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Crystallization and preliminary X-ray diffraction analysis of the cytosolic domain of a cation diffusion facilitator family protein

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The cation diffusion facilitator (CDF) family proteins are ubiquitously distributed in the three domains of life and transport metals such as zinc and various heavy metals. Prokaryotic CDF proteins consists of an N-terminal putative six-transmembrane domain followed by a C-terminal cytosolic domain. The cytosolic domain of the CDF-family protein from *Thermotoga maritima* has been overexpressed, purified and crystallized. The selenomethionine-substituted crystals diffracted X-rays to 2.5 Å resolution using synchrotron radiation, belonged to space group *R*32, with unit-cell parameters $a = b = 97.7$, $c = 83.4$ Å, and are expected to contain one molecule in each asymmetric unit.

1. Introduction

The cation diffusion facilitator (CDF) family proteins are ubiquitously distributed in the three domains of life and transport metals such as zinc and various heavy metals (Paulsen & Saier, 1997). They are found in the vacuolar membranes of both plants and yeast, the Golgi apparatus of animals and the bacterial cell membrane (Haney *et al.*, 2005). Most CDF proteins appear to be capable of transporting Zn^{2+}, but some can also transport divalent cations such as Co, Mn, Fe, Cd and Ni (Anton *et al.*, 1999; Delhaize *et al.*, 2003; Grass *et al.*, 2005; Munkelt *et al.*, 2004; Persans *et al.*, 2001). They are generally believed to play a role in the homeostasis of a wide range of divalent metal cations. The expression of one member of the CDF proteins, ZitB from *Escherichia coli*, is known to be inducible by Zn^{2+}, like many eukaryotic CDF proteins (Grass *et al.*, 2001).

Prokaryotic CDF proteins consist of an N-terminal putative six-transmembrane (TM) domain followed by a C-terminal cytosolic domain composed of about 100 amino-acid residues, and have been biochemically well characterized. They are generally homodimers (Wei *et al.*, 2004) that use proton antiport to transport substrate (Guffanti *et al.*, 2002; Chao & Fu, 2004*a*). Moreover, conserved metal-binding sites have been identified and a translocation pathway for metal ions at the presumed dimer interface has been proposed (Chao & Fu, 2004*b*; Wei & Fu, 2005, 2006). The cytosolic domain of a cation diffusion facilitator family protein, CzrB, has been proposed to play a role in metal-ion sequestration and transport (Spada *et al.*, 2002). Moreover, a preliminary X-ray analysis of the cytosolic domain of CzrB with zinc ions suggested that the cytosolic domain of CzrB contains zinc-binding sites (Höfer *et al.*, 2007). However, the three-dimensional structure of a CDF transporter has not yet been determined. Here, we report the crystallization of the cytosolic domain of a cation diffusion facilitator family protein from *Thermotoga maritima*.

2. Methods and materials

2.1. Cloning, expression and purification

The cytosolic domain of the cation diffusion facilitator family protein from *T. maritima* ($TM0876_{206–306}$; M_r 12 736) was cloned from genomic DNA into the *Nde*I and *Eco*RI sites of the pET-26b vector (Novagen). The domain boundary between the TM and cytosolic domains was predicted using the *PSIPRED* (McGuffin *et al.*, 2000)

and *TOPPRED* (von Heijne, 1992) programs. Selenomethionine-substituted (SeMet) protein containing a hexahistidine tag at the C-terminus was expressed in methionine-auxotroph *Escherichia coli* B834(DE3) cells grown in Core medium (Wako) with 50 µg ml^{-1} kanamycin and 30 µg ml^{-1} L-selenomethionine (Nakalai Tesque) and was induced at an optical density at 600 nm (A_{600}) of ∼0.5 with 0.5 m*M* IPTG for 20 h at 310 K. Cells were harvested by centrifugation (5000*g*, 15 min) and were resuspended in sonication buffer *A* (50 m*M* HEPES pH 7.0, 300 m*M* NaCl, 3 m*M* β-mercaptoethanol) supplemented with 1 m*M* phenylmethanesulfonyl fluoride. After sonication, the disrupted cells were harvested by centrifugation (20 000*g*) for 30 min. The cell lysate was agitated in a heat bath for 20 min at 343 K. After centrifugation (20 000*g*) for 30 min, the supernatant was loaded onto an Ni–NTA agarose column (Qiagen) pre-equilibrated in buffer *A* containing 20 m*M* imidazole, which was extensively washed with buffer *A* containing 50 m*M* imidazole. The protein was eluted in buffer *A* containing 300 m*M* imidazole and was dialysed against 50 m*M* HEPES buffer pH 7.0 containing 50 m*M* NaCl and 3 m*M* β-mercaptoethanol for ∼12 h. The sample was loaded onto a Mono Q 10/100 GL column (8 ml; GE Healthcare) and the flowthrough was concentrated using an Amicon Ultra 5K filter (Millipore). For further purification, the concentrated sample was applied onto a HiLoad 16/60 Superdex 200 (GE Healthcare) size-exclusion column equilibrated with 20 m*M* HEPES pH 8.0 containing 150 m*M* NaCl and 3 mM β-mercaptoethanol. The purified protein was concentrated to about 10 mg ml^{-1} using an Amicon Ultra 5K filter for crystallization screening. The protein concentration was estimated from the A_{280}.

2.2. Crystallization

Initial screening for crystallization conditions was performed with several screening kits purchased from Hampton Research, JB Screen kits (Jena Bioscience) and MemSys and MemStart kits (Molecular Dimensions). A Hydra II Plus One crystallization robot (Matrix Technologies) was used for an initial crystallization screen using the sitting-drop vapour-diffusion method at 293 K. Crystallization drops prepared by mixing 0.2 µl protein solution and 0.2 µl reservoir solution were equilibrated against 100 µl reservoir solution. Crystals of TM0876$_{206–306}$ were obtained using condition No. 32 (2.0 *M* ammonium sulfate) from Crystal Screen I (Hampton Research). To optimize crystallization conditions using the hanging-drop vapour-diffusion method, crystallization drops prepared by mixing 1 µl protein solution and 1 µl reservoir solution were equilibrated against 500 µl reservoir solution. After optimization, crystals appeared in a day using a reservoir solution containing 1.6–2.0 *M* ammonium sulfate (Fig. 1).

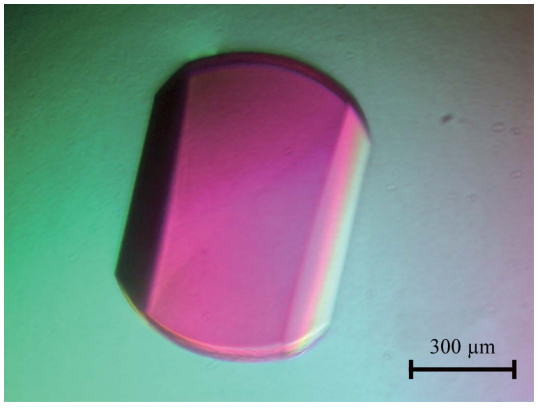

Figure 1
SeMet crystals of TM0876$_{206–306}$.

2.3. Preliminary crystallographic analysis

All of the X-ray diffraction data sets of TM0876$_{206–306}$ were collected at 100 K under a cold nitrogen stream using an ADSC Quantum 210 detector on beamline NW12 at the Photon Factory (Tsukuba, Japan). A clear selenium absorption edge was observed in the XAFS experiment, enabling us to determine the peak, inflection and high-remote wavelengths. The total oscillation ranges covered were 180° for all data sets, with an oscillation range per image of 1°. All diffraction data sets were processed with *DENZO*/*SCALEPACK* (Otwinowski & Minor, 1997). Before cryocooling in a nitrogen stream, the crystals were transferred into a cryoprotectant solution containing 2.2 *M* ammonium sulfate and 20% ethylene glycol.

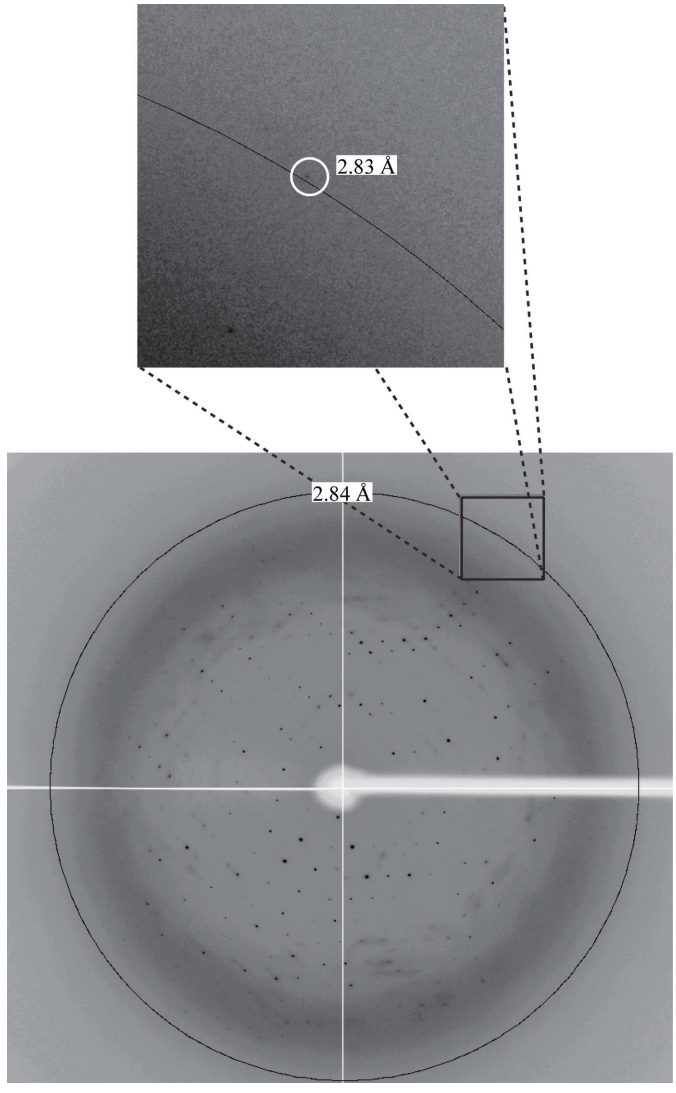

Figure 2
Diffraction pattern of TM0876$_{206–306}$. The value in the enlarged image (top) indicates the resolution to which diffraction was observed. The black circle is drawn at 2.84 Å resolution.

Table 1
Data-collection statistics.

Values in parentheses are for the last shell.

	Peak	Inflection	High remote
Wavelength (Å)	0.97923	0.97939	0.96416
Space group	*R*32		
Unit-cell parameters (Å)	$a = b = 97.7$, $c = 83.4$		
Resolution (Å)	50–2.5 (2.54–2.50)	50–2.84 (2.89–2.84)	50–2.84 (2.89–2.84)
Unique reflections	5396	3728	3717
Completeness (%)	99.4 (99.2)	99.6 (99.5)	99.3 (99.5)
Redundancy†	7.6	8.4	8.9
$I/\sigma(I)$	25.6 (2.4)	30.9 (4.9)	32.0 (4.6)
$R_{\rm sym}$‡	0.055 (0.449)	0.055 (0.319)	0.050 (0.331)

† Bijvoet pairs were not merged in the calculation of redundancy. ‡ $R_{\rm sym} = \sum_{hj} |I_{hj} - \langle I_h \rangle| / \sum_{hj} I_{hj}$, where I_{hj} is the jth measurement of the intensity of reflection h and $\langle I_h \rangle$ is its mean value.

3. Results and discussion

The crystals diffracted to 2.5 Å resolution and belong to space group *R*32, with unit-cell parameters $a = b = 97.7$, $c = 83.4$ Å. A diffraction image is shown in Fig. 2. The asymmetric unit is expected to contain one molecule, giving a Matthews coefficient of 3.01 $\text{Å}^3\,\text{Da}^{-1}$ and a solvent content of 59.1%. The data-collection statistics are summarized in Table 1. We have already identified the six selenium sites using the program *SnB* (Weeks & Miller, 1999). The number of selenium sites is equivalent to that for one $\text{TM0876}_{206–306}$ molecule. Structure determination is in progress.

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