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Heme oxygenase, steering dioxygen activation toward heme hydroxylation

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Abstract

The activation of dioxygen by heme oxygenase proceeds via formation of an obligatory ferric hydroperoxide intermediate (Fe^{III}–OOH), as is the case in the activation of dioxygen by monooxygenase enzymes. This review summarizes current understanding of the structural and dynamic properties in heme oxygenase that channel the reactivity of the Fe^{III}-OOH intermediate toward heme hydroxylation rather than oxoferryl formation. In addition, structural and electronic factors dictating the regiospecificity of heme oxygenation are analyzed in the context of recent X-ray and NMR spectroscopic studies. Differences in mechanism between heme hydroxylation, as carried out by heme oxygenase, and the coupled oxidation process, are also addressed.

Keywords: Heme oxygenase; Oxygen activation; Biliverdin; Coupled oxidation

1. Introduction and scope

The degradation of heme in mammalian cells is catalyzed by the enzyme heme oxygenase (HO) [1]. Two isoforms (HO-1 and HO-2) have been unambiguously identified [2]; HO-1 is the most actively investigated because it is induced by numerous stimuli such as heme, metals, hormones, and oxidizing agents [3]. HO-2 is a constitutively synthesized enzyme present in highest concentration in the testes and brain [3]. Not long ago, the HO system was regarded only in the context of the maintenance of cellular heme homeostasis as a catabolic enzyme. Furthermore, the products of HO activity, carbon monoxide, Fe and biliverdin, were considered toxic waste material. More recently this view has changed drastically after the discovery that the products of HO activity play important biological function: HO activity is crucial for the recycling of iron because only 1–3% of the daily iron requirement is obtained from dietary intakes [4]. Biliverdin and bilirubin are powerful antioxidants [2,5,6] and CO is thought to play a possible role as neural messenger [7]. Although the biological role of CO is a subject of controversy and continues to be debated [8], this diatomic molecule has been implicated as a factor in neuroendocrine regulation [9], as protective agent in hemorrhagic shock [10] and as a modulator of vascular tone [11,12].

Heme oxygenase has also been identified in bacteria and plants. A primary obstacle for successful bacterial colonization is the lack of available iron because the concentration of free iron in mammals is maintained at a very low level, $\sim 10^{-9}$ M [13]. Some bacterial pathogens are capable of utilizing heme as a sole source of iron, suggesting that bacterial HOs are integral part of a pathway to mine iron from host heme. Nevertheless, a relatively small number of bacterial heme-degrading enzymes have been identified so far [14–19]. The first bacterial heme oxygenase to be characterized by biophysical methods

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is the enzyme from *Corynebacterium diphtheriae*, hereafter referred to as *cd*-HO [14,16,20–22]. Shortly thereafter, detailed biophysical characterizations were reported for heme oxygenase enzymes from *Neisseria meningitidis* [23,24], hereafter *nm*-HO, and from *Pseudomonas aeruginosa* (*pa*-HO) [17,25]. As will become evident below, the study of these enzymes has provided an additional avenue to explore the mechanism of heme catabolism at the molecular level. Heme oxygenase also plays an important role in plants where it facilitates the synthesis of phycobilins and open-chain tetrapyrroles, which are produced from the final product of heme degradation, biliverdin [26,27]. Heme oxygenase enzymes have been found to be essential in the cyanobacterium *Synechocystis* sp. PCC6803, the red algae *Cyanidium caldarium* [28] and in the higher plant *Arabidopsis thaliana* [29], thus HO seems to be present ubiquitously in the plant kingdom. A recent study reported the bacterial expression and spectroscopic characterization of heme oxygenase from *Synechocystis* sp. PCC6803 [30] (Syn HO-1) and concluded that the heme binding site of this enzyme is likely to be more similar to that of the bacterial *cd*-HO than to the mammalian enzymes. Future detailed characterization of plant HOs is also likely to prove fruitful in the quest to understand the mechanism of dioxygen activation that leads to heme hydroxylation, as well as the mechanism followed by the subsequent reactions that convert *meso*-hydroxyheme to biliverdin.

The relatively rapid progress witnessed during the last decade toward the understanding of the mechanism of action of heme oxygenase has been catalyzed by the bacterial expression and characterization of a truncated, water soluble fragment of mammalian HO-1 [31,32]. It is now clear that the catalytic cycle of HO (Fig. 1) parallels that of cyt P450 in that the ferric enzyme is reduced to its ferrous state, followed by the formation of an oxyferrous complex (Fe^{II}–O_2), which accepts a second electron and is thereby transformed into an activated ferric hydroperoxy (Fe^{III}–OOH) oxidizing species [33]. Thereafter the mechanisms of heme hydroxylation and monooxygenation diverge. In heme oxygenase the Fe^{III}–OOH oxidizing species reacts with the heme macrocycle in order to give α-*meso* hydroxyheme, which undergoes a subsequent O_2-dependent elimination of the hydroxylated α-*meso* carbon as CO with the concomitant formation of verdoheme. Verdoheme is subsequently oxidized to Fe^{III}–biliverdin in a reaction that requires both O_2 and reducing equivalents [33,34]. It is therefore apparent that heme oxygenase is highly unusual in that it uses heme in a dual role of substrate and prosthetic group. There are several excellent reviews summarizing the rich chemistry and biochemistry [35–39] and the biology [2,3,40,41] of heme oxygenase enzymes, thus this review is in no way a comprehensive recapitulation of the immense field of heme oxygenase research. Rather, it will focus on the area of heme oxygenase chemistry that perhaps has seen the most advances in the last few years. Namely, the current understanding of the structural and dynamic properties inherent in heme oxygenase that allow this enzyme to channel dioxygen activation toward heme oxygenation and compete efficiently with the alternative process which leads to the formation of compound I or that of an oxoferryl species.

2. Heme oxygenase structure

The important role played by heme oxygenase in heme catabolism sparked intense interest in elucidating this pathway at the molecular level. Before the structure of HO was solved site-directed mutagenesis and spectroscopic studies revealed that the heme environment in the heme–HO complex is similar to that of myoglobin in that its heme is coordinated by a proximal, non-ionized His residue and by a distal H_2O or OH^- ligand [42–44]. Similar studies carried out before the structure of HO was solved suggested that His-132 would stabilize the distal water ligand and assist in the catalytic oxidation

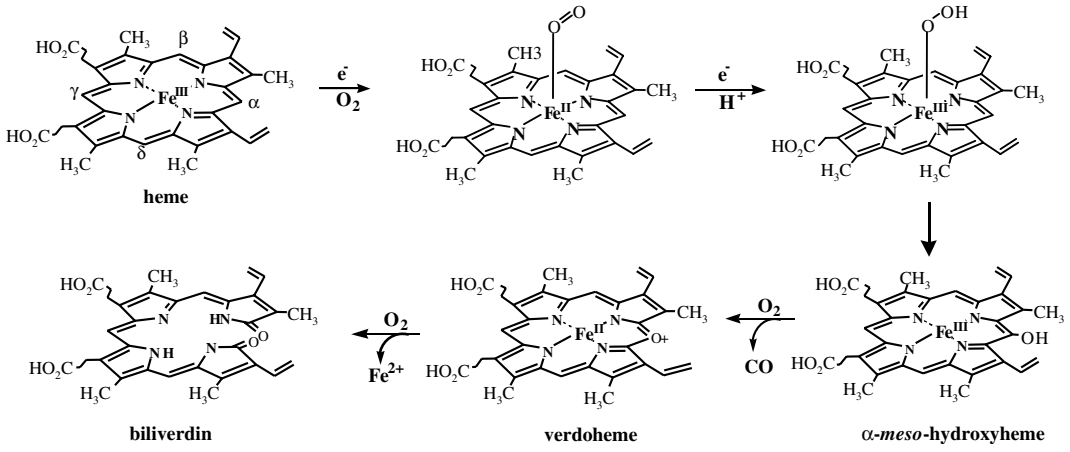

Fig. 1. Schematic representation of the heme oxidation path leading to the formation of biliverdin.