Biological Science

5A, 6A, 17A, NW12/2005G255

# Structure-function relationships of the heme oxygenase

Masaki Unno[1, 3, #], Takafumi Ueno[2], Norihiko Yokoi[2], Toshitaka Matsui[1], Tomoe Kawanami[1], Mari Iwasaki[1], Shusuke Kusama[1], Yoshihito Watanabe[2], and Masao Ikeda-Saito[1, 3]
[1]Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, 2-1-1 Katahira, Aoba, Sendai, Miyagi, Japan
[2]Department of Chemistry, Graduate School of Science, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8602, Japan
[3]Biometal Science Laboratory, RIKEN SPring-8 Center, Harima Institute, Sayo, Hyogo 679-5148, Japan

Heme oxygenase (HO) is known to catalyze the conversion of heme to biliverdin. HO first binds one equivalent heme to form a ferric heme-HO complex. The first electron donated from the reducing equivalent converts the heme iron to the ferrous state. Then O2 binds to reduced 5-coordinate heme to form a meta-stable oxy complex. A one-electron reduction of the oxy form generates a ferric hydroperoxo complex, which self-hydroxylates the α-*meso*-carbon of the porphyrin ring. The latter reaction is different from what occurs in P450 enzymes, in which O-O bond of the hydroperoxo complex is heterolytically cleaved to generate an actively hydroxylating, ferryl ($Fe^{4+}$=O) intermediate. Ferric a-meso-hydroxyheme is then converted to biliverdin by multiple oxidoreductive steps involving a verdoheme intermediate.

To elucidate the structure-function relationships, we solved some reaction intermediates of the HO catalysis using X-ray crystallography. For the crystallographic studies of the reaction intermediates, we have used HO from *Corynebacterium diphtheriae* called HmuO. In additio, we solved the crystal structures of HmuO those bind Schiff-base complexes, instead of heme, in the heme cavity of HmuO. Further, we are seeking the good inhibitor for mammalian HO. To this end, we solved the crystal structures of the rat HO-1 complexes with the two of the candidates of the inhibitors.

The resolutions of the solved structures and the used beamlines are listed in Table 1.

**Table 1: Solved structures**

| Structure | Beamline | Resolution (Å) | reference |
|---|---|---|---|
| Hydroperoxo intermediate (HmuO) | * | 1.9 | unpublished |
| hydroxyheme intermediate (HmuO) | PF NW12 | 1.5 | unpublished |
| Schiff-base complex 1 (HmuO) | PF BL5 | 1.35 | Ref. 1 |
| Schiff-base complex 2 (HmuO) | PF BL5 | 1.85 | Ref. 1 |
| Schiff-base complex 3 (HmuO) | PF BL5 | 1.75 | Ref. 1 |
| Inhibitor A complex (rat HO-1) | SPring-8 BL38B1 | 2.1 | unpublished |
| Inhibitor B complex (rat HO-1) | PF BL6A | 2.15 | unpublished |

*This structure was solved with data from 20 crystals. The intensity data were collected several beamlines including BL6A, NW12, BL5A, and BL17 of PF and BL38B1 and BL44B2 of SPring-8.

**Figure 1. Electron density map of a Schiff-base complex.**

Schiff-base complexes showed that Arg177 is one of the important residues controlling electron transfer required for the HO catalysis (Figure 1) [1].

The candidates of HO inhibitors are bound to heme iron like that the side chains replace the water molecules those consist of the hydrogen-bonding network [unpublished results].

Manuscripts are now prepared based on these structures, intermediate structures, and some biochemical data for further detailed discussion.

## Reference

T. Ueno et al., Proc. Natl. Acad. Sci. U. S. A., 103, 916-9421 (2006)

#unno19@tagen.tohoku.ac.jp