Structural and functional characterization of the dithiol glutaredoxins from *Saccharomyces cerevisiae*

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Glutaredoxins (Grxs) are small heat stable thiol-dependent oxido-reductases with at least one cysteine at their active sites. In *Saccharomyces cerevisiae*, seven Grxs isoforms were identified (Grx 1-7). Grx1-2 are dithiol glutaredoxins which contains the conserved CPYC motif in their active sites, whereas Grx3-7 are monothiolic isoforms. In spite of the fact that Grx1 and Grx2 share 85% of amino acid sequence similarity, we have shown before that Grx2 is fifteen times more active as oxidoreductase than Grx1. Characterization of the enzymatic activities through two-substrate kinetics analysis revealed that yGrx2 possesses both a lower K_M for glutathione and a higher turnover than yGrx1. To comprehend these biochemical differences, the pK_a of the N-terminal active site cysteines (Cys^{27}) of these proteins were determined. Since the pK_a values of the yGrx1 and yGrx2 Cys^{27} residues are very similar, these parameters cannot account for the difference observed between their specific activities. Therefore, crystal structures of yGrx2 in the oxidized form and with a glutathionyl mixed disulfide were determined. Through structural analysis we hypothesize that the substitutions of Ser^{23} and Gln^{52} in yGrx1 by Ala^{23} and Glu^{52} in yGrx2 could modify the capability of the active site C-terminal cysteine (Cys^{30}) to attack the mixed disulfide between the Cys^{27} and the glutathione molecule. Mutagenesis studies supported this hypothesis. To better understand the role of Ser/Ala^{23} and Gln/Glu^{52} residues in the activities of Grx1 and Grx2 we are currently studying the kinetic behavior and determining pK_a values for the thiolate of the cysteine residues of mutant proteins. The observed structural and functional differences between yGrx1 and yGrx2 may reflect variations in substrate specificity and non-redundant biological functions.

Palavras-chaves: Glutaredoxin, Saccharomyces cerevisiae.
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