Journal of Molecular Catalysis B: Enzymatic 60 (2009) 113–118

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Highly selective biotransformation of arbutin to arbutin-α-glucoside using amylosucrase from *Deinococcus geothermalis* DSM 11300

Dong-Ho Seo[a], Jong-Hyun Jung[a], Suk-Jin Ha[a], Myoung-Chong Song[a], Jaeho Cha[b], Sang-Ho Yoo[c], Tae-Jip Kim[d], Nam-In Baek[a], Cheon-Seok Park[a,*]

[a] *Graduate School of Biotechnology, and Institute of Life Science and Resources, Kyung Hee University, 1 Seocheon, Kiheung, Yongin 446-701, Republic of Korea*
[b] *Department of Microbiology, Pusan National University, Busan 609-735, Republic of Korea*
[c] *Department of Food Science and Technology, Sejong University, Seoul 143-747, Republic of Korea*
[d] *Department of Food Science and Technology, Chungbuk National University, Cheongju 361-763, Republic of Korea*

ARTICLE INFO

Article history:
Received 31 December 2008
Received in revised form 17 March 2009
Accepted 13 April 2009
Available online 23 April 2009

Keywords:
Amylosucrase
Arbutin
Deinococcus geothermalis
Stereoselective transglycosidation

ABSTRACT

Arbutin (Ab, 4-hydroxyphenyl β-glucopyranoside) is a glycosylated hydroquinone known to prevent the formation of melanin by inhibiting tyrosinase. An arbutin-α-glucoside was synthesized by the transglycosylation reaction of amylosucrase (AS) of *Deinococcus geothermalis* (DGAS) using arbutin and sucrose as an acceptor and a donor, respectively. The maximum yield of the arbutin transglycosylation product was determined to be over 98% with a 1:0.5 molar ratio of donor and acceptor molecules (sucrose and arbutin), in 50 mM sodium citrate buffer pH 7 at 35 °C. TLC and HPLC analyses revealed that only one transglycosylation product was observed, supporting the result that the transglycosylation reaction of DGAS was very specific. The arbutin transglycosylation product was isolated by preparative recycling HPLC. The structural analyses using ^{13}C and ^{1}H NMR proved that the transglycosylated product was 4-hydroxyphenyl β-maltoside (Ab-α-glucoside), in which a glucose molecule was linked to arbutin via an α-(1 → 4)-glycosidic linkage.

1. Introduction

Melanin is a class of compounds which function primarily as pigments in living organisms. The photochemical properties of melanin make it an excellent photoprotectant. It absorbs destructive UV-radiation and transforms the energy into harmless amounts of heat through an ultrafast internal conversion process [1]. In humans, however, excess melanin production or abnormal distribution can cause anomalous hyperpigmentation of the skin [2,3]. One of the key enzymes in mammalian melanine biosynthesis is tyrosinase [4,5]. Tyrosinase (monophenol monooxygenase; EC 1.14.18.1), a membrane-bound copper-containing glycoprotein [6], is an enzyme catalyzing the first two steps in melanin biosynthesis. Therefore, inhibition of tyrosinase activity has typically been targeted to improve or prevent hyperpigmentary disorders, such as melasma and age spots [7]. Many natural or synthetic tyrosinase inhibitors have been described, including arbutin, catechols, hinokitol, kojic acid, linoleic acid, and naturally occurring hydroquinones, but arbutin is the most popular and effective skin-whitening or depigmenting agent used because of its very low cytotoxicity [4,8,9].

Arbutin (Ab; hydroquinone-*O*-D-glucopyranoside) is a glycosylated benzoquinone extracted from bearberry plants in the genus *Arctostaphylos*. It is an extremely effective natural skin lightener because it inhibits the oxidation of L-tyrosine catalyzed by mushroom tyrosinase by competing for active binding sites in tyrosinase without being oxidized [10–12]. There are two anomeric (α- and β-) forms of Ab. Ab is naturally occurred anomer that inhibited both tyrosinase activities from mushroom and mouse melanoma, whereas α-Ab inhibited only the tyrosinase from mouse melanoma. However, the inhibitory effect of α-Ab on the activity of tyrosinase from human malignant melanoma cells was estimated to be ten times stronger than that of Ab [13]. Recently, a number of Ab-glucoside derivatives have been synthesized by enzymatic biotransformation and examined for their inhibitory effects on mushroom or human tyrosinase [14–16]. Various enzymes having transglycosylation activity have been employed to modify Ab. Cyclomaltodextrin glucanotransferase (CGTase) from *Bacillus macerans* was successfully employed to produce α-Ab-α-glucosides using α-Ab and soluble starch as an acceptor and a donor molecule, respectively [15]. Three Ab-β-glucosides were synthesized by β-glucosidase from hyperthermophilic *Thermotoga neapolitana* using Ab and cellobiose as an acceptor and a donor molecule, respectively [16]. α-Glucosidase from baker's yeast was used to synthesize α-Ab from hydroquinone and maltose as glucosyl donor by transglucosylation in a water system [17].

* Corresponding author. Tel.: +82 31 201 2631; fax: +82 31 204 8116.
E-mail addresses: cspark@khu.ac.kr, cspark67@gmail.com (C.-S. Park).

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doi:10.1016/j.molcatb.2009.04.006

Amylosucrase (EC 2.4.1.4; AS) is a member of family 13 of the glucoside hydrolases (the α-amylase), although its biological function is the synthesis of amylose-like polymers from sucrose [18]. Among the glucansucrases, AS is structurally unique because it does not have a circularly permuted $(\beta/\alpha)_8$-barrel assembly [19,20]. In addition, it synthesizes only α-1-4-glycosidic linkages to make a glucan polymer, whereas other glucansucrases make heterogeneous glycosidic bonds such as α-1-2-, α-1-3-, and α-1-6- in the final glucan polymer products [21,22]. Recently, the presence of AS-homologous genes in various microorganisms has been inferred by microbial whole genome sequencing projects, although ASs from only two genera, *Neisseria* and *Deinococcus*, have been reported [23–25]. When sucrose is used as a sole substrate, AS performs three different catalytic reactions: (1) a sucrose hydrolysis reaction to produce glucose and sucrose, (2) sucrose isomerization to form sucrose isomers (turanose and trehalulose), and (3) polymer synthesis to create a soluble maltooligosaccharide and insoluble glucan [25,26]. The polymerization activity of recombinant AS from *Neisseria polysaccharea* was used to glucosylate glycogen particles and thereby to synthesize carbohydrate-based dendritic nanoparticles [27].

Except for various starches and glycogen, other molecules are not examined as acceptors for the transglycosylation of AS. In this work, we have employed the transglycosylation activity of recombinant AS from *Deinococcus geothermalis* (DGAS) to synthesize Ab-glucoside. Unexpectedly, only a single product was formed with exceptionally high yield by DGAS. The structure of the resulting product was determined by nuclear magnetic resonance (NMR) and mass spectrometry (MS) analyses and its melanogenesis inhibitory activity was discussed.

2. Experimentals

2.1. Chemicals and enzymes

All chemicals including Ab used in this study were purchased from Sigma Chemical Co. and were of reagent grade. DNA-modifying enzymes such as restriction endonucleases, T4 DNA ligase and *Pfu* DNA polymerase were purchased from New England Biolabs (Beverly, MA, USA), Solgent (Seoul, Korea) or Promega (Madison, WI, USA). A Glutathione-Sepharose™ High Performance affinity column was obtained from Amersham Biosciences and used for the purification of glutathione S-transferase (GST)-fused DGAS.

2.2. Bacterial strains

The PCR method has been used to clone the gene corresponding to DGAS from the genomic DNA of *D. geothermalis* DSM 11300. The detailed cloning procedure for DGAS was described previously [23–25]. *Escherichia coli* DH10B [F^- *ara*D139 Δ(*ara leu*)7697 Δ*lac*X74 *gal*U *gal*K *rps*L *deo*R Φ80*lac*ZΔM15 *end*A1 *nup*G *rec*A1 *mcr*A Δ(*mrr hsdRMS mcrBC*)] was used for general recombinant DNA procedures while *E. coli* BL21 [F^-, *omp*T, *hsd*S_B(r_B-, m_B-), *dcm*, *gal*, λ(DE3)] was employed as a host for recombinant *dgas* expression studies. In general, recombinant *E. coli* cells were grown in Luria-Bertani (LB) medium containing 1% (w/v) bactotryptone, 0.5% (w/v) yeast extract, and 0.5% (w/v) NaCl, supplemented with ampicillin (100 μg/mL).

2.3. Preparation of recombinant DGAS

Recombinant *E. coli* BL21 cells harboring pGEX-DGAS, a plasmid for DGAS expression, were grown in 1 L of LB medium supplemented with 0.1 mg/mL ampicillin at 37 °C with vigorous agitation. When the optical density of the cells reached around 0.6, 1 mM (final concentration) of isopropyl-β-D-thiogalactopyranoside (IPTG) was added into the culture to induce *dgas* gene expression. After a 3 h induction, the cells were harvested and resuspended in phosphate-buffered saline (PBS, 5 mL/g (wet weight) of cells) buffer composed of 140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 1.8 mM KH_2PO_4 (pH 7.3). The crude enzyme solution was prepared by sonication (Sonifier 450, Branson, Danbury, CT, USA; output 4, 6 times for 10 s, constant duty), followed by centrifugation at 12,000 rpm for 10 min at 4 °C. The purified recombinant DGAS was obtained by affinity chromatography using a Glutathione-Sepharose™ High Performance affinity column as suggested by the manufacturer. The removal of fused GST protein from the purified recombinant DGAS was performed with thrombin treatment. The apparent homogeneity of recombinant DGAS was confirmed by SDS–PAGE analysis as previously mentioned [25] and protein concentration was determined with the Bradford reagents kit (Bio-Rad, Hercules, CA, USA) with bovine serum albumin as a standard. The specific activity of purified enzymes was 69 U/mg.

2.4. Determination of enzyme activity

In general, the activity assay of DGAS was performed with 40 mg/mL sucrose at 45 °C in 50 mM Tris–HCl (pH 8.0). One unit of DGAS activity was defined as the amount of enzyme that produces 1 μmol of fructose per minute in the reaction conditions [25]. Fructose concentration was determined by the dinitrosalicylic acid method using fructose as standard.

2.5. Transglycosylation reaction and purification of Ab-glucoside

An Ab transglycosylation product was obtained by incubating the reaction mixture (5 mL) containing 200 mg of sucrose, 250 mg of Ab, and 50 U of DGAS in 50 mM Tris–HCl buffer (pH 8) at 30 °C for 12 h. The reaction was stopped by boiling for 10 min, and freeze-dried for further use. The freeze-dried sample was dissolved in 2.5 mL of distilled water and purified by the preparative recycling HPLC system (LC-9104, JAI, Tokyo, Japan) equipped with refractive index detection. The transglycosylation reaction mixture (2.5 mL) was loaded onto a JAIGEL-W252 column (2 cm × 50 cm; Japan Analytical Industry Co., Ltd., Tokyo, Japan), a gel permeation chromatography column for water soluble and low molecular weight samples, which was connected to two JAIGEL-W251 columns (2 cm × 50 cm) and an RI detector RI-50 (JAI, Tokyo, Japan). Deionized water was used for the elution of the sample at a flow rate of 3 mL/min. The purification of the Ab transglycosylation product was analyzed by TLC. The purified products were lyophilized and used for NMR analysis.

2.6. Thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) analyses

TLC and HPLC analyses were carried out to detect Ab and the Ab transglycosylation product. A TLC silica gel plate (Whatman K5F, Whatman, Kent, UK) was activated at 110 °C for 30 min. The samples on the TLC plate were separated by a solvent of *n*-butanol:acetic acid: water (3:1:1, v/v/v). After allowing the solvent to ascend twice, the TLC plate was dried in a hood and then developed by being soaked in 0.3% (w/v) N-(1-naphthyl)-ethylenediamine and 5% (v/v) H_2SO_4 in methanol. Finally, the spots in the TLC plate were visualized by placing the plate in a 110 °C oven for 10 min. The yield of the Ab transglycosylation product was calculated from the reduction of the area of the Ab peak in HPLC analysis. For the HPLC analysis, the reaction mixtures were filtered through a 0.45 μm filter and analyzed using a SUPELCOSIL™ LC-NH_2 column (5 μm, 25 cm × 4.6 mm;

Supelco, Bellefonte, PA, USA) connected to a Shimadzu model SCL-10 system with Shimadzu LC10 pump and Shimadzu SPD-10A (at 280 nm). Separation of transglycosylation products was achieved isocratically using acetonitrile and water (3:1, v/v) at flow rate of 1.0 mL/min. All solvents were filtered, degassed and kept under pressure.

2.7. NMR and fast atom bombardment (FAB)-MS analyses

Twenty mg of samples (Ab and purified Ab derivative) were exchanged with D_2O and were dissolved in 0.5 mL of pure D_2O. They were then placed into 5 mm NMR tubes. ^{13}C and ^{1}H NMR spectra of Ab and the purified Ab derivative were acquired from a Varian Inova AS 400 MHz NMR spectrometer (Varian, Palo Alto, CA, USA). Tetramethylsilane (TMS) was dissolved in CD_2OD at 23 °C as an internal chemical shift reference. Both negative and positive FAB-MS were recorded on a JEOL JMS 700 (JEOL, Tokyo, Japan).

3. Results and discussion

3.1. Enzymatic synthesis of Ab-glucoside by DGAS

Ab was effectively used as an acceptor for the transglycosylation reaction of DGAS as shown in Fig. 1. Three spots appeared in the transglycosylation reaction of DGAS in TLC analysis. Two spots were evidently Ab and fructose, but one spot was not comparable to any standard molecule. Furthermore, one peak other than Ab was observed in the HPLC chromatogram when a UV detector was applied. These results indicated that an Ab transglycosylation product was synthesized by DGAS. When the transglycosylation reaction of CGTase from *B. macerans* was employed to synthesize α-Ab-glucosides (or Ab-glucosides) using α-Ab (or Ab) and soluble starch as an acceptor and a donor, respectively, two major products, 4-hydrophenyl α-maltoside and 4-hydrophenyl α-maltotrioside (or 4-hydrophenyl β-maltoside and 4-hydrophenyl β-maltotrioside), were isolated [14,15]. Three active compounds were synthesized through the transglycosylation reaction of *T. neapolitana* β-glucosidase with 15% Ab and 15% cellobiose as an acceptor and a donor, respectively. The products formed were β-D-glucopyranosyl-(1 → 6)-Ab, β-D-glucopyranosyl-(1 → 4)-Ab, and

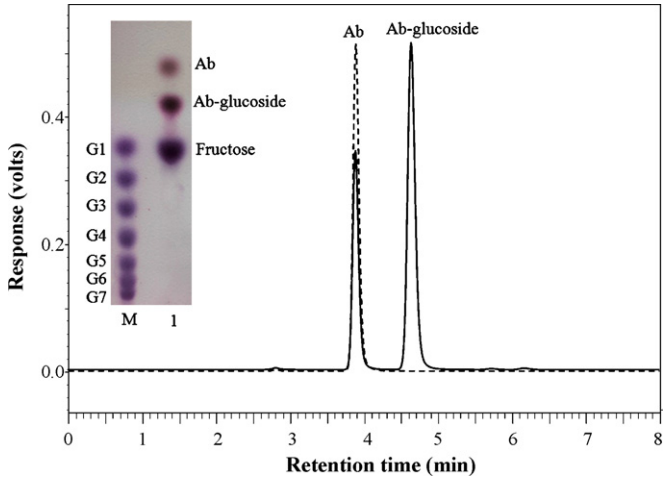

Fig. 1. TLC and HPLC analyses of transglycosylation reaction by DGAS with Ab and sucrose as an acceptor and a donor, respectively. For TLC analysis, lane M contained standard markers from G1 (glucose) to G7 (maltoheptaose); lane 1 contained the reaction product of the Ab transglycosylation reaction by DGAS. In HPLC, Ab was designated as a dotted line while the reaction product of the Ab transglycosylation reaction by DGAS was depicted as a straight line.

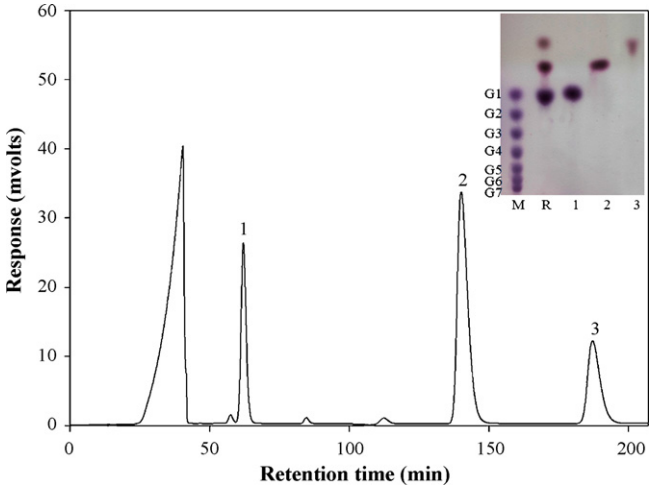

Fig. 2. Preparative recycling HPLC and TLC analysis of purified Ab-α-glucoside. Four percent sucrose and 5% arbutin were reacted with DGAS at 35 °C for 12 h. Insert shows the purified transglycosylation product. Lane M: standard marker from G1 (glucose) to G7 (maltoheptaose), lane R: reaction product of Ab transglycosylation reaction by DGAS, lane 1: fructose, lane 2: Ab-transglycosylated product, and lane 3: Ab.

β-D-glucopyranosyl-(1 → 3)-Ab [16]. In contrast to these reactions, only a single transglycosylation product was detected in the DGAS reaction, indicating that DGAS was very specific in its transglycosylation reaction and useful for making a sole transglycosylation product.

3.2. Isolation and structural analysis of Ab-glucoside synthesized by DGAS

The Ab-glucoside product synthesized by DGAS was purified by preparative recycling HPLC. The isolated Ab-transglycosylated product appeared as a single spot on TLC analysis, indicating that the purification was successful (Fig. 2). The FAB-MS of the isolated Ab-transglycosylated product showed a molecular-related ion peak at *m*/*z* 433.2, corresponding to the calculated molecular mass of Ab-glucoside (Fig. 3). In ^{13}C NMR analysis (Table 1), sixteen signals were observed and four of them were assigned to the *p*-hydroxyphenol group (δ 116.5–153.5). The remaining twelve

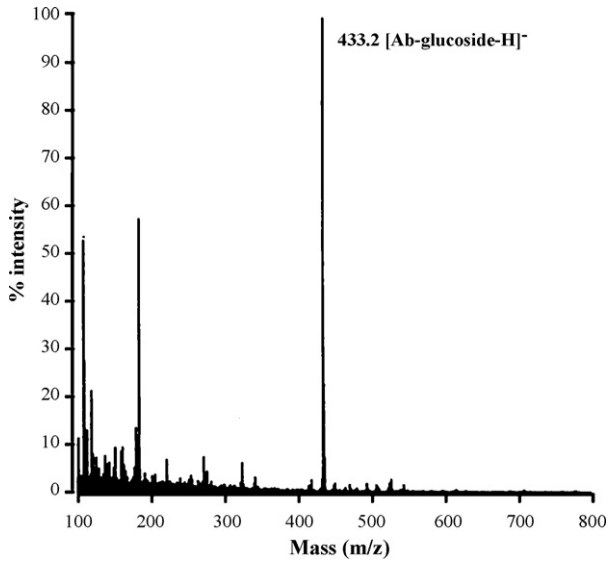

Fig. 3. FAB-MS analysis of purified Ab-α-glucoside.

Table 1
^{13}C (100 MHz) and ^{1}H NMR (400 MHz) data for Ab and Ab-glucoside (units: ppm, in CD_3OD).

		^{13}C NMR		^{1}H NMR	
		Ab	Ab-glucoside	Ab	Ab-glucoside
p-Hydroxyphenol	1	152.2	152.2		
	2	116.5	116.5	6.69 (d, *J* = 8.8 Hz)	6.69 (d, *J* = 9.2 Hz)
	3	119.2	119.3	6.96 (d, *J* = 8.8 Hz)	6.96 (d, *J* = 9.2 Hz)
	4	153.5	153.7		
	5	119.2	119.3	6.96 (d, *J* = 8.8 Hz)	6.96 (d, *J* = 9.2 Hz)
	6	116.5	116.5	6.69 (d, *J* = 8.8 Hz)	6.69 (d, *J* = 9.2 Hz)
β-D-Glucopyranose	1′	103.4	103.4	4.74 (d, *J* = 7.6 Hz)	4.76 (d, *J* = 7.6 Hz)
	2′	74.8	74.7		3.61 (dd, *J* = 7.6, 7.6 Hz)
	3′	77.8	77.6		
	4′	**71.2**	**80.9**	3.38 (dd, *J* = 7.2, 7.6 Hz)	3.62 (dd, *J* = 7.6, 7.6 Hz)
	5′	77.8	76.5		
	6′	62.4	62.0		
α-D-Glucopyranose	1″		102.8		**5.19 (d, *J* = 3.6 Hz)**
	2″		74.1		
	3″		75.0		
	4″		71.4		
	5″		74.5		
	6″		62.4		

signals were appointed to two glucose units, and six of them were inevitably assigned to the glucose molecule in arbutin. This result, combined with FAB-MS analysis, suggested that the Ab-transglycosylated product formed by DGAS had a basic molecular structure of Ab with a glucose molecule attached to it. When chemical shifts of Ab and the Ab-transglycosylated product were compared, a distinct chemical shift of C-4′ (from δ 71.2 to δ 80.9) in the glucose unit of Ab was observed, indicating that there is a 1,4 linkage between Ab and the transferred glucose unit. In addition, ^{1}H NMR analysis revealed that the glucose molecule was transferred to C-4′ in the glucose unit of Ab with α-anomeric configuration based on the coupling constant (*J* = 3.6 Hz) of the glucose anomeric proton signal observed at 5.19 ppm. These results together determined that the molecular structure of the Ab-transglycosylated product synthesized by DGAS was 4-hydroxyphenyl α-maltoside [α-D-glucopyranosyl-(1 → 4)-β-arbutin; Ab-α-glucoside] (Fig. 4). AS catalyses the synthesis of an amylose-like polysaccharide composed of only α-(1 → 4)-glycosidic linkages using sucrose as the only substrate [18]. This enzyme initiates polysaccharide formation by releasing a glucose molecule through sucrose hydrolysis. The resulting glucose molecule is subsequently used as the first acceptor molecule and is successively elongated at its non-reducing end to synthesize maltooligosaccharides until it reached a critical size to make an insoluble α-(1 → 4)-glucan. When Ab was used as an acceptor, obviously only α-(1 → 4)-glycosidic linkages formed, but it seemed that the following elongation did not occur efficiently. Finally, only Ab-α-glucoside was accumulated as a specific transglycosylation product in the DGAS reaction mixture.

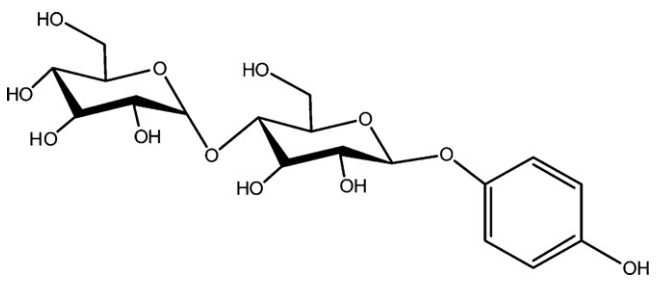

Fig. 4. The molecular structure of Ab-α-glucoside [α-D-glucopyranosyl-(1 → 4)-β-arbutin] synthesized by DGAS.

3.3. Effect of temperature and pH on the bioconversion of Ab to Ab-α-glucoside

Previously, it was found that DGAS catalyzed the transglycosylation reaction at a lower temperature than its optimum temperature for sucrose hydrolysis, which is 45 °C [25]. The effect of temperature and pH on the bioconversion of Ab to Ab-α-glucoside by DGAS was investigated Fig. 5. The maximum bioconversion of Ab to Ab-α-glucoside was observed to be 83.5% and 43.5% at 35 °C in donor to acceptor (D:A) ratios of 1:0.5 and 1:1, respectively. However, the bioconversion yields at 45 °C, an optimum temperature for sucrose hydrolysis, were 66.7% and 22.3% at the D:A ratios of 1:0.5 and 1:1, respectively. The result confirmed that the transglycosylation activity of DGAS was more efficient at a lower temperature (35 °C) than its hydrolysis activity. The maximum transfer of glucose to Ab to make Ab-α-glucoside was detected at pH 7 in both sodium citrate and Tris–HCl buffers. These results show that the maximum bioconversion yield can be obtained in sodium citrate buffers (pH 7) at 35 °C with a ratio of donor to acceptor molecules less than 1:1.

Time course of DGAS reaction for the bioconversion of Ab to Ab-α-glucoside revealed that the yield of Ab-α-glucoside was not increased although the donor (sucrose) was continuously degraded as reaction continued (Fig. 6). It implies that Ab is a relatively strong acceptor molecule in DGAS reaction and firstly used to make Ab-α-glucoside in the early stage of the reaction due to acceptor specificity of DGAS. However, at late stage of the reaction, the excess glucose molecules released from donor molecules are consumed to synthesize insoluble glucans by DGAS. Interestingly, the concentration of fructose was not decreased during the reaction, indicating that fructose was not a good acceptor molecule for DGAS reaction. This result is in accordance with the previous reports on the characterization of ASs from *Neisseria* and *Deinococcus* in which glucose was utilized to produce an insoluble glucan from sucrose while fructose was released in the reaction when ASs reacted with sucrose alone [18,24,25].

3.4. The yield of Ab-α-glucoside

The production of the Ab transglycosylation product by DGAS was analyzed with various donor and acceptor (D:A) ratios by TLC and HPLC (Fig. 7). As the molar ratios between donor and acceptor molecules were raised from 1:0.1 to 1:1, absolute amounts of Ab transglycosylation product increased. However, these amounts decreased as the concentration of acceptor molecules was further

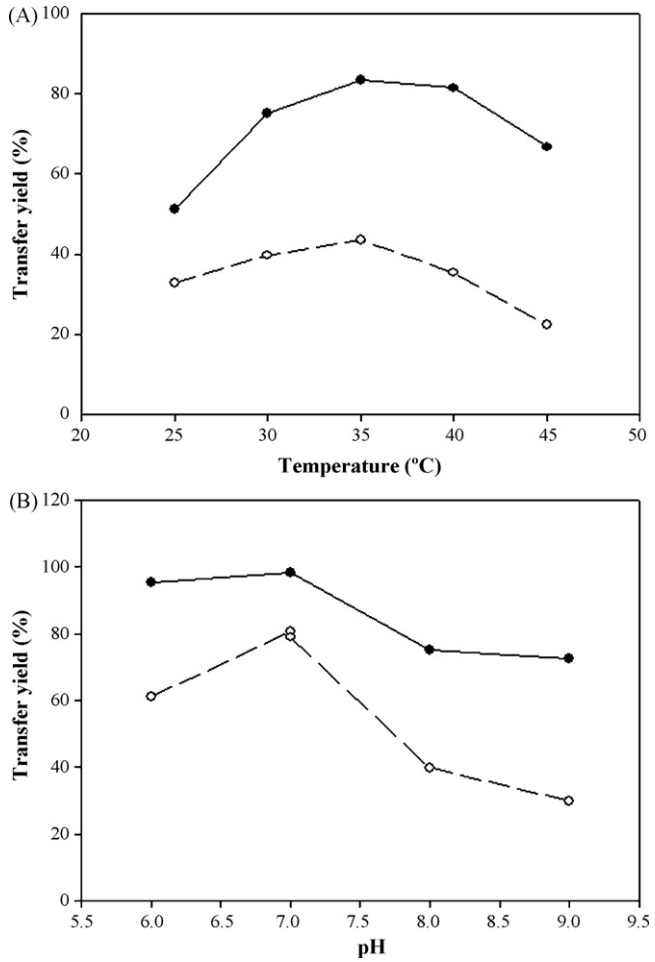

Fig. 5. Transfer yield of arbutin glycoside by DGAS on various temperatures (A) and pHs (B). (A) Ab transglycosylation reactions were performed in Tris–HCl pH 8.0 buffer at various temperatures. Closed circles represent the molar ratio of 1:0.5 between donor and acceptor molecules whereas open circles stand for the molar ratio of 1:1 of 1:1; (B) Ab transglycosylation reactions were performed at 35 °C in various pHs of 50 mM sodium citrate (●), (▲) and 50 mM Tris–HCl (○), (□) buffers. Circles are the molar ratio of 1:0.5 between donor and acceptor molecules while triangles are the molar ratio of 1:1.

increased. When the yield of Ab transglycosylation product was defined as the ratio of Ab transglycosylation product synthesized over Ab added, it was over 80% if the acceptor molecule was present less than 100% of donor molecule (Fig. 7). However, relatively lower yield was obtained with a higher proportion of acceptor molecules in the reaction. The yields were about 33% and 9% when D:A were 1:2, and 1:3, respectively (Fig. 8). The yield of α-Ab-α-glucosides (or Ab-α-glucosides) was estimated to be more than 70% of the applied acceptors (α-Ab or Ab) when CGTase was used to synthesize a series of α-Ab-α-glucosides (or Ab-α-glucosides) [14,15]. In this reaction, 50 mg of α-Ab and 0.5 g of soluble starch were used as acceptor and donor, respectively. Despite the difficulty in determining the molarity of soluble starch, it could be assumed that there were enough donor molecules in the CGTase reaction. The maximal molar yield was 98.2% and in these conditions DGAS form Ab-glucoside in concentration of 3.14 mg/mL. Even though it was assumed that the total transglycosylation yield of the CGTase reaction was comparable to that of DGAS, the yield of the single transglycosylation product was much higher in the DGAS reaction. The yield of total Ab-β-glucosides produced by *T. neapolitana* β-glucosidase was much lower than that of DGAS (personal communication). From these results, it is apparent that DGAS is a potent transglycosylation enzyme that can be applied to synthesize a specific α-(1 → 4)-glycosylated product with relatively high yield, as shown in Ab.

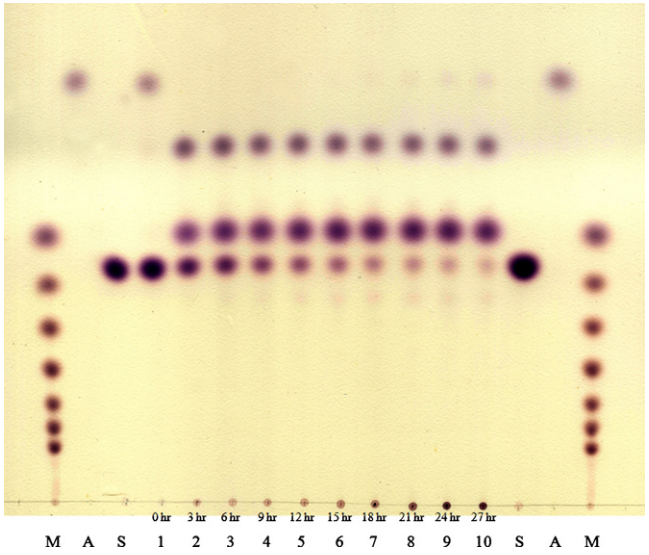

Fig. 6. Time course TLC analysis of transglycosylation reaction by DGAS with Ab and sucrose as an acceptor and a donor, respectively. Lane M: standard markers for G1 (glucose) to G7 (maltoheptaose), lane S: sucrose standard, lane A: Ab standard, lanes 1–10: reaction mixtures at different reaction times (h) as designated in the figure. The ratio of sucrose (donor) and Ab (acceptor) in the reactions was 1:0.5. Arbutin transglycosylation reaction was performed in sodium citrate buffer (pH 7.0) at 35 °C.

The inhibitory activities of Ab-α-glucoside on mushroom tyrosinase and the melanin production in melanoma cells were previously determined [16]. Ab-α-glucoside had a lower IC_{50} value (IC_{50} = 5 mM) on mushroom tyrosinase than Ab (IC_{50} = 6 mM). At 10 mM concentration, Ab-α-glucoside showed about 70% inhibition against mushroom tyrosinase. This result is similar to the previous report by Sugimoto et al., in which Ab-α-glucoside synthesized by CGTase of *B. macerans* exhibited IC_{50} at 5.7 mM on human tyrosinase [14]. In conclusion, the transglycosylation activity of DGAS can be usefully applied to synthesize Ab-α-glucosides. The DGAS reaction was very specific to produce a single compound as a transglycosylation product with very high yield. This reaction has an advantage in industrial use because it can use an inexpensive sucrose as a donor molecule.

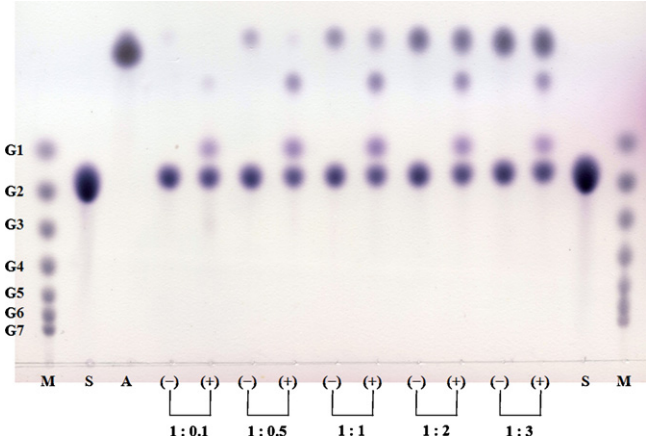

Fig. 7. TLC analysis of transglycosylation reaction of DGAS with the molar ratio between sucrose (donor) and Ab (acceptor). Lane M, standard marker from G1 (glucose) to G7 (maltoheptaose); lane S, sucrose; lane A, Ab; lanes (–), no enzyme (DGAS) added; lanes (+), enzyme (DGAS) was added in the reaction. The ratios of sucrose (donor) and Ab (acceptor) in the reactions were described as 1:0.1, 1:0.5, 1:1, 1:2, and 1:3. Ab transglycosylation reactions were performed in sodium citrate buffer (pH 7.0) at 35 °C.

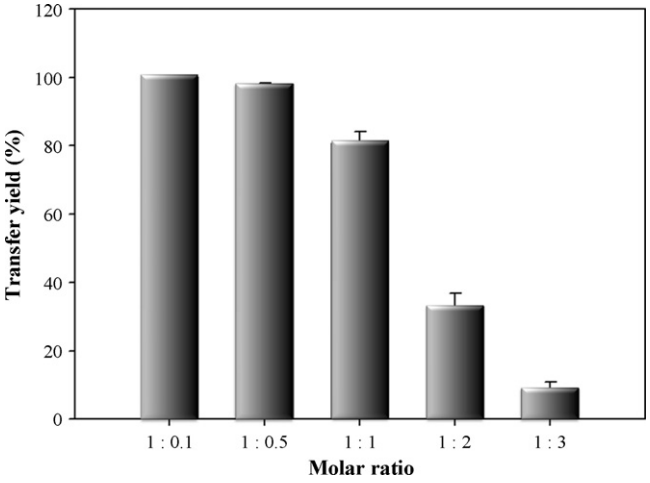

Fig. 8. The production yield of Ab-α-glucoside in the transglycosylation reaction with different molar ratios between sucrose (donor) and Ab (acceptor). Ab transglycosylation reactions were performed in sodium citrate buffer (pH 7.0) at 35 °C.

4. Conclusions

The enzymatic synthesis of arbutin-α-glucoside by means of direct transglycosylation of arbutin using amylosucrase from *D. geothermalis* DSM 11300 was investigated in the present study. A 98% of maximum molar conversion was obtained at a 1:0.5 molar ratio of donor (sucrose) and acceptor (arbutin) molecules in sodium citrate buffer (pH 7) at 35 °C. The results showed the amylosucrase transglycosylation reaction was highly specific and efficient to synthesize only one transglycosylation product, which was determined to be 4-hydroxyphenyl β-maltoside (Ab-α-glucoside).

Acknowledgement

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund) (KRF-2008-313-F00145).

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