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Cyclodextrin glucanotransferase (CGTase) catalyzed synthesis of dodecyl glucooligosides by transglycosylation using α-cyclodextrin or starch

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ABSTRACT

Dodecyl glucooligosides, a class of interesting non ionic surfactant molecules were synthesized by cyclodextrin glucanotransferase from *Bacillus macerans* using either α-cyclodextrin (α-CD) or soluble starch as glycosyl donor and dodecyl β-D-glucoside ($C_{12}G_1$) or dodecyl β-D-maltoside ($C_{12}G_2$) as acceptor substrates. The primary coupling products obtained in the respective reactions were identified as dodecyl glucoheptaoside and dodecyl maltooctaoside by mass spectrometry. Higher yields of coupling products were obtained using α-CD as donor, while more dispoportionation occurred with starch. Nearly 78% conversion of the acceptor substrate $C_{12}G_1$ into dodecyl glucooligosides could be achieved at 132 μg/ml of CGTase in 20 min, while 93% of $C_{12}G_2$ could be transformed into products at 17.6 μg/ml of enzyme in 120 min using soluble starch as donor substrate. For applications requiring pure compounds like $C_{12}G_7$, synthesis using α-CD is advantageous. However, for applications in which a mixture of elongated alkyl glycosides is needed, reactions employing starch are clearly competitive.

1. Introduction

The alkyl glycosides are biodegradable, non ionic detergents and find application in dishwashing, laundry detergents, cosmetics and cleaning products. Dermatologically, they represent a class of very mild surfactants and depending on the type of product, are suitable for use as sole surfactant or co-surfactant in the formulation of particularly gentle products. Dodecyl glycosides have been shown to be an effective booster for antibacterial agents like chlorohexidine (Von Rybinsky & Hill, 1998). The commercially available alkyl glycosides fall under two groups – alkyl polyglycosides (APGs) and anomerically pure alkyl glycosides, both of which are synthesized chemically.

The synthesis of pure alkyl glycosides with different degrees of polymerization (DP) by chemical means is difficult, tedious and involves multistage processes including the protection and deprotection steps, while enzymatic synthesis is relatively facile and ecofriendly. Acid catalyzed synthesis requires higher temperature, is more energy demanding and generally results in a mixture of isomers and generates secondary products like glucose polymers, ethers and colored impurities (Lai & Shafizadeh, 1974) while enzymes are found to produce anomerically pure alkyl glycosides in a single step (van Rantwijk, Woudenberg van Oosterom, & Sheldon, 1999). It is extremely difficult to chemically synthesize APGs with both long carbohydrate chain and long alkyl chain and APGs only up to maltotriosides are available in the market.

Cyclodextrin glucanotransferase (CGTase) from *B. macerans* belonging to family 13 of glycoside hydrolases catalyzes mainly three kinds of reactions: cyclization or intramolecular transglycosylation reaction, which involve the formation of α-(1,4) linked cyclomaltodextrins, mainly comprising of six D-glucose units (α-cyclodextrin, α-CD) and to a lesser extent cyclomaltodextrins with seven or eight glucose units from starch (French, 1957), intermolecular transglycosylation reactions which involve both coupling reactions primarily between α-CD and various carbohydrate acceptors (French et al., 1954) and disproportionation reactions that involve the transfer of a linear oligosaccharide chain to another linear oligosaccharide molecule that originate from coupling reactions to give rise to a series of sugar molecules of different sizes and hydrolytic reactions when water acts as an acceptor. CGTases form a variety of oligosaccharide products by an α-retaining double displacement mechanism which proceeds in two steps (Mc Carter & Withers, 1994). In the first step CGTase cleaves an α-(1, 4) glycosidic bond in its donor substrate and forms a covalent β-(1, 4) linked glycosyl-enzyme intermediate. In the second stage, the covalent bond of the intermediate is cleaved and an α-(1, 4) glycosidic bond is reformed with an acceptor such as the hydroxyl group at position 4 of another sugar residue. Svensson et al. has reported the synthesis of long carbohydrate chain alkyl glycosides by CGTase using α-CD as donor (Svensson, Ulvenlund, & Adlercreutz, 2009a, 2009b).

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The present study reports the optimization of the *B. macerans* CGTase catalyzed synthesis of alkylpolyglycosides containing dodecyl as the aglycone using starch or α-CD as the donor molecule.

2. Materials and methods

2.1. Materials

CGTase from *B. macerans* (EC 2.4.1.19) was obtained from Amano Enzyme Europe Ltd. (Oxfordshire, U.K.). α-CD and soluble starch were obtained from Sigma Chemical Co. (MO, USA). Dodecyl β-D-maltoside ($C_{12}G_2$) and dodecyl β-D-glucoside ($C_{12}G_1$) were purchased from Anatrace Inc. (OH, USA).

2.2. Methods

2.2.1. Transglycosylation reactions of $C_{12}G_1$ with α-CD as donor substrate

Reactions were carried out to investigate the transglycosylation ability of CGTase from *B. macerans* using dodecyl glucoside as acceptor substrate, to optimize primary coupling product formation and achieve high conversion of the acceptor substrate. The reactions were performed in 0.02 M citrate phosphate buffer (pH 6.0 unless otherwise stated) containing 5 mM $CaCl_2$ in a thermoshaker (Heiz Thermomixer HTMR-131, Bovenden, Germany) at 400 rpm.

The transglycosylation reactions employing α-CD as donor and $C_{12}G_1$ as acceptor were performed at 60 °C in a total volume of 1 ml containing α-CD (160 mM) and $C_{12}G_1$ (20 mM) at pH 6. Reactions were started by adding CGTase (protein concentration of 4.4 μg/ml) and samples were withdrawn at definite time intervals, inactivated by heat treatment in boiling water for 5 min and analysed by HPLC.

To study the effect of relative substrate concentrations on coupling, reactions were carried out at different donor (α-CD) to acceptor ($C_{12}G_1$) molar ratios of 2:1, 1:1, 0.5:1 and 0.25:1 at pH 6.0 and 60 °C for 20 min.

2.2.2. Optimisation of transglycosylation reactions with starch as donor

As α-CD is an expensive donor substrate, attempts were made to substitute α-CD with starch, a cheaper raw material. The reactions were carried out with soluble starch (10%, w/v) as donor, $C_{12}G_1$ or $C_{12}G_2$ (20 mM) as acceptor and CGTase (protein concentrations varying between 4 and 132 μg/ml) at pH 6 and 70 °C in a total reaction volume of 500 μl. Samples were withdrawn at regular intervals, heat inactivated and centrifuged at 14,500 rpm for 5 min before HPLC analyses.

2.2.3. High performance liquid chromatography

HPLC analysis was performed using a Merck-Hitachi Lachrom System comprising of L-7100 pump, L-7250 autosampler, online vacuum degassing system and evaporative light scattering detector (Alltech 500, Alltech Associates Inc., Deerfield, USA). A gas (air) flow rate of 2.67 SLPM (standard liters per minute) and temperature of 99 °C was employed in the detector. A C-18 column (4.6 mm × 250 mm, 3.5 μm, 100 Å; Kromasil, Akzo Nobel, Sweden) was used for the separation of the transglycosylation reaction products. The mobile phase employed was a binary system of acetonitrile (A) and milli Q water (B) at a flow rate of 1 ml/min. The gradient program developed for the separation of the coupling product is as follows: 35% A and 65% B at 0 min and increasing to 60% A in 23 min, held at 60% A for 1 min and back to 35% A in 3 min and equilibrated at the initial conditions for another 3 min. For the separation of the complex transglycosylation product mixture, the program was modified as follows. 35% A and 65% B in the beginning and increasing to 46% A in 20 min, and then to 67% A in another 6 min, holding at 67% A for 3 min, and then back to 35% in 2 min and equilibrating at 35% A for another 4 min prior to the next injection.

2.2.4. Isolation and purification of the primary coupling products

For preparative scale synthesis of the coupling product $C_{12}G_7$, transglycosylation reactions were performed in 10 ml reaction volume containing CGTase (88 μg/ml), 160 mM α-CD and 20 mM $C_{12}G_1$ at 60 °C and pH 4.6 for 20 min and inactivated by heat treatment in a boiling water bath for 5 min. For preparative synthesis of $C_{12}G_8$, reactions were performed similarly but with 20 mM $C_{12}G_2$ at pH 4 for 40 min. The primary coupling products, $C_{12}G_7$ and $C_{12}G_8$ formed in the respective CGTase catalyzed reactions were purified by flash chromatography using reverse phase bonded silica as adsorbent (45–75 μm) in step wise elution mode. The carbohydrates were eluted using 20:80 methanol–water and the primary coupling products were eluted in 65:35 methanol–water. The solvent was removed using a rotary evaporator and the products were dried in a vacuum dessicator. The calibration curves of the purified components presumed to be $C_{12}G_7$ and $C_{12}G_8$ were plotted for quantitative HPLC analyses.

2.2.5. LC–MS analyses

The transglycosylated product mixture was identified on a Perkin Elmer LC–MS System equipped with two series 200 micro pumps, autosampler and an API quadrupole TOF mass spectrometer (Q Star Pulsar i; AB Sciex Instruments) equipped with electrospray ionization (ESI).

The separation conditions were the same as that used for LC analysis of the product mixture. The electrospray mass data of the LC separated samples were acquired in the positive mode with a spray voltage of 5100 V. The dry gas temperature used was 250 °C and collision energy used was 20 eV. The scan range of the MS was from 300 to 3000 amu. The tandem mass spectra of the primary coupling products $C_{12}G_7$ and $C_{12}G_8$ were acquired by direct injection through syringe at a flow rate of 10 μl/min and the data were processed with Sciex Analyst Software QS 1.1 (Applied Biosystems, CA, USA).

3. Results and discusssion

$C_{12}G_1$ has a critical micelle concentration (CMC) of ~0.19 mM (Helenius, McCaslin, Fries, & Tanford, 1979) and a solubility of ≥0.008% in water at 20 °C. $C_{12}G_2$ on the other hand has a CMC of ~0.17 mM and a solubility of ≥20% at 0–5 °C (Van Aken, Foxall-Van Aken, Castleman, & Ferguson-Miller, 1986). α-CD is a cyclomaltohexaose molecule assuming cylindrical shape in aqueous solutions with a hydrophilic outer surface and hydrophobic internal cavity (Szejtli, 1989). No product other than α-CD and acceptor molecule was observed in control reactions performed without the addition of enzyme. Preliminary studies conducted earlier in our lab with *B. macerans* CGTase have shown the α-CD to be an efficient donor with high coupling activity compared to β- and γ-CDs. Increasing the degree of head group polymerization from one to many glucose units enhances considerably the solubility of the surfactant in water. For certain applications in the pharmaceutical industry, highly specific alkyl glucosides with long hydrophobic chain and long oligomeric head groups are desirable as cell toxicity studies with nonionic surfactants have shown the epithelial toxicity to decrease with increasing hydrophilic chain length (Ekelund et al., 2005). The purity of the primary coupling products obtained after purification by flash chromatography was determined to be 95–96% for $C_{12}G_7$ and $C_{12}G_8$ by HPLC analyses. (Purity was determined as the percentage of the area of the pure compound in the HPLC chromatogram, relative to the sum of the area of all the components in the HPLC chromatogram.)

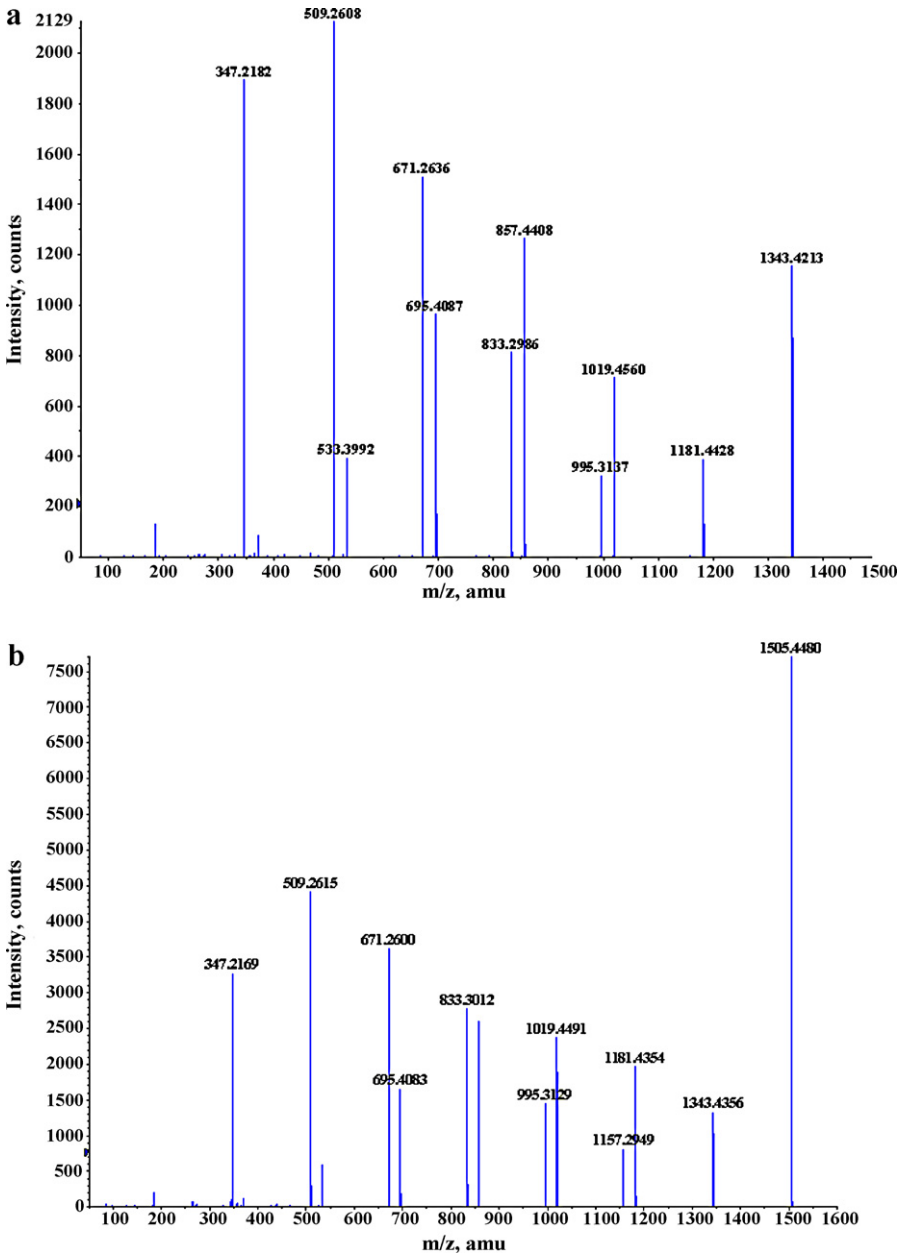

Fig. 1. (a) Tandem mass spectra of the sodium adduct of the primary coupling product, $C_{12}G_7$; (b) tandem mass spectra of the sodium adduct of the primary coupling product, $C_{12}G_8$.

3.1. Mass spectral analyses

ESI produced mainly the molecular ions $[M-H]^+$ and sodium adducts of the molecular ion. The complex mixture obtained by the reaction between soluble starch and dodecyl maltoside displayed $[M-H]^+$ ions corresponding to $C_{12}G_{13}$ (2293.9), $C_{12}G_{11}$ (2131.9), $C_{12}G_{10}$ (1807.8), $C_{12}G_9$ (1645.7), $C_{12}G_8$ (1483.6), $C_{12}G_7$ (1321.6), $C_{12}G_6$ (1159.5), $C_{12}G_5$ (997.5), $C_{12}G_4$ (835.4), $C_{12}G_3$ (673.3), $C_{12}G_2$ (511.3) and $C_{12}G_1$ (349.2).

The primary coupling product $C_{12}G_7$ gave base peak at 1343.4 (calculated, 1343.55) (Fig. 1a) corresponding to its $[M+Na]^+$ adduct with fragment ions at 1181.4 $[M+Na]^+$ of $C_{12}G_6$, 1019.4 $[M+Na]^+$ of $C_{12}G_5$, 857.4 $[M+Na]^+$ of $C_{12}G_4$, 695.4 $[M+Na]^+$ of $C_{12}G_3$, 533.3 $[M+Na]^+$ of $C_{12}G_2$ while $C_{12}G_8$ showed *m/z* ions corresponding to $[M+Na]^+$ at 1505.4 (calculated, 1505.61) (Fig. 1b) with fragment ions at 1343.4 $[M+Na]^+$ of $C_{12}G_7$, 1181.4, 1157.2, 1019.4, 995.3, 857.3, 833.3, 695.4, 671.2, 533.1, 509.2 and 347.2. The data from the tandem mass spectral analysis thus show that the isolated products are the expected primary coupling products $C_{12}G_7$ and $C_{12}G_8$ formed from α-CD and dodecyl-β-D-glucoside or dodecyl-β-D-maltoside, respectively.

3.2. Transglycosylation studies with α-CD as donor

CGTase from *B. circulans* which produces β-CD (7 glucose units) have been shown to possess a catalytic domain A with 9 subsites (+2 to −7) in the substrate binding groove. The +1 and +2 subsites have been shown to be involved in acceptor binding and −1 to −7 in binding the donor substrate. The non reducing end of the donor substrate always faces the negative subsite and the glycosidic bond is cleaved between +1 and −1 (Strokopytov et al., 1996). The number and composition of the subsites vary with the source of CGTase.

Van der Veen et al. have shown that the three transglycosylation reactions of CGTase differ in their kinetic mechanisms. According to them disproportionation proceeds by ping–pong mechanism, where in the cleaved off part of the donor substrate which occupies the subsites +1 and +2 must dissociate from the active site of the enzyme before the acceptor substrate can bind to the acceptor subsites. Coupling reactions have been shown to proceed by a random ternary complex mechanism and cyclization reactions with short amylose chains have been shown to follow Michaelis–Menten kinetics (Van der Veen, van Alebeek, Uitdehaag, Dijkstra, & Dijkhuizen, 2000).

The time profile studies performed with α-CD and $C_{12}G_1$, showed that the maximum primary coupling product ($C_{12}G_7$) formation occurred at 20 min with a molar yield of 11.7% based on the amount of $C_{12}G_1$ supplied (Fig. 2). The reaction rate was 63 μmol $C_{12}G_7$/min/mg protein at pH 6 and 60 °C. The coupling reaction catalyzed by CGTase might involve the binding of the donor substrate, α-CD and the acceptor substrate $C_{12}G_1$ simultaneously and in a random order to the active site cleft of CGTase as reported by van der Veen et al. The acceptor then makes a nucleophilic attack on the C1 of the maltohexaose unit with its C4 end, giving rise to α-(1-4) linkage and result in the concomitant release of $C_{12}G_7$. As the reaction proceeds, $C_{12}G_7$ (Fig. 3a) couples with another molecule of α-CD to form the secondary coupling product $C_{12}G_{13}$ (Fig. 3b). The coupling reaction of CGTase has the advantage of adding six glucose residues in a single step and is therefore advantageous over other enzymatic reactions where a single glucose residue is added in each step by the action of dextransucrase or alternansucrase (Richard, Morel, Willemot, Monsan, & Remaud-Simeon, 2003). Other product peaks in the chromatogram in the later stages correspond to dodecyl glycosides, which result

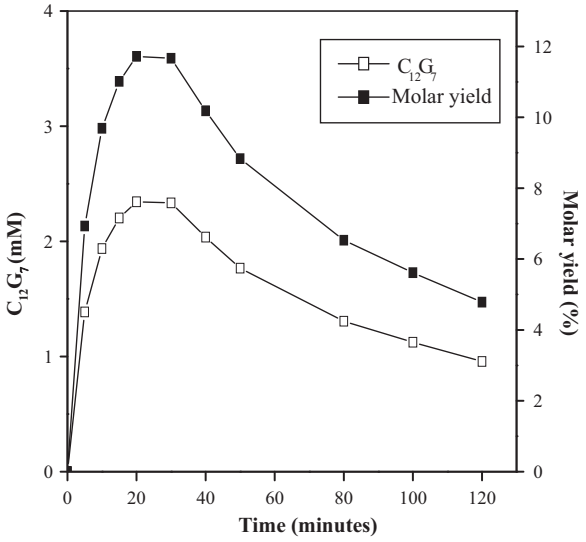

Fig. 2. Time course of $C_{12}G_7$ formation. (*Reaction conditions*: α-CD, 160 mM; $C_{12}G_1$, 20 mM; pH 6.0; CGTase, 04.4 μg/ml; and temperature, 60 °C.)

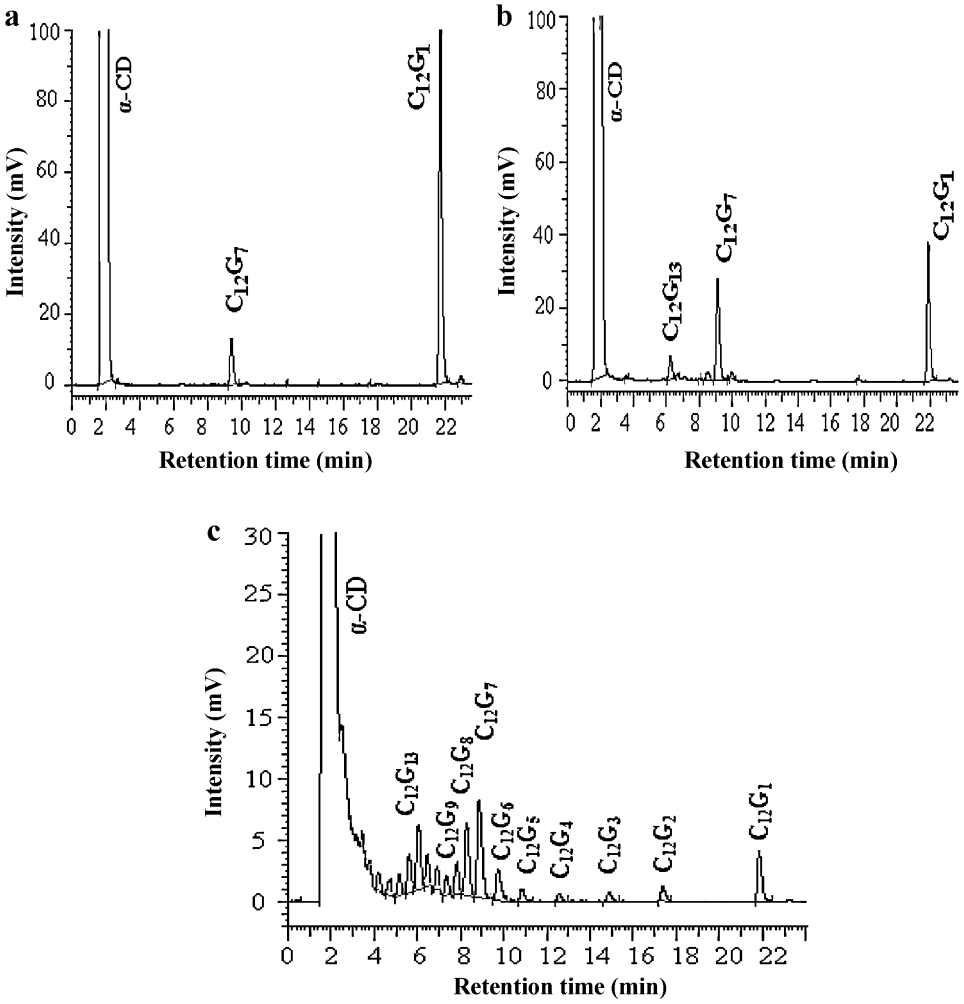

Fig. 3. HPLC chromatograms displaying the order of product formation at different time intervals. (a) 5 min, (b) 20 min, (c) 60 min. (*Reaction conditions*: α-CD, 160 mM; $C_{12}G_1$, 20 mM; pH 6.0; CGTase, 4.4 μg/ml, and temperature, 60 °C.)

Table 1
Influence of donor to acceptor ratio on transglycosylation reactions (*reaction conditions*: α-CD, 5–40 mM; $C_{12}G_1$, 20 mM; pH 6.0; CGTase, 88 μg/ml; and temperature, 60 °C). The results are expressed as area percentage.

C_nG_n	Donor to acceptor ratio			
	2:1	1:1	0.5:1	0.25:1
$C_{12}G_1$	41.4 ± 1.73	56.3 ± 0.41	70.1 ± 0.04	72.7 ± 0.47
$C_{12}G_2$	24.2 ± 0.40	23.6 ± 0.11	17.6 ± 0.11	16.9 ± 0.17
$C_{12}G_3$	12.5 ± 0.41	9.8 ± 0.11	6.8 ± 0.06	6.1 ± 0.07
$C_{12}G_4$	8.1 ± 0.31	5.2 ± 0.06	3.2 ± 0.00	2.5 ± 0.01
$C_{12}G_5$	6.0 ± 0.15	2.6 ± 0.11	1.3 ± 0.01	0.89 ± 0.00
$C_{12}G_6$	3.7 ± 0.13	1.4 ± 0.05	0.58 ± 0.00	0.61 ± 0.36
$C_{12}G_7$	2.2 ± 0.00	0.68 ± 0.05	0.29 ± 0.02	0.19 ± 0.02
$C_{12}G_8$	1.0 ± 0.00	0.32 ± 0.00		
$C_{12}G_9$	0.25 ± 0.00			
$C_{12}G_{10}$	0.37 ± 0.00			

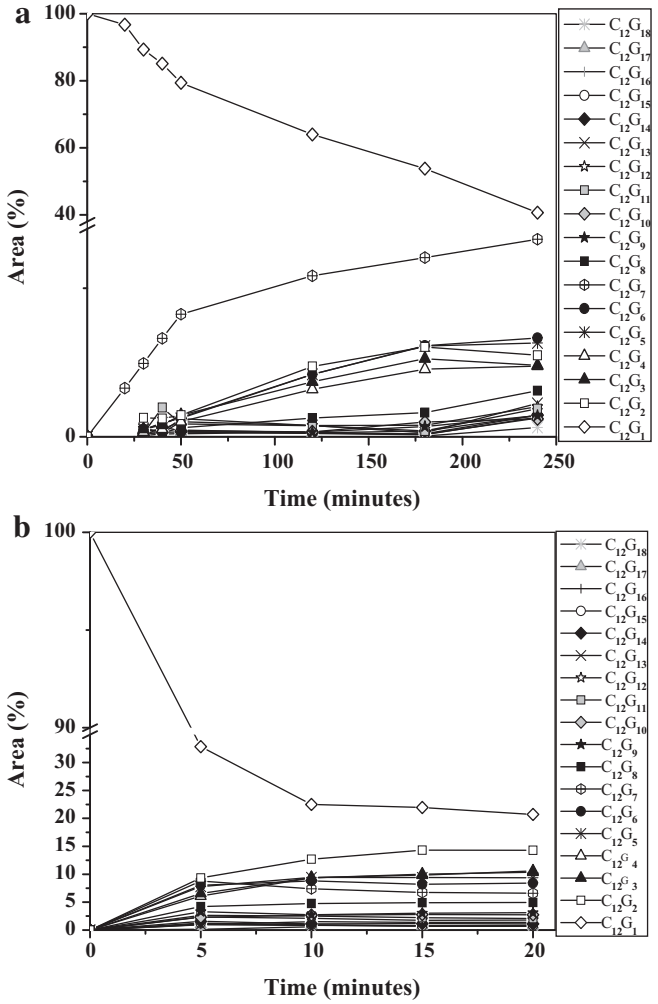

Fig. 4. (a) Influence of enzyme concentration and time on dodecyl glycoside formation (*reaction conditions*: soluble starch, 10% (w/v); $C_{12}G_1$, 20 mM; pH 6; CGTase, 8.8 μg/ml; and temperature, 70 °C); (b) CGTase, 132 μg/ml.

from disproportionation reactions (Fig. 3c). French et al. (1954) had shown that the coupling reactions between α-CD and radioglucose or sucrose proceed through a two stage process, the first stage involving the formation of an intermediate (primary coupling product) having a degree of polymerization (DP) (acceptor) + 6 and a second stage wherein the primary coupling product disproportionate to yield a broad distribution of chain lengths. Our studies have

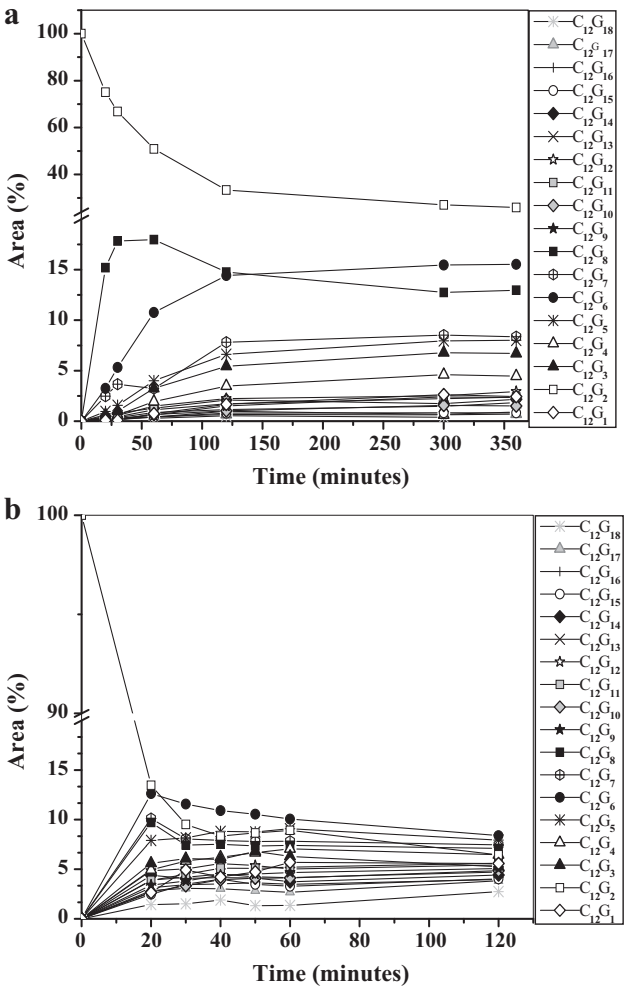

Fig. 5. (a) Influence of enzyme concentration and time on dodecyl glycoside formation (*reaction conditions*: soluble starch, 10% (w/v); $C_{12}G_2$, 20 mM; pH 6; CGTase, 4.4 μg/ml; and temperature, 70 °C); (b) CGTase, 17.6 μg/ml.

also confirmed the formation of the primary coupling product to be the first stage in the CGTase catalyzed reaction. With the declining levels of $C_{12}G_1$ and increasing concentration of $C_{12}G_7$, there is competition between α-CD and $C_{12}G_7$ which results in $C_{12}G_7$ being used as a donor substrate and starts to disproportionate giving rise to a wide range of alkyl glycosides with varying number of glucose residues. If $C_{12}G_7$ functions as donor and $C_{12}G_1$ as acceptor, the possible combinations of disproportionation products are $C_{12}G_6$–$C_{12}G_2$, $C_{12}G_5$–$C_{12}G_3$ and $C_{12}G_4$–$C_{12}G_4$. However, in reactions performed with α-CD and $C_{12}G_1$, in the late stages of coupling and early stages of disproportionation, $C_{12}G_8$ was one of the predominant products which possibly could have been generated from the disproportionation occurring when $C_{12}G_7$ functions as both acceptor and donor. The possible pairs with $C_{12}G_7$ as acceptor and donor are $C_{12}G_6$–$C_{12}G_8$, $C_{12}G_5$–$C_{12}G_9$ and $C_{12}G_4$–$C_{12}G_{10}$. From the results obtained at 60 min, $C_{12}G_8$ and $C_{12}G_6$ seems to be prominent among the disproportionation products (Fig. 3c; areas are related to the mass of the product). The seconday coupling product $C_{12}G_{13}$ can also function as an acceptor molecule forming a tertiary coupling product $C_{12}G_{19}$. Thus it can be seen, that it is possible to direct the synthesis of alkyl polyglycosides to a definite product of interest by kinetically controlling the reaction and stopping the reaction at the point of interest.

Studies performed at different donor (α-CD) to acceptor ($C_{12}G_1$) ratios showed that the products ranged from $C_{12}G_2$ to $C_{12}G_{10}$ and

Table 2
Product composition (area%) when maximal concentration of primary coupling product was achieved for different acceptor–donor combinations.

	G1	G2	G6	G7	G8	Sum G1–G2	Sum G3–G18
α-CD + $C_{12}G_1$[a]	52.2	0.38	1.10	37.5	2.44	52.5	47.4
α-CD + $C_{12}G_2$[b]	–	23.3	0.99	2.27	64.7	23.3	76.6
Starch + $C_{12}G_1$[c]	63.9	4.73	4.19	10.8	1.26	68.6	31.2
Starch + $C_{12}G_2$[d]	0.34	41.1	10.0	6.6	17.1	41.4	55.9

[a] *Reaction conditions*: α-CD, 160 mM; $C_{12}G_1$, 20 mM; pH 4.6; CGTase, 88 μg/ml; temperature, 60 °C; and time, 20 min.
[b] *Reaction conditions*: α-CD, 160 mM, $C_{12}G_2$, 20 mM; pH 4.6; CGTase, 88 μg/ml; temperature, 60 °C; and time, 20 min.
[c] *Reaction conditions*: Soluble starch, 10% (w/v); $C_{12}G_1$, 20mM; pH 6; CGTase, 8.8 μg/ml; temperature, 70 °C; and time, 120 min.
[d] *Reaction conditions*: Soluble starch, 10% (w/v); $C_{12}G_2$, 20 mM; pH 6; CGTase, 4 μg/ml; temperature, 70 °C; and time, 40 min.

the coupling product formation was very low at donor to acceptor ratio of 0.25:1 (Table 1). Higher α-CD content was necessary to enhance the formation of the coupling product.

3.3. Transglycosylation studies with starch as donor

In transglycosylation studies conducted with starch as glycosyl donor, $C_{12}G_1$ as acceptor and 4.4 μg/ml of CGTase at pH 6 and 70 °C, a reaction rate of 1.95 μmoles of $C_{12}G_7$/min/mg protein was obtained. Nearly 95% of the acceptor molecule remained, even at 240 min. When the enzyme concentration was raised to 8.8 μg/ml, nearly 60% of the acceptor substrate was converted into coupling and disproportionation products in 240 min (Fig. 4a). The maximum molar yield of $C_{12}G_7$ obtained was 6.3%. This is probably due to the comparatively low levels of α-CD formed in the starch reaction mixtures. The maltooligosaccharides resulting from starch breakdown can function as both acceptor and donor substrates, reducing the rate of $C_{12}G_7$ formation. Moreover, when acceptors like glucose or maltose are present in the mixture, they inhibit the formation of cyclodextrins as the glycosyl donor starch is deviated towards the acceptor reaction. However glucose as an acceptor has been shown to have acceleration effects on starch degradation activity (Kitahata, Okada, & Fukui, 1978) and in the presence of good acceptors, the reaction of water with CGTase is much less favorable and hydrolysis reaction is very minor. Unlike the transglycosylation reactions employing α-CD as donor, where $C_{12}G_8$ was a prominent disproportionation product, the lower glycosides $C_{12}G_2$, $C_{12}G_3$, $C_{12}G_4$, $C_{12}G_5$ and $C_{12}G_6$ were found to be more prominent when starch is used as donor. Though CGTase from *B. macerans* is a major α-CD producer, it produces low amounts of β- and γ-CD also, which can possibly undergo coupling to give rise to $C_{12}G_8$ and $C_{12}G_9$ but only in low amounts as they are very poor donor molecules in comparison to α-CD (Yoon & Robyt, 2002). However when maltooligosaccharides function as donors and $C_{12}G_1$ as acceptor they give rise to dodecyl glycosides mainly by disproportionation. At 132 μg/ml of CGTase, nearly 78% of the acceptor was transformed into coupling and disproportionation products in 60 min and the molar yield of $C_{12}G_7$ was 2.95% at 5 min reaction time due to extensive disproportionation (Fig. 4b).

The basic requirement for good acceptor performance with CGTase is the presence of a D- or L-glucopyranoside structure with equatorial unsubstituted hydroxyl groups at C-2, C-3 and C-4 (Rendleman, 1996). The K_{cat}/K_m value for maltose has been reported to be 20 times larger than for D-glucose, indicating that the acceptor binding site of CGTase can recognize at least two glucopyranose moieties (Nakamura, Haga, & Yamane, 1994). Maltose is a better acceptor substrate for CGTase probably because it can occupy the two acceptor binding subsites +1 and +2 more strongly than glucose. Reactions done with $C_{12}G_2$ as acceptor and 4.4 μg/ml of CGTase at pH 6 and 70 °C gave a reaction rate of 4.5 μmoles of $C_{12}G_8$/min/mg protein and a maximum molar yield of 4.8% (based on the amount of $C_{12}G_2$ supplied) for $C_{12}G_8$ in 20 min supporting the fact that $C_{12}G_2$ is a better substrate for CGTase than $C_{12}G_1$. The disproportionation products were observed in the reaction mixture even at 20 min unlike in the reaction with $C_{12}G_1$, where the amount of disproportionation products formed were comparably low showing that the reaction rate was much faster with $C_{12}G_2$. Among the disproportionation products, $C_{12}G_6$ was very prominent. Other major products were $C_{12}G_3$, $C_{12}G_4$, $C_{12}G_5$, $C_{12}G_7$, $C_{12}G_{11}$, $C_{12}G_{12}$ and $C_{12}G_{14}$. Nearly 73% of the acceptor was transformed in to products at 300 min (Fig. 5a) compared to $C_{12}G_1$ where only 5% conversion of the acceptor was observed at similar enzyme concentration and time. At lower enzyme concentration (4 μg/ml) the maximum molar yield of $C_{12}G_8$ obtained was 5.0% at 40 min. At 17.6 μg/ml, nearly 93.6% of the acceptor substrate $C_{12}G_2$ was transformed into products at 120 min (Fig. 5b).

Attempts made to substitute α-CD, an expensive donor substrate with starch, a cheap and abundant natural resource and capable of producing α-CD by the cyclization activity of CGTase have been successful, however the molar yields of the coupling products are lower in comparison to reactions employing pure α-CD as donor substrate. One possibility to enhance coupling is to increase the α-CD production from starch without product inhibition. A thermostable CGTase with high coupling activity is an alternate choice and the substrates also possess better solubility at higher temperatures. Under the present conditions of CGTase reaction, the maximum molar conversion observed was 6.3 and 5.0%, respectively, for $C_{12}G_7$ and $C_{12}G_8$ with soluble starch as donor substrate. A mixture of dodecyl glycosides with DP of 2–18 can be synthesized using starch as donor and this complex mixture of surface active compounds can be effectively used to lower the surface tension of aqueous solutions. Table 2 shows the production of the different transglycosylated products with different combinations of donor and acceptor substrate. CGTase is able to attack the starch directly under the reaction conditions employed, thus avoiding the use of α-amylase pretreatment for the liquefaction of starch, which facilitates a one pot synthesis of dodecyl glycosides using a single enzyme. Synthesis using α-CD is advantageous for producing pure compounds like $C_{12}G_7$ or $C_{12}G_8$. However, for applications in which all elongated alkyl glycosides (such as $C_{12}G_3$ and longer) are needed, reactions involving starch as glycosyl donor are clearly competitive (Table 2).

4. Conclusion

CGTase from *B. macerans* is able to utilise $C_{12}G_1$ and $C_{12}G_2$ as acceptor molecules and starch as donor substrate to give rise to a mixture of dodecyl glucooligosides. The yields of the coupling products obtained with starch are lower than that obtained with α-CD. Application of soluble starch and CGTase will provide significant process advantages with regard to economy and in reducing undesirable by-product formation. The commercially available APGs consist mainly of glucosides (C_nG_1), which limits their application while the present process of enzymatic synthesis give rise to a mixture of glycosides varying from $C_{12}G_1$ to $C_{12}G_{18}$.

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