Archives of Biochemistry and Biophysics 544 (2014) 87–95

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Review

Heme oxygenation and the widening paradigm of heme degradation

Angela Wilks *, Geoffrey Heinzl

Department of Pharmaceutical Sciences, University of Maryland, Baltimore, MD 21201-1180, USA

ARTICLE INFO

Article history:
Available online 23 October 2013

Keywords:
Heme degradation
Heme oxygenase
Biliverdin
Staphylobilin
Mycobilin
Coupled oxidation
Verdoheme

ABSTRACT

Heme degradation through the action of heme oxygenase (HO) is unusual in that it utilizes heme as both a substrate and cofactor for its own degradation. HO catalyzes the oxygen-dependent degradation of heme to biliverdin with the release of CO and "free" iron. The characterization of HO enzymes from humans to bacteria reveals a similar overall structural fold that contributes to the unique reaction manifold. The heme oxygenases share a similar heme-dependent activation of O_2 to the ferric hydroperoxide as that of the cytochrome P450s and peroxidases. However, whereas the P450s promote cleavage of the ferric hydroperoxide O—O bond to the oxoferryl species the HOs stabilize the ferric hydroperoxide promoting hydroxylation at the heme edge. The alternate reaction pathway in HO is achieved through the conformational flexibility and extensive hydrogen bond network within the heme binding site priming the heme for hydroxylation. Until recently it was believed that all heme degrading enzymes converted heme to biliverdin and iron, with the release of carbon monoxide (CO). However, the recent discovery of the bacterial IsdG-like heme degrading proteins of *Staphylococcus aureus*, *Bacillus anthracis* and *Mycobacterium tuberculosis* has expanded the reaction manifold of heme oxidation. Characterization of the heme degradation products in the IsdG-like reaction suggests a mechanism distinct from the classical HOs. In the following review we will discuss the structure–function of the canonical HOs as it relates to the emerging alternate reaction manifold of the IsdG-like proteins.

Introduction

The biological degradation of heme (iron-protoporphyrin IX) is catalyzed by a family of enzymes termed heme oxygenases that convert heme to biliverdin, with the release of carbon monoxide (CO) and iron (Fig. 1A) [1–4]. Although first described in mammals as a "housekeeping" enzyme for its role in the recycling of iron, HO[1] is now recognized for its role in antioxidant defense, cellular signaling and the biosynthesis of light-sensing bilins [5–10]. Furthermore, pathogenic bacteria utilize HOs as a means of acquiring iron that is essential for virulence and survival [11–15]. The HO-dependent conversion of heme to biliverdin consumes three molecules of oxygen and seven reducing equivalents. In mammals the reducing equivalents for the oxidation of heme are supplied by NADPH-cytochrome P450 reductase and the resulting biliverdin IXα (biliverdin BVIXα) is converted to bilirubin by the action of biliverdin reductase. In plants, algae and cyanobacteria a ferredoxin-dependent heme oxygenase generates biliverdin IXα as a precursor for the synthesis of light-harvesting pigments [16,17]. In the case of the canonical bacterial HOs no physiological electron donors have been identified [18–20].

The recent discovery of the *Staphylococcus aureus* and *Bacillus anthracis* IsdG-like proteins encoded within a cluster of genes termed iron-regulated surface determinants (*isd*) has expanded the reaction manifold of heme cleavage [21–23]. Although initially reported to convert heme to biliverdin [22,23] recent studies have shown the mechanism and reaction products of the IsdG-like proteins to be distinct from those of the canonical HOs [24,25]. The *S. aureus* IsdG reaction converts heme to a mixture of 5-oxo-bilirubin and 15-oxo-bilirubin, collectively termed "staphylobilins" (Fig. 1C) [25]. Moreover, recent studies have shown that oxidative cleavage of heme by IsdG leads to the release of the α-*meso* carbon as formaldehyde, precluding verdoheme as an intermediate [24]. Similarly, the structurally related *Mycobacterium tuberculosis* MhuD protein catalyzes oxidative ring cleavage with retention of the *meso*-carbon as an aldehyde suggesting the MhuD and IsdG-like proteins lie along the same reaction pathway (Fig. 1B) [26]. In contrast to the canonical HOs a physiological electron donor to the IsdG/I enzymes of *S. aureus* has recently been identified [27].

Although the canonical HOs and non-canonical IsdG-like proteins are mechanistically distinct from each other they belong to a unique class of heme enzymes that channel the activated

* Corresponding author. Address: Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, HSF II, 20 Penn Street, Baltimore, MD 21201-1140, USA. Fax: +1 410 706 5017.
E-mail address: awilks@rx.umaryland.edu (A. Wilks).

[1] *Abbreviations used:* CoPPIX, cobalt protoporphyrin; HO, heme oxygenase; hHO-1, human heme oxygenase; rHO-1 rat heme oxygenase; HmuO, *Corynebacterium diphtheriae* heme oxygenase; HemO, Pseudomonas heme oxygenase; Isd, iron surface determinant; Mhu, Mycobacterium heme utilization; EPR, electron paramagnetic resonance; NMR, nuclear magnetic resonance, ENDOR, electron-nuclear double resonance.

0003-9861/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.abb.2013.10.013

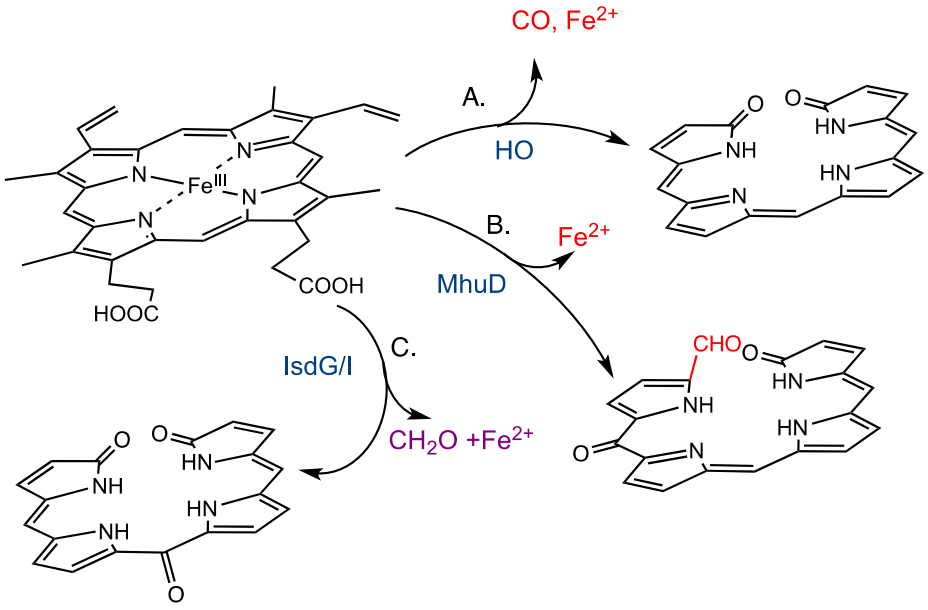

Fig. 1. Reaction products of the canonical HO and non-canonical IsdG/I-like heme oxygenases.

Fe(III)–OOH toward heme hydroxylation through modulation of the heme electronic configuration. This reaction is distinct from that of other monoxygenases such as the cytochrome P450s that diverge through an alternate pathway that promotes cleavage of the activated Fe(III)–OOH to the oxoferryl Fe(IV)=O species.

Structural diversity of heme oxidation

The canonical HO enzymes from bacteria to mammals have a similar overall α-helical structural fold (Fig. 2A) [28–30]. Heme is held between the proximal and distal helices and anchored in the pocket through interactions of the propionates with surface exposed Lys residues. Interestingly, the iron-regulated HemO from *Pseudomonas aeruginosa* gives rise to an altered regioselectivity from that of all other HO's as a consequence of an alternate seating of the heme within the active site. The in-plane rotation of the heme is a consequence of alternate propionate interactions with the protein scaffold [29]. In addition to the conserved proximal His ligand all canonical HO's retain an ordered hydrogen-bonding network required for proton delivery to the coordinated Fe(II)—O_2 to form activated Fe(III)–OOH (Fig. 3A).

In contrast the recently identified non-canonical IsdG/I and MhuD heme degrading enzymes of *S. aureus* and *M. tuberculosis*, respectively, have an overall structural fold distinct from that of the classical HOs (Fig. 2B) [31,32]. The Isd protein family are homodimers, with each monomer adopting a ferredoxin-like α/β-sandwich fold that comes together to form a β-barrel at the dimer interface. Each monomer binds one heme in a hydrophobic cleft on either side of the β-barrel with the coordinating His residue provided by the peripheral helix/loops surrounding the β-barrel. In the CoPPIX–IsdI complex the porphyrin is ligated through His-76 with the propionates being anchored in the back of the pocket through interactions with Arg-21 and Arg-25. Interestingly, the ordered hydrogen-bonding network found in the canonical HO enzymes is absent in the IsdG-like proteins (Fig. 3B). Moreover, the resting state heme in the Isd proteins undergoes significant distortion from planarity induced through steric interaction of the heme macrocycle with several residues within the heme pocket (Fig. 3B) [32]. The structural aspects of the respective proteins required for heme reactivity will be discussed in the following sections.

Steric versus electronic contributions to heme hydroxylation

The initial step in heme oxidation involves the reduction of the Fe(III)–heme complex to the Fe(II)–O_2 complex. Reduction of the Fe(II)–O_2 complex to the activated Fe(III)-peroxo species leads to the formation of α-*meso*-hydroxyheme, which in the presence of oxygen is rapidly converted to verdoheme (Fig. 4) [33]. Early studies by Ortiz de Montellano and Wilks further showed a molar equivalent of H_2O_2 could substitute for O_2 and reducing equiva-

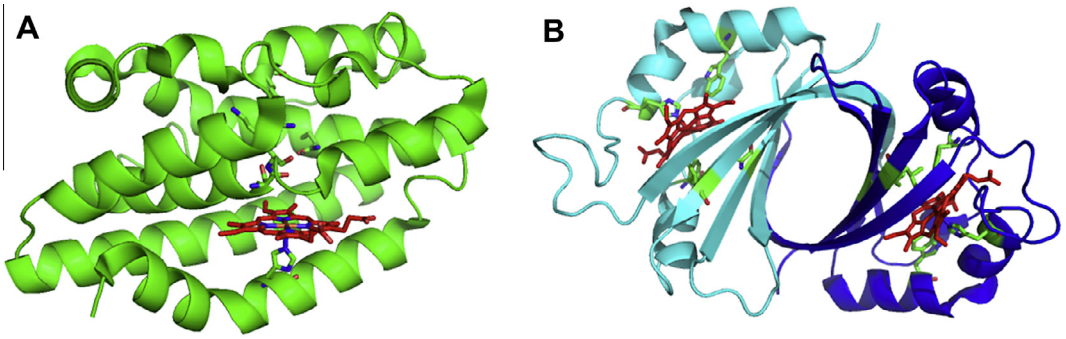

Fig. 2. Comparison of the oxygen activation pathways of HO and cytochrome P450.