J Incl Phenom Macrocycl Chem (2011) 70:359–368
DOI 10.1007/s10847-010-9888-z

ORIGINAL ARTICLE

Synthesis of alkyl glycosides from cyclodextrin using cyclodextrin glycosyltransferase from *Paenibacillus* sp. RB01

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Received: 15 June 2010 / Accepted: 10 October 2010 / Published online: 26 October 2010

Abstract Alkyl glycosides have potential use as biodegradable detergents due to their high surface activity with low toxicity. Recent progress in the application of enzymes to the preparation of these surface-active compounds demonstrates the advantages to the chemical synthesis. In this work, alkyl glycosides were, for the first time, synthesized from cyclodextrin (CD) and various soluble alcohols by transglycosylation reaction using cyclodextrin glycosyltransferase (CGTase) from *Paenibacillus* sp. RB01. Several alcohols (methanol, ethanol, 1-propanol, 2-propanol, 1-butanol and 2-butanol) as glycosyl-acceptor substrates were evaluated. It was found that the reaction products which were analyzed by TLC were maximum for 30% methanol, 20–30% ethanol, 10–20% 1-propanol, 10% 2-propanol, 8% 1-butanol and 5–10% 2-butanol. In addition, the increase in the yield of alkyl glycoside formation was achieved by using methanol as an acceptor. Optimal reaction conditions for methyl glycoside synthesis from CD were to incubate 1.2% (w/v) β-CD and 240 U/mL of CGTase in a water/methanol system containing 30% (v/v) methanol, pH 6.0 and a temperature of 40 °C. At least three main methyl glycoside products were formed having 1–3 monosaccharide units attached to methanol which were in accordance with the results of MS analysis.

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Keywords Alcohol · Alkyl glycoside · Cyclodextrin glycosyltransferase · *Paenibacillus* sp · Transglycosylation

Introduction

Alkyl glycosides are amphipathic molecules with hydrophobic and hydrophilic moieties which confer several abilities on surfactants. Especially, because they produce from sugars and fatty alcohols, they are rapidly biodegradable and nontoxic. Increased environmental awareness among consumers has prompted more serious consideration of biological surfactants as possible alternatives to existing products. Therefore, alkyl glycosides will undoubtedly constitute an important part not only in industrial chemicals widely used in almost every sector of various industries but also in household uses [1]. Although it has been shown that their chemical synthesis was well-developed, the method still employs toxic and expensive compounds to yield α- and β-configuration products via several steps. However, advances in enzyme technology have led an interest in the development of biological method to synthesize anomerically pure alkyl glycosides under mild conditions by a simple one-step reaction process [2]. Two types of enzymes have been used for the synthesis of alkyl glycosides, the glycosyl transferases (E.C. 2.4) and the glycohydrolases (E.C. 3.2). Most reports on enzymatic synthesis of alkyl glycosides have used various glycohydrolases including, β-glucosidase [3, 4], α-rhamnosidase [5] and β-galactosidase [6]. On the other hand, although glycosyl transferase enzymes have widely been studied for the synthesis of oligosaccharide by transfer reactions between donor and various kinds of acceptors [7–9], for alkyl glycoside synthesis, they have been explored to a minor extent. Only levansucrase has

been reported to synthesize methyl β-D-fructoside from sucrose and methanol [10].

Cyclodextrin glycosyltransferase (CGTase, E.C. 2.4.1.19) constitutes one of the most important carbohydrases for the industrial production of starch derivatives. CGTase produces cyclodextrins (CDs, circular α-(1, 4)-linked oligosaccharides) from starch with different sizes via cyclization [11]. They are used as stabilizers or solubilizers in food, pharmaceutical and cosmetic industries for their ability to form inclusion complexes with small hydrophobic molecules [12]. Furthermore, CGTase also presents a disproportionation reaction by transferring linear oligosaccharides to another oligosaccharide molecule, and a coupling reaction by opening the rings of CDs and transferring to acceptors [11]. The CGTase acceptors naturally are carbohydrates in the form of saccharides and glycosides from nature [13–15] or other compounds containing carbohydrate moiety [16–18]. These reactions proceed via a covalent glycosyl-enzyme intermediate [19]. The non-reducing end of a free oligosaccharide is subsequently used as an acceptor that cleaves the covalent enzyme-substrate bond, and a product is released. Thus, from this mechanism, CGTase also catalyzes transglycosylation to various compounds other than saccharides such as vitamin C [20–23] and (+) catechin [24] for the modification and development of their new functional properties. Water as well as acceptors added to the reaction medium can also be used by CGTase, which results in hydrolysis [11]. In general, the hydrolysis activity of CGTase is much lower than the transglycosylation activity [25].

The use of CGTase for alkyl glycoside synthesis is of great interest due to the fact that it can synthesize a glycoside product containing more than one monosaccharide residue whereas the transfer reaction catalyzed by glycohydrolases can only transfer one monosaccharide unit. Although CGTase has previously been used for alkyl glycoside synthesis, the coupling reaction was used to elongate the carbohydrate part of shorter alkyl glycosides [17, 18]. So far, there have been no reports on the use of alcohol as a glycosyl acceptor. In this work, we demonstrated the formation of alkyl glycosides from β-CD and alcohols by the transglycosylation action of CGTase from *Paenibacillus* sp. RB01. The most efficient alcohol acceptor was chosen and the glycoside products were identified. Optimized reaction conditions for maximum glycoside production were also investigated.

Experimental

Materials

β-CD and methyl α-D-glucopyranoside was obtained from Sigma-Aldrich Chemical Co., USA. Glucoamylase (amyloglucosidase) from *Aspergillus niger* was purchased from Fluka, Switzerland. Soluble starch (synthesis grade from potato) was from Scharlau Chemie S.A., Spain. Silica gel 60 F_{254} aluminium sheets (0.2 mm), methanol and ethanol were obtained from Merck, Germany. 1-Propanol, 2-propanol and 1-butanol were from Carlo Erba Reagents, Italy and 2-butanol was purchased from BDH Chemical, England. Other chemicals were of analytical grade.

Bacteria cultivation and enzyme production

Paenibacillus sp. RB01 isolated from hot spring area in Ratchaburi province in Thailand was characterized as thermotolerant bacteria with CGTase activity by our research group [26]. The bacteria were cultured as starter in Medium I containing 0.5% (w/v) beef extract, 1.0% (w/v) peptone, 0.2% (w/v) NaCl, 0.2% (w/v) yeast extract and 1.0% (w/v) soluble starch pH 7.2 at 37 °C until the A_{660} reached 0.3–0.5. Starter inoculum of *Paenibacillus* sp. RB01 (1.0%) was then transferred and cultured at 40 °C for 72 h in Horikoshi's medium pH 10.1 [27] containing 1.0% (w/v) soluble starch, 0.5% (w/v) peptone, 0.5% (w/v) yeast extract, 0.1%(w/v) K_2HPO_4, 0.02% (w/v) $MgSO_4{\cdot}7H_2O$ and 0.75% (w/v) Na_2CO_3. The crude enzymes were harvested by centrifugation at 6,500×*g* for 15 min at 4 °C and subjected to purification by starch adsorption [28] with slight modification [26]. Corn starch was oven dried at 120 °C for 30 min and cooled down to room temperature. It was then gradually sprinkled into stirring crude CGTase from the previous step to make 5% (w/v) concentration. After 3 h of continuous stirring, the starch cake was collected by centrifugation at 8,000×*g* for 30 min and washed twice with 10 mM Tris–HCl pH 8.5 containing 10 mM $CaCl_2$ (TB1). CGTase was eluted from the starch cake with 0.2 M maltose in TB1 (2 × 125 mL for starting broth of 1 L) with stirring for 30 min. The eluate was collected by centrifugation at 10,000 g for 30 min. The solution was dialyzed against three changes of distilled water at 4 °C.

Polyacrylamide gel electrophoresis and assay for protein

Native polyacrylamide gel electrophoresis (Native-PAGE) was used to identify CGTase in crude and adsorbed samples by running the samples on 7.5% polyacrylamide gel, and stained for starch degrading (dextrinizing) activity [29]. After electrophoresis, the gel was soaked in 10 mL of 2.0% (w/v) soluble starch in 0.2 M phosphate buffer, pH 6.0, at 40 °C for 10 min. It was then quickly rinsed several times with distilled water and immersed in 10 mL of I_2 staining reagent (0.2% (w/v) I_2 in 2.0% (w/v) KI) and left for color development at room temperature. The clear zone on the blue background indicates starch-degrading activity.

Protein concentration was determined by the method of Bradford [30] using serum bovine albumin as standard.

Assay for CGTase

CGTase activity was determined by the assay of starch-degrading (dextrinizing) activity [31] and assay of coupling activity [32, 33].

Dextrinizing activity measured the starch hydrolytic activity of CGTase spectrophotometrically by measuring the decrease in absorbance of starch-iodine complex at 600 nm. Enzyme sample (50 μL) was incubated with 0.15 mL starch substrate (0.2% (w/v) soluble starch in 0.2 M phosphate buffer pH 6.0) at 40 °C for 10 min. The reaction was stopped with 2 mL of 0.2 M HCl and 0.25 mL of iodine reagent (0.02% (w/v) I_2 in 0.2% (w/v) KI) was added. The mixture was adjusted to a final volume of 5 mL with distilled water and the absorbance at 600 nm was measured. For control tube, HCl was added before the enzyme sample. One unit of enzyme was defined as the amount of enzyme which produced 10% reduction in the intensity of the blue color of the starch-iodine complex per min under the described condition.

Coupling activity measured the disappearance of β-CD in the reaction mixture by the phenolphthalein method. A 250 μL of β-CD as standard or sample solution (in which CGTase was preincubated with β-CD and alcohol acceptors) was incubated with 750 μL of phenolphthalein solution for 15 min. The decrease in absorbance at 550 nm caused by complexion of the dye with β-CD was measured. Conversion of ΔA_{550} to μ moles β-CD was quantitated from the β-CD phenolphthalein calibration curve. The disappearance of β-CD in the reaction mixture was calculated from the difference between β-CD concentration at 0 and 24 h incubation with CGTase and acceptors. One unit of activity was defined as the amount of enzyme able to couple 1 μmole of β-CD to alcohol per minute [25].

Transglycosylation reactions

To determine the synthesis ability of CGTase and an appropriate alcohol acceptor, transglycosylation reactions with varying alcohol types and concentrations were performed using β-CD as a glycosyl donor. The reaction mixture (0.25 mL) consisted of final 0.6% (w/v) β-CD (1.5 mg), various concentrations and types of alcohols (methanol, ethanol, 1-propanol, 2-propanol: 10–60% (v/v), 1-butanol: 5–8% (v/v), 2-butanol: 5–20% (v/v)) and CGTase (final dextrinizing activity of 200 U/mL) in 50 mM acetate buffer pH 6.0. The reaction mixture was incubated at 40 °C for 24 h. Aliquots were withdrawn and analyzed by TLC. The amount of β-CD disappearance was measured by the phenolphthalein method.

Glucoamylase-catalyzed degradation of reaction products

Glucoamylase was introduced to catalyze the hydrolysis of glycosidic linkages between glucose residues in the reaction mixture. The transglycosylation reaction mixture containing methanol as an acceptor was heated to evaporate the remaining methanol and mixed with a solution of glucoamylase (final activity of 20 U/mL). After incubation at 40 °C for 1 h, glucoamylase was inactivated by heating at 100 °C for 5 min, after which the samples were analyzed by TLC and HPLC.

TLC analysis

Reactions were analyzed by applying 10 μL of samples on silica gel 60 F_{254} aluminium sheets, resolved once in a system composing of ethyl acetate/acetic acid/water (3:1:1 by vol) for the analysis of alkyl glycoside production. To analyze the methyl glycoside products, the TLC plates were developed twice in ethyl acetate/acetic acid/water 3:1.5:0.5 by vol. Chromatograms were visualized by spraying with conc. sulfuric acid–methanol (1:2) followed by heating at 110 °C for 10 min. The intensity of product spots of equal area was quantitated by scanning chromatograms with GeneTools program of SYNGENE, Synoptics Ltd., England.

HPLC analysis

Products in the reaction mixture were analyzed by high performance liquid chromatography (Shimadzu, Japan) connected with Asahipak amino column (4.6 × 250 mm) and a refractive index detector. The adsorbed compounds were eluted with 65% (w/v) acetonitrile in deionized water at a flow rate of 1.0 mL/min. Quantification was based on standard curve of methyl α-D-glucopyranoside.

MS analysis

The molecular mass of major methyl glycoside products was determined by mass spectrometry using a micrOTOF (Bruker Daltonics Inc., USA) at the Biological Service Unit of the National Center of Genetic Engineering and Biotechnology (BIOTECH, Thailand). The compounds were ionized by electrospray ionization on the positive-ion mode.

Results and Discussion

Purification of CGTase

CGTase was partial purified from *Paenibacillus* sp. RB01 using starch adsorption. The specific activity (dextrinizing

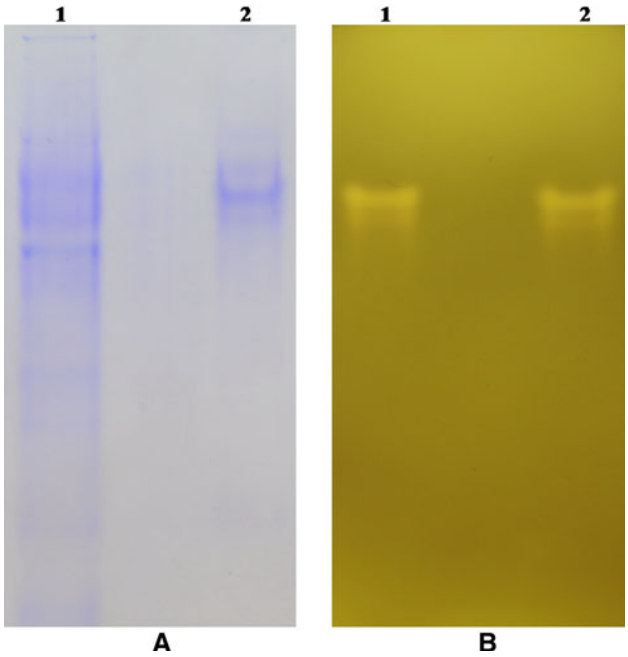

Fig. 1 Non-denaturing PAGE analysis of *Paenibacillus* sp. RB01 CGTase in crude and starch adsorbed samples on a 7.5% acrylamide gel. *Lane 1* crude enzyme, *Lane 2* starch adsorption. Coomassie blue staining (**a**) and dextrinizing activity staining (**b**)

activity) of the enzyme was 8,377 units/mg with 46.3 purification fold and 38% yield. The proteins in crude and starch adsorbed samples were electrophoresed on non-denaturing gel to determine the purity of CGTase. Protein staining revealed that the enzyme was successfully purified through one-step purification since less protein bands were observed (Fig. 1a). The mobilities of the active band as determined by dextrinizing activity staining (Fig. 1b) coincided with that stained with Coomassie brilliant blue (Fig. 1a). Thus, the enzyme obtained was sufficiently pure and was further used in this study.

Transglycosylation of CD to alcohols

Attempts were made to synthesize alkyl glycosides to investigate the capability of CGTase to transglycosylate alkyl alcohols. Different alcohols at which concentrations give a single phase were chosen as glycosyl-acceptor substrate according to previous report when β-glucosidase from Thai rosewood was used to synthesize alkyl glucoside [3]. The reaction was performed at the optimum pH (6.0) of the enzyme for 24 h. Products from transglycosylation of β-CD to 40% (v/v) methanol and ethanol, 10% (v/v) 1- and 2-propanol and 5% (v/v) 1- and 2-butanol were preliminary analyzed by TLC (Fig. 2). In order to confirm that the product observed as a spot on TLC was transglycosylation product by the action of CGTase, the reaction mixture without alcohol acceptors and reaction mixture containing

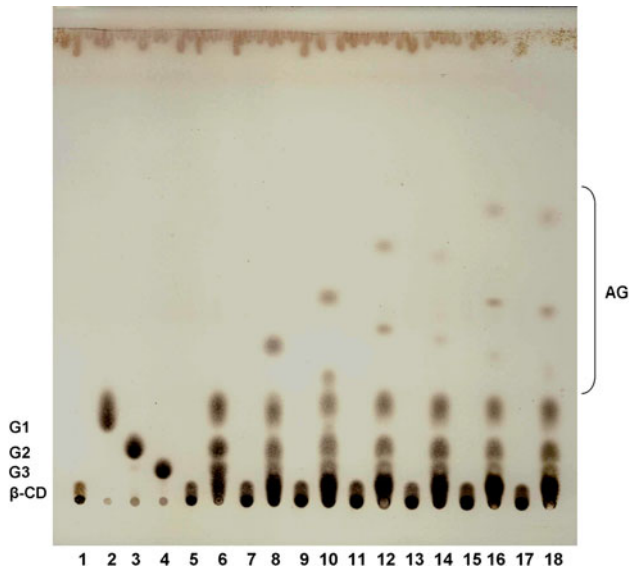

Fig. 2 TLC analysis of the transglycosylation products from β-CD to various alcohols by CGTase. *Lanes 1–4* β-CD, glucose (G1), maltose (G2) and maltotriose (G3), *Lanes 5–6* products from enzyme reaction without alcohol acceptor at 0 and 24 h, *Lanes 7–8* products from enzyme reaction between β-CD and 40% methanol at 0 and 24 h, *Lanes 9–10* products from enzyme reaction between β-CD and 40% ethanol at 0 and 24 h, *Lanes 11–12* products from enzyme reaction between β-CD and 10% 1-propanol at 0 and 24 h, *Lanes 13–14* products from enzyme reaction between β-CD and 10% 2-propanol at 0 and 24 h, *Lanes 15–16* products from enzyme reaction between β-CD and 5% 1-butanol at 0 and 24 h, *Lanes 17–18* products from enzyme reaction between β-CD and 5% 2-butanol at 0 and 24 h

both substrates incubated with CGTase at 0 h were used as control experiments. After 24-h incubation of CGTase with various concentrations of alkyl alcohol acceptors and β-CD donor, expected alkyl glycoside products (AG) which appeared after those of sugars due to the non polar alkyl chains of these compounds were detected by TLC (Fig. 2). Each alcohol acceptor gave several glycoside products in addition to hydrolysis products (glucose, maltose, and other oligosaccharides). No glycosylated products were seen under control conditions, only hydrolysis products were observed. The results suggested that CGTase from *Paenibacillus* sp. RB01 could transfer glucose residues from β-CD to short chain alkyl alcohols giving alkyl glycosides. Methanol gave at least one product while ethanol, 1- and 2-propanol gave at least two products. When 1-butanol and 2-butanol were used as acceptors, three alkyl glycosides were detected. These observations demonstrated that the number of spots appeared was dependent on polarity of the products which correlated with the length of alkyl chain and the number of glucose residues. However, it is possible that other alkyl glycosides were also produced in the reaction mixture containing methanol and ethanol as acceptors but they were probably shielded by hydrolysis products formed in large amounts with similar R_f values.

Influence of alcohol length and concentration on CGTase coupling activity

The coupling activity of CGTase was then investigated in buffer and in a mixture of buffer and alcohol solution with varying alcohol length and concentration at the optimum pH (6.0) of the enzyme (200 U/mL dextrinizing activity) for 24 h. The alcohol used here were 10–60% of methanol, ethanol, 1- and 2-propanol. However, for 1- and 2-butanol, their concentration used in a single phase reaction cannot exceed 10 and 20%, respectively due to their low solubility in water. The coupling activity of CGTase in buffer was 3.4×10^{-3} μmol/min/mg and was set to 100%. As can be seen in Fig. 3, the alcohol length and concentration had significant effect on CGTase activity. When the concentration of alcohol increased, the activity of the enzyme was dramatically decreased. The enzyme retained appreciable activity (more than 50%) only in the methanol-containing medium when its concentration was raised to 40% (v/v). The effect of alcohol on the activity of the enzyme has generally been observed. For example, the rate of starch hydrolysis by α-amylase from *Aspergillus oryzae* decreased with increasing concentration of methanol in the reaction mixture [34]. The rate of sucrose consumption was also found to be lowered with the rising concentration of methanol in the reaction catalyzed by levansucrase from *Rahnella aquatilis* [10]. This behavior is a consequence of the antagonistic influence of the water activity reduction which resulted in the loss of the enzyme activity in the non-aqueous medium [35]. In addition, the length of alcohol also had an important impact on the transglycosylation reaction. The longer the alkyl chain of the alcohol is the more inactivation of CGTase and thus, could result in a lower yield of the product. This has also been observed by many investigators [3–5].

The effect of alcohol length and concentration on the formation of alkyl glycoside was also investigated. Alcohols as well as water were nucleophiles that can react with the enzyme, thus alcohols competed with water as glycosyl acceptors. Sample from the above reactions were subjected to TLC analysis. The intensity of product spots of equal area was quantitated to determine acceptor specificity (Fig. 4). The degree of intensity of alkyl glycosides, which reflected the production yield, synthesized from primary alcohols was the highest in methanol, followed by ethanol and 1-propanol. Although the amount of alkyl glycosides

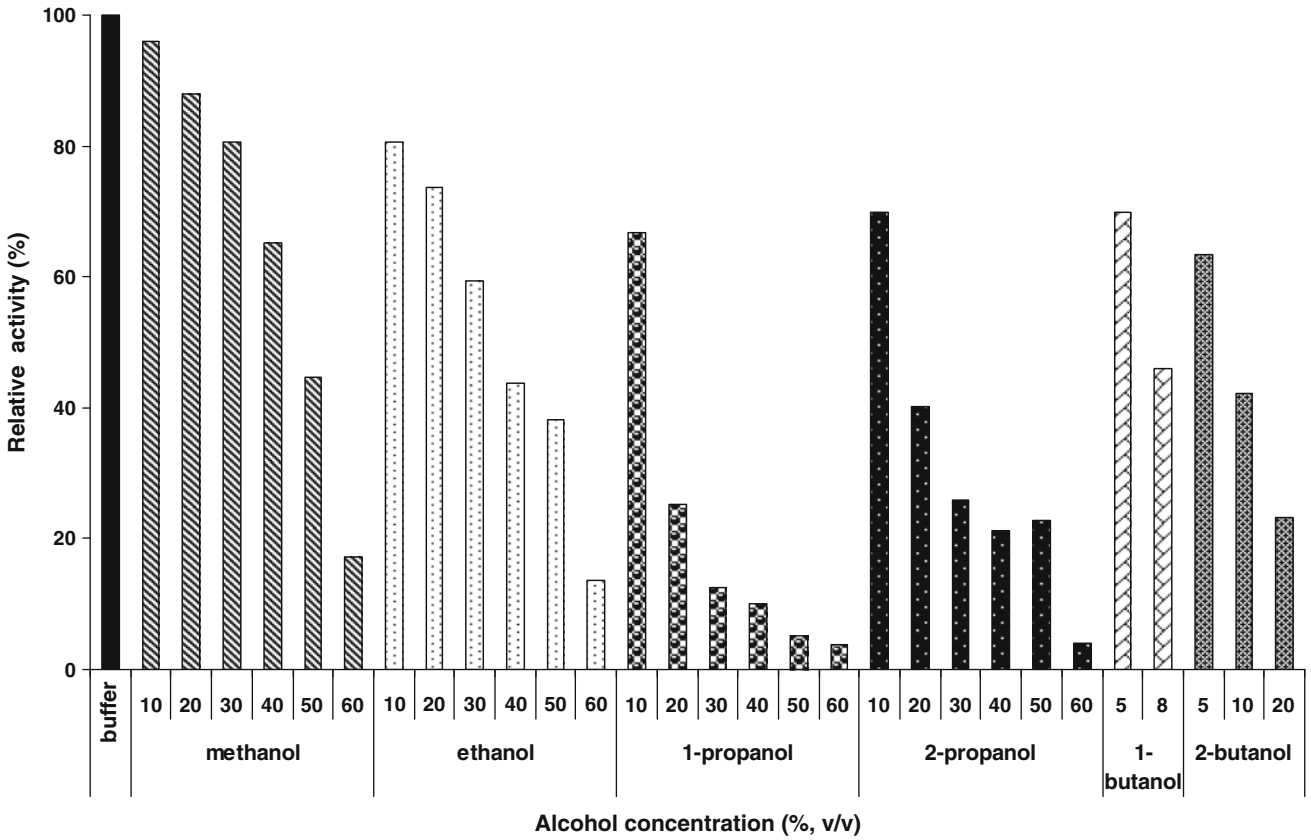

Fig. 3 Relative coupling activity of CGTase in alcohol solvent mixtures. Residual activity (%) was relative to the coupling activity of CGTase in 50 mM acetate buffer solution, pH 6.0. All values were average from two separate experiments

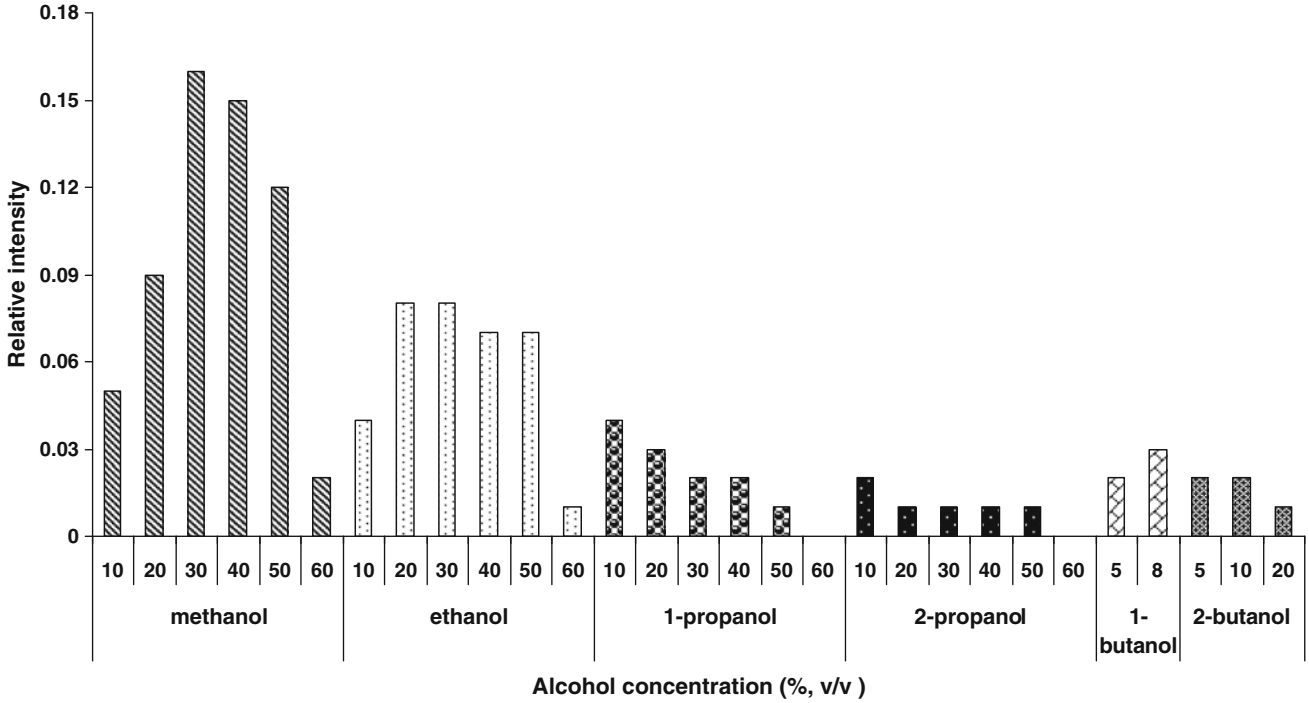

Fig. 4 Relative intensity of alkyl glycoside spots from transglycosylation reaction of β-CD to various alcohol acceptors. Alkyl glycosides of each spot were quantitated relative to glucose spot in the same TLC plate. The intensity of glucose spot was set to 1. All values were average from duplicate TLC plates

formed increased with increasing concentrations of alcohol, it was found that when their concentrations exceeded 50%, the yield was dramatically decreased which could be due to the inactivation of the enzyme. In the case of secondary alcohols, 2-propanol and 2-butanol, alkyl glycoside yield was much lower. This indicates that the length of alkyl group as well as the structure of alcohols affected the level of transglycosylation. In particular, for the alkyl glycoside production using 2-propanol as an acceptor, the yield was lower than that of 1-propanol although the enzyme activities in both reaction mixtures were approximately at the same level (see Fig. 3). The alcohol concentration for the production of alkyl glycosides has to be compromised between appropriate substrate concentration and enzyme activity for the transglycosylation reaction. From these results, methanol showed the highest acceptor specificity toward CGTase from *Paenibacillus* sp. RB01. Similarly, good yields of alkyl glucosides synthesized from Thai rosewood β-glucosidase were obtained with primary alcohol while little or no product was found with secondary and tertiary alcohols [3].

Characterization of methyl glycoside products

In order to elucidate the structure of alkyl glycosides synthesized by CGTase, the reaction with methanol as an acceptor was chosen here as a model. From TLC in Fig. 2 (lane 8), at least one methyl glycoside product was observed. However, there could be other glycoside products with similar R_f values with other oligosaccharides. Therefore, TLC condition was optimized. Under the optimum condition, three spots of the reaction products were observed (Fig. 5a, lane 6). Figure 5b shows an HPLC profile in which a peak of a product was observed before a peak of glucose, with the same retention time (Rt) as that of the methyl glucoside standard (methyl α-D-glucopyranoside, 3.9 min). Thus, the product was presumed to be methyl glucoside (with one sugar unit attached to methanol, MG_1). Furthermore, three more additional peaks at Rt 4.7, 5.9 and 7.6 min appeared with decreasing amounts. This result suggested that methyl glycosides with many glucose residues may also be formed in the reaction apart from MG_1. To confirm this, the products were treated with glucoamylase which cleaves all glycosidic bonds except those between alcohol and glucose. All products were converted to MG_1. From both TLC (Fig. 5a, lane 7) and HPLC analyses (Fig. 5b), after treatment with glucoamylase, those spots and peaks disappeared while the amounts of MG_1 and glucose (G1) increased. Thus, it seems likely that the peaks at 4.7, 5.9 and 7.6 min were the conjugate of methyl glucoside and oligosaccharides.

The molecular weights of synthesized products were analyzed by mass spectrometer with positive ions $[M + Na]^+$ to confirm the formation of methyl glycosides.

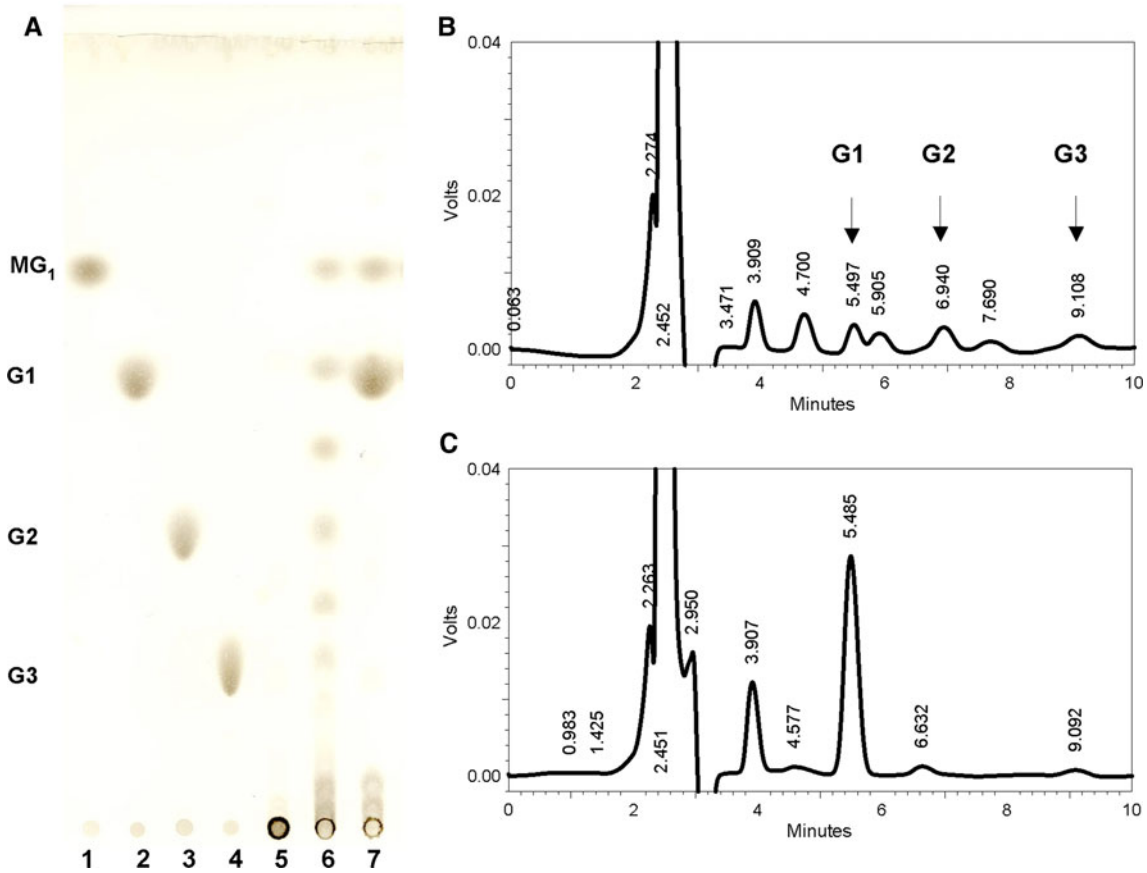

Fig. 5 **a** TLC analysis of the methyl glycoside before and after digestion with glucoamylase. *Lanes 1–4* methyl α-D-glucopyranoside (MG_1), glucose (G1), maltose (G2) and maltotriose (G3) as standards, *Lanes 5–6* products from enzyme reaction between *β*-CD and 30% methanol at 0 and 24 h, *Lane 7* products from enzyme reaction between *β*-CD and 30% methanol at 24 h after treatment with glucoamylase. **b** HPLC analysis of the products from enzyme reaction between *β*-CD and 30% methanol at 24 h. **c** HPLC analysis of the effect of glucoamylase on the digestion of methyl glycosides

From Fig. 6, the molecular weight of products in the reaction mixture were 194 Da (at *m/z* of 217), 356 Da (at *m/z* of 379) and 518 daltons (at *m/z* of 541) which are in good agreement with the calculated masses of the sodium adducted of the methyl monoglucoside, methyl maltoside and methyl maltotrioside, respectively.

Optimization of methyl glycoside synthesis

To obtain high yield of alkyl glycosides, several parameters affecting the production such as concentration of donor, acceptor and enzyme and also the reaction pH and temperature which affected enzyme activity have yet to be optimized. These parameters were studied by varying some of the conditions of standard transglycosylation reaction described in "Experimental". In order to analyze the total yield of methyl glycosides, the reaction mixtures were treated with glucoamylase (20 U/mL) to remove all glucose residues sequentially from the non-reducing end of oligosaccharide moiety conjugated to methanol. From Fig. 7a and b, the optimum pH and temperature for CGTase were still found to be at 6.0 and 40 °C, respectively. CGTase obtained from thermotolerant *Paenibacillus* sp. RB01 was found to be most stable at 40–65 °C and the pH optimum of 5–6 has previously been reported [26]. In optimizing the yields of reactions with methanol, the conditions were to incubate 1.2% (w/v) of *β*-CD with 240 U/mL CGTase in 30% (v/v) methanol at 40 °C, pH 6.0 for 24 h (Fig. 7). The yield of methyl monoglucoside product under optimized condition was 37% which was calculated from the mole ratio of product formed in comparison with *β*-CD used. Different enzyme catalyzing systems produced methyl glycoside products which differ in the type of linkages, the type of products formed and the production yield obtained. For example, Thai rosewood *β*-glucosidase [3] and levansucrase from *Rahnella aquatilis* [10] gave higher yields of methyl *β*-glucoside (97%) and methyl *β*-D-fructoside (70%), respectively in comparison with CGTase. However, only one monosaccharide residue was attached.

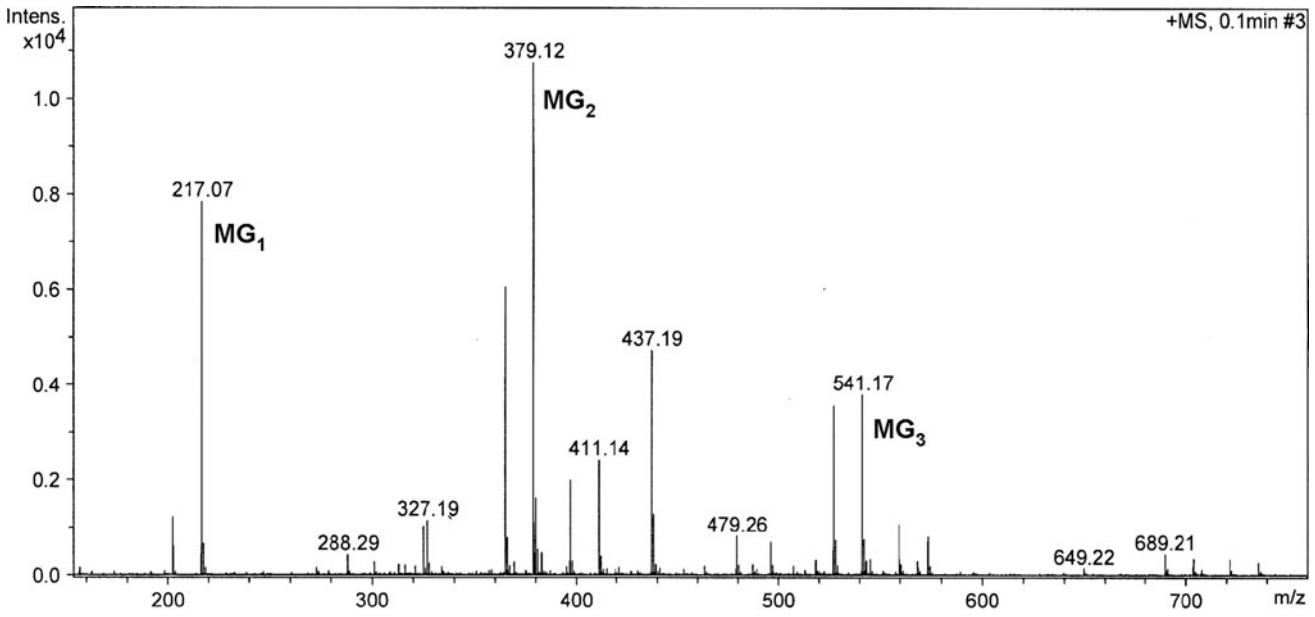

Fig. 6 Electrospray mass spectra (ESI-TOF) of the main transfer products from the enzyme reaction between β-CD and methanol with positive ions $(M + Na)^+$

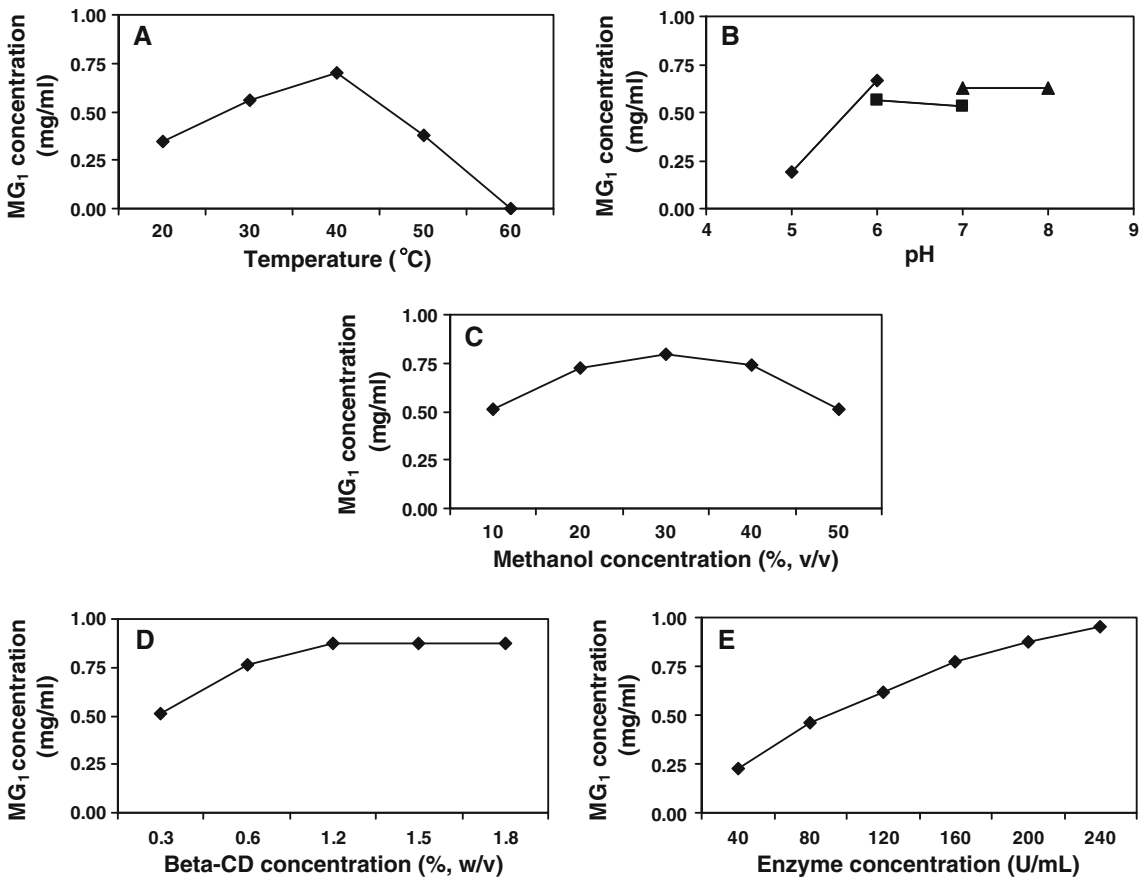

Fig. 7 Effect of **a** temperature, **b** pH: acetate buffer (*diamond*), phosphate buffer (*square*) and Tris–glycine buffer (*triangle*) were used, **c** methanol concentration, **d** β-CD concentration and **e** enzyme concentration on the production of methyl glycoside by CGTase. The amount of methyl monoglucoside was calculated on the basis of its standard curve (0–25 μg) of peak area, $y = 0.0063x$, $R^2 = 0.9969$

In this study, a new characteristic of CGTase in synthesizing alkyl glycoside in alcohol-buffer system was discovered. CGTase can use different alcohols as acceptors in transglycosylation reaction in which β-cyclodextrin is a glycosyl donor to produce various alkyl glycosides having one to several monosaccharide units, which are difficult to prepare via other routes. Studies on the synthesis of alkyl glycosides with more hydrophobic alcohols could be further by controlling water activity and by the use of immobilized enzyme [3].

Acknowledgments This research was supported by Ratchadapiseksompote Research Fund granted to Starch and Cyclodextrin Research Unit, Department of Biochemistry, Faculty of Science, Chulalongkorn University. The support from the Thai Government Stimulus Package 2 (TKK2555) under the project PERFECTA is also acknowledged.

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