Characterization of a Stress-Responsive PHGPx-homolgoue Gene in plant and its relation to defense Signaling.

Young Jun Jung, Yong Hun Chi, Seung Sik Lee, Jin Ho Park, Ji Hyun Jeong, Chae Oh Lim and Sang Yeol Lee

Environmental Biotechnology National Core Research Center, Division of Applied Life Science (BK21 program), Gyeongsang National University, Jinju, 660-701, Korea.

A cDNA, *PHCC-TPx,* specifying a protein highly homologous to known phospholipid hydroperoxide glutathione peroxidases was isolated from a Chinese cabbage cDNA library. *PHCC-TPx* encodes a preprotein of 232amino acids containing a putative N-terminal chloroplast targeting sequence and three conserved Cys residues (Cys107, Cys136, and Cys155). The mature form of enzyme without the signal peptide was expressed in *Escherichia coli,* and the recombinant protein was found to utilize thioredoxin (Trx) but not GSH as an electron donor. In the presence of a Trx system, the protein efficiently reduces H2O2 and organic hydroperoxides. Complementation analysis shows that overexpression of the PHCC-TPx restores resistance to oxidative stress in yeast mutants lacking GSH but fails to complement mutant lacking Trx, suggesting that the reducing agent of PHCC-TPx in vivo is not GSH but is Trx. Mutational analysis of the three Cys residues individually replaced with Ser shows that Cys107 is the primary attacking site by peroxide, and oxidized Cys107 reacts with Cys155-SH to make an intramolecular disulfide bond, which is reduced eventually by Trx. Tryptic peptide analysis by matrix-assisted laser desorption and ionization time of flight mass spectrometry shows that Cys155 can form a disulfide bond with either Cys107 or Cys136.