Visualization of the Human Manganese Superoxide Dismutase Product Inhibition Mechanism and Protonation States

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About 25% of known enzymes are oxidoreductases that catalyze the electron transfers that life depends on. To chemically accelerate redox reactions to the rates needed for life, oxidoreductases couple the transfer of electrons to the transfer of protons in a process called proton-coupled electron transfer (PCET). The biochemistry behind enzymes utilizing PCETs is not well understood as it requires precise definition of the proton donors/acceptors in tandem with electron transfer steps. Of particular note are oxidoreductases that regulate the concentration of reactive oxygen species (ROS) in cells through PCET-mediated redox reactions. ROS levels mediate mitophagy and programmed cell death and dysfunction of oxidoreductases responsible for limiting ROS concentrations contribute to cardiovascular disease, neurological disease, and cancer progression.

Human manganese superoxide dismutase (MnSOD) is an oxidoreductase found in the mitochondrial matrix that decreases $O_2^{\bullet-}$ concentrations using PCET reactions. MnSOD eliminates $O_2^{\bullet-}$ by oxidation to O_2 with a trivalent Mn ion (k_1), and reduction to H_2O_2 with a divalent Mn ion (k_2). MnSOD is the only means the mitochondrial matrix has to limit $O_2^{\bullet-}$ levels low enough to avoid damage to macromolecules and is a central axis to preserving mitochondrial function. In our past work, we were able to observe several changes in protonation and hydrogen bonding in conjunction with changes of the oxidation state of the metal using neutron protein crystallography of MnSOD at controlled oxidation states.

Neutron crystallography is particularly advantageous for studying PCET mechanisms because neutrons do not alter the electronic state of metals, unlike X-rays, and scattering of deuterium is on par with carbon, nitrogen, and oxygen.

$Mn^{3+} + O_2^{\bullet-} \leftrightarrow Mn^{2+} + O_2$ $\qquad k_1 = 1.5\ nM^{-1}\ s^{-1}$

Among all SODs, human MnSOD harbors the unique, though least known, attribute of product-inhibition that likely serves to limit the output of H_2O_2 within the mitochondrial matrix. Product inhibition is initiated from a divalent Mn to form a Mn-dioxygen complex of unknown electronic identities (k_3) and is thought to be relieved through two protonations that lead to trivalent Mn and H_2O_2 (k_4). Note that k_2 and k_3 are competing reactions, both initiated from a divalent Mn, with similar reaction rates.

$Mn^{2+} + O_2^{\bullet-} \leftrightarrow [X]$ $\qquad k_3 = 1.1\ nM^{-1}\ s^{-1}$

$[X] + 2H^{+} \leftrightarrow Mn^{3+} + H_2O_2$ $\qquad k_4 = 120\ s^{-1}$

The physiological purpose of product-inhibition may be explained by the role of H_2O_2 of as a redox signaling molecule. Mitochondria use H_2O_2-reactive ‘thiol switches’ to coordinate protein localization and activity. Slight changes in H_2O_2 steady-state concentrations stimulate apoptotic mitochondrial signaling pathways. Furthermore, abnormalities in H_2O_2 steady-state concentrations are hallmarks of cell proliferation and cancer progression. While the physiological role of MnSOD product-inhibition is vital to human health, the biochemical means in which product-inhibition is achieved and the identity of the product-inhibited complex is unknown. Structurally revealing the inhibited complex poses benefits for understanding catalysis of MnSOD and ultimately the mechanism in which H2O2 levels are regulated to maintain oxidative eustress.

Here, we sought to define the mechanism of human MnSOD product-inhibition in terms of PCET catalysis by (1) identifying the mode of product binding to the active site, (2) locating individual proton positions of the product-inhibited state, and (3) determining the electronic configuration of the complex. To pursue these goals, we utilized neutron crystallography to determine the position of every atom, including hydrogen positions, at the active site of product bound MnSOD variants, X-ray spectroscopy to identifying the electronic structure of the metal and its ligands, and quantum chemistry calculations to supplement our interpretations of the experimental data.