

## Structures and Reaction Mechanisms of Heme Oxygenases

Masaki Unno\*, Shusuke Kusama, Kouhei Omori, Toshitaka Matsui & Masao Ikeda-Saito  
Institute of Multidisciplinary Research for Advanced Materials (IMRAM), Tohoku University,  
Sendai, 980-8577, Japan

### Introduction

The biological process of heme degradation and turnover is initiated by a family of enzymes referred to as heme oxygenases (HO) that catalyze oxidative degradation of Fe(III) protoporphyrin IX (heme) to biliverdin IX, Fe(II) and CO in the presence of reducing equivalents.

Mammalian HO is a membrane-bound enzyme anchored to the microsomal membrane through a C-terminal hydrophobic tail and exists in two major isoforms, the inducible 33- kDa HO-1 and constitutive 36- kDa HO-2. HO-1 is induced by chemical agents and a variety of stress conditions, and is found in highest concentration in the spleen and liver. In contrast, HO-2 is not induced by exogenous stimuli and is found in highest concentration in the brain and testis. The primary functions of HO-1 are excess heme catabolism and antioxidant defence, whereas that of HO-2 has been proposed to be generation of CO as a physiological messenger molecule. HO-2 has also been reported to function as an oxygen sensor for a calcium-sensitive potassium channel in carotid cells.

Some pathogenic bacteria utilize HO to extrude iron from host heme so as to circumvent the low concentration of free extracellular iron. In comparison to the mammalian HO, none of them is membrane-bound. Instead, they are soluble and have smaller molecular masses. Despite their differences in size, the bacterial HO proteins have overall protein folds, heme environments, and catalytic mechanisms similar to those of the mammalian HO-1.

Heme degradation by HO proceeds via a multistep mechanism, in which HO oxidation consumes a total of three molecules of O<sub>2</sub> and seven electrons. The catalytic mechanism of HO includes several interesting aspects. First, HO is not a hemeprotein *per se* but utilizes heme as both a substrate and a cofactor. Second, the CO produced by HO does not appear to severely interfere with heme degradation, although CO generally binds to ferrous heme iron with a higher affinity than O<sub>2</sub> does. Third, the ferric hydroperoxo species (Fe–OOH) in HO self-hydroxylates the  $\alpha$ -*meso*-carbon of the porphyrin ring to generate  $\alpha$ -*meso*-hydroxyheme in HO. This is different from P450, peroxidases and nitric oxide synthase (NOS), where the O–O bond of the Fe–OOH species is heterolytically cleaved to generate an actively hydroxylating compound I, a ferryl (Fe<sup>4+</sup>=O) species coupled with a porphyrin  $\pi$ -cation radical. Fourth, heme cleavage by HO displays regioselectivity such that only the  $\alpha$ -*meso*-carbon is oxygenated.

In order to understand this unique enzyme catalytic reaction and to elucidate the mechanism for activation of mammalian HO-2, we have been encouraged to solve the structures of human HO-2 (2007G515) and reaction intermediates of HmuO, a heme oxygenase from *Corynebacterium diphtheriae* (2007G516) [1].

### Results and Discussion

#### *HmuO verdoheme intermediate*

We prepared the verdoheme-HmuO complex crystals by two strategies as follows:

- (i) The crystals of verdoheme intermediate were generated by the catalytic reaction from the crystals of ferric heme complex using ascorbic acid at low temperature (~ 6 °C) and the intermediate was trapped at 100 K using cold nitrogen stream. The spectra for these crystals were measured using microspectrophotometer in cryogenic nitrogen stream.
- (ii) Synthesized verdoheme was bound to HmuO and the reconstituted verdoheme complexes were purified and then crystallized. These procedures were performed in the nitrogen glove box.

The crystals prepared by method (i) were well diffracted and the structure was normally determined. The reconstituted verdoheme-HmuO (ii) was crystallized in the space group *C*2, which was different from ferric heme complex *P*2<sub>1</sub> crystals. Now we are analyzing these crystal structures.

#### *Human HO-2*

Recently the truncated and mutated human HO-2 structure was reported by another group [2]. However the structure did not contain any CP motifs, which is also named "heme regulatory motif (HRM)". Approximately 40 residues in the C-terminal regions were truncated in the reported structure from the expression system. In order to reveal the structure of the C-terminal region of HO-2, we crystallized enzyme that contains three CP motifs. We obtained data at 2.0 Å resolution and solved the structure by Molecular Replacement method but the C-terminal region has not been yet unambiguously determined.

### References

- [1] Unno, M. *et al.* (2007) *Nat. Pro. Rep.*
- [2] Bianchetti, C. M. *et al.* (2007) *J. Biol. Chem.*

\*unno19@tagen.tohoku.ac.jp