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Crystallization and preliminary X-ray crystallographic analysis of the α-2,6-sialyltransferase PM0188 from *Pasteurella multosida*

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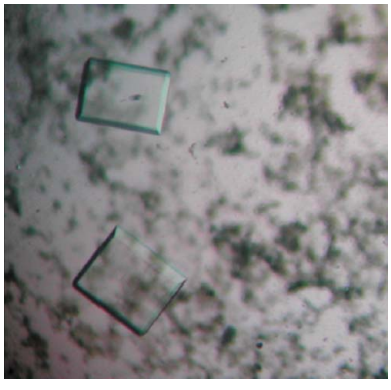
Sialyltransferase is an enzyme that transfers the sialic acid moiety from cytidine-5-monophospho-*N*-acetylneuraminic acid (CMP-NeuAc) to the carbohydrate group of various glycoproteins. These glycoproteins are involved in inflammation, embryogenesis, immune defence and metastasis of cancer cells by cell–cell interactions or cell–matrix interactions. The α-2,6-sialyltransferase PM0188 from *Pasteurella multocida* was purified using affinity-column chromatographic methods and crystallized using the hanging-drop vapour-diffusion method at 293 K. MAD data were collected to 1.9 Å resolution from an SeMet-substituted crystal. The crystal belongs to space group $P2_1$, with unit-cell parameters $a = 52.9$, $b = 61.0$, $c = 64.6$ Å, $\alpha = \gamma = 90$, $\beta = 112.3^\circ$. Assuming the presence of one molecule in the asymmetric unit, the solvent content is estimated to be about 45%.

1. Introduction

Sialic acids exist in a variety of mammalian glycoproteins and glycolipids. They are usually found at the non-reducing terminal positions of carbohydrate chains, which enables the sialylated oligosaccharides to play important roles in cell–cell recognition, cell differentiation and various receptor–ligand interactions (Harduin-Lepers *et al.*, 2001; Kelm & Schauer, 1997). In spite of their biological importance, the mechanism and molecular structures of these enzymes are still elusive. The protein PM0188 from *Pasteurella multocida* is an α-2,6-sialyltransferase that adds a terminal α-2,6-linked sialic acid to N-linked oligosaccharides of glycoproteins (Steenbergen *et al.*, 2005). *P. multocida* is a commensal and opportunistic pathogen of food animals, wildlife and pets, and a zoonotic cause of human infection arising from contact with these animals (Steenbergen *et al.*, 2005). It has also been increasingly associated with pulmonary disease, sepsis and meningitis in patients with impaired immune status (Berge *et al.*, 2002; Byrd & Roy, 2003; Kouppari *et al.*, 1999; Laupland *et al.*, 2002). Very interestingly, PM0188 is distinctive compared with other sialyltransferases. Firstly, it has no sialyl motif, whereas most sialyltransferases have two sialyl motifs: the L sialyl motif participates in the binding of the sugar donor CMP-NeuAc, whereas the S sialyl motif participates in the binding of both the donor and acceptor substrates (Datta & Paulson, 1995; Datta *et al.*, 1998). Secondly, it has very low sequence homology not only to other sialyltransferase families, but also to sialyltransferases from other sources. To date, only one sialyltransferase structure has been reported: the crystal structure of CstII from *Campylobacter jejuni* (Chiu *et al.*, 2004). From functional and structural points of view, however, PM0188 is quite different from CstII, which is a bifunctional α-2,3/2,8-sialyltransferase and is composed of a single Rossmann domain. In contrast, PM0188 is a α-2,6-sialyltransferase and is expected to consist of two Rossmann domains. From the structures published so far, glycosyltransferases have been categorized into two groups: the glycosyltransferase A (GTA) group, which consist of a single Rossmann domain, and the glycosyltransferase B (GTB) group, which consist of two Rossmann domains (Vrielink *et al.*, 1994; Ha *et al.*, 2000; Gibson *et al.*, 2002). PM0188 is supposed to belong to GTB.

In this study, PM0188 was purified by affinity and ion-exchange column chromatography. We also confirmed that PM0188 possesses



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α-2,6-sialyltransferase activity (data not shown). Here, we report the crystallization and preliminary X-ray crystallographic analysis of PM0188. The structural information on the PM0188 protein will explain the reaction mechanism of α-2,6-sialyltransferase and provide a structural basis for the design of novel antibacterial agents that function through inhibition of their action.

2. Materials and methods

2.1. Protein expression and purification

DNA encoding PM0188 (residues 25–412) from *P. multocida* strain PM70 was inserted into pET15b (Pharmacia Biotech) with a His tag at the C-terminus. The plasmid was transformed into *Escherichia coli* strain BL21 (DE3) competent cells and the transformants were selected on LB agar plates containing 100 µg ml^{-1} ampicillin. When the culture density (OD_{600}) reached 0.6, the culture was induced with 1 m*M* isopropyl-β-D-thiogalatopyranoside (IPTG) and grown for an additional 14 h at 298 K before the cells were harvested. The bacterial cell pellet was resuspended in buffer containing 20 m*M* Tris pH 7.5, 100 m*M* NaCl, 2 m*M* imidazole and homogenized by sonication. The lysate was centrifuged at 18 000*g* for 30 min and the supernatant was loaded onto an Ni–NTA column (Qiagen) and washed with the same buffer. PM0188 protein was eluted with buffer containing 20 m*M* Tris–HCl pH 7.5, 100 m*M* NaCl, 300 m*M* imidazole. Further purification was carried out using a Resource Q column which had been pre-equilibrated with buffer *A* (20 m*M* Tris–HCl pH 7.5). The PM0188 protein was then eluted from the column with a linear gradient to buffer *B* (20 m*M* Tris–HCl pH 7.5, 1 *M* NaCl) and was then concentrated to 10 mg ml^{-1} for crystallization. Purity was checked by SDS–PAGE (Fig. 1). The selenomethionine (SeMet) substituted protein was expressed in the methionine-auxotroph *E. coli* strain B834 (DE3) (Novagen) in a minimal medium supplemented with 50 mg ml^{-1} SeMet under the same conditions as the native. The purification method of the SeMet-substituted protein was identical to that of the native protein, except for the addition of 5 m*M* DTT to all buffers. The molecular weight of the SeMet-substituted protein was confirmed by mass spectrometry (data not shown).

2.2. Crystallization and data collection

Initial crystallization was performed with commercially available screening solutions (Hampton Research) by the microbatch method at 290 K. Further optimization of the crystallization conditions was preformed by hanging-drop vapour diffusion. 1.5 µl protein solution was mixed with 1.5 µl of a solution containing 26% PEG 2000, 0.1 *M* Bis-Tris pH 6.5. Under these conditions, crystals grew within 4 d to a sufficient size for data collection. SeMet-substituted PM0188 crystals were obtained using 25% PEG 3350, 0.1 *M* Bis-Tris pH 6.5 as precipitant (Fig. 2).

The crystals were transferred to a cryoprotectant solution containing 25% PEG 3350, 0.1 *M* Bis-Tris pH 6.5, 15% MPD. The crystals were mounted on a cryoloop in a 100 K nitrogen-gas stream. The native crystal was then rotated through a total of 180° (1° rotations) at a wavelength of 1.12710 Å. In addition, a three-wavelength MAD data set was collected from the SeMet-labelled crystal based on 180 images (1° rotations) at 0.97918 Å (edge wavelength), 0.97903 Å (peak wavelength) and 0.97137 Å (remote wavelength). All data were collected at beamline 6B of the Pohang Light Source (PLS), Pohang, South Korea using a Bruker AXS Proteum 300 CCD detector (Madison, WI, USA). The data were indexed, integrated and scaled using the *HKL*2000 suite (Otwinowski & Minor, 1997).

Table 1
X-ray data-collection statistics.

Values in parentheses are for the highest resolution shell.

		SeMet		
	Native	Edge	Peak	Remote
Wavelength (Å)	1.12710	0.97918	0.97903	0.97137
Resolution range (Å)	2.3 (2.38–2.3)	1.9 (1.97–1.9)	1.9 (1.97–1.9)	1.9 (1.97–1.9)
Space group	*P*2	$P2_1$		
Unit-cell parameters				
a (Å)	60.7	52.9		
b (Å)	70.6	61.0		
c (Å)	75.0	64.6		
α (°)	90	90		
β (°)	114.7	112.3		
γ (°)	90	90		
Total reflections	106811	375890	375814	378295
Unique reflections	17050	30177	30006	29913
Completeness (%)	93.6 (88.1)	98.4 (91.7)	98.8 (93.1)	98.5 (92.9)
R_{sym}† (%)	7.8 (18.3)	10.1 (44.6)	10.9 (46.5)	10.3 (49.7)
Average $I/\sigma(I)$	23.9 (6.5)	14.1 (1.7)	15.4 (2.1)	13.0 (1.6)

† $R_{sym} = \sum|I_{obs} - I_{avg}|/I_{obs}$, where I_{obs} is the observed intensity of an individual reflection and I_{avg} is the average over symmetry equivalents.

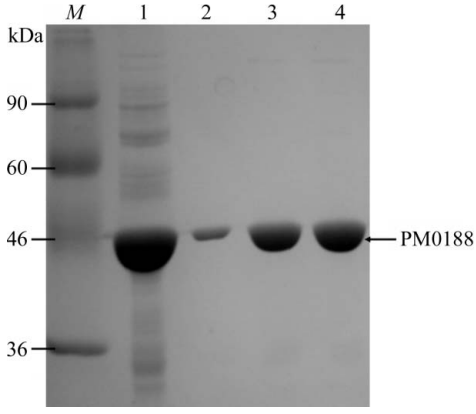


Figure 1
SDS–PAGE analysis of the expression and purification of PM0188 protein. Lane 1, total protein from *E. coli* BL21 (DE3) after induction; lanes 2 and 3, purified PM0188 protein using Ni–NTA affinity chromatography; lane 4, purified PM0188 protein after ion-exchange chromatography using a Resource Q column.

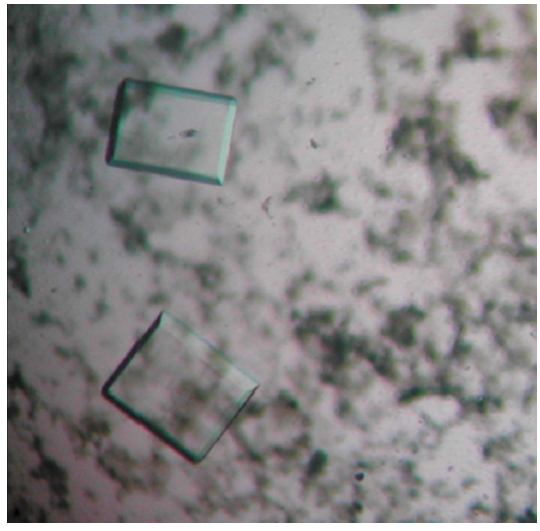
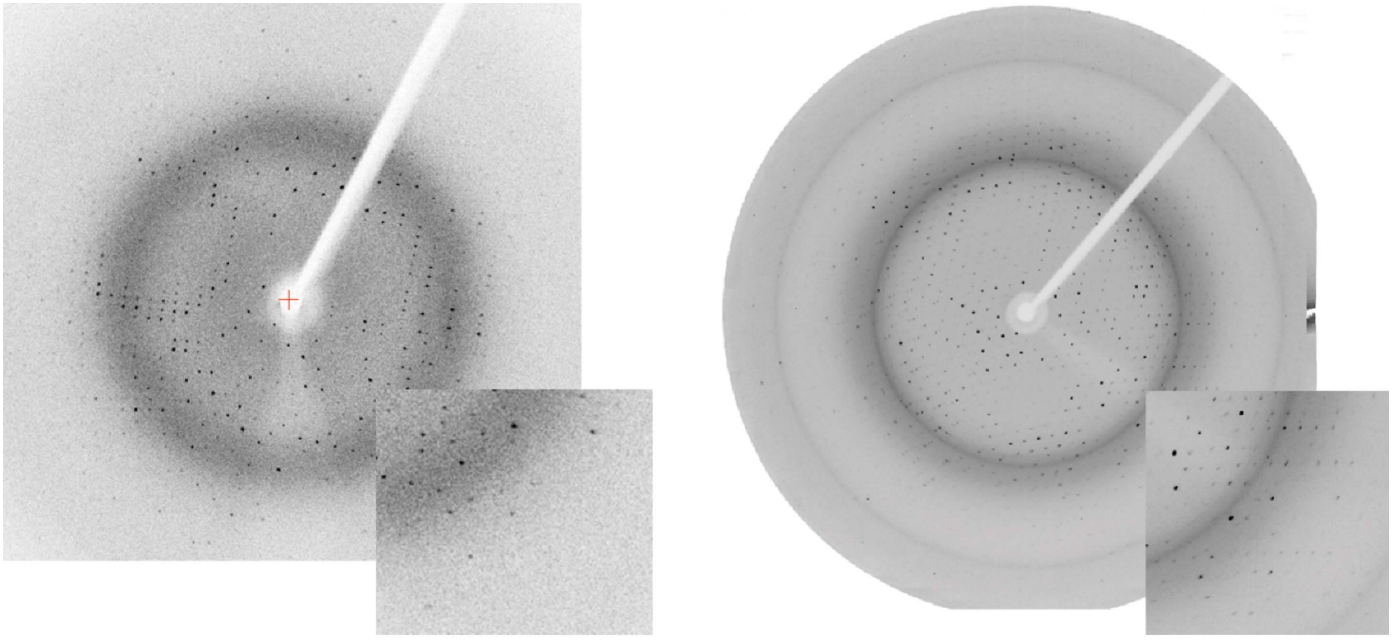


Figure 2
Crystals of SeMet-substituted PM0188 grown by the hanging-drop vapour-diffusion method. The approximate dimensions of the crystal are 0.3 × 0.25 × 0.07 mm.



(*a*) (*b*)

Figure 3
X-ray diffraction patterns of (*a*) native and (*b*) SeMet-labelled PM0188 crystals.

3. Results and discussion

The diffraction data indicated that the native PM0188 crystal belongs to space group $P2$, with unit-cell parameters a = 60.671, b = 70.625, c = 74.963 Å, $\alpha = \beta = 90$, $\gamma = 114.672^\circ$. The crystal of native PM0188 diffracted to 2.3 Å resolution (Fig. 3*a*). Assuming the presence of one molecule per asymmetric unit, the Matthews coefficient (V_M) was calculated to be 3.2 Å^3 Da^{-1}, corresponding to a solvent content of about 61.8% (Matthews, 1968). The SeMet-substituted crystals were determined to belong to space group $P2_1$, with unit-cell parameters a = 52.9, b = 61.0, c = 64.6 Å, $\alpha = \gamma = 90$, $\beta = 112.3^\circ$; V_M was 2.1 Å^3 Da^{-1}, corresponding to a solvent content of about 40.8% (Fig. 3*b*). The data-collection statistics are summarized in Table 1. Se sites were determined using the *SOLVE* program (Terwilliger, 2000) based on 2.3 Å anomalous data and six Se atoms were identified. The initial phases were then improved by *RESOLVE*. Model building is in progress.

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