BIOCHEMICAL AND STRUCTURAL CHARACTERIZATION OF A 1-CYS PEROXIREDOXIN FROM THE HUMAN OPPORTUNISTIC PATHOGEN ASPERGILLUS FUMIGATUS AND ITS INVOLVEMENT IN THE VIRULENCE OF THE FUNGUS

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Peroxiredoxin (Prx) are Cys-based, thiol-dependent peroxidases that contain one or two conserved Cys residues. To date, all characterized Prx display the universal motif Pro-X-X-Thr/Ser-X-X-Cys. Here, we aim to structurally and biochemically characterize AfPrxA, a 1-Cys Prx (Prx6 subfamily) from the human opportunistic pathogen Aspergillus fumigatus. Initially, the reactivity of AfPrxA towards H₂O₂ was determined through a HRP competitive assay as 3.7×10⁷ M⁻¹s⁻¹. Remarkably, AfPrxA presents a unique motif among Prx enzymes (Ser-X-X-X-His-X-X-Cys) and in spite of it still displays an extraordinary reactivity towards H₂O₂. Therefore, we are attempting to obtain the crystal structure of AfPrxA. Several crystals were obtained and to date, the best result was obtained with AfPrxA pre-reduced and treated with iodoacetamide (resolution 4Å). Additionally, we are investigating the reducing system of AfPrxA, which is still an elusive issue for enzymes belonging to the Prx6 subfamily. Therefore, we produced recombinant enzymes of the thioredoxin (Trx) system from Aspergillus fumigatus and none of them could reduce AfPrxA. In contrast, ascorbate and dihydrolipoic acid were able to support the peroxidase activity of AfPrxA. Finally, to investigate the possible involvement of AfPrxA in the virulence of A.fumigatus, we construct knockout strains of A.fumigatus for the AfPrxA gene as well as a strain harboring an AfPrxA allele without the catalytic cysteine (AfPrxAC28S). These strains will be used in future virulence assays in mice models. The unique characteristics of this protein might be important to the development of new therapeutical approaches for diseases caused by A.fumigatus as AfPrxA shares low identity with the human Prx (41%).

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