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Mini Review

The Heme Oxygenase System: Update 2005

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INTRODUCTION

TWO YEARS AGO I assembled a Forum in this Journal that was consecrated to the field of heme oxygenase (HO). Since then, the phenomenal rate of expansion of the field, which extends its reach into the very core of many systems, justifies this re-visitation. In this time span, over 1,300 articles have been published that directly or indirectly refer to the HO system and heme degradation products. The majority of this new information extends the topics that were addressed in the Forum including regulation of HO-1 gene expression (5, 18, 81, 84), antioxidant activity of bile pigments (8, 73), and the function of the HO system as a key component of cytoprotection against oxidative stress and free radical-mediated insult in various tissues—the brain (45, 66), kidneys (80), and the heart (54). Clearly, it would not be feasible to acknowledge the vast majority of the scholarly contributions that have been made to those topics in the past 2 years; a small minority is selected for this visitation. First, however, a brief introduction to the basics of the system is provided.

WHAT IS "HEME OXYGENASE" AND WHY IS IT NEEDED?

Not every system that degrades heme is a "heme oxygenase." Degradation of the heme molecule (Fe-protoporphyrin IX) can be accomplished both by enzymes and by chemical processes (38). The most effective mechanism for oxidative degradation of the molecule in the cell, and the only such process that results in formation of an equimolar ratio of CO and biliverdin IXα isomer is the HO system; the system consists of two isozyme, known as HO-1 and HO-2, or as heat shock protein 32 cognates (30, 43). Two years ago Sassa (65) addressed the question of why heme needs to be degraded by the HO system by aptly commenting that it is the only system that produces CO and biliverdin IXα and releases the chelated iron from the tetrapyrrole macromolecule. All the products of HO activity play vital roles in the cell. The crucial function of iron in the evolution of aerobic life, the signaling function of CO, and free radical quenching activity of bilirubin IXα all are now commonly acknowledged, and are discussed in many reports (1, 7, 12, 17, 56, 62, 64, 69, 71, 82).

The term "heme oxygenase," however, has been liberally used in reference to any cDNA having a sequence of nucleotides similar to that encoding the "heme-binding pocket" domain of HO-1 and HO-2, now known as the "heme oxygenase signature" (13, 37, 60); this is the case with a pseudogene variant of HO-2, referred to as HO-3, which is found only in the rat brain (49).

Although within each isozyme there is a good deal of conservation of the primary structure across species, beyond the heme-binding pocket the extent of sequence identity between HO-1 and HO-2 is less than 50%. In fact, there is no resemblance between the two forms at levels of gene structure, regulation, or tissue expression patters (38, 42).

Although the traditional perception of importance of the HO system is in reference to its function in the animal kingdom, with particular emphasis on hemoglobin and hemoprotein turnover, the importance of the pathway to all forms of life, including bacteria, is now acknowledged and was elaborated upon by Frankenberg-Dinkel (20).

REGULATION OF *HO-1* AND *HO-2* GENE EXPRESSION

Arguably, the number of stimuli responsible for activating *HO-1* gene transcription far exceeds that of any other single gene. The large array of stimuli of diverse structural features that have the ability to activate *HO-1* is reflective of multiple response elements within its promoter and multiplicity of interactions between components of different cell signaling cascades. This has been the subject of a number of recent reviews and reports (4, 5, 15, 28, 29, 31, 32).

The mitogen-activated protein kinase (MAPK) pathway was the first recognized as a link for extracellular stimuli of stress-mediated induction of *HO-1*. Many of the proteins linked to this pathway are members of the basic "leucine zipper" (bZip) family of transcription factors; the originally

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identified members were activator protein-1 (AP-1) and activating transcription factor (ATF)/cyclic AMP response element binding protein (CREB), CCAAT/enhancer protein, and Maf proteins (6); Maf transcription factors can be small or large. The more recently characterized bZip transcription factors include Bach-1 and -2, nuclear erythroid 2-related factor-2 [Nrf2 (NF-E2)], and biliverdin reductase (BVR) (32, 33, 36, 51, 79). Because bZip transcription factors can bind DNA in a homodimeric form or as a heterodimer with other bZip factors, their effector function in transmission of stress signals by way of the MAPK signaling pathways for regulation of gene expression is dependent on their dimerization partner.

The most recently identified member of bZip family of transcription factors is BVR (3, 33, 40). BVR functions in both major arms of the insulin/insulin-like growth factor (IGF)-1 signaling pathway (MAPK and phosphatidylinositol 3-kinase) (34, 50) and appears to serve as a mechanism for integrating both arms of the insulin/IGF signaling pathway for regulation of *HO-1* expression. This suggestion is supported by finding that phosphatidylinositol 3-kinase, which is directly downstream of the insulin–IGF signaling cascade, is involved in the Nrf2-mediated regulation of *HO-1* by heme (52).

BVR regulates a significant number of genes that mediate oxidative stress response of the cell, including that of *HO-1*. The identified target genes include ATF-2/CREB-2, c-Jun, heat shock transcription factor 1, Bcl2, cyclooxygenase-2, protein kinase Cα, heat shock proteins 90 and 27, and inducible signaling pathway protein 3. BVR activates ATF-2 at levels of transcription, protein, and phosphorylation, *i.e*., activation (33, 50).

ATF-2 forms a dimer with other bZip transcription factors, including c-Jun. Binding of ATF-2 in homo- or heterodimeric form to AP-1/cyclic AMP response element activates *HO-1* expression in response to oxidative stress. The Nrf2/ATF-4 heterodimer also has been shown to induce *HO-1* expression (26). It is reasonable to suspect that BVR regulation of *HO-1* gene expression extends to its heterodimeric complexes with various bZip factors, including ATF proteins 1–6.

What is emerging in the course of recent developments is the central role of the heme molecule in controlling transcriptional activity of bZip factors; heme binds directly to Bach-1 and triggers its nuclear export. This terminates transcriptional repressor activity of Bach-1 (22, 53, 75, 77, 78). Heme binding involves the specific coordination of the chelated iron with the cysteine residue in the cysteine-proline (CP) dipeptide motif of Bach-1. The latter plays a central role in mediating the response of *HO-1* to oxidative stress and hypoxia. By having the CP motif, Bach-1 becomes a member of a select group of proteins that possess this; HO-2 is another member of this exclusive group of proteins, all of which are linked to heme-regulated functions in the cell. The role of HO-2 in such a capacity is discussed below.

Nearly 2 decades ago a single criterion was found in common among inducers of *HO-1* gene expression, which is the ability to lower the intracellular thiol/disulfide (SH/S-S) ratio and to cause oxidative stress (36). Now it is well known that increased transcription of *HO-1* is the result of activation redox-sensitive transcription factors (AP-1 and Nrf2, and the repressor factor, Bach-1). Activation of *HO-1* by a wide range of stimuli, including by cobalt ion, the first oxidative stress-inducing agent shown to regulate *HO-1* expression (41), is now found to involve Nrf2 and nuclear export of Bach-1 (22, 75, 76). The ratio of reduced glutathione/oxidized glutathione in the cell dictates the efficiency of Nrf2 binding to antioxidant response element.

While the *HO-1* gene is a simple structure composed of five exons and four introns, mammalian *HO-2* has a decidedly more complex gene structure (35, 47). Five or more transcripts for *HO-2*, ranging in size from 1.3 to 2.4 kbp, are observed in mammals, with each showing distinct tissue-dependent abundance of patterns (47). Two transcripts of 1.3 and 1.9 kbp are the most widely expressed species, and use alternate polyadenylation sites (76). The only demonstrated functional response element in the promoter sequence of *HO-2* is the glucocorticoid response element (46, 58, 83). HO-2 is the prominent form of HO in the brain and testis, and *HO-2*'s insensitivity to stimuli that induce *HO-1* is not surprising (19, 57). It should be noted that the transcripts that use the second poly(A) signal are not effectively translated (76). The HO-2 transcripts are also sensitive to hypoxia and redox status of the cell (44, 47); this is manifested by changes in the ratio of the 1.3-kbp and 1.9-kbp transcripts.

FUNCTION OF THE HEME DEGRADATION PRODUCTS

The discovery of the links between the free radical quenching activity of bilirubin and the function of CO as an activator of soluble guanylate cyclase (sGC) (10, 74) has precipitated an outburst of reports on cytoprotective and signaling activities of those entities. Accordingly, there are many comprehensive reviews of the anti-inflammatory, anti-apoptotic, and anti-oxidant functions of HO activity products, as well as their participation in cell signaling and maintenance of vascular homeostasis (1, 7, 12, 62, 64, 69, 71, 82).

Not all reports, however, indicate a beneficial outcome to the cell as a consequence of induction of *HO-1*. For instance, although ample evidence for a cytoprotective function of bilirubin in the cell has been found, hyperbilirubinemia remains a matter of clinical concern. The condition has been implicated as a prominent factor in mortality caused by sepsis in patients with obstructive jaundice and in neonatal jaundice (25). Similarly, while HO-1 is typically considered to have an anti-apoptotic influence, an increase in its activity has been associated with apoptosis. Increased apoptosis can be viewed both positively or negatively: The negative effects are exemplified by bilirubin-induced apoptosis of developing neurons (23), while on the positive side, increased HO activity chaperones damaged neurons toward the path to apoptotic death rather than necrosis (55).

Heme iron presents a similar argument. Oxidative stress may be construed as a consequence of HO activity and liberation of chelated iron from the heme molecule, or the activity can be viewed as a means to generate an antioxidant and a vasodilator and to remove heme that can rather nonselectively bind to cellular constituents and alter their structure and function. Overall, provided that the cellular capacity to sequester

free iron in ferritin and transferrin is not compromised, increased HO-1 activity will be cytoprotective. Both heme and iron are catalysts for generation of the reactive oxygen radical (17, 56).

It appears, then, that the role of the HO system in cytoprotection may be dependent on the cellular milieu as to its being beneficial or detrimental to the cell. This aids in explaining the controversial reports encountered in the literature. Notably, three main conditions have been found in common between Alzheimer's disease and in the aging brain: iron accumulation in the mitochondria, increase in HO-1, and loss of complex IV (67, 70).

Other than catalyzing formation of free radicals, the ferrous ion plays a role in a feedback loop for synthesis of NO: The redox active iron activates inhibitory κB kinase (IKK) (86). An intimate, interwoven link for gene regulation and response to oxidative stress is indicated by the elucidation of the role of iron in IKK activation. For the most part, NOS activity is dependent upon the chelatable form of iron to activate IKK/nuclear factor-κB, and hence the availability of the redox active iron.

FUNCTION OF HO-2 AS THE INTRACELLULAR SENSOR OF O_2, NO, AND CO

In the past 2 years, attention has been somewhat shifted from focusing on HO-2 in heme oxidation context to its function as a sensor for gaseous signaling molecules, *i.e.*, O_2, CO, and NO. Although HO-1 and HO-2 catalyze the same heme degradation reaction (38), HO-2 has an additional function that stems from its being a hemoprotein (61). A major difference between the primary structures of HO-1 and HO-2 is the presence of cysteine residues in all HO-2 proteins and their absence in all HO-1 proteins (48, 59, 60, 68, 87). Cysteine is the axial ligand for heme iron in hemoproteins, cytochrome P450 forms, and nitric oxide synthase isozymes. As noted above, in a handful of proteins this residue is upstream of a proline residue, and together they form a CP dipeptide core flanked by positively charged residues N-terminal to cysteine and by the hydrophobic residue phenylalanine C-terminal to proline (88). This motif is known as the heme regulatory motif (HRM), and the few proteins that possess this motif all have a heme/oxygen regulatory function in the cell (88). Two copies of the HRM that are present in the carboxyl terminus of HO-2 tightly bind heme (48, 59, 61) and thus give the hemoprotein character to the enzyme; they are not required for heme oxidation activity of HO-2 (48).

NO, CO, and O_2 have high affinity for the chelated iron of heme. Of these, NO has the highest affinity (9, 16, 27); cysteine itself is the target for NO radicals (72). HO-2's function as an intracellular gas sensor was suggested by detection of high-affinity NO binding to its HRM-bound heme (16), implying a potential HRM function as a "sink" for the heme ligand and an intracellular sensor of oxygen (39). Recent reports confirm this function of HO-2 for a calcium sensitive potassium channel (85). Support for this concept is offered by finding that *HO-2* gene deletion blunts the hypoxic ventilatory response (2). Using a similar gene deletion approach, Chen and Regan (11) have shown that absence of *HO-2* increases astrocyte vulnerability to heme. This would be consistent with the suggestion that one function of HRMs is to sequester heme and protect against pathogenesis of the Fe-protoporphyrin complex. In addition, reduced levels of HO-2 have been detected in placental tissue of women with pregnancy-induced hypertension (14), which suggests a direct correlation between expression of HO-2 and production of CO, the vasodilator and activator of sGC.

Nearly 50 heme-based sensors have been identified in bacteria, plants, and animals (21), although, aside from HO-2, few other gas sensors have been identified in mammals, including sGC and (N)PAS2. NPAS, a rather recently identified transcription factor in the brain that possibly functions in neuronal signaling (24), binds heme, and formation of its complex with DNA is inhibited by low concentrations of CO (21). Therefore, it can be reasoned that HO-2 could influence NPAS transcriptional activity through control of heme levels needed for DNA-binding complex formation, by production of CO to inhibit DNA binding of the complex, and by HO-2 HRMs competing with NPAS for heme and CO.

PREDICTING FUTURE DIRECTION OF RESEARCH

The newly discovered role of BVR in the insulin/IGF signal transduction cascade and in control of oxidative response of the cell offers new uncharted areas for explorations to delineate the role of the heme metabolic pathway in context of human health and disease, and likely to the development of novel therapeutic approaches. Moreover, the open tetrapyrroles—biliverdin and bilirubin—and the heme molecule, often overlooked, yet the oracle in HO-related research, are predicted to assume their rightful center stage in this arena of research and to bridge two pathways: cell signaling and heme metabolism. Recent discoveries, including elucidation of the role of the heme molecule in controlling activity of transcription factors; that of biliverdin, the immediate product of heme oxidation, and bilirubin in modulating protein phosphorylation and kinase activity; plus the wide range of input of BVR in cell signaling pathways both as a kinase (63) and as a transcription factor, are predicted to spearhead this quest. Also, interest in HO-2 is likely to be investigated in context of its function as a cellular oxygen/gas sensor.

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ABBREVIATIONS

AP-1, activator protein-1; ATF, activating transcription factor; BVR, biliverdin reductase; bZip, basic "leucine zip-

per"; CP, cysteine-proline; CREB, cyclic AMP response element binding protein; HO, heme oxygenase; HRM, heme regulatory motif; IGF, insulin-like growth factor; IKK, inhibitory κB kinase; MAPK, mitogen-activated protein kinase; Nrf2 (NF-E2), nuclear erythroid 2-related factor-2; sGC, soluble guanylate cyclase.

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