Expression and Purification of cTPxIII and TrxI^{C33S} of *Saccharomyces cerevisiae* Aiming to Structural Studies of Protein-Protein Interactions

Santos ,V.F.[1], Netto, L.E.S.[2] & Oliveira M.A.[1]
[1]Departamento de Biologia, Universidade Estadual Paulista, Campus do Litoral Paulista, São Vicente-SP, Brazil.
[2]Departamento de Genética e Biologia Evolutiva, Universidade de São Paulo-SP, Brazil

Thioredoxin peroxidases (TPx) are antioxidant proteins able to decompose H_2O_2 and organic peroxides through highly reactive cysteines. In eukaryotes, TPx activity has been associated to different cellular processes such as differentiation, proliferation, hydrogen peroxide signaling, apoptosis and cancer. These proteins are widely distributed and conserved among organisms and can be found in several cellular compartments. *Saccharomyces cerevisiae* has five TPx isoforms that are located in nucleus (nTPx), mitochondria (mTPx) and cytoplasm (cTPx, I, II and III). In contrast with the cytosolic isoforms cTPxI and cTPxII, which possess high activity concerning the H_2O_2, the cTPxIII enzyme presents higher specificity towards organic hydroperoxide substrates and null-mutants are hypersensitive to hydroperoxides. Despite the biochemical differences concerning the substrate the *S. cerevisiae* cytosolic TPx uses reduced thioredoxin as an electron donor for the catalytic reduction. As the electrons are transferred by cysteine pairs present in the active sites of both proteins, the substitution of specific cysteines by serines can promote the formation of a stable protein complex binding by mixed disulfides. With the porpoise to investigate the structural aspects of the cTPxIII-TrxI interaction, we have expressed cTPxIII and the mutant TrxI^{C33S}. The cTPxIII protein was purified by immobilized metal ion affinity chromatography and TrxI^{C33S} by the boiling method. After purification, the proteins were treated with 50 equivalents of Diamide (cTPxIII) or DTT (TrxI^{C33S}), REDOX agents were removed and equimolar concentrations of the proteins were incubated for different times to promote complex formation and results were detected by non reducing SDS PAGE.

Key words:*reactive oxygen species, Saccharomyces cerevisiae, Thioredoxin, thioredoxin peroxidase*
Financial Support: FAPESP & CNPq.