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Review

Mechanisms of catalase activity of heme peroxidases

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ABSTRACT

In the absence of exogenous electron donors monofunctional heme peroxidases can slowly degrade hydrogen peroxide following a mechanism different from monofunctional catalases. This pseudo-catalase cycle involves several redox intermediates including Compounds I, II and III, hydrogen peroxide reduction and oxidation reactions as well as release of both dioxygen and superoxide. The rate of decay of oxyferrous complex determines the rate-limiting step and the enzymes' resistance to inactivation. Homologous bifunctional catalase-peroxidases (KatGs) are unique in having both a peroxidase and high hydrogen dismutation activity without inhibition reactions. It is demonstrated that KatGs follow a similar reaction pathway as monofunctional peroxidases, but use a unique post-translational distal modification (Met^{+}-Tyr-Trp adduct) in close vicinity to the heme as radical site that enhances turnover of oxyferrous heme and avoids release of superoxide. Similarities and differences between monofunctional peroxidases and bifunctional KatGs are discussed and mechanisms of pseudo-catalase activity are proposed.

Introduction

Hydrogen peroxide is known to effectively react with ferric states of heme-containing peroxidases and catalases in a two-electron redox reaction. Thereby, the O—O bond is cleaved heterolytically forming an electron deficient strong oxidizing "oxene" intermediate and water. The former transforms the heme protein to a redox intermediate called Compound I. In this fast reaction, Fe(III) is oxidized to an oxoferryl species [Fe(IV)=O] and the porphyrin (Por) to the corresponding π–cation radical ($Por^{\cdot+}$) (Reaction (Reaction 1)). The porphyrin radical can be quenched by the protein matrix (amino acid, aa) forming Compound I* [$aa^{\cdot+}$ Fe(IV)—OH Por] (Fig. 1). In any case Compound I or Compound I* are deficient of two electrons and in heme peroxidases (EC 1.11.1.7) reduction back to the native ferric state is accomplished by either two one-electron reductions via Compound II, usually an oxoferryl species, [aa Fe(IV)—OH Por] (Reactions (Reaction 2) and (Reaction 3)), or directly by a two-electron reduction step (Reaction (Reaction 4)). The nature of electron donor [organic or inorganic, cationic, neutral or anionic, low or high (e.g., protein) molar mass] and the actual substrate binding and oxidation site in heme peroxidases show great variability.

$$[\text{aa Fe(III) Por}] + H_2O_2 \rightarrow \text{aa Fe(IV)=O } Por^{\cdot+}] + H_2O \qquad k_1$$
(Reaction 1)

$$[\text{aa Fe(IV)=O } Por^{\cdot+}] + AH \rightarrow [\text{aa Fe(IV)—OH Por}] + A^{\cdot+} \qquad k_2$$
(Reaction 2)

$$[\text{aa Fe(IV)—OH Por}] + AH \rightarrow [\text{aa Fe(III) Por}] + H_2O + A^{\cdot+} \qquad k_3$$
(Reaction 3)

$$[\text{aa Fe(IV)=O } Por^{\cdot+}] + X^- + H^+ \rightarrow [\text{aa Fe(III) Por}] + H_2O + HOX \qquad k_4$$
(Reaction 4)

Typical or monofunctional heme catalases (hydrogen peroxide–hydrogen peroxide oxidoreductase, EC 1.11.1.6) are unique in using a second H_2O_2 molecule as reductant of Compound I (Reaction (Reaction 5)) thereby releasing dioxygen. Recent calculations indicate that Reaction (Reaction 5) does not proceed through a two-electron step but is better described as two one-electron transfer steps [1]. In any case, during the catalase or *catalatic* cycle (Reactions (Reaction 1) and (Reaction 5)) hydrogen peroxide is dismutated ($2\ H_2O_2 \rightarrow 2\ H_2O + O_2$).

$$[\text{aa Fe(IV)=O } Por^{\cdot+}] + H_2O_2 \rightarrow [\text{aa Fe(III) Por}] + H_2O + O_2 \qquad k_5$$
(Reaction 5)

Two (monofunctional) gene families evolved in the ancestral genomes capable of H_2O_2 dismutation [2], namely the typical heme catalases widely distributed among Bacteria, Archaea and Eukarya, as well as non-heme manganese-containing catalases (Mn-catalases) present only in Bacteria. All so far known sequences of these

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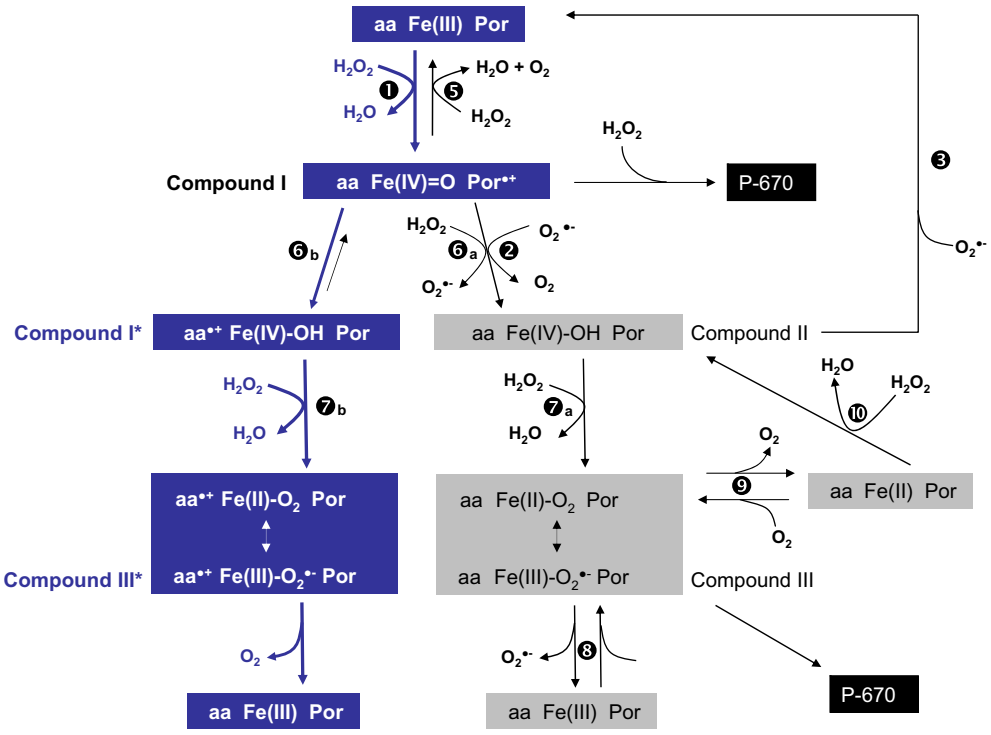

Fig. 1. Reaction intermediates formed upon incubation of heme peroxidases with excess hydrogen peroxide and in the absence of exogenous electron donor. Hydrogen peroxide dismutation by monofunctional catalases follows Reactions (Reaction 1) and (Reaction 5). By contrast, peroxidases show a pseudo-catalase activity following the reaction sequence ferric KatG → Compound I → Compound II (or Compound I*) → Compound III (or Compound III*) → ferric protein. Both Compound III or Compound III* in monofunctional peroxidases is catalytically incompetent and its decay represents the rate-limiting step. As a consequence the pseudo-*catalatic* activity is very low and both dioxygen and superoxide are produced. By contrast, catalase-peroxidases (KatGs) are proposed to follow the reaction sequence ferric KatG → Compound I → Compound I* → Compound III* → ferric KatG (pathway highlighted in blue). The trick of KatG is to keep the oxidation equivalent in Compound III* at the KatG-typical Met^{+}-Tyr-Try adduct, thereby enhancing significantly its turnover and release of only dioxygen.

two H_2O_2 dismutating protein families are collected and annotated in PeroxiBase [3] (http://peroxidase.toulouse.inra.fr).

Among heme catalases dismutation rates and peroxide affinities can differ significantly [4]. Generally, *catalatically* active enzymes do not follow Michaelis–Menten kinetics except at very low substrate concentrations [5], and different enzymes are affected differently at higher H_2O_2 concentrations [4]. Moreover, kinetic characterization of typical heme catalases from all three clades has revealed a wide range of *catalatic* efficiencies [6]. Thus, presentation and comparison of K_M and v_{max} values in the literature might be problematic [4,6]. Nevertheless, apparent values are reported to vary from 54,000 to 833,000 s^{-1} for k_{cat} and 38 to 600 mM for (apparent) K_M [4,6]. The H_2O_2 dismutation rates of Mn-catalases are significantly lower as is the substrate affinity. Apparent K_M values are reported to be around 220 mM [7] suggesting a modest catalytic efficiency at low H_2O_2 concentrations.

In addition to these two (monofunctional) catalase families, several heme-containing proteins, including metmyoglobin, methemoglobin and most heme peroxidases have been reported to exhibit some *catalatic* activity, albeit several orders of magnitude slower than monofunctional catalases [5]. However, there are two interesting exceptions, namely bifunctional catalase-peroxidases [2,5,8] and heme-thiolate haloperoxidases [9] with chloroperoxidase from the ascomycete *Caldariomyces fumago* being the best investigated representative [10,11]. Due to the very limited distribution of haloperoxidases in nature the multifunctional activity of this protein family will be not discussed here in more detail.

This review focuses on the two main heme peroxidase superfamilies (histidine as proximal ligand), namely the peroxidase-cyclooxygenase superfamily (formerly animal peroxidase superfamily) [12] and the peroxidase-catalase superfamily (formerly superfamily of enzymes from plants fungi and (archae)bacteria) [13] and discusses the mechanism of pseudo-catalase activity in these oxidoreductases (EC 1.11.1.7). Finally, it is discussed how (bifunctional) catalase-peroxidases (KatGs) have modified their heme cavity and substrate channel architecture to overcome this inefficient pseudo-*catalatic* activity thus being able to dismutate H_2O_2 at rates similar to monofunctional catalases.

Inhibition of monofunctional heme peroxidases by hydrogen peroxide and mechanism of catalase-like dioxygen production

Hydrogen peroxide, the oxidant of peroxidases and catalases, is also an inactivating agent of these metalloproteins [14,15]. With a large excess of H_2O_2 and in the absence of exogenous electron donors heme peroxidases are irreversibly inhibited with time. This process is accompanied by clear spectral changes. In plant-type enzymes (e.g., horseradish peroxidase, HRP) an absorption band at 940 nm (P-940) emerges that gradually decays, accompanied by the appearance of a new peak at 670 nm (verdohemoprotein, P-670). Organic hydroperoxides, such as hydroxymethyl hydroperoxide [16], *m*-chloroperoxybenzoic acid [17] or *m*-nitroperoxybenzoic acid [18] also inactivate the enzyme and cause similar spectral transitions.

In the presence of exogenous electron donors there is a competition for Compound I between the electron donor (AH in Reaction (Reaction 2) or X^- in Reaction (Reaction 4)) and H_2O_2. The number of catalytic cycles given by the peroxidase before its inactivation is a function of the [donor]/[H_2O_2] ratio, i.e., the peroxidase is protected from inactivation by its substrates (reducting agents) [14,19]. Besides HRP (Class III) this has been demonstrated for lignin-degrading peroxidases (Class II) [20] and ascorbate