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Structure of peroxiredoxin from the anaerobic hyperthermophilic archaeon *Pyrococcus horikoshii*

The crystal structure of peroxiredoxin from the anaerobic hyperthermophilic archaeon *Pyrococcus horikoshii* (PhPrx) was determined at a resolution of 2.25 Å. The overall structure was a ring-type decamer consisting of five homodimers. Citrate, which was included in the crystallization conditions, was bound to the peroxidatic cysteine of the active site, with two O atoms of the carboxyl group mimicking those of the substrate hydrogen peroxide. PhPrx lacked the C-terminal tail that forms a 32-residue extension of the protein in the homologous peroxiredoxin from *Aeropyrum pernix* (ApPrx).

1. Introduction

Peroxiredoxins (Prxs) are thiol-dependent peroxidases that reduce hydrogen peroxide and alkyl peroxides to water and to the corresponding alcohols, respectively. They play an important role in anti-oxidant defence mechanisms by removing hydrogen peroxide, which is a toxic reactive oxygen species, and they are also important in redox signalling. Prxs are widespread in all biological kingdoms and are classified in several ways. Classically, Prxs were classified as 1-Cys, typical 2-Cys and atypical 2-Cys Prxs on the basis of the number of redox-active Cys residues and the combination of disulfide bonds during the reaction cycle. However, the above classification method is considered to be too simplistic to accurately describe all Prxs. A sequence-based analysis classified Prxs into seven groups (Mizohata *et al.*, 2005), whereas the PREX (PeroxiRedoxin classification indEX) database contains six subfamilies that are divided on the basis of the structure of the active site (Soito *et al.*, 2010).

The activity of Prx depends on the peroxidatic cysteine (C_p; arrow 2 in Fig. 1), which is oxidized to its sulfenic acid form (C_p-SOH) in the first step of the reaction. The side chain of C_p has a lower pK_a value than that of a free thiol group because of its interaction with the base. According to a widely accepted Prx reaction mechanism, a highly conserved Arg residue (Fig. 1, arrow 3) near the C_p is the only basic residue that could support the oxidation of the C_p thiol group (Hall *et al.*, 2010). However, in a certain group of Prxs a His residue is conserved at a position eight residues before the C_p (arrows 1 and 2 in Fig. 1). According to the sequence-based classification, these Prxs are called 'archaeal Prxs' and '1-Cys Prxs' (Mizohata *et al.*, 2005), whereas they are listed as the Prx6 subfamily in the PREX database (Soito *et al.*, 2010). The involvement of this conserved His residue in the reaction mechanism has previously been proposed. During the oxidation of Prx from the archaeon *Aeropyrum pernix* K1 (ApPrx), a conformational change occurs so that the imidazole ring of His42 approaches the C_p (Cys50) and the side chain of the conserved Arg126 swings away from the C_p (Fig. 2; Nakamura *et al.*, 2006). The oxidized S^γ atom of C_p binds covalently to the N^{δ1} atom of His42 to form a hypervalent sulfurane intermediate (Nakamura *et al.*, 2008). The interaction between the conserved His and C_p is also observed in human Prx6 (formerly referred to as hORF6; Choi *et al.*, 1998). In the sulfenic acid form of human Prx6, three atoms, N^{δ1} of His39 and S^γ and O^δ of C_p47 (cysteine sulfenic acid), are linearly aligned, similar to

the arrangement in the sulfurane intermediate of ApPrx (Nakamura *et al.*, 2008). Prx6 also undergoes displacement of the conserved Arg132 side chain in the sulfenic acid form (Choi *et al.*, 1998). It is unknown whether the Prx subfamily with the conserved His residue uniquely functions through the reaction mechanism involving the imidazole side chain. Additional studies focusing on the structure-based reaction mechanism need to be performed in order to elucidate this.

Several Prxs from archaea are characterized by a CXDWWFC motif (boxed in Fig. 1) in the C-terminal arm domain (Nakamura *et al.*, 2006; Mizohata *et al.*, 2005) and by a conserved His residue neighbouring C_p (arrow 1 in Fig. 1). Among Prxs from archaea, ApPrx ('archaeal Prx' in the sequence-based classification and 'Prx6' in the PREX database) and BCP-PrxQ (PREX database) from *A. pernix* and the genus *Sulfolobus*, which are aerobic hyperthermophilic archaea, have been structurally characterized (Nakamura *et al.*, 2006; Mizohata *et al.*, 2005; Perkins *et al.*, 2012). On the other hand, no information on the tertiary structure of Prxs isolated from anaerobes is available. *Pyrococcus horikoshii*, an anaerobic hyperthermophilic archaeon, produces a Prx protein (PhPrx; Kashima & Ishikawa, 2003; Kawakami *et al.*, 2004) which is encoded by open reading frame (ORF) PH1217. PhPrx is induced by oxygen and plays an important role in the peroxide-scavenging

system of *P. horikoshii*. In this paper, we describe the crystallization and structural determination of PhPrx.

2. Materials and methods

2.1. Expression, purification and crystallization

The coding sequence of PhPrx was constructed from the sequence of the PH1217 ORF with codon optimization performed using *Escherichia coli* genes. The coding sequence was cloned into the *Nde*I and *Bam*HI sites of pET11 to generate an expression plasmid for PhPrx. *E. coli* Rosetta (DE3) cells harbouring the expression plasmid were cultivated at 310 K in LB medium containing 100 µg ml⁻¹ ampicillin. When the OD_{600 nm} reached approximately 0.6, isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mM and expression of PhPrx was induced at 298 K overnight. PhPrx was purified using the same procedure as previously described for ApPrx (Nakamura *et al.*, 2005).

The crystallization of PhPrx was performed using the hanging-drop vapour-diffusion method. 1 µl of protein sample at 20 mg ml⁻¹ in 20 mM Tris-HCl pH 8.1, 1 mM DTT was mixed with the same volume of reservoir solution and equilibrated against 400 µl reservoir solution at 293 K. After screening and optimization of the crystallization

Figure 1

Alignment of Prx6 proteins. The C-terminal tail of ApPrx is underlined. The CXDWWFC motif of archaeal Prxs is boxed. The sources of the Prx proteins are as follows: PhPrx, *P. horikoshii* (NP_143112); ApPrx, *A. pernix* (Q9Y9L0); Tmar, *Thermotoga maritima* (NP_228616); Ctet, *Clostridium tetani* (NP_781549); Prx6, *Homo sapiens* (BAA03496); Atha, *Arabidopsis thaliana* (AAT41856); Scer, *Saccharomyces cerevisiae* (NP_009489); the EMBL/GenBank/DBJ accession numbers are given in parentheses. All of these proteins belong to the Prx6 subfamily according to the PREX database (Soito *et al.*, 2010). In the sequence-based classification (Mizohata *et al.*, 2005) PhPrx, ApPrx, Tmar and Ctet belong to the archaeal Prxs, while Prx6, Atha and Scer belong to the 1-Cys Prxs.

Figure 2

Schematic representation of the oxidation process of ApPrx. The movement of the His side chain towards C_p and the displacement of the Arg side chain upon oxidation of C_p are schematically illustrated. For details, see the text and Nakamura *et al.* (2008).

conditions, the reservoir solution was found to consist of 100 mM sodium citrate buffer pH 5.2, 20%(w/w) polyethylene glycol 3350. The crystals appeared in 3 d (Fig. 3a).

2.2. Data collection, processing and structure determination

A single crystal of PhPrx was immersed in reservoir solution supplemented with 15%(v/v) ($\pm$)-2-methyl-2,4-pentanediol and was flash-cooled in a nitrogen stream at 100 K. Diffraction data were collected using synchrotron radiation on beamline BL44XU at SPring-8, Hyogo, Japan. The data were integrated and scaled using

(a)

(b)

(c)

Figure 3

Crystals and structure of PhPrx. (a) Crystals of PhPrx. (b) Two decameric rings in an asymmetric unit are shown within a unit cell. (c) Superimposition of the monomer structures of ApPrx (magenta) and PhPrx (green).

Table 1

Data-collection and refinement statistics.

Values in parentheses are for the outermost resolution shell.

Data collection	
X-ray source	BL44XU, SPring-8
Wavelength (Å)	0.9000
Space group	$P2_1$
Unit-cell parameters (Å, °)	$a = 125.35$, $b = 94.73$, $c = 228.20$, $\beta = 103.32$
Resolution range (Å)	50.00–2.25 (2.33–2.25)
Z	40
$R_{\text{merge}}^{\dagger}$ (%)	10.3 (35.6)
Completeness (%)	95.2 (89.5)
Total reflections	1252027
Unique reflections	245717
Multiplicity	5.4 (4.3)
$\langle I/\sigma(I) \rangle$	11.1 (4.7)
Wilson B factor (Å ²)	16.4
Refinement	
Resolution range (Å)	43.57–2.25 (2.39–2.25)
No. of reflections	223921 (35807)
$R_{\text{cryst}}^{\ddagger}/R_{\text{free}}^{\S}$ (%)	19.8 (23.2)/23.7 (26.6)
R.m.s.d., bond lengths (Å)	0.006
R.m.s.d., bond angles (°)	1.4
Protein atoms	34640
Water molecules	1026
Citrate molecules	20
Average B factor (Å ²)	25.9
Ramachandran plot, residues in (%) ¶	
Favoured region	89.6
Allowed region	10.0

$^{\dagger} R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the i th observation of reflection hkl and $\langle I(hkl) \rangle$ is the average of symmetry-related observations of reflection hkl . $^{\ddagger} R_{\text{cryst}} = \sum_{hkl} ||F_{\text{obs}}| - |F_{\text{calc}}|| / \sum_{hkl} |F_{\text{obs}}|$, where F_{obs} and F_{calc} are the observed and calculated structure-factor amplitudes, respectively. $^{\S} R_{\text{free}}$ was calculated using a randomly selected 5% of the data within the data set that was omitted from all stages of the refinement. ¶ Ramachandran plots were prepared for all residues other than Gly and Pro.

HKL-2000 (Otwinowski & Minor, 1997). The initial phase was determined by molecular replacement using *MOLREP* (Vagin & Teplyakov, 2010) from the *CCP4* suite (Winn *et al.*, 2011). ApPrx (Nakamura *et al.*, 2006) with the C-terminal 32 residues truncated was used as the search model. Structural refinement was performed using *CNS* (Brünger *et al.*, 1998). Data-collection and refinement statistics are summarized in Table 1. Superimposition of the protein structures was performed using *LSQKAB* (Kabsch, 1976) from the *CCP4* suite. Sequence alignment was performed using *ClustalW* (Higgins *et al.*, 1996).

3. Results and discussion

3.1. Structure determination

The crystal structure of PhPrx was determined at 2.25 Å resolution, with R_{cryst} and R_{free} values of 19.8 and 23.7%, respectively (Table 1). The asymmetric unit contained 20 polypeptides, *i.e.* two decameric rings (Fig. 3b), characterized by a Matthews coefficient and a solvent content of 2.31 Å³ Da^{−1} and 46.7%, respectively. Gel-filtration chromatography showed that PhPrx is a decamer in solution (data not shown). The decameric ring consists of five homodimers and the quaternary structure is similar to that of ApPrx. Fig. 3(c) shows a superimposition of the monomer structures of PhPrx and ApPrx. ApPrx has a C-terminal extension (underlined in Fig. 1) designated the C-terminal tail, whereas PhPrx does not. Except for the C-terminal tail, the monomer structures of PhPrx and ApPrx coincided well, with a root-mean-square deviation value of 0.88 Å over 209 C α atoms.

Figure 4

Close-up view around the peroxidatic cysteine. The orange map represents the σ_A -weighted $F_o - F_c$ electron-density map at the 3σ level, in which Cys46 (C_p), Arg121 and bound citrate were excluded from calculation of the structure factors. In addition, Val45, Pro44 and Thr43 are represented using stick models. Dashed lines represent possible hydrogen bonds.

3.2. Active-site structure

The C_p thiol group of PhPrx interacts with Arg121, which is invariant in all Prx sequences (Figs. 1 and 4). Citrate, which was included in the crystallization solution, is bound to the chain in the proximity of the active site. The carboxylic group tends to bind at this position of Prx chains in their crystal structures. Before the establishment of the structure of ApPrx bound to hydrogen peroxide (Nakamura *et al.*, 2010), the two O atoms of the bound carboxylic group had been considered to mimic the binding of the peroxide substrate to Prx (Declercq *et al.*, 2001; Karplus & Hall, 2007). In the PhPrx structure, OB2 of the citrate is in close proximity to Cys46 S^γ and N, Thr43 O^γ and Arg121 N^η , whereas OB1 is close to Val45 N (Fig. 4). These orientations correlate well with the interaction of the two O atoms of hydrogen peroxide bound to ApPrx (Nakamura *et al.*, 2010).

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