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Expression, purification, crystallization and preliminary X-ray analysis of the D-alanyl carrier protein DltC from *Staphylococcus epidermidis*

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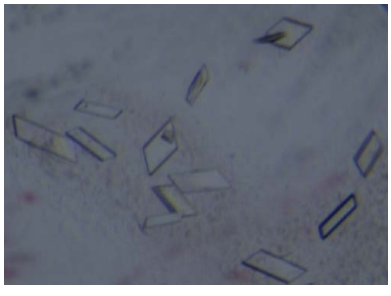
The D-alanyl lipoteichoic acids (D-alanyl LTAs) present in the cell walls of Gram-positive bacteria play crucial roles in autolysis, cation homeostasis and biofilm formation. The alanylation of LTAs requires the D-alanyl carrier protein DltC to transfer D-Ala onto a membrane-associated LTA. Here, DltC from *Staphylococcus epidermidis* (*Se*DltC) was purified and crystallized using the sitting-drop vapour-diffusion method. The crystals diffracted to a resolution of 1.83 Å and belonged to space group *P*2, with unit-cell parameters a = 66.26, b = 53.28, c = 88.05 Å, β = 98.22°. The results give a preliminary crystallographic analysis of *Se*DltC and shed light on the functional role of DltC in the alanylation of LTAs.

1. Introduction

Staphylococci are a frequent cause of nosocomial infections, which are associated with the use of medical devices in immunocompromised individuals (Otto, 2009). Like most Gram-positive bacteria, *S. epidermidis* modifies the teichoic acids (TAs) in the cell envelopes in multiple ways to protect against stressful conditions and harmful molecules. TAs are composed of wall TAs and lipoteichoic acids (LTAs). Generally, these two types of anionic polymers are modified with a D-alanyl ester or a glycosyl residue to control the activity of enzymes and the cation concentration in the cell envelope (Xia *et al.*, 2010).

The synthesis of D-alanyl LTA requires four genes (*dltA–D*) encoded by the *dlt* operon. D-Alanine:D-alanine carrier protein ligase (DltA) catalyzes the transfer of activated D-Ala to D-alanine carrier protein (DltC). The DltC-linked D-Ala is transported across the membrane by the integral membrane protein DltB. DltD subsequently catalyzes the incorporation of D-Ala into LTA (Neuhaus & Baddiley, 2003). In this sequential pathway, the incorporation of D-Ala into LTA is specifically required for DltC, which provides the essential link between DltA and D-alanyl LTA.

DltC is a homologue of the carrier proteins involved in the biosynthesis of fatty acids, polyketides and nonribosomal peptides. Similar to the peptidyl carrier proteins, DltC possesses a phosphopantetheine prosthetic group that is covalently attached to a conserved serine residue (Byers & Gong, 2007; Lai *et al.*, 2006). DltA recognizes the phosphopantetheine prosthetic group and then ligates D-alanine to DltC. To date, the only protein that is similar to *Se*DltC and that has had its structure determined by NMR is *Lc*DltC from *Lactobacillus casei* (Volkman *et al.*, 2001). However, because of the absence of the prosthetic group, the solution structure of *Lc*DltC does not provide sufficient information to elucidate the mechanism of the transfer of D-Ala to LTA by DltC. Therefore, the use of crystallography to determine the structure of *Se*DltC with the prosthetic modification was reasoned to be likely to prove to be a useful approach. Here, we report the cloning, expression, purification, crystallization and preliminary crystallographic analysis of *Se*DltC.



2. Materials and methods

2.1. Cloning, expression and purification

DltC was obtained by PCR amplification using *S. epidermidis* genomic DNA as a template and was cloned into a T7 promoter-driven expression system (pET-28b vector; Invitrogen) to allow the expression of a His_6-tagged recombinant DltC protein in *Escherichia coli* BL21 (DE3) cells. For the expression of DltC, cells were cultured in Luria–Bertani medium at 310 K and induced when an OD_{600} of 0.6 was attained by adding isopropyl β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 1.0 m*M*. To optimize the yield of soluble DltC, cell growth was continued at 293 K for a further 20 h after induction. The cells were harvested by centrifugation and the cell pellet was resuspended in buffer *A* (50 m*M* Tris–HCl pH 8.0, 500 m*M* NaCl, 5 m*M* imidazole). Subsequently, the cells were disrupted by sonication and the crude lysate was centrifuged at 20 000*g* for 90 min at 277 K. The clarified supernatant was applied onto Ni–NTA His-bind resin pre-equilibrated with binding buffer. Impurities were removed with Ni–NTA wash buffer (50 m*M* Tris–HCl, 500 m*M* NaCl, 10 m*M* imidazole pH 8.0) and the bound DltC was eluted with a 0–200 m*M* linear gradient of imidazole. The DltC-containing fractions were pooled, diluted in buffer *B* (50 m*M* Tris–HCl pH 8.0, 50 m*M* NaCl, 5 m*M* DTT) and loaded onto an ANX column (GE Healthcare) pre-equilibrated with buffer *B*. The sample was eluted with a linear NaCl gradient (0–0.5 *M*) in buffer *B*. Fractions containing DltC were pooled, concentrated by ultrafiltration using an Amicon Ultra-15 3K centrifugal filter device (Millipore; 3 kDa cutoff) and loaded onto a HiLoad 16/60 Superdex 200 size-exclusion column (GE Healthcare) equilibrated with gel-filtration buffer (50 m*M* Tris–HCl pH 8.0, 100 m*M* NaCl, 5% glycerol, 2 m*M* TCEP). The fractions containing His_6-tagged DltC were pooled and concentrated to 20 mg ml^{-1} for crystallization screening. The protein purity was determined to be >95% by scanning densitometry of Coomassie Blue-stained protein on a 12% sodium dodecylsulfate polyacrylamide gel (Fig. 1).

2.2. Crystallization

Initial crystallization trials were performed with commercially available kits from Hampton Research, Emerald BioStructures and Molecular Dimensions by using the sitting-drop vapour-diffusion method in 24-well VDX plates (Hampton Research). 1 µl protein solution (at 20 mg ml^{-1} in gel-filtration buffer) and 1 µl reservoir solution were mixed and equilibrated against 400 µl reservoir solution at 277 K. Initial crystals were grown in a precipitant consisting of 25% PEG 3350, 0.1 *M* bis-Tris pH 5.5, 0.2 *M* $MgCl_2$ (condition D5 of JBScreen JCSG++ 4 from Jena Bioscience). Further manual screens to refine the optimal crystallization conditions were then performed in a systematic manner by varying the pH and precipitant concentration. Diffraction-quality crystals were obtained using a reservoir solution consisting of 18% PEG 3350, 0.1 *M* bis-Tris pH 5.8, 0.2 *M* $MgCl_2$ (Fig. 2).

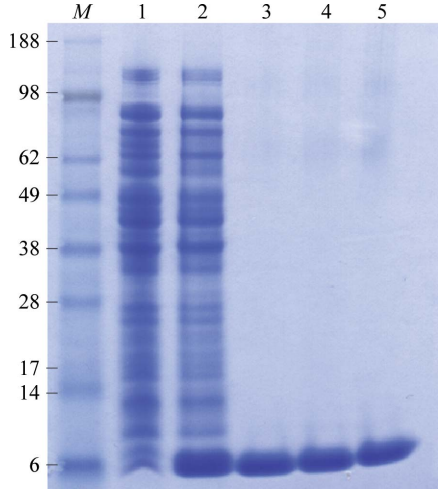


Figure 1
SDS–PAGE of purified DltC. Lane *M*, molecular-weight markers (labelled in kDa); lane 1, whole-cell lysate before IPTG induction; lane 2, whole-cell lysate after IPTG induction; lane 3, DltC purified with an Ni column; lane 4, DltC purified by an ANX column; lane 5, DltC purified by gel filtration.

2.3. Data collection

Crystals of *Se*DltC were successfully cooled in liquid nitrogen by flash-cooling them in mother liquor supplemented with 25% glycerol as a cryoprotectant for approximately 15 s. The crystals were then immediately flash-cooled by plunging them into liquid nitrogen. X-ray diffraction data were collected on BL13C1 at the National Synchrotron Radiation Research Center (NSRRC), Taiwan. The crystal was rotated through a total of 270° with consecutive 1° oscillations. The data were indexed and integrated using the *HKL*-2000 processing software (Otwinowski & Minor, 1997). An X-ray diffraction data image is shown in Fig. 3.

3. Results and discussion

The *dltC* gene of *S. epidermidis* consists of 234 bp encoding 78 amino-acid residues. The isoelectric point was calculated to be 3.93. The purified DltC protein contained an extra octapeptide LEH_6 at the C-terminal end and had a purity of >95%, with a single band of approximately 9 kDa on SDS–PAGE. Using gel-filtration analysis, we found that DltC exists as a monomer in solution.

A DltC crystal was obtained by the sitting-drop vapour-diffusion method in a buffer comprising 0.2 *M* $MgCl_2$, 0.1 *M* bis-Tris pH 5.8, 18% PEG 3350. Well diffracting crystals with dimensions of 0.25 × 0.25 × 2.0 mm were produced in one month. High-resolution diffraction data were obtained for the native DltC crystal, and the results of data processing indicated that the crystal belonged to the monoclinic space group *P*2. Data-collection statistics are presented in Table 1. Based on Matthews coefficient calculations, between six (39.92% solvent content) and eight (54.9% solvent content) molecules could be accommodated in the asymmetric unit, with an

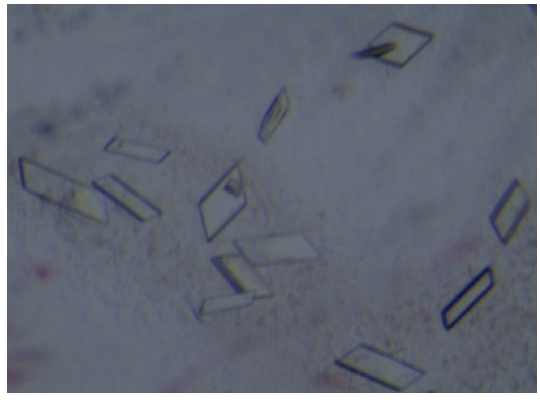


Figure 2
Diamond-shaped crystals of DltC from *S. epidermidis* grown by the sitting-drop vapour-diffusion method.

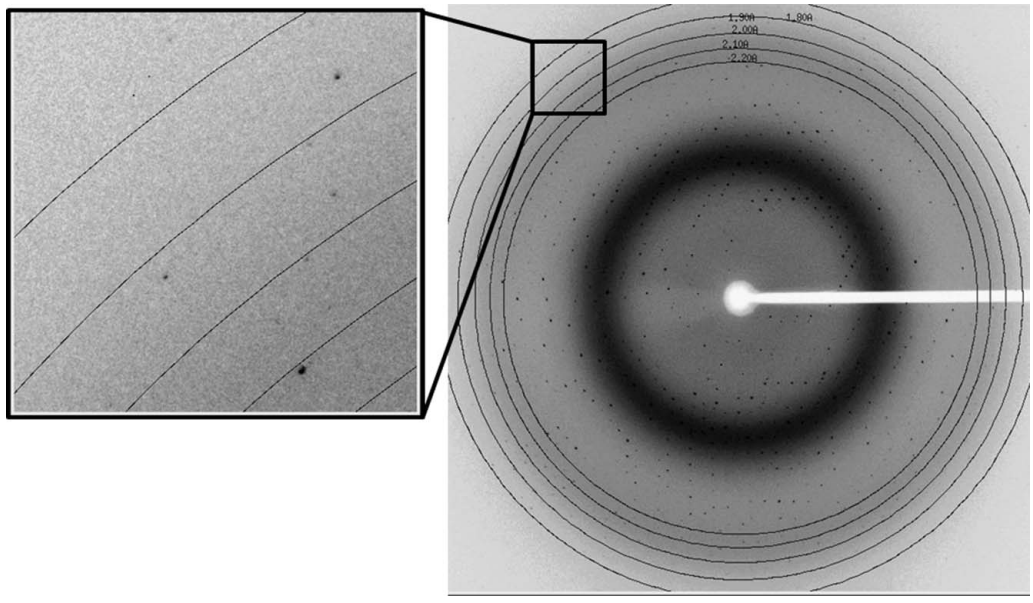


Figure 3
Diffraction pattern of DltC collected from a crystal flash-cooled in 25% glycerol on beamline 13C1 at the NSRRC.

Table 1
Data-collection statistics for the DltC crystal.

Values in parentheses are for the highest resolution shell.

X-ray wavelength (Å)	0.97622
Space group	*P*2
Unit-cell parameters (Å, °)	a = 66.26, b = 53.28, c = 88.05, β = 98.22
Resolution (Å)	30.0–1.83 (1.90–1.83)
No. of measured reflections	305348 (30077)
No. of unique reflections	54048 (5371)
Multiplicity	5.6 (5.6)
$R_{\rm merge}$ (%)	6.5 (34.2)
Data completeness (%)	99.9 (99.5)
$\langle I/\sigma(I)\rangle$	21.92 (5.05)

acceptable $V_{\rm M}$ in the range 2.05–2.73 Å^3 Da^{-1} (Matthews, 1968). Unfortunately, the structure of *Se*DltC could not be solved by molecular replacement (McCoy *et al.*, 2005) using *Lc*DltC (PDB entry 1hqb; 43% amino-acid sequence identity; Volkman *et al.*, 2011) as a search model. The lack of success in the molecular-replacement trials may have resulted from structural differences between *Se*DltC and *Lc*DltC. To attempt to resolve this difficulty, selenomethionine-derivatized DltC crystals have been produced with the goal of solving the structure employing the multiwavelength anomalous diffraction (MAD) method using selenomethionine-derivatized DltC protein (Hendrickson & Ogata, 1997).

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