Typical 2-Cys Peroxiredoxin (Prx) enzymes are a group of peroxidases ubiquitously distributed among the organisms that makes use of a reactive cysteine (peroxidatic cysteine – CysP–S–) to decompose hydroperoxides, culminating with an intermolecular disulfide bond formation with the so-called resolving cysteine (CysR). Disulfide is generated as a consequence of partial unfolding of CysP containing α-helix. Therefore, during its catalytic cycle, 2-Cys Prx alternates between two states: locally unfolded (LU) and fully folded (FF), but forces that drive this switch are still poorly understood. In this work, crystallographic structure of *Saccharomyces cerevisiae* Tsa1\textsuperscript{C47S} is presented, which gave insights on the redox dependent switches. The crystallographic structure is in the decameric form [(α2)\textsubscript{5}] with a reduced DTT molecule bound to the active site, the first typical 2-Cys Prx structure cocrystallized with a ligand. Glu50 and Arg146 residues stabilize Tsa1\textsuperscript{C47S} structure in FF state, through polar interactions with CysP. We postulate that upon oxidation of CysP to sulfenic acid, these polar interactions are lost and as consequence Tsa1 assumes LU state, enabling disulfide formation. Tsa1\textsuperscript{E50A} and Tsa1\textsuperscript{R146Q} mutants were able to decompose hydrogen peroxide, presenting a second-order rate constant one order of magnitude lower than the corresponding wild type protein. Remarkably, although Tsa1\textsuperscript{E50A} and Tsa1\textsuperscript{R146Q} were efficiently reduced by DTT, these mutants did not present thioredoxin-dependent peroxidase activity, indicating that Glu50 and Arg146 are important for Tsa1-thioredoxin interaction. Using size exclusion chromatography and structural analysis of known 2-Cys Prx structures, we also showed that there was a significant redox-dependent alteration in protein shape with the involvement of Glu50 and Arg146, which probably accounts at least partially for the ability of Trx to efficiently reduce Tsa1. We anticipate that these results may impact the knowledge of signaling pathways that depend on redox states of thioredoxin.

Keywords: Reactive Oxygen Species, *Saccharomyces cerevisiae*, Tsa1, thioredoxin

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