2-Cys Peroxiredoxins (Prx) are antioxidant proteins able to decompose hydroperoxides using a highly reactive cysteine named peroxidatic cysteine (CysP). As a consequence of peroxide decomposition, a disulfide is formed with a second Cys (resolving cysteine-CysR), which is frequently reduced by Trx. *Saccharomyces cerevisiae* possesses two cytosolic isoforms of Prx (Tsa1 and Tsa2) with high homology (86% of identity and 96% of similarity). However, there are significant differences concerning the pKa of CysP (Tsa1=5.4 and Tsa2=6.3) (Ogusucu et al., 2007, *Free Rad. Biol Med.*, 42: 326). Alignment of the amino acid sequences and molecular modeling of Tsa2, using Tsa1 coordinates, revealed that the Thr44 in Tsa1 is replaced by a serine in Tsa2 and may partially account to differences of the enzymes pKa values. Although the substitution of Thr for a Ser results in a loss of a single carbon atom in the amino acid side chain. Thr/Ser residues in position equivalent to the Thr44 of Tsa1 are conserved in all Prx, and are postulated to be involved in reactivity and substrate specificity. To investigate these aspects, we have generated the mutants Tsa1T44A, Tsa1T44S and Tsa1T44V and their reactivity over H2O2 were evaluated by NADPH (a peroxidatic Trx-linked assay), DTT oxidation assays and SDS-PAGE under oxidative and reductive conditions. Our preliminary results indicated that Thr→Ala substitution apparently enhanced the peroxidatic activity (8.18μM/s and 13.6μM/s, respectively), whereas the Thr→Val replacement abolished the Trx linked activity. Crystallization trials and competitive HRP assays aiming to determine the second order rates and the pKa of mutant proteins are in progress.

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