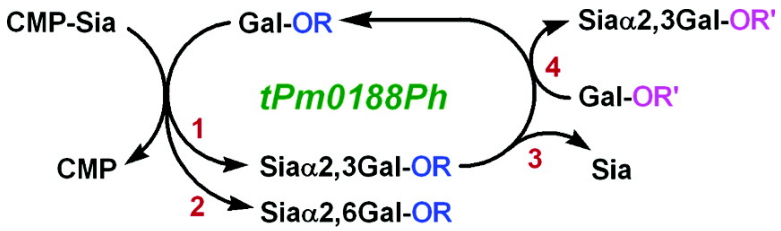
Communication

A Multifunctional *Pasteurella multocida* Sialyltransferase: A Powerful Tool for the Synthesis of Sialoside Libraries

Hai Yu, Harshal Chokhawala, Rebekah Karpel, Hui Yu, Bingyuan Wu, Jianbo Zhang, Yingxin Zhang, Qiang Jia, and Xi Chen

J. Am. Chem. Soc., **2005**, 127 (50), 17618-17619• DOI: 10.1021/ja0561690 • Publication Date (Web): 25 November 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 16 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Journal of the American Chemical Society is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

J|A|C|S
COMMUNICATIONS

Published on Web 11/25/2005

A Multifunctional *Pasteurella multocida* Sialyltransferase: A Powerful Tool for the Synthesis of Sialoside Libraries

Hai Yu,[†] Harshal Chokhawala,[†] Rebekah Karpel,[†] Hui Yu,[†] Bingyuan Wu,[‡] Jianbo Zhang,[§] Yingxin Zhang,[▽] Qiang Jia,[#] and Xi Chen*,[†]

Department of Chemistry, University of California, One Shields Avenue, Davis, California 95616

Received September 7, 2005; E-mail: chen@chem.ucdavis.edu

Sialic acid (Sia)-containing structures play important roles in cellular recognition and communication.[1] Chemical sialylation is considered as one of the most difficult glycosylation reactions due to the hindered tertiary anomeric center and the lack of a neighboring participating group in sialic acids.[2] Enzymatic sialylation catalyzed by sialyltransferases (SiaTs), especially those from bacterial sources,[2a,3] is an attractive alternative.[4] A highly active sialyltransferase with broad substrate specificity will be a powerful tool for broadening the application of chemoenzymatic approaches in synthesizing diverse naturally occurring or structurally modified sialosides. These compounds are invaluable probes for studying the important biological roles of sialosides.[5] Herein we report the discovery and the characterization of a highly soluble and extremely active multifunctional sialyltransferase from *Pasteurella multocida (Pm)*. Its application in a one-pot three-enzyme system for efficient synthesis of diverse sialoside libraries is also described.

BLAST search using the amino acid sequence of a *Photobacterium damsela* α2,6SiaT (Pd2,6SiaT)[6] 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 *Pm* 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 differences in the deduced protein (D105N, R135Q, G295E). The protein was named Pm0188Ph (Pm0188 *P*rotein *h*omologue).

Topology analysis identified a hydrophobic N-terminal domain (1−25 aa) in the Pm0188Ph. Ni^{2+}-column purified C-terminal His_6-tagged Pm0188Ph gave two protein bands in SDS−PAGE gels. The smaller size band represented 95% of the total amount of the purified protein. N-terminal amino acid sequencing of this band indicated that the N-terminal 25 amino acid residues of the full length protein had been cleaved off.

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

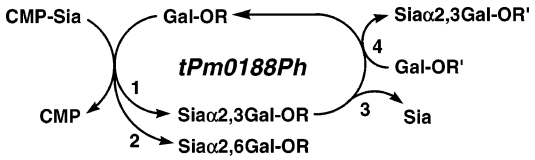


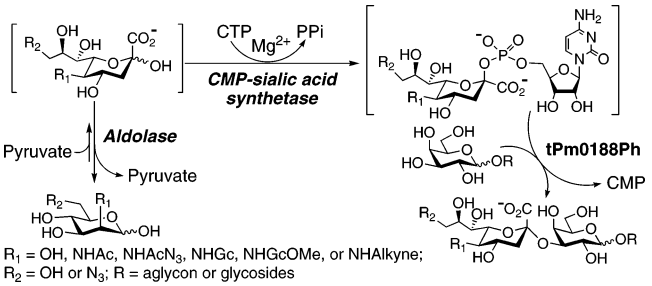
Figure 1. Multiple functions of a *Pm* sialyltransferase (tPm0188Ph).

was (1) an α2,3SiaT that transferred a Sia residue from CMP−Sia to galactosides to form α2,3-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 α2,6SiaT that formed α2,6-linkages much less efficiently at pH 4.5−7.0; (3) a sialidase that specifically cleaved α2,3-sialyl linkages but left α2,6-sialyl linkages intact (optimal pH = 5.0−5.5); and (4) a *trans*-sialidase that transferred the Sia residue from α2,3-linked sialyl galactosides (not α2,6-linked sialyl galactosides) to another galactoside (optimal pH = 5.5−6.5). A divalent metal ion, such as Mg^{2+} or Mn^{2+}, 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 α2,3SiaT 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-lactoside (LacMU, acceptor) per minute at 37 °C, pH 8.5]. The apparent kinetic data were obtained for CMP−Neu5Ac (K_M = 0.44 mM, k_{cat} = 32 s^{-1}) and LacMU (K_M = 1.4 mM, k_{cat} = 47 s^{-1}). The k_{cat}/K_M of LacMU (33 s^{-1} mM^{-1}) for the tPm0188Ph α2,3-SiaT was about 90-fold higher than that (22 min^{-1} mM^{-1}) of 8-aminopyrene-1,3,6-trisulfonic acid-labeled lactose for *N. meningitidis* α2,3SiaT.[3a]

The efficiency and the flexibility of the tPm0188Ph in the synthesis of α2,3-linked sialoside libraries were tested using a one-pot three-enzyme system (Scheme 1). In this system, CMP−Sia

Scheme 1. A One-Pot Three-Enzyme System for the Synthesis of Sialosides



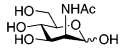
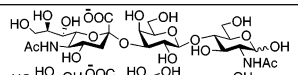
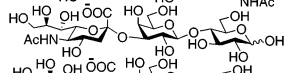
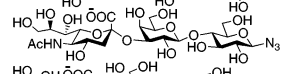
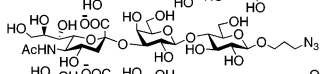
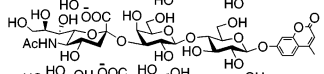
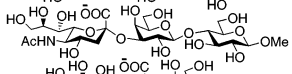
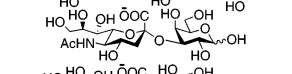
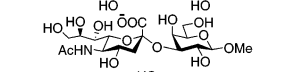
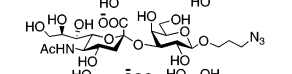
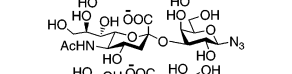
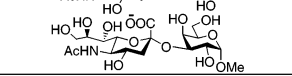
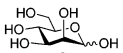
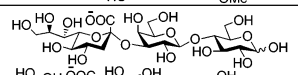
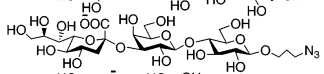
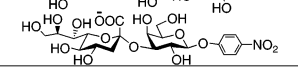
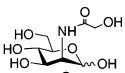
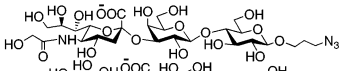
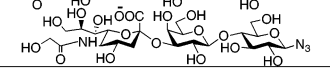
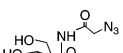
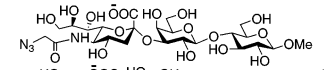
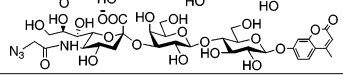
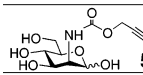
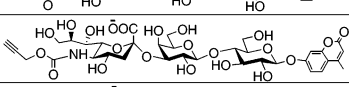
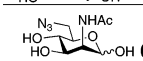
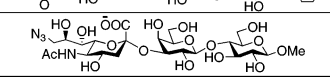
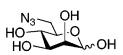
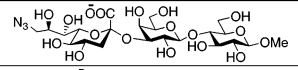
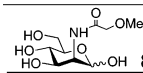
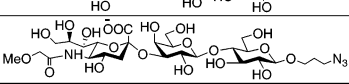
derivatives were generated in situ from sialic acid precursors catalyzed by a recombinant *E. coli* K12 sialic acid aldolase and a recombinant *N. meningitidis* CMP−Sia synthetase.[5a] Under reaction conditions used (37 °C, pH 8.5, 1−2 h), no significant sialidase or α2,6SiaT activity was observed.

As shown in Table 1, the tPm0188Ph had the most flexible donor substrate specificity among all bacterial SiaTs reported to date. It

[†] University of California, Davis.
[‡] Current address: Neose Technologies, Inc., Horsham, PA.
[§] Current address: Department of Chemistry, East China Normal University, China.
[▽] Current address: Incyte Corp., Experimental station, Wilmington, DE.
[#] Current address: Drug Source Company, Shanghai, China.

10.1021/ja0561690 CCC: $30.25

Table 1. Synthesis of Sialoside Libraries Using tPm0188Ph in a One-Pot Three-Enzyme System, as Shown in Scheme 1

Donor Precursors	Entry	Product	Yield (%)
 **1**	a		95
	b		79
	c		92
	d		84
	e		75
	f		91
	g		76
	h		79
	i		82
	j		84
	k		62
 **2**	l		80
	m		75
	n		75
 **3**	o		80
	p		81
 **4**	q		90
	r		82
 **5**	s		65
 **6**	t		91
 **7**	u		88
 **8**	v		90

[a] All reactions were carried out in preparative (50−150 mg) scales.

accepted CMP activated Neu5Ac, KDN (deaminoneuraminic acid), and Neu5Gc (*N*-glycolylneuraminic acid) obtained in situ from their corresponding precursors ManNAc (*N*-acetylmannosamine) **1**, mannose **2**, and ManNGc (*N*-glycolylmannosamine) **3**, respectively.[5a] In comparison, CMP−Neu5Gc and CMP−KDN were poor substrates for *N. gonorrheae* α2,3SiaT,[2a] and the *N. meningitidis* α2,-3SiaT could not use CMP−KDN as a donor.[4c] In addition, CMP−Sia derivatives with modifications at C-5 or C-9 in Neu5Ac or KDN obtained in situ from C-2 or C-6-modified ManNAc or mannose (**4**−**8**) were also acceptable by tPm0188Ph. For example, azide or alkyne groups introduced chemically onto ManNAc or mannose (**4**−**7**) were efficiently transferred to sialoside products, providing convenient chemical handles for further modifications via Staudinger ligation[7] or Click chemistry.[8]

The tPm0188Ph α2,3SiaT 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, j, and n) were good acceptors. Similar to that reported for the *N. meningitidis* α2,3SiaT,[3a] an α-galactoside (e.g., α-methylgalactoside, entry k) was an acceptor for tPm0188Ph. *N*-Acetylgalactosamine (GalNAc) and its derivatives, such as αGalNAcProN_3 and *β*GalNAcProN_3, however, were not acceptors.

By controlling the pH value of reaction, tPm0188Ph could be used in synthesizing α2,6-linked sialosides. For example, Neu5Acα2,-6LacMU was obtained in 5.5% 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, Neu5Acα2,-3LacMU was obtained in 36% yield from Neu5Acα2,3-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 α2,3SiaT, an α2,6SiaT, an α2,3-sialidase, and an α2,3-*trans*-sialidase also provides solid evidence for the complexity of bacterial sialylation.

Acknowledgment. This work was supported by Mizutani Foundation for Glycoscience, New Faculty Research Grant, and start-up funds from the Regents of the University of California.

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

(1) (a) Angata, T.; Varki, A. *Chem. Rev.* **2002**, *102*, 439−469. (b) Schauer, R. *Glycoconjugate J.* **2000**, *17*, 485−499.

(2) (a) Izumi, M.; Shen, G. J.; Wacowich-Sgarbi, S.; Nakatani, T.; Plettenburg, O.; Wong, C.-H. *J. Am. Chem. Soc.* **2001**, *123*, 10909−10918. (b) Boons, G. J.; Demchenko, A. V. *Chem. Rev.* **2000**, *100*, 4539−4566.

(3) (a) Gilbert, M.; Cunningham, A. M.; Watson, D. C.; Martin, A.; Richards, J. C.; Wakarchuk, W. W. *Eur. J. Biochem.* **1997**, *249*, 187−194. (b) Yamamoto, T.; Nakashizuka, M.; Kodama, H.; Kajihara, Y.; Terada, I. *J. Biochem.* **1996**, *120*, 104−110. (c) Kajihara, Y.; Yamamoto, T.; Nagae, H.; Nakashizuka, M.; Sakakibara, T.; Terada, I. *J. Org. Chem.* **1996**, *61*, 8632−8635. (d) Blixt, O.; Vasiliu, D.; Allin, K.; Jacobsen, N.; Warnock, D.; Razi, N.; Paulson, J. C.; Bernatchez, S.; Gilbert, M.; Wakarchuk, W. *Carbohydr. Res.* **2005**, *340*, 1963−1972. (e) Gilbert, M.; Bayer, R.; Cunningham, A. M.; DeFrees, S.; Gao, Y.; Watson, D. C.; Young, N. M.; Wakarchuk, W. W. *Nat. Biotechnol.* **1998**, *16*, 769−772.

(4) (a) Koeller, K. M.; Wong, C.-H. *Nature* **2001**, *409*, 232−240. (b) Blixt, O.; Allin, K.; Pereira, L.; Datta, A.; Paulson, J. C. *J. Am. Chem. Soc.* **2002**, *124*, 5739−5746. (c) Blixt, O.; Paulson, J. C. *Adv. Synth. Catal.* **2003**, *345*, 687−690.

(5) (a) Yu, H.; Yu, H.; Karpel, R.; Chen, X. *Bioorg. Med. Chem.* **2004**, *12*, 6427−6435. (b) Martin, R.; Witte, K. L.; Wong, C.-H. *Bioorg. Med. Chem.* **1998**, *6*, 1283−1292.

(6) Yamamoto, T.; Nakashizuka, M.; Terada, I. *J. Biochem. (Tokyo)* **1998**, *123*, 94−100.

(7) (a) Saxon, E.; Bertozzi, C. R. *Science* **2000**, *287*, 2007−2010. (b) Lin, F. L.; Hoyt, H. M.; van Halbeek, H.; Bergman, R. G.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2005**, *127*, 2686−2695.

(8) (a) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew Chem., Int. Ed.* **2001**, *40*, 2004−2021. (b) Fazio, F.; Bryan, M. C.; Blixt, O.; Paulson, J. C.; Wong, C.-H. *J. Am. Chem. Soc.* **2002**, *124*, 14397−14402.

JA0561690