6A, NW12/2002G139

Understanding of the reaction mechanism of Heme Oxygenase based on X-ray crystallography

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Introduction

Biological heme catabolism is conducted by a family of enzymes termed as heme oxygenase (HO), which catalyzes oxidative degradation of iron protoporphyrin IX (heme) to biliverdin IX, iron and CO in the presence of reducing equivalents. In pathogenic bacteria, HO is essential for heme-based iron acquisition from a host lacking in free extracellular iron.

HO is not a hemeprotein by itself but utilizes heme both a prosthetic group and a substrate. In its catalytic cycle, HO first binds 1 eq of heme to form a ferric heme-HO complex. The first electron donated from the reducing equivalent reduces the heme iron to the ferrous state. Then O_2 binds to it to form meta-stable oxy-complex. One-electron reduction of the oxy-form generates ferric hydroperoxo, which self-hydroxylates the α-*meso* carbon of the porphrin ring to form the ferric α-*meso*-hydroxyheme intermediate. This is different from P450 enzymes, in which the O-O bond of the hydroperoxyo is heterolytically cleaved to generate a ferryl (Fe^{4+}=O) hydroxylating active intermediate. Ferric α-*meso*-hydroxyheme in HO exists as a ferric oxopholin resonance structure that includes a ferrous porphyrin π neutral radical. Upon reaction with O_2 and one electron, ferric-α-*meso*-hydroxyheme is converted to ferrous verdoheme. This conversion has been proposed to be initiated by the dioxygen reaction with the ferrous porphyrin π neutral radical rather than that with the heme iron. The mechanism of the last oxygenation step, the conversion of verdoheme to biliverdin, is least understood, although the verdoheme iron appears to participate in the oxygen activation process in a manner similar to that described in the first oxygenation step.

HmuO, 24kDa bacterial HO, has the high sequence identity with mammalian HO-1, 33% sequence identity to the first 221 amino acids of human HO-1.

Site-directed mutagenesis studies have shown that Asp136 in HmuO is critical for enzyme activity. Mutation of Asp136 on the distal helix decreases or suppresses HO activity drastically and the formation of a ferryl species. In wild-type structure, Asp136 Oδ anchors water molecule W3 which is in part of the long range hydrogen bonding network (1).

To understand the role of Asp136 and oxygen activation mechanism, we have determined some mutants of HmuO-heme complexes.

Results

Crystals

We obtained D136E, D136N and D136A crystals in similar but different crystallization conditions from that for wild-type enzyme. D136A crystals belonged to space group $P2_1$ with almost same cell dimensions with wild-type crystals (1). D136E crystals belonged to space group *C*2 with cell dimensions a=106Å, b=64Å., c=79 Å, and β=130º degrees. For D136N crystals, both types of crystals belonged to space group $P2_1$ and *C*2 were obtained even in the same drops. The Intensity data statistics are summarized in Table 1.

Table 1: Data statistics

mutants	D136E-a	D136E-b	D136N	D136A
Max resolution (Å)	1.6	1.85	1.60	1.85
R_{sym} (%)	5.7	6.4	8.0	8.8
Redundancy	3.7	4.3	5.0	4.2

a, glycerol used as cryo-protectant
b, sucrose used as cryo-protectant

Phases

Phases were easily determined by Molecular Replacement method using the ferric wild-type structure as a search model.

Structures

Refinement statistics are summarized in Table2. In mutants, distal hydrogen bonding network were distorted especially in D136A, which mostly suppress the HO activity. It is revealed that mutation of D136 causes rearrangement of distal water molecules. The manuscript containing these results is now preparing.

mutants	D136E-a	D136E-b	D136N	D136A
R (R_{free}) (%)	17.2 (20.1)	14.8 (19.8)	20.7 (24.1)	16.8 (20.6)

References

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