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REVIEW

HEME OXYGENASE: ENZYME WITH FUNCTIONAL DIVERSITY

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In recent years role of Heme oxygenase (HO) has been considered in nearly all living system including plants, animals and other organisms. The common role of heme oxygenase is the degradation of heme, although there is a diversity of additional role of HO in organisms including iron acquisition, cellular signaling, defense against stress and biosynthesis during metabolism. Likewise, the function of HO is to provide cofactors for the photosynthetic apparatus in cyanobacteria. Heme concentration is variable in different plant species and found maximum in leguminous plant root nodules. Moreover HO has diverse isoforms in plant and animal systems. The review addressed important function of HO and focused on its functional diversity.

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In recent years role of Heme oxygenase (HO) has been considered in nearly all living system including plants, animals and other organisms. The common role of heme oxygenase is the degradation of heme, although there is a diversity of additional role of HO in organisms including iron acquisition, cellular signaling, defense against stress and biosynthesis during metabolism. Likewise, the function of HO is to provide cofactors for the photosynthetic apparatus in cyanobacteria. Heme concentration is variable in different plant species and found maximum in leguminous plant root nodules. Moreover HO has diverse isoforms in plant and animal systems. The review addressed important function of HO and focused on its functional diversity.

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Heme oxygenase (1.14.99.3) is a universal and active enzyme present in animals as well as in plant systems. It catabolizes free heme (Iron protoprophyrin IX) to Fe^{2+}, carbon monoxide (CO) and biliverdin (Shekhawat, Verma, 2010). The ubiquitous expressions of the heme oxygenase gene in the majority of living organism hint that HO may have evolutionary enzymatic role in living system. Substrate of heme oxygenase enzyme is molecule of heme which is highly conserved and exists in environment as a stable prosthetic group for hemoproteins, which act as carriers, electron transporters, hem-based gas sensors and catalysts of biodegradation or biosynthesis. Besides these important roles they can be cellular messengers as well. One of the very important aspects of heme oxygenase is that free enzyme catalyzes the production of free radicals. This enzyme was

originally identified as degrader of heme in rat and was characterized as a distinct protein entity in photosynthetic organism and higher plants. Heme oxygenase activity has been described in algae, cyanobacteria, red algae and cryptophytes. The enzymatic properties of algal heme oxygenase are different from those of animals (Troxler *et al*., 1979) and little bit different from higher plants.

In higher plant HO synthesizes phytocrome chromophore since biliverdin IX acts as precursor for phytocrome chromophrome synthesis (Elich *et al*., 1989) and for protection of cells against oxidative stress (Balestrasse *et al*., 2005; Shekhawat, Verma 2010) as well as attenuation of inhibition of seed germination and salt stress alleviation (Liu *et al*., 2007). Its role has been explored in developmental pathways as stromatal closure (Yu *et al*., 2007) and in leghemoglobin metabolism (Baudorin *et al*., 2004). Due to environmental unpredictable and rapid changes plants are facing different stress. Plants have strong inbuilt defense mechanism against abiotic stress through antioxidant network containing catalase, peroxidase and superoxide dismutase. And heme oxygenase has been recently shown to be an active part of this complex (Balestrasse *et al*., 2005).

Structural comparison of heme oxygenases

Genes encoding heme oxygenases have been found in a wide variety of organisms including mammals, higher plants, red algae, cryptophytes, cyanobacteria and pathogenic bacteria (Muramoto *et al*., 1999). Research on structure of heme oxygenases suggests that fold of HO is a single compact domain mostly assisting of α-helix. Crystal structures of Rat HO1 (rHO1), Synechocystis HO1 (SynHO1), Pea HO1 (PsHO1) are found to be almost similar to that of human HO1, but difference also exists in structure due to evolution. Crystal structure of mammals HO1 reveals that heme is sandwiched between proximal and distal helices with the *d*-meso edge (Unno *et al*., 2007). These two helices serve as a contact sites for heme group.

Similarity of crystal structures has been shown among the higher plants heme oxygenases. Structure of Pea HO1 and Arabidopsis HO1 (AtHO1) has been compared. In the PsHO1 molecule of ascorbate can be readily accommodated in the intramolecular space at suitable distance to interact with heme. Six amino acid residues: Glu96, Phe120, His207, Ile214, Tyr231 and Ser274 are also suitably placed and interact with ascorbate.

The amino acid sequences reported for higher plant heme oxygenases are found to be highly homologous to each other; for example, *Glycine max* HO1 (GmHO1) has 71.7% homology to AtHO1, and enzymes from other plant species have similar levels of homology. In contrast, the homology in amino acid sequences between plant heme oxygenases and enzymes from other biological species is quite low, for example 21% to SynHO1, 22% to rHO 1, 23% to *Corynebacterium diphtheriae* HmuO, and 21% to *Neisseria meningitides* HemO.

Gene family variety of Heme oxygenases

As a plastid-localized enzyme, it might be predicted that *A. thaliana* HO1 would be the most similar to the algal HOs that are encoded in the plastid genome (Reith, Munholland, 1995; Richaud, Zabulon, 1997) or the corresponding cyanobacterial enzyme (Cornejo *et al*., 1998). In higher plants, the gene for HO has been identified in moss plants, several angiosperms (maize, barley, cotton, tobacco, tomato, pea, soybean, rice, and sorghum) and a gymnosperm (loblolly pine), as well as *A. thaliana*

(The Arabidopsis Genome Initiative, 2000; Davis *et al*., 2001). Plant HOs comprise a small gene family with four members in total. This family can be categorized into two distinct classes on the basis of amino acid sequence alignments in HO proteins. One subfamily includes HO1-like genes (including HO3 and HO4 of Arabidopsis) and another includes HO2 genes (Davis *et al*., 2001; Emborg *et al*., 2006).

Four members of the HO family in Arabidopsis are transcriptionally active with substantially overlapping patterns of expression (Emborg *et al*., 2006). The recent results of Matsumoto *et al*. (2004) showed that HO1 is clearly the most highly expressed, followed by HO2, with both HO3 and HO4 expressed at low levels. The amino acid sequences are reported. In higher plants, a chloroplast location for HO1 was confirmed using a green fluorescent protein (GFP) reporter and immunoblot studies, which demonstrated that HO1 was presented predominantly in stroma (Muramoto *et al*., 1999). However, so far it is not clear whether HO1 has its origin in the plastid genome or has a different lineage. While HO1 has been shown to be localized in the chloroplast, the intracellular distribution of the other three heme oxygenases remains to be demonstrated. HO2, HO3, and HO4 appear to have an N-terminal transit peptide sequence for chloroplast import like HO1, but it remains possible that other destinations exist, including mitochondria that can use the encoded enzymes to metabolize hem-containing proteins found in high concentrations in this compartment. Recent evidence show that some N-terminal sequences can simultaneously target proteins to both mitochondria and chloroplasts (Silva-Filho, 2003; Rudhe *et al*., 2004) and one or more AtHOs can be directed to both compartments, but it does not appear to be the norm for other plant species (Shekhawat, Verma, 2010).

Functional diversity of Heme oxygenases

Heme oxygenases are important enzymes also due to their structural similarity and divergence among animal systems and lower as well as higher plant species.

Its major role has been established in animal system as second messenger (Maines *et al*., 1997) and as an antioxidant (Vogt *et al*., 1995). Heme oxygenase is responsible for the physiological breakdown of heme into equimolecular amounts of biliverdin, carbon monoxide, and iron. Three isoforms (HO1, HO2, and HO3) have been identified. HO1 is ubiquitous and its mRNA and activity can be increased several-fold by heme, other metalloporphyrins, transition metals, and stimuli that induce cellular stress. HO1 is recognized as a major heat shock/stress response protein. In contrast, HO2 is present chiefly in the brain and testes and is virtually uninducible. HO3 has very low activity; its physiological function probably involves heme binding.

During the last decade after initial establishing the role of heme oxygenase in plant, research has been explored on diversity of its role in plant system.

The enzymatic property of algal Heme oxygenase from *Cyanidium caldarium* is different from that of animal enzyme. Algal HO from *C. caldarium* has been enzymatic characterized as a soluble and ferredoxin dependent enzyme (Rhie, Beale, 1994). In contrast, animal heme oxygenase is a microsomal enzyme requiring NADPH-cytochrome P450 reductase for heme catabolism. Using data of amino acid sequence product of AtHO1 was predicted to be a soluble protein,

because it does not have a hydrophobic domain for microsomal membrane association at its C-terminus as it has been observed in animal HO1. Instead the AtHO1 protein contains a transit peptide that was sufficient for the transport of GFP in to the plastids. Experiments provide evidences that AtHO1 is accumulated in plastids so it is a soluble plastid protein (Beale, Cornejo, 1984).

Heme oxygenase, a rate-limiting enzyme responsible for carbon monoxide (CO) production, was regarded as a protective system maintaining cellular homeostasis. It was also established that metal ions are powerful HO-inducing agents and cobalt chloride ($CoCl_2$) was the first metal ion identified with an inducing property. Previous study suggests that $CoCl_2$ stimulates adventitious root formation in tomato and cucumber cuttings. It also predicted that both $CoCl_2$ and an inducer of HO1, hemin, could lead to the promotion of lateral root development, as well as the induction of HO1 protein expression, HO activity, or Tomato HO1/2 transcripts in lateral root initiation zone of tomato seedlings.

Beyond all these roles one more important fact about HO is that it is involved in the synthesis of phytochromophores. In higher plants the similarity of the phytochrome chromophore (PCB) to phycobilins and the fact that biliverdin was the precursor of PCB led to the proposal that a similar pathway might be utilized for PCB synthesis. Phycobilin pigments are structurally similar to biliverdin or bilirubin; they are attached to biliproteins and function as assessory photosystem antenna pigments. Light stable phytochrome may play a major role in photoperiodic induction of flowering in short day plants and inhibition in long day plants.

Recently role of heme oxygenase has been also explored as a member of antioxidant network. It shows strong activity against oxidative stress with other enzymes (catalase, peroxidase and superoxide dismutase). Another role of heme oxygenase has been established in developmental biology such as root development.

Future prospects of research on heme oxygenase in plant system

Due to different stress, caused by industrial and urban activities plant productivity is getting affected day by day. In the answer to all of these problems is to make variety of plants those can survive in these stress conditions. By understanding all mechanism of heme oxygenase at genetic and biochemical levels, we can make transgenic plants of HO. Those transgenic plants will be able to survive on the lands suffering from pollution due to metals or salts, or in crops affected by exposure to UV-b radiation. The key players of HO activity are biliverdin, CO and Fe^{++}. We will be able to know about the targets and interactions of these factors and their specific and separate role in plant physiology. By antisense RNA technology we can knockdown the HO gene in different parts of plants and it will help us to know about its regulatory functions in different parts of plants.

By knowing the role of HO in developmental biology we can modify important agricultural plants (*e.g.* crops) according to environmental changes.

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