An Overview of the Biological Oxygen-Oxygen Bond Splitting Mechanism in Heme-Containing Peroxide Reduction Enzymes

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Abstract

Peroxidases are enzymes which catalyze the reduction of hydrogen peroxide to water without a corresponding decomposition reaction involving oxygen evolution, as is typically seen on noble and transition metal catalysts. Such a reduction mechanism would be beneficial for the production of electricity from hydrogen peroxide, but nonetheless, such a catalyst has not been found which is active in relatively inert environments. This research area is of critical interest within Swift Enterprises, Ltd. in the development of biological fuel cells. This paper is intended to outline the closely related bond splitting mechanisms of peroxidase enzymes, with an emphasis on horseradish peroxidase, such that materials might be synthesized that replicate these mechanisms through knowledge of the environment surrounding the peroxide molecule.

Introduction

Enzymes catalyze reactions with surprising specificity and at high rates relative to many industrial chemical catalysts of today. One such category of enzymes are the peroxidases, catalyzing the reduction of hydrogen peroxide to water. There are many different peroxidases, and each enzyme differs in structure at the active site by only a small degree. Elucidation though various methods including extended x-ray absorption fine structure (EXAFS) and x-ray crystallography, has led to greater accuracy regarding structural differences between enzyme active sites. Coupling this with knowledge gained through computational methods such as Density Functional Theory (DFT), researchers are able to formulate relationships between structure and function for many different enzymes, including heme-containing peroxidases.[1]

This paper will address the different components that are of importance in the function of peroxidase enzymes, with a brief look at catalase, which decomposes hydrogen peroxide, in an attempt to elucidate the structural differences responsible for its fast kinetics.

Enzyme catalysis is very much a 3-dimensional process. It seems that the means to accomplish the goal of catalysis through enzymatic processes is through defined spatial arrangements of regions of charge, molecular orbital energies, and atomic spin. It is through these arrangements in defined 3-space that enzymes are able to catalyze with both specificity and speed. It is the goal of the present paper to reveal insight in the study of enzymatic catalysis, through an example of a typical and well known enzyme catalyst class, peroxidase.

Molecular Components

Enzymes are large macromolecules made up of amino acids, with molecular masses of 10^6 Daltons in many cases. The enzymes in question for this study are the peroxidases, but with a focus on horseradish peroxidase, due to an abundance of literature upon its structure-function relationships. Other peroxidases and oxidases can be studied in a similar manner.

The study in question is concerned only with the active site environment of the peroxidase enzyme, which is based around an iron-containing heme group, as seen in Fig. 1.

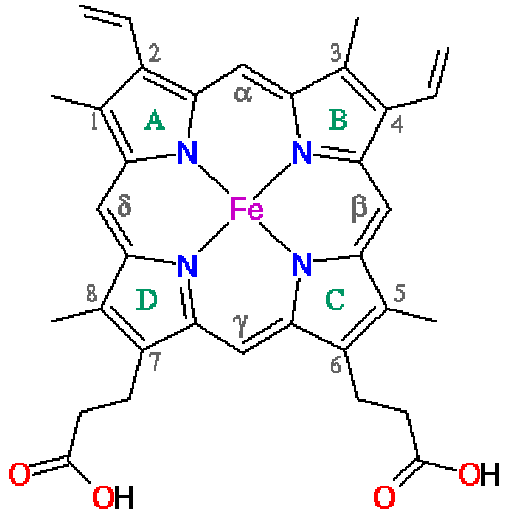

Fig. 1. The iron porphyrin heme-b. Other versions are similar (a, c, and d), centered around the iron porphyrin.[2]

The iron is bound on four sides by nitrogen, making up the heme center. Next in the construction of the active site of peroxidase is the perpendicular attachment of the amino acid histidine[3], to the iron of the planar porphyrin, thus occupying the 5th coordination position of the iron, Fig. 2.

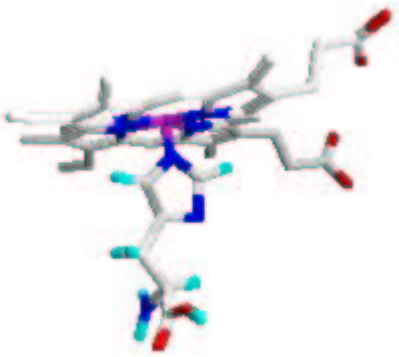

Fig. 2. Iron-containing heme-b porphyrin (Fig. 1), with a proximally located histidine residue. Model made with DTMM.[4]

On the distal (upper, in our views) side of the heme are 3 amino acid residues extending from the backbone of the protein, consisting of arginine, phenylalanine, and histidine[3]. These three amino acids comprise a ligand pocket for peroxide, and are conserved in other peroxidases, with divergence found via a substitution of tryptophan for phenylalanine.

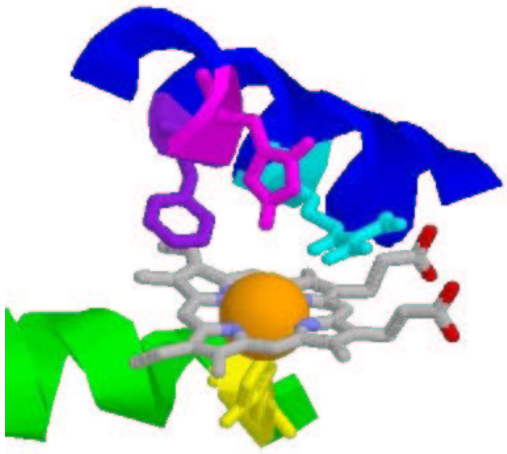

Fig. 3. View of active site of horseradish peroxidase, as seen from the distal region. Phenylalanine is purple, histidine is magenta, and arginine is aqua. The proximal histidine is below the heme in yellow.[5]

The primary molecules regarding the oxygen bond cleavage in hydrogen peroxide can be seen in Fig. 3. These include the heme porphyrin, which stabilizes a radical and acts as a region for charge transfer, the proximal histidine, which we will see acts as an electron donor, and distal arginine, phenylalanine, and histidine, which act as a ligand pocket for peroxide, as well as for the stabilization of reaction intermediates.

Additional to the above-mentioned structure, there also exists two calcium ions (not shown) lying just above and below the proximal and distal domains. These calcium ions provide structural stability as well as some control of enzyme activity[6], but their role will not be covered here.

Reaction Mechanism

Peroxidases catalyze the reduction of hydrogen peroxide with the oxidation of a 'reducing substrate', which is a fairly non-specific molecule, yielding the overall reaction[7]:

$$AH_2 + H_2O_2 \leftrightarrow 2H_2O + A$$

The half-reaction of hydrogen peroxide reduction can be written (assuming hydrogen as a proton donor):

$$H_2O_2 + 2H^+ + 2e^- \leftrightarrow 2H_2O$$

This half-reaction is the desired end-result for the production of electric power from hydrogen peroxide, so we must first understand the mechanism of peroxidase enzymes.

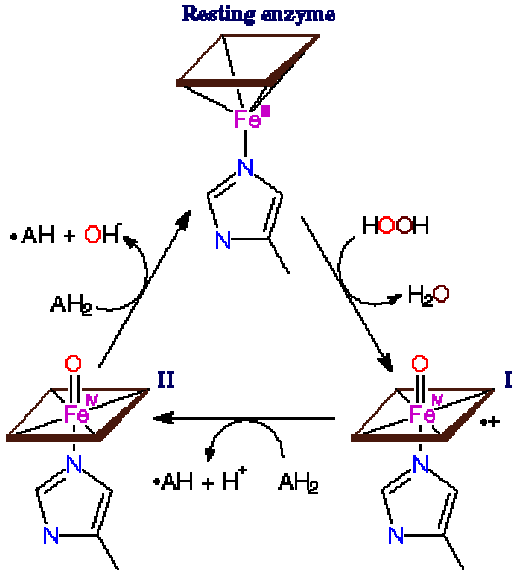

Fig. 5. In a 3-step process, hydrogen peroxide acts as electron acceptor and oxygen donor, in the oxidation of species AH_2, which comprises many possible reducing substrates.[2]

The complex reaction cycle can be written in three steps, where HPase stands for a generic peroxidase[8]:

1. Complex I formation:

$H_2O_2 + (HPase)^+ \leftrightarrow (Complex\ I)^+\bullet + H_2O$

2. Formation of Complex II from Complex I:

$(Complex\ I)^+\bullet + AH_2 \leftrightarrow Complex\ II + (AH)\bullet$

3. Reduction of Complex II to resting state, and oxidation of AH_2:

$Complex\ II + AH_2 \leftrightarrow HRP + (AH)\bullet + H2O$

In a fourth reaction step, (AH) • can react through separate mechanisms to produce stable products.

In the first step of this mechanism, peroxide is first attracted to the polar region of the active site, relative to the proteins' hydrophobic surroundings. The peroxide is lined up with the distal histidine and porphyrin-bound iron, as diagramed in Fig 6. Following incorporation of peroxide is the cleavage of the O-O bond through a proposed 2-intermediate "push-pull" mechanism.[9,10]

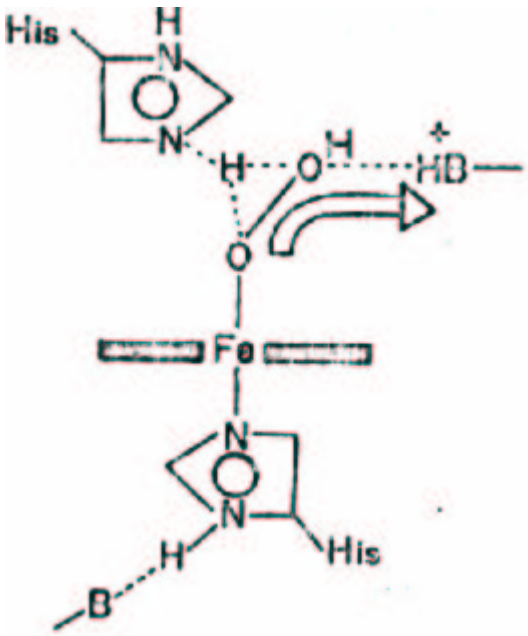

Fig. 6. Diagram of the coordination of hydrogen peroxide relative to the proximal and distal histadines and heme. Also shown is the direction of electron transfer within the "push-pull" mechanism of O-O bond splitting leading to Compound I formation.[9]

Compound I formation

The resting iron is in the ferric (Fe^{III}) state, and carries a +1 charge. Coordination of HOOH to the Fe^{III} resting state produces iron-oxo Fe^{IV}=O Compound I through cleavage of the O-O bond. Compound I is two oxidation states above the resting heme group, and contains a pi-cation radical, an oxyferryl iron group, and a +1 charge. One oxidation equivalent is on the Fe^{IV} group, and the other is held by the heme. Three unpaired electrons in Compound I result from the magnetic coupling of the (spin = 1) Fe^{IV} and the porphyrin pi-cation (s = ½). Compound I formation (the "push-pull" mechanism, Fig. 7) is set up by histidine H-bonding strongly with

neighboring groups on the proximal side, creating short porphyrin Fe-N bond distances and a good region of charge for electron-donating from the heme component. Initially then, the peroxide displaces water and donates a proton to the distal histidine, while the OOH fragment is coordinated to the heme iron, transferring negative charge to the proximal side. The proximal histidine is then deprotonated, leaving a net negative charge on the proximal side. On the distal side are two positively-charged residues (histidine and arginine), with histidine donating the first proton to the non-iron-bound oxygen. Negative charge then transfers from the proximal side to the distal side. In this "pull", the (H)OH group separates from Fe^{IV}=O, as oxygen-oxygen bond distance within the O-O(H)H fragment increases with the addition of negative charge. DFT has also determined the transition energies of the intermediates leading to Compound I formation.[11] In the starting position of the sequence, a proton is donated from peroxide to distal histidine, with ΔE = -5.3 kcal/mol, thus giving off energy. Next, the proton is transferred to the non-iron oxygen, with ΔE = 0 kcal/mol. The next, rate-determining, step in Compound I formation occurs with the cleavage of the oxygen-oxygen bond corresponding to 'Transitional Complex II' in Fig. 7, and ΔE = 10.4 kcal/mol. Compound I then rests at a relative energy of ΔE = -13.3 kcal/mol.

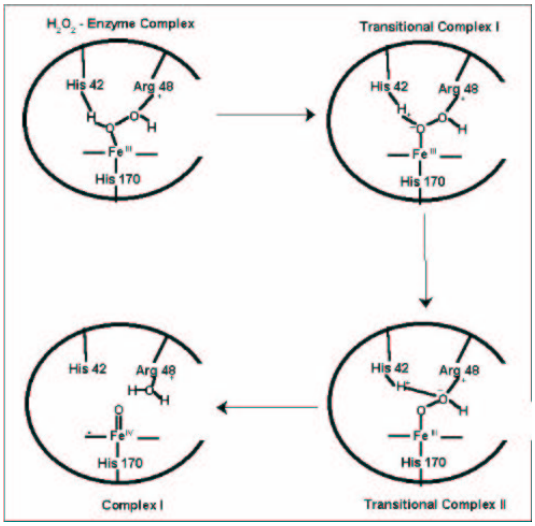

Fig. 7. O-O bond cleavage to form Compound I in a heme peroxidase.[5]

Compound II Formation and Reduction

Compound I accepts one electron from the reducing substrate to form Compound II, in generally the rate-determining step of the full reaction sequence. Compound II is therefore one oxidation equivalent above the native state and has no charge, yet carries the same Fe^{IV}=O group, but no porphyrin radical cation. Compound I is both a good oxidant as well as oxygen donor, in the path to Compound II. Reactions in this sequence take place at the heme edge, and the environment of the heme plays a crucial role in determining the function of the enzyme in this step.[12] In general, a buried heme edge at the γ position suppresses substrate oxidation activity, whereas an available heme edge at this location promotes it. Also, oxo-transfer reactions occur if the oxo-ferryl group is exposed to the reducing substrate.[12,13] The reaction proceeding from Compound II back to the resting state follows similar pathways and characteristics, again dependent upon the environment at the heme edge.

Catalase

A hypothesis can be constructed regarding the possibility of *de novo* synthesis of a catalyst species for hydrogen peroxide reduction. First, however, it is illustrative to gain insight into the similar enzymatic structure of hydrogen peroxide decomposition enzymes, called catalases, which catalyze the decomposition reaction:

$$H_2O_2 + H_2O_2 \rightarrow 2H_2O + O_2$$

There exist few gross structural differences between catalases and peroxidases, but they exhibit different reaction schemes and rates. Catalase comprises a heme group much like peroxidases, as well as proximal and distal regions that are similar.[14] The proximal and 5th iron coordinate bond of catalase is made up of tyrosine, as seen in Fig. 8.

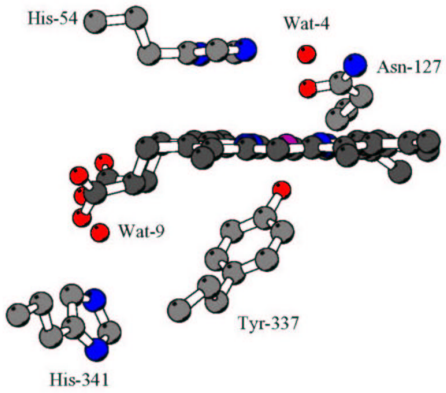

Fig. 8. Catalase active site. A proximal tyrosine phenolate (Tyr-337) binds the 5th iron coordinate in the proximal position, whereas the distal heme comprises histidine (His-54), asparagine (Asp-127), and water. A few catalases also are derived from heme-d.[15]

Proximally, catalases do not have a polar residue at the same position as arginine in peroxidase, and the tyrosine comprises a full proximal negative charge instead of a partial charge. Additionally, catalase lacks phenyl-alanine in the distal region.

The reaction cycle of catalase proceeds to Compound I in much the same way as peroxidase, wherein the lack of a positive arginine is made up for by the strong electron-donating tyrosine. However, catalase doesn't proceed through to Compound II, instead oxidizing a second molecule of hydrogen peroxide to form molecular oxygen, Fig. 9.

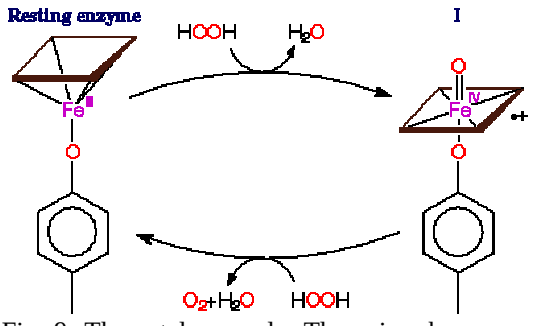

Fig. 9. The catalase cycle. The spin, charge, and radical states of Compound I are similar to that of peroxidase Compound I, but subsequent reduction to Compound II does not occur. Oxygen has been colored red.[15]

Some catalases and peroxidases do, under certain conditions, catalyze reactions of one another, giving rise to the notion that much is the same between the molecules. In the design of a catalyst *de novo*, it would be useful to mimic the rate-enhancing structural characteristics of catalase, which nears diffusion limits in activity.

Catalyst Hypothesis

In the development of an appropriate hydrogen peroxide reduction catalyst, the molecules can be arranged in such a way that the charge and molecular-orbital characteristics of the biological species under investigation can be mimicked as closely as possible. Additionally, such factors may be included to enhance reaction rates.

The first task is the mimicking of an easily oxidizable and reducible metal center. Lower transition metals are chosen instead of late transition metals, due to the strong oxidative character of these metals. In this case, iron would be a good choice, as well as other lower transition metals.

Also desirable is the construction of a structure capable of stabilizing radicals, and charge distribution over a wide molecular space. Critical in the stabilization of Compound I is the capability of having 3 unbound electrons. DFT has shown that two of the unpaired electrons reside in the Fe^{IV}=O group, along with a portion of the third shared with the porphyrin structure.[11] Additionally in cytochrome c peroxidase, the radical found on the porphyrin heme in other peroxidases is located on the amino acid tryptophan in the proximal region, thus releasing the heme porphyrin from stabilization of this radical, via its aromatic structure. Studies also show that the electron transfer pathway of the radical from the active site is through the proximal region (through tryptophan, in cytochrome c peroxidase), to the reducing substrate.[11] Compound I formation in horseradish peroxidase and cytochrome c peroxidase are similar, perhaps leading to the notion that radical stabilization need only occur near the metal center.[16,17] Thus a redox region of a metal, along with non-bonded electron

stabilization, and charge transfer properties might all be sufficient conditions for reduction catalysis. Many aromatic structures might suit these requirements, in the roles of the heme porphyrin as well as distal radical stabilization and charge transfer. The aromatic structure should also comprise sites for binding to a mechanistic substrate or backbone for proton and charge transfer. There are a number of materials that might be postulated, including liquid crystal polymers, that could carry out the role of charge and radical stabilization, that need not be either aromatic, or have mechanical substrate binding sites.

Adjacent to the charge- and radical-stabilizing region surrounding the metal center a full negatively charged moiety, or one with strong partial negative charge capable of becoming fully negatively charged and donating electrons to the active region through some manner, would need to be present. Many materials also have this property, including some polymers, and semi-conductors exposed to appropriate wavelengths of light.

Requirements regarding the spatial separation of these groups have not been stressed until now, as there is probably some variation possible given the strength of the charged group, the radical-stabilizing ability of the groups, and the redox properties of the metal center.

The most difficult requirement will perhaps be the construction of a 3-space ligand binding site for hydrogen peroxide. This site requires a proton accepting and donating group, such as a weak acid moiety, functioning in the same manner as the distal histidine in peroxidase and catalase. The identity and properties of this group have been found to be critical for the reaction rate of Compound I formation, though its position is not critical.[16,17,18] The possibility to circumvent the necessity for additional positive charge regions in the distal region other than the distal histidine might be done through fortuitous additions of proton-donating polymer electrolyte additions to the distal region around what might correspond to the γ heme position, to mimic reducing substrate additions later in the reaction sequence, or alternatively, additions of dendritic charge-transfer centers through the mechanical substrate. The strict spatial arrangement adhered to in peroxidases restricts random formation of this region. Possible catalyst structures might therefore be formed by tailoring of molecular sieves and zeolites with appropriate charge-centers and conducting capabilities, though mainly this exists only as a method of immobilization.[19]

Conclusions and Recommendations

Studies of the mechanism expressed in biological systems (peroxidases) shows that the complexities of these systems can be narrowed down to a few charge-transfer and stabilizing regions, but a procedure for the fabrication of a reduction catalyst for hydrogen peroxide has not been constructed to date. It might be possible to mimic these regions through creative uses of advanced materials technology, utilizing surface-localized separations of positive and negative charge-accepting and donating regions, respectively. Additionally, it is feasible that an incorporation of redox metal centers surrounded by radical stabilizing structures could also be constructed. Thus the final hurdle to overcome would be a somewhat strict spatial arrangement of a ligand binding pocket for hydrogen peroxide opposite of the negative charge donating region from the redox metal center.

Work at Swift Enterprises, Ltd. is proceeding in this direction.

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