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Crystallization and preliminary X-ray analysis of vicenisaminyltransferase VinC

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A recombinant glycosyltransferase, VinC, from *Streptomyces halstedii* HC34 has been crystallized at 293 K using PEG 3350 as precipitant. The diffraction pattern of the crystal extends to 2.0 Å resolution at 100 K using synchrotron radiation at SPring-8. The crystals are orthorhombic and belong to space group *I*222, with unit-cell parameters a = 98.21, b = 130.39, c = 140.11 Å. The presence of two molecules per asymmetric unit gives a crystal volume per protein weight (V_M) of 2.43 Å^3 Da^{-1} and a solvent content of 49.5% by volume.

1. Introduction

Vicenistatin is an antitumour β-glycosidic antibiotic produced by *Streptomyces halstedii* HC34. It comprises the amino sugar vicenisamine and the 20-membered macrocycle vicenilactam (Fig. 1; Shindo *et al.*, 1993). We have identified the gene cluster for the biosynthesis of vicenistatin and clarified that the cluster contains putative genes for vicenilactam biosynthesis; these include four modular polyketide synthases (PKSs), glutamate mutase, acyl-CoA ligase and AMP ligase (Ogasawara *et al.*, 2004). Also found in the cluster are genes for the expression of NDP-hexose-4,6-dehydratase and aminotransferase, which are involved in vicenisamine biosynthesis.

VinC has been identified as a vicenisaminyltransferase that catalyzes the transfer of vicenisamine from dTDP-vicenisamine to vicenilactam in the last step of vicenistatin biosynthesis (Ogasawara *et al.*, 2004). We recently demonstrated that VinC is able to accept structurally diverse aglycons (glycosyl acceptors) to form the respective vicenisamides (Minami, Kakinuma *et al.*, 2005; Minami, Uchida *et al.*, 2005). Furthermore, VinC displays glycosyltransfer activities with various dTDP-sugars, including anomers of both D- and L-sugars (Minami & Eguchi, 2007). Thus, VinC is thought to be an attractive glycosyltransferase for the generation of various glycosides because of its broad substrate specificity. In fact, previous studies have shown that new compounds could be constructed with VinC using unnatural glycosyl donors and acceptors (Minami & Eguchi, 2007). Moreover, if the structural determinants of substrate specificity can be understood, directed genetic modification of the glycosyltransferases could be exploited to yield a broader diversity of products.

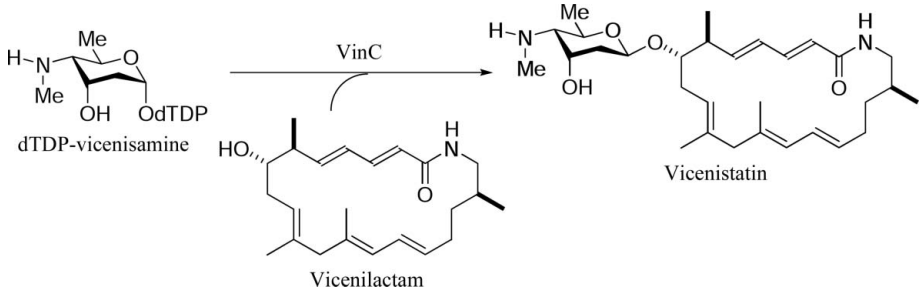

Figure 1
The transfer of vicenisamine from dTDP-α-D-vicenisamine to vicenilactam catalysed by VinC.

In order to understand the issues underlying catalysis and substrate specificity, we have undertaken structural analysis of VinC. In this study, we report the crystallization and preliminary X-ray diffraction analysis of VinC.

2. Experimental

2.1. Crystallization

The *vinC* gene was cloned, inserted into the vector pET30b(+) and transformed into *Escherichia coli* strain BL21 (DE3) (Ogasawara *et al.*, 2004). The expressed VinC was purified using ammonium sulfate precipitation and DEAE Sepharose Fast Flow column chromatography (Minami & Eguchi, 2007). Typical yields are 84 mg homogeneous VinC from approximately 5 g cell paste. VinC was obtained in an electrophoretically homogeneous state and its molecular weight was estimated to be 46 kDa using SDS–PAGE analysis, which was in agreement with the calculated weight of 46 066 Da. The purified enzyme was prepared in 5 m*M* Tris–HCl pH 8.0 and crystallization was carried out using the hanging-drop vapour-diffusion method at 293 K. A 2 µl droplet of 11 mg ml^{-1} protein solution mixed with the same volume of reservoir solution [20%(*w*/*v*) PEG 3350, 200 m*M* $MgCl_2$ and 100 m*M* Tris–HCl pH 8.5] was equilibrated against 1 ml reservoir solution to obtain VinC crystals. Small needle-shaped crystals appeared in 3–6 d in this condition and grew to maximum dimensions of 0.02 × 0.02 × 0.1 mm. Microseeding was used in order to obtain larger crystals. To set up the drops for seeding, 2 µl protein solution at 11 mg ml^{-1} was mixed with 2 µl precipitant solution and equilibrated against the same condition as used above for 2 d. Several needle-shaped crystals were removed from their crystallization drop and transferred to a 10 µl drop of precipitant solution, where they were crushed with a spatula to generate seed crystals. The seed suspension was diluted with precipitant solution at a 1:5(*v*:*v*) ratio. Seeds were then transferred to the equilibrated drops from the seed suspension with a cryoloop (0.1–0.2 mm diameter). Crystals grew to maximum dimensions of 0.2 × 0.2 × 0.6 mm within a week of seeding.

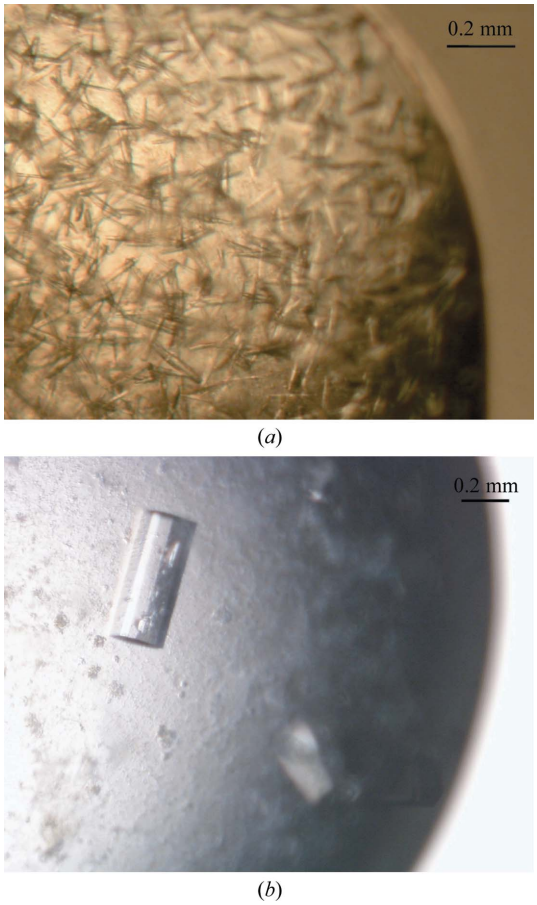

Figure 2
Crystals of VinC. (*a*) Initial crystals of VinC. (*b*) Single crystals obtained by seeding. The average dimensions of these crystals were 0.2 × 0.2 × 0.6 mm.

2.2. Data collection

A drop containing the crystals (approximately 4 µl) was supplemented with 2 µl 20%(*v*/*v*) polyethylene glycol (PEG) 400 (the final concentration in the drop was approximately 11% PEG 400) because crystals broke if transferred directly to the cryosolution. After 10–20 s, the crystals were transferred to reservoir solution supplemented with 20% PEG 400 as cryoprotectant. The crystal, mounted in a cryoloop, was flash-cooled in a liquid-nitrogen stream at 100 K. Data collection was performed at 100 K using an X-ray wavelength of 1.0 Å on BL26B1 of SPring-8 (Harima, Japan) using a Rigaku/MSC Jupiter210 detector system. Diffraction images were integrated and scaled using the programs *DENZO* and *SCALEPACK* from the *HKL* suite (Otwinowski & Minor, 1997).

3. Results and discussion

Preliminary screening was carried out using commercially available screening kits (Hampton Research Crystal Screens I and II and PEG/Ion Screen). The crystals obtained without seeding were small needles, but grew to maximum dimensions of 0.35 × 0.35 × 1.0 mm (Fig. 2) after seeding. The crystals diffracted X-rays to 2.0 Å resolution (Fig. 3). The space group was determined to be *I*222, with unit-cell parameters a = 98.21, b = 130.39, c = 140.11 Å. Assuming the presence of one dimeric subunit in the asymmetric unit, the Matthews

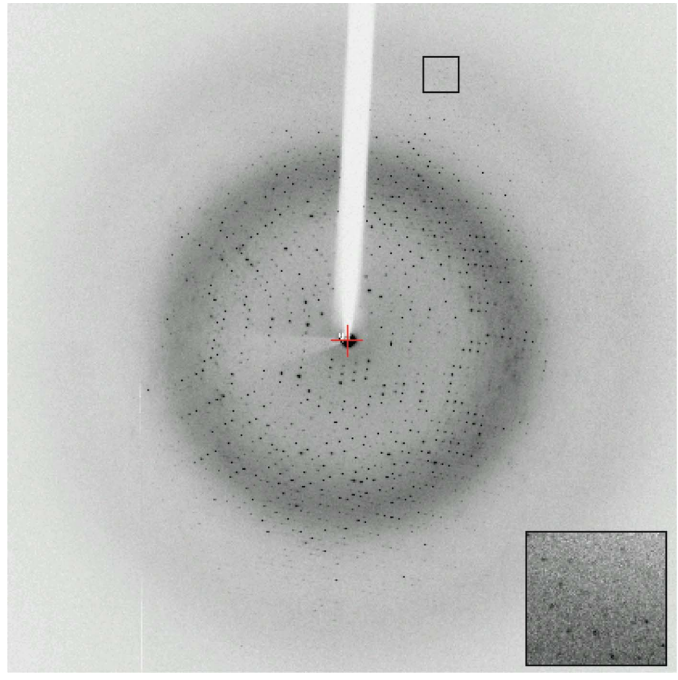

Figure 3
Diffraction pattern of a VinC crystal. An enlarged image of the area around 2.0 Å is shown.

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

Space group	*I*222
Unit-cell parameters (Å)	$a = 98.21$, $b = 130.39$, $c = 140.11$
Wavelength (Å)	1.0
Resolution (Å)	100.00–2.00 (2.07–2.00)
Total observations	549280
Unique reflections	60204
$\langle I/\sigma(I)\rangle$	12.9
$R_{\rm merge}$† (%)	5.6 (32.7)
Data completeness (%)	98.5 (91.2)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the ith intensity measurement of reflection hkl, including symmetry-related reflections, and $\langle I(hkl)\rangle$ is its average.

coefficient $V_{\rm M}$ (Matthews, 1968) was calculated to be 2.43 Å^3 Da^{-1}, with an estimated solvent content of 49.5%.

Scaling and merging of the crystallographic data resulted in an overall $R_{\rm merge}$ of 5.6% and an $R_{\rm merge}$ in the highest resolution shell of 32.7%. Complete data-collection statistics are given in Table 1.

We have attempted to carry out molecular replacement for phase determination using *MOLREP* (Vagin & Teplyakov, 1997) with structures of homologous glycosyltransferases (PDB codes 1rrv, 1iir and 1pnv), but have not been successful. Therefore, we are preparing seleno-L-methionine-labelled VinC for structure determination.

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