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

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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 Fe^3+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 Fe^3+OOH intermediate. This was performed on the whole system (solvated protein) using well-tempered metadynamics at the QM/MM (B3LYP/AmbertFF99SB) level. Enabled by the development of a new set of force field parameters for the active site, a conformational MM study of the Fe^3+OOH species gave insights into its solvation pattern, in addition of generating the two starting conformations for the \textit{ab initio} metadynamics setup. Two different protonation mechanisms for the Fe^3+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-solvatation 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.