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Note

Glucosylation of Sucrose Laurate with Cyclodextrin Glucanotransferase

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Sucrose monolauroyl esters were found to serve as substrates for cyclodextrin glucanotransferase (CGTase)-catalyzed transglucosidation reactions, affording new sucrose esters that have an additional 1-3 glucose residues on the pyranose ring of the sucrose moiety in the ester.

Key words: cyclodextrin glucanotransferase; CGTase; sucrose monolaurate; surfactant

Fatty acid esters of sugars have wide use in a variety of fields, including food technologies,[1,2] cosmetics,[3] and pharmaceutical production.[4] Their physicochemical properties are primarily dependent on the structure of the sugar part and the hydrophobic lipid group. The desired structure and properties of such compounds are naturally different in different practical uses. Most of the approaches to produce sugar esters with different properties have been directed to the esterification of sugars, including mono- and disaccharides with different fatty acids.[5] Another approach in tuning the hydrophilic/lipophilic balance in non-ionic surfactans is altering the size-length of the saccharide chain by adding or reducing the number of sugar units in the glycosides.

Cyclodextrin glucanotransferase (CGTase) catalyzes the transfer of dextrin units from cyclodextrin or longer dextrins to polyols such as sugars and flavonoids.[6] However, it has been said that when the substrate polyols are rather hydrophobic, they may not be a good substrate for the enzyme. In fact, there appears to be no example in which saccharides bearing highly lipophilic substituents serves as good substrates for CGTase. Hayashibara Biochemical Laboratories, Inc., developed a new CGTase produced by *Bacillus stearothermophilus* with improved catalytic activity. This enzyme has been used practically by the company to increase the water solubility of hesperidine making it feasible as a food additive. Fatty acid esters of sugars are an important class of non-ionic amphiles or surfactants. Within them, sucrose laurates in particular have notable biological functions, including anti-tumoral and insecticidal activity,[7] as well as wide utility in the food and cosmetics industries. The ester also has potential usefulness in topical drug delivery systems.[7] Therefore, varying the number of glucose units in the sugar part is of great importance in preparing such sugar esters having desired properties. There appears to be, however, no example of glucan (dextrins including glucose) transfer to such lipophilic sugar substrates so far. In the present study, we examined CGTase-catalyzed transglucosylation from dextrin to a mixture of sucrose esters, (**1**), (**2**), and (**3**) in Scheme 1, having a hydrophobic medium chain lauroyl group (C_{12}-carbons).

CGTase-catalyzed transglucosylation was conducted as follows, using commercially available monolauroylsucrose that was a mixture of **1** and **2**: A solution of the monolauroylsucrose, dextrin, and CGTase solution in an acetate buffer was stirred at 50 °C for 3 h, followed by inactivation of the enzyme in boiling water for 5 min. The reaction mixture was analyzed by silica gel TLC (Fig. 1). The fractions, A ($R_f = 0.7$), B ($R_f = 0.4$), C ($R_f = 0.6$) and D ($R_f = 0.2$), were isolated by silica gel column chromatography eluted with $CHCl_3/CH_3OH$ (3:1) to afford almost pure product. ^1H, ^{13}C NMR (CD_3OD) and ESI-MS data for the products are given in Table 1. As shown in entry 1, for fraction A, the ratio of the sum of the signal intensity for all the protons on the sugar rings without the hydroxy protons and that of the lauroyl group was 0.98, approximately equal to the value (0.91) calculated from the structure **4**. This indicates that one glucose unit was added to the sucrose moiety with a lauroyl group. Moreover, two ^1H signals (doublet, $J = 3.80$ Hz) were observed at δ 5.4 and 5.7, indicating that there were two anomeric protons in the product obtained from fraction A, with an α-glucosidyl

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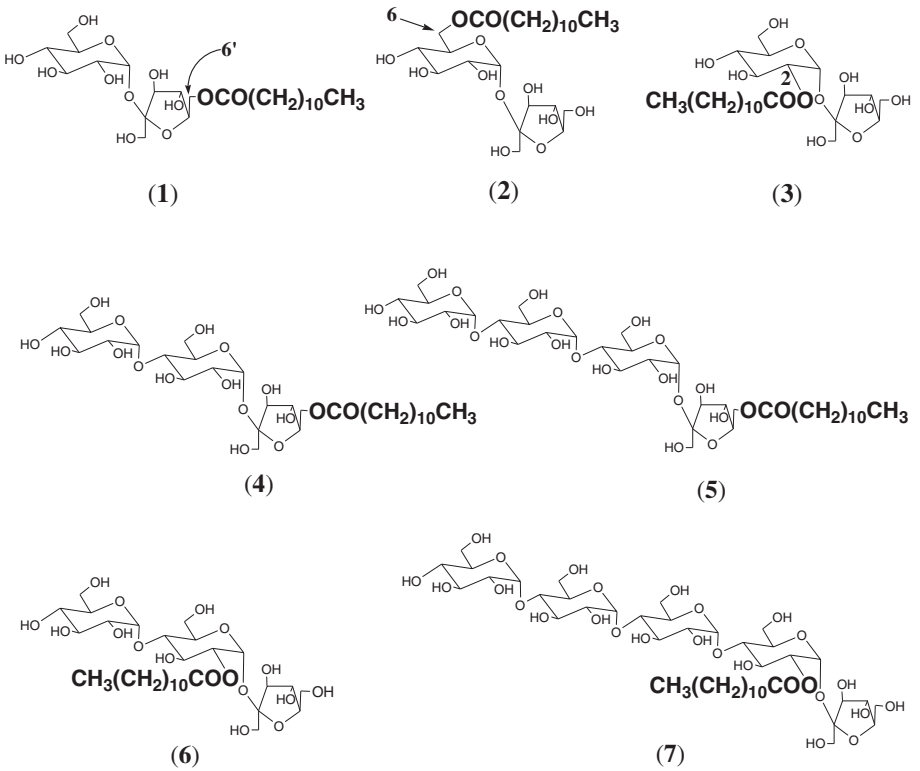

Scheme 1. Structure of Sucrose Monolaurates **1–3** and Reaction Products **4–7** by CGTase Catalyzed Glucanotransfer Reactions.

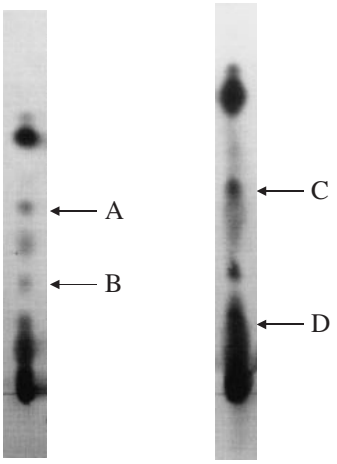

Fig. 1. TLC of the Reaction Mixture Obtained by CGTase-Catalyzed Transglucosidation from Dextrin to Sucrose Mono-Laurate.

Left, Reaction mixture using a mixture of 6- and 6′-monolauroylsucrose. Right, Reaction mixture using 2-monolauryolsucrose. TLC, Silica gel TLC plate F_{254} developed by chloroform/methanol/water (65:25:4) detected with 10% sulfuric acid in ethanol.

linkage. It is not incompatible with the general feature that CGTase catalyzes cleavage or formation of a glucosidic bond in an α-1,4 manner. The ^{13}C NMR (CD_3OD) spectrum also indicated two anomeric signals, at δ 101.5 and 101.8. ESI-MS (positive mode) of fraction A gave a molecular ion peak of m/z 687.2, which coincided with m/z 687.3, calculated from a protonated form of glucosylated sucrose monolaurate (**4**). It is known that when the hydroxy group at the 6-position of the pyranose ring in sucrose is acylated, the ^1H signal of a proton at 6-position shifts to a lower field around δ 4.5. In the ^1H spectrum, however, no signal was found at that region suggesting that the lauroyl group was attached at the 6′-position of the furanose ring rather than at the 6-position of the pyranose ring. Combining all of these spectral data, the compound in fraction A should have mono-glucosylated structure (**4**) in which the lauroyl group was attached to 6′-OH.

On the other hand, fraction B, had a much lower R_f value (0.4), indicating that it was more hydrophilic than A, and it showed no ^1H signal at δ 4.5. As shown in entry 2 in Table 1, the ratio of sum of the intensities of proton signals on sugar carbons to that of the lauroyl group was 1.22, which coincided well with that (1.22) calculated for structure (**5**) indicating that two glucose units were introduced into mono-lauroyl sucrose (**1**). In addition, three ^1H signals (doublet, $J = 3.80$ Hz) were observed at δ 5.05, 5.09, and 5.24, indicating that there were three anomeric protons in the molecule of the fraction B. These data unambiguously indicate that the compound in fraction B had structure (**5**). ^{13}C and ESI-MS spectra also confirmed structural integrity. Other fractions on TLC were also isolated, but their structure could not be characterized due to their insufficient purity.

A second substrate in the present study was 2-lauroylsucrose (**3**), which was synthesized according to

Table 1. ^{1}H, ^{13}C NMR, ESI MS Data and Chemical Yields in the CGTase-Catalyzed Glucanoyl Transfer Reaction from Dextrin to Monolauroyl Sucrose

Entry	Sugar ester	Sug./Lip.* found	Sug./Lip.* calculated	A.P.S** (δ, ppm)	A.C.S*** (δ, ppm)	m/z found	m/z $(M+H)^+$ calculated	Yield (%)
1	(**4**)	0.98	0.91	5.06 5.29	101.5 101.8	687.2	687.3	20
2	(**5**)	1.22	1.22	5.05 5.09 5.24	100.8 101.0 101.4	850.0	849.9	21
3	(**6**)	0.99	0.91	5.08 5.28	100.8 102.1	687.2	687.3	3
4	(**7**)	1.51	1.47	5.02 5.06 5.13 5.25	100.6 100.9 101.9 102.0	1033.4†	1033.4††	6

*Number of protons on the sugar carbons/number of protons on lauroyl carbon chain.
**Chemical shift of anomeric proton signals (300 MHz, Varian Mercury 300).
***Chemical shift of anomeric carbon signals (90 MHz, Varian Mercury 300).
†Observed molecular mass in ESI MS (Perkin Elmer Model API III by positive ion mode).
††Mass number calculated for $(C_{42}H_{74}O_{27}+Na)^+$.

the reported method.[8] Under the conditions described above, this substrate was subjected to CGTase catalyzed transglucosidation, and two fractions, C and D, on TLC were isolated in pure form. As shown in entry 3 in Table 1, the fraction C ($R_f = 0.6$) showed signals of two anomeric protons at δ 5.08 and 5.28, with 3.90 Hz of coupling constant and two carbon signals at δ 100.8 and 102.1, as shown in entry 3 in Table 1. The ratio of the sum of the signal intensities of all the protons on the sugar rings without the hydroxy protons to that of the lauroyl group was 0.99, approximately equal to the calculated value (0.91) based on structure (**6**). The ESI-MS spectrum gave a molecular ion peak at m/z 687.2, which coincided with the m/z 687.3, of a protonated form of glucosylated sucrose mono-laurate (**6**).

As shown in entry 4 in Table 1, fraction D ($R_f =$ 0.2), isolated in pure form showed signals of four anomeric protons at 5.02, 5.06, 5.13, and 5.25, and four carbon signals at δ 100.6, 100.9, 101.9, and 102.0. The ratio of the sum of the signal intensities of all the protons on the sugar rings without the hydroxy protons to those of the lauroyl group was 1.51, approximately equal to the value (1.47) calculated from structure (**7**). In the ESI-MS spectrum, a signal was detected at m/z 1033.4 which coincided to the value (m/z 1033.4) calculated for (**7**) as a complex with Na^+. The above proton and carbon NMR data suggest the reliability of the structure (**7**) for the fraction with three additional glucose units. The isolated yield for (**6**) and (**7**) were 3 and 6%, respectively (Table 1). These values are much lower than (**4**), at 20% and (**5**), at 21%. In the substrate **3**, the lauroyl group was much closer to the reaction site (4-hydroxy group) of the sugar pyranose than to that at 2′-position in the furanose ring of (**1**). This might be reflect in the large difference in the observed chemical yields. All the yields described above are for the isolated product alone, and, since there should be additional products with different numbers of glucose units in the reaction mixture, the total yield might have been much higher, although estimation was not easy.

In conclusion, this study constitutes the first example that acylated glucose with medium chain lipophilic group can serve as a substrate for the CGTase-catalyzed glucan transfer reaction. If the reaction conditions can be optimized to improve the chemical yield, this enzymatic system might give a new opportunity for environmentally safe production of saccharide-based more water-soluble amphiles. The fatty acid sucrose esters prepared so far have the problem that their water solubility was not enough to obtain the desired surfactant property and sulfonation of the sugar hydroxy groups using sulfur trioxide[9] is one of the way to solve this problem. Sulfur trioxide is known to react violently with water, affording sulfuric acid.

Experimental

^{1}H and ^{13}C NMR spectra were recorded on Varian Mercury 300 using CD_3OD, and ESI MS spectra were on API III, Perkin Elmer (Ontario, Canada), by direct infusion using a mixture of $THF/CH_3OH/H_2O$ (15:4:1) with 0.1% HCOOH as a solvent in positive mode.[10]

CGTase-catalyzed transglucosylation was conducted as follows: A mixture of monolauroylsucroses **1** and **2** (400 mg, 0.76 mmole), dextrin (1.2 g, a mixture of oligomers with the number of glucose units = 10–15) and CGTase solution (1.2 ml, 1,500 units/ml) in an acetate buffer (8 ml, pH 6.5, mM) was stirred at 50 °C for 3 h, followed by inactivation of the enzyme in boiling water for 5 min. The reaction mixture was analyzed by silica gel TLC (Fig. 1). Some of the fractions were isolated by silica gel column chromatography eluted with $CHCl_3/CH_3OH$ (3:1) to afford almost pure product, and structural analyses were conducted using ^{1}H, ^{13}C NMR and ESI MS.

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10) (**4**): ^{1}H NMR δ_H (CD_3OD): 0.84 (3H, t, $J = 6.9$ Hz, CH_3), 1.24 (16H, m, $8 \times CH_2$), 1.56 (2H, m, β-CH_2 from C=O), 2.29 (2H, m, α-CH_2–C=O), 3.20–4.33 (27H, m, protons on sugar moiety), 5.13 (1H, d, $J = 3.9$ Hz, an anomeric proton on the first pyranose ring from the glucose in the sucrose structure), 5.29 (1H, d, $J = 3.9$ Hz, an anomeric proton on the glucose next to the furanose ring). ^{13}C NMR δ_C (CD_3OD): 13.2 (CH_3), 21.6–28.6 ($8 \times CH_2$), 30.9 (β-CH_2 from C=O), 32.9 (α-CH_2–C=O), 59.3–78.6 (15 carbons in the sugar moiety), 101.5–101.8 (2 anomeric carbons), 104.5 (2′-carbon in the furanose ring), 173.5 (C=O). (**5**): ^{1}H NMR δ_H (CD_3OD): 0.79 (3H, t, $J = 6.9$ Hz, CH_3), 1.19 (16H, m, $8 \times CH_2$), 1.51 (2H, m, β-CH_2 from C=O), 2.24 (2H, m, α-CH_2–C=O), 3.18 (1H, dd, $J = 9.3$ Hz, proton at the non-reducing terminal on the second pyranose ring from the sucrose), 3.19–4.28 (27H, m, protons in sugar moiety), 5.06 and 5.09 (2H, d, $J = 3.9$ Hz, anomeric protons on the first and second pyranose rings from the glucose in the sucrose structure), 5.24 (1H, d, $J = 3.9$ Hz, an anomeric proton on the glucose next to the furanose ring). ^{13}C NMR δ_C (CD_3OD): 13.2 (CH_3), 21.6–28.7 ($8 \times CH_2$), 30.9 (β-CH_2 from C=O), 32.9 (α-CH_2–C=O), 58.1–79.4 (20 carbons in the sugar moiety), 100.8–101.4 (3 anomeric carbons), 104.1 (2′-carbon in the furanose ring), 173.5 (C=O). (**6**): ^{1}H NMR δ_H (CD_3OD): 0.79 (3H, t, $J = 6.9$ Hz, CH_3), 1.19 (16H, m, $8 \times CH_2$), 1.52 (2H, m, β-CH_2 from C=O), 2.26 (2H, m, α-CH_2–C=O), 3.16 (1H, m, proton at the non-reducing terminal of the glucose moiety next to sucrose), 3.20–4.53 (18H, m, protons on sugar moiety), 5.03 (1H, d, $J = 3.9$ Hz, an anomeric proton on the first pyranose ring from the glucose in the sucrose structure), 5.28 (1H, d, $J = 3.9$ Hz, an anomeric proton on the glucose next to the furanose ring). ^{13}C NMR δ_C (CD_3OD): 12.9 (CH_3), 21.7–28.8 ($8 \times CH_2$), 31.1 (β-CH_2 from C=O), 33.0 (2H, m, α-CH_2–C=O), 59.6–94.3 (15 carbons in the sugar moiety), 100.8–102.1 (2 anomeric carbons), 103.5 (2′-carbon in the furanose ring), 173.5 (C=O). (**7**): ^{1}H NMR δ_H (CD_3OD): 0.78 (3H, t, $J = 6.9$ Hz, CH_3), 1.16 (16H, m, $8 \times CH_2$), 1.49 (2H, m, β-CH_2 from C=O), 2.26 (2H, m, α-CH_2–C=O), 3.13–4.23 (28H, m, protons on sugar carbons), 3.98 (1H, m, a proton at 5-carbon in pyranose ring of sucrose), 4.26 (1H, d, $J = 12$ Hz, a proton at 3-carbon in pyranose ring of sucrose), 4.54 (1H, m, a proton at 2-carbon in pyranose ring of sucrose), 5.02 and 5.06 (2H, d, $J = 3.9$ Hz, anomeric protons on the second and third pyranose rings from the glucose in the sucrose structure), 5.13 (1H, d, $J = 3.9$ Hz, an anomeric proton on the first pyranose ring from the glucose in the sucrose structure), 5.25 (1H, d, $J = 3.9$ Hz, anomeric proton on the glucose next to the furanose ring). ^{13}C NMR δ_C (CD_3OD): 12.5 (CH_3), 21.7–28.7 ($8 \times CH_2$), 31.0 (β-CH_2 from C=O), 32.9 (α-CH_2–C=O), 59.6–94.2 (25 carbons in the sugar moiety), 100.6–102.0 (4 anomeric carbons), 103.4 (an anomeric carbon in the third pyranose ring from the sucrose), 173.5 (C=O).