“Structural and Functional Characterization of the Ascorbate-Peroxidatic Activity in 1-Cys Peroxiredoxins”

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Peroxiredoxins (Prx) are peroxidases described as strictly thiol-dependent, and can be divided in two groups based on the number of cysteine residues that participate in their catalytic cycle. Both groups decompose peroxynitrite, organic and hydrogen peroxides, by the oxidation of a peroxidatic cysteine into a sulfenic acid. In 2-Cys group, there is also a resolving cysteine forming a stable disulfide bond within one molecule (atypical 2-Cys) or between two molecules (typical 2-Cys). In 1-Cys Prxs, the sulfenic acid is the stable oxidized form, as they lack the resolving cysteine. The physiological reductant for 1-Cys is not known, but in 2007, Monteiro *et al.* described the reduction of the Prx sulfenic acid by ascorbate, changing the thiol-specific paradigm for these proteins. To further characterize this activity, we have previously described docking assays that suggested the involvement of some residues in the interaction with ascorbate. Here, we describe *in silico* and enzymatic assays using rat peroxiredoxin 6 (Prdx6) mutants (Prdx6 H39S, Prdx6 T44A, Prdx6 T48A), according to the docking analysis, Prdx6 H39S and Prdx6 T44A mutants had their ascorbate-dependent activity strongly reduced compared with the wild type, In contrast, the thiol peroxidase activity was only slightly affected. This data supported the idea of the importance of these residues for the ascorbate-sulfenic acid interaction in Prdx6, and can be used for comparative studies in other proteins possessing sulfenic acid oxidized forms during their catalytic cycle or in activity modulation. Currently, other site specifics mutants of Prdx6 are being investigated.
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