Understanding the influence of the metal ions (Fe or Mn) in the active site of the cambialistic enzyme Superoxide Dismutase (SOD) of *Trichoderma reseei*

Mendoza, E.[1]; Ferreira-Junior, J.R.[2]; James. E. Penner-Hahn.[3]; Garratt, R.[1]

[1]Instituto de Física de São Carlos, Universidade de São Paulo, SP, Brazil.
[2]Escola de Artes, Ciências e Humanidades, Universidade de São Paulo, SP, Brazil
[3]Chemistry and Biophysics, University of Michigan, Michigan, EE.UU.

Superoxide dismutases (SODs) are crucial metalloenzymes that protect cells against oxidative stress[1,2]. SOD from *Trichoderma reesei* was classified in the manganese- and iron-containing family of SODs and presents 65–90% similarity with MnSODs from other filamentous fungi[3]. Based on computational analyses several residues (including positions M27 and G73) have been suggested to be important for the fine-tuning of the redox potential of the metal in the active site and thereby the catalytic activity. The main objective is to characterize and solve the crystal structure of SOD from *T. reesei* (TrSOD) in order to use it as the basis for selecting point mutations aimed at altering the metal ion selectivity. Enzymes were cloned, expressed and characterized by X-ray crystallography. Iron and manganese cofactors were identified using electron paramagnetic resonance spectroscopy and quantified by atomic absorption spectroscopy. It was shown that the protein is able to use either manganese or iron for catalysis suggesting it to be properly classified as cambialistic. However, by performing metal fidelity studies, the enzyme was found to be more catalytically active with manganese than iron (inhibition rates of 90% and 22% respectively at 0.05mg/mL). Interestingly, the mutant G73ASOD does not show catalytic activity with either metal. This loss of activity may result from the alanine side chain inducing structural changes that affect the redox potential of the active site metal ion via the solvent ligand to the metal. In contrast, FeM27VSOD results in an increase in relative activity for the iron substituted enzyme. Crystals of cambialistic SOD from *Trichoderma reesei* and the G73A and M27V mutants were used to collect diffraction data to 2.3Å, 2.8Å and 1.4Å resolution, respectively. They are currently undergoing crystallographic refinement. These results contribute to our understanding of the structural details associated with the fine-tuning of metal ion specificity in the active site.

Keywords: Superoxide dismutases, metalloenzymes, crystallography.
Acknowledgements: FAPESP, Banco Santander.