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Purification, crystallization and preliminary crystallographic characterization of the α2,6-sialyltransferase from *Photobacterium* sp. JT-ISH-224

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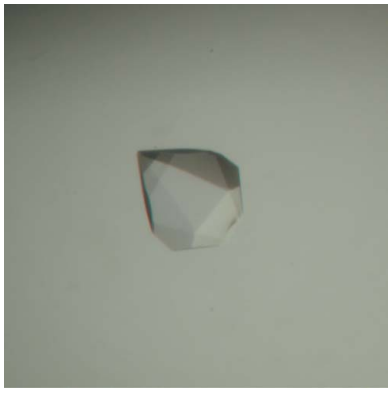
Sialyltransferases transfer sialic acid from cytidine-5-monophospho-*N*-acetylneuraminic acid (CMP-NeuAc) to the nonreducing termini of the oligosaccharyl structures of various glycoproteins and glycolipids. The newly cloned α2,6-sialyltransferase from *Photobacterium* sp. JT-ISH-224 (from the *Vibrionaceae* family) is composed of two domains: an unknown N-terminal domain and a catalytic C-terminal domain which shares significant homology with the *Pasteurella multocida* multifunctional sialyltransferase. The putative mature form of JT-ISH-224 α2,6-sialyltransferase was overproduced in *Escherichia coli*, purified and crystallized using the hanging-drop vapour-diffusion method at 293 K. The crystal belonged to space group $P3_121$ or $P3_221$, with unit-cell parameters $a = b = 90.29$, $c = 204.33$ Å. X-ray diffraction data were collected to 2.5 Å resolution.

1. Introduction

Sialic acids are of great interest for the development of pharmaceuticals and other applications because sialylated molecules play important roles in cell–cell interactions, cell differentiation and various receptor–ligand interactions (Schauer, 2000; Angata & Varki, 2002). In some mucosal pathogens, the sialylation of lipopolysaccharides is a widely conserved structural modification and plays an important role in resistance to the killing effects of normal human serum (Vimr & Lichtensteiger, 2002; Vimr *et al.*, 2004). Furthermore, it has been demonstrated that sialyltransferase (STase) activity is crucial for bacterial pathogenicity. In general, bacterial STases are also important for the enzymatic modification of glycoconjugates and the enzymatic synthesis of glycans because they are more stable and productive than mammalian enzymes. Since α2,3-STase was first cloned from *Neisseria meningitidis* and *N. gonorrhoeae* (Gilbert *et al.*, 1996), bacterial STases have been cloned from several microorganisms (Gilbert *et al.*, 2000; Yamamoto *et al.*, 1998; Bozue *et al.*, 1999; Shen *et al.*, 1999). To date, the crystal structures of three bacterial STases have been reported: the bifunctional STase CstII from *Campylobacter jejuni* (Chiu *et al.*, 2004), the α2,3-STase CstI from *C. jejuni* (Chiu *et al.*, 2007) and the multifunctional STase Δ24PmST1 from *Pasteurella multocida* (Ni *et al.*, 2006, 2007). Very recently, α2,6-STase from *Photobacterium* sp. JT-ISH-224 (ISH224 α2,6-STase) has been cloned in our laboratory (Tsukamoto *et al.*, 2007). The deduced amino-acid sequence of this enzyme shows 35.6% identity to the multifunctional STase from *P. multocida*, but no significant homology to the bifunctional STase from *C. jejuni*. Interestingly, the STases from *C. jejuni* and *P. multocida* have bifunctional (α2,3/α2,8-STase) and multifunctional (α2,3/α2,6-STase, α2,3 sialidase and α2,3-trans-sialidase) activities, respectively, but ISH224 α2,6-STase shows only α2,6-STase activity. Therefore, it is of great interest to elucidate the molecular structure of this enzyme in order to help understand its catalytic mechanism and α2,6 reaction specificity. In this study, we report the preparation, crystallization and preliminary X-ray analysis of α2,6-STase from *Photobacterium* sp. JT-ISH-224.



2. Materials and methods

2.1. Preparation of the expression plasmid and recombinant enzyme

DNA encoding α2,6-STase (Δ2–17, lacking the N-terminal hydrophobic region) from *Photobacterium* sp. JT-ISH-224 was inserted into pTrc99A (Pharmacia; Tsukamoto *et al.*, 2007). The resulting expression plasmid was transformed into *Escherichia coli* TB1 competent cells. The transformant was cultured in 6 ml LB medium containing 100 µg ml^{-1} ampicillin at 303 K for 8 h. The culture was transferred into 300 ml LB medium containing 100 µg ml^{-1} ampicillin and 1 m*M* IPTG and cultured at 303 K for 16 h. The cells were harvested by centrifugation and suspended in buffer *A* (20 m*M* bis-Tris buffer pH 6.0 containing 0.336% Triton X-100) and disrupted by sonication. The cell debris was removed by centrifugation (100 000*g* for 1 h) and the supernatant was filtered through a 0.45 µm cellulose acetate membrane. The obtained crude enzyme solution was loaded onto a HiLoad 26/10 Q-Sepharose HP column (GE Healthcare). The adsorbed protein was washed with buffer *A* and eluted with a linear gradient of buffer *A* with an increasing concentration of NaCl up to 1 *M*. The pooled fractions showing α2,6-STase activity were diluted with four volumes of buffer *B* (20 m*M* potassium phosphate buffer pH 6.0 containing 0.336% Triton X-100) and applied onto a CHT20-I column (Bio-Rad). The adsorbed protein was washed with the same buffer and eluted with a linear gradient of buffer *B* with an increasing concentration of potassium phosphate up to 0.5 *M*. The active fractions were pooled and applied onto a Mono Q 10/100 GL column (GE Healthcare) equilibrated with buffer *A*. The enzyme was eluted with an increasing NaCl gradient from 0 to 1 *M* in buffer *A*. Finally, the active fractions were pooled and applied onto a Superdex 200 HR column (GE Healthcare) equilibrated with 25 m*M* MES buffer pH 6.0 containing 100 m*M* NaCl. The eluted α2,6-STase protein was concentrated to 10 mg ml^{-1} for crystallization. The protein determination was performed using a protein assay (Bio-Rad) with bovine serum albumin as a standard. The activity of α2,6-STase was measured according to the method of Yamamoto *et al.* (1996).

2.2. Crystallization and data collection

Initial crystallization trials were carried out by the hanging-drop vapour-diffusion method in a 24-well plate at 293 K using the Crystal Screen, Crystal Screen 2 and Index Screen kits (Hampton Research). Each drop was prepared by mixing 1 µl protein solution (10 mg ml^{-1}) containing 10 m*M* cytidine monophosphate (CMP) and 10 m*M* lactose and an equal volume of reservoir solution. After 5 d, a small crystal of α2,6-STase was observed using a reservoir condition consisting of 0.2 *M* lithium sulfate monohydrate, 0.1 *M* Tris–HCl pH 8.5 and 30%(*w*/*v*) PEG 4000 (Fig. 1). This result was found to be reproducible. Under these conditions, crystals grew within 10 d to a sufficient size for data collection. Prior to data collection, the crystal was transferred stepwise into cryoprotectant solution consisting of the crystallization condition containing 10% glycerol and flashed-cooled in a nitrogen-gas stream at 100 K. X-ray diffraction data were collected using synchrotron radiation (1.000 Å wavelength) with a Jupiter 210 detector (Rigaku/MSC Corporation) at beamline BL38B1 of SPring-8 (Hyogo, Japan). The oscillation angle was 1.0° and the exposure time was 10.0 s per frame. A total of 180 diffraction images were collected at a camera distance of 150 mm and were processed using *HKL*-2000 (Otwinowski, 1993).

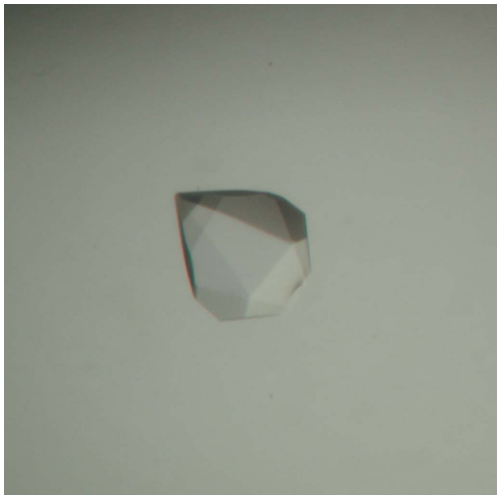


Figure 1
A crystal of ISH224 α2,6-STase obtained using the hanging-drop vapour-diffusion method. The approximate dimensions of the crystal are 0.3 × 0.3 × 0.3 mm.

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell (2.59–2.50 Å).

Space group	$P3_121$ or $P3_221$
Unit-cell parameters (Å)	$a = b = 90.29$, $c = 204.33$
Beamline	BL38B1, SPring-8
Wavelength (Å)	1.000
Resolution range (Å)	50.0–2.5
No. of observed reflections	184556
No. of unique reflections	34177
Redundancy	5.4 (5.5)
R_{sym}†	0.082 (0.432)
$I/\sigma(I)$	20.0 (3.78)
Completeness (%)	99.8 (100.0)

† $R_{sym} = \sum I - \langle I \rangle / \sum \langle I \rangle$, where I is the intensity measurement for a given reflection and $\langle I \rangle$ is the average intensity for multiple measurements of this reflection.

3. Results and discussion

The putative mature form of ISH224 α2,6-STase was expressed in *E. coli* and purified by a combination of chromatographies. The final yield of the pure protein was approximately 24 mg from 300 ml culture, corresponding to 21% recovery. The molecular weight of the α2,6-STase was determined to be 55 kDa by SDS–PAGE and 59 kDa by gel filtration, suggesting that α2,6-STase exists as a monomer in solution.

A crystal of ISH224 α2,6-STase in a complex with CMP and lactose diffracted to 2.5 Å and belongs to the hexagonal space group $P3_121$ or $P3_221$, with unit-cell parameters $a = b = 90.29$, $c = 204.33$ Å. The data statistics are summarized in Table 1. We have attempted molecular-replacement methods for phase determination, but have not succeeded. The crystal structure is now being solved by the MAD method with selenium as the anomalous scattering atom using synchrotron radiation.

Recently, Ni and coworkers reported the crystal structure of the multifunctional STase Δ24PmST1 from *P. multocida* (Ni *et al.*, 2006, 2007). Since ISH224 α2,6-STase shows only α2,6-STase activity, structural analysis of the α2,6-STase would explain the functional difference between Δ24PmST1 and ISH224 α2,6-STase. Furthermore, from primary sequence analysis of ISH224 α2,6-STase, it was revealed that the enzyme is composed of an unknown N-terminal domain and a catalytic C-terminal domain. Structural information on the α2,6-STase will also provide new information about the function of the N-terminal domain.

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