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Crystallization and preliminary X-ray data of a bifunctional peroxiredoxin from poplar

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Two variants (wild type and V152C mutant) of a bifunctional poplar peroxiredoxin have been overexpressed in *Escherichia coli* cells. The two recombinant enzymes were purified and crystallized using the hanging-drop vapour-diffusion technique. Data sets were collected to 1.62 and 2.48 Å resolution using X-ray synchrotron-source radiation from two crystal forms of wild-type peroxiredoxin which belonged to the monoclinic space group $P2_1$ (with unit-cell parameters a = 59.26, b = 68.80, c = 75.71 Å, β = 93.45°) and to the orthorhombic space group $P2_12_12$ (with unit-cell parameters a = 64.70, b = 130.73, c = 35.59 Å), respectively. Data were also collected to 2.17 Å resolution using a home X-ray source from a V152C peroxiredoxin crystal which belongs to the triclinic space group ($P1$), with unit-cell parameters a = 36.65, b = 41.53, c = 58.06 Å, α = 70.52, β = 93.45, γ = 64.31°. Phases have been obtained using molecular replacement with the structure of human peroxiredoxin V (PDB code 1hd2) as a search model. Refinement of the structures is in progress.

1. Introduction

Peroxiredoxins (PRX) are non-haem peroxidases which catalyse the conversion of alkylhydroperoxides into the corresponding alcohol and water in the presence of an adequate electron/proton donor according to the equation

$$ROOH + R'(SH)_2 \rightarrow ROH + H_2O + R'SS.$$

Peroxiredoxins are ubiquitous enzymes from bacteria to mammals and are generally overexpressed when the cells are subjected to oxidative stress, a condition where H_2O_2 is produced in massive amounts (Kang, Chae *et al.*, 1998). They also play a role in apoptosis or in the regulation of transcription factors (Zhang *et al.*, 1997; Zhou *et al.*, 2000). As the proton donor is mostly the small ubiquitous protein thioredoxin, it has also often been called thioredoxin peroxidase. Other variants have been named AhpC and tryparedoxin peroxidase (Poole, 1996; Montemartini *et al.*, 1998). Several classes of peroxiredoxins can be differentiated based on their catalytic mechanisms and sequences. All the peroxiredoxins possess a conserved catalytic cysteine surrounded by conserved amino acids with the consensus sequence FT(P/F)(V/G/T)C(P/T/S). In the course of catalysis, this catalytic cysteine is transformed into a sulfenic acid which is regenerated into a thiol group, generally in the presence of thioredoxin as an electron donor (Kang, Baines *et al.*, 1998). In the so-called 2-Cys peroxiredoxins, catalysis proceeds through the formation of either an intramolecular disulfide between the catalytic cysteine and another conserved cysteine or of an intermolecular disulfide between two head-to-tail peroxiredoxin monomers (Kong *et al.*, 2000). In another type, called the 1-Cys peroxiredoxins, only the catalytic cysteine is necessary and the thiol-regenerating system is either thioredoxin or an unidentified thiol component that can be replaced by dithiothreitol *in vitro* (Kang, Baines *et al.*, 1998; Pedrajas *et al.*, 2000). A third type, recently characterized in poplar (*Populus trichocarpa*), is an atypical 2-Cys peroxiredoxin which contains the conserved catalytic Cys and another Cys, the position of which is conserved in this third group. It differs however from the position of the second Cys of the 2-Cys peroxiredoxins and, in addition, it is not necessary for catalysis (Rouhier *et al.*, 2002). This protein is a bifunctional enzyme which can use thioredoxin as a proton donor but exhibits preference for a related protein, glutaredoxin, which is itself reduced by NADPH, glutathione reductase and glutathione, instead of NADPH and thioredoxin reductase as required for thioredoxin (Rouhier *et al.*, 2001).

In mammals, there are at least six genes coding for peroxiredoxin and the corresponding proteins play a prominent role in fighting oxidizing species, since they are produced at very high levels in several tissues (Seo *et al.*, 2000). Currently, six structures are known for peroxiredoxins: three are of human

proteins (PDB codes 1prx, 1h40, 1qmv), a fourth is a rat enzyme (PDB code 1qq2) and the two remaining are enzymes from *Crithidia fasciculata* (PDB code 1e2y) and *Mycobacterium tuberculosis* (PDB code 1knc) (Choi *et al.*, 1998; Hirotsu *et al.*, 1999; Alphey *et al.*, 2000; Schröder *et al.*, 2000; Declercq *et al.*, 2001). All are X-ray structures that are related to the thioredoxin fold. Although biochemical evidence indicates that peroxiredoxins function either as dimers or as monomers, several of these structures reveal that this enzyme can organize as decamers. Whether this is of physiological significance remains to be determined. Of the known structures, five correspond to 2-Cys peroxiredoxins and one to a 1-Cys peroxiredoxin.

The poplar enzyme recently identified and characterized is a very interesting protein because it is the only enzyme that is able to use glutaredoxin efficiently as an electron donor. It is thus likely that the sites of protein–protein interaction should be different on the poplar enzyme compared with the other peroxiredoxins described so far. In addition, unlike mammalian or bacterial enzymes, there are no structural data concerning peroxiredoxins in plants. Interestingly, we have also shown that the simple mutation V152C transforms the bifunctional peroxiredoxin into an enzyme which can use only thioredoxin as an electron donor (Rouhier *et al.*, 2002). In this respect, the poplar V152C mutant behaves more like its human homologue, peroxiredoxin V (Seo *et al.*, 2000; Rouhier *et al.*, 2002). We report here preliminary crystallization data concerning the wild type (WT) and the V152C mutant of poplar peroxiredoxin.

2. Results and discussion

2.1. Cloning, expression and purification

The cloning and expression of WT and V152C poplar peroxiredoxin is described in Rouhier *et al.* (2002). A high-yield expression was obtained by cloning the peroxiredoxin cDNA in expression plasmid pET-3d and cotransforming the BL21 *Escherichia coli* cells with the recombinant plasmid and the helper plasmid pSBET in order to compensate for the presence of unfavourable Arg codons. Typically, a single ampicillin- and kanamycin-resistant colony was selected and successively amplified in LB medium at 310 K to a final volume of 5 l. The protein production was promoted by the addition of 100 µ*M* IPTG for 3 h at 310 K. After breaking the cells by sonication, the protein was purified by a combination of ammonium sulfate precipitation, ACA 44 gel filtration and DEAE Sephacel chromatography. Typically, 300 mg of homogeneous protein were obtained and stored frozen in 1 ml aliquots in 30 m*M* Tris–HCl pH 8.0 containing 1 m*M* EDTA and 14 m*M* β-mercaptoethanol at a concentration of 15 mg ml^{-1} at 248 K. The purity of the fractions was monitored by SDS–PAGE.

Table 1
Data-collection statistics.

Values in parentheses refer to the highest resolution shell.

Crystal	WT form I	WT form II	V152C
Space group	$P2_1$	$P2_12_12$	$P1$
Unit-cell parameters (Å, °)	a = 59.26, b = 68.80, c = 75.71, β = 93.45	a = 64.70, b = 30.73, c = 35.59, $\alpha = \beta = \gamma$ = 90	a = 36.65, b = 41.53, c = 58.06, α = 70.52, β = 84.68, γ = 64.31
Resolution (Å)	1.62 (1.68–1.62)	2.48 (2.58–2.48)	2.17 (2.25–2.17)
Total reflections	294901	44949	73395
Unique reflections	79011	11600	13900
Completeness (%)	95.8 (83.5)	93.8 (90.4)	90.8 (79.5)
$R_{\rm merge}$†	0.062 (0.19)	0.044 (0.11)	0.042 (0.11)
Mean $I/\sigma(I)$‡	15 (4)	10 (5)	10 (5)
Mosaicity	0.67	0.37	0.48
Crystal dimensions (µm)	900 × 300 × 200	700 × 100 × 50	400 × 300 × 200

† $R_{\rm merge} = \sum(I_j - \langle I_j\rangle)/\sum I_j$, where I is the intensity of observation I and $\langle I\rangle$ is the mean intensity of the reflection. ‡ $I/\sigma(I)$ is the intensity to background ratio.

2.2. Crystallization

The initial screenings on the two PRX variants were carried out using the sparse-matrix sampling approach proposed by Jancarik & Kim (1991) and the hanging-drop vapour-diffusion method (McPherson, 1999). Drops were prepared for these initial crystallization experiments at 293 K by mixing 2 µl of protein solution (15.6 mg ml^{-1} WT PRX or 10 mg ml^{-1} V152C PRX) and 2 µl of reservoir solution (800 µl). A first monoclinic crystal form (form I) was obtained at 293 K in 30% polyethyleneglycol (PEG) 4000, 0.1 *M* Tris–HCl pH 8.0 and 0.2 *M* Li_2SO_4 for the WT protein but with a very low reproducibility. Although crystals were always obtained in this condition, it was extremely rare to obtain single crystals. Most of the time, the crystals were dendritic and not suitable for X-ray analysis. The use of these crystals to seed pre-equilibrated droplets allowed us to reproducibly obtain single crystals, but with poor diffraction pattern. This crystal condition was further optimized by an extensive search around this condition and by testing additives (Hampton Research). Very reproducible crystals, in an orthorhombic form, were finally obtained in 26% PEG 4000, 0.1 *M* Tris–HCl pH 8.0, 0.2 *M* Li_2SO_4 and 0.02 *M* L-Cys at 293 K (with an equal volume of protein and precipitant agent in the drops). Both crystal forms reached their final dimensions within a week. V152C PRX crystallized at 293 K in a similar condition (22.5% PEG 4000, 0.075 *M* Tris–HCl pH 8.0 and 0.15 *M* Li_2SO_4) to the WT PRX orthorhombic form but with a different ratio of protein to reservoir solution (3:1) in the drops and without reducing reagent. Reproducible mutant PRX crystals appeared within 5–6 d as thick plates.

2.3. Data collection and analysis

Data were collected from nitrogen flash-frozen crystals (100 K). Prior to this, the crystals were quickly soaked in a cryogenic buffer composed of the reservoir solution plus 20% glycerol and mounted in a nylon loop. Complete data sets were collected from the two different WT PRX forms on beamline DW32 at LURE (λ = 0.948 Å, Orsay France), whereas the V152C PRX data collection was performed on our home area detector (DIP2030) with a Φ goniometer using Cu $K\alpha$ radiation from a rotating-anode generator (Nonius BV, model FR591). Indexing, integration and merging of the data were carried out with the *HKL* suite (Otwinowski & Minor, 1996). The WT crystals (form I, obtained without seeding) belong to the monoclinic space group $P2_1$, with unit-cell parameters a = 59.26, b = 68.80, c = 75.71 Å, β = 93.45°. The crystal diffracted to 1.62 Å, with overall data completeness and $I/\sigma(I)$ of 95.8% and 15, respectively, and values of 83.5% and 4, respectively, for the 1.68–1.62 Å shell. The $R_{\rm merge}$ of the data set was 0.062% overall and 0.19% for the 1.8–1.6 Å shell (Table 1). The WT PRX orthorhombic crystals (form II) diffracted to 2.48 Å and belong to space group $P2_12_12$, with unit-cell parameters a = 64.70, b = 130.73, c = 35.59 Å (Table 1). The Matthews coefficients $V_{\rm M}$ (Matthews,

1968) for the monoclinic and the orthorhombic forms were 2.22 and 2.17 Å^3 Da^{-1}, respectively, which correspond to 42.4 and 41.1% solvent content, respectively, assuming four and two molecules per asymmetric unit. The mutant V152C PRX crystals belong to the triclinic space group (*P*1), with unit-cell parameters a = 36.65, b = 41.53, c = 58.06 Å, α = 70.52, β = 93.45, γ = 64.31°. They diffracted to 2.17 Å resolution (Table 1). Assuming two molecules per asymmetric unit, the Matthews coefficient V_M is 2.10 Å^3 Da^{-1} (39.2% solvent content).

2.4. Phase determination

Molecular-replacement trials using the program *AMoRe* (Navaza, 1994) were performed with different modifications of human red blood cell PRX coordinates (PDB code 1qmv) or rat PRX coordinates (PDB code 1qq2) used as a model (28 and 25% identity, respectively), but were unsuccessful. Several attempts were also made to obtain either xenon or bromide derivatives. Finally, release of the coordinates of human peroxiredoxin V (PDB code 1hd2; 42% identity) allowed the determination of the phases by molecular replacement using *AMoRe*. The search model used is the human PRX refined structure in which all the non-aligned residues (except Gly and Pro residues) between the poplar PRX and human PRX V have been replaced by alanines. Using data in the resolution range 12–4 Å and an integration radius of 25 Å, a clear solution was obtained with two molecules in the asymmetric unit for the WT orthorhombic form, with a correlation coefficient and an R factor of 44.9 and 44.5%, respectively (next best solution: 33.8 and 48.8%, respectively). Model building and refinement of these three structures is currently under way.

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