

Formation of H₂O₂ in Superoxide Reductase: Protonation or Dissociation First? A QM/MM Metadynamics mechanistical study.

Anne Milet (Universite Grenoble Alpes), Rolf David, H el ene Jamet, Vincent Nivi ere, Yohann Moreau.

Superoxide reductase is a mononuclear iron enzyme involved in superoxide radical detoxification in some bacteria. Its catalytic mechanism is associated with the remarkable formation of a ferric hydroperoxide Fe³⁺-OOH intermediate, which is specifically protonated on its proximal oxygen to generate the reaction product H₂O₂. Here we present a computational study of the protonation mechanism of the Fe³⁺-OOH intermediate. This was performed on the whole system (solvated protein) using well-tempered metadynamics at the QM/MM (B3LYP/AmberFF99SB) level. Enabled by the development of a new set of force field parameters for the active site, a conformational MM study of the Fe³⁺-OOH species gave insights into its solvation pattern, in addition of generating the two starting conformations for the *ab initio* metadynamics setup. Two different protonation mechanisms for the Fe³⁺-OOH intermediate have been found depending on the starting structure. Whereas a possible mechanism involves at first the protonation of the hydroperoxide ligand and then dissociation of H₂O₂, the most probable one starts with an unexpected dissociation of the HOO ligand from the iron, followed by its protonation. This favored reactivity was specifically linked to the influence of both the nearby conserved lysine 48 residue and the micro-solvation on the charge distribution of the oxygens of the HOO ligand. These data highlight the crucial role of the whole environment; solvent and protein, to describe accurately this second protonation step in superoxide reductase.