Characterization of Ohr Active Site: Identification of Aminoacid Residues Involved in Interaction with Substrates

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Ohr (organic hydroperoxide resistance protein) possesses a thiol-dependent peroxidase activity to detoxify organic hydroperoxides endowed by a reactive cysteine (Cys61 in Ohr from *Xylella fastidiosa*). It is well known that sulfhydryl groups with low pK_a (present in physiological conditions mainly as thiolate anion) are strong nucleophiles. In contrast, sulfhydryl group of free cysteine has a relatively high pK_a (8,5) been relatively inert for redox reactions in physiological conditions. Arg19 and Glu51 have been postulated to contribute to the stabilization of the Cys61 in the thiolate anion form. Previously, we reported a molecule of PEG (polyethylene glycol) co-crystallized in Ohr active site making hydrophobic interactions with the side-chain of several aminoacids. Therefore, assuming that the molecule of PEG may mimic the endogenous substrates, such as peroxides derived from long chain fatty acids, these hydrophobic interactions might be relevant for Ohr activity. At this work, through site directed mutagenesis we have cloned and purified recombinant Ohr with mutations in several residues postulated to be involved in interactions with biological substrates. We tested the peroxidase activity from mutants and compared with Ohr WT. From the five Ohr mutants tested, we found a high decrease in activity in OHR V36S and Ohr G95S. We calculated the pK_a from Cys_p61 from all mutants and observed pK_a values (approximately 5,5) very close from that observed for Ohr WT. These data indicate that Valine 36 and Glycine 95 are important residues involved in catalysis interacting directly with Ohr substrate.

Keywords: cysteine, thiolate, Ohr, peroxide

Supported by FAPESP and CNPQ (Instituto do Milênio – Redoxome)