

5.1: Catalase and Peroxidase

Description of the Enzymes

Catalase and peroxidase are heme enzymes that catalyze reactions of hydrogen peroxide.^{94,95} In catalase, the enzymatic reaction is the disproportionation of hydrogen peroxide (Reaction 5.82) and the function of the enzyme appears to be prevention of any buildup of that potentially dangerous oxidant (see the discussion of dioxygen toxicity in Section III).



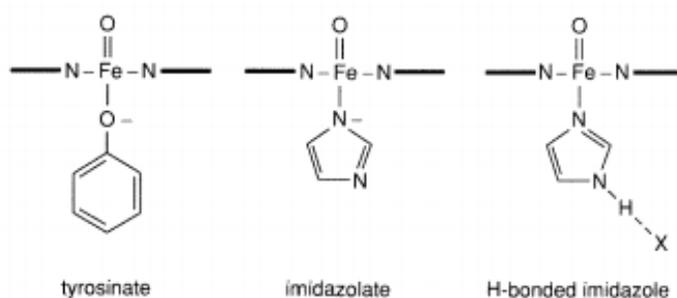
Peroxidase reacts by mechanisms similar to catalase, but the reaction catalyzed is the oxidation of a wide variety of organic and inorganic substrates by hydrogen peroxide (Reaction 5.83).



(The catalase reaction can be seen to be a special case of Reaction 5.83 in which the substrate, AH_2 , is hydrogen peroxide.) Some examples of peroxidases that have been characterized are horseradish peroxidase, cytochrome c peroxidase, glutathione peroxidase, and myeloperoxidase.^{94,95}

X-ray crystal structures have been determined for beef-liver catalase⁸⁰ and for horseradish peroxidase⁹⁶ in the resting, high-spin ferric state. In both, there is a single heme b group at the active site. In catalase, the axial ligands are a phenolate from a tyrosyl residue, bound to the heme on the side away from the active-site cavity, and water, bound to heme within the cavity and presumably replaced by hydrogen peroxide in the catalytic reaction. In horseradish peroxidase, the axial ligand is an imidazole from a histidyl residue. Also within the active-site cavity are histidine and aspartate or asparagine side chains that appear to be ideally situated to interact with hydrogen peroxide when it is bound to the iron. These residues are believed to play an important part in the mechanism by facilitating O—O bond cleavage (see Section VI.B below).

Three other forms of catalase and peroxidase can be generated, which are referred to as compounds I, II, and III. Compound I is generated by reaction of the ferric state of the enzymes with hydrogen peroxide. Compound I is green and has spectral characteristics very similar to the $Fe^{IV}(P^{\bullet-})(O)^+$ complex prepared at low temperatures by reaction of ferric porphyrins with single-oxygenatom donors (see Section V.C.1.a). Titrations with reducing agents indicate that it is oxidized by two equivalents above the ferric form. It has been proposed (see 5.84) that the anionic nature of the tyrosinate axial ligand in catalase may serve to stabilize the highly oxidized iron center in compound I of that enzyme,⁸⁰ and furthermore that the histidyl imidazole ligand in peroxidase may deprotonate, forming imidazolate,^{52,97} or may be strongly hydrogen bonded,⁹⁸ thus serving a similar stabilizing function for compound I in that enzyme.



(5.84)

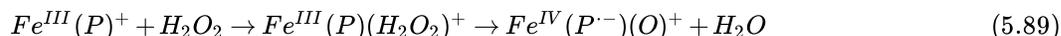
Reduction of compound I by one electron produces compound II, which has the characteristics of a normal ferryl-porphyrin complex, analogous to 2, i.e., $(L)Fe^{IV}(P)(O)$. Reaction of compound II with hydrogen peroxide produces compound III, which can also be prepared by reaction of the ferrous enzyme with dioxygen. It is an oxy form, analogous to oxy myoglobin, and does not appear to have a physiological function. The reactions producing these three forms and their proposed formulations are summarized in Reactions (5.85) to (5.88).



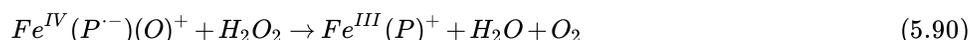


Mechanism

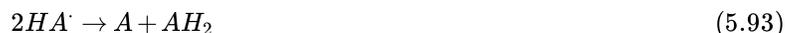
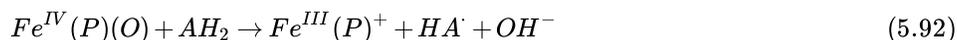
The accepted mechanisms for catalase and peroxidase are described in Reactions (5.89) to (5.94).



catalase:



peroxidase:



or



In the catalase reaction, it has been established by use of $H_2^{18}O_2$ that the dioxygen formed is derived from hydrogen peroxide, i.e., that O—O bond cleavage does not occur in Reaction (5.90), which is therefore a two-electron reduction of compound I by hydrogen peroxide, with the oxo ligand of the former being released as water. For the peroxidase reaction under physiological conditions, it is believed that the oxidation proceeds in one-electron steps (Reactions 5.91 and 5.92), with the final formation of product occurring by disproportionation (Reaction 5.93) or coupling (Reaction 5.94) of the one-electron oxidized intermediate.^{94,95}

Comparisons of Catalase, Peroxidase, and Cytochrome P-450

The proposal that these three enzymes all go through a similar high-valent oxo intermediate, i.e., **3** or compound I, raises two interesting questions. The first of these is why the same high-valent metal-oxo intermediate gives two very different types of reactions, i.e., oxygen-atom transfer with cytochrome P-450 and electron transfer with catalase and peroxidase. The answer is that, although the high-valent metal-oxo heme cores of these intermediates are in fact very similar, the substrate-binding cavities seem to differ substantially in how much access the substrate has to the iron center. With cytochrome P-450, the substrate is jammed right up against the location where the oxo ligand must reside in the high-valent oxo intermediate. But the same location in the peroxidase enzymes is blocked by the protein structure so that substrates can interact only with the heme edge. Thus oxidation of the substrate by electron transfer is possible for catalase and peroxidase, but the substrate is too far away from the oxo ligand for oxygen-atom transfer.^{99,124}

The second question is about how the high-valent oxo intermediate forms in both enzymes. For catalase and peroxidase, the evidence indicates that hydrogen peroxide binds to the ferric center and then undergoes heterolysis at the O—O bond. Heterolytic cleavage requires a significant separation of positive and negative charge in the transition state. In catalase and peroxidase, analysis of the crystal structure indicates strongly that amino-acid side chains are situated to aid in the cleavage by stabilizing a charge-separated transition state (Figure 5.14).

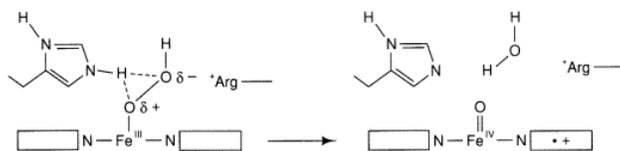


Figure 5.14 - Schematic representation of the mechanism for heterolytic O—O bond cleavage to form Compound I in peroxidase, as proposed in Reference 96. The histidyl imidazole aids in transfer of a proton from the oxygen atom of hydrogen peroxide that is bound to the iron to the departing oxygen atom, while the positive charge of the arginyl side chain stabilizes the developing negative charge on the departing oxygen, thus facilitating heterolytic O—O bond cleavage.

In cytochrome P-450, as mentioned in Section V.C.1, no such groups are found in the hydrophobic substrate-binding cavity. It is possible that the cysteinyl axial ligand in cytochrome P-450 plays an important role in O—O bond cleavage, and that the interactions found in catalase and peroxidase that appear to facilitate such cleavage are therefore not necessary.

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