Hypervalent Intermediate in the Oxidation of Archaeal Peroxiredoxin

Increased levels of reactive oxygen species, such as hydrogen peroxide and alkyl peroxides, cause damage to biological macromolecules in aerobic cells, including lipids, proteins, and nucleic acids. Therefore, aerobic organisms have evolved mechanisms that protect them from oxidative damage. As a result, most cells have developed antioxidation proteins such as superoxide dismutase, catalase, thioredoxin, and peroxidase.

Peroxiredoxins (Prxs) are thiol-dependent peroxidases that reduce hydrogen peroxide and alkyl peroxides to water and the corresponding alcohols, respectively [1]. In addition to antioxidant functions, Prxs maintain the intracellular level of hydrogen peroxide that affects signal mediators such as protein tyrosine phosphatase and the lipid phosphatase PTEN through its self-inactivation mechanism [2]. These two distinct functions make Prx a switching molecule that regulates the cellular H_2O_2 level. In general, the oxidation of a cysteine side chain of protein is initiated by the formation of cysteine sulfenic acid (Cys-SOH) (Fig. 1). The same oxidation mechanism has been reported also for the oxidation process of Prx proteins. Prx contains a redox active peroxidatic cysteine (C_p) residue in its N-terminal region. It has been reported that the first step of the reaction of Prx is the formation of the single oxidation intermediate, cysteine sulfenic acid (C_p-SOH). Cysteine sulfenic acid is prone to further oxidation in aqueous solution, leading to a self-inactivation process by overoxidation to form cysteine sulfinic acid (C_p-SO_2H) and cysteine sulfonic acid (C_p-SO_3H). In the peroxidase process of Prx, the single oxidation intermediate is followed by disulfide linkage formation or reaction with a cellular reductant.

To gain insight into the oxidation mechanism of the archaeal Prx, we determined crystal structures of ApTPx in multiple oxidation states [3], using beamline **BL41XU**, and compared them with the reduced form [4] (Fig. 2). In the reduced state, the C_p side chain interacts with Arg126, which is invariant in all known Prxs. With the addition of hydrogen peroxide, the conformation around the C_p changes so that Arg126 swings away and His42 moves near to C_p50, while the side chain of C_p50 is still reduced. Oxidation of C_p50 results in the single oxidation form, in which an extra oxygen atom bonds covalently to the S^{γ} atom. In addition, the S^{γ} atom is situated near $N^{\delta 1}$ of His42. The distance between these atoms is so close (2.2 Å) that they are considered to form a covalent bond rather than a hydrogen bond. The bond angle formed around the S^{γ} atom shows a characteristic feature where the $N^{\delta 1}$, S^{γ}, and O^{δ} atoms are aligned linearly and the C^{β} atom of C_p50 bonds to the S^{γ} from the perpendicular direction (Fig. 3(a)). Simulation with quantum chemical calculations revealed that the single oxidation form represents a sulfurane derivative, which is a hypervalent sulfur compound (Fig. 3(b)). The crystal structure is considered to represent a mixture of the sulfurane derivatives with either a neutral or protonated imidazole moiety.

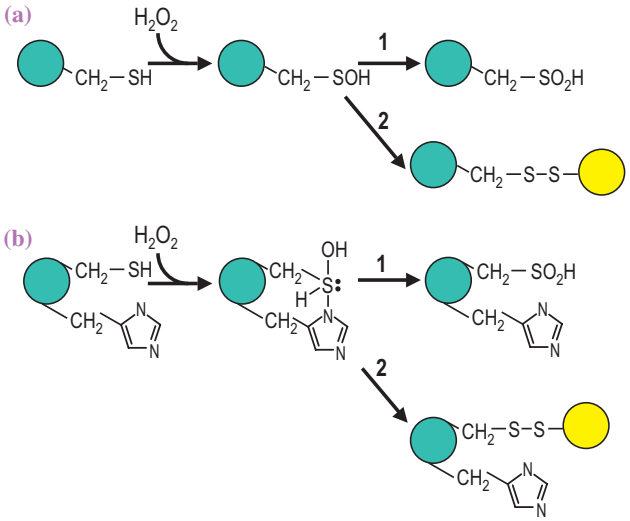


Fig. 1. Mechanism of thiol oxidation in proteins. Panel **(a)** represents the conventional mechanism of thiol oxidation involving cysteine sulfenic acid and panel **(b)** the novel mechanism involving hypervalent sulfur intermediate. Arrows 1 and 2 indicate further oxidation and disulfide bond forming pathways, respectively.

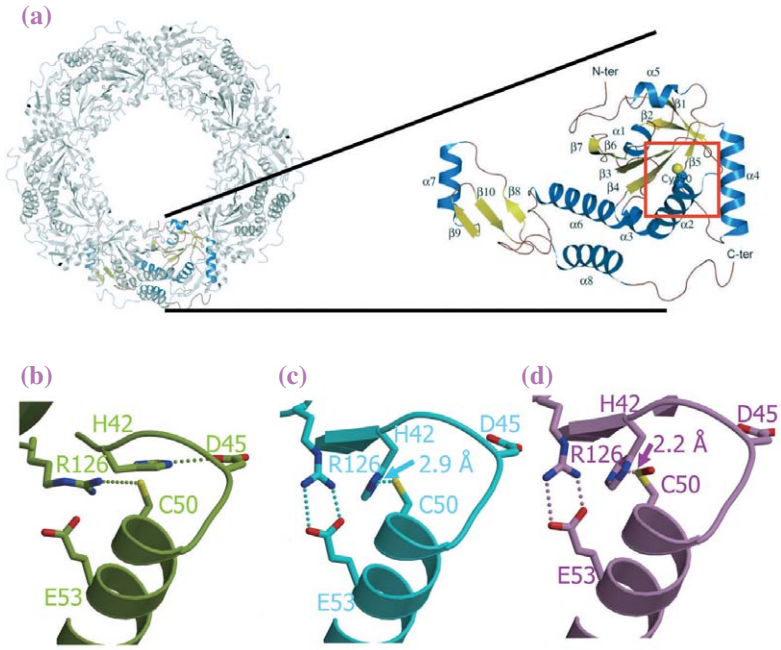
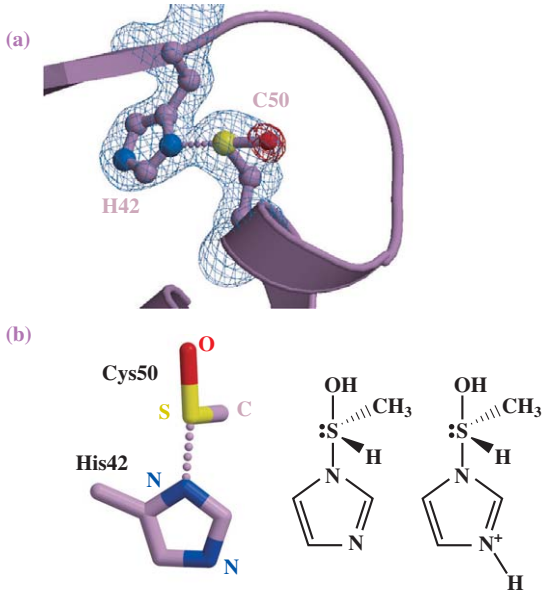


Fig. 2. Crystal structure of ApTPx in multiple oxidation forms. **(a)** shows decamer and monomer structures of ApTPx. **(b)**, **(c)** and **(d)** show the structure around the C_p residue (red box in a) in the reduced, preoxidation, and single-oxidation forms, respectively.



Angle (O-S-C) (˚)	**81.3 ± 2.8**	91.5	93.7
Angle (O-S-N) (˚)	**170.2 ± 4.5**	170.3	172.4
Angle (N-S-C) (˚)	**91.0 ± 3.7**	88.2	89.0
Distance (O-S) (Å)	**1.72 ± 0.01**	1.80	1.68
Distance (S-N) (Å)	**2.21 ± 0.13**	1.97	2.39

Fig. 3. Structure of the hypervalent sulfur intermediate. **(a)** Crystal structure around C_p50 and His42 of ApTPx in the single-oxidation form. **(b)** List of bond angles and lengths. Values for the crystal structure (shown on the left) are the average ± SD of the 10 subunits. The right columns show the calculated values of the neutral and protonated sulfurane models.

This study shows for the first time that sulfurane is involved in a biochemical reaction. Hypervalent compounds have been studied in the field of organic synthetic chemistry for about 50 years. Nowadays, hypervalent compounds are known as active reagents such as the Wittig reagent, a hypervalent phosphorus compound. This study expands the importance of hypervalent compounds with respect to the field of biochemistry by demonstrating that a sulfurane derivative occupies a crucial position in biochemical processes. The architecture of hypervalent sulfur in proteins will provide useful guidelines for methodological studies towards the synthesis of novel hypervalent compounds.

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