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Heme Oxygenase

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Abbreviations.

CO: Carbon monoxide.
DNA: Deoxyribo nucleic acid.
Fe^{2+} : Ferrous iron.
HO: Heme oxygenase.
IL: Interleukin.
IRE:Iron responsive element.
IRP: Iron regulatory protein.
kDa: Kilodalton.
NADPH: Nicotinamide adenine dinucleotide phosphate (reduced).
ROS: Reactive oxygen species.

Abstract

Heme oxygenase is an enzyme found in the endoplasmic reticulum. It catalyzes the conversion of heme to carbon monoxide, iron and biliverdin. This biliverdin formed is immediately reduced to bilirubin. Three isoforms of heme oxygenase (HO) is known, HO-1, HO-2, and HO-3. HO-1 is inducible and is identical to one of the heat shock proteins (HSP 32). The constitutive forms HO-2 and HO-3 are highly concentrated in neurons, spleen and liver. The induction of HO-1 in response to an oxidative stress, suggests its proposed role in protecting cells against oxidative stress related injury. The overall effect of HO is to remove a pro-oxidant (heme) while generating an antioxidant (bilirubin) and pro-oxidant (iron, Fe^{2+}). The balance between the

injurious and protective roles of HO is dependant on the actions of heme, bilirubin, CO and Fe_2^+. This review addresses the proposed antioxidant and pro-oxidant functions of HO enzyme.

Introduction

An imbalance between production of ROS and cellular antioxidant defense mechanisms may result in oxidative stress, which may be implicated in the pathogenesis of several human diseases. The induction of intracellular antioxidant proteins may act as cytoprotective defense against oxidative stress. These stress proteins include the heat shock proteins, metallothionein and hemeoxygenase -1. The microsomal 32 kDa protein heme- oxygenase catalyzes the rate-limiting step in the conversion of heme to bilirubin. (see Figure 1).

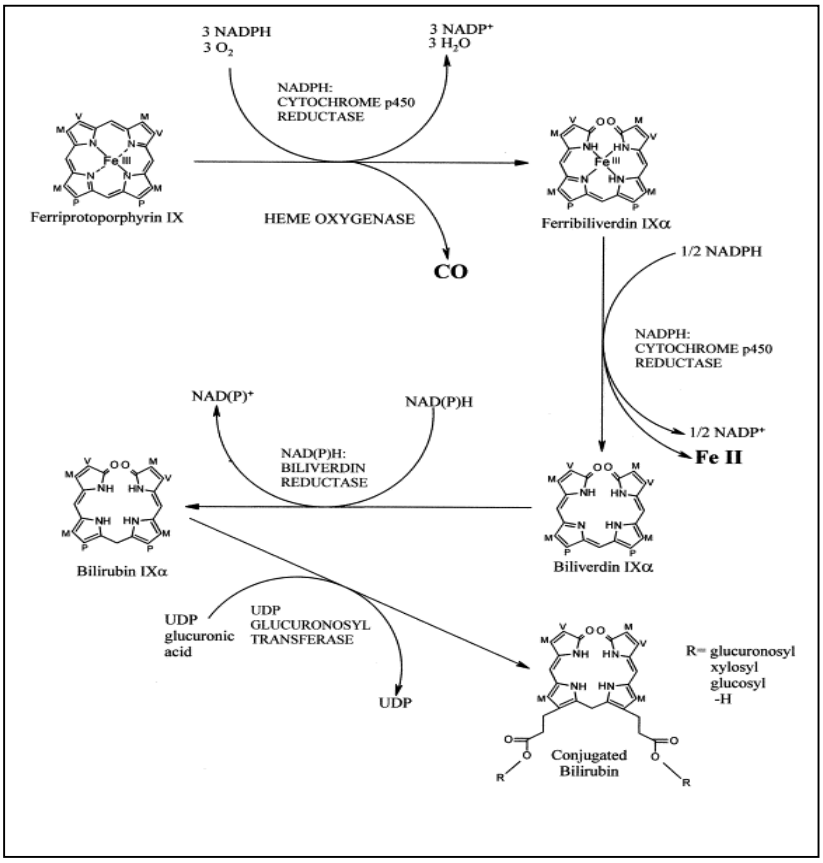

Figure1. Heme degradation. Microsomal heme oxygenase, catalyzes the rate limiting step in heme metabolism. Both heme oxygenase isozymes (HO-1 and HO-2) oxidize heme (ferriprotoporphyrin IX) to the bile pigment biliverdin-IXα (BV), in a reaction requiring 3 moles of molecular oxygen. NADPH:cytochrome p-450 reductase, reduces the ferric heme iron as a prerequisite for each cycle of oxygen binding and oxygen activation. The cleavage of the heme ring frees the α-methene bridge carbon as carbon monoxide, and generates the biliverdin-iron complex (BV-Fe-III). An additional NADPH dependent reduction releases Fe-II from BV. The BV is reduced to BR by NAD(P)H:biliverdin reductase. Heme side chains are marked: M = methyl; V = vinyl; P = propionate [1].

Heme oxygenase cleaves the α mesocarbon bridge of b type heme molecules to yield equimolar quantities of biliverdin IXa, carbon monoxide, and free iron. Biliverdin is subsequently converted to bilirubin by the action of biliverdin reductase and free iron formed is promptly sequestered to ferritin.

Forms of heme oxygenase

Three forms of heme oxygenase have been identified, HO-1, HO-2 and HO-3. HO-1 is a 32 kDa protein that is inducible by an extraordinary array of stimuli, including hydrogen peroxide, irradiation, heavy metals, chemotherapeutic agents, microbial products such as endotoxin, hypoxia, hyperoxia, hormones, various cytokines and heme itself [2]. HO-2 is constitutively synthesised 36 kDa protein existing in brain and testes [2]. HO-3 is recently cloned gene product, 33 kDa in size and is involved in catalyzes of heme degradation.

Functional significance.

The functional significance of HO-1 induction following oxidative stress is not well understood. Nevertheless, recent studies are supportive of the hypothesis that HO-1 induction plays an important role in cellular protection against both heme and non heme mediated oxidant injury. Many toxins that induce HO-1 expression promote oxidation reactions that destabilize intracellular heme proteins, releasing the heme moeity. The actions of HO-1 help the cell to get rid of pro-oxidants and allows the cells to withstand further exposure to noxious stimuli [3].

Role of heme oxygenase in tissue injury and protection.

Though studies have shown that HO1 has a cytoprotective role, the exact mechanism in which HO-1 inhibits inflammation and protects endothelium and smooth muscles from oxidative stress is only partly known (See Figure:2).

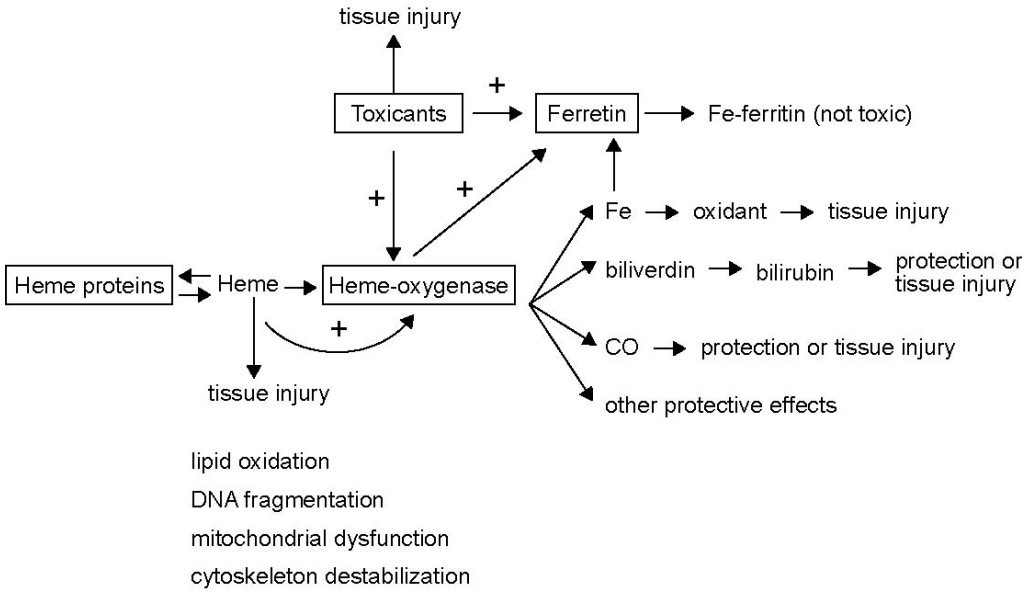

Figure:2. Shows the role of heme oxygenase in tissue injury and protrection. Heme released from heme proteins can induce cellular injury. Heme oxygenase, induced by endotoxins, oxidants and cytokines protects against tissue injury by: metabolising heme; fostering the synthesis of iron sequestering protein ferritin; producing carbon monoxide (CO), which is vasodilatory; promoting production of bilirubin, an antioxidant ; and triggering cellular protection against toxicants through pathways as yet undefined. This figure is adapted from [3].

The mechanism by which HO-1 can mediate the cytoprotective function may be through the major catalytic by-products of heme degradation, carbon monoxide, ferritin, and bilirubin. Carbon monoxide is considered lethal at high concentrations, but at low concentrations it can regulate vasomotor tone as well as neurotransmission [4]. This regulatory action may account for the anti inflammatory effects of HO-1 expression in endothelial and smooth muscle cells because vasodilation may allow maintenance of basal blood flow at sites of inflammation.

Bilirubin has shown to be a potent antioxidant in the brain and functions as a chain breaking antioxidant, and is found to be efficient in scavenging the peroxyl radicals [5]. Other antioxidant effects of bilirubin include reaction with $O_2^{\bullet-}$ and HOCl, quenching of $^1O_2$, inhibition of photoxidative damage to protein and inhibition of chemiluminescence in active macrophages [6]. Biliverdin administration to rodents has also shown to provide protection against ishaemic heart injury in studies involving rat model [6].

It is important to note that one of the consequences of heme degradation is the formation of Fe_2^+. HO-1 activity recruits ferritin, which sequesters iron, thus avoiding iron dependant oxidative stress [3]. The ability of cells expressing HO-1 to decrease iron content has recently been suggested to account for the antiapoptotic effects of HO-1 [7].

Pro-oxidant consequences of HO activity.

The carbon monoxide , bilirubin and Fe_2^+ released during the HO mediated degradation of heme can be toxic to the cell. Carbon monoxide is a lethal gas at high concentration and it can increase apoptosis [8]. Bilirubin has been implicated in tissue injury in kernicteurus (pathological accumulation of bilirubin in brain) and in renal injury that accompanies hepatic failure [9]. The HO activity releases reduced iron directly into the endoplasmic reticulum. The pro-oxidant effects of iron include the stimulation of lipid peroxidation, generation of $HO^{\bullet}$ through Fenton reaction, formation of hypervalent oxidizing complexes, the promotion of oxidative DNA damage, and the sensitisation of cells to various oxidants [10]. We can infer that HO enzyme function has both indirect antioxidative and potential pro-oxidant consequences, depending on the magnitude of induction.

Regulation of Heme oxygenase

Studies suggest that the modulation of the rate of gene transcription is the principal mechanism by which most of the inducing agents regulate HO-1 production. HO-1 is induced by ROS, sulfhydryl-reactive agents, heavy metals, and by depletion of glutathione levels. Various regulatory elements have been identified within the 5′ flanking region of HO-1 genes. In most cases, these motifs are equivalent to or variations of recognition sites for known DNA binding protein, including those for the Fos /Jun (AP-1) and NF kB/Rel family of proteins - the two major oxidative stress responsive transcription factors in mammalian cells [11].

The various gene elements that mediate inducer dependant gene activation are as follows:

1. The stress response elements : Inducer responsive elements have been identified in 3 regions of HO-1 gene, a proximal enhancer region and two distal enhancer regions. Most inducing agents except hypoxia acts by expressing this element [11].
2. IL-6 and hyperoxia response elements: Several cytokines, including IL-6, activate HO-1 gene transcription. Hyperoxia also activate gene expression by the same regulatory elements [11].
3. Hypoxia response elements: These elements are located directly downstream of the stress response elements [11].
4. Heat shock response elements: Induction of HO-1 gene by hyperthermia has been demonstrated in rat models.The gene elements for this are located in the proximal enhancer region [11].
5. Prostaglandin response element: Type A and J prostaglandins are suggested to have a role in inducing these elements [11].

6. TPA and cadmium response elements: The TPA associated element is localized to the promoter region and the cadmium associated element is located in the distal enhancer region [11].

In summary, activation of mammalian HO-1 gene is mechanistically complex because these loci are under control of inducer specific elements.

Role of heme oxygenase in pathological conditions.

Studies have shown the increased expression of HO-1 levels in variety of pathological conditions like atherosclerosis, acute renal failure, alzheimers disease, cancer, transplant rejection, lung injury. The induction of HO-1 has been demonstrated in many models of lung injury including hyperoxia and endotoxemia . Studies on rodent model by Suttner and Dennery [12] have shown that moderate overexpression of HO-1 in fibroblast is protective against oxidative injury, whereas high levels of HO-1 expression can be associated with significant oxygen toxicity. The cellular cytotoxicity in this situation is attributed to the reactive iron released from the catalyzes of heme.

Summary

Heme oxygenase degrades heme to form bile pigments and carbon monoxide. It is readily inducible in oxidative stress. It would seem that molecules such as HO are not exclusively cytoprotective or exclusively cytotoxic but rather they may contribute in many complex ways, to the balance of inflammatory and reparative pathways that determine the fate of cells and tissues.

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