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Crystallization and preliminary X-ray diffraction studies of a protein disulfide oxidoreductase from *Aeropyrum pernix* K1

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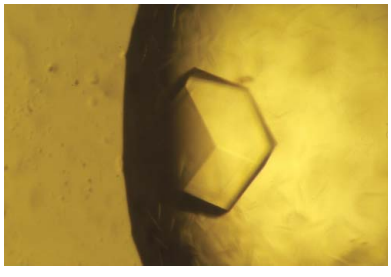

A protein disulfide oxidoreductase from the archaeon *Aeropyrum pernix* K1 has been overexpressed in *Escherichia coli* and crystallized at 298 K using the hanging-drop vapour-diffusion method. Crystals belong to the space group *I*222 or $I2_12_12_1$, with unit-cell parameters a = 90.59, b = 102.43, c = 128.96 Å. A complete data set has been collected at the Elettra synchrotron source in Trieste to 1.93 Å resolution using a single frozen crystal.

1. Introduction

Protein disulfide oxidoreductases are ubiquitous redox enzymes that catalyse dithiol–disulfide exchange reactions. These enzymes, including the thioredoxin (Trx), glutaredoxin (Grx), protein disulfide isomerase (PDI), disulfide-bond forming (Dsb) families and their homologues, share a C*XX*C sequence motif at their active site (Loferer & Hennecke, 1994). The two cysteines can undergo reversible oxidation/reduction by shuttling between a dithiol and a disulfide form in the catalytic process. While Trx and Grx mainly catalyse the reduction of disulfides, PDI and Dsb catalyse the formation or rearrangement of disulfide bridges in protein-folding processes.

Protein disulfide oxidoreductases have been well characterized in bacteria and eukarya. In contrast, little is known about the structure and function of these proteins in archaea. Archaeal protein disulfide oxidoreductases have been isolated from *Sulfolobus solfataricus* (SsPDO; Guagliardi *et al.*, 1994), *Pyrococcus furiosus* (PfPDO; Guagliardi *et al.*, 1995), *P. horikoshii* (PhPDO; Kashima & Ishikawa, 2003), *Methanococcus jannaschii* (Bult *et al.*, 1996) and *Methanobacterium thermoautotrophicum* (McFarlan *et al.*, 1992). Of these, PfPDO is the best characterized with respect to structure and function (Ren *et al.*, 1998; Pedone *et al.*, 2004). This protein has an unusual molecular weight of about 26 kDa, compared with the small size of most glutaredoxins and thioredoxins, and its amino-acid sequence shows no overall sequence similarity to previously studied protein disulfide oxidoreductases. Interestingly, it presents two active sites with the conserved C*XX*C sequence motif. The three-dimensional structure of PfPDO has recently been solved, providing important conformational details on this enzyme and suggesting that this protein may be related to the multidomain PDI, which is currently known only in eukaryotes (Ren *et al.*, 1998).

Comparison of the genomes of archaea and bacteria showed the existence of a group of redox proteins with two C*XX*C active-site motifs and molecular weights similar to that of *Pf*PDO. The peculiar features of these enzymes and their exclusive presence in extremophiles suggested that they could constitute a new family of protein disulfide oxidoreductases which could have a special role in adaptation to extreme conditions (Pedone *et al.*, 2004). We have recently identified a new member of this enzyme family, isolated from the archaeon *Aeropyrum pernix* K1. This 243-residue protein (calculated M_r = 27 334.4), named *Ap*PDO, presents two redox sites, CETC at the N-terminal active site, which has never been observed in any other protein disulfide oxidoreductase, and CPYC at the C-terminus, a sequence conserved in the glutaredoxin family and identical to that found in *Pf*PDO. In a general research project aimed at providing insights into the function, structural diversity and evolution of protein disulfide oxidoreductases, we have undertaken a structural and functional study on *Ap*PDO. Here, we report its overexpression,

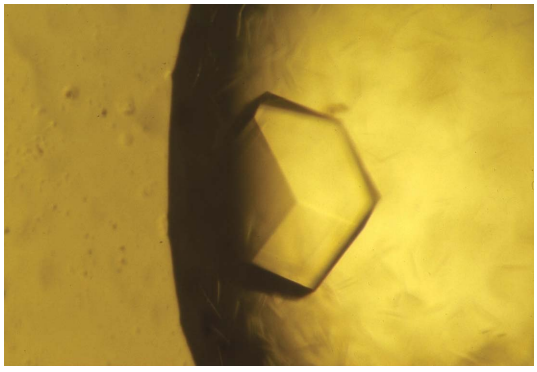

Figure 1
Crystals of *Ap*PDO. Crystal dimensions are 0.25 × 0.25 × 0.15 mm.

crystallization and preliminary X-ray crystallographic analysis as a first step towards structure determination.

2. Results

2.1. Expression and purification

The *Ap*PDO-encoding gene was amplified by PCR and cloned between the *Nco*I and *Bam*HI sites of the pTrc99A vector (Novagen). This vector was then transformed into *Escherichia coli* strain RB791. Cells were grown to an OD_{600} of approximately 1 in Luria–Bertani media containing 0.1 mg ml^{-1} ampicillin (Sigma) at 310 K and the expression of *Ap*PDO was induced by 1 m*M* isopropyl-β-D-thiogalactoside (IPTG; Inalco). After 18 h induction, cells were harvested and resuspended in 10 m*M* Tris–HCl pH 8.0 (buffer *A*). After sonication and subsequent centrifugation, the cell extract was heated and kept at 353 K for 10 min. After centrifugation at 35 000*g* for 30 min, the supernatant was loaded onto a S75 Superdex gel-filtration column (Amersham Pharmacia Biotech) previously equilibrated with buffer *A* containing 200 m*M* KCl. *Ap*PDO was further purified using a Resource Q column (Amersham Pharmacia Biotech) in buffer *A* and eluted with a linear gradient of 0–0.2 *M* NaCl. The protein purity was checked on 12.5% SDS–PAGE gels stained with silver nitrate.

2.2. Crystallization and X-ray data analysis

The purified protein was concentrated to 10 mg ml^{-1} in 10 m*M* Tris–HCl pH 8.0 and crystallized at 298 K. Crystallization experiments were carried out using the hanging-drop vapour-diffusion method (McPherson, 1999). The search for initial crystallization conditions was performed using Hampton Research Crystal Screens I and II (Jancarik & Kim, 1991; Cudney *et al.*, 1994). Drops containing equal volumes (1 µl) of protein and reservoir solution were equilibrated against 1 ml reservoir solution. Initial crystals with irregular shape were obtained using a reservoir solution consisting of 2 *M* ammonium sulfate, 2%(*v*/*v*) PEG 400, 0.1 *M* HEPES pH 7.5. Several parameters such as buffer composition, pH and protein concentration were varied in order to improve the crystal quality. Optimal conditions for crystallization were achieved at 298 K with reservoir solutions consisting of 2 *M* ammonium sulfate, 2%(*v*/*v*) PEG 400, 0.1 *M* HEPES pH 8. The crystals grew to maximum dimensions of 0.25 × 0.25 × 0.15 mm within one week (Fig. 1). At room temperature, diffraction from the crystals under investigation was found to fade rapidly; however, it was possible to collect a complete data set at 100 K using a single flash-cooled crystal. Since the crystallization solution was not suitable for providing cryoprotection, the crystals were quickly washed in a solution of 2 *M* ammonium sulfate, 2%(*v*/*v*) PEG 400, 0.1 *M* HEPES pH 8 and 15%(*v*/*v*) glycerol and immediately flash-frozen in the nitrogen-gas stream from an Oxford Cryosystems Cryostream cooler operated at a temperature of 100 K. Data were collected at the Elettra synchrotron source in Trieste using a MAR CCD detector and were processed using the *HKL* crystallographic data-reduction package (Otwinowski & Minor, 1997). Data-collection statistics are outlined in Table 1. The protein crystals diffract to 1.93 Å resolution and belong to space group *I*222 or $I2_12_12_1$, with unit-cell parameters a = 90.59, b = 102.43, c = 128.96 Å. Packing-density considerations (Matthews, 1968) for a monomer weight of 27 334.4 Da suggest the presence of two monomers in the asymmetric unit (V_M = 2.7 Å^3 Da^{-1}, corresponding to a solvent content of 55%).

Attempts to solve the structure using molecular-replacement technique are currently under way in both the *I*222 and $I2_12_12_1$ space groups.

Table 1
Crystal and data-collection parameters.

Values in parentheses are for the outermost resolution shell.

Space group	*I*222 or $I2_12_12_1$
Unit-cell parameters (Å)	
a	90.59
b	102.43
c	128.96
Resolution limits (Å)	20.00–1.93 (2.00–1.93)
Wavelength (Å)	1.20
Temperature (K)	100
Total reflections	1502136
Unique reflections	45416
Completeness (%)	98.1 (87.5)
R_{sym}† (%)	3.4 (29.7)
Mean $I/\sigma(I)$	36.9 (2.9)

† $R_{sym} = \sum |I_i - \langle I \rangle| / \sum I_i$ over all reflections.

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