Identification of Mn/FeSODs structural determinants necessary to metal specificity

Stelmastchuk, L.B.F.[1]; Bachega, J.F.R.[2]; Garratt, R.C. [2]; Ferreira Júnior, J.R.[1]

[1]School of Arts, Sciences and Humanities - EACH-USP, SP, Brazil; [2]Physics Institute of São Carlos - IFSC-USP, SP, Brazil

Superoxide dismutases (SODs) are metalloenzymes that convert the superoxide anion in molecular oxygen (O_2) and hydrogen peroxide (H_2O_2). The metal in the catalytic center of such enzymes is directly related to their catalysis mechanisms and tridimensional structures.
Evolutionarily, FeSOD and MnSOD may have evolved from a common ancestor, because both proteins have homologous primary sequences and superposable crystallographic structures.
However, at the catalytic level, both proteins diverged sufficiently to prevent interchange of their metallic centers, which would generate non-functional enzymes, indicating that these proteins have high metal specificity. The objective of this work is to identify structural determinants of Fe/MnSODs necessary to metal specificity and oligomeric state for Trypanosoma brucei iron superoxide dismutase (TbFeSODB2) by X-ray crystallography. We intend to use statistical coupling analysis (SCA) to select amino acid residues for site-directed mutagenesis in TbFeSODB2. Mutant genes were constructed and their proteins expressed, purified and submitted to crystallization.
Classics II and PEG II NeXtal suites were used for condition screen in a sitting drop vapour diffusion method at 18ºC. Hanging drop method was used for optimization. The enzymatic activities was qualitatively analysed by in-gel SOD assay. The following stages of the project will involve SOD activity assay with xantine oxidase method as well as electron paramagnetic resonance (EPR) for metal coordenation analysis. We hypothesize that SCA is useful to indentify amino acid candidates for site-directed mutagenesis to design new SODs with intermediated Fe/Mn specificity, and even metal specificity interconversion, by studying the evolutionary history of these proteins.

Palavra chave: metalloprotein, superoxide dismutase, metal specificity
Patrocínio: CAPES