

**Experiment title:**

Oxidized form of human peroxiredoxin 5

Experiment**number:**

MX-457

Beamline: ID14 3	Date of experiment: from: 20/6/2005 16:00 to: 20/6/2005 24:00	Date of report: 27/10/2005 <i>Received at ESRF:</i>
Shifts: 1	Local contact(s): Elspeth GORDON	

Names and affiliations of applicants (* indicates experimentalists):

DECLERCQ Jean-Paul*	Université Catholique de Louvain, Belgium
SMEETS Aude	Université Catholique de Louvain, Belgium
EVRARD Christine*	Université Catholique de Louvain, Belgium

Report:

Peroxiredoxins (PRDXs) constitute a family of ubiquitous peroxidases found in all biological kingdoms [1]. PRDXs reduce hydrogen peroxide, alkyl hydroperoxides and peroxynitrite by the use of reducing equivalents derived from thiol-containing donor molecules such as thioredoxin.

All PRDXs exhibit a conserved peroxidatic cysteine residue in their N-terminal region that attacks the peroxide and is consequently oxidized to cysteine sulfenic acid (Cys-SOH). Mammalian PRDXs are now divided into three subgroups referred to as typical 2-Cys (PRDX1-4), atypical 2-Cys (PRDX5) and 1-Cys (PRDX6) PRDXs [2]. In typical 2-Cys subgroup, the second redox-active cysteine, the resolving cysteine, is localized to the C-terminal region of the enzyme in a conserved domain. In atypical 2-Cys PRDXs, the C-terminal resolving cysteine is contained within the same polypeptide chain and the reaction with the peroxidatic cysteine results in the formation of an intramolecular disulfide bond. PRDX5 is the only mammalian atypical 2-Cys PRDX identified so far [1,2,3].

In the past, we have determined the crystal structure of human PRDX5 in its reduced form [4,5]. The structure revealed that peroxidatic Cys47 and resolving Cys151 were too distant to form an intramolecular disulfide bond upon oxidation without important conformational changes. Recently, a new crystal form of PRDX5 was obtained in our laboratory [6]. The asymmetric unit contains three polypeptide chains. Surprisingly, beside two reduced chains, the third one is oxidized although the enzyme was crystallized under initial reducing conditions in presence of 1 mM 1,4-dithio-DL-threitol. The oxidized polypeptide chain forms an homodimer with a symmetry related one through intermolecular disulfide bonds between Cys47 and Cys151. The formation of these disulfide bonds is accompanied by the partial unwinding of the N-terminal parts of the $\alpha 2$ helix which, in the reduced form, contains the peroxidatic Cys47 and the $\alpha 6$ helix which is sequentially close to the resolving residue Cys151. In each monomer of the oxidized chain, the C-terminal part including the $\alpha 6$ helix is completely reorganized and is isolated from the rest of the protein on an extended arm. In the oxidized dimer, the arm belonging to the first monomer now appears at the surface of the second subunit and vice-versa. The availability of the crystal structures of the reduced monomer and the

oxidized dimer of PRDX5 allowed to suggest a modelled conformation of the oxidized monomeric form of PRDX5.

Crystals of the oxidized form of PRDX5 have an hexagonal shape with dimensions 200*200*50 μm . Despite these comfortable dimensions, the crystals diffract very poorly. Data were collected at 100K on beam line ID14-3 to a resolution of 2.95 \AA . A total of 73,204 reflections were collected of which 14,572 unique. $R_{\text{merge}} = 11,3 \%$, completeness is 99.2 % and the Wilson B-factor is 77 \AA^2 . The data can be processed in the hexagonal space group $P6_x22$ but all the statistics indicate the presence of nearly perfect twinning, making de-twinning impossible. All the attempts to solve the structure by classical molecular replacement methods in any of the ten possible space groups ($P6_2$, $P6_4$, $P6_222$, $P6_422$, $P3_1$, $P3_2$, $P3_121$, $P3_221$, $P3_112$, $P3_212$) failed. Finally, the program PHASER [8] succeeded to solve the problem using the modelled oxidized monomer [6]. The crystals are trigonal, space group $P3_121$ with 4 molecules in the asymmetric unit. The molecules are associated in dimers, in which two monomers are related by an NCS twofold axis parallel to the additional crystallographic twofold axis present in hexagonal space groups. As shown on figure 1, the two dimers are related by a translation $(1/2, 0, 0)$ although the corresponding peak in the Patterson function was rather weak (6.3σ). The refinement was initiated with SHHELXL [9], because this program is able to deal with perfect twinning. After some initial refinement, we obtained $R = 0.27$ and $R_{\text{free}} = 0.32$ before any manual adjustment. The refined value of the twin fraction is 0.47.

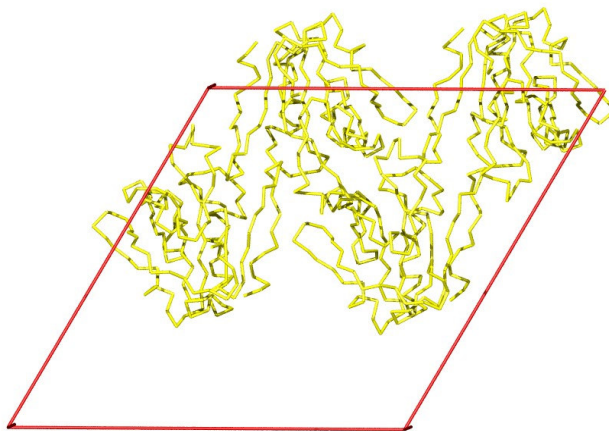


Figure 1. C^α trace of the four molecules of the asymmetric unit presented in the trigonal unit-cell.

References

- [1] S.G. Rhee, S.W. Kang, T.S. Chang, W. Jeong and K. Kim, *IUBMB Life*, 52, 35 (2001)
- [2] Z.A. Wood, E. Schröder, J.R. Harris and L.B. Poole, *Trends Biochem. Sci.* 28, 32 (2003)
- [3] B. Knoop, A. Clippe, C. Bogard, K. Arsalane, R. Wattiez, C. Hermans, E. Duconseille, P. Falmagne, and A. Bernard, *J. Biol. Chem.* 274, 30451 (1999)
- [4] J.-P. Declercq, C. Evrard, A. Clippe, D. Vander Stricht, A. Bernard and B. Knoop, *J. Mol. Biol.* 311, 751 (2001)
- [5] J.-P. Declercq and C. Evrard, *Acta Crystallogr. sect. D*, 57, 1829 (2001).
- [6] C. Evrard, A. Capron, C. Marchand, A. Clippe, R. Wattiez, P. Soumillion, B. Knoop and J.-P. Declercq, *J. Mol. Biol.* 337, 1079 (2004)
- [7] M.S. Seo, S.W. Kang, K. Kim, I.C. Baines, T.H. Lee and S.G. Rhee, *J. Biol. Chem.* 275, 20346 (2000)
- [8] A. J. McCoy, R. W. Grosse-Kunstleve, L. C. Storoni and R. J. Read, *Acta Crystallogr. sect. D*, 61, 458 (2005)
- [9] G.M. Sheldrick, *SHELXL97*, University of Göttingen (1997)