SUPPORTING INFORMATION

O-Glycoligases, a new synthetic category of mutant glycosidases, catalyse facile syntheses of isoprimeverosides

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Experimental Procedures and NMR data

Materials – Pwo polymerase was purchased from Roche (Germany) and restriction enzymes from Fermentas (Germany). α-D-Xylopyranosyl fluoride (αXylF) was synthesized according to literature procedures. 1 4-Nitrophenyl β-D-glucopyranoside (pNPβGlc), 4-nitrophenyl β-D-galactopyranoside, 4-nitrophenyl β-D-mannopyranoside, 4-nitrophenyl β-D-xylopyranoside, 4-nitrophenyl β-D-N-acetyl glucosamine, 4-nitrophenyl β-D-N-acetyl galactosamine and 4-nitrophenyl α-D-xylopyranoside were purchased from Sigma Chemical Co.

General experiments – All 1H and 13C nuclear magnetic resonance spectra were recorded at 400 MHz using a Bruker AV-400 spectrometer. Mass spectrometry for small molecules was recorded using a PE-Sciex API 300 triple quadrupole mass spectrometer (Sciex, Thornhill, ON, Canada) equipped with an electrospray ionization ion source. Thin layer chromatography (TLC) was performed on aluminum-backed sheets of silica gel 60F254 (Merek) of thickness 0.2 mm. The plates were visualized using UV light (254 nm) and/or by exposure to 10 % sulfuric acid in methanol followed
by charring. The SEP-PAK C18-cartridge for solid phase extraction of phenyl compounds was purchased from Waters Corporation (Division of Millipore, Milford, MA, USA). Silica flash column chromatography was carried out using Siliaflash F60 (230 +/- 400 mesh). The analyses of DNA sequences were carried out by the Nucleic Acid and Proteins Service Unit in the Michael Smith Laboratories at the University of British Columbia.

**Site-directed mutagenesis of the +1 subsite of wild type YicI and YicI-D482A** — Substitutions of an Ala residue for Trp8, Asp49, and Asp185 of YicI were carried out using the mega-primer method. The primers used in the mutation of W8A (Trp8 →Ala) were BLATG primer (5’-ATC GAC TTT GTA GGG T-3’), T7-terminator primer (5’-TCG TAG TTA TTG CTC AGC GG-3’), and W8A-rev primer (5’-GAG GCC AGG TTG AAT CAA CGC GTT TCC ATC-3’). BLATG primer and W8A-rev primer were used first, and the resulting PCR product was purified by QIAquick gel extraction kit (Qiagen) on a 1% agarose gel. For amplification of the full sized gene, 20 ng of the quantitated PCR product as a mega primer and T7 terminator primer were used. The resulting PCR product was purified and subcloned into pTKNd119 after digestion with NdeI and XhoI. Primers D49A-rev (5’-AAG GCG TCG CAA GCT GCC AGG TAC GTT CAC-3’) and D185A-rev (5’-TGT GCC GCC CGC CCG GTT CCA GGT CTC TAC-3’) were used for a mutagenesis for D49A (Asp49 →Ala) and for D185A (Asp185→Ala), respectively, instead of the W8A-rev primer. Plasmid pBLYicI(His) were used as templates.

**Purification of YicI and its mutant enzymes** — To purify wild type YicI and its mutants from *E. coli* TOP10 cells harboring the corresponding genes on pTKNd119, affinity chromatography using nickel-nitrotriacetate agarose (QIAGEN) was conducted as described previously. The desalting and concentration of purified enzyme solutions were carried out using an Amicon Ultra-4 filter unit (10,000 Da cut-off, Millipore). The buffer used in enzyme purification was changed to 50 mM potassium phosphate buffer/pH 7. Protein concentrations were determined by the Bradford method using
bovine serum albumin as a standard.

**Kinetic analysis for hydrolysis catalyzed by YicI and its derivatives** – All kinetic studies were performed at 30 °C in pH 7.0, 100 mM phosphate buffer. Twenty microliters of wild type YicI (or one of its variants) was added to 100 μL of buffer containing varying amounts of pNPαXyl. Hydrolysis activity for 4-nitrophenyl-β-D-isoprimervoside (pNPβIP) was measured by a coupled assay adopting a β-glucosidase from *Agrobacterium* sp.³ as an auxiliary enzyme. In this coupled assay, YicI and its derivatives produce D-xylose and pNPβGlc, which is further hydrolyzed by the β-glucosidase to generate p-nitrophenol (pNP). The release of pNP was monitored at 400 nm using a microplate reader (SPECTRAMax plus, Molecular Devices Corporation). Upon using αXylF as the substrate, an Orion fluoride electrode (model 96-09BN) interfaced with a Fischer Scientific Accumet 925 pH/ion meter was used to monitor fluoride release during reaction. All enzymatic rates were corrected for the spontaneous hydrolysis rate of αXylF. The values of $K_m$ and $k_{cat}$ were determined by fitting the initial velocity curves to the Michaelis-Menten equation using non-linear regression with the program GraFit (Erithacus Software Ltd., Staines, UK).

**Kinetic analysis for transglycosylation catalyzed by YicI-D482A and its derivatives** – The concentration of either αXylF or pNPβGlc was fixed and that of the counterpart was varied to allow $K_m$ and $k_{cat}$ determinations. The amount of released fluoride ion was determined using the Orion fluoride electrode. All enzymatic rates were corrected for the spontaneous hydrolysis rate of the xylosyl fluoride. The program GraFit was used to calculate kinetic parameters.

**Transglycosylation and isolation of transfer products with YicI-D482A** – The transglycosylation reactions were carried out with 0.1 mg YicI-D482A per μmol of acceptor at room temperature in 100 mM sodium phosphate buffer, pH 7.0. Reactions were monitored by TLC. Upon completion, the reaction mixtures were subjected to a C18 SEP PAK cartridge (Waters) to remove non-aryl sugars. The cartridge was washed with 6 mL of water and then the pNP-sugars were eluted with 4 mL of 50 % (v/v) methanol.
4-Nitrophenyl [(2,3,4-tri-O-acetyl-α-D-xylopyranosyl)-(1→6)-O-2,3,4-tri-O-acetyl-β-D- glucopyranoside (1). A mixture of the αXylF (29.9 mg 196 μmol) and pNPGlc (37.6 mg, 125 μmol) in phosphate buffer (5 mL of 0.1 M, pH 7.0) was treated with YicID482A (12 mg) and the mixture was incubated (42 h, 25 °C). The aryl glycoside products were purified using a C18 SEP PAK cartridge, then the solvent was evaporated under reduced pressure. The residue was acetylated in pyridine (2 mL) and Ac₂O (2 mL) overnight at RT. The solvent was co-evaporated with MeOH under reduced pressure. The residue was chromatographed on Siliaflash F60 (230 +/ 400 mesh) using (petroleum ether/EtOAc = 6:4) to give 1 (82.6 mg, 98 %). ¹H NMR (CDCl₃, 400 MHz): δ 8.30 (m, 2H, Ar-H), 7.12 (m, 2H, Ar-H), 5.47 (t, 1H, J₃',₄' 10.0 Hz, H-3’), 5.31 (t, 1H, J₃,₄ 8.8 Hz, H-3), 5.26 (t, 1H, J₂,₃ 9.2 Hz, H-2), 5.17 (d, 1H, J₁,₂ 7.6 Hz, H-1), 5.00 (t, 1H, J₄,₅ 10.0 Hz, H-4), 4.97 (d, 1H, J₁',₂' 3.2 Hz, H-1’), 4.88 (dt, 1H, J₄',₅'a = J₄',₅'b 6.0 Hz, H-4’), 4.78 (dd, 1H, J₂',₃' 10.0 Hz, H-2’), 3.97 (m, 1H, H-5), 3.78 (dd, 1H, J₅,a₃₅,b 8.0 Hz, J₆a₃₅,b 10.4 Hz, H-6a), 3.45 (dd, 1H, J₄',₅'a = J₄',₅'b 6.0 Hz, H-5’a), 3.43 (dd, 1H, J₅,a₃₅,b 2.4 Hz, H-6b); 2.08-1.73 (6 s, 18 H, 6 × CH₃CO). ¹³C NMR (CDCl₃, 100 MHz): δ 170.16, 170.06, 170.03, 169.70, 169.40, 169.19, 161.24, 143.32, 126.22 (2C), 116.50 (2C), 98.14, 95.69, 73.36, 72.41, 70.97, 70.95, 68.99, 68.85, 68.72, 66.22, 58.41, 20.81, 20.74, 20.64, 20.61, 20.56, 20.52, 20.16. ESIMS: Calcd for [C₂₉H₃₅NO₁₇+Na]⁺: 692.2. Found: 692.2.

4-Nitrophenyl [(2,3,4-tri-O-acetyl-α-D-xylopyranosyl)-(1→6)-O-2,3,4-tri-O-acetyl-β-D- mannopyranoside (2). A mixture of the αXylF (12.8 mg, 84 μmol) and pNPMan (15 mg, 50 μmol) in phosphate buffer (3 mL of 0.1 M, pH 7.0) was treated with YicID482A (5 mg) and the mixture then incubated (48 h, 25 °C). The aryl glycoside products were purified using a C18 SEP PAK cartridge, then the solvent was evaporated under reduced pressure. The residue was acetylated in pyridine (2 mL) and Ac₂O (2 mL) overnight at RT. The solvent was co-evaporated with MeOH under reduced pressure. The residue was chromatographed on Siliaflash F60 (230 +/ 400 mesh) using (Petroleum same as before ether/EtOAc = 8:2→6:4) to give 2 (32 mg, 96 %). ¹H NMR (CDCl₃, 400 MHz): δ 8.32 (m, 2 H, Ar-H), 7.16 (m, 2 H, Ar-H), 5.71 (dd, 1 H, J₂,₃ 2.0
Hz, H-3), 5.52 (t, 1 H, J₃',₄' 10.0 Hz, H-3’), 5.35 (d, 1 H, J₁,₂ 0.8 Hz, H-1), 5.20 (t, 1 H, J₃,₄ 9.0 Hz, H-4), 5.17 (dd, 1 H, H-3), 5.02 (d, 1 H, J₁',₂' 3.6 Hz, H-1’), 4.90 (ddd, 1 H, H-4'), 4.81 (dd, 1 H, J₂',₃' 10.4 Hz, H-2’), 3.96 (m, 1 H, H-5), 3.91 (dd, 1 H, J₅,₆a 8.4 Hz, H-6a), 3.68 (dd, 1 H, J₄',₅'ₐ 5.6 Hz & J₅'ₐ,₅'ₐ 10.8 Hz, H-5’a), 3.51 (t, 1 H, J₄',₅'ₐ = J₅'ₐ,₅'ₐ 10.8 Hz, H-5’b), 3.46 (dd, 1 H, J₅,₆b 1.2 Hz, J₆ₐ,₆ₐ 10.0 Hz, H-6), 2.25-1.74 (6 s, 18 H, 6 × CH₃CO). ¹³C NMR (CDCl₃, 100 MHz): δ 170.42, 170.39, 170.23, 170.06, 169.95, 169.89 , 161.36, 143.48, 126.43 (2 C), 116.63 (2 C), 96.29, 95.84, 74.13, 71.28, 70.85, 69.16, 69.13, 68.69, 66.68, 66.24), 58.70), 21.00, 20.97 (2 C), 20.87, 20.73, 20.37. ESIMS: Calcd for [C₂₉H₃₅NO₁₇+Na]⁺: 692.2. Found: 692.2.

References