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α-Thioglycoligase-based synthesis of *O*-aryl α-glycosides as chromogenic substrates for α-glycosidases

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ABSTRACT

α-Thioglycoligases are retaining α-glycosidase mutants, with modification of their general acid/base catalytic residue to an inactive amino acid residue, catalyzing the formation of *S*-glycosidic linkages using a sugar donor with an excellent leaving group and suitable sugar acceptors with a thiol group as the substrate. In this study, we describe the enzymatic synthesis of *O*-aryl α-glycosides catalyzed by α-thioglycoligases. An α-xylosidase mutant (YicI-D482A) efficiently catalyzed the synthesis of *O*-aryl α-xylosides in near-quantitative yields (up to 99%) using 4-methylumbelliferone and nitrophenols. Synthesis did not occur with those acceptors having a nitro group at the ortho-position. The conversion yields of 3-nitrophenol markedly increased at pH 8.0, whereas those of other aryl compounds were nearly independent of pH, ranging from pH 6.0 to 8.0. The *O*-aryl α-xylosides were prepared on a preparative scale with yields of up to 96%. Upon employing the *O*-aryl α-xylosides as the substrate for the wild-type YicI, Brønsted relationships of log k_{cat} versus pK_a and log (k_{cat}/K_M) versus pK_a both showed a linear monotonic dependence on the leaving group pK_a with low β_{lg} values of 0.39 and 0.38, respectively. In addition, synthesis of *O*-aryl α-glucosides was successfully conducted by an α-glucosidase mutant (MalA-D416A) in the same fashion with high yields. Therefore, this strategy can be used for the synthesis of *O*-aryl α-glycosides using an acid/base mutant of retaining α-glycosidases that hydrolyze the glycosides.

1. Introduction

A wide variety of natural products, such as antibiotics and functional glycosides, consist of phenols and sugars that are connected through a glycosidic bond [1]. In addition, synthetic *O*-aryl glycosides with chromogenic or fluorogenic aglycons are invaluable substrates for the study of enzyme kinetics [2–4]. Therefore, the chemical synthesis of *O*-aryl glycosides is an interesting topic in carbohydrate chemistry. However, in many cases, these chemical reactions lead to a mixture of two stereoisomers that differ in the configuration of the anomeric center. Of the stereoisomers, 1,2-*trans*-glycosides (for example: β-glucosides, β-xylosides, and α-mannoside) were obtained almost exclusively through glycosylation procedures based on the neighboring group participation by a 2-*O*-acyl functionality [5]. In contrast, the introduction of 1,2-*cis*-glycosidic linkages using activated glycosyl donors with a nonassisting functionality at C-2 leads to the formation of an anomer mixture [6]. Recently, several stereoselective syntheses of 1,2-*cis*-glycosides, such as aryl α-glucosides and aryl α-galactosides, have been developed, but these reactions require laborious protection/deprotection processes and sometimes costly reaction conditions, including low temperatures (<0 °C) or reflux for several hours [7–9]. In the case of aryl α-xylosides, the general synthesis pathway has not yet been reported, and only 4-nitrophenyl α-D-xylopyranoside (4-NPαXyl) is commercially available.

On the other hand, enzymatic synthesis has emerged as a potent alternative to conventional chemical methods due to its high stereoselectivity and generally mild reaction conditions [10–13]. Although glycosyltransferases are necessary for the naturally occurring production of oligosaccharides and glycosides, glycosidases have attracted much attention in oligosaccharide synthesis due to the utilization of cheaper substrates than glycosyltransferases, which use expensive activated nucleotide sugars as substrates. Thus far, various glycosidases have been applied to the synthesis of oligosaccharides, glycosides and glycoconjugates [14–16], and several strategies for glycosidase engineering have addressed the problem of rehydrolysis of the glycosylated products by wild-type glycosidases [12,17–19]. The first successful engineered glycosidases termed glycosynthases are retaining glycosidase mutants in which the catalytic nucleophile has been converted to a non-nucleophilic residue [12,20]. These mutants catalyze the formation of a new *O*-glycosidic bond with the same

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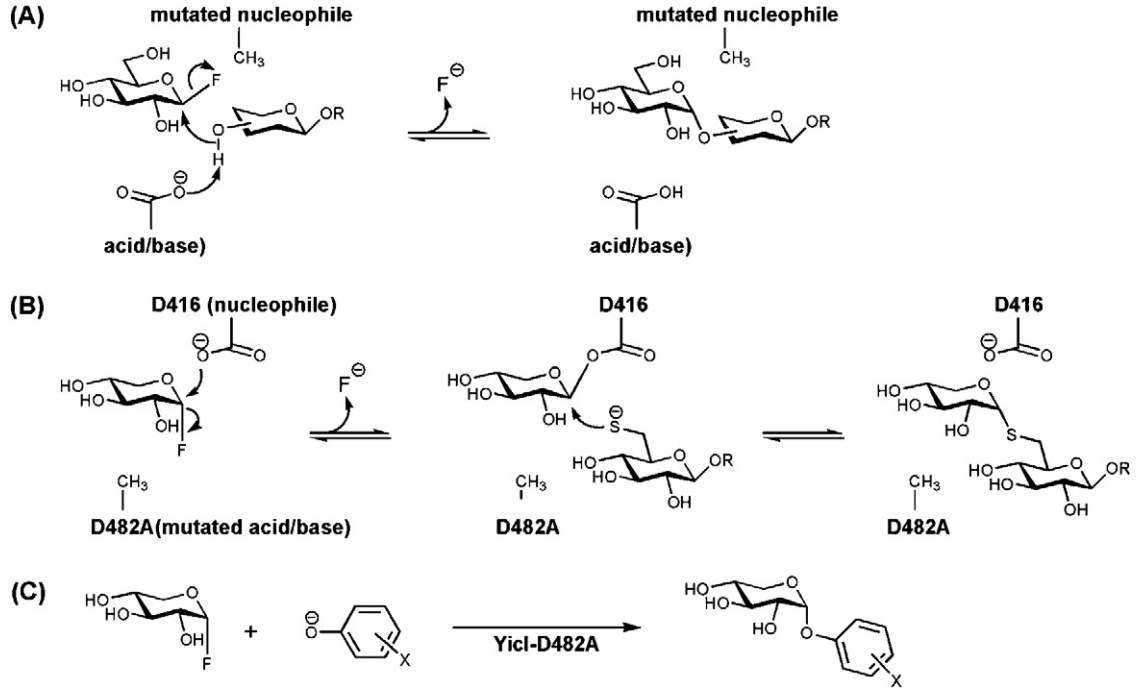

Fig. 1. Mechanism of transglycosylation catalyzed by a α-glycosynthase derived from a α-glucosidase (A [20]), YicI-D482A as a α-thioglycoligase (B [18]), and YicI-D482A for synthesis of *O*-aryl α-xylosides (C).

stereochemistry as the original substrates when glycosyl fluorides with the anomeric configuration opposite to that of the natural substrate, thereby mimicking the glycosyl enzyme intermediate, are employed as donor substrates (Fig. 1A). In contrast, thioglycoligases catalyze the formation of the *S*-glycosidic bond (Fig. 1B) [18,21]. The glycosidase mutants whose general acid/base catalyst is mutated to an inactive amino acid residue are capable of forming glycosyl enzyme intermediates using substrates with a good leaving group, such as dinitrophenol and fluoride. Upon employing suitable sugar acceptors with a more nucleophilic thiol group than the hydroxyl group, the intermediate turnover to free enzyme by nucleophilic attack of the thio-sugar acceptor results in the formation of *S*-glycosidic linkages.

Given the transglycosylation mechanism of thioglycoligases, aryl compounds possessing a hydroxyl group with a pK_a value lower than normal sugars would be used as the sugar acceptor, resulting in the formation of *O*-aryl glycosides instead of the thio-glycosides. Recently, two α-thioglycoligases (YicI-D482A and MalA-D416A) derived from a α-xylosidase from *Escherichia coli* (YicI) and a α-glucosdiase from *Sulfolobus solfataricus* (MalA), respectively, have been reported [18], raising the question of whether such mutants will function as described for the synthesis of *O*-aryl α-glycosides. This report describes the facile enzymatic synthesis of *O*-aryl α-glycosides using α-thioglycoligases (Fig. 1C). Two α-thioglycoligases (YicI-D482A and MalA-D416A) synthesized *O*-aryl α-glycosides with excellent yields, and kinetic analysis was conducted using *O*-aryl α-xylosides as a substrate for the wild-type enzyme.

2. Materials and methods

2.1. Materials and general analysis

α-D-Xylosyl fluoride (αXylF) and α-D-glucosyl fluoride (αGlcF) were synthesized as described previously [22]. 2-Nitrophenol (2-NP), 2,4-dinitrophenol (2,4-DNP), 2,5-dinitrophenol (2,5-DNP), 3-nitrophenol (3-NP), 3,4-dinitrophenol (3,4-DNP), 4-nitrophenol (4-NP), 4-methylumbelliferone (MU), 4-NPαXyl, 4-nitrophenyl α-D-glucopyranoside (4-NPαGlc), 4-methylumbelliferyl α-D-glucopyranoside (MUαGlc), an α-glucosidase from *Saccharomyces cerevisiae*, and silica gel (200–425 mesh) for flash chromatography were purchased from Sigma–Aldrich Co., Ltd. High-performance liquid chromatography (HPLC) was carried out using a YL9100 HPLC system (Younglin, Anyang, Korea) equipped with a Nova-Pak® C18 column (3.9 mm × 150 mm, Waters). The products were eluted at 0.8 mL/min with acetonitrile–water (20:80, v/v) and analyzed using an ultraviolet (UV) detector at 310 nm. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on a 300 MHz spectrometer from Varian Technologies Ltd. Mass spectra for aryl xylosides were recorded using an API 3200 triple quadrupole mass spectrometer (AB Sciex, Singapore). Thin-layer chromatography (TLC) was performed on aluminum-backed sheets of silica gel 60F_{254} (Whatmann, USA) of 0.2-mm thickness using 17.5:2:0.5 (v/v/v) ethyl acetate/methanol/water. The plates were visualized using UV light (254 nm) and/or by exposure to 10% sulfuric acid in methanol followed by charring. Wild-type YicI and two thioglycoligases (YicI-D482A and MalA-D416A) were obtained as described previously [18,23]. The protein concentration was determined by a Bradford method using serum albumin as a standard [24].

2.2. Transglycosylation of aryl compounds using mutant glycosidases

The transglycosylation reaction with 1 mM αXylF and an aryl compound (1 mM, 2 mM and 3 mM) as the donor and acceptor, respectively, and YicI-D482A (final concentration 5 mg/mL) was conducted in 200 mM sodium phosphate buffer at pH 6.0, 7.0 and 8.0. The reactions were carried out at 25 °C for 4 h. After monitoring by TLC, the reactions were terminated by adding a 19-fold volume of acetonitrile (v/v) followed by centrifugation of the mixtures at 10,000 rpm for 15 min to remove precipitated enzymes and buffer salts. To calculate the reaction yield, the samples were subjected to HPLC analysis. Standard curves for the aryl glycosides were determined by HPLC using the corresponding aryl glycosides

purified from the enzymatic reactions on a preparative scale as the standards. The reaction conditions for MalA-D416A (final concentration 5 mg/mL) were conducted in 200 mM sodium phosphate buffer (pH 8.0) containing αGlcF (1 mM) and an aryl compound (2 mM) as the donor and acceptor, respectively. In order to confirm the anomeric configuration of the aryl glucosides, a α-glucosidase from *S. cerevisiae* (Sigma) was added in the reaction mixture (final 2 U/mL). After 2 h incubation at 37 °C, the reaction mixtures were analyzed by TLC.

2.3. Kinetic analysis of hydrolysis catalyzed by wild-type YicI

Kinetic analysis was performed by measuring the enzymatic activity using the aryl xylosides as the substrates at 30 °C in 50 mM phosphate buffer (pH 7.0). Fifty microliters of wild-type YicI, preincubated at 30 °C, were added to 50-μL buffer solution containing the aryl xylosides ranging from 0.2 to 2.5 mM, which were also preincubated at the corresponding temperature. The release of phenolic compounds was monitored at 400 nm using a plate reader (SPECTRA^{Max}, Molecular Devices Corporation, USA). Standard curves for the aryl compounds were determined by measuring the absorbance of the corresponding free aryl compounds at 400 nm at pH 7.0 and 30 °C. In the case of MUαXyl, the amount of released MU was measured using a spectrofluorimeter (VICTOR^3 1420 Multilabel plate readers; Perkin-Elmer, USA) with excitation and emission wavelengths of 365 and 450 nm, respectively. The standard curve for MU was determined by measuring the fluorescence signal using the spectrofluorimeter at pH 7.0 and 30 °C using MU as the standard. Kinetic parameters were obtained by a direct fit of the data to the Michaelis–Menten equation using GraFit version 7.0 software (Leatherbarrow, R.J., Erithacus Software Ltd., Staines, UK).

2.4. Production of aryl xylosides using YicI-D482A on a preparative scale

A mixture of αXylF (0.13 mmole) and an aryl compound (0.26 mmole) in phosphate buffer (5 mL of 0.2 M, pH 8.0) was treated with YicI-D482A (5 mg/mL). The mixture was then incubated at 25 °C for 4 h. To purify the aryl xylosides, the reaction mixtures were subjected to a C18 SEP PAK cartridge (Waters) to remove free sugars, enzyme and salts, and the solvent was then evaporated under reduced pressure. Transfer products were purified by silica-gel flash chromatography (EtOAc/MeOH/H_2O = 17.5:2:0.5). The isolated products were analyzed and identified by NMR and ESI-MS.

2.4.1. 4-Nitrophenyl-α-D-xylopyranoside (4-NPαXyl)

White powder (32.7 mg, 93%). ^{1}H NMR (CD_3OD, 300 MHz): 8.25 (m, 2H, Ph-H), 7.30 (m, 2H, Ph-H), 5.63 (d, 1H, J = 4.5 Hz, H-1), 3.80–3.50 (m, 5H, the rest protons of sugar ring). ESI-MS: calc. for $C_{11}H_{13}NO_7 + Na^+$ = 294.2; found 294.2.

2.4.2. 3-Nitrophenyl-α-D-xylopyranoside (3-NPαXyl)

White powder (31.5 mg, 89%). ^{1}H NMR (CD_3OD, 300 MHz): 7.98 (m, 1H, Ph-H), 7.90 (m, 1H, Ph-H), 7.58 (m, 1H, Ph-H), 7.53 (m, 1H, Ph-H), 5.58 (d, 1H, J = 3.9 Hz, H-1), 3.70–3.50 (m, 5H, the rest protons of sugar ring). ESI-MS: calc. for $C_{11}H_{13}NO_7 + Na^+$ = 294.2; found 294.2.

2.4.3. 3,4-Dinitrophenyl-α-D-xylopyranoside (3,4-DNPαXyl)

Yellow powder (36.4 mg, 89%). ^{1}H NMR (CD_3OD, 300 MHz): 8.14 (d, 1H, J = 9.0 Hz, Ph-H), 7.66 (d, 1H, J = 2.4 Hz, Ph-H), 7.49 (dd, 1H, J = 2.7 Hz, J = 9.0 Hz, Ph-H), 5.70 (d, 1H, J = 3.9 Hz, H-1), 3.80–3.60 (m, 5H, the rest protons of sugar ring). ESI-MS: calc. for $C_{11}H_{12}N_2O_9 + Na^+$ = 339.2; found 339.2.

2.4.4. 4-Methylumbelliferyl-α-D-xylopyranoside (MUαXyl)

Yellow powder (38.2 mg, 96%). ^{1}H NMR (CD_3OD, 300 MHz): 7.73 (m, 1H, Ph-H), 7.15 (m, 1 H, Ph-H), 7.11 (m, 1H, Ph-H), 5.60 (d, 1H, J = 3.9 Hz, H-1), 3.70–3.60 (m, 5H, the rest protons of sugar ring). ESI-MS: calc. for $C_{15}H_{16}O_7 + Na^+$ = 331.2; found 331.2.

3. Results and discussion

3.1. Transglycosylation of 4-nitrophenol by YicI-D482A

First, the transglycosylation activity of wild-type YicI and YicI-D482A were compared using αXylF and 4-NP as the donor and acceptor, respectively. αXylF rapidly disappeared in the reaction catalyzed by YicI, and the transglycosylation product was rarely detected by TLC analysis. In contrast, the reaction catalyzed by YicI-D482A yielded one transglycosylation product (4-NPαXyl), which was detected by TLC analysis. Given the previous kinetic analysis of YicI-D482A, the YicI mutant should be capable of hydrolyzing α-XylF with the catalytic efficiency (k_{cat}/K_M) with three orders of magnitude lower than the wild-type enzyme, whereas 4-NPαXyl was an inert substrate for YicI-D482A [19]. YicI-D482A formed a covalent xylosyl-enzyme intermediate when αXylF was used as the substrate. This was followed by a transfer of the xylose moiety to the hydroxyl group of 4-NP, and the transfer product then accumulated in the reaction mixture due to an absence of hydrolysis of the expected product. Due to the spontaneous and enzymatic hydrolysis of αXylF, use of an equimolar ratio of αXylF and 4-NP was not sufficient to achieve the maximum yield of the transfer product. To improve the transglycosylation yield, the amount of 4-NP was increased to overcome the competition with water molecules. Use of a 1:2 ratio of αXylF to 4-NP resulted in improved production of the product, whereas the addition of more 4-NP (molar ratio of αXylF to 4-NP up to 1:3) decreased the quantity of product (Fig. 2). Previously, it was reported that YicI-D482A possessed not only thioglycoligase activity but also highly regiospecific transglycosylation activity, termed *O*-glycoligase activity, to transfer the xylose moiety from αXylF to the 6-OH group of both glucoside and mannoside but not to that of galactoside and xyloside lacking the 6-OH group [19]. Given the sugar-acceptor specificity of YicI-D482A no second transfer product was observed by TLC or HPLC analysis. The transfer product was purified using flash column chromatography and subjected to detailed structural investigation by ^{1}H NMR analysis. The NMR data described in Section 2 revealed a chemical shift from 9 to 7 ppm, which represents the phenolic group,

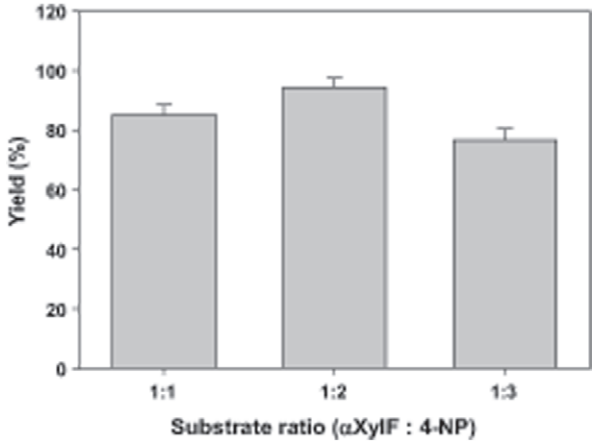

Fig. 2. Effect of the αXylF to 4-NP ratio on the 4-NPαXyl yield using YicI-D482A.

Table 1
Transglycosylation catalyzed by YicI-D482A and yields of the transfer products at different pH.[a]

Aryl compound	pK_a	Product	Conversion yield (%)[b]		
			pH 6	pH 7	pH 8
4-NP	7.15	4-NPαXyl	92	93	99
3-NP	8.39	3-NPαXyl	23	28	94
3,4-DNP	5.36	3,4-DNPαXyl	95	95	97
MU	7.79	MUαXyl	95	97	99

[a] Reaction condition: 1 mM αXylF, 2 mM aryl compound, 5 mg/mL YicI-D482A in 200 mM phosphate buffer at 25 °C for 4 h.
[b] The conversion yields were calculated using the amount of the product determined by HPLC analysis based on the amount of the employed αXylF.

while the xylose moiety showed a chemical shift at high-field from 6 to 3 ppm. The chemical shift of H-1 harbored by 4NPαXyl showed typical double peaks around 5.63 ppm. It is worth noting that the ^{1}H NMR spectra exhibited a small coupling constant for H-1 signal (J = 4.5), revealing that the aryl product is a 1,2-*cis*-xyloside, as expected for an α-anomer.

3.2. Acceptor specificity of YicI-D482A for the synthesis of aryl xylosides

To investigate sugar-acceptor specificity, various aryl compounds were tested at pH 7.0. Given the results from the reaction using 4-NP as the sugar acceptor, the ratio of αXylF to an aryl compound was fixed to 1:2. TLC analysis revealed that 3,4-DNP, 4-NP, and a structurally different acceptor, MU, were excellent sugar acceptors with yields up to 97%, while 3-NP was a weaker acceptor with a poor yield (<30%, Table 1). Unexpectedly, three kinds of ortho-substituted nitrophenols, such as 2-NP, 2,4-DNP and 2,5-DNP, did not function as the sugar acceptor (data not shown). This was likely caused by the steric hindrance between the ortho-nitro group and the active site of YicI-D482A. Such acceptor specificity can be properly explained by previous analysis of the complex structure of YicI with a thio-linked substrate analog [18]. Given the structure of the +1 subsite, YicI-D482A is not expected accommodate enough space for binding of the ortho-nitro group of the unsuccessful nitrophenols in the +1 subsite. The ortho-nitro group would clash with either Asp185 and Arg466 or Phe277 and Trp380 of YicI (Fig. S1, Supplementary Information).

3.3. pH effect on aryl xyloside synthesis and preparative production

To determine the optimum pH conditions, reactions using the four aryl compounds as acceptors were conducted at pH 6.0, 7.0 and 8.0. HPLC analysis revealed that the yields of transfer products from the reaction using 3,4-DNP, 4-NP and MU slightly increased with pH. Interestingly, the yield of 3-NPαXyl markedly increased at pH 8.0 (94%, Table 1). Of the aryl compounds that yielded transfer products, 3-NP had the highest pK_a value (8.39), suggesting that the improved yield for 3-NPαXyl at pH 8.0 was due to enhancement of the nucleophilicity of the hydroxyl group of 3-NP at a basic pH.

Based on the optimization results, a 5-mL scale reaction was conducted with 26 mM αXylF and 52 mM each of the aryl compounds (3-NP, 4-NP, 3,4-DNP, and MU) as the substrates at pH 8.0. The reactions were completed within 4 h. The transfer product was purified using flash column chromatography and subjected to detailed structural investigation by ^{1}H NMR analysis. As described in Section 2, the ^{1}H NMR spectra of all transfer products showed a typical double peak of H-1 with the chemical shift around 5.6 ppm and a small coupling constant (J = 3.9 or 4.5), indicating that these aryl xylosides were α-anomers.

3.4. Kinetic analysis of aryl α-xylosides as wild-type YicI substrates

The aryl glycosides bearing different leaving groups are chromogenic substrates that can be used for detailed kinetic analysis of retaining glycosides [2–4]. The kinetic parameters of YicI were measured for the hydrolysis of the aryl α-xyloside substrates. Hydrolysis rates over substrate concentrations were fit to the Michaelis–Menten equation (Fig. S2, Supplementary Information). From this, the kinetic parameters for these substrates were determined (Table 2). Comparison of the K_M values for the aryl α-xylosides revealed that there was no significant difference in the apparent binding of these substrates (0.99–1.5 mM). The main difference between these substrates was in their k_{cat} values, with 3,4-DNPαXyl being ~7-fold greater than that of 4-NPαXyl, which was 11-fold greater than that of MUαXyl. A Brønsted plot representing the relationship of the rate of cleavage of a series of aryl xylosides with the pK_a of the corresponding phenol allows us to understand the mechanism of glycosidases. The kinetic parameters of the aryl xylosides were plotted in the form of Brønsted relationships (Fig. 3). The log k_{cat} versus pK_a and log (k_{cat}/K_M) versus pK_a plots clearly revealed a dependence, albeit shallow (β_{lg} = −0.39 and −0.38, respectively) rate on the leaving group ability, suggesting that the rate-limiting step involves breakage of the aryl glycoside bond to form the covalent glycosyl enzyme intermediate.

In the case of retaining β-glycosidases, a biphasic relationship is quite common in Brønsted plots, that is one of evidences on the mechanism of retaining β-glycosidases via formation of the covalent glycosyl enzyme intermediate [3]. Although any break in these plots for YicI, one of retaining α-glycosidases was not observed, such linear monotonic dependence on leaving group pK_a with low β_{lg} values of ~−0.4 is consistent with previous studies on α-1,4-glucan lyase belonging to the same glycoside hydrolase family as YicI [2].

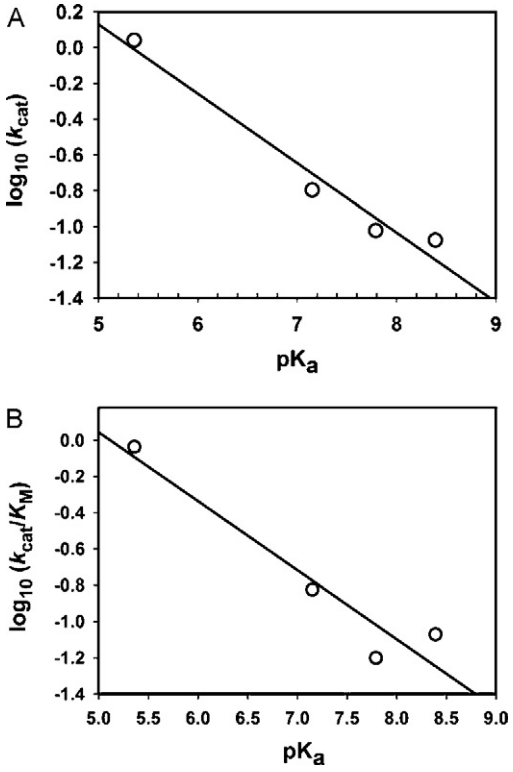

Fig. 3. Brønsted plot constructed from the data in Table 2 showing the relationship between the rate of cleavage of a series of aryl xylosides with the pK_a of the corresponding phenol. (a) Log k_{cat} vs. pK_a and (b) log (k_{cat}/K_M) vs pK_a.

Table 2
Kinetic parameters for hydrolysis of aryl α-xylosides by YicI.[a]

	pK_a [b]	k_{cat} (s^{-1})	K_M (mM)	k_{cat}/K_M (s^{-1} mM^{-1})	Ref.
αXylF	–	75 ± 3	0.97 ± 0.13	77	[23]
4-NPαXyl	7.15	0.16 ± 0.006	1.08 ± 0.04	0.15	This study
3-NPαXyl	8.39	0.084 ± 0.003	0.99 ± 0.06	0.085	This study
3,4-DNPαXyl	5.36	1.1 ± 0.034	1.20 ± 0.14	0.92	This study
MUαXyl	7.79	0.095 ± 0.006	1.51 ± 0.09	0.063	This study

[a] Reaction condition: 50 mM phosphate buffer (pH 7.0) at 30 °C.
[b] pK_a value of the aglycon of the corresponding xyloside.

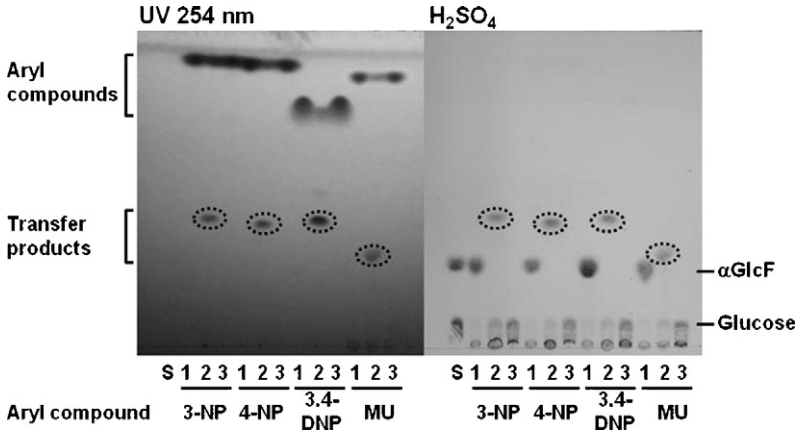

Fig. 4. TLC analysis of MalA-D416A-catalyzed reactions. The TLC plate was visualized by UV at 254 nm (left) and by sulfuric acid followed by charring (right). Transfer products are indicated by dotted circles. Lane S: standard including αGlcF and glucose; lane 1: blank reaction without MalA-D416A; lane 2: reaction mixture with MalA-D416A; lane 3: hydrolysis reaction mixture with α-glucosidase.

3.5. Synthesis of O-aryl α-glucosides using MalA-D416A

The successful synthesis of *O*-aryl α-xylosides using YicI-D482A raised the question as to whether such a strategy can be expanded to the synthesis of other *O*-aryl α-glycosides. Therefore, transglycosylation reactions catalyzed by another α-thioglycoligase, MalA-D416A, derived from an α-glucosidase [18], were carried out with αGlcF and aryl compounds as the sugar donors and acceptors, respectively, in 0.2 M phosphate buffer (pH 8.0). As shown in Fig. 4, MalA-D416A also catalyzed the synthesis of aryl α-glucosides. The aryl compounds that were transformed by MalA-D416A were 3,4-DNP, 3-NP, 4-NP, and MU as sugar acceptors (Fig. 4, lane 2 of each aryl compound), whereas the ortho-substituted nitrophenols, including 2-NP, 2,4-DNP and 2,5-DNP did not function as the sugar acceptors (data not shown). These results were consistent with those of YicI-D482A. From this, YicI and MalA, which belong to the same glycoside hydrolase family 31, share the same active site architecture [25] that would lead to such acceptor specificity. Given the mechanism of retaining α-glucosidase and the results of YicI-D482A experiments, the synthesized aryl glucosides were expected to be α-anomers. Although NMR analysis can be used to determine the anomeric configuration of these aryl glucosides, the direct evidence can arise from the result of the hydrolysis of the products by wild-type α-glucosidase. Upon employment of α-glucosidase (2 U/mL) in the reaction mixture, TLC results showed that the spots of each product disappeared completely, while those corresponding to glucose were detected (Fig. 4, lane 3 for each aryl compounds). This suggests that the anomeric configuration of all aryl glucosides synthesized by MalA-D416A was that of α-anomer. Using standard curves of the glucosides purchased from Sigma, the yields of the 4-NPαGlc and MUαGlc, as determined by HPLC, were 83% and 75%, respectively, based on the donor sugar employed.

4. Conclusions

We demonstrate here for the first time the facile enzymatic synthesis of *O*-aryl α-glycosides, which was developed using α-thioglycoligases derived from two retaining α-glycosidases. These α-glycosidase mutants convert non-ortho-substituted nitrophenols and MU to the corresponding aryl sugars with near quantitative yields. Glycosylation of the aryl compounds catalyzed by the engineered glycosidases was dependent of the nucleophilicity of the hydroxyl group to which the glycosyl moiety would be transferred. Such thioglycoligase-based aryl glycoside synthesis strategies can be used to synthesize *O*-aryl glycosides using an acid/base mutant of retaining glycosides that hydrolyze the glycosides.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molcatb.2012.10.008.

References

[1] S.H. Kim, C. Naveen Kumar, H.J. Kim, D.H. Kim, J. Cho, C. Jin, Y.S. Lee, Bioorg. Med. Chem. Lett. 19 (2009) 6009–6013.
[2] S.S. Lee, S. Yu, S.G. Withers, Biochemistry 42 (2003) 13081–13090.
[3] T. Bravman, G. Zolotnitsky, V. Belakhov, G. Shoham, B. Henrissat, T. Baasov, Y. Shoham, Biochemistry 42 (2003) 10528–10536.
[4] F.S. Shidmoossavee, L. Cheng, J.N. Watson, A.J. Bennet, Biochemistry 49 (2010) 6473–6484.
[5] G.J. Boons, Contemp. Org. Synth. 3 (1996) 173–200.
[6] A.V. Demchenko, Synlett (2003) 1225–1240.
[7] S.S. Chang, C.C. Lin, Y.K. Li, K.K. Mong, Carbohydr. Res. 344 (2009) 432–438.
[8] J. Kadokawa, T. Nagaoka, J. Ebana, H. Tagaya, K. Chiba, Carbohydr. Res. 327 (2000) 341–344.
[9] H.M. Chen, S.G. Withers, ChemBioChem 8 (2007) 719–722.
[10] A. Trincone, Recent Pat. Biotechnol. 4 (2010) 30–47.
[11] A. Trincone, A. Giordano, Curr. Org. Chem. 10 (2006) 1163–1193.
[12] S.M. Hancock, M.D. Vaughan, S.G. Withers, Curr. Opin. Chem. Biol. 10 (2006) 509–519.
[13] P. Bojarová, V. Kren, Trends Biotechnol. 27 (2009) 199–209.
[14] H.S. Lee, J.H. Auh, H.G. Yoon, M.J. Kim, J.H. Park, S.S. Hong, M.H. Kang, T.J. Kim, T.W. Moon, J.W. Kim, K.H. Park, J. Agric. Food Chem. 50 (2002) 2812–2817.
[15] C.H. Choi, S.H. Kim, J.H. Jang, J.T. Park, J.H. Shim, Y.W. Kim, K.H. Park, J. Sci. Food Agric. 9 (2010) 1179–1184.
[16] L.X. Wang, W. Huang, Curr. Opin. Chem. Biol. 13 (2009) 592–600.
[17] G. Osanjo, M. Dion, J. Drone, C. Solleux, V. Tran, C. Rabiller, C. Tellier, Biochemistry 46 (2007) 1022–1033.
[18] Y.W. Kim, A.L. Lovering, H. Chen, T. Kantner, L.P. McIntosh, N.C. Strynadka, S.G. Withers, J. Am. Chem. Soc. 128 (2006) 2202–2203.
[19] Y.W. Kim, R. Zhang, H. Chen, S.G. Withers, Chem. Commun. 46 (2010) 8725–8727.
[20] M. Okuyama, H. Mori, K. Watanabe, A. Kimura, S. Chiba, Biosci. Biotechnol. Biochem. 66 (2002) 928–933.
[21] M. Jahn, J. Marles, R.A.J. Warren, S.G. Withers, Angew. Chem. Int. Ed. 42 (2003) 352–354.
[22] M. Hayashi, S. Hashimoto, R. Noyori, Chem. Lett. 10 (1984) 1747–1750.
[23] A.L. Lovering, S.S. Lee, Y.W. Kim, S.G. Withers, N.C.J. Strynadka, J. Biol. Chem. 280 (2005) 2102–2125.
[24] M. Bradford, Anal. Biochem. 72 (1976) 248–254.
[25] H.A. Ernst, L. Lo Leggio, M. Willemoës, G. Leonard, P. Blum, S. Larsen, J. Mol. Biol. 358 (2006) 1106–1124.