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# Structural analysis of reaction intermediates and mutants of heme oxygenase

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## Introduction

Heme oxygenase (HO) catalyzes oxidative conversion of iron protoporphyrin IX (heme hereafter) to biliverdin IX, iron, and CO. The enzyme is not a heme protein by itself and uses heme as both the prosthetic group and substrate. In catalytic cycle of mammalian enzymes, HO first binds one equivalent of heme to form a ferric heme-HO complex. The first electron supplied by NADPH through NADPH-cytochrome P450 reductase converts the heme iron to the ferrous state. Then $O_2$ binds to reduced penta-coordinate heme to form a meta-stable oxy-complex. One-electron reduction of the oxy form generates a ferric hydroperoxo species, which self-hydroxylates the α-*meso* carbon of the porphyrin ring. Ferric α-*meso*-hydroxyheme is then converted to biliverdin by multiple oxidoreductive steps involving a verdoheme intermediate.

Mutational studies on polar residues in the distal helix of mammalian heme oxygenase (HO-1) have uncovered a critical residue, Asp140, whose replacement have lead to dominant formation of the inactive ferryl heme. The distal Asp is located far from the heme iron (~8 Å) but forms a H-bond network to an iron-bound water ligand through intervening water molecule(s). Since this distal Asp mutation is not expected to disrupt the distal helix structure, its has been proposed that Asp140, its carboxylate moiety in particular, is crucial in activation of Fe-OOH through the intervening waters. The distal Asp is highly conserved in most mammalian, plant and bacterial HOs, whereas HemO from *Neisseria meningitidis* and PigA from *Pseudomonas aeruginosa* lack this distal Asp. Absence of carboxyl residue in their distal heme pockets has been confirmed in the crystal structures of HemO and PigA. Despite the absence of the distal Asp residue, both HemO and PigA are considered to utilize Fe-OOH in hydroxylation of the heme *meso*-carbon to form *meso*-hydroxyheme as mammalian HO. These findings have raised questions about the general importance of the distal Asp carboxylate proposed for mammalian HO-1, and thus, its reassessment is needed to accurately delineate how Fe-OOH hydroxylates the heme *meso*-carbon.

## Result and discussion

To confirm the mutation-induced changes in the water network, crystal structures of the Asp136 HmuO (HO from *Cryneabacterium diphteriae*) mutants have been examined. The D136N and D136A mutants as well as the wild type crystallized in a space group $P2_1$ and their asymmetric units consisted of three protein molecules. Only the D136E mutant gave *C*2 crystals containing two protein molecules in the asymmetric unit. Overall structures and positions of heme group are almost identical among the wild type and Asp136 mutants (r.m.s. deviations of $C_\alpha$ are within 0.3 Å), and the major structural changes are limited to distal heme pockets.

The longer Glu side chain in D136E HmuO directed to the heme center to diminish its direct interaction with Tyr53 and Arg132 present in the wild type HmuO structure. Arg132 in D136E HmuO rotated away from heme and forms a H-bond with a new water molecule (W7) without considerable movement of Tyr53. This structural change of Arg132 should have little effect on the HO catalysis because rat HO-1 mutants at the corresponding Arg (Arg136) catalyzed the heme degradation properly. The water molecules in D136E were clearly seen as in the wild type except for replacement of W2 by the carboxylate group of Glu136. The W1 water in the mutant is further from W0, 4.2 Å, compared to that in the wild type, and now connected through another water molecule.

To our surprise, the side chain of Asn136 in D136N HmuO flipped away from the heme center and formed a new water bridged H-bond with Tyr53. This "open" conformation of Asn136 may be partly due to incorporation of a few iodide ions, a component of the crystallization solution, in the distal pocket of D136N HmuO; however, wild type and D136E HmuO did not include the large ions at the similar condition. Thus, it is likely that Asn136 intrinsically prefers, to a certain extent, the "open" conformation. Any attempts to remove the large ions were failed to prevent us from discussing the water network for this mutant.

D136A HmuO had similar protein conformation to those of D136E and D136N HmuO including the position of Tyr53 and the moved Arg132 side chain (Fig. 6d). Nevertheless, the water network in D136A HmuO is totally different from those in wild type and D136E HmuO. Electron density of W1 was no longer observed in molecule A of D136A HmuO, while W2 and W3 remained at their original positions. The molecule B of D136A HmuO also lacks W1, whereas the nearby water was found at 3.1 Å from W0 in the C molecule. Partial disappearance of W1 is consistent with the small shift of ν(N–O), high reactivity with $H_2O_2$ and the ~50 % formation of ferryl heme in the Ala mutant. These results support significance of the nearby water in activating Fe–OOH to promote *meso*-hydroxylation by HmuO.

## References

[1] T. Matsui, et al., J. Biol. Chem. 280, 2981 (2005).

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