Characterization of High Valent Iron Porphyrins in the Peroxidase Catalytic Cycle

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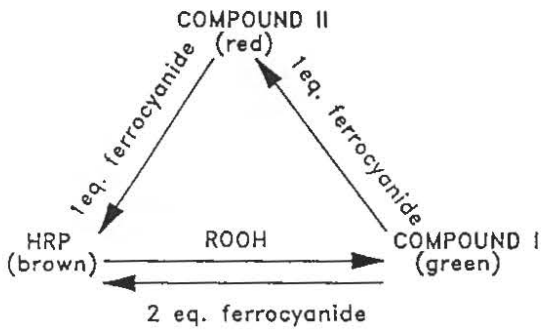
The oxygen activation and transfer reaction of heme proteins is a subject of continuing interest. High valent iron porphyrin species (oxidation states above iron(III)) are proposed in various catalytic cycles [1]. Peroxidases and catalases (CAT) are two heme-containing enzymes which belong to this class. Peroxidases catalyze the oxidation of numerous organic and inorganic compounds at the expense of H_2O_2 or other peroxides (ROOH). Catalases are related to peroxidases in that they catalyze the reduction of H_2O_2 with another molecule of H_2O_2. Cytochrome c peroxidase (CcP), Chloroperoxidase (ClP), and Horseradish peroxidase (HRP) are peroxidase which have been studied extensively. The oxidized intermediates of HRP are all stable and have been well characterized.

The resting state of HRP, with iron(III)porphyrin as reactive center, is brown in color. Two-electron oxidation generates the green species, Compound I of HRP (HRP-I). One electron reduction of HRP-I yields the red species, Compound II of HRP (HRP-II) (see the scheme below).

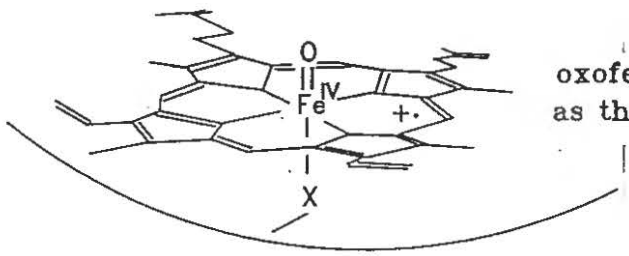


Mössbauer results compared with other synthetic iron porphyrin data [2] are consistent with an iron(IV), ferryl, configuration in both HRP-I and HRP-II. Similar results were found for Compound I of ClP(ClP-I), and CcP(CcP-I). The second oxidation equivalent in HRP-I is thought to reside as a porphyrin π-cation radical. A very broad EPR line with 0.7 ± 0.4 unpaired electron per heme of HRP was observed [3]. The signal is attributed to a porphyrin π-cation radical which couples magnetically with the spin $s = 1$ of ferryl center. A similar broad signal due to porphyrin π-cation radical was also detected for ClP-I. A sharp signal for a stable free radical without coupling to ferryl center was found for CcP-I. Further studies have shown that the radical is not located at the porphyrin but at an amino acid residue of the peptide chain.

Evidence of porphyrin π-cation radicals also can be obtained through UV-Vis spectroscopic studies [4]. The characteristics of forming π-cation radical on porphyrins are, a blue shift of Soret band which is broadened and lowered in intensity; as well as a broad band in visible α, β-region. These results have been found for Compound I of CAT (CAT-I), HRP-I, ClP-I and synthetic analogues but not for CcP-I. In fact, the spectrum of CcP-I is quite close to that of HRP-II.

Recently, EXAFS and Resonance Raman (RR) data [5,6] of HRP-I and HRP-II together with several synthetic metalloporphyrins have been reported. Both spectroscopic studies suggested that a iron-oxo (Fe=O) moiety is present in the compounds studied. The RR study found Fe=O stretching bands at higher energy than those of ferrimyoglobin hydroxide and oxyhemoglobin. This indicated there is an iron-oxygen bond order higher than one in the oxidized intermediates of HRP. EXAFS data suggested a short ca. 1.64 Å Fe-O bond is with a high valent iron in both HRP-I and HRP-II, and synthetic analogues.

Through studies of high valent iron porphyrins in either natural systems or synthetic analogues, it has become evident that Compound I of peroxidases and catalases (i.e., HRP-I, ClP-I and CAT-I) have a common moiety, the oxoferryl (Fe^{IV} = O) porphyrin π-cation radical (see figure below).



oxoferryl porphyrin π cation radical as the reaction center of Compound I

The oxidation state of CcP-I is formally equivalent to HRP-I, ClP-I, or CAT-I. However, the second oxidizing equivalent resides not on the porphyrin but on an amino acid residue remote from heme.

Although these enzymes have a common active site in the oxidized intermediates, the fifth ligand to iron is different, nitrogen(histidine) for HRP and CcP, oxygen(tyrosine) for CAT, and sulfur(cysteine) for ClP. They also have different heme site environments due to different amino acid sequences of peptide chains. This means the enzymes can use the same oxidant to form similar oxidized intermediates which have slightly different activities and are able to react with different substrates [7].

References

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