Mimicking Evolution from Inactive *Bacillus subtilis* SOD-like Protein to Active Mutants

Vito Calderone[§‡], L. Banci[‡], M. Benvenuti[§], I. Bertini[*,‡], A. Fantoni[‡], S. Mangani[§‡], M. Migliardi[‡], M. S. Viezzoli[‡], D. E. Cabelli[$], [§]*Dept of Chemistry, Univ. of Siena, Italy.* [‡]*Dept of Chemistry and CERM, Univ of Florence, Italy.* [$]*Chemistry Dept, BNL, Upton, USA.* E-mail: vito.calderone@unisi.it

Cu,Zn superoxide dismutases (Cu,ZnSOD) are metalloenzymes that catalyze the dismutation of the superoxide anion into oxygen and hydrogen peroxide. These enzymes, for a long time considered peculiar of eukaryotic organisms have been found to be present also in bacteria. From an analysis of their protein sequences we can observe that, with few exceptions, the ligands of metal sites are conserved. Among the bacterial proteins the only one which does not conserve two of the residues able to bind copper is the protein from *Bacillus subtilis*.

The BsSOD protein may be thought as a step of the evolution line from a no-Cu,ZnSOD world to the fully active Cu,ZnSODs. With this in mind we have tried to reconstitute SOD's activity through an artificial evolution obtained by introducing the copper ligands with site-directed mutagenesis. We have cloned the wild type, the two mutants P104H and Y88H-P104H which reintroduce one or both of the copper binding histidines respectively, reestablishing in the first case the ability to bind copper and in the second case the standard copper site of Cu,ZnSOD. We report the structural and biochemical characterization of the three proteins showing the restoration in the double mutant of a partially active Cu,ZnSOD and the resulting mechanistic and physiological implications.

Keywords: bacillus subtilis SOD, CuZn SOD, SOD mutants