency of the glucosidic linkages synthesized.[12,17,18] Dextransucrase (DS) (E.C. 2.4.1.5) from *L. mesenteroides* NRRL B-512F synthesizes a glucan, called dextran, mainly composed of α-(1 → 6)-linked glucosyl residues in the main linear chain.[19] DS from *L. mesenteroides* NRRL B-1299 catalyses the formation of a polymer containing between 27 and 35% of α-(1 → 2) branched linkages in addition to α-(1 → 6) ones.[20–24] Another enzyme of particular interest is alternansucrase (AS) (E.C. 2.4.1.140) which catalyses the synthesis of a glucan (called alternan) consisting of alternating α-(1 → 6) and α-(1 → 3) glucosidic linkages in the main chain.[25,26] This enzyme is secreted by *L. mesenteroides* NRRL B-1355 together with DS.[27,28] Last, a mutant strain from *L. mesenteroides* NRRL B-1355 was used in this work: *L. mesenteroides* NRRL B-23192 which mainly produces AS.[29]

In the presence of acceptor, an additional compound introduced in the reaction mixture, GS can catalyse acceptor glucosylation to the detriment of glucan synthesis: this reaction is named 'acceptor reaction'.[18,19] Usually, during this reaction, the specificity of GS is conserved. Besides, several compounds, mainly carbohydrates, have been analysed with regard to their acceptor efficiency,[17,30] and were divided into two classes: (1) strong acceptors, such as maltose[21,25] and isomaltose,[17] yielding products which are in turn glucosylated leading to series of oligosaccharides, whereas glucan synthesis is strongly limited; (2) weak acceptors for which only one glucosylated product is formed; fructose belongs to this last class, and may be glucosylated to form leucrose (α-D-glucopyranosyl-(1 → 5)-D-fructopyranose). Furthermore, the sucrose/acceptor molar ratio (S/A) is an important parameter of the reaction: it enables to control the average DP of the glucooligosaccharides produced, the average DP increasing with the S/A ratio.[19,20,25,31,32]

Up to now, few alkylglucopyranosides have been tested as acceptors. α-Methylglucopyranoside was shown to be glucosylated by several DS[33] or AS[26] from *L. mesenteroides*, whereas β-octylglucopyranoside was not an efficient acceptor.[34] The present work describes for the first time the enzymatic glucosylation of α-AG containing more than two carbons in the alkyl chain. For this purpose, GS were used to lengthen the glucosidic moiety of the molecule. To study the influence of the alkyl chain length, two distinctive α-AG were tested as acceptor: α-butylglucopyranoside (α-BG, **1**) and α-octylglucopyranoside (α-OG, **2**) (see Fig. 1). Different GS from *L. mesenteroides* NRRL B-512F, B-1299, B-1355 and B-23192 selected on the basis of their specificity, were tested as catalysts at various S/A ratio.

2. Results and discussion

2.1. Glucosylation of α-BG

2.1.1. Enzyme screening. The ability of the GS from *L. mesenteroides* B-512F, B-1299, B-1355 and B-23192 to catalyse the transfer of glucosyl residues to α-BG (as acceptor) was first tested using a S/A ratio equal to 1:1. As shown on Figs. 2 and 3, all GS catalysed sucrose consumption correlated with fructose release, the for-

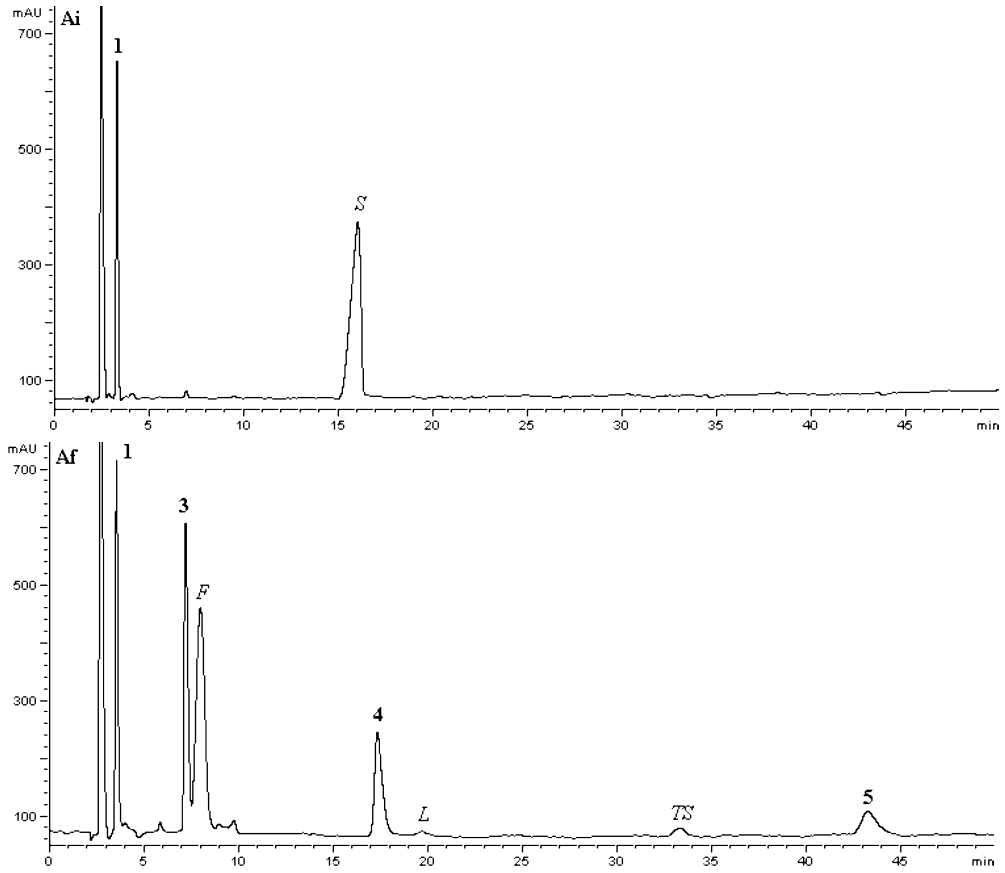

Fig. 2. HPLC analysis on NH_2 column of α-BG **1** acceptor reaction by DS NRRL-B512F at initial Ai and final Af state. Peak identification: F: fructose; S: sucrose; L: leucrose; TS: trisaccharide; **1**: α-BG; **3**: α-D-glucopyranosyl-(1→6)-*O*-butyl-α-D-glucopyranoside, [MNa^+]: $m/z = 421.2$; **4**: α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-*O*-butyl-α-D-glucopyranoside, [MNa^+]: $m/z = 583.3$; **5**: α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-*O*-butyl-α-D-glucopyranoside, [MNa^+]: $m/z = 745.4$.

mation of leucrose, and the transglucosylation from sucrose to α-BG leading to the synthesis of products **3**, **4**, **5**. In some cases, additional peaks were observed indicating the presence of additional compounds. Consequently, all GS recognize α-BG as acceptor. However, the chromatograms of the acceptor reaction differ for each enzyme indicating that different compounds are synthesized.

2.1.2. Structural characterization. Compounds **3**, **4**, **5** were the only products obtained when B-512F DS was used as catalyst. Their retention times on NH_2 column increased compared to α-BG one indicating that they probably correspond to glucosylated products. Thus, LCMS analyses revealed that **3** is a DP 2, **4** a DP 3, **5** a DP 4 (see Fig. 4). In addition, the kinetic study of the formation of each of these compounds (data not shown) showed that **3**, **4** and **5** are synthesized successively. Last, a correlation between DP and α-BG retention times was observed, as shown on Fig. 5 (a similar correlation was also observed with glucosylation products of maltose in the presence of B-1299 DS).[21]

For a complete characterization, products **3** and **4** were isolated in higher quantities for NMR and HRMS (high resolution mass spectrometry) analyses.

2.1.2.1. Compound 1. Firstly, the spectrum of α-BG was carried out for being used as reference. On ^1H NMR spectrum, one doublet at 5 ppm is present, which was assigned to the anomeric proton. The corresponding J_{1-2} coupling constant 3.8 Hz is characteristic of an α linkage. The other protons of the sugar ring have a chemical shift between 3.45 and 4.00 ppm, and at lower chemical shifts for the alkyl chain. Comparison with HMBC and HSQC spectra allowed to identify the various protons and carbons. Their chemical shifts are gathered in Table 1.

2.1.2.2. Compound 3. The ^1H NMR spectrum showed two doublets in the region 5.0–5.1 ppm, integrating for two protons. These protons correspond to the anomeric positions, and integration indicates a disaccharide structure. This is in accordance with MS data which resulted in a molecular weight of 421.2 g/mol. More-

Table 1
^{1}H and ^{13}C NMR Chemical shifts of α-BG

Molecule	Ring [a]	δ (ppm)	Carbohydrate ring							Alkyl chain [b]				
			1	2	3	4	5	6		α		β	γ	δ
								6a	6b	αa	αb			
1	I	^{1}H	5.01	3.65	3.80	3.51	3.81	3.87	3.96	3.65	3.85	1.71	1.49	1.02
		^{13}C	98.50	71.80	73.64	70.13	72.18	61.07	68.50	31.18	19.15	13.48		
3	I	^{1}H	5.03	3.68	3.79	3.61	3.95	4.08	3.82	3.68	3.87	1.73	1.49	1.02
		^{13}C	98.57	71.93	73.88	70.02	70.69	66.13	68.70	31.15	19.15	13.50		
	II	^{1}H	5.05	3.65	3.79	3.54	3.83	3.96	3.84					
		^{13}C	98.31	71.73	73.57	70.02	72.27	60.98						
4	I	^{1}H	4.84	3.65	3.65	3.45	3.83	3.66	3.90	3.48	3.66	1.53	1.30	0.83
		^{13}C	98.35	71.66	73.54	69.67	70.26	65.69	68.44	30.92	18.97	13.31		
	II	^{1}H	4.87	3.65	3.65	3.45	3.83	3.66	3.90					
		^{13}C	97.95	71.56	73.65	69.67	70.42	65.69						
	III	^{1}H	4.87	3.65	3.65	3.34	3.64	3.70	3.76					
		^{13}C	97.95	71.43	73.25	69.67	72.00	60.63						

[a] Rings are numbered from the alkyl chain.
[b] Carbons of alkyl chain are indexed from the glucose residue.

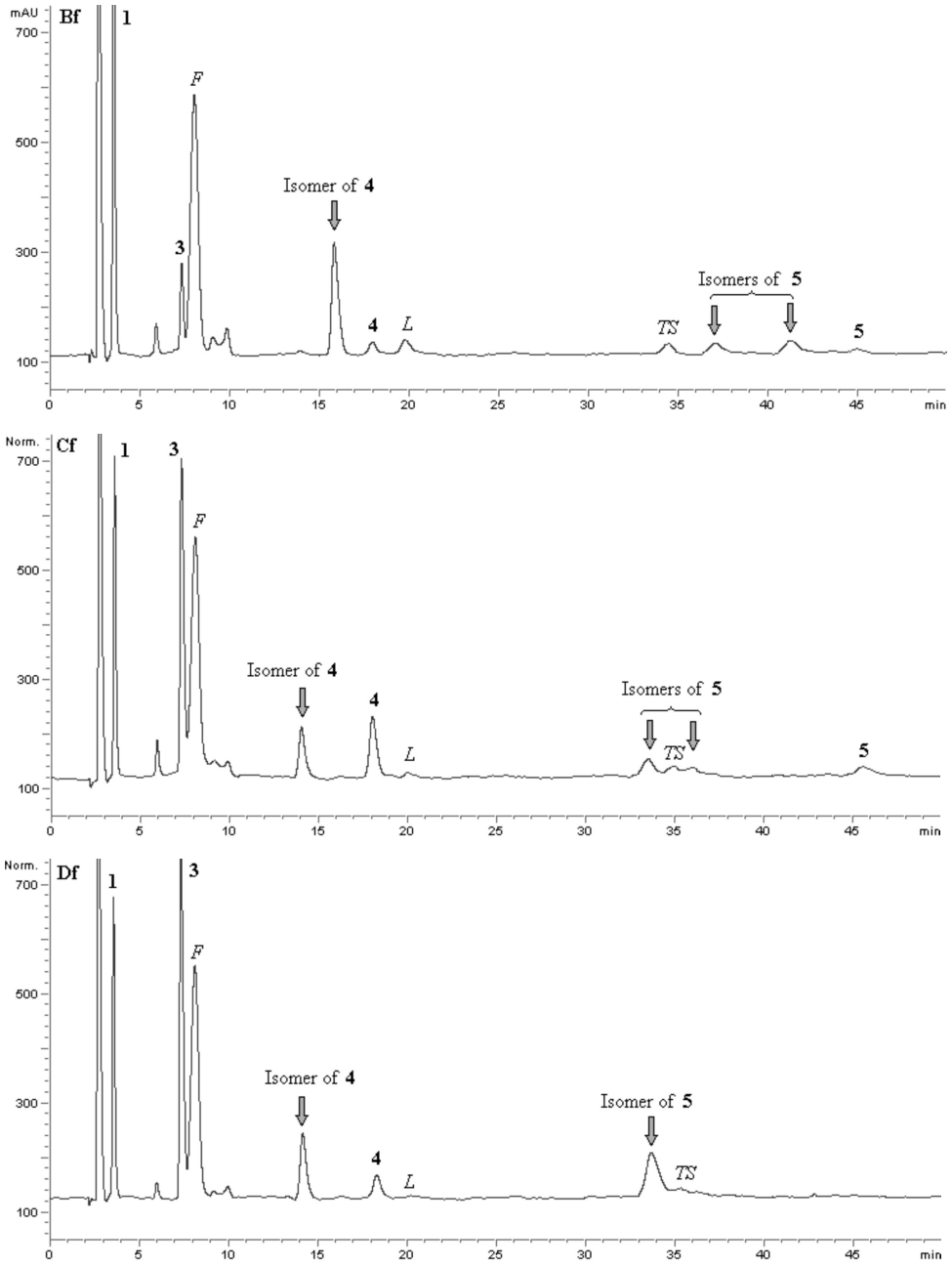

Fig. 3. HPLC analysis on NH_2 column of reaction mixtures after glucosylation of α-BG **1**. The catalysts used are *L. mesenteroides* B-1299 DS (Bf), B-1355 (Cf) and B-23192 AS (Df). Peak identification: S: sucrose; F: fructose; L: leucrose; TS: trisaccharide; **3**: [MNa^+]: $m/z = 421.2$; **4** isomers: [MNa^+]: $m/z = 583.3$; **5** isomers: [MNa^+]: $m/z = 745.4$

over, both doublets exhibit weak J_{1-2} coupling constants (3.8 Hz) which demonstrate the presence of α linkages. As for the disaccharide structure, the coupling between H-1 of unit II and C-6 of unit I on HMBC spectrum is an evidence of the formation of an α-(1→6) linkage. Last, a coupling between anomeric proton H-1 of unit I and C-α of the alkyl chain shows that the alkyl chain remains attached to the glucosyl residue at the reducing end. The other assignments of signals on ^1H and ^{13}C spectra are summarized in Table 1. From the NMR study, it can be deduced that compound **3** is α-D-glucopyranosyl-(1→6)-*O*-butyl-α-D-glucopyranoside.

2.1.2.3. Compound 4. The ^1H NMR spectrum showed three doublets around 5 ppm integrate for three protons, corresponding to the three anomeric protons of a trisaccharide structure. MS data are in agreement with these results: the experimental molecular weight thus obtained is 583.2 g/mol. The $^{13}C-^1H$ HMBC spectrum

showed strong cross-peaks between H-1 of unit II (unit III) and C-6 of unit I (unit II) demonstrating that glucose unit I (unit II) is α-(1→6)-linked to unit II (unit III). The chemical shifts of the spectrum are given in Table 1. Thus, product **4** is α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-*O*-butyl-α-D-glucopyranoside.

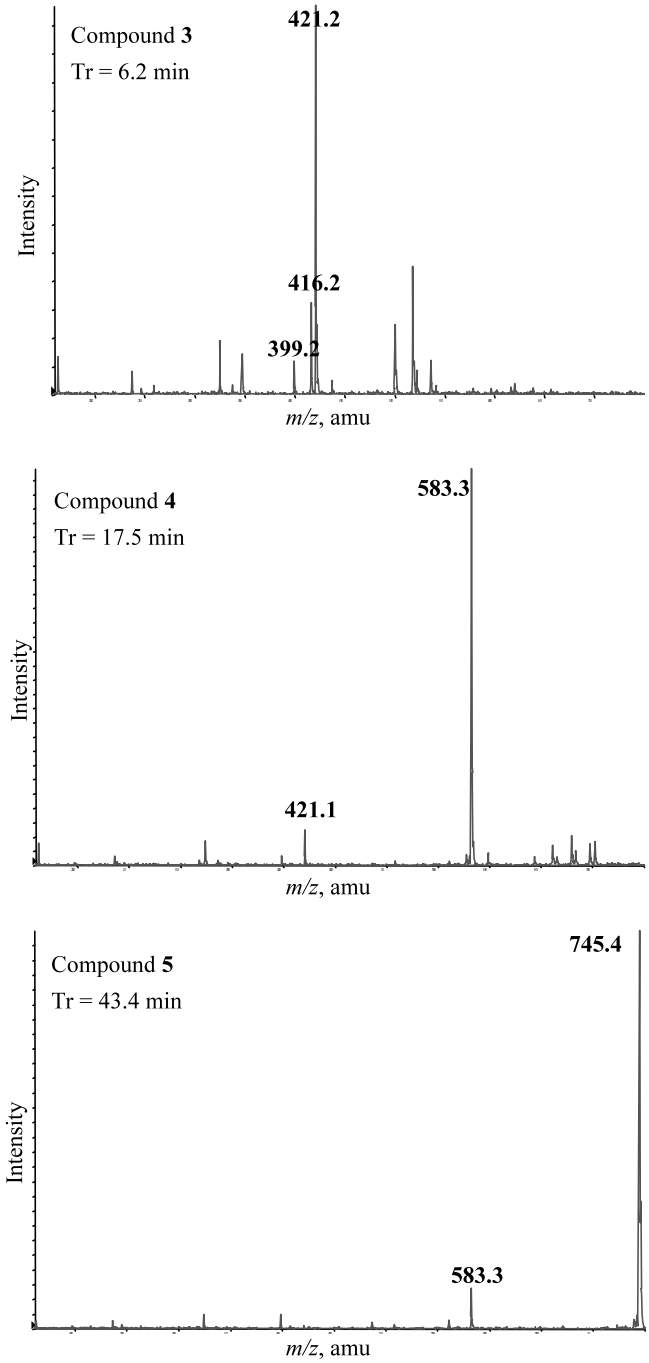

Fig. 4. Mass spectrum of chromatographic peaks corresponding to products **3**, **4** and **5** of Fig. 2; these give evidence that **3**, **4** and **5** correspond, respectively to α-butyloligosaccharides of DP 2 ([MNa^+]: $m/z = 421$), DP 3 ([MNa^+]: $m/z = 598$) and DP 4 ([MNa^+]: $m/z = 745$).

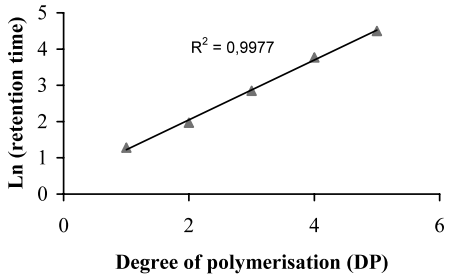

Fig. 5. Retention time analysis of APGs synthesized by glucosylation of α-BG **1** in the presence of *L. mesenteroides* NRRL B-512F DS.

Compounds **3**, **4**, **5**, previously demonstrated to be α-butyl-di-, tri-, and tetraglucopyranoside, respectively are also synthesized by B-1299 DS, B-1355 and B-23192 AS as shown by LCMS data (data not shown) and retention time comparison (see Fig. 3). But, additional peaks are present on the chromatograms. LCMS analysis showed that these peaks corresponded to additional glucosylated products and possessed the same DP as **4** and **5**. This indicates that several regioisomers are synthesized with these enzymes and may be related to the different regiospecificities of the catalysts.

2.1.3. Conversion rates study. Conversion rates were calculated from α-BG depletion. Several S/A ratio of 1:2, 1:1 or 2:1 were tested for each GS (Table 2). For all enzymes, the conversion rates increased with the S/A ratio from 10 to 20% when S/A ratio is doubled. Indeed, at high S/A ratio, more glucosyl residues are available to be transferred to α-BG. It must also be pointed out that among the GS tested, AS preparations from *L. mesenteroides* B-1355 and B-23192 are the most efficient GS for glucosylation of α-BG: an improvement of the α-BG conversion rate of 10–15% is observed with these enzymes in comparison with DS from *L. mesenteroides* B-512F and B-1299.

2.1.4. DP distribution of APG. The final DP repartition of the APG synthesized with B-512F DS as a function of S/A ratio was then determined. Results of Table 3 show that increased S/A ratio turns the reaction towards the synthesis of APG with longer glucosidic moiety. Roughly, doubling S/A ratio induces an increase of the average DP of 0,5. Consequently, the average DP of the final mixture can be controlled by the choice of initial S/A ratio. This correlation is perfectly in agreement with the results obtained by Remaud and co-workers[35] for glucosylation of maltose catalysed by *L. mesenteroides* NRRL B-1299 DS.

2.2. Glucosylation of α-butyltriglucopyranoside: influence of the polymerization degree

After isolation, α-butyltriglucopyranoside (**4**) was tested as an acceptor for B-512F DS at a S/A ratio of 2:1 (in

Table 2
α-BG conversion rates as a function of sucrose/acceptor ratio and GS used

S/A ratio (mol/mol)	Acceptor concentration (mol/L)	*L. mesenteroides*			
		B-512F (%)	B-1299 (%)	B-1355 (%)	B-23192 (%)
1:2	0.22	32	40	52	44
1:1	0.22	54	47	65	67
2:1	0.11	70	68	76	81

Table 3
Average DP of glucosylated of α-butyloligoglucopyranoside synthesized by *L. mesenteroides* B-512F DS as a function of sucrose/acceptor ratio

S/A ratio (mol/mol)	Compound				AverageDP [a]
	1 DP = 1 (%)	**3** DP = 2 (%)	**4** DP = 3 (%)	**5** DP = 4 (%)	
1:2	68	22	8	2	1.4
1:1	46	28	18	8	1.9
2:1	30	29	26	15	2.6

[a] Average DP calculated from the Schulz's law.[4]

order to obtain the best conversion rates as demonstrated above).

From HPLC analysis (data not shown), it can be concluded that **4** was used as acceptor but did not react as a glucosyl donor since no DP 2 nor DP 1 was formed. Thus, the peak corresponding to **4** decreased, whereas new peaks with higher retention times appeared, the first one being **5** (DP 4) and the second **6** (DP 5). All compounds obey to the correlation between retention time and DP previously pointed out (Fig. 5).

From a quantitative aspect, the conversion rate reached 78%, a little higher than the one obtained in the same conditions with α-BG acceptor. Consequently, reaction products are better acceptors than initial acceptors.

2.3. Glucosylation of α-OG: influence of the alkyl chain length

α-OG was tested as acceptor. To increase its solubility, reactions were carried out at 40 °C.

2.3.1. Enzyme screening. The only glucansucrase able to glucosylate α-OG is AS from *L. mesenteroides* B-1355 (and B-23192). New glucosylated products were formed as shown on Fig. 6. Indeed, *L. mesenteroides* B-512F DS was not stable during the course of the reaction and *L. mesenteroides* B-1299 DS consumed sucrose but did not catalyse transglucosylation from sucrose to α-OG. Consequently, *L. mesenteroides* B-1299 DS is much more affected by the increase of alkyl chain length than AS: the presence of a longer alkyl chain (and thus higher hydrophobicity) may prevent a correct docking of the acceptor in the enzyme active site.

2.3.2. Conversion rate study. Acceptor reactions were carried out with α-OG at various S/A ratio in the presence of AS preparations from *L. mesenteroides* B-1355 and B-23192 (Fig. 6). The presence of new peaks is correlated with the disappearance of the acceptor. Since more glucosylated the product is, lower the retention time will be, it can be concluded that the products corresponding to the new peaks derived from α-OG glucosylation.

The best conversion rates are again obtained with B-23192 AS (Table 4). This observation emphasizes the better efficiency of AS to catalyse glucosylation of α-AG. However, the use of α-OG **2** as acceptor induced a 20% conversion rate decrease for both enzymes (compared to α-BG conversion rate) indicating that increasing the alkyl chain length diminishes the glucosylation efficiency.

For the first time, the enzymatic glucosylation of α-BG and α-OG was carried out by using GS from *L. mesenteroides*. Conversion rates were improved by using a high S/A ratio and AS as catalyst. Thus, 66% of α-OG and 81% of α-BG were converted, a difference resulting from a difference in hydrophobicity.

Moreover, each GS conserved its own specificity during α-AG glucosylation, leading to different regioisomers. In the meantime, S/A ratio is a key parameter which influences the final average DP of APG. Consequently, by selecting the initial conditions of synthesis (i.e., enzyme, S/A ratio), it is possible to have

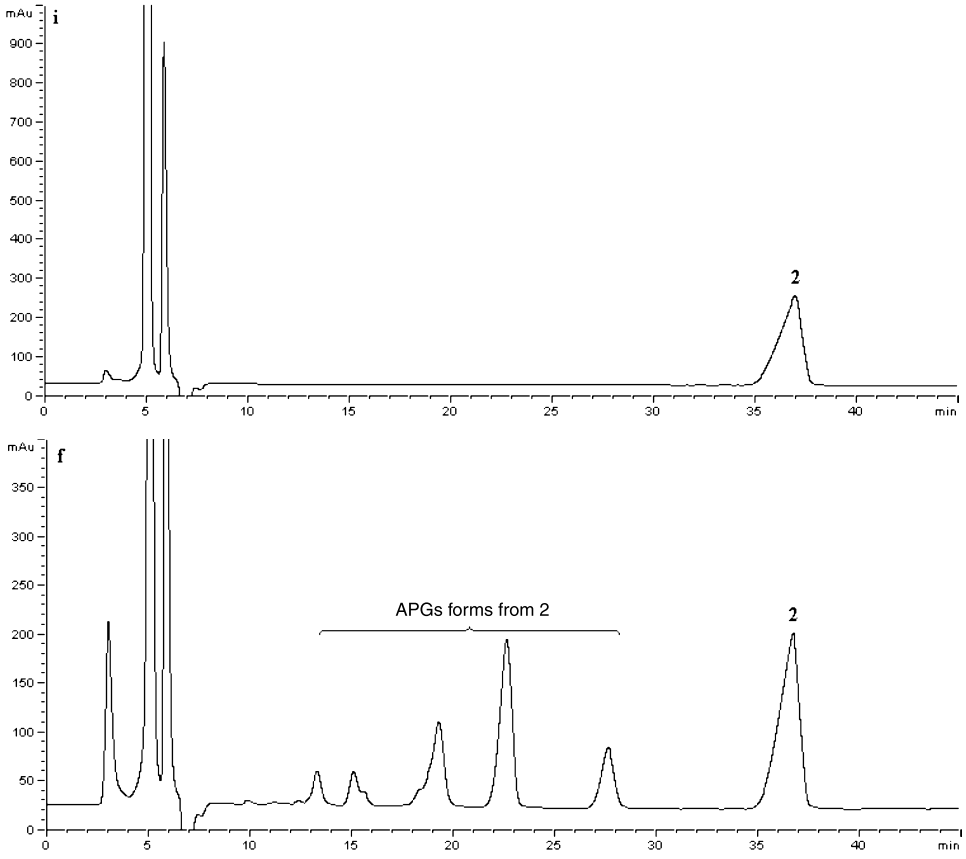

Fig. 6. HPLC analyis on C18 column of reaction mixtures after glucosylation of α-OG **2** with AS from *L. mesenteroides* NRRL B-1355 (similar chromatograms are obtained with B-23192 enzyme) S/A ratio equal to 1:1 (i, initial state; f, final state).

Table 4
α-OG conversion rates as a function of sucrose/acceptor ratio used during glucosylation catalysed by AS preparations

S/A ratio (mol/mol)	Acceptor concentration (mol/L)	*L. mesenteroides*	
		B-1355 (%)	B-23192 (%)
1:2	0.22	12	27
1:1	0.22	31	42
2:1	0.11	51	66

access to various APG of controlled size and structure. The physico-chemical properties of these products issued from green chemistry must be now investigated to develop new applications.

3. Experimental

3.1. Materials

L. mesenteroides NRRL B-512F, B-1299, B-1355 and B-23192 were supplied by the NRRL Peoria (IL), USA. The production of the enzymes from these strains was detailed previously.[28,29,36,37] α-BG was a gift from Solabia (France), and α-OG was purchased from Aldrich.

3.2. Analytical methods

Melting points were measured with a Köfler hot bench and are uncorrected. ^{1}H (400.13 MHz) and ^{13}C NMR (100.612 MHz) were recorded on a Bruker-ARX 400 spectrometer equipped with an ultrashim system. Samples were studied as solutions in deuterium oxyde (20 mg in 0.75 mL of solvent) at 25 °C. Total assignment of the carbon and proton signal was made through COSY, HMQC and HMBC experiment. HPLC analyses were performed using a Hewlett Packard 1050 series consisting of a pump, an automatic injector and a HP 1047A refractometer. Analysis of the glucosylation of α-BG was done on an analytical NH_2 column (Spherisorb

Amino 5 μm, Bischoff Chromatography, 250 × 4.0 mm) thermostated at 30 °C and with water–MeCN (20/80 v/v) as eluant at a constant flow of 1.0 mL/min. Analysis of the glucosylation of α-OG was performed on an analytical C18 column (Nucleosil, Bischoff Chromatography, 250 × 4.6 mm) with water–MeCN (70/30 v/v) as eluent and a constant flow of 0.5 mL/min. Each sample was analysed for a period of 60 min at 30 °C. Samples were diluted before injection in order to ensure a total concentration lower than 10 g/L. LCMS was run on a Perkin–Elmer SCIEX model API 365 system connected to a NH_2 column. Liquid chromatography was performed with the same column described before and with water–MeCN (20/80 v/v) as eluent at 0.8 mL/min. As for mass spectrometry, molecules were ionised by Ion Spray technique with a source at 360 °C, separated by a quadripole and detected on the positive mode. HRMS analyses were performed on a high resolution Autospec Micromass (35 keV, EI^+).

3.3. Alkylpolyglucopyranoside synthesis

The glucosylation reaction was performed in AcONa buffer (20 mM, pH 5.2), supplemented with sucrose at various concentrations (owing depending on S/A molar ratio). With α-BG (0.23 M), the mixture was thermostated at 30 °C, whereas with α-OG (0.12 M), the temperature was 40 °C (due to the limited solubility of the acceptor in the buffer). For α-AG glucosylation, enzyme activity[1] was fixed to 1 U/mL. The acceptor reactions were carried out for 24 h. The reaction was stopped by dilution in MeCN–water for α-BG, or by heating at 95 °C for 5 min with α-OG. Then, the samples were centrifuged for 10 min at 10,000 rpm at room temperature before HPLC analysis.

3.4. APG purification

In order to characterize glucosylated products of α-BG, the APG synthesis described above was performed at preparative scale at S/A equal to 2:1 (16 g = 47 mmol in sucrose and 5.6 g = 23 mmol in **1** for 100 mL of buffer). After a 72 h reaction time, an equal volume of EtOH was added to the mixture in order to precipitate the soluble dextran. The medium was centrifuged at 8000 rpm for 20 min at 4 °C, the supernatant collected and evaporated and the solute obtained dissolved in 100 mL of ultra-pure water (with a final concentration lower than 100 g/L). Then, isolation of glucosylated products was performed by gel permeation using Bio-Gel® P-2 (polyacrylamide gel; fine particles: ∅ = 45–90 μm; 91 × 10 cm) as the stationary phase, ultra-pure water as eluent at a constant flow rate of 14 mL/min. APG detection was carried with a refractometer, and each peak was collected separately, concentrated and reinjected into an analytical HPLC system to check the purity of the compounds. Thus, were obtained 2.7 g = 6.7 mmol in **3**, 3.4 g = 6.0 mmol in **4**, 2.5 g = 3.5 mmol in **5**, and 1.7 g = 6.9 mmol remaining in **1**.

3.4.1. α-D-Glucopyranosyl-(1 → 6)-*O*-butyl-α-D-glucopyranoside (3). Mp 81 °C; HRMS: Anal. Calcd for $C_{16}H_{30}O_{11}$: 421.1686 [MNa^+], Found: 421.1692; ^1H and ^{13}C NMR data in Table 1.

3.4.2. α-D-glucopyranosyl-(1 → 6)-α-D-glucopyranosyl-(1 → 6)-*O*-butyl-α-D-glucopyranoside (4). Mp 130 °C; HRMS: Anal. Calcd for $C_2H_{40}O_{16}$: 583.2214 [MNa^+], Found: 583.2209; ^1H and ^{13}C NMR data in Table 1.

3.5. Final composition in APGs

As for the average DP of the mixture, it was calculated thanks to Schulz's law[4] (Eq. (1)).

$$\overline{DP} = \frac{p_1}{100} \times 1 + \frac{p_2}{100} \times 2 + \cdots = \sum_{i=1}^{\infty} \frac{p_i}{100} \times i \quad (1)$$

Eq. (1)—Calculation of the average DP according to the Schulz's law;[4] with i as the individual DP and p_i the molar percentage of APG in the mixture.

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References

1. Milius, B.; Brancq, B. *Ol., Corps Gras, Lipides*. **1995**, *3*, 177–182.
2. Hill, K. *Agro-Food-Ind. Hi-tech*. **1998**, *9-10*, 9–15.
3. Weuthen, M.; Kawa, R.; Hilland, K.; Ansmann, A. *Fett. Wiss. Technol*. **1995**, *6*, 209–211.
4. Schulz, P. *Chim. Oggi*. **1992**, *8–9*, 33–38.
5. Hill, K.; Von Rybinski, W.; Stoll, G., Eds. *Alkyl Polyglucosides–Technology, Properties*; Weinheim: New York, 1997
6. Garcia, M.T.; Ribosa, I.; Campos, E.; Gonzalez, J. World Surfactants Congress, 4th, 1996; 228–232.
7. Brancq, B. *Actual. Chim*. **1996**, *2–3*, 63–65.
8. Knaut, J.; Kreinfeld, G. *Chim. Oggi*. **1993**, *9*, 41–46.
9. Balzer, D. *Tenside, Surfactants, Deterg*. **1991**, *28-6*, 419–427.

[1] Enzymatic activity is defined as the quantity of enzyme involved for hydrolysis of 1 μmol of sucrose in 1 min (expressed in unit per ml).

10. Hill, K. In *Carbohydrates as Organic Raw Materials II*; Descotes, G. L. Ed.; Weinheim VCH, New York, 1993; pp 163–184.
11. Waldhoff, J.; Scherler, M.; Schmitt M. World Surfactants Congress., 4th, 1996; pp 507–518.
12. Sidebotham, R. L. *Dextrans. Adv. Carbohydr. Chem. Biochem.* **1974**, *30*, 371–444.
13. Mayer, R. M. *Methods Enzymol.* **1987**, *138*, 649–661.
14. Hehre, E. J. *Methods Enzymol.* **1995**, *1*, 178–184.
15. Henrissat, B. *Biochem. J.* **1991**, *280*, 309–316.
16. Coutinho, P.M.; Henrissat, B. http://afmb.cnrs-mrs.fr/ ~ pedro/CAZY/db.html.
17. Koepsell, H. J.; Tsuchiya, H. M.; Hellman, N. N.; Kasenko, A.; Hoffman, C. A.; Sharpe, E. S.; Jackson, R. W. *J. Biol. Chem.* **1952**, *200*, 793–801.
18. Monchois, V.; Willemot, R. M.; Monsan, P. *FEMS Microbiol. Rev.* **1999**, *23*, 131–151.
19. Robyt, J. F.; Walseth, T. F. *Carbohydr. Res.* **1978**, *61*, 433–445.
20. Seymour, F. R.; Knappk, R. D. *Carbohydr. Res.* **1980**, *81*, 105–129.
21. Dols, M.; Remaud-Simeon, M.; Willemot, R. M.; Vignon, M. R.; Monsan, P. *Carbohydr. Res.* **1998**, *305*, 549–559.
22. Bourne, E. J.; Sidebotham, R. L.; Weigel, H. *Carbohydr. Res.* **1974**, *34*, 279–288.
23. Kobayashi, M.; Mitsuhishi, Y.; Tagagi, S.; Matsuda, K. *Carbohydr. Res.* **1984**, *127*, 305–317.
24. Mitsuhishi, Y.; Kobayashi, M.; Matsuda, K. *Carbohydr. Res.* **1984**, *127*, 331–337.
25. Remaud-Simeon, M.; Lopez-Munguia, A.; Pelenc, V.; Paul, F.; Monsan, P. *Appl. Biochem. Biotechnol.* **1994**, *44*, 101–116.
26. Côté, G. L.; Robyt, J. F. *Carbohydr. Res.* **1982**, *111*, 127–142.
27. Côté, G. L.; Robyt, J. F. *Carbohydr. Res.* **1982**, *101*, 57–74.
28. Lopez-Munguia, A.; Pelenc, V.; Remaud, M.; Biton, J.; Michel, J. M.; Lang, C.; Paul, F.; Monsan, P. *Enzyme Microb. Technol.* **1993**, *15*, 77–85.
29. Smith, M. R.; Zahnley, J. *Appl. Environ. Microbiol.* **1997**, *63*, 581–586.
30. Robyt, J. F.; Elkund, S. H. *Carbohydr. Res.* **1983**, *68*, 95–111.
31. Su, D.; Robyt, J. F. *Carbohydr. Res.* **1993**, *248*, 339–348.
32. Robyt, J. F.; Eklund, S. H. *Carbohydr. Res.* **1983**, *121*, 279–286.
33. Jones, R. W.; Jeanes, A.; Stringer, C. S.; Tsuchiya, H. M. *J. Am. Chem. Soc.* **1956**, *78*, 2499–2502.
34. Demuth, K.; Jordening, H. J.; Buccholz, K. *Carbohydr. Res.* **2002**, *337* (20), 1811–1820.
35. Remaud, M.; Paul, F.; Monsan, P. J. *Carbohydr. Chem.* **1992**, *11* (3), 359–378.
36. Auriol, D. Doct. Ing. Thesis; INSA Toulouse, France; 1984; 144.
37. Dols, M.; Remaud-Simeon, M.; Monsan, P. *Enzyme Microbiol. Technol.* **1997**, *20*, 523–530.