Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

On-chip biosynthesis of GM1 pentasaccharide-related complex glycans

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Experimental materials and methods

Materials. Lactose, 4-(2-aminoethyl)aniline, whole cholera toxin (ctxAB_5), uridine 5′-diphospho-N-acetylgalactosamine (UDP-GalNAc) disodium salt, and uridine 5′-diphosphogalactose (UDP-Gal) disodium salt were purchased from Sigma-Aldrich (St. Louis, MO, USA). Streptavidin−Cy5™ and goat anti-rabbit conjugated Alexa Fluor® 546 were purchased from Invitrogen (Carlsbad, CA, USA). Rabbit polyclonal anti-ctx IgG was purchased from Abcam (Cambridge, MA, USA). Cytidine-5′-monophospho-*N*-acetylneuraminic acid (CMP-Neu5Ac) sodium salt and cell lysates containing PmST were obtained from GeneChem Inc. (Daejeon, Korea). Other glycans were purchased as follows: GM1 pentasaccharide from Alexis Biochemicals (San Diego, CA, USA), GM2 pentasaccharide from Elicityl Oligotech (Crolles, France), and α2,3-sialyllactose (GM3 trisaccharide) from GeneChem Inc. (Daejeon, Korea). Rhodamine-conjugated *Ricinus communis* agglutinin I (RCA_{120}), soybean agglutinin (SBA), peanut agglutinin (PNA), and biotinylated *Maackia amurensis* lectin II (MAL II) were purchased from Vector Laboratories (Burlingame, CA, USA).

Glycan modification. To immobilize glycans onto *N*-hydroxysuccinimide (NHS)-functionalized glass slides (GmbH, Jena, Germany), the glycans were modified as described previously.[1] Briefly, GM1 pentasaccharide, GM2 tetrasaccharide, GM3 trisaccharide, and lactose were dissolved in deionized water at a final concentration of 100 mM, and 4-(2-aminoethyl)aniline was dissolved in 100 mM acetic acid. The glycans and 4-(2-aminoethyl)aniline solutions were mixed in a volume ratio of 1:1 and incubated in a sealed tube at 37 °C for 1 h. Next, freshly prepared reducing agent, 100 mM dimethylamine borane,

was added to each reaction solution, and the tubes were incubated at room temperature for 1 h. Each product was then evaporated under a nitrogen gas stream with heating at 50 °C for 1 h. Without further purification steps, all modified carbohydrates were dissolved in 150 mM phosphate buffer containing 5% (v/v) glycerol, 0.1 mg mL^{-1} BSA, and 0.04% (v/v) Tween 20.

Preparation of glycan chip. NH_2-modified glycans were spotted onto NHS-activated glass slides (GmbH) using a Microsys 5100 microarrayer (Cartesian Technologies, Ann Arbor, MI, USA) with a Chip Marker 2 pin (Telecom International, Sunnyvale, CA, USA) at 75% humidity in a class 10,000 clean room and incubated overnight under the same humidity conditions to achieve tight immobilization.

Plasmid construction. The CgtB-coding gene was amplified by polymerase chain reaction (PCR) from the genome of *Campylobacter jejuni* NCTC 11168.[2] The forward primer (5'-GCG CCA TAT GAG TCA AAT TTC CAT CAT ACT AC-3') and backward primer (5'-GCG CCT CGA GCG GAA TTA AAT TAT ATA AAA ATT TTT TC-3') were designed to contain *Nde*I and *Xho*I restriction sites, respectively. The PCR product was digested with *Nde*I and *Xho*I, and the digested DNA fragment was ligated into a pET22b(+) vector (Novagen, Darmstadt, Germany), which was predigested with *Nde*I and *Xho*I, to make the recombinant plasmid pET-CgtB. The amino acid sequence of CgtA from *C. jejuni* ATCC 33560 is different from that of a characterized CgtA from *C. jejuni* ATCC 43456. The Thr^{282} (^{844}acc^{846} codon) of CgtA from *C. jejuni* ATCC 33560 was converted to Ala^{282} (^{844}gcg^{846} codon) by using overlap extension PCR. Two pairs of primers were used: forward primer (5'-GCG CCA TAT GCT TAA AAA AAT CAT TTC-3') and backward primer (5'-GCG CCT

CGA GTT TTA TCT CTC CTT GAA ATT TC-3'), which contain *Nde*I and *Xho* I restriction sites, respectively, and forward primer (5'-TTA GAA GAA TTT CAG **GCG** TTT CAT CGT AAG AGC-3') and the backward primer (5'-GCT CTT ACG ATG AAA **CGC** CTG AAA TTC TTC TAA-3') containing a mutation codon marked in bold. The resulting PCR-amplified gene was digested with *Nde*I and *Xho*I restriction enzymes and then ligated with *Nde*I- and *Xho*I-digested pET22b(+) to generate the recombinant plasmid pET-CgtA.

Culture condition. Each recombinant plasmid, pET-CgtA and pET-CgtB, was transformed into *E. coli* BL21 (DE3) (Novagen). Transformants were grown to an optical density at 600 nm (OD_{600}) of approximately 0.6–0.8 at 37 °C in shake flasks containing 400 mL Luria-Bertani (LB) medium (0.5% yeast extract, 1% tryptophan, and 1% NaCl) with 50 μg mL^{-1} ampicillin (Sigma-Aldrich). Protein expression was induced by adding isopropyl β-D-thiogalactopyranoside (IPTG; Sigma-Aldrich) to a final concentration of 0.1 mM. The recombinant cells were grown for an additional 24 h at 20 °C. The cells were harvested by centrifugation at 4,000 × *g* for 10 min. The cell pellets were resuspended in 1× phosphate-buffered saline (PBS) buffer (pH 8.0) and disrupted with a sonic dismembrator (Fisher Scientific, Atlanta, GA, USA) at 20 % power (3 s pulse on and 7 s pulse off). The soluble and insoluble fractions were then separated by centrifugation at 10,000 × *g* for 10 min at 4 °C. The crude soluble enzyme fractions were used for on-chip biosyntheses of GM1 pentasaccharide and GM2 tetrasaccharide.

Glycosyltransferase activity assays with HPLC. Glycosyltransferase activity assays were performed under previously reported protocols.[2,3] An α2,3-sialyltransferase activity assay of PmST was conducted in a total volume of 50 μL in Tris-HCl buffer (100 mM; pH 8.5)

containing $MgCl_2$ (20 mM), CMP-Neu5Ac (10 mM), lactose-AB (1 mM), and PmST (35 μg) at 37 °C for 30 min. The reaction was quenched by the addition of ice-cold 12% acetonitrile.[3] For the glycosyltransferase activity assay of recombinant CgtA, the reaction was performed in a volume of 100 μL of 100 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES) buffer (pH 7) containing α2,3-sialyllactose (1 mM), UDP-GalNAc (5 mM), $MnCl_2$ (1 mM), and crude soluble CgtA fraction (10 μL) at 37 °C for 5 h.[2] A recombinant CgtB activity assay was carried out under the following conditions: 100 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer (pH 6) containing GM2 tetrasaccharide (1 mM), UDP-Gal (1 mM), and crude soluble CgtB fraction (10 μL) at 37 °C for 12 h.[2] The reactions were terminated by adding 100% ethanol, and the soluble protein samples were removed by centrifugation at 100,000 × *g* for 10 min at 4 °C. The CgtA- and CgtB-mediated reaction products were derivatized with 2-aminobenzamide (2-AB) using a Signal™ labeling Kit (Glyko, San Leandro, CA, USA) and purified using a GlycoClean™ S cartridge (Glyko). Three 2-AB-derivatized glycan products were analyzed by normal-phase high-performance liquid chromatography (HPLC; LC-10Avp; Shimadzu, Kyoto, Japan) on an amide-80 column (2 mm × 250 mm; Tosoh Biosep, Montgomeryville, PA, USA). Samples were eluted using a linear gradient of acetonitrile (50–80%, v/v) in 10 mM ammonium formate (pH 7.0) and monitored by fluorescence (λ_{ex} = 330 nm, λ_{em} = 420 nm).

Assay of glycosyltransferase activity on lactose-immobilized surface. Each prepared glycan chip was treated with blocking solution (50 mM ethanolamine in 50 mM sodium borate buffer; pH 8.0) for 1 h to deactivate the remaining functional groups and to block nonspecific interactions. Next, each slide was removed from the blocking solution and rinsed with washing buffer I (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, and

0.5% (v/v) Tween 20; pH 7.5) and washing buffer II (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, and 1.4 mM KH_2PO_4; pH 7.5). To analyze enzymatic glycosylation, each glycosyltransferase was treated on defined blocks of the corresponding acceptor glycan-immobilized surface by Geneframe® (Thermo Scientific, San Jose, CA, USA). After the slides were washed with washing buffers I and II, the slides were incubated with fluorescence dye-labeled lectins to check the products of enzymatic reactions, rhodamine-labeled *Ricinus communis* agglutinin I (RCA_{120}) and soybean agglutinin (SBA), and the complex of biotin-conjugated *Maackia amurensis* lectin II (MALII) with Cy5-labeled streptavidin.

Enzymatic synthesis of GM1 pentasaccharide-related complex glycans on lactose-immobilized surface. For biosynthesis of GM1 pentasaccharide, GM2 tetrasaccharide, and GM3 trisaccharide on a chip, the lactose-immobilized surface was separated onto three different blocks by a Geneframe®. A solution of PmST (35 μg), CMP-Neu5Ac (10 mM), and $MgCl_2$ (20 mM) was dropped into three blocks and the slide was reacted for 12 h at 37 °C in a humid chamber. The slide was washed with washing buffers I and II. For *N*-acetylgalactosylation of GM3 trisaccharide, a solution of crude soluble CgtA fraction (10 μL), UDP-GalNAc (5 mM), and $MnCl_2$ (1 mM) was dropped into two of the three blocks, and the slide was reacted for 4-6 days at 37 °C in a humid chamber. The slide was washed with the same buffers. For galactosylation of GM2 tetrasaccharide, a solution of crude soluble CgtB fraction (10 μL) and UDP-Gal (1 mM) was dropped into one of the three blocks, and the slide was reacted for 4-6 days at 37 °C in a humid chamber. The slide was washed with the abovementioned buffers. The slide was sequentially incubated with $ctxAB_5$, rabbit anti-ctx IgG, and anti-rabbit IgG conjugated Alexa Fluor 546. A confocal laser scanner (GSI Lumonics, Wilmington, MA, USA) was used for image acquisition.

References

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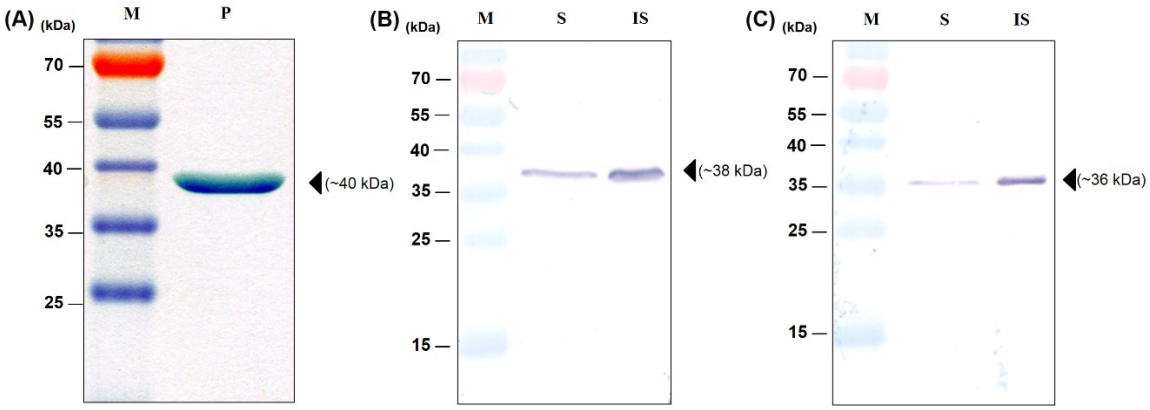

Figure S1. (A) Coomassie blue-stained analysis of purified *P. multocida* PmST and Western blot analyses for recombinant expression of (B) *C. jejuni* CgtA and (C) *C. jejuni* CgtB. Lane: M, molecular weight; P, purified fraction; S, soluble fraction; IS, insoluble fraction.

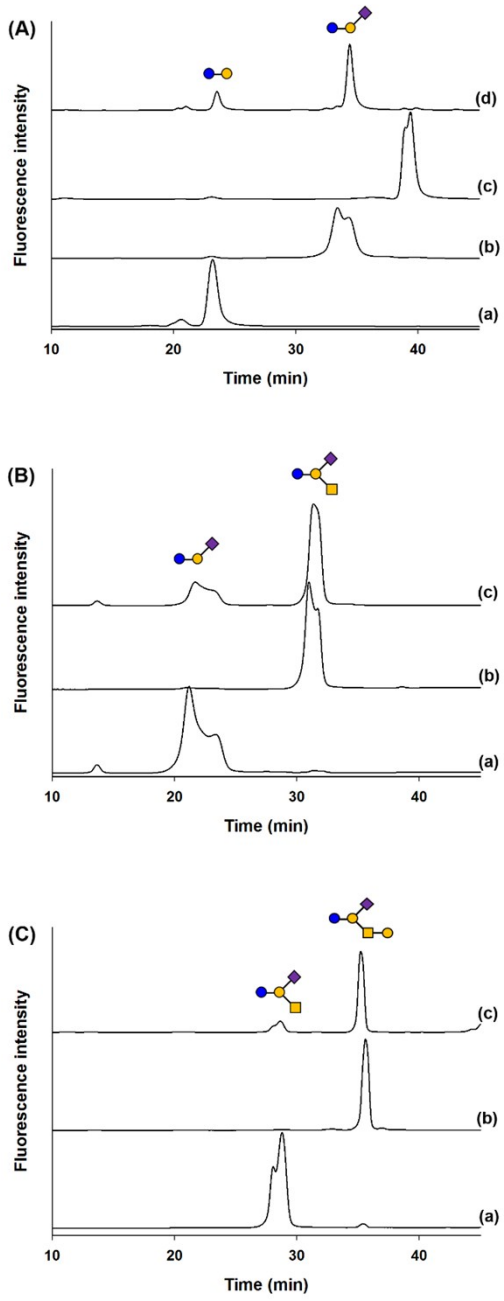

Figure S2. Normal-phase HPLC analyses for the enzyme activities of three glycosyltransferases. (A) Sialyltransferase activity of PmST at pH 8.5. Graphs: (a), lactose-AB standard; (b), α2,3-sialyllactose (GM3)-AB standard; (c), α2,6-sialyllactose-AB standard; (d), lactose-AB and CMP-Neu5Ac incubated with 35 μg PmST at pH 8.5 and 37 °C for 30 min. (B) *N*-acetylgalactosaminyltransferase activity of recombinant CgtA. Graphs: (a), GM3-AB standard; (b), GM2-AB standard; (c), GM3-AB and UDP-GalNAc incubated with the soluble CgtA fraction at pH 7.0 and 37 °C for 5 h. (C) Galactosyltransferase activity of recombinant CgtB. Graphs: (a), GM2-AB standard; (b), GM1-AB standard; (c), GM2-AB and UDP-Gal incubated with the soluble CgtB fraction at pH 6.0 and 37 °C for 12 h. Symbols: blue circle, Glc; yellow circle, Gal; yellow square, GalNAc; purple square, Neu5Ac.