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Crystallization and preliminary X-ray diffraction analysis of the metalloregulatory protein DtxR from *Thermoplasma acidophilum*

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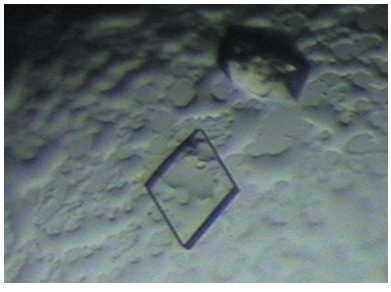

The diphtheria toxin repressor (DtxR) is a metal-ion-dependent transcriptional regulator which regulates genes encoding proteins involved in metal-ion uptake to maintain metal-ion homeostasis. DtxR from *Thermoplasma acidophilum* was cloned and overexpressed in *Escherichia coli*. Crystals of N-terminally His-tagged DtxR were obtained by hanging-drop vapour diffusion and diffracted to 1.8 Å resolution. DtxR was crystallized at 296 K using polyethylene glycol 4000 as a precipitant. The crystals belonged to the orthorhombic space group $P2_12_12$, with unit-cell parameters a = 61.14, b = 84.61, c = 46.91 Å, $\alpha = \beta = \gamma = 90^\circ$. The asymmetric unit contained approximately one monomer of DtxR, giving a crystal volume per mass (V_M) of 2.22 Å^3 Da^{-1} and a solvent content of 44.6%.

1. Introduction

Metal-ion homeostasis is essential for cellular living organisms. One third of all proteins require transition-metal ions for biological activity, including respiration, nerve transmission and defence against toxic agents (Rosenzweig, 2002). Metal-ion homeostasis in prokaryotes is maintained by metalloregulatory proteins that bind metal ions and regulate the transcription of genes encoding proteins involved in metal detoxification, sequestration, efflux and uptake. A large number of metalloregulatory proteins have been identified and extensively characterized (Reyes-Caballero *et al.*, 2011). The prokaryotic metalloregulatory proteins have been classified into five structural families: MerR, AsrR, DtxR, Fur and NikR (Pennella & Giedroc, 2005). Of these, the DtxR family contains a large group of Fe/Mn-dependent metalloregulators.

The diphtheria toxin repressor (DtxR) represses the transcription of the diphtheria toxin and other genes associated with ferrous-ion homeostasis in *Corynebacterium diphtheriae* (Boyd *et al.*, 1990; Schmitt & Holmes, 1991). The crystal structure of DtxR from *C. diphtheriae* revealed an N-terminal winged-helix–turn–helix DNA-binding domain, two distinct metal-binding sites, a dimerization-interface domain and a C-terminal SH3-like domain (Qiu *et al.*, 1995, 1996; Schiering *et al.*, 1995). The crystal structures of DtxR homologues from different species, including *Mycobacterium tuberculosis* IdeR, *Streptococcus gordonii* ScaR and *Bacillus subtilis* MntR, have been determined with a variety of divalent metal ions (Feese *et al.*, 2001; Stoll *et al.*, 2009; Glasfeld *et al.*, 2003). Mutational studies have revealed that both metal-binding sites are important for the biological activity of the diphtheria toxin repressor (Tao & Murphy, 1993, 1994; Ding *et al.*, 1996; Goranson-Siekierke *et al.*, 1999; D'Aquino *et al.*, 2005). Several transition-metal ions such as Fe^{II}, Ni^{II}, Co^{II}, Mn^{II}, Zn^{II} and Cd^{II} can activate DtxR *in vitro* (Schmitt & Holmes, 1993). However, DtxR is only activated by Fe^{II} *in vivo* (Schmitt *et al.*, 1992). The activated DtxR binds to its cognate *tox* operator region as a double dimer (White *et al.*, 1998; Pohl *et al.*, 1999).

The DtxR homologue (TA0872) from *Thermoplasma acidophilum* has been identified and encodes a protein of 220 amino-acid residues with 27% sequence identity to DtxR from *C. diphtheriae* (Ruepp *et al.*, 2000). Further sequence comparisons of *T. acidophilum* DtxR with IdeR (*M. tuberculosis*), ScaR (*S. gordonii*) and MntR (*B. subtilis*) showed 27, 26 and 15% sequence identity, respectively, and there are some notable differences in the two metal-binding sites. Metal

binding site 1 of *C. diphtheriae* DtxR consists of residues His79, His98, Glu170 and Gln173, which correspond to Glu83, Asp101, Arg195 and Tyr198, respectively, in *T. acidophilum* DtxR. Metal-binding site 2 of *C. diphtheriae* DtxR is composed of Met10, Cys102, Glu105 and His106, which correspond to Asp13, Met105, Glu108 and His109, respectively, in *T. acidophilum* DtxR. Met10 and Cys102 in *C. diphtheriae* DtxR, which correspond to Asp13 and Met105, respectively, in *T. acidophilum* DtxR, have been reported to be responsible for metal selectivity in the *C. diphtheriae* protein (Guedon & Helmann, 2003; Glasfeld *et al.*, 2003). Here, as a first step in structure determination, we report the cloning, purification, crystallization and preliminary X-ray diffraction data of *T. acidophilum* DtxR.

2. Materials and methods

2.1. Expression and purification

The gene encoding DtxR (TA0827) was amplified by the polymerase chain reaction using the genomic DNA of *T. acidophilum* as a template. It was inserted into the *Nde*I/*Xho*I-digested expression vector pET-28b(+) (Novagen), resulting in a 20-residue hexahistidine-containing tag at the N-terminus. The recombinant *T. acidophilum* DtxR protein was expressed in *Escherichia coli* BL21 (DE3) Star pLysS cells (Invitrogen). The cells were grown at 310 K to an OD_{600} of ∼0.5 in Luria–Bertani medium containing 30 µg ml^{-1} kanamycin and chloramphenicol. Protein expression was induced using 1.0 m*M* isopropyl β-D-1-thiogalactopyranoside (IPTG). Cell growth was continued at 303 K for 4 h after IPTG induction and the cells were harvested by centrifugation at 7000*g* for 10 min at 277 K.

The cell pellet was resuspended in ice-cold lysis buffer [20 m*M* Tris–HCl pH 8.0, 500 m*M* NaCl, 10%(*v*/*v*) glycerol, 1 m*M* phenylmethylsulfonyl fluoride] and was homogenized using an ultrasonic processor. The crude cell extract was centrifuged at 40 000*g* for 60 min at 277 K and the recombinant protein in the supernatant fraction was purified in three chromatographic steps. The first step was metal-chelate chromatography on Ni–NTA resin (GE Healthcare). The protein was eluted with lysis buffer containing 300 m*M* imidazole and the eluted sample was diluted fivefold with buffer *A* [20 m*M* Tris–HCl pH 8.0, 10%(*v*/*v*) glycerol, 2 m*M* EDTA, 1 m*M* DTT]. The diluted sample was applied onto a Q Sepharose ion-exchange column (GE Healthcare) which had previously been equilibrated with buffer *A*. The protein was eluted with a linear gradient of 0.1–1.0 *M* NaCl in buffer *A*. The next step was gel filtration on a Superdex 200 column (GE Healthcare) employing an elution buffer consisting of 0.1 *M* NaCl, 20 m*M* Tris–HCl pH 8.0, 1 m*M* DTT, 5 m*M* $MgCl_2$ and 5%(*v*/*v*) glycerol. The purified protein was concentrated to 12 mg ml^{-1} using Centricon YM-10 (Millipore) and aliquots of the protein were stored at 193 K.

2.2. Crystallization

Initial crystallization was performed by the sitting-drop vapour-diffusion method using 96-well CrystalQuick plates (Greiner Bio-One) and commercial screens from Hampton Research, Qiagen and Emerald BioSystems at 296 K. Each sitting drop, which was prepared by mixing 0.2 µl each of the protein solution and the reservoir solution using a Mosquito Crystallization Robot (TTP LabTech), was placed over 0.1 ml reservoir solution. Initial crystals were obtained in several polyethylene glycol (PEG) 4000 and ammonium acetate conditions, which were further optimized to 20%(*v*/*v*) PEG 4000, 0.1 *M* sodium acetate pH 5.0, 0.35 *M* ammonium acetate. Crystals of *T. acidophilum* DtxR used for data collection were grown using hanging-drop vapour-diffusion methods by mixing equal volumes (4 µl) of the protein and reservoir solution and equilibrating the mixed solutions against 600 µl reservoir solution in a 24-well plate at 296 K.

2.3. X-ray data collection

Crystals were transferred into a cryoprotectant solution consisting of reservoir solution augmented with 20%(*v*/*v*) glycerol and then flash-cooled in liquid nitrogen. X-ray diffraction data were collected at 100 K with an ADSC Quantum 270 CCD image-plate detector using synchrotron radiation on the NE-3A beamline of the Photon Factory, Japan. The data were collected using a 1° oscillation per image with a crystal-to-detector distance of 213.9 mm. Data were

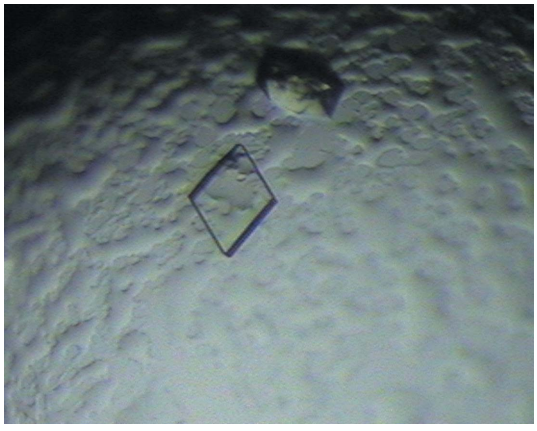

Figure 1
Orthorhombic crystals of *T. acidophilum* DtxR. Their approximate dimensions are 0.2 × 0.2 × 0.05 mm.

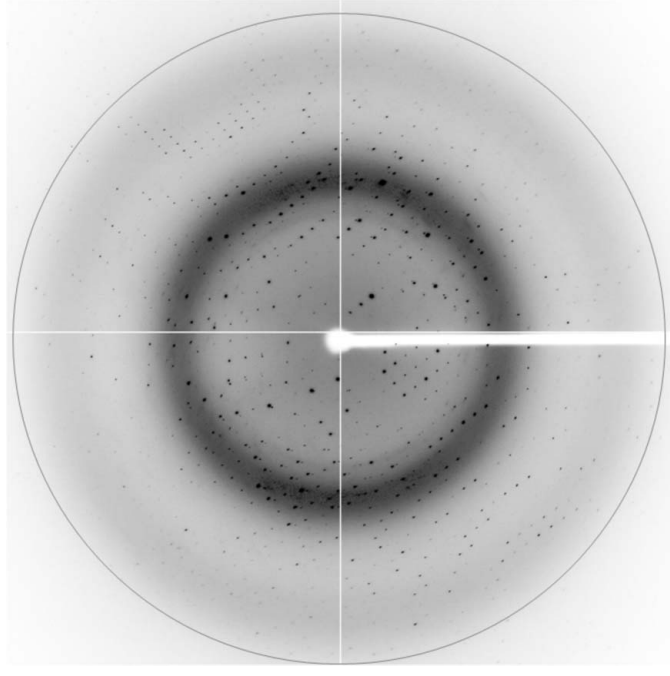

Figure 2
X-ray diffraction image from a crystal of *T. acidophilum* DtxR. The black circle is drawn at the resolution of the detector edge (1.80 Å).

Table 1
Data-collection statistics for the *T. acidophilum* DtxR crystal.

Values in parentheses are for the highest resolution shell.

Wavelength (Å)	1.000
Temperature (K)	100
Oscillation range (°)	1
Resolution range (Å)	50.0–1.80 (1.86–1.80)
No. of observations	274722
Unique reflections	22713
Space group	$P2_12_12$
Unit-cell parameters (Å, °)	a = 61.14, b = 84.61, c = 46.91, $\alpha = \beta = \gamma = 90$
Data completeness (%)	97.1 (98.7)
Multiplicity	12.1 (13.4)
Average $I/\sigma(I)$	34.5 (13.5)
$R_{\rm merge}$† (%)	5.8 (25.8)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the ith observation of reflection hkl, $\sum_{hkl}$ is the sum over all reflections and $\sum_i$ is the sum over i measurements of reflection hkl.

processed and scaled using *DENZO* and *SCALEPACK* from the *HKL*-2000 program suite (Otwinowski & Minor, 1997).

3. Results and discussion

The recombinant *T. acidophilum* DtxR protein was overexpressed in *E. coli* and purified to give a final yield of ∼20 mg per litre of culture. The best crystals of the N-terminally His-tagged DtxR were obtained by hanging-drop vapour diffusion at 296 K. The optimized reservoir solution for crystallization was 20%(*v*/*v*) PEG 4000, 0.1 *M* sodium acetate pH 5.0, 0.35 *M* ammonium acetate. Crystals grew reproducibly to maximum dimensions of approximately 0.2 × 0.2 × 0.05 mm within a week (Fig. 1). Crystals were transferred into a cryoprotectant solution consisting of reservoir solution augmented with 20%(*v*/*v*) glycerol. A crystal was flash-cooled in liquid nitrogen and mounted at the NE-3A experimental station of the Photon Factory. A total of 22 713 unique reflections were measured with a multiplicity of 12.1 (Fig. 2). The merged data set was 97.1% complete to 1.8 Å resolution with an $R_{\rm merge}$ (on intensity) of 5.8%. The crystals belonged to the orthorhombic space group $P2_12_12$, with unit-cell parameters a = 61.14, b = 84.61, c = 46.91 Å, $\alpha = \beta = \gamma = 90^\circ$. The asymmetric unit contained approximately one monomer of DtxR, giving a crystal volume per mass ($V_{\rm M}$) of 2.22 Å^3 Da^{-1} and a solvent content of 44.6% based on a partial specific volume of 0.5 cm^3 g^{-1} (Matthews, 1968; Kantardjieff & Rupp, 2003). Table 1 summarizes the statistics of data collection. Molecular replacement was attempted using the structures of several DtxR homologues [*C. diphtheriae* DtxR, PDB entry 2dtr, 27% sequence identity (Qiu *et al.*, 1996); *M. tuberculosis* IdeR, PDB entry 1fx7, 27% sequence identity (Feese *et al.*, 2001); *S. gordonii* ScaR, PDB entry 3hrs, 26% sequence identity (Stoll *et al.*, 2009)] with the program *Phaser* (McCoy *et al.*, 2007), but was not successful. To determine the crystal structure, selenomethionine-substituted *T. acidophilum* DtxR has been purified and crystallized. We are presently attempting to collect a three-wavelength MAD data set.

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References

Boyd, J., Oza, M. N. & Murphy, J. R. (1990). *Proc. Natl Acad. Sci. USA*, **87**, 5968–5972.
D'Aquino, J. A., Tetenbaum-Novatt, J., White, A., Berkovitch, F. & Ringe, D. (2005). *Proc. Natl Acad. Sci. USA*, **102**, 18408–18413.
Ding, X., Zeng, H., Schiering, N., Ringe, D. & Murphy, J. R. (1996). *Nature Struct. Biol.* **3**, 382–387.
Feese, M. D., Ingason, B. P., Goranson-Siekierke, J., Holmes, R. K. & Hol, W. G. (2001). *J. Biol. Chem.* **276**, 5959–5966.
Glasfeld, A., Guedon, E., Helmann, J. D. & Brennan, R. G. (2003). *Nature Struct. Biol.* **10**, 652–657.
Goranson-Siekierke, J., Pohl, E., Hol, W. G. & Holmes, R. K. (1999). *Infect. Immun.* **67**, 1806–1811.
Guedon, E. & Helmann, J. D. (2003). *Mol. Microbiol.* **48**, 495–506.
Kantardjieff, K. A. & Rupp, B. (2003). *Protein Sci.* **12**, 1865–1871.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
McCoy, A. J., Grosse-Kunstleve, R. W., Adams, P. D., Winn, M. D., Storoni, L. C. & Read, R. J. (2007). *J. Appl. Cryst.* **40**, 658–674.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–325.
Pennella, M. A. & Giedroc, D. P. (2005). *Biometals*, **18**, 413–428.
Pohl, E., Holmes, R. K. & Hol, W. G. (1999). *J. Mol. Biol.* **292**, 653–667.
Qiu, X., Pohl, E., Holmes, R. K. & Hol, W. G. (1996). *Biochemistry*, **35**, 12292–12302.
Qiu, X., Verlinde, C. L., Zhang, S., Schmitt, M. P., Holmes, R. K. & Hol, W. G. (1995). *Structure*, **3**, 87–100.
Reyes-Caballero, H., Campanello, G. C. & Giedroc, D. P. (2011). *Biophys. Chem.* **156**, 103–114.
Rosenzweig, A. C. (2002). *Chem. Biol.* **9**, 673–677.
Ruepp, A., Graml, W., Santos-Martinez, M. L., Koretke, K. K., Volker, C., Mewes, H. W., Frishman, D., Stocker, S., Lupas, A. N. & Baumeister, W. (2000). *Nature (London)*, **407**, 508–513.
Schiering, N., Tao, X., Zeng, H., Murphy, J. R., Petsko, G. A. & Ringe, D. (1995). *Proc. Natl Acad. Sci. USA*, **92**, 9843–9850.
Schmitt, M. P. & Holmes, R. K. (1991). *Infect. Immun.* **59**, 1899–1904.
Schmitt, M. P. & Holmes, R. K. (1993). *Mol. Microbiol.* **9**, 173–181.
Schmitt, M. P., Twiddy, E. M. & Holmes, R. K. (1992). *Proc. Natl Acad. Sci. USA*, **89**, 7576–7580.
Stoll, K. E., Draper, W. E., Kliegman, J. I., Golynskiy, M. V., Brew-Appiah, R. A., Phillips, R. K., Brown, H. K., Breyer, W. A., Jakubovics, N. S., Jenkinson, H. F., Brennan, R. G., Cohen, S. M. & Glasfeld, A. (2009). *Biochemistry*, **48**, 10308–10320.
Tao, X. & Murphy, J. R. (1993). *Proc. Natl Acad. Sci. USA*, **90**, 8524–8528.
Tao, X. & Murphy, J. R. (1994). *Proc. Natl Acad. Sci. USA*, **91**, 9646–9650.
White, A., Ding, X., vanderSpek, J. C., Murphy, J. R. & Ringe, D. (1998). *Nature (London)*, **394**, 502–506.