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The *Hd0053* gene of *Haemophilus ducreyi* encodes an α2,3-sialyltransferase

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Abstract

Haemophilus ducreyi is a Gram-negative bacterium that causes chancroid, a sexually transmitted genital ulcer disease. Different lipooligosaccharide (LOS) structures have been identified from *H. ducreyi* strain 35000, including those sialylated glycoforms. Surface LOS of *H. ducreyi* is considered an important virulence factor that is involved in ulcer formation, cell adhesion, and invasion of host tissue. Gene *Hd0686* of *H. ducreyi*, designated *lst* (for lipooligosaccharide sialyltransferase), was identified to encode an α2,3-sialyltransferase that is important for the formation of sialylated LOS. Here we show that *Hd0053* of *H. ducreyi* genomic strain 35000HP, the third member of the glycosyltransferase family 80 (GT80), also encodes an α2,3-sialyltransferase that may be important for LOS sialylation.

Keywords

bacterial sialyltransferase; glycosyltransferase; *Haemophilus ducreyi*; GT80; lipooligosaccharides; sialyltransferase

Introduction

Haemophilus ducreyi is a Gram-negative human mucosal pathogen that causes chancroid, a highly contagious sexually transmitted genital ulcer disease [1]. Chancroid is common in developing countries. Although less common in the United States, chancroid has been associated with increased risk for developing other sexually transmitted diseases including human immunodeficiency virus (HIV) [2]. Sialylated LOS structures have been identified from *H. ducreyi* strains and may be expressed by the bacteria to mimic host cell surface glycans to evade the host's immune system as other Gram-negative mucosal pathogens, such as *Haemophilus influenzae*, *Neisseria meningitidis*, and *Neisseria gonorrhoeae* [3]. The *lst* gene in *H. ducreyi* (*Hd0686*) has been proven to be a lipooligosaccharide (LOS) sialyltransferase gene by complementation studies [4]. It shares homology to *cpsK* gene, a capsular polysaccharide sialyltransferase gene, in GBS strains [5] and *Hi0871* gene of *H. influenzae*, which encodes a hypothetic lipooligosaccharide sialyltransferase [6]. *H. ducreyi* is suggested to use a sialic acid scavenging mechanism for LOS sialylation which is similar to that described for *H. influenzae* and *Haemophilus somnus* [7-10]. This precursor scavenging pathway involves the uptake of host sialic acid as the precursor. Production of activated sugar nucleotide donor CMP-sialic acid and subsequent transfer of sialic acid to appropriate membrane

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acceptors are then accomplished by CMP-sialic acid synthetase(s) and sialyltransferase(s) produced by the bacteria [8]. While the LOS from the *H. ducreyi neuA* (a CMP-*N*-acetylneuraminic acid synthetase gene) mutant lacked detectable sialic acid, the LOS from the *lst* mutant still contained some sialic acid [4]. This indicates that another sialyltransferase may exist that contributes to the sialylation of LOS in *H. ducreyi*.

BLAST search using the amino acid sequence of a *Photobacterium damsela* α2,6-sialyltransferase (Pd2,6ST or sialyltransferase 0160) (GenBank accession number: BAA25316) [11,12] identified two homologous proteins encoded by gene *Pm0188* from *Pasteurella multocida* genomic strain Pm70 and gene *Hd0053* from the *H. ducreyi* genomic strain 35000HP, respectively. These three proteins do not share sequence homology with any other reported bacterial or mammalian glycosyltransferases. They have now been classified into a new glycosyltransferase family GT80 on the Carbohydrate-Active enzymes (CAZy) database (http://afmb.cnrs-mrs.fr/CAZY/) [13]. We have shown previously that *Pm0188* encodes a multifunctional sialyltransferase having α2,3-sialyltransferase, α2,6-sialyltransferase, α2,3-sialidase, and α2,3-trans-sialidase functions [14]. Here, we report the cloning and biochemical characterization of the *Hd0053* gene product. The recombinant enzyme was expressed as a His-tagged fusion protein and purified to homogeneity using a Ni-NTA affinity column. NMR studies of the product produced by the reaction catalyzed by the enzyme confirm that the *Hd0053* gene encodes an α2,3-sialyltransferase. This is the second α2,3-sialyltransferase identified from *H. ducreyi*.

Materials and methods

Bacterial strains, plasmids, and materials.

E. coli electrocompetent DH5α and chemically competent BL21 (DE3) cells were from Invitrogen (Carlsbad, CA). Genomic DNA of *H. ducreyi* 35000HP was from American Type Culture Collection (ATCC, Manassas, VA) (ATCC#700724D). Vector plasmids pET15b and pET22b(+) were purchased from Novagen (EMD Biosciences, Inc. Madison,WI). Ni^{2+}–NTA agarose (nickel–nitrilotriacetic acid–agarose), QIAprep spin miniprep kit, and QIAEX II gel extraction kit were from Qiagen (Valencia, CA). Herculase enhanced DNA polymerase was from Stratagene (La Jolla, CA). T4 DNA ligase, 1 kb DNA ladder, and *BamH*I restriction enzyme were obtained from Promega (Madison, WI). *Nde*I restriction enzyme was from New England Biolabs, Inc. (Beverly, MA). Precision Plus Protein Standards, and BioGel P-2 fine resin were from Bio-Rad (Hercules, CA). Bicinchoninic acid (BCA) protein assay kit was from Pierce Biotechnology, Inc. (Rockford, IL). CTP, D-*N*-acetylmannosamine (ManNAc), and pyruvate were purchased from Sigma (St. Louis, MO). CMP-Neu5Ac was synthesized enzymatically from ManNAc, pyruvate, and CTP by a one-pot two-enzyme system using a recombinant sialic acid aldolase cloned from *E. coli* K12 and a recombinant *N. meningitidis* CMP-sialic acid synthetase as described previously [15]. 4-Methylumbelliferyl β-D-lactoside (LacMU) and 3-azidopropyl β-D-lactoside (LacβProN_3) were synthesized from lactose through a hepta-*O*-acetyllactosyl trichloroacetimidate intermediate as reported [14].

Cloning.

Hd0053 was cloned as an N-His_6-tagged or a C-His_6-tagged fusion protein using genomic DNA of *H. ducreyi* strain 35000HP as the template for polymerase chain reactions (PCR). The primers used to clone the N-His-tagged protein in pET15b vector were: forward primer 5'GATCCATATGCTGATTCAACAAAATCTTG (*Nde*I restriction site is underlined) and reverse primer 5' CGCGGATCCTTAATTATGTATTGTACACATAAATGC 3' (*BamH*I restriction site is underlined). To clone the C-His-tagged protein in pET22b(+) vector, the same forward primer was used, and the reverse primer used was 5' CGCGGATCCTTA*GTGATGATGATGATGATG*ATTATGTATTGTACACATAAATGC

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3' (*BamH*I restriction site is underlined, sequence that encodes the hexahistidine tag is in italics.). PCR reactions for amplifying the target gene were performed in a 50 μL reaction mixture containing genomic DNA (1 μg), forward and reverse primers (1 μM each) , 10 × Herculase buffer (5 μL), dNTP mixture (1 mM), and 5 units (1 μL) of Herculase enhanced DNA polymerase. The reaction mixture was subjected to 35 cycles of amplification with an annealing temperature of 52 °C. The resulting PCR product was purified and digested with *Nde*I and *BamH*I restriction enzymes. The purified and digested PCR product was ligated with predigested pET15b or pET22b(+) vector and transformed into electrocompetent *E. coli* DH5αcells. Selected clones were grown for minipreps and characterization by restriction mapping and DNA sequencing performed by Davis Sequencing Facility in the University of California-Davis.

Expression.

Positive plasmid was selected and transformed into BL21 (DE3) chemically competent cells. The plasmid-bearing *E. coli* strain was cultured in LB rich medium (10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl) supplemented with ampicillin (100 μg/mL). Overexpression of the target protein was achieved by inducing the *E. coli* culture with 0.1 mM of isopropyl-1-thio-β-D-galactopyranoside (IPTG) when the $OD_{600\ nm}$ of the culture reaches 0.8−1.0 and incubating at 15 °C for 48 h with vigorous shaking at 250 rpm in a C25KC incubator shaker (New Brunswick Scientific, Edison, NJ).

Purification.

His_6-tagged target proteins were purified from cell lysate. To obtain the cell lysate, cell pellet harvested by centrifugation at 4,000 rpm for 2 hrs was resuspended in lysis buffer (pH 8.0, 100 mM Tris–HCl containing 0.1% Triton X-100) (20 mL per liter cell culture). Lysozyme (50 μg/mL) and DNaseI (3 μg/mL) were then added and the mixture was incubated at 37 °C for 50 min with vigorous shaking. Cell lysate was obtained by centrifugation at 11,000 rpm for 30 min as the supernatant. Purification of His-tagged proteins from the lysate was achieved using an AKTA FPLC system (GE Healthcare) equipped with a HisTrap™ FF 5 mL column. The column was pre-equilibrated with 10 column volumes of binding buffer (5 mM imidazole, 0.5 M NaCl, 50 mM Tris–HCl, pH 7.5) before the lysate was loaded. Followed by washing with 8 column volumes of binding buffer, 10 column volumes of washing buffer (50 mM imidazole, 0.5 M NaCl, 50 mM Tris–HCl, pH 7.5), the protein was eluted with a linear gradient of elute buffer containing 50−250 mM imidazole in Tris-HCl buffer (50 mM, pH 7.5, 0.5 M NaCl). The fractions containing the purified enzymes were collected and stored at 4 °C.

pH Profile by HPLC.

Typical enzymatic assays were performed in a total volume of 20 μL in a buffer (200 mM) with pH varying from 5.0−11.0 containing CMP-Neu5Ac (2 mM), LacMU (1 mM), and the recombinant enzyme (0.3 μg). The buffers used were: MES, pH 5.0−6.0; Hepes, pH 7.0; Tris–HCl, pH 7.5−9.0; and CAPS, pH 10−11. Reactions were allowed to proceed for 60 min at 37 °C and quenched by adding ice-cold 12% acetonitrile (780 μL) to make 40-fold dilution. The samples were then kept on ice until aliquots of 10 μL were injected and analyzed by a Shimadzu LC-2010A system equipped with a membrane on-line degasser, a temperature control unit and a fluorescence detector. A reverse phase Premier C18 column (250 × 4.6 mm I.D., 5 μm particle size, Shimadzu) protected with a C18 guard column cartridge was used. The mobile phase was 12% acetonitrile. The fluorescent compounds LacMU and Neu5Acα2,3LacMU were detected by excitation at 325 nm and emission at 372 nm [16]. All assays were carried out in duplicate.

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Effects of metal ions, EDTA, and dithiothreitol (DTT).

EDTA (5 mM), different concentrations (5 mM, 10 mM, and 20 mM) of $MgCl_2$ or $MnCl_2$, and various concentrations of DTT (0 mM, 0.2 mM, 1 mM, and 5 mM) were used in a Tris–HCl buffer (pH 8.5, 100 mM) to analyze their effects on the α2,3-sialyltransferase activity of Hd0053 (0.35 μg). Reaction without EDTA, DTT, and metal ions was used as a control.

Kinetics by HPLC assays.

The enzymatic assays were carried out in a total volume of 20 μL in a Tris-HCl buffer (100 mM, pH 8.5) containing CMP-Neu5Ac, LacMU and the recombinant protein (0.3 μg). Reactions were allowed to proceed for 60 min at 37 °C. Apparent kinetic parameters were obtained by varying the CMP-Neu5Ac concentration from 0.1−4.0 mM (0.1mM, 0.25 mM, 0.4 mM, 1 mM, 2 mM, and 4 mM) and a fixed concentration of LacMU (1 mM), or a fixed concentration of CMP-Neu5Ac (1 mM) and varied concentrations of LacMU (0.1 mM, 0.25 mM, 0.4 mM, 1 mM, 2 mM, and 4 mM). The double reciprocal Lineweaver-Burk plots were obtained from the average values of duplicate or triplet assay results. One unit of enzyme is defined as the amount of enzyme that synthesizes the formation of 1 μmole of Neu5Acα2,3LacMU per minute at 37 °C under the assay conditions.

Enzymatic synthesis of Neu5Acα2,3LacβProN$_3$.

The synthesis was carried out in a total volume of 10 mL in Tris-HCl buffer (100 mM, pH 8.5) containing LacβProN$_3$ (27 mg, 0.063 mmol), CMP-Neu5Ac (50 mg, 0.076 mmol), and Hd0053 (25 μg). The reaction was allowed to proceed at 37 °C for 2 hrs and further reacted at room temperature for overnight when TLC analysis (developing solvent used was EtOAc : MeOH : H_2O : HOAc = 4 : 2 : 1 : 0.1) indicated the completion of the reaction. The reaction was stopped by adding an equal volume of ice-cold ethanol. The mixture was mixed well and kept on ice for 30 min. After the precipitated protein was removed by centrifuging at 5000 × *g* for 30 min, the supernatant was concentrated and purified by Bio-Gel P2 size exclusion chromatography and lyophilized to give Neu5Acα2,3LacβProN$_3$ (38 mg, 82% yield) as white powder. ^{1}H-NMR, ^{1}H-^{1} H COSY, and ^{1}H-^{13}C HSQC experiments were carried out at 26 °C in D_2O on a Bruker DRX-600 and ^{13}C-NMR experiment was carried out at Varian mercuryplus 300 spectrometer. The proton chemical shifts were referenced to the HOD signal at 4.79 ppm and the ^{13}C chemical shifts were referenced to the methyl resonance of the internal deuterated acetone at 30.89 ppm.

Results

Expression and purification of Hd0053 protein

Hd0053 from *H. ducreyi* strain 35000HP was cloned as both an N-His$_6$-tagged and a C-His$_6$-tagged recombinant proteins using pET15b and pET22b(+) vectors, respectively. The DNA sequence of the cloned gene matches to that reported in GenBank (Accession number AE017143). The optimized expression condition was incubating at 15 °C for 48 h with vigorous shaking (250 rpm) after induction with 0.1 mM of IPTG. Under the same expression conditions, the expression level of N-His$_6$-tagged form is higher than that of the C-His$_6$-tagged one. Therefore, only the N-His$_6$-tagged Hd0053 is characterized in detail. The SDS-PAGE analysis of the protein expression (Fig. 1.) indicates that Hd0053 was expressed in a large amount which consists of about 70% of the total protein extracts of the *E. coli* host cells. The solubility of the recombinant protein, however, was quite low. Only a small portion of the target protein was observed in the lysate, which is the soluble portion of the protein. Ni-Column purification using the AKTA FPLC system provided purified protein showing a molecular weight of about 42 kD in the SDS-PAGE. This molecular weight is close to that (48.7 kD) calculated for the Hd0053 protein.

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pH Profile of Hd0053

The pH profile of the α2,3-sialyltransferase activity of Hd0053 (Fig. 2A) indicates that the enzyme catalyzes the transfer of Neu5Ac from CMP-Neu5Ac to an acceptor such as LacMU under basic condition (pH > 7.0) with a optimal activity at pH 8.0. More than 90% of the activity was observed at pH 9.0. The enzyme activity declines quickly when pH is below 7.5 or above 9.0. The activity drops to about 16% and 18% when the pH is at 5.0 and 11.0, respectively.

Effect of metal ions, EDTA, and dithiothreitol (DTT) on the activity of Hd0053

The effects of various metal ions, the chelating agent EDTA, and DTT on the enzyme activity of Hd0053 were examined at pH 8.5. As shown in Fig. 2B, a divalent metal ion is not required for the α2,3-sialyltransferase activity of the enzyme, as 5 mM of EDTA does not affect the enzyme activity. The addition of Mg^{2+} up to 20 mM does not affect the activity of the enzyme. Increasing the concentration of Mn^{2+} in the reaction mixture, however, decreases the activity of the enzyme. The detrimental effect of the Mn^{2+} is similar to that was shown for the α2,3-sialyltransferase activity of PmST1, but is not as dramatic [14]. The metal effect is different from that reported for the *N. meningitidis* (MC58 and 406Y) Lst, for which the sialyltransferase activity can be stimulated by Mg^{2+} (2-fold) or Mn^{2+} (3-fold) [17].

There are four non-conserved cysteine residues in the Hd0053 protein sequence. The effect of DTT on the α2,3-sialyltransferase activity of the enzyme was studied. Addition of DTT up to 5 mM does not significantly increase the activity of Hd0053 (Fig. 2B), indicating disulfide formation is not required for the sialyltransferase activity of the Hd0053.

Kinetics.

The apparent K_M values obtained for the α2,3-sialyltransferase activity of Hd0053 are 0.05 mM and 5.3 mM for CMP-Neu5Ac and LacMU, respectively (Table 1). This K_M value of CMP-Neu5Ac (0.05 mM) is comparable to that for recombinant *N. meningitidis* α2,3-sialyltransferase (20 μM) [18], rat liver Galβ1,4GlcNAc α2,6-sialyltransferase (50 μM), and Galβ1,4(3)GlcNAc α2,3-sialyltransferase (70 μM) [19]. It is one magnitude less than that of PmST1 (0.44 mM) [14] and Cst II (0.46 mM) [20]. The K_M value of LacMU (5.3 mM) for Hd0053 is three fold to that for the PmST1 (1.4 mM), indicating a weaker binding of the LacMU to Hd0053 than to PmST1 [14]. The k_{cat}/K_M for LacMU is 0.13 s^{-1} mM^{-1} (7.8 min^{-1} mM^{-1}) for the Hd0053, which is about three fold less than that (22 min^{-1} mM^{-1}) obtained for APTS (8-aminopyrene-1,3,6-trisulfonic acid)-labeled lactose for the *N. meningitidis* α2,3-sialyltransferase [18]. It is 254 fold less than that of the α2,3-sialyltransferase activity of the PmST1 (33 s^{-1} mM^{-1}) [14].

Enzymatic synthesis and characterization of sialylation product

Using Lacβ$ProN_3$ as the acceptor and CMP-Neu5Ac as the donor, the synthesis of sialylated Lacβ$ProN_3$ was achieved in high yield (82%) at pH 8.5. The purified sialylated product was characterized by NMR spectrometry. As listed in Table 2, the NMR data match well to those reported for α2,3-sialosides [14,21]. For example, the chemical shift of the proton at C-3 of Gal in the sialylated product (4.09 ppm) is 0.39−0.46 ppm downfield to that in Neu5Acα2,6Lacβ$ProN_3$ [12] and Lacβ$ProN_3$ [14]. The difference of the chemical shifts of two protons at C-6 of Gal in the sialylated product (0.05 ppm) is similar to that in Lacβ$ProN_3$ [14], but is much smaller than that in Neu5Acα2,6LacβProN (0.35 ppm) [12]. In the ^{13}C NMR spectrum, the chemical shifts of C-3 and C-2 on the Gal of the sialylated product are 2.7 ppm downfield and 1.5 ppm upfield, respectively, compared to those in LacβN_3 (72.9 ppm). All other ^{13}C chemical shifts for the Lacβ$ProN_3$ moiety in the sialylated product are very similar (within 0.5 ppm difference) to those in Lacβ$ProN_3$ [14]. These data strongly confirm that the sialylation product catalyzed by Hd0053 is α2,3-linked sialoside Neu5Acα2,3Lacβ$ProN_3$.

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Discussion

Sialic acids are commonly found as terminal carbohydrate residues on cell surface glycoconjugates (glycoproteins and glycolipids) of higher animals. As the terminal carbohydrate residue, sialic acid is one of the first molecules encountered in cellular interactions and has been found to play important roles in cellular recognition and communication [22,23]. Cell surface sialic acids have also been found in relatively few microorganisms, mainly pathogenic bacteria, and their presence is often associated with virulence [22-24]. Sialic acids on LOS of several mucosal pathogens, including *N. meningitidis*, *N. gonorrhoeae*, *H. ducreyi* and *H. influenzae* strains are considered important LOS components for molecular mimicry to allow them to evade the host's immune system or as modulators of microbial interactions with host cells [7,8,23,25,26]. Some of these pathogenic bacteria present their LOS structure in a phase-variable manner and the degree of sialylation is related to growth conditions of the bacteria [26-28].

Cloning, expression, and biochemical characterization of the *Hd0053* gene product shown here confirmed that the *Hd005*3 gene sequence encodes an active α2,3-sialyltransferase. It is the second sialyltransferase that has been identified in *Haemophilus ducreyi* 35000HP (the first one is Lst) [4]. Considering the existence of four sialyltransferase genes (*siaA*, *lic3A*, *lic3B*, and *lsgB*) described in *H. influenzae* [6,26,29] and three putative sialyltransferase genes predicted in *P. multocida* [30], the presence of multiple sialyltransferase genes seems to be a common phenomenon for the *Haemophilus-Actinobacillus-Pasteurella* (HAP) group bacteria. The presence of multiple sialyltransferases in these pathogenic bacteria indicates that bacterial sialylation is a very complex process.

Hd0053 shares 32% identity and 52% similarity to the amino acid sequence of PmST1. Compared to the amino acid sequence of Pd2,6ST, the sequence of Hd0053 has 26% identify and 44% similarity. PmST1, Pd2,6ST, and Hd0053 represent a new family of sialyltransferases since they lack sequence similarity to any other reported sialyltransferases, either from bacterial or mammalian sources. They have now been classified into glycosyltransferase family GT80 on the CAZy database (http://afmb.cnrs-mrs.fr/CAZY/) [13]. Despite the sequence homology shared among these three proteins, they have different activities. While both Pd2,6ST and Hd2,3ST have a single but different ST activity, PmST1 is a multifunctional enzyme for which four different functions have been identified, including an α2,3-sialyltransferase, an α2,6-sialyltransferase, an α2,3-sialidase, and an α2,3-trans-sialidase activities. These three enzymes, thus, provide an excellent model system for structure-function relationship studies of sialyltransferases.

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References

1. Morse SA. Chancroid and *Haemophilus ducreyi*. Clin. Microbiol. Rev 1989;2:137–157. [PubMed: 2650859]
2. Annan NT, Lewis DA. Treatment of chancroid in resource-poor countries. Expert. Rev. Anti. Infect. Ther 2005;3:295–306. [PubMed: 15918786]
3. Melaugh W, Campagnari AA, Gibson BW. The lipooligosaccharides of *Haemophilus ducreyi* are highly sialylated. J. Bacteriol 1996;178:564–570. [PubMed: 8550483]
4. Bozue JA, Tullius MV, Wang J, Gibson BW, Munson RS Jr. *Haemophilus ducreyi* produces a novel sialyltransferase. Identification of the sialyltransferase gene and construction of mutants deficient in the production of the sialic acid-containing glycoform of the lipooligosaccharide. J. Biol. Chem 1999;274:4106–4114. [PubMed: 9933604]

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5. Chaffin DO, McKinnon K, Rubens CE. CpsK of *Streptococcus agalactiae* exhibits α2,3-sialyltransferase activity in *Haemophilus ducreyi*. Mol. Microbiol 2002;45:109–122. [PubMed: 12100552]
6. Jones PA, Samuels NM, Phillips NJ, et al. *Haemophilus influenzae* type b strain A2 has multiple sialyltransferases involved in lipooligosaccharide sialylation. J. Biol. Chem 2002;277:14598–15611. [PubMed: 11842084]
7. Mandrell RE, McLaughlin R, Aba Kwaik Y, Lesse A, Yamasaki R, Gibson B, Spinola SM, Apicella MA. Lipooligosaccharides (LOS) of some *Haemophilus* species mimic human glycosphingolipids, and some LOS are sialylated. Infect. Immun 1992;60:1322–1328. [PubMed: 1372291]
8. Vimr E, Lichtensteiger C. To sialylate, or not to sialylate: that is the question. Trends Microbiol 2002;10:254–257. [PubMed: 12088651]
9. Schilling B, Goon S, Samuels NM, Gaucher SP, Leary JA, Bertozzi CR, Gibson BW. Biosynthesis of sialylated lipooligosaccharides in *Haemophilus ducreyi* is dependent on exogenous sialic acid and not mannosamine. Biochemistry 2001;40:12666–12677. [PubMed: 11601991]
10. Inzana TJ, Glindemann G, Cox AD, Wakarchuk W, Howard MD. Incorporation of *N*-acetylneuraminic acid into *Haemophilus somnus* lipooligosaccharide (LOS): enhancement of resistance to serum and reduction of LOS antibody binding. Infect. Immun 2002;70:4870–4879. [PubMed: 12183531]
11. Yamamoto T, Nakashizuka M, Terada I. Cloning and expression of a marine bacterial beta-galactoside alpha2,6-sialyltransferase gene from *Photobacterium damsela* JT0160. J. Biochem. (Tokyo) 1998;123:94–100. [PubMed: 9504414]
12. Yu H, Huang S, Chokhawala H, Sun M, Zheng H, Chen X. Highly efficient chemoenzymatic synthesis of naturally occurring and non-natural alpha-2,6-linked sialosides: a *P. damsela* alpha-2,6-sialyltransferase with extremely flexible donor-substrate specificity. Angew. Chem. Int. Ed. Engl 2006;45:3938–3944. [PubMed: 16721893]
13. Coutinho PM, Deleury E, Davies GJ, Henrissat B. An evolving hierarchical family classification for glycosyltransferases. J. Mol. Biol 2003;328:307–317. [PubMed: 12691742]
14. Yu H, Chokhawala H, Karpel R, Yu H, Wu B, Zhang J, Zhang Y, Jia Q, Chen X. A multifunctional *Pasteurella multocida* sialyltransferase: a powerful tool for the synthesis of sialoside libraries. J. Am. Chem. Soc 2005;127:17618–17619. [PubMed: 16351087]
15. Yu H, Yu H, Karpel R, Chen X. Chemoenzymatic synthesis of CMP-sialic acid derivatives by a one-pot two-enzyme system: comparison of substrate flexibility of three microbial CMP-sialic acid synthetases. Bioorg. Med. Chem 2004;12:6427–6435. [PubMed: 15556760]
16. Kajihara Y, Kamiyama D, Yamamoto N, Sakakibara T, Izumi M, Hashimoto H. Synthesis of 2-[(2-pyridyl)amino]ethyl beta-D-lactosaminide and evaluation of its acceptor ability for sialyltransferase: a comparison with 4-methylumbelliferyl and dansyl beta-D-lactosaminide. Carbohydr. Res 2004;339:1545–1550. [PubMed: 15178399]
17. Gilbert M, Watson DC, Cunningham AM, Jennings MP, Young NM, Wakarchuk WW. Cloning of the lipooligosaccharide alpha-2,3-sialyltransferase from the bacterial pathogens *Neisseria meningitidis* and *Neisseria gonorrhoeae*. J. Biol. Chem 1996;271:28271–28276. [PubMed: 8910446]
18. Gilbert M, Cunningham AM, Watson DC, Martin A, Richards JC, Wakarchuk WW. Characterization of a recombinant *Neisseria meningitidis* alpha-2,3-sialyltransferase and its acceptor specificity. Eur. J. Biochem 1997;249:187–194. [PubMed: 9363771]
19. Gross HJ, Rose U, Krause JM, Paulson JC, Schmid K, Feeney RE, Brossmer R. Transfer of synthetic sialic acid analogues to N- and O-linked glycoprotein glycans using four different mammalian sialyltransferases. Biochemistry 1989;28:7386–7392. [PubMed: 2510824]
20. Chiu CP, Watts AG, Lairson LL, Gilbert M, Lim D, Wakarchuk WW, Withers SG, Strynadka NC. Structural analysis of the sialyltransferase CstII from *Campylobacter jejuni* in complex with a substrate analog. Nat. Struct. Mol. Biol 2004;11:163–170. [PubMed: 14730352]
21. Berman E. Structural and conformational analysis of sialyloligosaccharides using carbon-13 nuclear magnetic resonance spectroscopy. Biochemistry 1984;23:3754–3759. [PubMed: 6477894]
22. Angata T, Varki A. Chemical diversity in the sialic acids and related alpha-keto acids: an evolutionary perspective. Chem. Rev 2002;102:439–469. [PubMed: 11841250]

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23. Schauer R. Achievements and challenges of sialic acid research. Glycoconj. J 2000;17:485–499. [PubMed: 11421344]
24. Smith H, Parsons NJ, Cole JA. Sialylation of neisserial lipopolysaccharide: a major influence on pathogenicity. Microb. Pathog 1995;19:365–377. [PubMed: 8852278]
25. Mandrell RE, Apicella MA. Lipo-oligosaccharides (LOS) of mucosal pathogens: molecular mimicry and host-modification of LOS. Immunobiology 1993;187:382–402. [PubMed: 8330904]
26. Bouchet V, Hood DW, Li J, et al. Host-derived sialic acid is incorporated into *Haemophilus influenzae* lipopolysaccharide and is a major virulence factor in experimental otitis media. Proc. Natl. Acad. Sci. U. S. A 2003;100:8898–8903. [PubMed: 12855765]
27. Jennings MP, Srikhanta YN, Moxon ER, Kramer M, Poolman JT, Kuipers B, van der Ley P. The genetic basis of the phase variation repertoire of lipopolysaccharide immunotypes in *Neisseria meningitidis*. Microbiology 1999;145(Pt 11):3013–3021. [PubMed: 10589709]
28. Burch CL, Danaher RJ, Stein DC. Antigenic variation in *Neisseria gonorrhoeae*: production of multiple lipooligosaccharides. J. Bacteriol 1997;179:982–986. [PubMed: 9006061]
29. Fox KL, Cox AD, Gilbert M, et al. Identification of a bifunctional lipopolysaccharide sialyltransferase in *Haemophilus influenzae*. Incorporation of disialic acid. J. Biol. Chem 2006;281:40024–40032. [PubMed: 17071616]
30. St Michael F, Vinogradov E, Li J, Cox AD. Structural analysis of the lipopolysaccharide from *Pasteurella multocida* genome strain Pm70 and identification of the putative lipopolysaccharide glycosyltransferases. Glycobiology 2005;15:323–333. [PubMed: 15537789]

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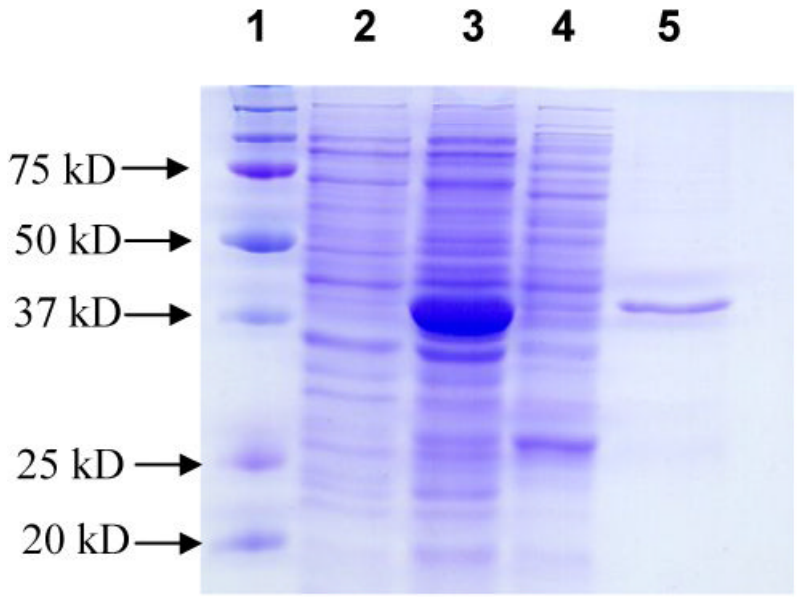

Fig. 1.
SDS-PAGE analysis for Hd0053 protein expression and purification. Lanes: 1, protein standards; 2, whole cell extraction before induction; 3, whole cell extraction after induction; 4, lysate after induction; 5, Ni-column purified Hd0053 protein.

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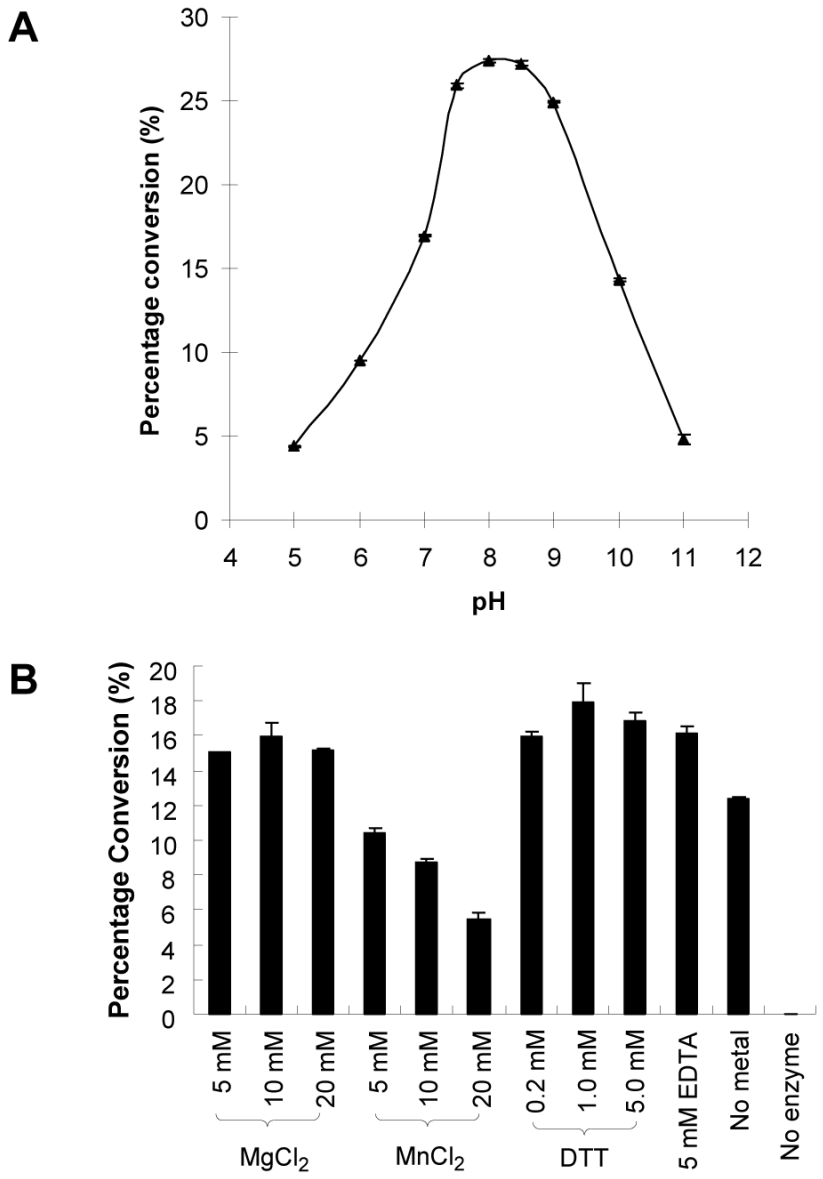

Fig. 2.

The pH profile and the effects of metal ions, EDTA, and DTT on the α2,3-sialyltransferase activity of Hd0053 by quantitative HPLC analysis. (A) The pH profile. Activity was measured at indicated pH at 37 °C for 60 min. Buffers (200 mM) used were: MES, pH 5.0−6.0; Hepes, pH 7.0; Tris–HCl, pH 7.5−9.0; and CAPS, pH 10−11. (B) Effects of metal ions, EDTA, and DTT. The activity was determined in Tris-HCl buffer (100 mM, pH 8.5) at 37 °C for 60 min.

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Table 1

Apparent kinetic parameters for the α2,3-sialyltransferase activity of Hd0053.[a]

Substrates	**CMP-Neu5Ac**	**LacMU**
K_M (mM)	0.05 ± 0.008	5.3 ± 0.46
V_{max} (mM s^{-1})	(3.0 ± 0.05) × 10^{-5}	(2.0 ± 0.08) × 10^{-4}
k_{cat} (s^{-1})	0.10 ± 0.002	0.67 ± 0.03
k_{cat}/K_M (s^{-1} mM^{-1})	2.0	0.13

[a] Assays were performed in triplicate in Tris-HCl buffer (100 mM, pH 8.5) with either varied concentrations of CMP-Neu5Ac (0.1, 0.25, 0.4, 1, 2, 4 mM) and a fixed concentration of LacMU (1 mM) or a fixed concentration of CMP-Neu5Ac (1 mM) and varied concentrations (0.1, 0.25, 0.4, 1, 2, and 4 mM) of LacMU. Hd0053 was used at the same concentration (0.3 μM) for these assays.

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Table 2

Chemical shifts (ppm) and assignments of Neu5Acα2,3LacβProN$_3$, the sialylation product catalyzed by *H. ducreyi* α2,3-sialyltransferase Hd0053.[a]

Sugar	Position	H	C
Glc	1	4.46	102.3
	2	3.29	72.9
	3	3.63	74.5
	4	3.64	78.3
	5	3.58	74.9
	6	3.96	60.2
	6'	3.80	
Propyl azide	-CH_2-CH_2-CH_2-N_3	3.99	67.5
		3.76	
	-CH_2-CH_2-CH_2-N_3	1.89	28.4
	-CH_2-CH_2-CH_2-N_3	3.43	48.0
Gal	1	4.50	102.8
	2	3.55	69.5
	3	4.09	75.6
	4	3.94	67.6
	5	3.69	75.3
	6	3.74	61.2
	6'	3.69	
Neu5Ac	1		174.0
	2		99.9
	3_{ax}	1.77	39.8
	3_{eq}	2.73	
	4	3.67	68.5
	5	3.84	51.8
	6	3.62	73.0
	7	3.57	68.2
	8	3.88	71.9
	9	3.86	62.7
	9'	3.66	
	N-CO-CH_3	2.01	22.2
	N-CO-CH_3		175.1

[a] NMR spectra were recorded at 299 K in D_2O at neutral pH. Chemical shifts (ppm) are given relative to HOD (4.79 ppm) for ^{1}H NMR or the methyl resonance of the internal deuterated acetone (30.89 ppm) for ^{13}C NMR. The peaks were assigned using ^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC with reference to [21].