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Crystallization and preliminary X-ray diffraction analysis of two extracytoplasmic solute receptors of the DctP family from *Bordetella pertussis*

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DctP6 and DctP7 are two *Bordetella pertussis* proteins which belong to the extracytoplasmic solute receptors (ESR) superfamily. ESRs are involved in the transport of substrates from the periplasm to the cytosol of Gram-negative bacteria. DctP6 and DctP7 have been crystallized and diffraction data were collected using a synchrotron-radiation source. DctP6 crystallized in space group $P4_12_12$, with unit-cell parameters $a = 108.39$, $b = 108.39$, $c = 63.09$ Å, while selenomethionyl-derivatized DctP7 crystallized in space group $P2_12_12_1$, with unit-cell parameters $a = 64.87$, $b = 149.83$, $c = 170.65$ Å. The three-dimensional structure of DctP7 will be determined by single-wavelength anomalous diffraction, while the DctP6 structure will be solved by molecular-replacement methods.

1. Introduction

DctP6 and DctP7 are two periplasmic proteins from *Bordetella pertussis*, the whooping cough agent. These two proteins of ∼320 amino-acid residues are 42% identical and belong to the extracytoplasmic solute receptor (ESR) superfamily. DctP6 and DctP7 are homologous to DctP from *Rhodobacter capsulatus*, which is the periplasmic component of the prototypical tripartite ATP-independent periplasmic (TRAP) transporter DctPQM (Forward *et al.*, 1997). TRAP transport systems are involved in the translocation of specific substrates from the periplasm to the cytoplasm of Gram-negative bacteria (Rabus *et al.*, 1999). In the TRAP system of *R. capsulatus*, the solute is first captured by DctP and then transferred specifically to the integral inner membrane proteins DctQ and DctM, which then convey the molecule to the cytosol using an electrochemical gradient as the driving force (Kelly & Thomas, 2001). DctP6 and DctP7 are encoded by the BP1887 and BP1991 genes, respectively, which are part of tripartite operons that include genes for DctM and DctQ homologues. These two operons potentially code for two new TRAP systems, TRAP6 and TRAP7, which are found in the vicinity of the *fha–fim* locus, which encodes important virulence factors (Locht *et al.*, 2001).

In *B. pertussis*, however, the TRAP6 and TRAP7 systems are likely to be non-functional, since the genes coding for the large membrane components DctM of the respective transporters, BP1885 and BP1888, both harbour frameshift mutations. This suggests that DctP6 and DctP7 might be part of uptake transporters whose membrane components are encoded elsewhere in the genome. Alternatively, these proteins might have other roles in *B. pertussis*. Several studies have shown that ESRs may also be involved in signal transduction in association with two-component systems (Antoine *et al.*, 2005; Tam & Saier, 1993; Winnen *et al.*, 2003). In addition, the high level of similarity between the two proteins may indicate that they have partially redundant functions.

Solving the three-dimensional structures of DctP6 and DctP7 will allow progress in the characterization of the solutes that they may bind. This will in turn help shed light on the possible functions of these two ESRs in *B. pertussis*. In this paper, we describe the overexpression, purification and crystallization of DctP6 and DctP7.

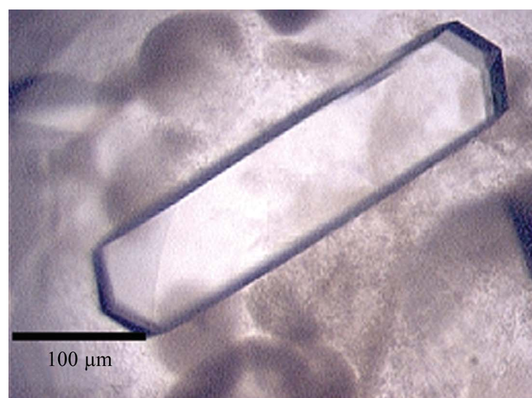
2. Materials and methods

2.1. Cloning

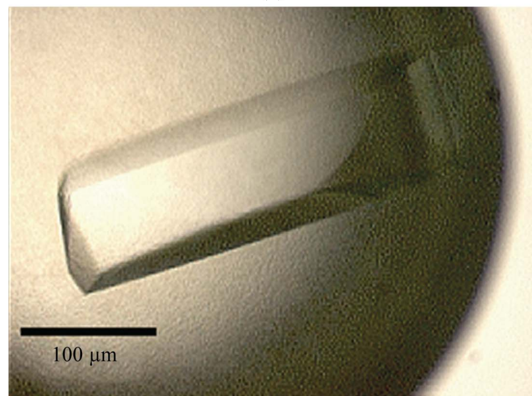
The open reading frames coding for DctP6 and DctP7 were amplified by PCR using genomic DNA extracted from *B. pertussis* Tohama I. The primers used were DctP6-UP (5′-AT**AGATCT**-CAGACCAAATGGGATCTGC-3′) and DctP6-LO (5′-AT**AAG-CTT**CAAGCGATCCAGGAAACG-3′) for DctP6 and DctP7-UP (5′-AT**AGATCT**GCCACCAGCTGGACCATGA-3′) and DctP7-LO (5′-TA**AAGCTT**ATGGCGTCGTCCGGCTTAc-3′) for DctP7, containing *Bgl*II and *Hin*dIII restriction sites, respectively (in bold). In order to place a hexahistidine tag at the N-terminus of the recombinant proteins, the respective PCR products were cloned into the pQE30 vector (Qiagen Inc.) restricted by *Bam*HI and *HindI*II. This resulted in the addition of 12 residues (MRGSHHHHHHGS) at the N-terminus of DctP6 and DctP7. The recombinant plasmids were sequenced in order to ensure that no mutations had occurred during the amplification reaction.

2.2. Protein expression and purification

B. pertussis DctP6 and DctP7 were overexpressed and purified using identical protocols. Electro-competent SG13009 (*pREP4*) *Escherichia coli* cells were transformed with the respective recombinant plasmids and transformants were selected on LB agar plates containing 100 µg ml^{-1} ampicillin and 25 µg ml^{-1} kanamycin. From a single colony, an overnight culture (20 ml) was grown at 310 K in LB media supplemented with 100 µg ml^{-1} ampicillin and 25 µg ml^{-1} kanamycin. This overnight culture was used to inoculate 1 l of the same media at a 1/100 dilution. The resulting culture was grown to an



(*a*)



(*b*)

Figure 1
Crystals of (*a*) DctP6 in space group $P4_12_12$ and (*b*) DctP7 in space group $P2_12_12_1$.

Table 1
Data-collection and reduction statistics.

Values in parentheses are for the last resolution shell.

	DctP6	DctP7
Wavelength (Å)	0.97985	0.97942
Space group	$P4_12_12$	$P2_12_12_1$
Unit-cell parameters (Å)	*a* = 108.39, *b* = 108.39, *c* = 63.09	*a* = 64.87, *b* = 149.83, *c* = 170.65
Molecules per ASU	1	4
V_M (Å^3 Da^{-1})	2.88	3.04
Resolution limits (Å)	20–1.8 (1.9–1.8)	10–2.2 (2.3–2.2)
$I/\sigma(I)$	29.84 (6.6)	15.12 (4.67)
Observations	298371	752078
Independent reflections	35219	161484
R_{merge}† (%)	4.0 (18.0)	9.8 (32.4)
Completeness‡ (%)	99.5 (99.4)	99.9 (100)

† $R_{merge} = \sum_h \sum_j |I(h)_j - \langle I(h)\rangle| / \sum_h \sum_j I(h)_j$, where $I(h)_j$ is the observed intensity and $\langle I(h)\rangle$ is the mean intensity of reflection *h*. ‡ Completeness is the ratio of the number of reflections to the number of possible reflections.

OD_{600} value of 0.7 and was then treated with 1 m*M* isopropyl β-D-thiogalactoside and grown for a further 3 h at 310 K. Cells were harvested by centrifugation at 5000*g* for 20 min at 277 K and stored frozen at 253 K.

Frozen cell pellets were resuspended in buffer *A* (20 m*M* Tris–HCl pH 8.0, 250 m*M* sodium chloride, 10 m*M* imidazole) supplemented with 10 mg ml^{-1} DNAseI and Complete EDTA-free inhibitor cocktail (Roche). The cells were disrupted by two passages through a French press. The lysate was clarified by centrifugation at 12 000*g* for 40 min at 277 K. All subsequent steps were carried out at 277 K.

The crude extract was applied onto a HisTrap column (Amersham) equilibrated in buffer *A*. The protein of interest was eluted in buffer *B* (buffer *A* containing 250 m*M* imidazole). The eluate was dialysed against buffer *C* (10 m*M* Tris–HCl buffer pH 8.0, 250 m*M* sodium chloride), concentrated to 25 mg ml^{-1} and stored at 277 K prior to crystallization trials. Protein concentrations were determined using the Bio-Rad Laboratories protein-assay kit (Bradford, 1976) and bovine serum albumin as standard.

Selenomethionine (SeMet) labelled DctP7 was produced using standard procedures (Doublié, 1997) and the protein was purified using the same protocol as above except that 10 m*M* DTT and 2 m*M* EDTA were added to the protein eluted from the HisTrap column and to buffer *C*.

2.3. Crystallization

Protein purity was assessed by SDS–PAGE and DctP crystallization was screened at 277 K. Crystallization screening was carried out in 96-well plates (Greiner) in a sitting-drop vapour-diffusion setup using a CyBi-HTPC crystallization robot (CyBio). The following commercial kits were used for initial screening: Cryo Kits I and II (Sigma), based on the sparse-matrix screen (Jancarik & Kim, 1991), and Cations Suite (Nextal), based on a mini-grid approach combined with the use of salts as precipitating agents. Crystals were obtained within a few days and the crystallization conditions were transposed to larger drop volumes (1 µl protein at 25 mg ml^{-1} plus 1 µl crystallization reagent equilibrated against a 0.5 ml reservoir volume) using the hanging-drop vapour-diffusion setup.

The first DctP7 hanging-drop crystallization trials yielded poorly diffracting crystals which appeared after three weeks. Further screening showed that DctP7 crystal growth was highly pH-dependent. The best crystals, with approximate dimensions of 70 × 80 × 200 µm, were obtained within 5 d (Fig. 1*a*) at 277 K in 3.8 *M* potas-

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DctP6   MYKKLSLLAGTIVLAFGAGAQAQTKWDLPTAYPASNLHVENLTQFVKDVDSLSGGKLKIT 60
DctP7   MKTTFAVLA--AALALSTGAQAATSWTMTAEQPDANYLTQNARQFADEVKAATAGALEIK 58
        * ..:::**   .**:.:**** *.* :.:   * :*   .:*  **..:*.: :.* *:*.

DctP6   LHNNASLYKAPEIKRAVQGNQAQIGEILLTNFANEDPVYELDGLPFLATGYDASFKLYQA 120
DctP7   VQSNSTLLKRPEVKRGVQQGVVQIGEVLVSALGNEDPLFEIDSVPFLASSFNESEKLWKA 118
        ::.*::* * **:**.** . .****:*:: :.****::*:*.:****:.:: * **::*

DctP6   QKPFLEKKLASQGMMLLYSVAWPPQGIFANRDIKQVSDMKGLKWRAYSPVTAKIAELVGA 180
DctP7   TRPLLAQRLDKQGIVLVYGSPWPPQGIYTKKPVAALADLKGTRFRAYSASTSHMAALMGA 178
         :*:* ::* .**::*:*. .******:::: :  ::*:** ::****. *:::* *:**

DctP6   QPVTVQQAELAQAMATGVIDSYMSSGSTGFDTKTYEYIKKFYDTEAWLPKNAVLVNKKAF 240
DctP7   VPTTVQTPEVPQAFSTGVIDAMLTSPATGVDSQAWDYVKYYYDAQAFIPQSFVIANKRAF 238
         *.*** .*:.**::*****: ::* :**.*:::::*:* :**::*::*:. *:.**:**

DctP6   DALDPATQQALKKAGAQAEERGWKLSQEKNSWYKEQLAKNGMAIIAPTAELKSGLTEVGK 300
DctP7   QRLPAEVRQAVLDAGAKAEIRGWQTARAKTRELTDTLARNGMSVEPLPPQLAKELQAIGA 298
        : * . .:**: .***:** ***: :: *.   .: **:***:: . ..:* . *  :*

DctP6   RMLDDWLKKAGADGQAMIDAYRKQ                                     324
DctP7   TMVSDWSKKAGADGQQLLDAYRK-                                     321
         *:.** ******** ::*****
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Figure 2
The sequences of DctP6 and DctP7 show 42% identity when aligned using *ClustalW* (Thompson *et al.*, 1994).

sium acetate and 0.1 *M* sodium citrate pH 4.0. SeMet DctP7 crystallized under the same conditions as the native protein.

Initial DctP6 crystallization attempts produced a large number of crystalline plates in many drops. After optimizing crystallization buffer pH and precipitating agent concentration, single DctP6 crystals with approximate dimensions of 50 × 50 × 250 µm were obtained within two weeks (Fig. 1*b*) at 277 K in 36%(*w*/*v*) polyethylene glycol 2000 MME and 0.1 *M* PIPES pH 6.5.

2.4. Data collection and X-ray analysis

Data collection was carried out on the BM30A beamline at the ESRF (Grenoble) using a MAR CCD detector. DctP7 crystals were cryoprotected by soaking in a solution composed of 5%(*v*/*v*) polyethylene glycol 200, 3.8 *M* potassium acetate and 0.1 *M* sodium citrate pH 4.0 and were flash-cooled in a 100 K nitrogen-gas stream. A single-wavelength anomalous diffraction (SAD) data set was collected from a selenomethionyl crystal at a wavelength of 0.97942 Å, with a completeness of 99.7% in the last resolution shell (2.0 Å). DctP7 crystals belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters $a = 64.87$, $b = 149.83$, $c = 170.65$ Å. The Matthews coefficient (V_M; Matthews, 1968) was determined to be 3.04 $Å^3$ Da^{-1} and the estimated solvent content was 59.5%, assuming the presence of four molecules of DctP7 in the asymmetric unit. Native DctP6 crystals diffracted to 1.8 Å with a completeness of 99.8% in the last resolution shell and the data were processed in the tetragonal space group $P4_12_12$, with unit-cell parameters $a = 108.39$, $b = 108.39$, $c = 63.09$ Å. The V_M was determined to be 2.88 $Å^3$ Da^{-1}, with a solvent content of 57%, assuming the presence of one molecule of DctP6 in the asymmetric unit. Diffraction data were processed using *XDS* (Kabsch, 1993). A summary of data-collection and processing statistics is given in Table 1.

We are currently in the process of solving the three-dimensional structure of DctP7 using SAD phasing. The three-dimensional structure of DctP6 will subsequently be solved by molecular replacement using DctP7 as a model, given the high percentage of identity between the two proteins (Fig. 2).

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