·LECTURE·

Heme Oxygenase: a Heme Catabolic Enzyme and More

Phyllis A. Dennery, Yi-Hao Weng, David K. Stevenson, Guang Yang

Department of Pediatrics, Stanford University, School of Medicine, Stanford, California, USA

[CLC number] R722.17 [Document Code] A [Article ID] 1008-8830(2001)04-0442-04

Introduction

Heme oxygenase (HO) is the first and rate-limiting enzyme in the formation of bilirubin. This enzyme allows for the degradation of heme from hemoglobin of other heme containing proteins to form biliverdin. This process is energy requiring because NADPH donates electrons via the cytochrome cP450 system and molecular oxygen is consumed for the liberation of iron from the porphyrin ring of heme, the release of carbon monoxide (CO), as well as the formation of biliverdin (Figure 1). Biliverdin reductase, a microsomal enzyme, allows for the reduction of biliverdin to bilirubin. A microsomal assay demonstrated increased HO activity in the presence of hemin, the substrate for the reaction[1]. Other compounds were found to upregulate HO activity such as cobalt chloride and various heavy metals[1,2]. Maines[3] identified a developmental pattern of heme catabolic enzymes thereby suggesting an explanation for the overproduction of bilirubin in neonatal jaundice. In addition, better purification and characterization of the 32 kd HO-1 protein was accomplished and, with the synthesis of metalloporphyrins, HO activity could be inhibited leading to a further understanding of the cellular consequences of HO activity and to the development of strategies for the prevention of neonatal hyperbilirubinemia. In the 1980's characterization of the constitutive form, HO-2 was achieved[4]. In the late 1980's, the regulation of the HO-1 gene was addressed. Shibahara and others demonstrated the induction of HO-1 at the transcriptional level[5]. As the field advanced, HO-1 gene induction was noted to be a generalized marker of oxidative stress[6]. This ubiquitous nasture of the HO-1 inducible response is now clear. As molecular biology and molecular genetics techniques improve, the biochemistry, role and regulation of HO are becoming more clearly understood.

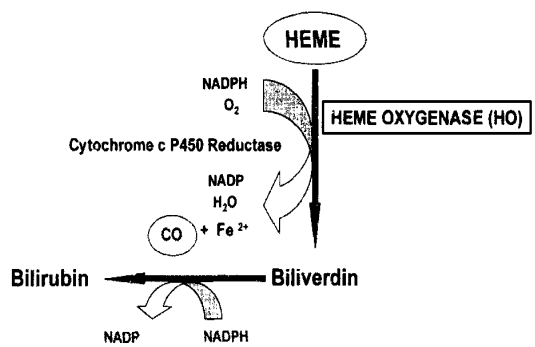

Figure 1 Pathway of heme degradation
Heme is metabolized by heme oxygenase (HO) to form carbon monoxide (CO) and iron (Fe^{2+}). This reaction is energy requiring as the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) and molecular oxygen (O_2) are metabolized by cytochrome cP450 reductase to the oxidized form of nicotinamide adenine dinucleotide phosphate (NADP) and water (H_2O_2). Biliverdin is further reduced to bilirubin by biliverdin reductase and NADPH is also oxidized to NADP in this step.

[Received] April 28, 2001
[A Brief Introduction to the First Author] Phyilis A. Dennery, M.D., Associate Professor of Pediatrics.

Regulation of HO

The organization of the HO-2 gene remains relatively obscure. Although HO-2 is referred to as the constitutive isoform of HO, it has been shown to be up-regulated by a few factors to date such as with corticosterone in fetal rat brain[7], and with the NOS inhibitor, N (G)-nitro-L-arginine and the HO inhibitor, zinc mesoporphyrin (ZnMP) in cultured rat aortic cell[8]. A consensus sequence of the glucocorticoid response element (GRE) in the promoter region of the HO-2 gene has been demonstrated and specific binding of glucocorticoid receptor protein to the GRE was also observed[7]. It is not known whether other factors can induce HO-2 but preliminary observations in the HO-1 knockout mice reveal increased lung HO-2 mRNA at baseline[9], suggesting a compensatory up-regulation of HO-2 in the absence of HO-1.

Much more is known about the regulation of the HO-1 gene and its promoter. The inducibility of HO-1 is explained by the configuration of the HO-1 gene. The 6.8 kilobase (kb) HO-1 gene is organized into 4 introns and 5 exons. A promoter sequence (not TATAA) is located approximately 28 base pairs upstream from the transcription initiating site. There are several transcriptional enhancer elements in the 5' flanking region, including heat shock elements and metal regulatory elements[10]. Inducer responsive sequences have been identified in the proximal enhancer directly upstream of the promoter. Two or more distal enhancers located 4 kb and 10 kb upstream of the transcription initiantion site also exist[11] (Figure 2). The many factors that affect HO-1 gene regulation do so via different binding regions on the HO-1 gene. The HO-1 promoter region contains and antioxidant response element ARE with the consensus sequence GCnnnGTA as with other antioxidant enzymes[12]. Heme treatment results in a specific and marked increase in the NF-kappa B and AP-2 transription factors, a sequence upstream between positions - 3.5 and 12.5 kb is required for the induction by cadmium (CdRE) and the distal enhancer regions are important in regulation of HO-1 in inflammation[10]. This demonstrates that HO-1 is readily inducible.

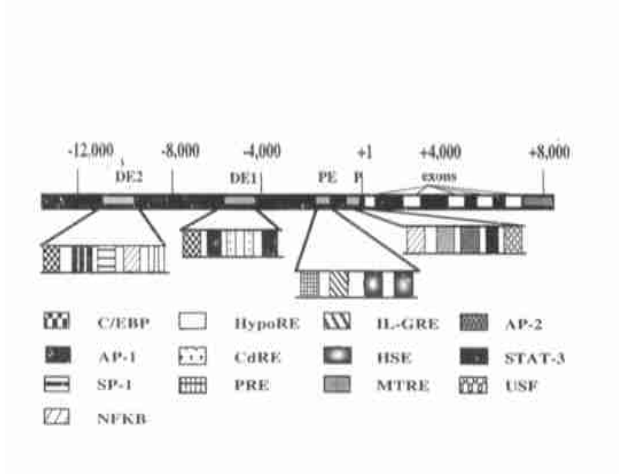

Figure 2 Illustration of the HO-1 gene. This gene contains 5 exons (white boxes) and 4 introns (black boxes between introns). There is promoter (P), a proximal enhancer (PE) and 2 distal enhancers (DE1 and DE2). Each of these regions contain transcription factor binding sites (patterned boxes within the brackets). C/EBP: C CAT enhancer binding protein; AP-1 activator protein-1, SP-1: stress protein-1; NF-KB: nuclear factor kappa B; HypoRE: hypoxia response element; CdRE: cadmium response element; IL-GRE: glucocorticoid response element; HSE: heat shock element; MTRE: metal response element; AP-2: Activator protein-2; STAT-3: signal transducer and activator of transcription; USF: upstream stimulatory factor

Cytoprotective Role of Heme Oxygenase

Because of its oxidative regulation, HO-1 is not merely catalytic but a synthetic enzyme for the formation of potent antioxidant bile pigments and the important neurotransmitter carbon monoxide (CO). Due to the production of bilirubin, a potent antioxidant surpassing vitamin E[13] and the sequestration of heme, a pro-oxidant molecule, the overall effect of the HO-1 reation may be cytoprotective. Nonetheless, recent evidence suggests that HO-1 is not always cytoprotective and that there may be a beneficial threshold of HO-1 induction in cells. In a model system allowing for regulation of HO-1 in a dose-dependent fashion, HO was a pro-oxidant at higher levels (greater than 5-fold increased activity) due to the associated release of reactive iron as a consequence of increased HO activity[14]. Furthermore, the association of HO-1 protein with neurons in degenerative brain diseases and the protection of these neurons by HO inhibitors[15] suggest a detrimental role for HO-1 over-

expression in certain disease processes.

With the availability of HO knockouts, the understanding of the function of HO has improved. Null mutants for HO-2 have increased susceptibility to hyperoxia despite induction of HO-1[16] and have ejaculatory abnormalities[17]. The neurons of these animals are also more susceptible to oxidative injury[18]. As to HO-1 null mutants, these animals age faster than their wild type counterparts and demonstrate anemia and iron deposition in the kidneys and liver[19]. Additionally, these animals are more likely to demonstrate cardiac xenograft rejection[20], have abnormal inflammatory responses[19] and increased renal injury in response to an ischemic insult[21]. Despite the strong evidence to suggest a protective role for HO-1 in many situations, HO-1 disruption in the lung was not associated with increased susceptibility to hyperoxia[16] nor was HO-1 overexpression[22].

Role of CO in Cellular Function

Another by-product of the HO reaction that has received considerable attention is carbon monoxide. This diatomic gas is known to be extremely toxic at high concentrations as it interferes with the delivery of oxygen to tissues by binding tightly to hemoglobin[23]. Nonetheless, CO has recently been shown, like nitric oxide (NO), to be a physiologic regulator guanosine 3', 5'-monophosphate (cGMP). This results in both vasodilatory effects and altered neurotransmission[24]. Additionally, CO at low concentrations both in vivo and in vitro, selectively inhibited the expression of pro-inflammatory cytokines and increased the expression of the anti-inflammatory cytokine interleukin-10 through a pathway involving the mitogen-activated protein kinases (MAPK) rather than through a guanylyl cyclase-cGMP or NO pathway. These investigators have also demonstrated that expression of HO-1 or exposure of endothelial cells to exogenous CO enhanced p38 MAPK activation by TNF-alpha resulting in decreased apoptosis. Furthermore, specific inhibition of p38 MAPK activation abrogated the antiapoptotic effect of HO-1[25]. Phosphorylation of this kinase and subsequent downstream events occur early in response to cellular stress. Some have demonstrated a clearly cytotoxic effect of this event[26] whereas others demonstrate a protective role for this kinase[25]. These contradictory findings further illustrate the complexity of understanding the physiologic role of the HO/CO system.

Nonenzymatic Roles for HO

Previously it had been suggested that HO was found in only in the microsomal fraction attached to the smooth endoplasmic reticulum. However, recent investigations have demonstrated that HO can be found in the nuclear fraction suggesting that HO may play a signaling role. In fact, we have recently demonstrated that transfection of HO-2 into NIH 3T3 cells expressing an HO-1 promoter attached to the luciferase gene resulted in increased HO-1 gene expression. It appears that this effect is due to the presence of the HO-2 protein itself as truncation of this protein altered the HO-1 regulatory effect. Others also suggest a signaling role for HO-2 as they demonstrate that this enzyme can be phosphorylated via the protein kinase C pathway[27]. Furthermore, there appears to be an interaction of HO-1 and HO-2 protein within cells and a binding site for the HO-1/HO-2 protein complex on the HO-1 gene. Therefore, HO-1 mey be involved in the regulation of its own gene transcription. HO-1 serves to degrade heme, yet, in the absence of heme, there is still a fair amount of HO-1 present in tissues. Perhaps a pathway that allowed for the upregulation of HO-1 in the absene of heme would permit constitutive tissue expression of HO-1 when no stress is present.

Others suggest that HO-1 may be involved in the regulation of other genes such as superoxide dismutase[28]. This could also allude to a role for HO-1 as a signaling molecule.

From its humble beginnings as a catabolic enzyme, HO appears to be an important molecule with roles in oxidative stress, inflammation, immunity as well as cellular regulation and signaling.

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(本文编辑:俞燕)