Crystallization and preliminary X-ray diffraction
analysis of thioredoxin peroxidase from the aerobic
hyperthermophilic archaeon *Aeropyrum pernix* K1Tsutomu Nakamura,^{a,*} Hiroyoshi
Matsumura,^b Tsuyoshi Inoue,^b
Yasushi Kai,^b Koichi Uegaki,^a
Yoshihisa Hagihara,^a Mitsuo
Ataka^a and Kazuhiko Ishikawa^a^aNational Institute of Advanced Industrial
Science and Technology, Ikeda,
Osaka 563-8577, Japan, and ^bDepartment of
Materials Chemistry, Graduate School of
Engineering, Osaka University, Suita,
Osaka 565-0871, Japan

Correspondence e-mail: nakamura-t@aist.go.jp

Received 23 November 2004

Accepted 17 February 2005

Online 24 February 2005

Thioredoxin peroxidase is a member of the peroxiredoxin family and plays a dominant role in a hydrogen peroxide metabolism. A recombinant form of the hyperthermostable thioredoxin peroxidase from the aerobic hyperthermophilic archaeon *Aeropyrum pernix* K1, a polypeptide consisting of 250 amino acids, was purified. The C207S mutant protein was crystallized by the hanging-drop vapour-diffusion method using potassium sodium tartrate as the precipitant at 298 K. Diffraction data were collected and processed to 2.7 Å resolution. The crystal belongs to space group *P*1, with unit-cell parameters $a = 126.2$, $b = 126.3$, $c = 213.7$ Å, $\alpha = 80.4$, $\beta = 80.3$, $\gamma = 70.7^\circ$. Calculation of the self-rotation function showed that the protein quaternary structure includes a fivefold axis and five twofold axes.

1. Introduction

The peroxiredoxin (Prx) family includes a number of antioxidant peroxidases that reduce hydrogen peroxide and alkyl hydroperoxides to water and the corresponding alcohols, respectively (Rhee *et al.*, 2001; Wood, Schroder *et al.*, 2003). To date, the three-dimensional structures of Prxs from various organisms have been reported. Distinct quaternary structures, *i.e.* monomers (Declercq *et al.*, 2001), dimers (Choi *et al.*, 1998, 2003; Hirotsu *et al.*, 1999) and toroid-shaped decamers (Alphey *et al.*, 2000; Schroder *et al.*, 2000; Wood *et al.*, 2002; Wood, Poole *et al.*, 2003), were observed depending on the subtype and redox state of the protein.

The thioredoxin system is a cellular function that removes toxic hydrogen peroxide. In this system, hydrogen peroxide is neutralized through the oxidation of one of the Prx-family proteins, thioredoxin peroxidase (TPx). The resulting TPx containing a disulfide bond is then reduced by thioredoxin. Subsequently, thioredoxin in the oxidized form is reduced by NADPH through the catalytic function of thioredoxin reductase. We previously identified the gene for TPx encoding a polypeptide of 250 amino-acid residues with a molecular weight of 28 703 Da in the genome of an aerobic hyperthermophilic archaeon, *Aeropyrum pernix* K1, and produced the recombinant protein (Jeon & Ishikawa, 2003). The recombinant TPx from *A. pernix* K1 (ApTPx) exhibited thioredoxin peroxidase activity and constituted the thioredoxin system together with two other proteins from *A. pernix*: thioredoxin and thioredoxin reductase (Jeon & Ishikawa, 2002). Mutational analyses showed that Cys50 and Cys213 participate in the intersubunit disulfide bonding upon oxidation by hydrogen peroxide and that another cysteine residue (Cys207) is not essential for the peroxidase function. Interestingly, transmission electron microscopy revealed a twofold toroid-shaped structure of ApTPx. This unique quaternary structure of ApTPx contributes to its hyperthermostability, as indicated by mutational analysis (Jeon & Ishikawa, unpublished results). Here, we report the crystallization and preliminary X-ray analysis of the C207S mutant of ApTPx, which should shed some light on the origin of the thermostability of this oligomeric protein.

© 2005 International Union of Crystallography
All rights reserved

2. Materials and methods

2.1. Expression and purification of the recombinant protein

Escherichia coli Rosetta (DE3) cells harbouring an expression plasmid for ApTPx (C207S mutant; Jeon & Ishikawa, 2003) were grown to an A_{600} of ~ 0.6 in LB medium at 310 K and expression of the recombinant protein was then induced by the addition of 1 mM (final concentration) isopropyl-1-thio- β -D-galactopyranoside and further incubation for 15 h at 298 K. *E. coli* cells containing the recombinant protein were disrupted by sonication in buffer A (50 mM Tris-HCl pH 8.0, 1 mM DTT, 0.1 mM EDTA) and a soluble

fraction was obtained by centrifugation at 31 000g for 30 min. The soluble materials were heat-treated at 358 K for 30 min and then centrifuged. Streptomycin sulfate was added to the supernatant to a final concentration of 2% (w/v) and the solution was then stirred on ice for 30 min. After the centrifugation, the supernatant was dialyzed against buffer A and then loaded onto a HiTrap Q column (Amersham Biosciences, Sweden). The bound protein was eluted with a linear gradient of 0–1 M NaCl in buffer A. The fraction containing ApTPx was subjected to gel filtration on a Superdex 200 PG column (Amersham Biosciences) with buffer A containing 150 mM NaCl. The purified protein was precipitated with 80% saturated ammonium sulfate and then dialyzed against 20 mM Tris-HCl pH 7.5. The protein concentration of the final preparation was 25 mg ml⁻¹, as determined from the absorbance at 280 nm (Edelhoch, 1967). The molecular weight of the purified protein was confirmed by matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) with a Voyager DE-STR instrument (Applied Biosystems, USA).

Figure 1
Crystal of ApTPx (C207S).

2.2. Crystallization and data collection

The purified protein was crystallized by the hanging-drop vapour-diffusion method at 298 K. A Crystal Screen kit (Hampton Research, USA) was used for initial screening. Good crystals were grown from condition No. 2 (0.4 M potassium sodium tartrate) in a drop consisting of 2 μ l protein in 20 mM Tris-HCl pH 7.5 and 2 μ l reservoir solution equilibrated against 0.5 ml of the reservoir solution. Preliminary X-ray diffraction data were collected at room temperature in-house on a Rigaku rotating-anode Cu K α X-ray generator operated at 50 kV and 100 mA. The data were processed with HKL2000 (Otwinowski & Minor, 1997). The self-rotation function was calculated with the MOLREP program from the CCP4 suite (Collaborative Computational Project, 1994).

Figure 2
Self-rotation function calculated in the (a) $\chi = 72^\circ$ and (b) $\chi = 180^\circ$ sections.

Table 1

Data-collection statistics.

Values in parentheses are for the highest resolution shell.

Wavelength (Å)	1.54178
Detector	Rigaku R-AXIS IV ⁺⁺
Space group	<i>P</i> 1
Unit-cell parameters (Å, °)	<i>a</i> = 126.2, <i>b</i> = 126.3, <i>c</i> = 213.7, <i>α</i> = 80.4, <i>β</i> = 80.3, <i>γ</i> = 70.7
Resolution range (Å)	30–2.7 (2.8–2.7)
<i>R</i> _{merge} [†] (%)	9.9 (34.4)
<i>I</i> / <i>σ</i> (<i>I</i>)	5.8 (2.2)
Total reflections	496291
Unique reflections	334804
Completeness (%)	86.2 (79.3)

[†] $R_{\text{merge}} = \sum |I - \langle I \rangle| / \sum I$, where *I* represents the intensity of the reflection and *I* the averaged intensity.

3. Results and discussion

We selected the C207S mutant of ApTPx for crystallographic study as this mutant exhibits significant peroxidase activity and its quaternary structure is more homogeneous than that of the wild-type protein (Jeon & Ishikawa, 2003). The purified protein lacked the N-terminal methionine residue, as revealed on MALDI–TOF MS (data not shown). Crystals of dimensions 0.2 × 0.2 × 0.5 mm were obtained by the vapour-diffusion method within one week (Fig. 1). A room-temperature X-ray diffraction data set was collected to 2.7 Å resolution with an overall completeness of 86.2%. The detailed conditions and measurement results are summarized in Table 1. The results of self-rotation function calculations are presented in Fig. 2. A distinct peak was observed in the $\chi = 72^\circ$ section. In the $\chi = 180^\circ$ section, five peaks were found in directions perpendicular to the fivefold axis. These indicate the existence of a fivefold axis and five twofold axes in the crystal structure, implying that the toroid-shaped ring includes ten rather than eight subunits. Assuming four decamers (4 × 10 × 28 571 Da) in the asymmetric unit, the crystal volume per protein weight (*V*_M) and solvent content can be calculated as 2.75 Å³ Da^{−1} and 55.3%, respectively (Matthews, 1968). On the basis of gel-filtration and sedimentation-equilibrium measurements, we have

previously reported that ApTPx exists as a hexadecamer consisting of twofold toroid-shaped octamers (Jeon & Ishikawa, 2003). However, the self-rotation function of the current crystal form of ApTPx indicates the presence of a fivefold axis rather than that of a fourfold axis and showed that the single ring is likely to consist of five homodimers of the protein.

Phase determination with selenomethionine derivatives is in progress.

This work was supported by the National Project on Protein Structural and Functional Analyses.

References

- Alphey, M. S., Bond, C. S., Tetaud, E., Fairlamb, A. H. & Hunter, W. N. (2000). *J. Mol. Biol.* **300**, 903–916.
- Choi, H. J., Kang, S. W., Yang, C. H., Rhee, S. G. & Ryu, S. E. (1998). *Nature Struct. Biol.* **5**, 400–406.
- Choi, J., Choi, S., Cha, M. K., Kim, I. H. & Shin, W. (2003). *J. Biol. Chem.* **278**, 49478–49486.
- Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* **D50**, 760–763.
- Declercq, J. P., Evrard, C., Clippe, A., Stricht, D. V., Bernard, A. & Knoop, B. (2001). *J. Mol. Biol.* **311**, 751–759.
- Edelhoch, H. (1967). *Biochemistry*, **6**, 1948–1954.
- Hirotsu, S., Abe, Y., Okada, K., Nagahara, N., Hori, H., Nishino, T. & Hakoshima, T. (1999). *Proc. Natl Acad. Sci. USA*, **96**, 12333–12338.
- Jeon, S. J. & Ishikawa, K. (2002). *Eur. J. Biochem.* **269**, 5423–5430.
- Jeon, S. J. & Ishikawa, K. (2003). *J. Biol. Chem.* **278**, 24174–24180.
- Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
- Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
- Rhee, S. G., Kang, S. W., Chang, T. S., Jeong, W. & Kim, K. (2001). *IUBMB Life*, **52**, 35–41.
- Schroder, E., Littlechild, J. A., Lebedev, A. A., Errington, N., Vagin, A. A. & Isupov, M. N. (2000). *Structure Fold. Des.* **8**, 605–615.
- Wood, Z. A., Poole, L. B., Hantgan, R. R. & Karplus, P. A. (2002). *Biochemistry*, **41**, 5493–5504.
- Wood, Z. A., Poole, L. B. & Karplus, P. A. (2003). *Science*, **300**, 650–653.
- Wood, Z. A., Schroder, E., Harris, J. R. & Poole, L. B. (2003). *Trends Biochem. Sci.* **28**, 32–40.