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Enzymatic synthesis of alkyl α-2-deoxyglucosides by alkyl alcohol resistant α-glucosidase from *Aspergillus niger*

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Abstract—*Aspergillus niger* α-glucosidase (ANGase) was used for an efficient syntheses of alkyl α-D-2-deoxyglucosides (A2DGs) and for regioselectivity studies of alkoxy-hydro additions of D-glucal in the presence of alkyl alcohols. ANGase showed a high stability with respect to the high concentration of alkyl alcohols. The reaction conditions were optimized for pH, temperature, alkyl alcohol concentration, and D-glucal concentration. On the basis of MS and NMR analyses, A2DGs were confirmed to have only an α-2-deoxyglucosidic bond and the two-dimensional NMR (HMBC) spectra showed to be made up of 2-deoxyglucosyl and alkyl moieties.

1. Introduction

Some glycosidases possess the hydrolytic activity for glycosides as well as the hydration activity toward D-glycals.[1,2] The stereochemical studies of the hydration reactions with D-glycals have provided insight into the specificity and function of glycosidases. The hydrations of D-glucal performed in D_2O by α-glucosidase and β-glucosidase produce 2-deoxy-[2(*a*)-^{2}H]-α-D-glucose and 2-deoxy-[2(*e*)-^{2}H]-β-D-glucose through *trans*-addition (Fig. 1). The product-anomeric configuration of each enzyme in the hydrolytic reaction is also maintained in the hydration reaction of D-glucal. α-Glucosidase family I enzymes cannot hydrate D-glucal, but α-glucosidase family II enzymes, such as *Aspergillus niger*, pig serum, rice, buckwheat, and sugar beet, are able to catalyze the hydration of the double bond in D-glucal and produce the α-anomer of 2-deoxyglucose.[1]

α-Glucosidase [EC 3.2.1.20, α-D-glucoside glucohydrolase] is a group of typical *exo*-type carbohydrases, which catalyze the split of α-glucosyl linkages to liberate α-glucose from the non-reducing terminal of substrate. Various types of α-glucosidases are distributed widely in microorganisms, plants and animal tissues, and their substrate specificities are diverse.[3] α-Glucosidases are divided into two groups, α-glucosidase families I and II based on their amino acid sequences.[4] The primary structures of α-glucosidase families I and II are members of families 13 and 31 of glycosyl hydrolases, respectively.[5,6] α-Glucosidase family I, for example, *Saccharomyces carlsbergensis* (*Saccharomyces cerevisiae*) α-glucosidase,[7] *Bacillus cereus* α-glucosidase,[8] and honeybee α-glucosidase I, II, and III,[9,10] has the same four amino acid sequence regions as conserved in α-amylase and its related enzymes.[11] On the other hand, the enzymes from origins such as human lysosomal,[12] *A. niger* (ANGase),[13] and *Schizosaccharomyces pombe*[14,15] belong to α-glucosidase family II, which has regions A and B including critical residues for activity.[14] There is no homology in the primary structures between α-glucosidase families I and II.

Alkyl α- or β-2-D-deoxyglucosides are known to occur in many antibiotics.[16] Some β-glycosidases have been reported to produce β-2-deoxyglycosides from D-glycal and alcoholic or phenolic compounds.[17,18] The anomerically selective enzymatic synthesis of α-2-D-deoxyglucosides is an interesting approach because their chemical synthesis gives two stereoisomers of α- and β-configurational compounds.[19] We found that α-glucosidase family II enzymes catalyze the transfer of 2-deoxyglucosyl groups from D-glucal to various alcohols as acceptors thus synthesizing various alkyl α-D-2-deoxyglucosyl derivatives

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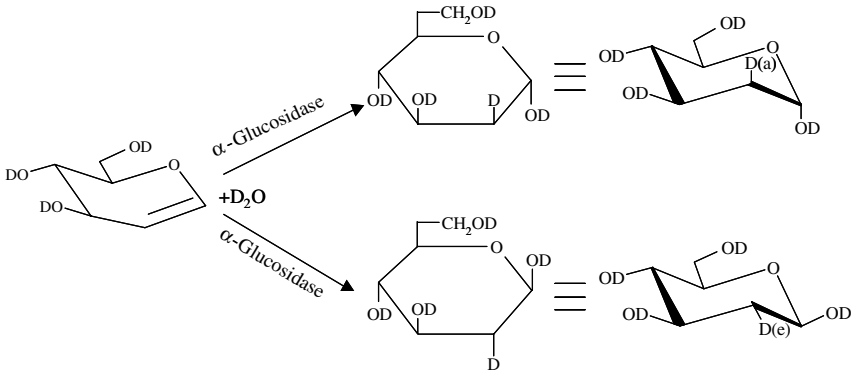


Figure 1. Reaction scheme for D-glucal by α- and β-glucosidases in D_2O.

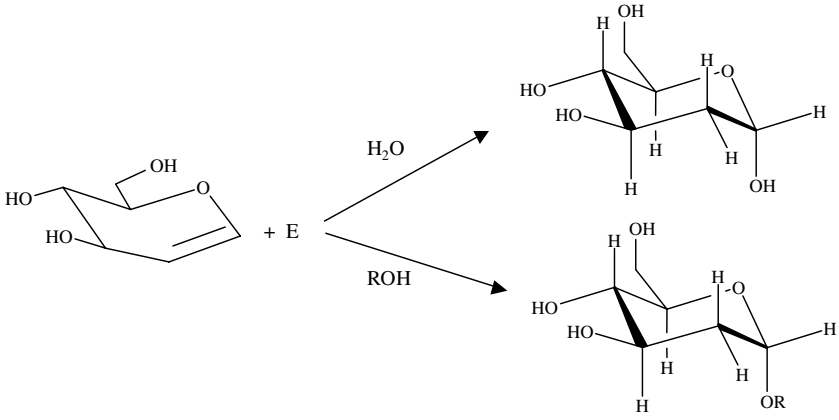
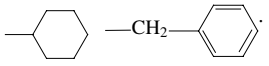


Figure 2. Synthesis of alkyl α-D-2-deoxyglucoside and α-2-deoxyglucose from D-glucal by ANGase. E: ANGase; R: $-CH_3$, $-(CH_2)_nCH_3$ [n = 1–7], $-CH(CH_3)CH_3$, $-CH_2CH(CH_3)CH_3$, $CH(CH_3)CH_2CH_3$, $-CH_2CH{=}CH_2$,  .

(Fig. 2). Herein we report (i) the screening of α-glucosidase from various origins having high stability in high concentration of alkyl alcohols, (ii) the conditions suitable for the synthetic reaction of alkyl α-2-D-deoxyglucosides using D-glucal, and (iii) the determination of structures of newly synthesized α-D-2-deoxyglucosides by HPAEC, MS, and NMR.

2. Results and discussion

2.1. Screening for stable enzymes in alkyl alcohol

In order to select the most suitable enzyme for synthesizing the A2DG in alcohols, we compared the stabilities of α-glucosidases from different origins such as *A. niger*, pig serum, buckwheat, and *S. pombe* in 50% methanol (Fig. 3). Buckwheat α-glucosidase was rapidly inactivated in 0.5 h. *S. pombe* and pig serum α-glucosidases retained 6% and 20% of original activity after 4 h, respectively, but *A. niger* α-glucosidase (ANGase) retained 91% of initial activity after 4 h. As ANGase was the most stable of four enzymes, it was selected as the enzyme suitable for synthesizing A2DGs in alkyl alcohols. The residual activity of ANGase in the various concentrations of methanol (0–70%, v/v) was investigated at 35 °C (Fig. 4). ANGase showed 91% of initial activity in 50% methanol for 4 h, and ANGase maintained 75% of initial activity even in 70% methanol. ANGase was suitable for synthesis of alkyl α-glucosides

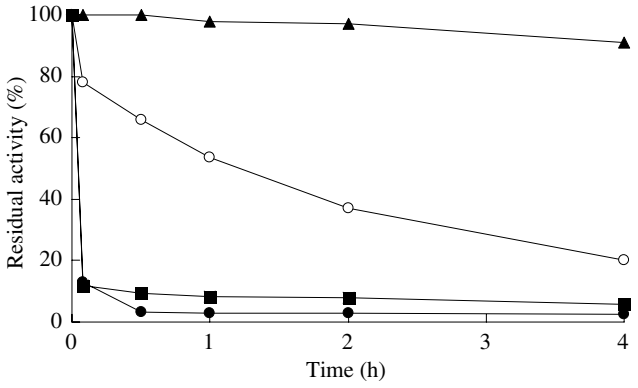


Figure 3. Stability of α-glucosidases in 50% (v/v) methanol. Each reaction mixture (0.1 mL) containing ANGase (▲, 0.68 U, pH 4.3), pig serum α-glucosidase (○, 0.65 U, pH 7.0), *S. pombe* α-glucosidase (●, 0.65 U, pH 4.5), or buckwheat α-glucosidase (■, 0.66 U, pH 5.0), 50% (v/v) methanol and 20 mM buffer was incubated at 35 °C. In the indicated time, aliquots of 10 μL were used for measuring the residual activities.

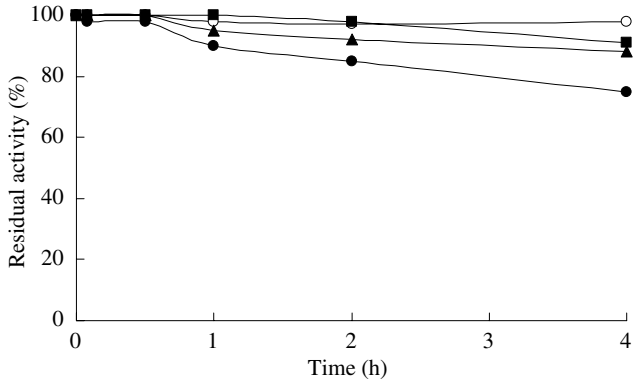


Figure 4. Stability of ANGase in methanol. Reaction mixture (0.1 mL) containing ANGase (0.68 U), 0 (○), 30 (■), 50 (▲), or 70 (●)% (v/v) methanol in 20 mM sodium acetate buffer (pH 4.3) was incubated at 35 °C. In the indicated time, aliquots of 10 μL were taken out from the reaction mixture for measuring the residual activities.

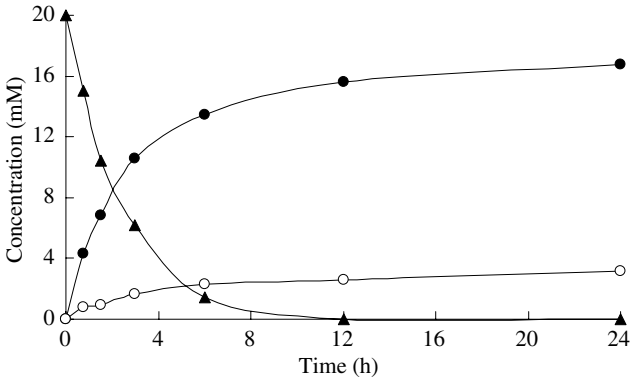


Figure 5. Time course of M2DG and 2-deoxyglucose syntheses by ANGase from D-glucal. Reaction mixture (0.1 mL) containing 20 mM D-glucal, 20 mM sodium acetate buffer (pH 4.3), and ANGase (0.26 U) in 50% (v/v) methanol was incubated at 35 °C for 24 h. At the indicated times, the reaction mixture (10 μl) was pipetted out. Concentration of each carbohydrate was measured by HPAEC. ▲, D-Glucal; ○, 2-deoxyglucose; ●, M2DG.

in alkyl alcohols. Pelenc et al., Monsan et al., and Bousquet et al. have reported on industrial process for butyl α-glucoside production using ANGase and *Talaromyces duponti* α-transglucosidases.[20–23] They found that residual activity of ANGase was maintained over 50% of initial activity in 9% (v/v) of 1-butanol for 20 days.

How can ANGase retain high residual activity in high concentration alkyl alcohols than other α-glucosidases? Two possibilities were considered for the resistance in alkyl alcohols. The first possibility is that ANGase is a glycoprotein containing 25–27% carbohydrate most of which is mannose.[24] It is known that sugar chains on the surface of proteins protect the enzyme from denatured conditions. ANGase consisting of P1 and P2 subunits has 16 Asn residues putatively *N*-glycosylated.[13,24] Subsequent ANGase was treated with Endo H for the deglycosylation of the sugar chain, however, the deglycosylated ANGase showed the same residual activity as that of native ANGase in 30%, 50%, and 70% methanol for 4 h, meaning that the sugar chain is not a critical factor to keep the stability. ANGase has also five residues linked by putative *O*-glycosylated sugar chain,[9] but its role remains obscure.

The finding also implies that the protein moiety is important, that is, the second possibility. Even if α-glucosidase family II enzymes have homology in their sequence, the amino acids exposed to surface of protein are considered to be different. ANGase is thought to have hydrophilic residues in the surface area of protein to keep native protein conformation from denaturing by alkyl alcohols. Even if we elucidated the properties and amino acid sequence of crystalline ANGase,[24–29] its 3D structure is not available. It was reported that the modification of chymotrypsin by some hydrophilic reagents showed the strong stabilization of the enzyme against denaturation by organic solvents.[30]

2.2. M2DG from D-glucal by ANGase

The time course for synthesis of methyl 2-deoxyglucoside (M2DG) by ANGase in 50% methanol is shown in Figure 5. At the initial stage up to 5 h, the formation of M2DG, as well as 2-deoxyglucose (by-product made by hydration), was observed along with a sharp decrease in D-glucal on HPAEC. After 24 h incubation, the yield of M2DG reached 83%, while the yield of 2-deoxyglucose was 16%. Reaction conditions of temperature, pH, and D-glucal concentration were examined in 50% (v/v) methanol in order to increase the productivity of M2DG. The reaction mixture was incubated at temperatures of 25–60 °C for 6 h, and M2DG was obtained in the maximum at 35 °C. The optimal pH was observed around pH 4.5. The effects of D-glucal concentrations on the synthesis of M2DG were examined at various concentrations of D-glucal in the 50% methanol for 24 h. The formation of M2DG was proportional to the concentration of D-glucal up to 500 mM. D-Glucal concentration showed no effect on the final yield of M2DG (about 80%). This result implies that the high concentration of substrate is better for mass production of M2DG in the industrial field. Figure 6 shows the effects of methanol concentrations on the synthesis of M2DG. The production of M2DG was increased up to 70% (v/v) methanol, and 93% of D-glucal was converted into M2DG after 24 h. The productivity was steeply decreased more than 70% (v/v) of methanol.

ANGase showed 93% maximum yield production of M2DG at pH 4.5 and 35 °C in 70% methanol (Table 1). Produced M2DG was purified by a silica gel column chromatography to be homogenous on TLC and HPAEC. FD/MS and NMR analyses were preformed to confirm the structure of isolated compound. The molecular mass of M2DG was 178 by FD/MS spectrometry. The NMR spectra of M2DG indicated the formation of only α-2-deoxyglucosidic bond and the other signals were in accord with the proposed structure. Particularly, 2-deoxy form of M2DG was confirmed by the signals of H_{eq} and H_{ax} of the C_2 methylene observed at 2.13 and 1.71 ppm, respectively, which correspond into ^{13}C NMR signal of C_2 at 39.4 ppm. Consequently, isolated compounds were identified as methyl

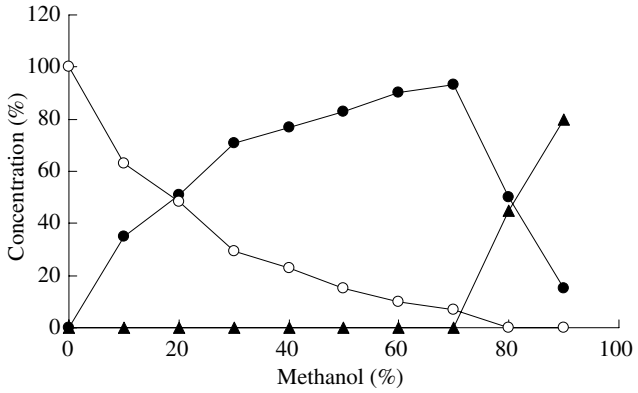


Figure 6. M2DG production from D-glucal by ANGase in methanol. Reaction mixture (0.1 mL) containing different concentration (10–90%, v/v) of methanol, 20 mM sodium acetate buffer (pH 4.3), 20 mM D-glucal and ANGase (0.26 U) was incubated at 35 °C for 24 h. At the indicated times, the reaction mixture (10 μL) was pipetted out. The reaction was stopped by heating for 5 min in boiling water. Concentration of each carbohydrate was measured by HPAEC. ▲, D-Glucal, ○, 2-deoxyglucose; ●, M2DG.

Table 1. Production of various A2DG

Alcohol	Water solubility	Product (%)	
		A2DG	2-Deoxyglucose
Methanol[a]	Miscible	93	6
Ethanol[b]		91	6
1-Propanol[b]		88	11
1-Pentanol[b]	Immiscible	83	16
1-Heptanol[b]		45	54

[a] The reaction mixture contained 70% alcohol.
[b] The reaction mixture contained 90% alcohol. Analytical method of products was described in the experimental section.

α-2-deoxyglucoside. From these results it was concluded that methyl α-2-deoxyglucoside was synthesized by the ANGase-catalyzed addition of methanol to D-glucal.

2.3. Synthesis of novel A2DG

The analytical scale synthesis of various A2DGs by ANGase was investigated by HPAEC (Table 1). The production of ethyl, 1-propyl, 1-pentyl, and 1-heptyl α-D-2-deoxyglucosides increased with increasing ethanol, 1-propanol, 1-pentanol, and 1-heptanol concentrations of up to 90% (v/v) from 20 mM D-glucal in 24 h-reaction, giving yields of 91%, 88%, 83%, and 45%, respectively. Newly synthesized A2DGs were obtained in high yield.

Enzymatic preparative-scale syntheses were performed using 70–90% alkyl alcohols as described in the experimental section. A2DGs were isolated according to two kinds of methods depending on the properties of alkyl moieties; (1) from the reaction mixture containing alcohols miscible with water, A2DGs were purified by a silica gel column chromatography and (2) from the reaction mixture containing alcohols immiscible with water, A2DGs were separated by liquid/liquid extraction. The latter method is simple and effective for isolating alcohol-soluble product, because A2DG having water-immiscible alkyl group remains in the organic phase. Each A2DG was obtained as follows: methyl 2DG (11.8 mg), ethyl 2DG (13.6 mg), 1-propyl 2DG (9.5 mg), 1-butyl 2DG (20.6 mg), 1-pentyl 2DG (20.2 mg), 1-hexyl 2DG (12.1 mg), 1-heptyl 2DG (10.3 mg), 1-octyl 2DG (5.2 mg), 2-methyl-1-propyl 2DG (20.6 mg), 2-propyl 2DG (20.4 mg), 2-butyl 2DG (10.4 mg), allyl 2DG (20.4 mg), cyclohexyl 2DG (6.5 mg), and benzyl 2DG (10.5 mg). The molecular mass of A2DGs was analyzed by FD/MS as described in the experimental section. ^{1}H NMR and ^{13}C NMR analyses of all A2DGs were performed. In particular, 1-butyl α-D-2-deoxyglucoside in D_2O and 1-pentyl α-D-2-deoxyglucoside in $(CD_3)_2SO$ were analyzed by secondary NMR (COSY, HMBC, HSQC). NMR study of A2DGs indicated the formation of only α-2-deoxyglucosidic bond and all signals in accord with the proposed structures. Alkyl group connected at the C_1 of 2-deoxyglucosyl residue was confirmed by the two dimensional NMR (HMBC) spectra.

It is difficult to synthesize glycosides by carbohydrases in two solvent phases of water and hydrophobic alcohols. However, in this experiment, the formation of A2DG was preceded in the water-insoluble alcohol system. Furthermore, most of the formed A2DGs remained in the organic layer, resulting in easy separation of product.

The addition and hydration of D-glucal occurs at the same time, meaning the two reactions are competitive with each other. If hydration is reduced, the addition becomes the main reaction. The alcohol itself, which also acts as substrate, results in shifting the thermodynamic equilibrium toward synthesis not only by the increase of alcohol (reactant) concentration but also by the low concentration of water (reducing hydrolysis). The enzymatic synthesis of A2DG from D-glucal was first examined in 70% methanol, 90% ethanol, 90% 1-propanol by ANGase, and A2DGs were obtained in the high yields of 93%, 91%, and 88%, respectively. It was found that ANGase had four benefits: (i) the simplicity of one step reaction, (ii) the high stability in alcohols, (iii) its strong addition activity, (iv) the wide acceptability of various alcohols.

ANGase shows the hydrolytic activity to M2DG, but its magnitude is very small, implying that A2DGs are candidates for inhibitors of α-glucosidase. Currently, we analyze the inhibition ability of A2DGs to α-glucosidase as well as glucoamylase.

3. Experimental

3.1. Materials

3.1.1. Enzyme and chemicals. The α-glucosidases from *A. niger*[24] pig serum,[31] buckwheat,[32] and *S. pombe*[14] were purified as previously reported. Maltose was further purified by repeated recrystallization. Fucose and 2-deoxyglucose were purchased from Nacalai Tesque

Chemical Inc. (Kyoto, Japan) and tri-*O*-acetyl-D-glucal from Sigma Chem. Co., (St. Louis, Mo, USA). All organic solvents used in this study were of the highest quality available from Nacalai Tesque Chemical Inc.

3.1.2. D-Glucal preparation. The 3,4,6-tri-*O*-acetyl-D-glucal (5 mmol) was deacetylated by freshly prepared sodium methoxide (0.025 mL) in 40 mL of dry methanol.[1] Mixture was maintained at 25 °C until reaction was completed as judged by TLC, then 3 g of silica gel 60 (Kieselgel 60) was added to the mixture. The suspension was dried under vacuum, and the dried powder was put on a 3 × 30 cm column of silica gel 60, followed by subjecting to chromatography with ethyl acetate/ethanol (5:1, v/v) as solvent. Fractions containing D-glucal were collected, and the solvent was evaporated to syrup. The syrup was further dried with ethanol and benzene, and then kept for 1–2 days in the vacuum desiccator over calcium sulfate (yield, 45–50%). D-Glucal (0.74 mg/mL) was kept in a dry methanol solution at −20 °C.

3.2. Biochemical assays

3.2.1. Measurements of enzyme activity. The enzyme concentration was measured spectrophotometrically taking $E^{1\%}_{1cm}$ at 280 nm to be 17.7, 12.9, 2.46, and 8.1 for α-glucosidase from *A. niger*[24] buckwheat,[32] *S. pombe*,[14] and pig serum,[31] respectively. The reaction mixture containing 0.2 mL of 0.5% maltose, 0.2 mL of 0.1 M buffer and 0.1 mL of enzyme solution was incubated at 35 °C. The sodium acetate buffer was used for α-glucosidase *A. niger* (pH 4.3), *S. pombe* (pH 4.5), and buckwheat (pH 5.0). For pig serum α-glucosidase, 0.1 M sodium phosphate buffer (pH 7.0) was used. The glucose liberated was measured by the Tris–glucose oxidase–peroxidase method with 'Glucose ARII-Test Wako' (Wako Pure Chemical Ind., Ltd, Japan).[3] One unit of α-glucosidase activity was defined as the amount of enzyme that hydrolyzed 1 μmol of maltose per minute under the above conditions.

3.2.2. Enzyme stability in methanol. Reaction mixture (0.1 mL), containing 50% of methanol, and enzyme of ANGase (0.68 U, pH 4.3), *S. pombe* α-glucosidase (0.65 U, pH 4.5), buckwheat α-glucosidase (0.66 U, pH 5.0) in 20 mM sodium acetate buffer, or pig serum α-glucosidase (0.65 U, pH 7.0) in 20 mM sodium phosphate buffer, was incubated at 35 °C for 4 h. At the indicated time, 10 μL were pipetted out and diluted 50–100 times with 20 mM buffer used. Then, 0.1 mL of each diluted enzyme solution, 0.2 mL of 0.5% maltose, and 0.2 mL of 20 mM sodium acetate buffer or 20 mM sodium phosphate buffer were incubated at 35 °C. Glucose liberated was measured by glucose oxidase–peroxidase method.[2] One hundred percent of the residual activity was defined as the enzyme activity without the methanol treatment.

3.2.3. Reaction conditions for formation of M2DG (methyl α-2-deoxyglucoside). For analysis of pH effects, reaction mixture (0.1 mL) containing 20 mM D-glucal, 20 mM sodium acetate buffer (pH 3.6–5.5) or 20 mM sodium phosphate buffer (pH 5.5–8.0), and ANGase (15 μg) in methanol (50%, v/v) was incubated at 35 °C for 6 h. The effects of D-glucal concentrations on the synthesis of M2DG were examined in 50% methanol with various concentrations of D-glucal for 24 h. Reaction mixture was incubated between 25 and 60 °C for 6 h for obtaining optimum temperature.

3.3. Deglycosylation of ANGase by Endo H

ANGase (200 μg) was treated with 50 munit (1.25 μg) of Endo H (Roche Diagnostics GmbH, Mannheim, Germany) in 50 mM sodium acetate buffer (pH 5.5) at 28 °C for overnight. The deglycosylation of ANGase by Endo H was confirmed by decreasing molecular mass in SDS-PAGE.[33]

3.4. Production and purification of A2DG

To synthesize A2DG, ANGase (0.51 U) in 20 mM sodium acetate buffer (pH 4.3) was incubated with 20 mM D-glucal and 70–90% (v/v) of alcohols at 35 °C in a final volume of 1 mL. After incubation, 0.02 mL of the medium was withdrawn, and heated in boiling water for 10 min to stop the reaction. The products were analyzed by TLC and HPAEC.

Fourteen kinds of alcohols were checked for preparative scale-synthesis of A2DG. In the synthetic reactions, for methanol, 1-butanol, 1-pentanol, ally alcohol, and 1-octanol, 70% (v/v) of concentration was used, and 90% (v/v) of concentration for ethanol, 1-propanol, 1-hexanol, 1-heptanol, cyclohexanol, 2-butanol, 2-methyl-1-propanol, benzyl alcohol, and 2-propanol. Reaction mixture (4 mL) containing ANGase (2.55 U), D-glucal (40 mM), in 40 mM sodium acetate buffer (pH 4.3) and 70% or 90% (v/v) alcohol as described above was incubated at 35 °C for 2 days on a rotary shaker (180 rpm).

In the reactions using alcohols highly soluble in water, the produced A2DGs were purified by a silica gel column (1.5 × 30 cm) with a mixture of acetonitrile–water (85:15, v/v) at 0.35 mL/min. In the reactions using poorly soluble alcohol in water, A2DG was isolated from the excess of 2-deoxyglucose and D-glucal by liquid–liquid extraction in the following manner: from the reaction mixture (4 mL) forming two-layer of aqueous phase and alcohol phase, the alcohol phase containing A2DG as well as small amounts of 2-deoxyglucose and D-glucal was transferred into new test tube, and then the equal volume of water was added. From the layers made again, the aqueous phase containing D-glucal and 2-deoxyglucose was discarded, and the same procedures were repeated 5–10 times.

3.5. Analysis of AD2G and carbohydrates

3.5.1. Thin layer chromatography (TLC). The reaction mixtures were put on TLC plate (Kiesel Gel G, Type 60, Merck Co.), and developed two times using a solvent system (acetonitrile:water = 85:15, v/v) with 2-deoxy-D-glucose (R_f: 1.62; R_f for glucose being 1.0) and D-glucal (R_f: 2.38) as the standards. Among the produced

A2DGs, methyl, ethyl, and propyl α-D-2-deoxyglucosides were migrated in the positions between 2-deoxyglucose and D-glucal, and other A2DGs showed the higher mobilities than D-glucal. The carbohydrates were visualized on the TLC plate by dipping into 0.03% (w/v) *N*-(1-naphthyl)ethylenediamine, 5% (v/v) H_2SO_4 in methanol, followed by heating at 120 °C for 5 min.[34]

3.5.2. High performance anion exchange chromatography (HPAEC). The reaction mixture (0.02 mL) was taken out at the indicated time, and diluted to 200 times. The sample (50 μL), filtered by Millex®-HV unit (Millipore, Bedford, MA, USA), was injected onto the HPAEC (Dionex, Osaka, Japan) equipped with a CarboPac PA-1 column (4 × 250 mm, Dionex). The carbohydrates were eluted in 16 mM NaOH with fucose as internal standard at a flow rate of 1 mL/min with monitoring by a pulsed amperometric detector (Dionex). Each carbohydrate and its amount were determined by the retention time and by the area ratio between a fucose and carbohydrate (0–500 μM), respectively.

3.5.3. MS and NMR analysis. The molecular mass of each A2DG was determined by FD–MS (JEOL JMS-SX102A, Tokyo, Japan). Isolated A2DG (approx. 10 mg) was dissolved in D_2O (0.5 mL, 99.98% D_2O) or $(CD_3)_2SO$ (0.5 mL, 99.96% of D). NMR spectra were recorded with a Bruker AMX-500 spectrometer operated at 500 MHz for the ^1H and at 125 MHz for ^{13}C nucleus. Chemical shifts (δ) were expressed in ppm relative to the external standard of TSP.

Methyl α-D-2-deoxyglucopyranoside; FD–MS: *m*/*z* 178, ^1H NMR δ_H (D_2O): δ 4.9, 3.85, 3.84, 3.76, 3.59, 3.35, 2.13 (H_{eq}), 1.89, 1.71 (H_{ax}); ^{13}C NMR δ_C (D_2O): δ 101.1, 74.9, 73.8, 70.9, 63.5, 57.2, 39.4.

Ethyl α-D-2-deoxyglucopyranoside; FD–MS: *m*/*z* 192, ^1H NMR δ_H (D_2O): δ 5.02, 3.86, 3.84, 3.77, 3.63, 3.35, 3.52, 2.12 (H_{eq}), 1.71 (H_{ax}), 1.19; ^{13}C NMR δ_C (D_2O): δ 99.6, 74.9, 73.8, 71.1, 65.9, 63.5, 39.5, 16.8.

Propyl α-D-2-deoxyglucopyranoside; FD–MS: *m*/*z* 206, ^1H NMR δ_H (D_2O): δ 5.01, 3.88, 3.87, 3.77, 3.74, 3.62, 3.34, 3.46, 2.13 (H_{eq}), 1.71 (H_{ax}), 1.59, 0.9; ^{13}C NMR δ_C (D_2O): δ 99.7, 74.98, 73.8, 72, 71.1, 63.5, 39.5, 24.7, 12.7.

Butyl α-D-2-deoxyglucopyranoside; FD–MS: *m*/*z* 220, ^1H NMR δ_H (D_2O): δ 4.99, 3.87, 3.84, 3.76, 3.61, 3.68, 3.34, 3.49, 2.12 (H_{eq}), 1.7 (H_{ax}), 1.56, 1.35, 0.89; ^{13}C NMR δ_C (D_2O): δ 99.8, 75, 73.8, 71.1, 70.1, 63.4, 39.5, 33.4, 21.6, 15.9.

Pentyl α-D-2-deoxyglucopyranoside; FD–MS: *m*/*z* 234, ^1H NMR δ_H (DMSO-d_6): δ 4.99, 3.86, 3.83, 3.75, 3.66, 3.61, 3.49, 3.34, 2.12 (H_{eq}), 1.7 (H_{ax}), 1.59, 1.32, 1.32, 0.87; ^{13}C NMR δ_C (DMSO-d_6): δ 99.7, 74.9, 73.8, 71.1, 70.4, 63.4, 31, 30.5, 24.6, 16.1.

Hexyl α-D-2-deoxyglucopyranoside; FD–MS: *m*/*z* 248, ^1H NMR δ_H (DMSO-d_6): δ 4.75, 3.59, 3.53, 3.44, 3.35, 3.25, 2.99, 2.29, 1.84 (H_{eq}), 1.41 (H_{ax}), 1.48, 1.26, 0.85; ^{13}C NMR δ_C (DMSO-d_6): δ 96, 73.1, 71.7, 68, 66.1, 61, 38.1, 31.1, 29, 25.5, 22.1, 13.9.

Heptyl α-D-2-deoxyglucopyranoside; FD–MS: *m*/*z* 262, ^1H NMR δ_H (DMSO-d_6): δ 4.76, 3.59, 3.53, 3.45, 3.28, 2.99, 2.44, 1.47, 1.84 (H_{eq}), 1.41 (H_{ax}), 1.26, 0.85; ^{13}C NMR δ_C (DMSO-d_6): δ 96.4, 73.1, 71.7, 67.4, 66.1, 38.1, 31.1, 29.1, 28.8, 28.7, 25.8, 22.1, 13.97.

Octyl α-D-2-deoxyglucopyranoside; FD–MS: *m*/*z* 276, ^1H NMR δ_H (DMSO-d_6): δ 4.76, 3.53, 2.99, 3.29, 3.44, 3.62, 3.0, 3.3, 1.48, 1.84 (H_{eq}), 1.4 (H_{ax}), 1.25, 0.86, 0.84; ^{13}C NMR δ_C (DMSO-d_6): δ 96.4, 73.1, 71.7, 67.4, 66.1, 61, 38.1, 31.1, 29.1, 28.8, 28.7, 25.8, 22.1, 13.97.

Benzyl α-D-2-deoxyglucopyranoside; FD–MS: *m*/*z* 254, ^1H NMR δ_H (DMSO-d_6): δ 7.35, 7.28, 4.77, 4.37, 3.68, 3.65, 3.48, 3.41, 3.05, 1.93 (H_{eq}), 1.48 (H_{ax}); ^{13}C NMR δ_C (DMSO-d_6): δ 138.2, 128.3, 127.7, 127.5, 95.9, 73.5. 71.8, 68.1, 68, 61.1, 37.9.

Cyclohexyl α-D-2-deoxyglucopyranoside; FD–MS: *m*/*z* 246, ^1H NMR δ_H (DMSO-d_6): δ 4.94, 3.59, 3.37, 3.33, 2.99, 1.62, 1.79 (H_{eq}), 1.42 (H_{ax}), 1.26, 1.18; ^{13}C NMR δ_C (DMSO-d_6): δ 9 4.4, 73.2, 71.9, 67.9, 61.1, 34, 33, 31.1, 25.3, 23.7, 23.5.

Allyl α-D-2-deoxyglucopyranoside; FD–MS: *m*/*z* 204, ^1H NMR δ_H (D_2O): δ 5.89, 5.23, 5.12, 4.82, 4.13, 4.06, 3.86, 3.61, 3.44, 3, 1.87 (H_{eq}), 1.44 (H_{ax}); ^{13}C NMR δ_C (D_2O): δ 134.9, 116.2, 95.9, 73.2, 71.6, 67.9, 66.6, 60.9, 37.8.

2-Methyl-1-propyl α-D-deoxyglucopyranoside; FD–MS: *m*/*z* 220, ^1H NMR δ_H (DMSO-d_6): δ 4.75, 3.61, 3.59, 3.44, 3.32, 3.29, 2.99, 1.77, 1.86 (H_{eq}), 1.42 (H_{ax}), 0.86, 0.84; ^{13}C NMR δ_C (DMSO-d_6): δ 96.5, 73.1, 72.7, 71.6, 67.9, 60.9, 38, 27.9, 19.4, 19.3.

2-Propyl α-D-2-deoxyglucopyranoside; FD–MS: *m*/*z* 206, ^1H NMR δ_H (D_2O): δ 5.14, 3.97, 3.84, 3.75, 3.66, 3.33, 1.89, 2.06 (H_{eq}), 1.72 (H_{ax}), 1.17; ^{13}C NMR δ_C (D_2O): δ 97.5, 75, 73.9, 72.1, 71.1, 63.5, 39.8, 25.1, 22.9.

2-Butyl α-D-2-deoxyglucopyranoside; FD–MS: *m*/*z* 220, ^1H NMR δ_H (D_2O): δ 4.87, 3.59, 3.56, 3.43, 3.37, 3.3, 2.99, 1.8 (H_{eq}), 1.35 (H_{ax}), 1.35, 1.06, 0.84; ^{13}C NMR δ_C (D_2O): δ 96.1, 93.2, 73.8, 70.8, 67.9, 61, 38.4, 28.4, 20.7, 19.4.

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