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Review

Heme Catabolism by Heme Oxygenase and Model Reaction: What We Have Learned About Reaction Mechanism

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ABSTRACT

HO enzyme catalyzes the degradation of free heme to biliverdin, CO, and Fe in three consecutive steps. The upregulated activity of heme oxygenase is thought to be correlated with the antioxidant role of HO-1 in an oxidative stress environment. HO enzyme regiospecificity oxidizes heme at α meso position in a three oxygenation steps process. Although hydroperoxy-ferric heme, generated in the first step, has been indicated as an intermediate in most heme enzyme, heme degradation is only occurred in HO reaction. Therefore, a different mechanism from the other heme enzymes must be responsible for the heme hydroxylation in HO-catalyzed reaction in the first step. In the second step of the process, there are also uncertainties regarding electron requirement and a binding site for O₂ molecule. In this article, we review researchers' attempts to elucidate complicated heme catabolism mechanism, especially in the less known process steps which have been done to shed light on the determinants of specificity and better contribute to developing new medicines. Special focus is placed on the experimental and theoretical studies on the structural characteristics and electronic configurations of heme catabolism intermediates catalyzed by HO. The fascinating electronic communication between the metal and the ring in this process has been discussed, as well. An overview of the non- precedent route for the direct conversion of oxophlorin to biliverdin is also presented.

Keywords: Heme Degradation, Heme Oxygenase, Heme, Oxophlorin, Verdoheme, Biliverdin, DFT, QM/MM.

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1-Introduction

The heme catabolism catalyzed by Heme Oxygenase (HO) enzyme is one of the most important biological processes. Heme Oxygenase (HO) enzyme is a membrane-bound protein [1, 2] found in three isoforms. Conversion of unwanted heme to biliverdin in birds, plants, and mammalian is known as the essential role of HO. In addition, different types of physiological functions, including cellular signaling [3-5], iron homeostasis [6, 7], and cytoprotective effects are catalyzed by HO. In oxidative stress environment, heme degradation is known as a cellular defense system [8-12]. Heme catabolism by HO enzyme results in generation of biliverdin, Iron, and CO in three oxygenation steps. For the products of heme degradation important biological functions have been reported, as well [13-19]. HO-1 excessive activation in disorders like jaundice [20-23], cancer[24], and brain disorders [25] are treated by inhibitors mostly metalloporphyrins which competitively inhibit degradation of Fe protoporphyrin by the HO enzyme [26-29]

To elucidate the complicated heme catabolism mechanism biological [30-44] and coupled oxidation [45-60] studies have been conducted. Intermediates of the process, oxophlorin, verdoheme, and biliverdin, are very versatile compounds, and they readily take part in the oxidative/ reductive reaction. Therefore, the structure and electronic configuration of reactive intermediates have not been completely characterized by experimental works. Theoretical calculations [61-79] in combination with experimental approaches, shed light on details of the intermediates electronic structure, and provided a great deal of understanding into the mechanism of all three steps. As a result, considerable advances have been made in characterizing the intermediates, determining the role of HO enzyme architecture in the heme degradation process, and the unique heme degradation mechanism. In this Article, we review experimental and theoretical investigations that have been conducted to isolate the process intermediates and to characterize their structures. This Article also includes a recent understanding of heme catabolism intriguing mechanism in all three steps.

2- Heme Oxygenase

2-1-Heme Oxygenase Biological Function

Heme Oxygenase (HO) is the rate-limiting enzyme in heme degradation. Three isoforms of HO denoted as HO-1, HO-2, and HO-3 have been identified in mammals. HO-3 is the least

investigated isoform of HO family, therefore significant functions of HO-3, are still elusive [80, 81]. HO-1 mostly found in the liver and spleen and it is inducible by various stimuli while HO-2 is a constitutive enzyme, and is mainly expressed in brain. HO-2 is probably the source of CO and involved in modulation of neuronal signaling pathways in brain [82].

The process of removing old and damaged erythrocytes from the circulation leads to release of the free heme, highly cytotoxic agent. The toxicity of unbound heme is attributed to the iron atom. Degradation of free heme prevents the harmful effects such as oxidation of protein, the formation of cytotoxic lipid peroxide and oxidation of DNA. In mammals, in addition to heme catabolism as the essential role of HO-1, this enzyme involves in a variety of physiological functions, including iron homeostasis, antioxidant defense, cellular signaling. CO as the product of ring cleavage process has been found in the highest concentration in testis, brain, and the vascular systems. The modulation of various physiological functions has been reported as CO biological role. In brain, CO is identified as a neural messenger molecule; in the vascular systems, the regulation of vascular tone is attributed to CO, additionally, anti-apoptosis and anti-inflammatory effects of CO have been reported [3-5, 83, 84]. The iron requirement in mammals is partly acquired by dietary; the majority of iron is provided by HO-mediated iron recycling within the spleen and bone marrow through heme degradation mechanism [6, 7].

Under oxidative stress condition caused by various stimuli such as light exposure[11], hydrogen peroxide [85], free radicals [86], heavy metals [87], inflammation[88], and increased amounts of protoheme IX (heme), heme oxygenase (HSP32) is up-regulated [89] and subsequently plays its antioxidant roles[90, 91]. However, the heme oxygenase overexpression in some disease such as hypoxia, Alzheimer, Parkinson, ischemia, and transplant rejection has been documented [20-25]. A significant part of the cytoprotective effects of HO-1 is due to the functions of heme degradation products, CO, ferrous iron (Fe^{II}), biliverdin. In addition to anti-oxidant activity of biliverdin, cytoprotective roles of bilirubin against oxidative stress have also been identified, as well [13-18]. The important functions of HO have also been reported for plants and cyanobacteria [92, 93]. In these species, derived α -biliverdin is involved in the biosynthesis of phytochrome chromophore and phycobilin as light-harvesting pigments [94]. Furthermore, iron is an essential requirement for pathogenic bacteria to survive and infect, bacteria acquire it by the degradation of host heme [95].

2-2-The Heme Oxygenase Structure

Heme Oxygenase enzyme is not identified as a hemoprotein. However, heme-enzyme complex generated by binding of one heme molecule to the active site of HO exhibits spectral properties similar to that of hemoproteins[96]. The heme in the active site of HO has both cofactor and substrate roles. HO-1 with ~33 kDa molecular mass and HO-2 with higher molecular mass, calculated to be ~36 kDa, are HO isoforms with only 45% similarity between their amino acid sequences, but essentially reveal identical enzymatic activities[97]. The HO enzyme is a microsomal membrane-bound protein [98]. The bounded structure which limits access to the enzyme has made mechanistic studies complicated; construction the truncated form of HO in which membrane binding terminal is deleted, but enzyme catalytic activity is preserved, resolved the complexity [45, 99]. The crystal structure of Bacterial HO enzymes such as Neisseria meningitides HemO [100], Corynebacterium diphtheriae HmuO [30, 31], and Pseudomonas aeruginosa PigA [101] - and rat HO-1 [102], show similarity to human HO-1 structure[32, 103].

The active site of the heme oxygenase system consists of a heme molecule sandwiched between the proximal and distal helices (Figure 1). The spectroscopic data, later confirmed by crystal structure, indicated that heme is ligated to His25, side chain of the proximal helix, and a water molecule as a distal ligand [32, 43, 104-107] (Figure 1). His25 has a significant role in HO-1 catalytic activity, although no important effect on the heme binding capability has been reported. The Fe – His stretching mode[60, 105, 106], v_{Fe-His} , which is comparable to the heme in myoglobin[108, 109], and differs from that of imidazolate characteristic in peroxidase enzymes[42] implies that His25 has a neutral characteristic rather than the protonated form of imidazolate . This characteristic could be due to strength of the hydrogen bonding between His with Glu29 in the proximal helix which must be weaker than that of the peroxidase enzymes [32]. It is supposed that the O-O bond cleavage is under the control of hydrogen bonding strength on the proximal side so the formation of ferryl species by O-O bond heterolytic cleavage -push effect- must be ruled out as a mechanism in heme degradation. Analysis of the human and rat heme oxygenase crystal structure reveals that Gly-139 and Gly-143, as well as Asp-140 are polar side chains that are capable of hydrogen bonding to the water molecule, through which they contribute in the heme degradation catalytic mechanism. However, for far polar groups located on 5.9 Å, hydrogen bonding is not possible; their main role is to regulate the polarity of the active site, which involves in the regiospecificity [32, 102]. Mutagenesis studies reveal the importance of distal Glycine residues in the HO activity. The Gly-139 mutants lost their HO activity and through the formation of the ferryl species exhibit peroxidase activity; the Gly-143 mutants do not show heme oxygenase or peroxidase activity. However, the regiospecificity of the reaction in the mutants was not perturbed [110]. The conserved sequence of Glycine residues (Gly-139 and Gly-143) in the distal pocket provide a kinked structure -about 50° right over the heme- which allows flexibility of HO [32] (Figure 2). The flexibility of the distal and proximal helices allows easy entry of the substrate in the increased form while the closed active site provides better conformation for the reaction.



Figure 1: X-ray structure of the human heme oxygenase active site with heme (PDB 1N3U)[103].

The extensive distal hydrogen-bonding cluster has great importance in the catalytic activity of HO. This network involves some water molecules, catalytically important Asp140, and the other protein residues which serve as proton donors or acceptors. Water molecules in strong H-bonding network serve as a water channel which transfers protons to active site to activate the O–O bond by the formation of hydroperoxo species. Stabilization of the hydroperoxy intermediate is another role of water molecules [103, 111-113] (Figure 1). Additionally, strong hydrogen bonding between ligated oxygen and distal residues causes a decrease in oxygen dissociation rate[114].

3- The First Step of Heme Degradation: Conversion of heme to Oxophlorin

3-1- Intermediates of the first step: Activated Oxygen Species

The first step of heme degradation (Scheme 1) initiated by the reduction of ferric-heme bound to HO to ferrous form by NADPH-cytochrome P450 reductase. Ferriheme species are completely unreactive, so reduction to ferrous form facilitates oxygenation. The absorption spectra of the oxygenated form of ferrous-heme, absorption maxima at 412, 540, and 575 nm[44] resembles of oxymyoglobin spectrum. However, Raman spectra of $Fe^{II}O_2$ - HO differ from oxymyoglobin. Based on an abnormal oxygen isotope shift pattern, it was suggested that the Fe-O-O is bent and the distal oxygen atom is brought to a closer distance to the heme to facilitate heme hydroxylation [33]. Crystal structure of dioxygen-bound heme in HmuO revealed that the interaction between O₂ and hydrogen-bonding network, as well as distal helix residues is responsible for the bent Fe-O-O bond angle[30].



Scheme 1: Three subsequent steps of heme catabolism catalyzed by Heme Oxygenase[66].

The reduction of oxygenated form of heme-HO complex, $Fe^{II}=O_2$, is vital to proceed to hydroxylation of heme, $Fe^{II}=O_2$ complex is stable at neutral pH and without reducing agents[44]. Although it was proposed that the nonprotonated peroxo form could be the reactive form of oxygen in the first step[115], in natural HO-1 system this species has not been detected even near 4 K[116]. In a low temperature – 77 K – radiolytic cryoreduction of O₂-bound complex, hydroperoxy-Fe^{III} heme has been directly observed. The source of proton seems to be the water molecules in the distal pocket that stabilizes the O₂ moiety of oxy-HO [116-119].

The spectral similarity between heme-HO-1 and heme-HO-2 in reaction with H_2O_2 suggested a similar catalytic mechanism for two isoforms in heme degradation [120] . H_2O_2 is known as a suitable substitute for molecular oxygen and two electrons in heme hydroxylation [45]. In an anaerobic condition, the reaction of heme-HO-1 with H_2O_2 led to the first direct and effective observation of hydroxyheme which could only be converted to verdoheme in the presence of O_2 [46]. However, except for ethyl hydroperoxide [121], most other alkyl and acyl hydroperoxides including tert-butylhydroperoxide, or cumene hydroperoxide [45] generate ferryl hemes, an unreactive intermediate in hydroxylation.

3-2- Regioselectivity

The bilirubin of mammalian biles is exclusively identified as bilirubin Ixa with minor traces of the other isoforms [122, 123]. Several studies have been conducted to explain the specificity in heme degradation mechanism. Earlier works suggested that α bridge was intrinsically more reactive and the other produced isomers are catabolized by alternative routes [124]. The generation of a mixture of the four isomers, denoted as α , β , γ and δ , in chemical heme degradation in aqueous pyridine indicated that α –meso-regioselectivity of hydroxylation is not inherent in the heme group [125]. However, when degradation of a mixture of four oxyprotohaem IX isomers-reconstituted into rat HO-1 was studied, only the α -isomer of meso-oxyprotohaem IX was converted to biliverdin Ixa [48]. Moreover, investigation on heme cleavage in various kinds of hemeprotein indicated that biliverdin Ixa is not the only generated isomer; biliverdin IXa is exclusively generated in the presence of myoglobin, although about 60% of α isomer and 40% of β isomer obtained through heme cleavage process with haemoglobin[34, 36, 126, 127]. Heme Oxygenase of drosophila melanogaster catalyzes the degradation of hemin to three isomers of biliverdin (IX α , β and δ) and Neisserial Heme Oxygenase [35] produces mixtures of β - and δ -isomers of biliverdin without production of α isomer. Based on the results of various model systems, it was proposed that regioselectivity in heme degradation is under steric control of apo-proteins.



Figure 2: Structure of the distal pocket over the dioxygen bound [30].

The distal Gly residues restrict access of distal Fe-O-O oxygen atom to all the meso-positions except for the α -meso-carbon. Thus, bent Fe-O-O is directed, by an angle of 110°, toward the α -meso-carbon atom and allows van der Waals contact of the terminal oxygen with the α -mesocarbon [30, 32, 37] (Figure 2). In addition, strong hydrogen bonding between Fe-O-O and distal pocket water molecules holds terminal oxygen in distance suitable for regiospecific oxygenation [31, 33, 38]. Mutagenesis studies have supported steric control by heme oxygenase structure. In replacement of Arg-183 with Glu-183 in rat HO-1 biliverdin β and δ isomers are also generated, In HO mutants in which the interactions between the side chains of heme and distal residues are removed, β and δ positions are accessible by ligated O₂ and therefore corresponding biliverdin is generated[39, 40].

To further explore the regiospecificity in heme catabolism, meso-substitute models have been studied. Enhancement of reaction at the position of methyl-substituted carbon and generation of exclusively corresponding biliverdin isomers revealed that regiospecificity is under electronic rather than steric control. These finding could not be described by a steric mechanism [128]. The hypothesis of electronically controlled heme oxidation was supported by regiospecificity observed in meso-formyl mesoheme oxidation, as well. The α -meso-formyl mesoheme, an electron withdrawing substituent, is mostly oxidized at a non-formyl-substituted meso-carbon [129]. These results rule out the theory of regioselectivity due to the ruffling of heme structure, although Montellano suggested that meso substitution might decrease the steric control and allow the electronic properties to determine the oxidation regiochemistry[130, 131]. Furthermore, meso-methyl and meso-formyl substitution studies confirmed the previously proposed mechanism as the electrophilic addition of oxygen ligand to the meso position of porphyrin ring [132].

3-3- Mechanism of the First Step

Hydroperoxy-ferric heme is observed as an intermediate in most heme enzymes. However, heme degradation only occurs in HO-catalyzed reaction which must be due to a completely different mechanism of the other heme enzymes. In P450 and catalase as well as peroxidase, a high-valent ferryl porphyrin π -cation radical, compound I (O =Fe^{IV}(Por ^{+ ·})) is formed as an active oxidant species that is not capable of heme degradation. Synthesized compound I, generated in the reaction of heme with bulky alkyl- and acyl hydroperoxides such as meta-chloroperbenzoic

acid, tert-butylhydroperoxide, and cumene hydroperoxide, is not capable of heme oxidation to either verdoheme or biliverdin. In this reaction, instead of biliverdin formation the ferryl species is converted to the ferric state through an oxo ferryl porphyrin, compound II [45]. These results confirmed the non-involvement of ferryl species as an intermediate in the reaction catalyzed by heme oxygenase. In contrast, regiospecifically α -meso-oxygenation with ethyl hydroperoxide which results in α -meso-ethoxyheme provides strong evidence for electrophilic oxidation mechanism, electrophilic addition of Fe (III)-OOH distal oxygen to porphyrin with concomitant cleavage of the dioxygen bond [121, 133]. This experiment ruled out nucleophilic addition of the unprotonated form of Fe (III)-OOH species proposed as a mechanism for the first step [45]. The results of cryoreduction/annealing experiments implied that hydroperoxoferri-HO species was converted to α -meso-hydroxyheme through a first-order reaction, without a detectable intermediate [118]. In addition, concerted mechanism for hydroxylation by Fe-OOH intermediate in the first step was suggested by kinetic experiments (Scheme 2, 3A). Kinetic isotope solvent effects, SKIE(215K)=2.3, indicated proton transfer is critical in the FeOOH activation. Furthermore secondary isotope effect, sec-KIE(215K)=0.7, upon deuteration of α -meso position revealed rehybridization of the sp² heme α -mesocarbon to an sp³ hydroxylated intermediate[134].



Scheme 2: Concerted mechanism proposed for α -meso-hydroxylation by HO [134].

However, theoretical studies proposed the stepwise mechanisms are in contradiction to the suggested concerted pathway. According to theoretical studies the concerted pathway has a much higher energy barrier than a proposed stepwise mechanism which involves prior O-O bond breaking. Shaik and co-workers proposed a stepwise mechanism initiated by O-O bond homolysis and generation of Fe^{IV}=O (compound II) plus a hydroxyl. Subsequent bonding of OH radical to α -meso position generates α -meso hydroxyheme [61-63] (Scheme 3B). A different stepwise mechanism through O-O bond heterolysis and formation of compound I plus hydroxide was

proposed by Yoshizawa group [64, 65] (Scheme 3C). It was shown that synthesized Cmpd I could not generate verdoheme in HO system and therefore involvement of this compound as intermediate in the heme degradation must be ruled out [133, 135]. By contrast, some experiments including the solvent isotopic experiments which revealed the role of the water molecule as proton transfer are in agreement with heterolytic mechanism and Cmpd I generation. Moreover, the heterolytic pathway is supported by the specific method of generating Cmpd I which followed by meso position nucleophilic attack in verdoheme formation [134, 136]. These findings along with other experiments suggested that involvement of Cmpd I in heme degradation could be considered.



Scheme 3: Proposed mechanisms for heme oxidation by HO. (A) Concerted mechanism, (B) stepwise mechanism, Homolytic cleavage, and (C) stepwise mechanism, Heterolytic cleavage [136].

3-4- Chemistry of Oxophlorin (hydroxyheme)

3-4-1- Chemical analogues of Oxophlorin

In the earlier studies on heme degradation with different model systems, hydroxyheme as the first intermediate in heme degradation process was introduced. Conversion of meso-hydroxylated heme reconstituted with HO to biliverdin has also approved the involvement of this species in a

natural pathway [48, 49]. In the enzymatic conversion of the heme-hHO1 complex with H_2O_2 , direct formation of α -meso- hydroxyheme was reported [46].

The studies on heme degradation process have been extensively carried out on different model systems via the coupled oxidation procedure. Coupled oxidation has been extensively used to mimic heme degradation process by oxidation of heme aerobically in the absence of NADPH and cytochrome P450 reductase and using other reducing agents such as ascorbic acid or hydrazine. In this procedure heme is oxidized in the active site of HO-1[41, 45, 46, 48-51], other hemoproteins like myoglobin, hemoglobin [47, 51, 127], and in a coordinating solution[47, 52-58].

The iron α -meso-hydroxyheme-HO1 complex in anaerobic environment shows a rhombic EPR signal at g=6.07 and 5.71 and another signal at g=2.008 which respectively are attributed to high spin Fe^{III} phenolate, keto resonance structures, and π neutral radical species. Resonance Raman spectra of ferric α -hydroxyheme are not typical of hemoprotein structure. The absence of isotope effect on the resonance Raman lines suggested that the α -meso-hydroxy group must be deprotonated species [50]. The resonance equilibrium of ferric phenolate (1), ferric keto anion (2), and ferrous keto π neutral radical (3) structures has been proposed as the electronic structures of deprotonated ferric α -meso-hydroxyheme, depicted in scheme 4 [50, 52]. In the atmosphere of CO, resonance equilibrium shifts toward the π neutral radical species- soret band 408 nm- as dominant species, while the signal at g=6.07 and 5.71 disappears[46, 50, 66, 137]. This observation provides strong evidence for the suggested resonance equilibrium. The EPR results of α -meso-hydroxyheme-HO-1 complex imply that Fe^{III} in the α -hydroxyheme-HO complex is five-coordinated [45, 46, 50], the EPR spectrum is similar to that of meso-hydroxyheme-myoglobin [138, 139].



Scheme 4: Resonance structures of five coordinated oxophlorin [137].

However, to elucidate the intermediate electronic structures and the heme degradation mechanism, the six-coordinated complex [(py)₂ Fe(OEPO)], bis pyridine octaethyloxophlorin has been studied in coupled oxidation. The complex is a chemical model exclusively used as an analogous of hydroxylated heme intermediate in the biological system. Spectral observations of [(py)₂ Fe(OEPO)] in aqueous or pure pyridine solution, supported the proposed oxophlorin structure and resonance equilibrium. The characteristic ¹H-NMR spectrum of this intermediate shows unusually far up-field shifts for the meso proton, which is in contrast to the spectrum of any other iron porphyrin compounds with iron-centered paramagnetism. The unique ¹HNMR pattern of [(py)₂ Fe(OEPO)] have been interpreted as delocalization of electron spin on the porphyrin π system, the findings were further confirmed by the UV-visible spectra as well [47, 52-54, 140].

3-4-2- Electronic structure of Oxophlorin with different axial ligands

In addition to $[(py)_2Fe(OEPO)]$, electronic structure of oxophlorin with other axial ligands such as Imidazoles [52, 54-56], isocyanides[54, 55], cyano[57] and in oxidation by dibromine and dichlorine[58] has been studied by spectroscopic and X-ray diffraction methods. These studies demonstrated that electron distribution on macrocycle and central metal was influenced by the nature of the axial ligand. Fe (III) α -meso-hydroxyheme reveals oxophlorin-like characteristic in different solvents with different pK_a. The population of oxophlorin resonance structures is proportional to basicity of the axial ligands [52]. To further elucidate the electron distribution between iron and the porphyrin macrocycle, electronic structure and reactivity of oxophlorin with different axial ligands has been evaluated by theoretical studies, DFT methods.



Figure 3: Energy diagram for reorientation of R_{Py} from perpendicular to parallel [67].

As shown in Figure 3, DFT study demonstrated that pyridine ligands in ${}^{2}[(Py)_{2} Fe^{III}(PO)]^{0}$ complex in the ground state are in perpendicular orientation and doublet state. On the energy diagram of the complex quartet and sextet species (with $d_{xy}^{2} d_{xz}^{1} d_{yz}^{1} \sigma_{z}^{*1}$ $d_{xy}^{1} d_{xz}^{1} d_{yz}^{1} \sigma_{z}^{*1} \sigma_{x}^{*1} \sigma_{z}^{*1} \sigma_{z$ its high reactivity [67]. Another result of this electron transfer is shortening of the C–O bond by about 0.03 Å which is in contradiction to the experimental results by Balch et al. [56], it is supposed that C-O bond disorder could be the origin of this contrast.



Figure 4. Orbital occupations for a) ${}^{2}[(Py)_{2} Fe^{III}(PO)]_{xy}$ b) ${}^{2}[(Py)_{2} Fe^{II}(PO)]_{a2u}$ [67].

In experimental works by Balch et al. it is reported that spin state and orientation of axial ligands of $[(Py)_2 Fe^{III}(OEPO)]$ in solution and solid phase are in contradiction. It exists in the high spin state with parallel pyridine ligands in the solid state, but in solution, the complex exists in a low-spin form [56]. DFT study in the gas phase reported that sextet and quartet in a parallel orientation, are more stable than doublet species (Figure 3) hence, to re-orient pyridines from perpendicular to parallel, a spin crossover from the doublet in perpendicular to the quartet and sextet spin surface in parallel orientation is required. In ${}^2R_{Py}$, $\pi_{xz}^2 \pi_{yz}^2 d_{xy}^1$, with perpendicular pyridines, strong orbital overlaps of d orbitals of iron with π^* orbitals of ligands led to a high rotation barrier, 6.95 kcal/mol, to rotate around Z-axis to generate high spin parallel conformation, $d_{xz}^1 d_{yz}^1 d_{xy}^1 d_{z^2}^1 d_{x^2-y^2}^1$. However, what causes the reorientation of the pyridines from perpendicular to the parallel one has not been identified [67].

The energy diagrams, represented in Figure 5, for complexes of oxophlorin with Imidazole, Pyridine, and (tert -butyINC) ligands illustrate the influence of axial ligands on the electronic structure of oxophlorin. The axial ligand effect is attributed to π -accepting and σ -donating ability of ligand; for R_{Im}: ²[(Im)₂ Fe^{III}(PO)] complex strong σ -donating ability of imidazole resulted in $d_{xy}^2 \pi_{yz}^2 \pi_{xz}^1$ electronic configuration while good π -acceptor ability of (tert -butyINC) changed the electron configuration of R_{t-BuNC}: [(tert -butyINC)₂ Fe^{II}(PO[•])] species to $\pi_{xz}^2 \pi_{yz}^2 d_{xy}^2 a_{2u}^1$. Thus, π dianion radical nature of ${}^{2}R_{t-BuNC}$ due to electron shifting from a_{2u} orbital to iron could describe its facile conversion to verdoheme, as documented by experimental reports [55]. In addition, the HOMO –LUMO gaps represented in energy diagrams provide a qualitative explanation for temperature-dependent behavior of magnetic moments of ${}^{2}R_{Im}$ complex observed in experimental study, and demonstrate why such behavior is not observed for ${}^{2}R_{t-BuNC}$ [55, 56].



Figure 5. Energy diagram for molecular orbital of (a) R_{Im} , (b) R_{Py} , and (c) R_{t-BuNC} in the ground state [67].

3-4-3- oxidation/reduction of oxophlorin

The essential requirement to answer the uncertainties in the mechanism of the second step, conversion of the oxophlorin to verdoheme, is to characterize the reactive form of oxophlorin.

The electrochemical study reveals that $[(Py)_2 \text{ Fe}^{III}(OEPO)]$ (species (1), see Figure 6, and Scheme 5) could be converted to $[(Py)_2\text{Fe}^{III}(OEPO^{\bullet})]^+$ (species (2), see Figure 6, and Scheme 5)

by a remarkably low potential [141]. In agreement with experimental results, DFT study calculated such a low potential for this conversion (with ionization potential= 3.95 kcal/mol), as well [68]. The configuration of the oxidized and reduced form of six-coordinated oxophlorin is depicted in Figure 6.



Scheme 5: Reversible, one electron reduction and oxidation reaction of the complex 1. $(1)^{2}[(Py)_{2} Fe^{II}(PO)]^{0}$, $(2)^{2}[(Py)_{2} Fe^{II}(PO^{*})]^{+}$, $(3)^{1}[(Py)_{2} Fe^{II}(POH)]$ [68].

In the oxidation process, ${}^{2}[(Py)_{2} Fe^{III}(PO)]^{0}$ complex converts to ${}^{2}[(Py)_{2} Fe^{III}(PO^{*})]^{+}$ species (Figure 6, complex 2) by removing one electron from ring a_{2u} orbital. In ${}^{2}[(Py)_{2} Fe^{III}(PO^{*})]^{+}$ species with a $d_{xy}{}^{2}d_{xz}{}^{2}a_{2u}{}^{1}d_{yz}{}^{1}$ electronic configuration, alignments of unpaired electrons spin results in two different spin states, singlet and triplet. The ground state of this oxidized form is in singlet state in which unpaired electron of d_{yz} orbital of central atom is antiferromagnetically coupled to unpaired electron of a_{2u} orbital. This structure with unpaired electron on the iron and the macrocycle can describe the high reactivity of this intermediate. Whenever unpaired electrons coupled ferromagnetically, triplet state of ${}^{2}[(Py)_{2} Fe^{III}(PO^{*})]^{+}$ is generated which is populated at ambient temperature and is only 0.82 kcal/mol more energetic than ground state (Figure 6). Therefore, spin admixture of singlet and triplet states due to small differences in their energies led to appear partial paramagnetic character in this compound [68]. The electronic configuration of $[(Py)_{2}Fe^{III}(PO^{*})]^{+}$ resulted in the theoretical study could clarify ¹HNMR spectrum at -20 °C and EPR-silent spectra [141, 142]. As shown in Figure 6, although d_{xy} and a_{2u} orbitals are degenerates, removing one electron from d_{xy} orbital of Fe which has high charge density (1.57) must be ruled out. Ionization potential to oxidize d_{xy} orbital calculated to be 21.15 kcal/mol [68].



Figure 6: Orbital occupation and redox reaction profile of ${}^{2}[(Py)_{2}Fe^{III}(PO)]^{0}$. $(1)^{2}[(Py)_{2}Fe^{III}(PO)]^{0}$, $(2)^{2}[(Py)_{2}Fe^{III}(PO^{*})]^{+}$, $(3)^{1}[(Py)_{2}Fe^{II}(POH)]$ [68].

As shown in (Figure 6, 2) small gaps between HOMO and LUMO orbitals of $[(Py)_2 \text{ Fe}^{III} (PO^{\bullet})]^+$, calculated to be 1.42 eV, could describe spin crossover behavior with temperature in oxidized species [141].

In reduction process which at low potential occurs (reduction potential = -32.06 kcal mol⁻¹), in accordance with electrochemical results by Balch et al [56, 141], by adding one electron to the Fe d_{xy} orbital, $[(Py)_2Fe^{II}(PO)]^-$ (complex 3, see Figure 6 and Scheme 5) is generated. In the subsequent step, rapid protonation of reduced species resulted in ¹[(Py)₂Fe^{II}(POH)]. In $[(Py)_2$ Fe^{II}(POH)] strong π –back-interaction led to a tight bonding between iron and ligand, and therefore, the access of O₂ molecules to Fe is limited. Such structure for $[(Py)_2 Fe^{II}(POH)]$ makes this species air stable, therefore reduced form could not be an active intermediate in heme degradation. Figure 6 could demonstrate diamagnetic and temperature invariant characteristic of this reduced form of oxophlorin [68].

As presented, oxidation of iron oxophlorin by O_2 through changing the electronic configuration and spin states of iron oxophlorin results in the generation of an oxidized species

which is more reactive than iron oxophlorin. Therefore, in coupled oxidation, conversion of oxophlorin to oxidized form in the process prior subsequent oxidation to verdoheme could be considered [68].

DFT study showed that all spin states in both orientations of pyridine, parallel and prependecular, in oxidized species $[(Py)_2Fe^{III}(PO^{\bullet})]^+$ are degenerate so reorientation of axial ligands in this complex is a barrierless reaction [68]. However, as outlined in Figure 3 in ${}^2[(Py)_2Fe^{III}(PO)]^0$ the rotation barrier in the gas phase is about 6.95 kcal mol⁻¹ [66]. In addition the structural parameters of the oxidized species, calculated in DFT study, [68] are in good agreement with x-ray structure results [56]. Therefore, along with other ideas that proposed the strong crystal packing forces as the origin of pyridine ligands reorientation in the solid state, the generation of oxidized form $[(Py)_2Fe^{III}(PO^{\bullet})]^+$ during crystallization in the presence of oxygen molecules needs to be considered.

Redox potential energies of oxophlorin complexes with Imidazole and (tert -butylNC) ligands were calculated as well. The [(tert -butylNC)₂Fe^{II}(PO[•])] complex due to the π -back-bonding ability of the (tert-butylNC) ligand undergoes difficult oxidation and reduction process while [(Im)₂Fe^{III}(PO)] is easily oxidized and reduced to corresponding monocation and anion respectively. These findings demonstrate the effect of the axial ligand on redox potential. In the solution phase, H₂O and CH₃CN, considerably lower oxidation potentials than those of the gas phase have been calculated, which are indicative of a more facile heme degradation [68].

3-4-4- Theoretical study on the electronic structure of oxophlorin in the hemeoxygenase system.

The hydroxyheme –HO-1 complex is five coordinated, the fifth site of central atom (iron) is taken by His25, as discussed in previous section. The proposed resonance structures for five coordinated oxophlorin intermediate are depicted in scheme 6. DFT study on 5-coordinated oxophlorin [(Im)Fe(PO)] calculated the 6 [(Im)-Fe^{II}(PO•)]_{a2u}, (2) species as the ground state of the deprotonated form of [(Im)-Fe^{II}(POH)], (1). As shown in Figure 7, removing one electron from a_{2u} orbital (-1.60 eV) of ferrous hydroxy complex (1) results in 6 [(Im)-Fe^{II}(PO•)]_{a2u} (2) form. Whenever one electron is removed from d_{xy} orbital (-2.04 eV) ferric keto anion 6 [(Im) Fe^{III}(PO)]_{xy} (3) is generated (Figure 7 and Scheme 6). Ionization potential for generation of 6 [(Im) Fe^{III}(PO)]_{xy} (3) is higher than ${}^{6}[(Im)-Fe^{II}(PO\bullet)]_{a2u}$ (2) complex, (8.66 and 12.08 kcal/mol, respectively) kcal/mol at low positive potentials (with ionization energies = 8.66 kcal/mol). ${}^{6}[(Im) Fe^{III}(PO)]_{xy}$ (3) is a closely-lying excited state, which lies 3.42 kcal/mol above ${}^{6}[(Im)-Fe^{II}(PO\bullet)]_{a2u}$ resonance form in energy diagram [66].



Scheme 6: Proposed resonance structures for oxophlorin intermediate. (1) [(Im)-Fe^{II}(POH)], (2) 6 [(Im)Fe^{II}(PO•)]_{a2u}, (3) 6 [(Im) Fe^{III}(PO)]_{xy}. [66].

Calculations on ${}^{6}[(Im)Fe^{III}(PO)]_{xy,a2u}$ in the protein and polar environments, revealed the chameleonic nature of iron oxophlorin systems. In protein environment, simulated by electrical field the character of the ground state is changed from an oxophlorin-based radical (2) to an oxophlorin-based trianion (3), whereby with a dielectric constant 5.7 for protein surrounding, ${}^{6}[(Im)Fe^{III}(PO)]_{xy}$ is the ground state and ${}^{6}[(Im)Fe^{II}(PO•)]_{a2u}$ is 1.47 kcal/mol less stable. This instability reaches to 2.58 kcal/mol for polar (ethanol) environment (ϵ = 24.55) [66]. Theoretical results on oxophlorin are in good agreement with experimental findings regarding high-spin iron(III) and radical signal [46, 50, 137].



Figure 7: Energy potential plots for the oxidation of the ferrous hydroxyheme complex from a_{2u} and d_{xy} orbital. (1)[(Im)-Fe^{II}(POH)], (2)⁶[(Im)-Fe^{II}(PO•)]_{a2u}, (3)⁶[(Im) Fe^{III}(PO)]_{xy} [66]

As reviewed, in the active site of heme oxygenase there is a H-bonded network involving of water molecules, and the distal ligand of heme is usually identified as a water molecule [32, 103, 111-113]. To simulate and evaluate the flexibility of the HO moiety, electronic and structural changes in iron hydroxyheme through approaching of H₂O molecule to the vacant coordination site of $[(Im)(H_2O)Fe^{III}(PO)]$, (4), (see scheme 6) in different orientations has been studied. The hydroxyheme shows a radical characteristic whenever water molecule is located on a distance of 2.70 Å from iron atom. This could be a possible mechanism to change hydroxyheme to a more reactive intermediate toward O₂ molecule through a spin-allowed path (Figure 8).



Figure 8: Electronic configuration diagram of $[(Im)(H_2O)Fe^{II}(PO\bullet)]_{a2u}$ complex in the presence of distal H₂O [66].

DFT results express that coordination of CO molecule to hydroxyheme results in generation of [(Im)(CO)Fe^{II}(PO')] complex (6) with $d_{xy}^2 \pi_{xz}^2 \pi_{yz}^2 a_{2u}^1$ electronic configuration. The ground state of CO-bound complex calculated to be doublet; quartet and sextet lie 20.14 and 15.04 kcal/mol, respectively, above than ground state[66]. Therefore, in the presence of CO, released in the second step of heme degradation, the resonance structure of oxophlorin shifts toward an active π -neutral radical form with further reactivity as supported by experimental studies [46, 50, 137].

4-The second step of heme degradation: Conversion of hydroxyheme to Verdoheme

4-1-The Second Step Mechanism

4-1-1- Experimental study on second step mechanism

Based on the experimental studies, contradictory mechanisms for transformation of α -hydroxyheme to verdoheme have been proposed [47, 50, 54, 143]. There has been question

centered on whether or not the additional reducing equivalents are required in the second step. Some experiments suggested that external electron is needed to reduce Fe^{III} to Fe^{II} prior to O_2 binding, while the other works reported that electron is just required to reduce Fe^{III} -verdoheme. It has been documented by some groups that in the reaction of oxygen with the ferric α -hydroxyheme complex, the exogenous electron is not required to generate Fe^{III} -verdoheme [46, 47]. Noguchi et al. also showed that $Fe^{III} \alpha$ -hydroxyheme-rHO could be converted to Fe^{II} -verdoheme without any requirement for exogenous electrons [137]. However, Matera et al. and Migita et al. revealed that in addition to dioxygen, one reducing equivalent is necessary to generate verdoheme in the second step [50, 142].

Furthermore, the activation site of dioxygen is also matter of debate; whether the reaction is started by the attack of dioxygen on the iron atom or binding site for O_2 is the porphyrin ring of the Fe (III) hydroxyheme. In spite of some mechanisms which proposed activation of O_2 on ferrous iron, other studies suggested that ferrous iron is not involved in the oxygen activation in Fe- α -meso-hydroxyheme and oxygen attacks on the 'activated' heme ring and the observed signal is believed to attribute to a ferrous heme peroxy radical intermediate [47, 50, 143]. Therefore, intermediate characteristics of the heme degradation second step have remained elusive.

4-1-2- Theoretical study on the second step mechanism: QM approach

To clarify uncertainties regarding electron requirement, the mechanism of the second step was investigated with DFT calculations, (outlined in Figure 9) [69].

Iron (III) superoxo oxophlorin complex [(Im) (O₂ ') Fe^{III} (PO')] (²1) (Figure 9), as the starting point of this mechanism is generated by reaction of O₂ with a sextet (ground state) oxophlorin dianion radical [(Im)Fe^{II} (PO')] [66]. The Fe(III) superoxo compound has two close-lying states, doublet and quartet, in which doublet, ²[(Im) (O₂ ') Fe^{III} (PO')],(²1) calculated to be the ground state, whereas the quartet lies 0.32 kcal mol⁻¹ higher. The ground species has $\delta_{xy}^2 \pi_{xz}^{*2} \pi_{yz}^{*1} \pi_{00}^{*0} \, \varphi_{1\,ring}^1$ (tri-radicaloid) electronic configuration, whereby the unpaired electrons antiferromagnetically coupled. Another orbital occupation in the doublet state, $\delta_{xy}^2 \pi_{xz}^{*2} \pi_{yz}^{*2} \pi_{00}^{*0}$ (closed-shell) is higher in energy than tri-radicaloid form by 3.65 kcal mol⁻¹. Exchange interactions [144] of single electrons of d orbitals in tri-radicaloid states (²1) make this species more stable than

closed shell doublet state. In accordance with theoretical studies, a reactive intermediate with a radical type EPR signal at g= 2.004 was also identified in experimental works [47, 50].



Figure 9: DFT potential energy profiles for conversion of ^{2,4}1 to ^{2,4}4. The energy values in square brackets and curly brackets are respectively related to dielectric constant of $\varepsilon = 5.7$ and $\varepsilon = 24.55$ [69].

The energy profile for the second step mechanism provided by DFT study is presented in Figure 9. The proposed mechanism is initiated by the attack of the distal oxygen atom of the superoxo in complex (1) to carbon adjacent to the keto group, which leads to intermediate (2) (Figure 9). This step has the highest barrier, calculated to be 12.15 kcal mol⁻¹, in the whole process. Subsequently, homolytic cleavage of dioxygen bond generates ferric- oxophlorin alkoxy radical (3). Fe in species (3) is in Fe(III) oxidation state; this oxidation state can be generated due to the electron transfer from macrocycle to metal after O-O bond hemolysis step. The reaction proceeds as well as some other transition metal complexes via two-state reactivity (TSR) pattern [74, 75]. As depicted in the energy profile (Figure 9) this reaction for doublet and quartet spin states is on

competition. Although, the reaction is initiated in doublet spin state, the ground state for complex (3) is changed to quartet spin state (⁴3). The last step is an exothermic reaction, by 14.56 kcal mol⁻¹, whereby intermediate (3) through a 12.15 kcal mol⁻¹ activation barrier is converted to Fe(III)–oxo verdoheme with simultaneously CO molecule release. In solution phase, bulk polarity (ϵ = 5.7 and 24.55) effect decreases the rate determining barrier respectively to 9.4 and 5.1 kcal mol⁻¹, which is in the range for the enzymatic process [69].

In the rate limiting step distortion of the oxophlorin ligand resulted in high barrier energy which led to intermediate 2 (Figure 10) in which carbon atom hybridization is changed from sp^2 to sp^3 . In the next step, results show that ²TS2 (Figure 10) is generated by one electron shifting from d_{xz} to O₂ orbital. The small barrier of this step, only 2.85 kcal mol⁻¹, is due to ²TS2 stability. It is supposed that ²TS2 stability is related to exchange interaction in the complex [69].



Figure 10: Electronic configuration diagrams for ²2 and ²TS2 derived from DFT study [69].

Involvement of the sextet state of Fe (III)-superoxo oxophlorin complex which is 7.1 kcal mol^{-1} less stable than the doublet species in reaction mechanism must be ruled out. Occupation of Fe antibonding orbital, d_{z^2} , in the sextet state results in longer Fe-O bond and consequently shorter O–O bond, therefore oxygen–oxygen bond requires more energy to break in this species.

Fe–oxo verdoheme (4) (Figure 9) in the doublet state can be viewed as two resonance structures shown in Figure 11. The energy levels for $\varphi_{2 \text{ ring}}$ and π^* orbitals are so close that electron transfer from the ring into the Fe(IV) atom to generate Fe(III) oxidation state is possible. This

result is an evidence which confirms non-involvement of ferryl intermediate in the heme degradation process [69].



Figure 11: Two electronic resonance structures of the iron verdoheme (4) [69].

To answer the question regarding reactivity of reduced form of Fe(III)–superoxo oxophlorin, the second step mechanism was examined with Fe(III)–superoxo oxophlorin tri-anion with $\delta_{xy}^2 \pi_{xz}^{*2} \pi_{yz}^{*1} \pi_{00}^{*0} \varphi_{1} r_{ing}^2$ electronic configuration by DFT calculation, as well. Fe(III)– superoxo oxophlorin tri-anion is generated by filling the φ_1 ring orbital of Fe(III)–superoxo oxophlorin with one electron in triplet state. The results represented an endothermic path, by 5.46 kcal mol⁻¹, with high energy barrier, 22.55 kcal mol⁻¹, for this mechanism. In addition, another reduced form, Fe(II) superoxo with a $\delta_{xy}^2 \pi_{xz}^{*2} \pi_{yz}^{*2} \pi_{00}^{*0} \varphi_1$ ring¹ electronic structure was also calculated and found to be higher in energy around 7.55 kcal mol⁻¹.

As a result, theoretical studies demonstrated that the reduced form of Fe(III)–superoxo oxophlorin could not be a favorable intermediate in heme degradation, although, involvement of the oxidized form of Fe(III)–superoxo oxophlorin could be considered. These findings support the theory that exogenous electron is not required to transform iron oxophlorin to Fe^{III}-verdoheme [69].

4-1-3- Theoretical study on the second step mechanism: QM/MM approach

Hybrid calculations, QM/MM, Quantum Mechanics / Molecular Mechanics, and big-QM demonstrate the effects of surrounding protein on the mechanism.



Figure 12: Active site model (QM region) used in QM/MM studies on second step mechanism[76].

Figure 12 depicted the QM region in the QM/MM study on conversion of Fe(III) superoxooxophlorin to verdoheme [76]. QM/MM calculation proceeds through a mechanism similar to that of suggested by QM-cluster [69] calculations. However, there are some differences between two mechanisms in the later stages. In QM/MM calculations, bond formation between distal oxygen of superoxo and carbon atom adjacent to the keto group of the oxophlorin generated a closed structure $(3\rightarrow 4)$; subsequently, in the next step $(4\rightarrow 5)$, CO molecule is released, (Scheme 7), whereas, homolytically broken of the bond between keto carbon and its adjacent carbon and CO release $(3\rightarrow 4)$ occur simultaneously in the QM-cluster calculations (Figure 9). QM/MM calculation shows strong hydrogen bonds of all intermediates with water cluster[76].



Scheme 7. The suggested QM/MM energy profile for the second step heme degradation mechanism [76].

In QM-cluster study [69], as mentioned above the reaction has two-state-reactivity (TSR) pattern in which doublet state is slightly more stable (Figure 9). However, in QM/MM approach [76] for the doublet state, the first step (TS1) is rate-limiting but for the quartet spin state, the second step of the mechanism (TS2) is rate-limiting step (Scheme 7). In QM/MM method the O– O bond in quartet state requires more energy to cleave (second reaction step) than the bond formation between the terminal Oxygen atom and ring carbon (first step). However, notable differences in energy of the quartet and doublet state in energy profile for intermediate 2, transition states (TS1 and TS2), and the product (6), calculated to be 11-16 kcal/mol, suggest that mechanism on the quartet potential energy surface (PES) must be ruled out (Scheme 7) [76]. The same results have been calculated for the sextet surface in QM/MM study [76], as was observed in QM study [69]. The calculated pathway by QM/MM approach is more exothermic (Δ Etot= -34.3 kcal/mol) with higher energy barrier, 19 kcal/mol, compared with QM-cluster data (with activation barrier by 12 kcal/mol and Δ Etot = -14.82 kcal/mol).

Another difference between QM [69] and QM/MM [76] calculation was observed in their structures. In spite of the gas phase, the orientation of His25 in QM/MM calculation is constrained to the orthogonal orientation by a hydrogen bond to Glu29.

QM/MM results show that Van-der-Waals interactions (MM), EMM, are rather small positive values, 0–4 kcal/mol. However, the electrostatic effect from the surroundings is large, up to 13 kcal/mol whereby decreases the activation energy barrier by 6 kcal/mol. Water cluster, six water molecules (not considered in the QM study [69]), is effective in decreasing the reaction and barrier energies, by 2–7 kcal/mol. The porphyrin side chains, Arg127, Asp131, have a large effect on reaction energies, up to 14 kcal/mol. It seems that the QM/MM calculations which involve the steric and electrostatic effects of the enzyme environment provide more reliable and better description of the energetic of mechanism.

5- The Third Step of Heme Degradation: Conversion of Verdoheme to Biliverdin and Fe^{II}

In the final step of heme degradation porphyrin macrocycle of Fe(III) verdoheme is cleaved to ferrous iron and biliverdin. This conversion requires molecular Oxygen and reducing equivalents [145]. This step is initiated by the reduction of Fe(III)-verdoheme to Fe(II)-verdoheme and proceeds through binding of one molecular oxygen, and second electron transfer. To dissociate biliverdin from enzyme environment the prior reduction of Fe(III) to Fe(II)-biliverdin is necessary; the biliverdin dissociation is catalyzed by biliverdin reductase enzyme [43, 146-148]. In physiological condition, converson of verdoheme to Fe (III) biliverdin is the rate-limiting step in the overall heme degradation process [146]. However, in coupled oxidation reactions, dissociation of biliverdin from enzyme cavity is the rate-limiting step. As the first step in heme degradation, the third step is also stereoselective for the α -isomer substrate [145].

5-1- Mechanism of the third step

The path by which verdoheme is converted to biliverdin is the least known in the overall heme degradation process. Different mechanisms have been suggested for this step in aerobic condition[149]. However, the nature of the reactive intermediates, the binding sites of O_2 and H_2O_2

are ambiguities which have not been resolved. Verdoheme non-enzymatically has been converted to biliverdin via oxidation by O_2 and H_2O_2 [47, 150, 151], nucleophilic addition, and hydrolysis reactions[152-155]. Theoretical studies along these experimental investigations provide a great deal of insights into third step [70-73].

5-1-1- conversion of Verdoheme to Biliverdin: Hydrolysis pathway

Investigations on HO enzyme system showed that O₂ is exclusively utilized for conversion of verdoheme to biliverdin and therefore hydrolytic mechanism could be ruled out as an enzymatic pathway [147, 150, 156]. However coupled oxidation studies by Balch et al. represented that reaction of verdoheme with nucleophile ions results in the ring opening [152-155]; therefore hydrolysis pathway could be considered as a possible biological mechanism in conversion of verdoheme to biliverdin. To clarify the argument, a DFT study has been conducted on ring opening of five and six coordinated verdoheme through hydrolysis mechanism [70]. The mechanism studied for ring opening of six-coordinated verdoheme, bis imidazole iron (II) veroheme, is depicted in Figure (13). This mechanism involves nucleophilic attack of hydroxyl ion to the carbon atom of verdohem macrocycle (adjacent to the 5-oxo group). This process is -2.26 kcal/mol exothermic with an activation energy of 9.76 kcal/mol. The hydrolysis of six-coordinated verdoheme is a spin forbidden reaction, and a spin crossover could occur during the reaction. In addition, a small difference in energy of closed ring intermediate (Figure 13, species 2), and open chain biliverdin (Figure 13, species 3) suggests that in this slow pathway the closed ring intermediate (2) could be observed in experimental works [70]. However, intermediates have not been distinguished by spectroscopy and are not still fully characterized [152]. Electronic configuration of Biliverdin analogous in the triplet state $d_{xy}^2 d_{xz}^1 d_{yz}^1 LUMO_{macrocylce}^1 d_{z^2}^1$ interpreted this species as iron (III) π anion radical in which unpaired electron on the macrocycle has inverse spin [70].



Figure 13: DFT potential energy profile for hydrolysis of six-coordinated verdoheme to biliverdin in the singlet and triplet state surface. Energy in nunit of kcal/mol and bond distances in unit of Å [70].

In regard to the five-coordinated verdoheme resonance structures, two different mechanisms could be possible [70]. One of the possible pathway involves nucleophilic attack of OH⁻ on the iron atom first, and subsequent OH⁻ transfer to the macrocycle through a rebound mechanism. The other possible mechanism is concerned to direct attack of OH⁻ on macrocycle to the positive carbons adjacent to the 5-oxo position, without any involvement of the iron center. Ring opening in the five-coordinated pathway is spin allowed. The small barrier (4.18 kcal/mol) of direct nucleophilic attack on the macrocycle in comparison to rebound mechanism (32.68 kcal mol⁻¹ for the triplet) indicated the direct attack of a nucleophile to the carbon adjacent to 5-oxo position of macrocycle as the possible mechanism.

The nucleophile, (OH⁻), transfers one electron into the LUMO of ring which has π^* character and located on the C-O bond; therefore the C-O bond is weakened and through bond dissociation open chain iron biliverdin is formed. The electronic structure of five-coordinated verdoheme and [(Im)Fe (OH)] (OP)] indicated that the coordination of OH⁻ to Fe led to destabilization of the metal-based orbital [70].

To clarify the effect of axial ligands in the protein environment on the reactivity of the heme and heme degradation mechanism, conversion of five-coordinated verdoheme complexes with different axial ligands, imidazole (IM), Py, water (H₂O), hydroxyl (OH⁻), CN⁻, phenolate (OPh⁻), chloride (Cl⁻), thiolate (SMe⁻), and imidazolate (IMT⁻) to biliverdin have been studied by DFT method. The results indicated that H₂O, imidazole and Pyridine as neutral ligands and CN⁻ facilitate heme degradation, while thiolate (SMe⁻) and Imidazolate (IMT⁻) retard this process [71].

5-1-2- conversion of Verdoheme to Biliverdin: Nucleophilic reactions

In addition to hydrolysis pathway, verdoheme ring opening has been observed in reaction of verdoheme and its analogues, Co(II) and Zn(II) verdoheme, with other ions like methoxy [152, 153], thiolate, and amide [154].

In agreement with hydrolysis path, DFT results confirmed that direct attack of nucleophiles on the macrocycle leads to generate a more stable species by 12 kcal mol⁻¹ than nucleophile attack on the central atom. It was suggested that the only role of central atom is to increase the positive charge on carbon adjacent to oxygen to facilitate nucleophilic attack. Nucleophilic attack of NH⁻₂ on the carbon atoms adjacent to the oxygen in the verdoheme complex generates a closed geometry species which by passing over a small barrier, 1.55 kcal mol⁻¹ is converted to open ring biliverdin analogous (product) which is 183 kcal mol⁻¹ more stable [72]. These results describe that easily generation of open chain biliverdin of the closed ring intermediate could be the reason of unsuccessful attempt to isolate closed ring intermediate in experimental studies[155].

The nucleophilic addition of NMe_2^- and OH^- ions to $[Zn^{II}(OP)]^+$ resulted in species with higher energy than that of NH_2^- . The most unstable species is the product of nucleophilic addition of OH^- to verdoheme. Instability observed in the OH^- case could be due to lower nucleophilic strength of OH^- relative to other nucleophiles [72].

5-1-3-conversion of Verdoheme to Biliverdin: oxygenation pathway

In HO-catalyzed system, verdoheme is converted to biliverdin through oxygenation mechanism. Labeling studies indicated that two oxygen atoms involved in biliverdin derived from two different oxygen molecules not H₂O molecules [150, 156]. In addition to O₂-dependent reaction , verdoheme ring opening by H₂O₂ was reported as well, supposed by the author that it could be a defensive response in vivo under severe oxidative stress [149, 150, 157]. Although H₂O₂ is not a suitable substitute for catalytically activated dioxygen in the conversion of Fe⁺³-verdoheme [45], reaction of Fe⁺²-verdoheme with H₂O₂ in the presence of reducing agent results in biliverdin. The H₂O₂-dependent reaction products indicated that H₂O₂ is a more efficient oxygen source than O₂, calculated to be 40 times faster than O₂-dependent reaction.



Scheme 8: resonance structure of ferrous verdoheme [149].

Matsui et al. based on the resonance structures of verdoheme (scheme 8) proposed three pathways for conversion of verdoheme to biliverdin in reaction with H_2O_2 and O_2 (Figure 14). The first mechanism proposed that iron is the binding site for O_2 or H_2O_2 , (Figure 14, A), and in the subsequent step OH is transferred to α -pyrrole carbon, although in second mechanism the partially cationic α -pyrrole carbon suggested being the binding site, (Figure 14, B). Deprotonation of both intermediates, generated on Fe or verdoheme ring, and conversion to a bridged species was proposed in the third mechanism, C (Figure 14)[149]. Verdoheme ring-opening reaction with small alkyl peroxides like CH3OOH results in methoxy biliverdin (MeOBV) generation[157]. On the basis of this finding a mechanism analogous to meso-hydroxylation of heme in the first step is proposed for verdoheme degradation and Fe is identified as the binding site for O_2 or H_2O_2 , as proposed by the A mechanism in Figure 14 [157]. In addition, mutagenesis study, Asp140 mutation, in which the O_2 - and H_2O_2 -dependent verdoheme ring opening is significantly lowered supported the formation of Fe-OOH intermediate and reveals the significant role of H-bonding net on third step mechanism[149]. Verdoheme ring opening in the presence of iron-coordinating ligands such as CO, cyanide, and azide is retarded [46].

To clarify the mechanism of this step and characterize the electronic structure of intermediates, theoretical approaches along with experimental studies have been conducted.



Figure 14: Proposed mechanisms for the verdoheme ring opening process in reaction with H_2O_2 (blue) and ROOH (Red) [157].

In the DFT/B3LYP study on conversion of Fe(II) verdoheme to Fe(III) biliverdin in the presence of O_2 three possible pathways with three different intermediates have been investigated [73]. As depicted in scheme 9, in the first route, conversion of Fe-OO⁻ -verdoheme, (compound 3), to iron (III) biliverdin (compound 5) was examined; Fe-OO⁻ -verdoheme was generated by one electron addition to the ferrous verdoheme-O₂ complex, (compound 2). In the second path,

Fe(II)-O₂ verdoheme complex, (compound 2), without an external electron, was proceeded to iron (III) biliverdin, (compound 7). Direct attack of O₂ on the macrocycle resulted in the generation of compound (2a) which is 20 kcal mol⁻¹ less stable that the compound 2.



Scheme 9: Reaction mechanism for iron-(II) verdoheme ring opening [73].

Finally, in the third path iron hydroperoxy verdoheme complex, Fe-OOH-verdoheme, as a reactive intermediate in the ring-opening process was studied. The verdoheme cleavage paths studied in DFT method are two-steps pathways. In the first step, distal oxygen of coordinated ligand (O_2 , peroxide & Hydroperoxide) through a concerted rebound mechanism is transferred to macrocycle carbon (Figure 15). The reaction with Fe-OO⁻ -verdoheme is the most exothermic pathway; oxidation products generated in this path is exothermic by 32.7 kcal mol⁻¹, in comparison with 21.8 kcal mol⁻¹ energy for O_2 -supported and 1.13 kcal mol⁻¹ energy for hydroperoxide-supported reactions. However, the barrier for rate-limiting step of ferric peroxide path is 8.7 kcal mol⁻¹, which is 12.3 kcal mol⁻¹ less than ferric hydroperoxide verdoheme cleavage reaction (Figure 15).



Figure 15: Energy diagram for conversion of Fe-OOH-verdohem to iron biliverdin[73].

In spite of some experimental works which proposed iron in biliverdin is in high oxidation state like as compound I [158, 159], the theoretical study revealed that in conversion of verdoheme to biliverdin, iron remains at Fe(III) oxidation state which could be due to the electron transfer from macrocycle on the central atom in biliverdin (Scheme 8, compounds 5&7).

However, the QM/MM calculation by Shaik group on FeOOH-verdoheme and FeHOOH-verdoheme pathways specified the effects of water cluster in the third step mechanism as in the first oxidation step. Homolytic O-O bond cleavage step has the higher barrier in the ring-opening process. Highly reactive OH^{-}/OH^{-} species stabilized by water cluster regioselectively attack on macrocycle α position [77].

5-1-4-Direct Conversion of Oxophlorin to Biliverdin

Simultaneous formation of verdoheme and biliverdin in the subsequent step of α -meso hydroxyheme generation, have been reported by Balch et al. [160]. Thus, a question could be set on the possibility of direct formation of biliverdin from oxophlorin. To answer this question, oxygenation of six-coordinated hydroxyheme has been studied by QM and QM/MM approaches.

Different studies suggested that verdoheme is generated through the reaction of a five-coordinated hydroxyheme and O₂. On the basis of the theoretical results, a new mechanism in which biliverdin is directly generated from oxophlorin has been suggested (Scheme 10)[78].



Scheme 10: The QM/MM mechanism for the conversion of [(Im)(CO)Fe^{II}(PO')] to Biliverdin [78].

In accordance with experimental data [46, 50, 137], DFT study [66] on [(Im)(CO)FeII(PO•)] complex (scheme 10, complex 1) showed that by binding CO to oxophlorin complex the resonance structures of oxophlorin shifts toward active π -neutral radical form. CO molecule is mostly released in the second step of heme degradation,

As discussed above [(Im)(CO)Fe^{II}(PO^{*})] (scheme 10, compound 1) have a π -radical characteristic which shows high reactivity in reaction with O₂ molecule. In direct attack of molecular O₂ on the carbon atoms adjacent to the keto group of the six-coordinated oxophlorin ring through a pericyclic cycloaddition reaction the HOMO of O₂ directly interacted with the LUMO of the ring of complex (1), (both have π^* character), as a result in (scheme 10, compound 2), two σ bonds are formed, simultaneously with no barrier. The species (scheme 10, compound 2) has $\delta_{xy}^2 \pi_{xz}^2 \pi_{yz}^2 \varphi_{ring+00}^1$ electronic configuration. In the second step (2 \rightarrow 3), an electron transfer from π_{xz}^2 orbital of Fe(II) in compound 2 to $\varphi_{ring+00}^1$ orbital which has σ^* character on O-O bond led to O–O bond cleavage, and iron biliverdin [(Im)(CO)Fe^{III}(BV)] (scheme 10, compound 3) formation. The energy barrier of this step, (2 \rightarrow 3), is 7 kcal/mol, while in solution medium (ϵ = 25) energy barrier decreased to 3 kcal/mol.

In addition to CO, reaction mechanism was studied by QM/MM and QM-cluster approach for H₂O and O₂ ligands as well. QM/MM study indicated the reaction of species with water ligand as the most exothermic (60 kcal/mol) process with the lowest calculated energy barrier (3 kcal/mol).

Although energy barriers of both steps for these species are quite similar to the QM calculation results, the process in QM-cluster study (85 kcal/mol) [66] calculated to be more exothermic than QM/MM method.

Therefore, in comparison with the conversion of five-coordinated oxophlorin to verdoheme process studied by QM approach (exothermic by 14.5kcal/mol with an activation barrier of 12kcal/mol in the gas phase, and 5–9 kcal/mol in solution) [69], the proposed mechanism for direct conversion of oxophlorin to biliverdin could be a feasible path.

6- Heme Oxygenase-1 Inhibitors

Despite the biological importance of HO activity, previously reviewed, excessive activation of the HO system has cytotoxic effects by generating its biologically active metabolites, which could involve in tissue injury. Excessive accumulation of bilirubin has cytotoxic effects. In neonatal jaundice, high levels of unconjugated bilirubin for too long may cause a type of brain damage[20-23]. Metalloporphyrins are heme derivatives like tin-protoporphyrin (SnPP), zinc-protoporphyrin (ZnPP), which compete with heme for binding to the enzyme and inhibit heme degradation. MPs (Metalloporphyrins) have been applied as a useful clinical method in the treatment of hyperbilirubinemia in neonatal (jaundice) [26-29]. In addition, use of HO-1 inhibitors has been considered as a therapeutic application for the treatment of cancers and infectious diseases as well [19, 161, 162].

DFT studies of verdoheme ring opening mechanism have a significant contribution to elucidate the inhibitory role of tin metal. Hydrolysis of tin-verdoheme in the six, five, and four-coordinate complexes by evaluation the effect of axial ligands has been investigated. Results of computational studies show that conversion of six coordinated tin (IV) verdoheme to open chain macrocycle is just thermodynamically favorable. In four and five verdoheme complexes whenever hydroxide ion is bound to the central metal, the reaction is completed at the verdoheme stage and will not continue any longer. A metal-centered inhibition due to high affinity of Sn to increase its coordination state is the proposed mechanism for blocking the ring opening [163, 164].

7- Conclusion

Heme degradation by HO has been greatly studied over the last 4 decades. Details of the reactive intermediates nature could open the route for investigation of this mechanism. Along with experimental studies, theoretical studies - QM (DFT) and QM/MM calculations- are suited to study on mechanism of heme enzymes catalyzed process like heme degradation by heme oxygenase (HO). Theoretical investigation on properties and reactivity of the iron oxophlorine clarified ambiguities in view of electron distribution between the metal and oxophlorin ring. Finally, a new enzymatic mechanism to conversion of oxophlorin to biliverdin has been proposed, which demonstrated that the conversion of oxophlorin to biliverdin could be a feasible route which must be considered in heme degradation process. Extensive studies have been carried out on Heme Oxygenase field, however in attempt to devise new drugs for disorders attributed to Heme oxygenase activity it is crucial to improve understandings of irritating or inhibitory effects of different metals and ligands on the mechanism of heme degradation.

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Conflict of Interest

All authors have participated in approval of the final version.

References:

- 1. Yoshida, T., and Sato, M. *Posttranslational and direct integration of heme oxygenase into microsomes, Biochemical and Biophysical Research Communications, 163*, 1086 (1989).
- 2. McCoubrey, W. K., and Maines, M. D. *Domains of Rat Heme Oxygenase-2: The Amino Terminus and Histidine 151 Are Required for Heme Oxidation, Archives of Biochemistry and Biophysics, 302*, 402 (1993).
- 3. Dennery, P. A. Signaling function of heme oxygenase proteins, Antioxidants & redox signaling, 20, 1743 (2014).
- 4. Bilban, M., Haschemi, A., Wegiel, B., Chin, B. Y., Wagner, O., and Otterbein, L. E. *Heme* oxygenase and carbon monoxide initiate homeostatic signaling, Journal of Molecular Medicine, 86, 267 (2008).

- 5. Motterlini, R., and Otterbein, L. E. *The therapeutic potential of carbon monoxide, Nature reviews Drug discovery, 9,* 728 (2010).
- 6. Ganz, T. Systemic Iron Homeostasis, Physiological Reviews, 93, 1721-1741, 2013).
- 7. Gozzelino, R., and Arosio, P. *Iron homeostasis in health and disease, International journal of molecular sciences*, *17*, 130 (2016).
- 8. Naito, Y., Takagi, T., and Higashimura, Y. *Heme oxygenase-1 and anti-inflammatory M2 macrophages, Archives of Biochemistry and Biophysics, 564,* 83 (2014).
- 9. Nath, K. A. Heme oxygenase-1 and acute kidney injury, Current opinion in nephrology and hypertension, 23, 17 (2014).
- 10. Barone, E., Di Domenico, F., Mancuso, C., and Butterfield, D. A. *The Janus face of the heme oxygenase/biliverdin reductase system in Alzheimer disease: it's time for reconciliation, Neurobiology of disease, 62*, 144 (2014).
- 11. Tian, F., Zhang, F., Lai, X., Wang, L., Yang, L., Wang, X., Singh, G., and Zhong, J. L. L. *Nrf2-mediated protection against UVA radiation in human skin keratinocytes, Bioscience trends*, *5*, 23 (2011).
- 12. Wu, M.-L., Ho, Y.-C., and Yet, S.-F. *A central role of heme oxygenase-1 in cardiovascular protection, Antioxidants & redox signaling, 15,* 1835 (2011).
- 13. Jansen, T., Hortmann, M., Oelze, M., Opitz, B., Steven, S., Schell, R., Knorr, M., Karbach, S., Schuhmacher, S., and Wenzel, P. *Conversion of biliverdin to bilirubin by biliverdin reductase contributes to endothelial cell protection by heme oxygenase-1—evidence for direct and indirect antioxidant actions of bilirubin, Journal of molecular and cellular cardiology, 49*, 186 (2010).
- 14. Gazzin, S., Vitek, L., Watchko, J., Shapiro, S. M., and Tiribelli, C. *A novel perspective on the biology of bilirubin in health and disease, Trends in Molecular Medicine*, *22*, 758 (2016).
- 15. Hamoud, A.-R., Weaver, L., Stec, D. E., and Hinds Jr, T. D. *Bilirubin in the liver–gut signaling Axis, Trends in Endocrinology & Metabolism, 29*, 140 (2018).
- 16. Ziberna, L., Martelanc, M., Franko, M., and Passamonti, S. *Bilirubin is an endogenous antioxidant in human vascular endothelial cells, Scientific reports, 6*, 29240 (2016).
- 17. Zelenka, J., Muchova, L., Zelenkova, M., Vanova, K., Vreman, H. J., Wong, R. J., and Vitek, L. Intracellular accumulation of bilirubin as a defense mechanism against increased oxidative stress, Biochimie, 94, 1821 (2012).
- 18. Müllebner, A., Moldzio, R., Redl, H., Kozlov, A. V., and Duvigneau, J. C. *Heme Degradation by Heme Oxygenase Protects Mitochondria but Induces ER Stress via Formed Bilirubin*, *Biomolecules*, *5*, 679 (2015).
- 19. Chau, L.-Y. *Heme oxygenase-1: emerging target of cancer therapy, Journal of Biomedical Science,* 22, 22 (2015).
- 20. Roy-Chowdhury, N., Wang, X., and Roy-Chowdhury, J. Bile pigment metabolism and its disorders, In *Emery and Rimoin's Principles and Practice of Medical Genetics and Genomics*, 507 (2020).
- 21. Watchko, J. F., and Tiribelli, C. *Bilirubin-Induced Neurologic Damage Mechanisms and Management Approaches, New England Journal of Medicine*, *369*, 2021 (2013).
- 22. Watchko, J. F. *Bilirubin-induced neurotoxicity in the preterm neonate, Clinics in perinatology, 43,* 297 (2016).
- 23. Amin, S. B., Saluja, S., Saili, A., Orlando, M., Wang, H., Laroia, N., and Agarwal, A. *Chronic auditory toxicity in late preterm and term infants with significant hyperbilirubinemia, Pediatrics, 140*, e20164009 (017).
- 24. Chiang, S.-K., Chen, S.-E., and Chang, L.-C. A dual role of Heme Oxygenase-1 in cancer cells, International journal of molecular sciences, 20, 39 (2019).

- 25. Schipper, H., and Song, W. A heme oxygenase-1 transducer model of degenerative and developmental brain disorders, International journal of molecular sciences, 16, 5400 (2015).
- 26. Pittala, V., Salerno, L., Romeo, G., N Modica, M., and A Siracusa, M. *A focus on heme oxygenase- 1 (HO-1) inhibitors, Current Medicinal Chemistry, 20*, 3711 (2013).
- 27. Schulz, S., Wong, R. J., Vreman, H. J., and Strevenson, D. K. *Metalloporphyrins–an update*, *Frontiers in pharmacology*, *3*, 68(2012).
- 28. Wagner, K., Hua, Y., Broderick, J., Nishimura, R., Lu, S., and Dwyer, B. *Tin-mesoporphyrin, a potent heme oxygenase inhibitor, for treatment of intracerebral hemorrhage: in vivo and in vitro studies, Cellular and molecular biology (Noisy-le-Grand, France), 46*, 597(2000).
- 29. Bhutani, V., Poland, R., Meloy, L., Hegyi, T., Fanaroff, A., and Maisels, M. *Clinical trial of tin mesoporphyrin to prevent neonatal hyperbilirubinemia, Journal of Perinatology, 36*, 533(2016).
- 30. Unno, M., Matsui, T., Chu, G. C., Couture, M., Yoshida, T., Rousseau, D. L., Olson, J. S., and Ikeda-Saito, M. *Crystal Structure of the Dioxygen-bound Heme Oxygenase from Corynebacterium diphtheriae: IMPLICATIONS FOR HEME OXYGENASE FUNCTION, Journal of Biological Chemistry*, 279, 21055(2004).
- 31. Hirotsu, S., Chu, G. C., Unno, M., Lee, D.-S., Yoshida, T., Park, S.-Y., Shiro, Y., and Ikeda-Saito, M. The Crystal Structures of the Ferric and Ferrous Forms of the Heme Complex of HmuO, a Heme Oxygenase of Corynebacterium diphtheriae, Journal of Biological Chemistry, 279, 11937(2004).
- 32. Schuller, D. J., Wilks, A., Ortiz de Montellano, P. R., and Poulos, T. L. *Crystal structure of human heme oxygenase-1, Nature Structural Biology, 6*, 860(1999).
- 33. Takahashi, S., Ishikawa, K., Takeuchi, N., Ikeda-Saito, M., Yoshida, T., and Rousseau, D. L. Oxygen-Bound Heme-Heme Oxygenase Complex: Evidence for a Highly Bent Structure of the Coordinated Oxygen, Journal of the American Chemical Society, 117, 6002(1995).
- 34. O'Carra, P. Porphyrins and metalloporphyrins, KM Smith ed, 123(1975).
- 35. Ratliff, M., Zhu, W., Deshmukh, R., Wilks, A., and Stojiljkovic, I. *Homologues of Neisserial Heme Oxygenase in Gram-Negative Bacteria: Degradation of Heme by the Product of thepigA Gene of Pseudomonas aeruginosa, Journal of Bacteriology,* 183, 6394(2001).
- 36. Docherty, J. C., and Brown, S. B. *Haem degradation in human haemoglobin in vitro. Separation of the contribution of the alpha- and beta-subunits, The Biochemical journal, 222*, 401(1984).
- 37. Lad, L., Wang, J., Li, H., Friedman, J., Bhaskar, B., Ortiz de Montellano, P. R., and Poulos, T. L. *Crystal Structures of the Ferric, Ferrous, and Ferrous–NO Forms of the Asp140Ala Mutant of Human Heme Oxygenase-1: Catalytic Implications, Journal of Molecular Biology, 330*, 527(2003).
- 38. Chu, G. C., Tomita, T., Sönnichsen, F. D., Yoshida, T., and Ikeda-Saito, M. *The Heme Complex of Hmu O, a Bacterial Heme Degradation Enzyme from Corynebacterium diphtheriae : STRUCTURE OF THE CATALYTIC SITE, Journal of Biological Chemistry, 274,* 24490 (1999).
- 39. Zhou, H., Migita, C. T., Sato, M., Sun, D., Zhang, X., Ikeda-Saito, M., Fujii, H., and Yoshida, T. Participation of Carboxylate Amino Acid Side Chain in Regiospecific Oxidation of Heme by Heme Oxygenase, Journal of the American Chemical Society, 122, 8311(2000).
- 40. Zeng, Y., Deshmukh, R., Caignan, G. A., Bunce, R. A., Rivera, M., and Wilks, A. *Mixed Regioselectivity in the Arg-177 Mutants of Corynebacterium diphtheriae Heme Oxygenase as a Consequence of in-Plane Heme Disorder, Biochemistry,* 43, 5222(2004).
- 41. Brown, S. B., Chabot, A. A., Enderby, E. A., and North, A. C. T. *Orientation of oxygen in oxyhaemoproteins and its implications for haem catabolism, Nature, 289*, 93(1981).

- 42. Smulevich, G., Mauro, J. M., Fishel, L. A., English, A. M., Kraut, J., and Spiro, T. G. *Heme pocket interactions in cytochrome c peroxidase studied by site-directed mutagenesis and resonance Raman spectroscopy, Biochemistry,* 27, 5477(1988).
- 43. Colas, C., and Ortiz de Montellano, P. R. *Autocatalytic Radical Reactions in Physiological Prosthetic Heme Modification, Chemical Reviews,* 103, 2305(2003.
- 44. Yoshida, T., Noguchi, M., and Kikuchi, G. *Oxygenated form of heme. heme oxygenase complex and requirement for second electron to initiate heme degradation from the oxygenated complex, Journal of Biological Chemistry,* 255, 4418(1980).
- 45. Wilks, A., and Ortiz de Montellano, P. R. *Rat liver heme oxygenase. High level expression of a truncated soluble form and nature of the meso-hydroxylating species, Journal of Biological Chemistry, 268, 22357*(1993).
- 46. Liu, Y., Moënne-Loccoz, P., Loehr, T. M., and de Montellano, P. R. O. *Heme Oxygenase-1, Intermediates in Verdoheme Formation and the Requirement for Reduction Equivalents, Journal of Biological Chemistry, 272*, 6909(1997).
- 47. Sano, S., Sano, T., Morishima, I., Shiro, Y., and Maeda, Y. *On the mechanism of the chemical and enzymic oxygenations of alpha-oxyprotohemin IX to Fe.biliverdin IX alpha, Proceedings of the National Academy of Sciences, 83*, 531(1986).
- 48. Yoshinaga, T., Sudo, Y., and Sano, S. *Enzymic conversion of α-oxyprotohaem IX into biliverdin IXα by haem oxygenase, Biochemical Journal, 270*, 659(1990).
- 49. Yoshida, T., Noguchi, M., Kikuchi, G., and Sano, S. *Degradation of mesoheme and hydroxymesoheme catalyzed by the heme oxygenase system: involvement of hydroxyheme in the sequence of heme catabolism, Journal of Biochemistry, 90,* 125(1981).
- 50. Matera, K. M., Takahashi, S., Fujii, H., Zhou, H., Ishikawa, K., Yoshimura, T., Rousseau, D. L., Yoshida, T., and Ikeda-Saito, M. *Oxygen and One Reducing Equivalent Are Both Required for the Conversion of -Hydroxyhemin to Verdoheme in Heme Oxygenase, Journal of Biological Chemistry*, 271, 6618(1996).
- 51. Hildebrand, D. P., Tang, H.-l., Luo, Y., Hunter, C. L., Smith, M., Brayer, G. D., and Mauk, A. G. *Efficient Coupled Oxidation of Heme by an Active Site Variant of Horse Heart Myoglobin, Journal of the American Chemical Society, 118*, 12909(1996).
- 52. Morishima, I., Fujii, H., Shiro, Y., and Sano, S. *Studies on the Iron(II) meso-Oxyporphyrin .pi.-Neutral Radical as a Reaction Intermediate in Heme Catabolism, Inorganic Chemistry, 34*, 1528 (1995).
- 53. Morishima, I., Fujii, H., Shiro, Y., and Sano, S. *NMR studies of metalloporphyrin radicals. Iron(II)* oxophlorin radical formed from iron(III) meso-hydroxyoctaethylporphyrin, Journal of the American Chemical Society, 108, 3858(1986).
- 54. Masuoka, N., and Itano, H. A. *Radical intermediates in the oxidation of octaethylheme to octaethylverdoheme, Biochemistry*, *26*, 3672(1987).
- 55. Rath, S. P., Olmstead, M. M., and Balch, A. L. *The Effects of Axial Ligands on Electron Distribution and Spin States in Iron Complexes of Octaethyloxophlorin, Intermediates in Heme Degradation, Journal of the American Chemical Society, 126*, 6379(2004).
- 56. Rath, S. P., Olmstead, M. M., and Balch, A. L. *Electron Distribution in Iron Octaethyloxophlorin Complexes. Importance of the Fe(III) Oxophlorin Trianion Form in the Bis-pyridine and Bis-imidazole Complexes, Inorganic Chemistry,* 45, 6083(2006).
- 57. Balch, A. L., Koerner, R., Latos-Grażyński, L., Lewis, J. E., St. Claire, T. N., and Zovinka, E. P. Coupled Oxidation of Heme without Pyridine. Formation of Cyano Complexes of Iron Oxophlorin and 5-Oxaporphyrin (Verdoheme) from Octaethylheme, Inorganic Chemistry, 36, 3892 (1997).

- 58. Balch, A. L., Latos-Grazynski, L., Noll, B. C., Szterenberg, L., and Zovinka, E. P. *Chemistry of iron* oxophlorins. 2. Oxidation of the iron(III) octaethyloxophlorin dimer and observation of stepwise, two-electron oxidation of the oxophlorin macrocycle, Journal of the American Chemical Society, 115, 11846 (1993).
- 59. Hashimoto, S., Teraoka, J., Inubushi, T., Yonetani, T., and Kitagawa, T. *Resonance Raman study* on cytochrome c peroxidase and its intermediate. Presence of the Fe(IV) = O bond in compound ES and heme-linked ionization, Journal of Biological Chemistry, 261, 11110(1986).
- 60. Takahashi, S., Wang, J., Rousseau, D. L., Ishikawa, K., Yoshida, T., Takeuchi, N., and Ikeda-Saito, M. Heme-Heme Oxygenase Complex: Structure and Properties of the Catalytic Site from Resonance Raman Scattering, Biochemistry, 33, 5531 (1994).
- 61. Sharma, P. K., Kevorkiants, R., de Visser, S. P., Kumar, D., and Shaik, S. Porphyrin Traps Its Terminator! Concerted and Stepwise Porphyrin Degradation Mechanisms Induced by Heme-Oxygenase and Cytochrome P450, Angewandte Chemie International Edition, 43, 1129 (2004).
- 62. Kumar, D., de Visser, S. P., and Shaik, S. *Theory Favors a Stepwise Mechanism of Porphyrin* Degradation by a Ferric Hydroperoxide Model of the Active Species of Heme Oxygenase, Journal of the American Chemical Society, 127, 8204 (2005).
- 63. Chen, H., Moreau, Y., Derat, E., and Shaik, S. Quantum Mechanical/Molecular Mechanical Study of Mechanisms of Heme Degradation by the Enzyme Heme Oxygenase: The Strategic Function of the Water Cluster, Journal of the American Chemical Society, 130, 1953 (2008).
- 64. Kamachi, T., and Yoshizawa, K. *Water-Assisted Oxo Mechanism for Heme Metabolism, Journal of the American Chemical Society*, *127*, 10686(2005).
- 65. Kamachi, T., Nishimi, T., and Yoshizawa, K. *A new understanding on how heme metabolism occurs in heme oxygenase: water-assisted oxo mechanism, Dalton Transactions, 41,* 11642(2012).
- 66. Gheidi, M., Safari, N., and Zahedi, M. *Chameleonic Nature of Hydroxyheme in Heme Oxygenase* and Its Reactivity: A Density Functional Theory Study, Inorganic Chemistry, 53, 2766(2014).
- 67. Gheidi, M., Safari, N., and Zahedi, M. *Effect of Axial Ligand on the Electronic Configuration, Spin States, and Reactivity of Iron Oxophlorin, Inorganic Chemistry,* 51, 7094 (2012).
- 68. Gheidi, M., Safari, N., and Zahedi, M. *Structure and Redox Behavior of Iron Oxophlorin and Role of Electron Transfer in the Heme Degradation Process, Inorganic Chemistry,* 51, 12857(2012).
- 69. Gheidi, M., Safari, N., and Zahedi, M. *Density functional theory studies on the conversion of hydroxyheme to iron-verdoheme in the presence of dioxygen, Dalton Transactions, 46*, 2146 (2017).
- 70. Gheidi, M., Safari, N., Bahrami, H., and Zahedi, M. *Theoretical investigations of the hydrolysis pathway of verdoheme to biliverdin, Journal of Inorganic Biochemistry*, *101*, 385(2007).
- 71. Jamaat, P. R., Safari, N., Ghiasi, M., Naghavi, S. S.-a.-d., and Zahedi, M. *Noninnocent effect of axial ligand on the heme degradation process: a theoretical approach to hydrolysis pathway of verdoheme to biliverdin, JBIC Journal of Biological Inorganic Chemistry, 13*, 121(2008).
- 72. Bahrami, H., Zahedi, M., and Safari, N. *Theoretical investigations of the reactivity of verdoheme* analogues: Opening of the planar macrocycle by amide, dimethyl amide, and hydroxide nucleophiles to form helical biliverdin type complexes, Journal of Inorganic Biochemistry, 100, 1449(2006).
- 73. Gheidi, M., Safari, N., and Zahedi, M. *Theoretical investigation of the ring opening process of verdoheme to biliverdin in the presence of dioxygen, Journal of Molecular Modeling, 16,* 1401(2010).

- 74. Shaik, S., de Visser, S. P., Ogliaro, F., Schwarz, H., and Schröder, D. *Two-state reactivity mechanisms of hydroxylation and epoxidation by cytochrome P-450 revealed by theory, Current Opinion in Chemical Biology, 6*, 556 (2002).
- 75. Shaik, S., Kumar, D., de Visser, S. P., Altun, A., and Thiel, W. *Theoretical Perspective on the Structure and Mechanism of Cytochrome P450 Enzymes, Chemical Reviews*, *105*, 2279 (2005).
- 76. Alavi, F. S., Zahedi, M., Safari, N., and Ryde, U. *QM/MM Study of the Conversion of Oxophlorin into Verdoheme by Heme Oxygenase, The Journal of Physical Chemistry B*, *121*, 11427 (2017).
- 77. Lai, W., Chen, H., Matsui, T., Omori, K., Unno, M., Ikeda-Saito, M., and Shaik, S. *Enzymatic Ring-Opening Mechanism of Verdoheme by the Heme Oxygenase: A Combined X-ray Crystallography and QM/MM Study, Journal of the American Chemical Society, 132*, 12960 (2010).
- Alavi, F. S., Gheidi, M., Zahedi, M., Safari, N., and Ryde, U. A novel mechanism of heme degradation to biliverdin studied by QM/MM and QM calculations, Dalton Transactions, 47, 8283-8291, 2018).79. Alavi, F. S., Zahedi, M., Safari, N., and Ryde, U. QM/MM study of the conversion of biliverdin into verdoheme by heme oxygenase, Theoretical Chemistry Accounts, 138, 72 (2019).
- 80. McCoubrey Jr, W. Isolation and Characterization of a cDNA from the Rat Brain that Encodes Hemoprotein Heme Oxygenase-3, J. Biochem, 247, 725(1997).
- 81. Hayashi, S., Omata, Y., Sakamoto, H., Higashimoto, Y., Hara, T., Sagara, Y., and Noguchi, M. *Characterization of rat heme oxygenase-3 gene. Implication of processed pseudogenes derived from heme oxygenase-2 gene, Gene, 336*, 241 (2004).
- 82. Ewing, J. F., and Maines, M. D. *Histochemical localization of heme oxygenase-2 protein and mRNA expression in rat brain, Brain Research Protocols,* 1, 165 (1997).
- 83. Peng, Y.-J., Makarenko, V. V., Nanduri, J., Vasavda, C., Raghuraman, G., Yuan, G., Gadalla, M. M., Kumar, G. K., Snyder, S. H., and Prabhakar, N. R. *Inherent variations in CO-H2S-mediated carotid body O2 sensing mediate hypertension and pulmonary edema, Proceedings of the National Academy of Sciences, 111*, 1174 (2014).
- 84. Barañano, D. E., and Snyder, S. H. *Neural roles for heme oxygenase: Contrasts to nitric oxide synthase, Proceedings of the National Academy of Sciences, 98,* 10996(2001).
- 85. Zhang, H., Liu, Y.-y., Jiang, Q., Li, K.-r., Zhao, Y.-x., Cao, C., and Yao, J. Salvianolic acid A protects RPE cells against oxidative stress through activation of Nrf2/HO-1 signaling, Free Radical Biology and Medicine, 69, 219 (2014).
- 86. Jomova, K., and Valko, M. Importance of iron chelation in free radical-induced oxidative stress and human disease, Current Phar maceutical Design, 17, 3460 (2011).
- 87. Shen, Q., Jiang, M., Li, H., Che, L. L., and Yang, Z. M. *Expression of a Brassica napus heme oxygenase confers plant tolerance to mercury toxicity, Plant, Cell & Environment, 34*, 752 (2011).
- 88. Lee, I.-S., Lim, J., Gal, J., Kang, J. C., Kim, H. J., Kang, B. Y., and Choi, H. J. *Anti-inflammatory activity of xanthohumol involves heme oxygenase-1 induction via NRF2-ARE signaling in microglial BV2 cells, Neurochemistry International, 58*, 153 (2011).
- 89. Sharp, F. R., Zhan, X., and Liu, D.-Z. *Heat shock proteins in the brain: role of Hsp70, Hsp 27, and HO-1 (Hsp32) and their therapeutic potential, Translational stroke research, 4*, 685-692, 2013.
- 90. Ryter, S. W., and Choi, A. M. *Targeting heme oxygenase-1 and carbon monoxide for therapeutic modulation of inflammation, Translational Research, 167, 7*(2016).
- 91. Poss, K. D., and Tonegawa, S. *Reduced stress defense in heme oxygenase 1-deficient cells*, *Proceedings of the National Academy of Sciences*, *94*, 10925 (1997).

- 92. Mahawar, L., and Shekhawat, G. S. *Haem oxygenase: a functionally diverse enzyme of photosynthetic organisms and its role in phytochrome chromophore biosynthesis, cellular signalling and defence mechanisms, Plant, Cell & Environment, 41, 483 (2018).*
- 93. Fujita, Y., Tsujimoto, R., and Aoki, R. *Evolutionary aspects and regulation of tetrapyrrole biosynthesis in cyanobacteria under aerobic and anaerobic environments, Life, 5*, 1172(2015).
- 94. Carvalho, R. F., Campos, M. L., and Azevedo, R. A. The role of phytochromes in stress tolerance, In *Salt Stress in Plants* Springer 283 (2013).
- 95. Wilks, A., and Ikeda-Saito, M. *Heme utilization by pathogenic bacteria: not all pathways lead to biliverdin, Accounts of Chemical Research, 47,* 2291(2014).
- 96. Yoshida, T., and Kikuchi, G. *Purification and properties of heme oxygenase from rat liver microsomes, Journal of Biological Chemistry,* 254, 4487(1979).
- 97. Rotenberg, M. O., and Maines, M. D. *Characterization of a cDNA-encoding rabbit brain heme oxygenase-2 and identification of a conserved domain among mammalian heme oxygenase isozymes: possible heme-binding site?*, *Archives of Biochemistry and Biophysics*, *290*, 336(1991).
- 98. Maines, M. D., Trakshel, G. M., and Kutty, R. K. *Characterization of two constitutive forms of rat liver microsomal heme oxygenase. Only one molecular species of the enzyme is inducible, Journal of Biological Chemistry*, *261*, 411(1986).
- 99. Ishikawa, K., Sato, M., Ito, M., and Yoshida, T. *Importance of histidine residue 25 of rat heme oxygenase for its catalytic activity, Biochemical and Biophysical Research Communications, 182,* 981(1992).
- 100. Schuller, D. J., Zhu, W., Stojiljkovic, I., Wilks, A., and Poulos, T. L. *Crystal structure of heme* oxygenase from the gram-negative pathogen Neisseria meningitidis and a comparison with mammalian heme oxygenase-1, Biochemistry, 40, 11552(2001).
- 101. Friedman, J., Lad, L., Li, H., Wilks, A., and Poulos, T. L. Structural basis for novel δ-regioselective heme oxygenation in the opportunistic pathogen Pseudomonas aeruginosa, Biochemistry, 43, 5239 (2004).
- 102. Sugishima, M., Omata, Y., Kakuta, Y., Sakamoto, H., Noguchi, M., and Fukuyama, K. *Crystal* structure of rat heme oxygenase-1 in complex with heme, FEBS Letters, 471, 61(2000).
- 103. Lad, L., Schuller, D. J., Shimizu, H., Friedman, J., Li, H., de Montellano, P. R. O., and Poulos, T. L. *Comparison of the heme-free and-bound crystal structures of human heme oxygenase-1, Journal of Biological Chemistry, 278*, 7834(2003).
- 104. Wilks, A., Black, S. M., Miller, W. L., and Ortiz de Montellano, P. R. *Expression and characterization of truncated human heme oxygenase (hHO-1) and a fusion protein of hHO-1 with human cytochrome P450 reductase, Biochemistry, 34*, 4421(1995).
- 105. Sun, J., Wilks, A., Ortiz de Montellano, P. R., and Loehr, T. M. *Resonance Raman and EPR spectroscopic studies on heme-heme oxygenase complexes, Biochemistry*, *32*, 14151(1993).
- 106. Takahashi, S., Wang, J., Rousseau, D. L., Ishikawa, K., Yoshida, T., Host, J. R., and Ikeda-Saito, M. *Heme-heme oxygenase complex. Structure of the catalytic site and its implication for oxygen activation, Journal of Biological Chemistry, 269*, 1010(1994).
- 107. Sun, J., Loehr, T. M., Wilks, A., and Ortiz de Montellano, P. R. *Identification of histidine 25 as the heme ligand in human liver heme oxygenase, Biochemistry, 33*, 13734(1994.
- 108. Kitagawa, T. Biological applications of raman spectroscopy, Biological Applications of Raman Spectroscopy, 3, 97(1988).
- 109. Kincaid, J., Stein, P., and Spiro, T. *Absence of heme-localized strain in T state hemoglobin: insensitivity of heme-imidazole resonance Raman frequencies to quaternary structure, Proceedings of the National Academy of Sciences, 76*, 549 (1979).

- 110. Liu, Y., Lightning, L. K., Huang, H.-w., Moënne-Loccoz, P., Schuller, D. J., Poulos, T. L., Loehr, T. M., and De Montellano, P. R. O. *Replacement of the distal glycine 139 transforms human heme oxygenase-1 into a peroxidase, Journal of Biological Chemistry*, *275*, 34501(2000).
- 111. Li, Y., Syvitski, R. T., Auclair, K., Wilks, A., de Montellano, P. R. O., and La Mar, G. N. Solution NMR characterization of an unusual distal H-bond network in the active site of the cyanideinhibited, human heme oxygenase complex of the symmetric substrate, 2, 4dimethyldeuterohemin, Journal of Biological Chemistry, 277, 33018 (2002).
- 112. Li, Y., Syvitski, R. T., Auclair, K., Ortiz de Montellano, P., and La Mar, G. N. Solution 1H, 15N NMR spectroscopic characterization of substrate-bound, cyanide-inhibited human heme oxygenase: Water occupation of the distal cavity, Journal of the American Chemical Society, 125, 13392(2003).
- 113. Lightning, L. K., Huang, H.-w., Moënne-Loccoz, P., Loehr, T. M., Schuller, D. J., Poulos, T. L., and De Montellano, P. R. O. *Disruption of an active site hydrogen bond converts human heme oxygenase-1 into a peroxidase, Journal of Biological Chemistry, 276*, 10612(2001).
- 114. Migita, C. T., Matera, K. M., Ikeda-Saito, M., Olson, J. S., Fujii, H., Yoshimura, T., Zhou, H., and Yoshida, T. *The oxygen and carbon monoxide reactions of heme oxygenase, Journal of Biological Chemistry*, *273*, 945(1998).
- 115. Noguchi, M., Yoshida, T., and Kikuchi, G. *A stoichiometric study of heme degradation catalyzed* by the reconstituted heme oxygenase system with special consideration of the production of hydrogen peroxide during the reaction, The Journal of Biochemistry, 93, 1027(1983).
- 116. Davydov, R., Chemerisov, S., Werst, D. E., Rajh, T., Matsui, T., Ikeda-Saito, M., and Hoffman, B. M. Proton transfer at helium temperatures during dioxygen activation by heme monooxygenases, Journal of the American Chemical Society, 126, 15960(2004).
- 117. Davydov, R. M., Yoshida, T., Ikeda-Saito, M., and Hoffman, B. M. *Hydroperoxy-heme oxygenase* generated by cryoreduction catalyzes the formation of α -meso-hydroxyheme as detected by EPR and ENDOR, Journal of the American Chemical Society, 121, 10656(1999).
- 118. Davydov, R., Kofman, V., Fujii, H., Yoshida, T., Ikeda-Saito, M., and Hoffman, B. M. *Catalytic mechanism of heme oxygenase through EPR and ENDOR of cryoreduced oxy-heme oxygenase and its Asp 140 mutants, Journal of the American Chemical Society, 124*, 1798(2002).
- 119. Garcia-Serres, R., Davydov, R. M., Matsui, T., Ikeda-Saito, M., Hoffman, B. M., and Huynh, B. H. Distinct reaction pathways followed upon reduction of oxy-heme oxygenase and oxy-myoglobin as characterized by Mössbauer spectroscopy, Journal of the American Chemical Society, 129, 1402(2007).
- 120. Ishikawa, K., Takeuchi, N., Takahashi, S., Matera, K. M., Sato, M., Shibahara, S., Rousseau, D. L., Ikeda-Saito, M., and Yoshida, T. *Heme oxygenase-2 properties of the heme complex of the purified tryptic fragment of recombinant human heme oxygenase-2, Journal of Biological Chemistry*, *270*, 6345(1995).
- 121. Wilks, A., Torpey, J., and de Montellano, P. O. *Heme oxygenase (HO-1). Evidence for electrophilic oxygen addition to the porphyrin ring in the formation of alpha-meso-hydroxyheme, Journal of Biological Chemistry, 269*, 29553(1994).
- 122. Noguchi, M., Yoshida, T., and Kikuchi, G. *Identification of the Product of Heme Degradation Catalyzed by the Heme Oxygenase System as Biliverdin IX*α *by Reversed-Phase High-Performance Liquid Chromatography, The Journal of Biochemistry, 91*, 1479(1982).
- 123. Blumenthal, S. G., Taggart, D. B., Ikeda, R., Ruebner, B., and Bergstrom, D. *Conjugated and unconjugated bilirubins in bile of humans and rhesus monkeys. Structure of adult human and rhesus-monkey bilirubins compared with dog bilirubins, Biochemical Journal, 167*, 535(1977).

- 124. Lemberg, R. *The chemical mechanism of bile pigment formation, Rev. pure appl. Chem, 6,* 23(1956).
- 125. Bonnett, R., and McDonagh, A. F. *The meso-reactivity of porphyrins and related compounds. Part* VI. Oxidative cleavage of the haem system. The four isomeric biliverdins of the IX series, Journal of the Chemical Society, Perkin Transactions 1, 881(1973).
- 126. Colleran, E. *HAEM catabolism and coupled oxidation of haemproteins*, *FEBS Letters*, *5*, 295(1969).
- 127. Docherty, J. C., Masters, B. S. S., Firneisz, G. D., and Schacter, B. A. *Heme oxygenase provides α*-selectivity to physiological heme degradation, Biochemical and Biophysical Research Communications, 105, 1005(1982).
- 128. Torpey, J., and de Montellano, P. R. O. *Oxidation of the meso-Methylmesoheme Regioisomers by Heme Oxygenase ELECTRONIC CONTROL OF THE REACTION REGIOSPECIFICITY, Journal of Biological Chemistry, 271,* 26067(1996).
- 129. Torpey, J., and de Montellano, P. R. O. *Oxidation of α-meso-Formylmesoheme by Heme Oxygenase ELECTRONIC CONTROL OF THE REACTION REGIOSPECIFICITY, Journal of Biological Chemistry*, *272*, 22008 (1997).
- 130. Senge, M. O., Ema, T., and Smith, K. M. *Crystal structure of a remarkably ruffled nonplanar* porphyrin (pyridine)[5, 10, 15, 20-tetra (tert-butyl) porphyrinato] zinc (II), Journal of the Chemical Society, Chemical Communications, 733(1995).
- 131. Ortiz de Montellano, P. R., and Wilks, A. Heme oxygenase structure and mechanism, In *Advances in Inorganic Chemistry*, pp 359 (2000).
- 132. Torpey, J., Lee, D. A., Smith, K. M., and Ortiz de Montellano, P. R. Oxidation of an α-mesomethyl-substituted heme to an α-biliverdin by heme oxygenase. A novel heme cleavage reaction, Journal of the American Chemical Society, 118, 9172(1996).
- 133. Ortiz de Montellano, P. R. *Heme oxygenase mechanism: evidence for an electrophilic, ferric peroxide species, Accounts of Chemical Research, 31*, 543 (1998).
- 134. Hydroxylation, H., DAVYDOV, R., MATSUI, T., FUJII, H., IKEDA-SAITO, M., and HOFFMAN, B. M. *VC Bioinorganic Chemistry and Structural Biology of Heme Proteins, J. Am. Chem. Soc, 125*, 16208 (2003).
- 135. Matsui, T., Kim, S. H., Jin, H., Hoffman, B. M., and Ikeda-Saito, M. *Compound I of heme oxygenase cannot hydroxylate its heme meso-carbon, Journal of the American Chemical Society, 128*, 1090 (2006).
- 136. Garcia-Bosch, I., Sharma, S. K., and Karlin, K. D. A Selective Stepwise Heme Oxygenase Model System: An Iron(IV)-Oxo Porphyrin π -Cation Radical Leads to a Verdoheme-Type Compound via an Isoporphyrin Intermediate, Journal of the American Chemical Society, 135, 16248 (2013).
- 137. Sakamoto, H., Omata, Y., Palmer, G., and Noguchi, M. *Ferric α*-hydroxyheme bound to heme oxygenase can be converted to verdoheme by dioxygen in the absence of added reducing equivalents, Journal of Biological Chemistry, 274, 18196 (1999).
- 138. Ikeda-Saito, M., Hori, H., Andersson, L., Prince, R., Pickering, I., George, G., Sanders, C. n., Lutz, R., McKelvey, E., and Mattera, R. *Coordination structure of the ferric heme iron in engineered distal histidine myoglobin mutants, Journal of Biological Chemistry*, *267*, 22843 (1992).
- 139. Bogumil, R., Maurus, R., Hildebrand, D. P., Brayer, G. D., and Mauk, A. G. Origin of the pHdependent spectroscopic properties of pentacoordinate metmyoglobin variants, Biochemistry, 34, 10483 (1995).
- 140. Balch, A. L., Noll, B. C., Reid, S. M., and Zovinka, E. P. *Coordination patterns for oxophlorin ligands. Pyridine-induced cleavage of dimeric manganese (III) and iron (III) octaethyloxophlorin complexes, Inorganic Chemistry, 32*, 2610 (1993).

- 141. Balch, A. L., Koerner, R., Latos-Grażyński, L., and Noll, B. C. A role for electron transfer in heme catabolism? Structure and redox behavior of an intermediate, (pyridine) 2Fe (octaethyloxophlorin), Journal of the American Chemical Society, 118, 2760 (1996).
- 142. Migita, C. T., Fujii, H., Mansfield Matera, K., Takahashi, S., Zhou, H., and Yoshida, T. *Molecular* oxygen oxidizes the porphyrin ring of the ferric alpha-hydroxyheme in heme oxygenase in the absence of reducing equivalent, Biochimica et Biophysica Acta, 1432, 203 (1999).
- 143. Yoshida, T., and Migita, C. T. *Mechanism of heme degradation by heme oxygenase*, *Journal of Inorganic Biochemistry*, *82*, 33 (2000).
- 144. Carter, E. A., and Goddard III, W. A. *Relationships between bond energies in coordinatively unsaturated and coordinatively saturated transition-metal complexes: a quantitative guide for single, double, and triple bonds, The Journal of Physical Chemistry, 92,* 5679 (1988).
- 145. Zhang, X., Fujii, H., Matera, K. M., Migita, C. T., Sun, D., Sato, M., Ikeda-Saito, M., and Yoshida, T. Stereoselectivity of each of the three steps of the heme oxygenase reaction: hemin to mesohydroxyhemin, meso-hydroxyhemin to verdoheme, and verdoheme to biliverdin, Biochemistry, 42, 7418 (2003).
- 146. Liu, Y., and de Montellano, P. R. O. *Reaction intermediates and single turnover rate constants for the oxidation of heme by human heme oxygenase-1, Journal of Biological Chemistry, 275*, 5297 (2000).
- 147. YOSHIDA, T., and NOGUCHI, M. Features of intermediary steps around the 688-nm substance in the heme oxygenase reaction, The Journal of Biochemistry, 96, 563 (1984).
- 148. Yoshida, T., and Kikuchi, G. *Features of the reaction of heme degradation catalyzed by the reconstituted microsomal heme oxygenase system, Journal of Biological Chemistry, 253*, 4230 (1978).
- 149. Matsui, T., Nakajima, A., Fujii, H., Matera, K. M., Migita, C. T., Yoshida, T., and Ikeda-Saito, M. O2-and H2O2-dependent Verdoheme Degradation by Heme Oxygenase REACTION MECHANISMS AND POTENTIAL PHYSIOLOGICAL ROLES OF THE DUAL PATHWAY DEGRADATION, Journal of Biological Chemistry, 280, 36833 (2005).
- 150. Saito, S., and Itano, H. A. Verdohemochrome IX alpha: preparation and oxidoreductive cleavage to biliverdin IX alpha, Proceedings of the National Academy of Sciences, 79, 1393 (1982).
- 151. Nguyen, K. T., Rath, S. P., Latos-Grażyński, L., Olmstead, M. M., and Balch, A. L. Formation of a highly oxidized iron biliverdin complex upon treatment of a five-coordinate verdoheme with dioxygen, Journal of the American Chemical Society, 126, 6210 (2004).
- 152. Koerner, R., Latos-Grażyński, L., and Balch, A. L. *Models for verdoheme hydrolysis. Paramagnetic products from the ring opening of verdohemes, 5-oxaporphyrin complexes of Iron (II), with methoxide ion, Journal of the American Chemical Society, 120, 9246 (1998)..*
- 153. Latos-Grażyński, L., Johnson, J., Attar, S., Olmstead, M. M., and Balch, A. L. *Reactivity of the verdoheme analogues, 5-oxaporphyrin complexes of cobalt (II) and zinc (II), with nucleophiles: Opening of the planar macrocycle by alkoxide addition to form helical complexes, Inorganic Chemistry, 37*, 4493 (1998).
- 154. Johnson, J. A., Olmstead, M. M., and Balch, A. L. *Reactivity of the verdoheme analogues. Opening of the planar macrocycle by amide and thiolate nucleophiles to form helical complexes, Inorganic Chemistry, 38*, 5379 (1999).
- 155. Johnson, J. A., Olmstead, M. M., Stolzenberg, A. M., and Balch, A. L. *Ring-opening and meso substitution from the reaction of cyanide ion with zinc verdohemes, Inorganic Chemistry,* 40, 5585 (2001).

- 156. Docherty, J. C., Schacter, B. A., Firneisz, G. D., and Brown, S. *Mechanism of action of heme oxygenase. A study of heme degradation to bile pigment by 180 labeling, Journal of Biological Chemistry, 259,* 13066 (1984).
- 157. Matsui, T., Omori, K., Jin, H., and Ikeda-Saito, M. *Alkyl peroxides reveal the ring opening mechanism of verdoheme catalyzed by heme oxygenase, Journal of the American Chemical Society, 130,* 4220 (2008).
- 158. Lad, L., Friedman, J., Li, H., Bhaskar, B., Ortiz de Montellano, P. R., and Poulos, T. L. *Crystal structure of human heme oxygenase-1 in a complex with biliverdin, Biochemistry, 43*, 3793 (2004).
- 159. Lad, L., de Montellano, P. R. O., and Poulos, T. L. *Crystal structures of ferrous and ferrous–NO forms of verdoheme in a complex with human heme oxygenase-1: catalytic implications for heme cleavage, Journal of Inorganic Biochemistry, 98*, 1686 (2004).
- 160. Balch, A. L., Latos-Grazynski, L., Noll, B. C., Olmstead, M. M., and Safari, N. *Isolation and characterization of an iron biliverdin-type complex that is formed along with verdohemochrome during the coupled oxidation of iron (II) octaethylporphyrin, Journal of the American Chemical Society, 115*, 9056 (1993).
- 161. Loboda, A., Jozkowicz, A., and Dulak, J. *HO-1/CO system in tumor growth, angiogenesis and metabolism—Targeting HO-1 as an anti-tumor therapy, Vascular pharmacology,* 74, 11 (2015).
- 162. Salerno, L., Floresta, G., Ciaffaglione, V., Gentile, D., Margani, F., Turnaturi, R., Rescifina, A., and Pittalà, V. *Progress in the development of selective heme oxygenase-1 inhibitors and their potential therapeutic application, European journal of medicinal chemistry*, (2019).
- 163. Davari, M. D., Bahrami, H., Zahedi, M., and Safari, N. *Theoretical investigations on the hydrolysis pathway of tin verdoheme complexes: elucidation of tin's ring opening inhibition role, Journal of Molecular Modeling, 15, 1299 (2009).*
- 164. Davari, M. D., Bahrami, H., Zahedi, M., and Safari, N. *Effect of the axial ligands on the structure and reactivity of tin verdoheme in the ring opening process, Inorganica Chimica Acta, 363*, 1577 (2010).

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