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Crystallization and preliminary diffraction studies of the C-terminal domain of the DipZ homologue from *Mycobacterium tuberculosis*

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Protein disulfide-bond formation is poorly understood in the pathogenic bacterium *Mycobacterium tuberculosis*. Rv2874 is the *M. tuberculosis* homologue of the disulfide-bond electron transporter DsbD from *Escherichia coli*. Both proteins share a core central transmembrane domain and a C-terminal thioredoxin domain. To investigate the possible role of Rv2874 in disulfide-bond formation in *M. tuberculosis*, the C-terminal domain of Rv2874 has been cloned, expressed, purified and crystallized. The crystals belong to space group $P2_12_12_1$, with unit-cell parameters $a = 109.7$, $b = 118.3$, $c = 122.9$ Å, and diffract to at least 3.0 Å.

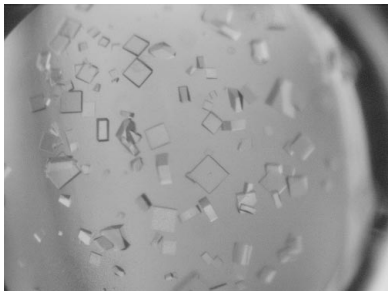
1. Introduction

Disulfide-bond formation is an important process in the folding of many secreted proteins. The formation of disulfide bonds between two cysteine residues is a redox reaction requiring the removal of electrons. Conversely, electrons must be added to break a protein disulfide bond. The best characterized mechanism of protein disulfide-bond formation involves five Dsb proteins in the periplasm of *Escherichia coli* (Kadokura *et al.*, 2003). DsbA and DsbB catalyse the formation of disulfide bonds in folding proteins and the transport of the resulting electrons out of the periplasm (Bader *et al.*, 1999). DsbC and DsbG catalyse the rearrangement of disulfide bonds that may form between incorrect pairs of cysteines as proteins fold (Kadokura *et al.*, 2003). The integral membrane protein DsbD (also known as DipZ) transports electrons from cytoplasmic thioredoxin to DsbC and DsbG in the periplasm (Katzen & Beckwith, 2000). DsbD has three domains: an N-terminal immunoglobulin-like (Ig-like) domain (Goulding *et al.*, 2002; Haebel *et al.*, 2002), a central transmembrane domain with eight predicted transmembrane regions (Stewart *et al.*, 1999) and a C-terminal thioredoxin-like domain (Kim *et al.*, 2003; Rozhkova *et al.*, 2004). All three domains work cooperatively to reduce the periplasmic disulfide-bond isomerases DsbC and DsbG (Katzen & Beckwith, 2000).

Protein disulfide-bond formation in the pathogenic bacterium *Mycobacterium tuberculosis* is much less well understood. There are two proteins in *M. tuberculosis* which show homology to the Dsb family of proteins. Rv2878c is a thioredoxin-like protein which has recently been shown to function as a non-specific oxidase *in vitro*, with oxidative properties similar to those of DsbA (Goulding *et al.*, 2004). Rv2874 is a homologue of DipZ (DsbD).

Both Rv2874 and DsbD share a common domain structure, with an N-terminal domain followed by a central transmembrane region and then a C-terminal extra-cytoplasmic domain (Juarez *et al.*, 2001; Katzen & Beckwith, 2000). The C-terminal region of Rv2874 is approximately 350 residues in length and is predicted to contain a thioredoxin fold (Juarez *et al.*, 2001) with the conserved thioredoxin active-site motif C*xx*C (reisdues 437–440). In addition to the predicted thioredoxin fold, the C-terminal domain of Rv2874 contains a further 150 residues of unknown function. A 312-residue version of Rv2874 (residues 384–695) has been expressed, but no disulfide reductase activity was detected (Juarez *et al.*, 2001).

To investigate the possible role of Rv2874 in the formation of disulfide bonds in *M. tuberculosis*, we have cloned a longer 329-residue C-terminal domain of Rv2874 (residues 366–695). Here, we describe the cloning, expression and purification of the C-terminal



region of Rv2874 and its subsequent crystallization and preliminary X-ray diffraction analysis.

2. Methods and results

2.1. Cloning, expression and purification

The DNA fragment encoding Rv2874-C (residues 366–695) was amplified by PCR from the genomic DNA of *M. tuberculosis* H37Rv using the primers Rv2874-C-term-fwd (5′-C ACC GCC ATG GAG ATA CGG GAA CAA CTG AAC C-3′) and R2874-r (5′-CCG ACA TGA TTT CTT AGG ATC CGT CCG GGT-3′). The resulting PCR product was digested with *Nco*I/*Bam*HI and cloned into the linearized expression vector pProEX-Hta. The construct was verified by DNA sequencing.

The plasmid encoding Rv2874-C was transformed into *E. coli* BL21 (DE3) pRI cells for expression. Cells were grown in Luria–Bertani broth containing the antibiotics ampicillin (100 µg ml^{-1}) and chloramphenicol (34 µg ml^{-1}) at 310 K until mid-log phase ($OD_{600\,nm} \simeq$ 0.5–0.7). Protein expression was induced by the addition of 0.5 m*M* isopropyl-β-D-thiogalactopyranoside (IPTG) at 301 K. Cells were harvested by centrifugation (15 min, 4000*g*) and resuspended in 20 m*M* HEPES pH 7.5, 200 m*M* NaCl before being lysed using a cell disruptor (Constant Systems) at 124 MPa. The resulting lysate was centrifuged at 20 000*g* for 30 min at 277 K to remove cellular debris. The soluble fraction was collected and oxidized glutathione (GSSG) was added to a final concentration of 1 m*M* to oxidize any potential disulfide bonds.

Rv2874-C was purified from the resulting cell lysate by Ni^{2+}-affinity chromatography using a 5 ml Hi-Trap chelating column (Amersham Biosciences). After loading the lysate, the column was washed with 50 ml 20 m*M* HEPES pH 7.5, 200 m*M* NaCl, 60 m*M* imidazole to remove non-specifically bound protein. Rv2874-C was then eluted with 20 m*M* HEPES pH 7.5, 200 m*M* NaCl, 300 m*M* imidazole. Fractions containing Rv2874-C were pooled and then concentrated prior to further purification by size-exclusion chromatography using a HiLoad Superdex 200 16/60 PG column (Amersham Biosciences) equilibrated with 10 m*M* HEPES pH 7.5, 150 m*M* NaCl. The purity of Rv2874-C in the remaining fractions was assessed by SDS–PAGE. Rv2874-C migrated as a single band at ∼37 kDa. Fractions eluting at approximately 85 ml contained Rv2874-C and were pooled before being concentrated to 20 mg ml^{-1} for crystallization. Rv2874-C was stored at 277 K.

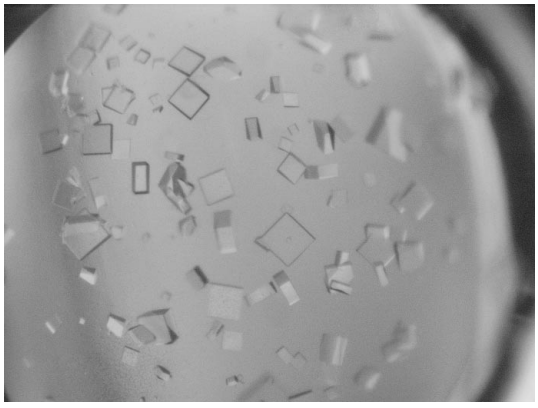


Figure 1
Typical crystals of Rv2874-C grown in 20% PEG 4000, 0.1 *M* sodium citrate pH 5.6. Crystals appeared after 3 d and grew to maximum size after 14 d (typically 0.1 × 0.1 × 0.02 mm).

Table 1
Data-collection statistics.

Values in parentheses are for the outermost shell of data collected.

Space group	$P2_12_12_1$
Wavelength (Å)	1.5418
Unit-cell parameters (Å)	a = 109.75, b = 118.32, c = 122.92
Mosaicity (°)	0.41
Resolution range (Å)	50–3.0 (3.11–3.0)
No. of reflections	517155
Unique reflections	40020
Completeness (%)	99.9 (100)
Mean $I/\sigma(I)$	8.7 (3.3)
R_{merge}† (%)	20.7 (53.6)

† $R_{merge} = \sum(I_i - \langle I\rangle)/\sum I_i$, where I_i and $\langle I\rangle$ are the measured and mean values of reflection *hkl*, respectively.

2.2. Crystallization

All crystallization trials were carried out using the sitting-drop vapour-diffusion method in 96-well Intelli-Plates containing 100 µl reservoir solution. Drops consisted of 1 µl protein solution plus 1 µl reservoir solution. Crystallization trays were maintained at 291 K. Precipitant screens were initially carried out using Crystal Screens 1 and 2 (Jancarik & Kim, 1991), the PEG/Ion screen (Hampton Research) and the Top67 screen (Page *et al.*, 2003) as well as PEG and salt screens developed in our laboratory. None of these screens resulted in any promising conditions. A systematic trial of 192 conditions consisting of eight different molecular-weight PEGs between PEG 400 and PEG 8000 at six different pH values between pH 4 and pH 9 yielded crystals in 20%(*w*/*v*) PEG 4000, PEG 6000 and PEG 8000, 0.1 *M* sodium citrate pH 5.6. Despite further screening around these conditions, varying the PEG concentration and the pH, crystals only grew using the original conditions.

The crystals appeared over 2–3 d as square blocks (Fig. 1) and were only ever observed growing either on the surface of the drop or against the wall of the dish. Crystals grew to typical dimensions of 0.1 × 0.1 × 0.02 mm and to maximum dimensions of 0.25 × 0.25 × 0.1 mm. When viewed under plane-polarized light, crystals appeared to be weakly birefringent. Crystals grown in 20%(*w*/*v*) PEG 4000, 0.1 *M* sodium citrate pH 5.6 were used for X-ray diffraction experiments.

2.3. Data collection and processing

Individual crystals were transferred into a cryoprotectant buffer solution consisting of 20%(*w*/*v*) PEG 4000, 0.1 *M* sodium citrate pH 5.6, 20%(*v*/*v*) glycerol for approximately 10–30 s before being flash-frozen at 110 K in a stream of nitrogen. X-ray diffraction data were collected from native crystals on a Rigaku RU-H3R copper rotating-anode generator equipped with a MAR 345 image-plate detector.

Native crystals diffracted to a maximum resolution of 3.0 Å. Crystals were shown to belong to space group $P2_12_12_1$, with unit-cell parameters a = 109.7, b = 118.3, c = 122.9 Å. The Matthews coefficient suggests that there are between three and six Rv2874-C monomers in the asymmetric unit, with a corresponding solvent content of between 34.7 and 67.3% ($V_M \simeq$ 3.8–1.9 Å^3 Da^{-1}). All data were processed using the *HKL* program package (Otwinowski & Minor, 1997). Data-collection statistics are given in Table 1. Owing to the weak nature of the diffraction, the collection of higher quality data using synchrotron-radiation sources is required.

Structure determination is currently in progress.

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References

Bader, M., Muse, W., Ballou, D. P., Gassner, C. & Bardwell, J. C. (1999). *Cell*, **98**, 217–227.
Goulding, C. W., Apostol, M. I., Gleiter, S., Parseghian, A., Bardwell, J., Gennaro, M. & Eisenberg, D. (2004). *J. Biol. Chem.* **279**, 3516–3524.
Goulding, C. W., Sawaya, M. R., Parseghian, A., Lim, V., Eisenberg, D. & Missiakas, D. (2002). *Biochemistry*, **41**, 6920–6927.
Haebel, P. W., Goldstone, D., Katzen, F., Beckwith, J. & Metcalf, P. (2002). *EMBO J.* **21**, 4774–4784.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Juarez, M. D., Torres, A., Espitia, C., Hewinson, R. G., Michell, S. L., Russell, W. P., McAdam, R. A. & Jacobs, W. R. Jr (2001). *FEMS Microbiol. Lett.* **203**, 95–102.
Kadokura, H., Katzen, F. & Beckwith, J. (2003). *Annu. Rev. Biochem.* **72**, 111–135.
Katzen, F. & Beckwith, J. (2000). *Cell*, **103**, 769–779.
Kim, J. H., Kim, S. J., Jeong, D. G., Son, J. H. & Ryu, S. E. (2003). *FEBS. Lett.* **543**, 164–169.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Page, R., Grzechnik, S. K., Canaves, J. M., Spraggon, G., Kreusch, A., Kuhn, P., Stevens, R. C. & Lesley, S. A. (2003). *Acta Cryst.* D**59**, 1028–1037.
Rozhkova, A., Stirnimann, C. U., Frei, P., Grauschopf, U., Brunisholz, R., Grutter, M. G., Capitani, G. & Glockshuber, R. (2004). *EMBO J.* **23**, 1709–1719.
Stewart, E. J., Katzen, F. & Beckwith, J. (1999). *EMBO J.* **18**, 5963–5971.