Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray diffraction studies of a protein disulfide oxidoreductase from *Aquifex aeolicus*

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Received 13 July 2004
Accepted 10 September 2004

A protein disulfide oxidoreductase from the thermophilic bacterium *Aquifex aeolicus* has been overexpressed in *Escherichia coli* and crystallized at 298 K using the hanging-drop vapour-diffusion method. Crystals belong to space group *R*32, with unit-cell parameters $a = b = 161.1$, $c = 153.1$ Å. A complete data set has been collected to 2.4 Å using synchrotron radiation. Packing-density considerations agree with the presence of 2–4 monomers in the asymmetric unit, with a corresponding solvent content of 66–32%.

1. Introduction

Protein disulfide oxidoreductases are ubiquitous redox enzymes that catalyse dithiol–disulfide exchange reactions. These enzymes share a C*XX*C sequence motif at their active site. A growing number of proteins belonging to this superfamily have been identified, including the thioredoxin (Trx), glutaredoxin (Grx), protein disulfide isomerase (PDI) and DsbA families and their homologues. While Trx and Grx mainly catalyse the reduction of disulfide, PDI and DsbA catalyse the formation or rearrangement of disulfide bridges in the protein-folding processes.

In 1995, a hyperthermostable protein disulfide oxidoreductase was purified from the archaeon *Pyrococcus furiosus* (*Pf*PDO; Guagliardi *et al.*, 1995). This protein had an unusual molecular weight of 26 kDa, compared with the small size of most glutaredoxins and thioredoxins, and its amino-acid sequence showed no overall sequence similarity to previously studied protein disulfide oxidoreductases. Interestingly, it presented two active sites with the conserved C*XX*C sequence motif. A CPYC sequence, which is conserved in the glutaredoxin family, was located in the C-terminal part of the enzyme, while a CQYC sequence, which had never been observed in any other protein disulfide oxidoreductase, was found in its N-terminal part. The resolution of the enzyme's three-dimensional structure revealed important conformational details that suggested that *Pf*PDO may be related to the multidomain PDI, which is currently known only in eukaryotes (Ren *et al.*, 1998). Recently reported functional studies confirmed that this protein may represent an ancestor of the eukaryotic PDI (Pedone *et al.*, 2004).

Comparison of the genomes from archaea and bacteria showed the existence of a group of redox proteins with two C*XX*C active sites and a molecular weight similar to that of *Pf*PDO. The unusual features of these enzymes suggested that they could constitute a new family of protein disulfide oxidoreductases. The exclusive presence of *Pf*PDO-like proteins in extremophiles could suggest that these proteins play a particular role in adaptation to extreme conditions (Pedone *et al.*, 2004). We have recently focused our attention on a new protein isolated from the thermophilic bacterium *Aquifex aeolicus* that belongs to this putative new enzyme family (Pedone *et al.*, 2004). This 229-residue enzyme, named *Aa*PDO, presents two redox sites: CESC, which has never been observed in any other protein disulfide oxidoreductase, at the N-terminus and CGYC, a sequence found in DsbC, a protein disulfide-bond isomerase from *Escherichia coli*, at the C-terminus. Moreover, it shows 34% sequence identity with *Pf*PDO. In order to obtain insights into the function, structural diversity and evolution of protein disulfide oxidoreductases, we have undertaken a structural and functional study on this protein. As a first step toward its structure determination, we report here its overexpression, crystallization and preliminary X-ray crystallographic analysis.

2. Results

2.1. Protein expression and purification

The *Aa*PDO-encoding gene was amplified by PCR and cloned between the *Nde*I and *Xho*I sites of the pET30(a) vector (Novagen) in frame with a C-terminal eight-residue tag (LEHHHHHH). This vector was then transformed into *E. coli* strain BL21 (DE3) RIL. 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 *Aa*PDO was induced by 1 m*M* isopropyl-β-D-thiogalactoside (IPTG;

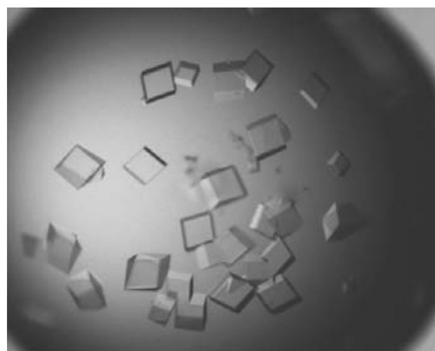

Figure 1
Crystals of *Aa*PDO. Crystal dimensions are 0.1 × 0.2 × 0.2 mm.

Inalco). After 18 h induction, cells were harvested and resuspended in 10 m*M* Tris–HCl pH 8.0. After sonication and subsequent centrifugation, the supernatant was loaded onto an Ni–NTA column (Qiagen). *Aa*PDO was further purified using a Resource Q column (Amersham Pharmacia Biotech) in 10 m*M* Tris–HCl pH 8.0 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 and the protein was concentrated to approximately 10 mg ml^{-1} for crystallization.

2.2. Crystallization and X-ray data analysis

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). The wells contained 500 µl precipitant solution and the drops were composed of 1 µl reservoir solution and 1 µl protein solution at a concentration of 10 mg ml^{-1}. Crystals were obtained using a reservoir solution consisting of 0.1 *M* sodium chloride, 1.6 M ammonium sulfate, 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 0.1 *M* sodium chloride, 1.4 *M* ammonium sulfate, 0.1 *M* HEPES pH 7.5. The crystals grew to maximum dimensions of 0.1 × 0.2 × 0.2 mm within in one week (Fig. 1).

Table 1
Crystal and data-collection parameters.

Values in parentheses are for the outermost data shell (2.49–2.40 Å).

Space group	*R*32
Unit-cell parameters	
a (Å)	161.1
c (Å)	153.1
Resolution limits (Å)	20.0–2.4
Wavelength (Å)	1.000
Temperature (K)	100
Total reflections	376674
Unique reflections	29957
Completeness (%)	98.3 (88.5)
R_{sym}† (%)	7.7 (36.5)
Mean $I/\sigma(I)$	13.9 (2.6)

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

X-ray diffraction experiments were carried out at 100 K. Since the crystallization solution was not suitable for providing cryoprotection, the crystals were quickly washed in reservoir solution containing 25%(*v*/*v*) glycerol and immediately flash-frozen in a nitrogen-gas stream at 100 K. X-ray diffraction data were collected to 2.4 Å resolution at the Elettra synchrotron source in Trieste using a MAR CCD detector. The data were processed using *DENZO* and scaled with *SCALEPACK* (Otwinowski & Minor, 1997). Table 1 summarizes the crystallographic data and data-collection statistics. Packing-density considerations (Matthews, 1968) for a monomer weight of 26 700 Da suggested the presence of 2–4 monomers in the asymmetric unit (V_M = 3.6–1.8 Å^3 Da^{-1}, corresponding to a solvent content of 66–32%). Attempts to solve the structure using the molecular-replacement technique are currently under way.

We would like to thank Maurizio Amendola for technical assistance and Sincrotrone Trieste CNR/Elettra for giving us the opportunity to collect data at the Crystallographic Beamline. This work was supported by grants from MIUR (PRIN 2002).

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