Efforts to Obtain a Stable Mixed Disulfide Protein Complex Between Thioredoxin Peroxidase I (cTPxI) and Thioredoxin (TrxII) of *Saccharomyces cerevisiae*

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Thioredoxin peroxidases (TPx) are a ubiquitous family of peroxidases widely distributed among the organisms. In *Saccharomyces cerevisiae* five isoforms have been identified: the cytosolics (cTPx I-III), mitochondrial (mTPx) and nuclear (nTPx). cTPxI, also called TSA1, is the most abundant TPx in the yeast, making up to 0.7% of the cellular soluble proteins. cTPxI contains very reactive cysteines (Cys^{47} and Cys^{170}) used to reduce the peroxides. In this process, the catalytic cysteines of cTPxI become oxidized to a disulfide. Thioredoxin II (TrxII) is a monomeric protein that contains in it active site two cysteines (Cys^{31} and Cys^{34}) used to reduce the cTPxI disulfide. Since the electrons are transferred through cysteine pairs of both proteins, the substitution of specific cysteines by serines can promote the formation of protein complexes binding by mixed disulfides. Despite the existence of several studies regarding the biochemical activities of both proteins, data concerning structural relationships between the proteins are inexistent. To shed a light on the structural aspects of the cTPxI-TrxII interactions we have expressed the $cTPxI^{C170S}$ and TrxII as recombinant proteins. The yeast $cTPxI^{C170S}$ was purified by IMAC and TrxII by the boiling method. After purification, the proteins were treated with 50 equivalents of DTNB ($cTPxI^{C170S}$) or DTT (TrxII). Equimolar quantities were incubated for different times to promote complex formation and the results were analyzed by non reducing SDS PAGE. Our goal is to achieve large amounts of soluble protein complexes with high purity degree to proceed to the protein crystallization and structure determination.

Key words: *peroxiredoxin, reactive oxygen species, Saccharomyces cerevisiae, thioredoxin.*
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