

Structural insights into the peroxidase activity and inactivation of human peroxiredoxin 4

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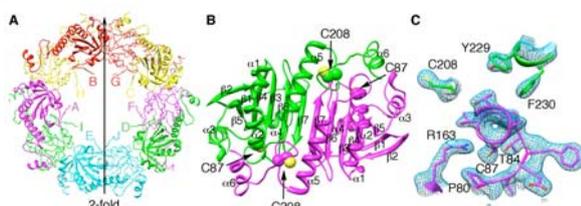
Abstract

Prx4 (peroxiredoxin 4) is the only peroxiredoxin located in the ER (endoplasmic reticulum) and a proposed scavenger for H₂O₂. In the present study, we solved crystal structures of human Prx4 in three different redox forms and characterized the reaction features of Prx4 with H₂O₂. Prx4 exhibits a toroid-shaped decamer constructed of five catalytic dimers. Structural analysis revealed conformational changes around helix α 2 and the C-terminal region with a YF (Tyr-Phe) motif from the partner subunit, which are required for interchain disulfide formation between Cys87 and Cys208, a critical step of the catalysis. The structural explanation for the restricting role of the YF motif on the active site dynamics is provided in detail.

Crystal structure of reduced Prx4

In the presence of DTT Prx4 was crystallized into space group *C*2 with five chains in an asymmetric unit. The overall structure resembles a toroid-shaped decamer from two asymmetric units (**Fig 1A**). The toroid has a maximal diameter of ~120 Å and an inside diameter of ~60 Å. Each subunit exhibits a typical extended Trx fold ($\beta\beta\alpha\beta\alpha\beta\alpha\beta\beta\alpha\alpha$) (**Fig 1B**). The peroxidatic Cys87 is located in the first turn of helix α 2 and surrounded by three conserved residues, Pro80, Thr84 and Arg163. The resolving Cys208, located on a flexible loop between α 5 and α 6, is ~13.0 Å apart from Cys87 of the partner chain (**Fig 1B and C**). As the distance is obviously unfavorable for the disulfide formation, conformational changes are required for the resolving process.

Fig 1

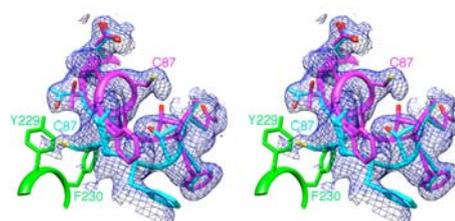


Locally unfolded conformation of oxidized Prx4

In the absence of DTT Prx4 was crystallized in a different unit cell, and the averaged 2Fo-Fc omit map of four chains in one asymmetric unit clearly showed that the region around Cys87 exists in mixed conformations (**Fig 2**). One is fully folded, quite similar to the reduced structure. The other is locally unfolded. Notably Cys87

shifted to the position that was occupied by the aromatic rings of the partner YF motif in the reduced structure, suggesting that the YF motif, which is absent in AhpC, has to move to allow the inter-chain disulfide formation between Cys87 and Cys208.

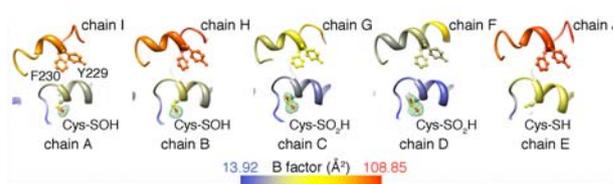
Fig 2



Oxidation of Cys87 by H₂O₂

Treatment with 1 mM H₂O₂ of reduced Prx4 crystal yielded a 2.4 Å structure. Cys87 are assigned in -SOH form in chain A and B, in -SO₂H form in chain C and D, and remains reduced in chain E (**Fig 3**). The B factors of helix α 2 with Cys87 in -SO₂H form and its partner C-terminal region (chains C/G and D/F) are much lower than those in the -SOH and the reduced forms (**Fig 3**), showing much lower flexibility. The low flexibility of helix α 2 in chain C and D as well as their partner C-terminal region would inevitably restrict conformational changes and thus gave Cys87 more chance to be over-oxidized. In this structure the crystal packing endowed subunits in the decamer with diverse flexibility around these regions leading to distinct oxidation states of Cys87, thus enabled us to address the necessity of conformation changes during the catalysis.

Fig 3.



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