Insights in the interaction with substrates of Tsa1 and Tsa2

Breyer, C. A. Netto, L.E.S. and Oliveira, M. A.

1 PPG em Biotecnologia, UFSCar, São Carlos, SP. 2 LABIMES, UNESP, São Vicente, SP. 3 IB, USP, São Paulo, SP breyer@clp.unesp.br

2-Cys peroxiredoxins (Prx) are homodimers that constitute a group of antioxidant proteins able to decompose several types of hydroperoxides, using a reactive cysteine (CysP). As consequence of the hydroperoxide reduction, a disulfide is formed between the CysP and a second cysteine (CysR), which is frequently reduced by the thioredoxin enzyme (Trx). However, at high hydroperoxide levels, overoxidation of CysP overoxidation (CysP-SO₂H) occurs, resulting in the loss of the peroxidatic activity, and assembling of 2-Cys Prx in high molecular weight complexes, which possess molecular chaperone function, in a process dependent of Trx. In Saccharomyces cerevisiae, two cytosolic Prx (Tsa1 and Tsa2) and two Trx isoforms (Trx1 and Trx2) are found, which share high primary structure similarity (96 and 89%, respectively). However, since the switch of the peroxidase to chaperone function is dependent on Trx, the rates of peroxiredoxin reduction by this oxido-reductase may be a pivotal step in this process. Tsa1 was already crystallized and structural model was generated but Tsa2 structural studies were not performed. Structural analysis revealed that majority of the divergent amino acids is located at protein surfaces, which may be related to protein – protein interactions among Prxs and Trxs. The research goal is the determination of the structure of Tsa2 and investigation of the molecular determinants involved in the interaction with substrates of Tsa1 and Tsa2. To investigate interaction with substrates differences surface residues of Trx2 or Tsa2 were mutated (Tsa2D150N and Tsa2K97N, Trx2F27Y, Trx2S62G and Trx2A72S). At the present the expression and purification conditions of Tsa2 mutants were established and determination of the Trx2F27Y and Trx2A72S expression conditions are in progress. Concerning the efforts to Tsa2 structure determination, crystallization screenings of DTT reduced and H₂O₂ oxidized enzyme (192 conditions) revealed favorable results in only one mother liquor solution (0,1M sodium acetate trihydrate pH 4.6 and 8% polyethylene glycol 4000). Refinement of the initial hit resulted in high quality crystals with dimensions of 0.5 × 0.4 × 0.3 mm when the crystallization solution consisted in 0,1M sodium acetate trihydrate pH 3.6 and 12% PEG 4000. At the present X-ray diffraction experiments are in progress. We believe that the results of this study may contribute significantly to the understanding of the function of Tsa1 and Tsa2.

Word Keys: 2-Cys peroxiredoxins, thioredoxin, overoxidation, chaperone.
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