Sialic acid (Sia)-containing structures play important roles in cellular recognition and communication. Chemical sialylation is considered as one of the most difficult glycosylation reactions due to the hindered tertiary anomic center and the lack of a neighboring participating group in sialic acids. Enzymatic sialylation catalyzed by sialyltransferases (SiaTs), especially those from bacterial sources, is an attractive alternative. A highly active sialyltransferase with broad substrate specificity will be a powerful tool for broadening the application of chemoenzymatic approaches to the synthesis of sialosides. Herein we report the isolation and characterization of a multifunctional sialyltransferase from Pasteurella multocida (Pm). Its application in a one-pot three-enzyme system for efficient synthesis of diverse sialosides libraries is also described.

BLAST searching amino acid sequence of a Photobacterium damselae α,2,6SiaT (Pd2,6SiaT) as a probe identified a putative SiaT (GenBank accession number AAK02272) encoded by gene Pm0188 from Pasteurella multocida genomic strain Pm70. The protein shared 37% identity and 57% amino acid similarity to the sialyltransferase domain of the Pd2,6SiaT. A homologue of gene Pm0188 was amplified from the chromosome DNA of Pasteurella multocida strain P-1059 (ATCC 15742) and cloned into a PET23a(+) vector. DNA sequencing indicated that the obtained gene had 10 base differences compared to the published Pm0188 gene sequence, which led to three amino acid changes in the deduced protein (D105N, R135Q, G295E). The protein was named Pm0188Ph (Pm0188 Protein homologue).

Topology analysis identified a hydrophobic N-terminal domain (1–25 aa) in the Pm0188Ph. The N-terminal amino acid sequence of this domain was compared to the N-terminal 25 amino acid residues of the full-length protein. A truncated protein (tPm0188Ph) lacking the N-terminal 2–25 amino acid residues of the Pm0188 Ph was sub-cloned as a C-His 6 -tagged protein. The obtained tPm0188Ph was highly soluble and could be expressed (37 °C for 3 h with shaking at 250 rpm) in high yields in E. coli by isopropyl-1-thio-β-d-galactopyranoside (0.1 mM) induction. About 100 mg of tPm0188Ph could be routinely purified from the cell lysate obtained from a 1 L E. coli culture. SDS–PAGE and gel filtration analyses indicated that the tPm0188Ph existed as a monomer in solution.

The recombinant tPm0188Ph was a multifunctional enzyme for which four types of functions (Figure 1) have been identified. It was (1) an α,2,3SiaT that transferred a Sia residue from CMP–Sia to galactosides to form α,2,3-sialyl linkages efficiently in a wide pH range (pH 6.0–10.0) with an optimal activity at pH 7.5–9.0; (2) an α,2,6SiaT that formed α,2,6-linkages much less efficiently at pH 4.5–7.0; (3) a θ-sialidase that specifically cleaved α,2,3-sialyl linkages but left α,2,6-sialyl linkages intact (optimal pH = 5.0–5.5); and (4) a trans-sialidase that transferred the Sia residue from α,2,3-linked sialyl galactosides (not α,2,6-linked sialyl galactosides) to another galactoside (optimal pH = 5.5–6.5). A divalent metal ion, such as Mg²⁺ or Mn²⁺, was not required for any of these four activities.

The expression level (6000 U/L purified protein) and the specific activity (60 U/mg protein) of the tPm0188Ph α,2,3SiaT activity were the highest among all SiaTs known to date [1 U = 1 μmol of product formed from CMP-N-acetylneuraminic acid (CMP–Neu5Ac, donor) and 4-methylumbelliferyl-β-d-galactoside (LacMU, acceptor) per minute at 37 °C, pH 8.5]. The apparent kinetic data were obtained for CMP–Neu5Ac (Kₐf = 0.44 mM, kcat = 32 s⁻¹) and LacMU (Kₐf = 1.4 mM, kcat = 47 s⁻¹). The kₐf/Kₐs of LacMU (33 s⁻¹ mM⁻¹) for the tPm0188Ph α,2,3-SiaT was about 90-fold higher than that (22 min⁻¹ mM⁻¹) of 8-aninopyrene-1,3,6-trisulfonic acid-labeled lactose for N. meningitidis α,2,3SiaT.

The efficiency and the flexibility of the tPm0188Ph in the synthesis of α,2,3-linked sialoside libraries were tested using a one-pot three-enzyme system (Scheme 1). In this system, CMP–Sia was the Synthesis of Sialoside Libraries.

![Figure 1. Multiple functions of a Pm sialyltransferase (tPm0188Ph).](image-url)
The tPm0188Ph α,2,3SiaT also had relaxed acceptor specificity. As shown in Table 1, N-acetyllactosamine (entry a), lactose (entries b and l), and β-lactosides with methyl (entries f, q, t, and u), azido (entries c and p), azidopropyl (entries d, m, o, and v), or 4-methylumbelliferyl (entries e, r, and s) aglycons were excellent acceptors. Galactose (entry g) and β-galactosides (entries h, i, and j) were also acceptable by tPm0188Ph. For example, azide or 8-methylumbelliferyl (entries e, r, and s) aglycons were excellent acceptors. Similar to that reported for the N. meningitidis α,2,3SiaT, an α-galactoside (e.g., α-methylgalactoside, entry k) was an acceptor for tPm0188Ph. N-Acetylgalactosamine (GalNAc) and its derivatives, such as αGalNAcProN₂ and βGalNAcProN₂, however, were not acceptors.

By controlling the pH value of reaction, tPm0188Ph could be used in synthesizing α,2,6-linked sialosides. For example, Neu5Ac(2→6)LacMU was obtained in 55% yield using a one-pot two-step reaction in which CMP—Neu5Ac was synthesized at pH 8.8 in the first step catalyzed by the sialic acid aldolase and the CMP—Sia synthetase. After acidification of the reaction to pH 5.5, tPm0188Ph was added and the trisaccharide was produced in the second step without isolating the CMP—Neu5Ac intermediate.

The application of the trans-sialidase activity of tPm0188Ph in the synthesis of sialosides was also tested. In this case, Neu5Ac(2→3)LacMU was obtained in 36% yield from Neu5Ac(2→3)-lactose and LacMU.

In conclusion, due to its broad substrate specificity, high solubility, high expression level, and multifunctionality, the newly discovered P. multocida SiaT(tPm0188Ph) is an extremely powerful tool for synthesizing structurally diverse sialosides to understand their important biological functions. The unusual multifunctionality of the enzyme as an α,2,3SiaT, an α,2,6SiaT, an α,2,3-sialidase, and an α,2,3-trans-sialidase also provides solid evidence for the complexity of bacterial sialylation.

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Supporting Information Available: Experimental details for cloning, expression, purification, and characterization of tPm0188Ph, and details for chemoenzymatic synthesis of sialosides. This material is available free of charge via the Internet at http://pubs.acs.org.

References


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