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Structural basis of activation of mammalian heme peroxidases

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ABSTRACT

The mammalian heme peroxidases including lactoperoxidase (LPO), myeloperoxidase (MPO), eosinophil peroxidase (EPO) and thyroid peroxidase (TPO) contain a covalently linked heme moiety. Initially, it was believed that the heme group was fully cross-linked to protein molecule through at least two ester linkages involving conserved glutamate and aspartate residues with 1-methyl and 5-methyl groups of pyrrole rings A and C respectively. In MPO, an additional sulfonium ion linkage was present between 2-vinyl group of pyrrole ring A of the heme moiety and a methionine residue of the protein. These linkages were formed through a self processing mechanism. Subsequently, biochemical studies indicated that the heme moiety was partially attached to protein. The recent structural studies have shown that the covalent linkage involving glutamate and 1-methyl group of pyrrole ring of heme moiety was partially formed. When glutamate is not covalently linked to heme moiety, its side chain occupies a position in the substrate binding site on the distal heme side and blocks the substrate binding site leading to inactivation. However, an exposure to H_2O_2 converts it to a fully covalently linked state with heme. Thus in mammalian heme peroxidases, the Glu-heme linkage is essential for catalytic action.

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1. Introduction

The heme peroxidases are an important class of heme-containing enzymes which are widely distributed in biological systems. The prosthetic heme group is a derivative of protoporphyrin IX (Fig. 1). It is an essential component of the catalytic scheme in heme peroxidases. There are two superfamilies of heme peroxidases that seem to have arisen independently (Zederbauer et al., 2007). These two superfamilies are broadly divided as non-animal and mammalian heme peroxidases. The heme moiety is non-covalently associated with the non-animal heme peroxidases (Huang et al., 2006; Poulos et al.; Kunishima et al., 1994; Schuller et al., 1996; Wada et al., 2003) while it is covalently linked to protein in the superfamily of mammalian heme peroxidases (Rae and Goff, 1998; Colas et al., 2002; Colas and Ortiz de Montellano, 2003). This is one of the major differences between the members of two superfamilies. The family of mammalian heme peroxidases includes lactoperoxidase (LPO), myeloperoxidase (MPO), eosinophil peroxidase (EPO) and thyroid peroxidase (TPO). The role of covalent

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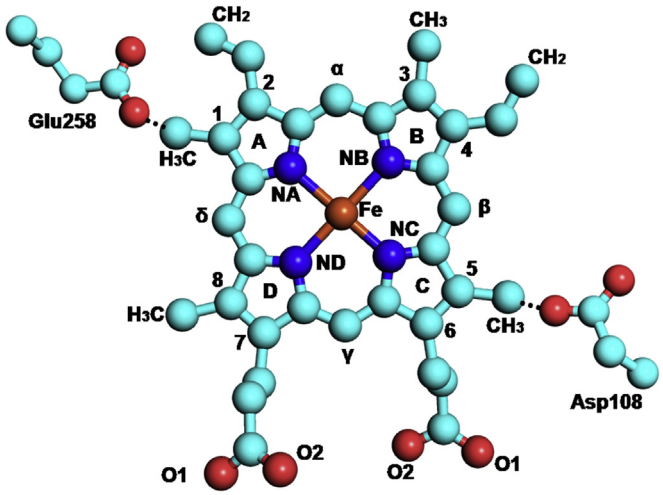

Fig. 1. The prosthetic heme moiety is shown with numbering scheme. The covalently interacting groups with Glu258 and Asp108 are also indicated.

linkages in mammalian heme peroxidases raises an important question as to what are the additional advantages of covalent linkages. Since the heme moiety plays a critical role in the catalytic actions of heme peroxidases, it is of great interest to get further insights into the purpose of covalent linking.

Among the mammalian heme peroxidases, the detailed three-dimensional structures of LPO (Singh et al., 2008; Sheikh et al., 2009) and MPO (Zeng and Fenna, 1992; Fenna et al., 1995) are known but the purpose of the chemical attachment of the heme moiety in these proteins has not yet been clearly stated. According to the current understanding, there are two ester linkages between heme group and protein involving conserved glutamate and aspartate residues with 1-methyl and 5-methyl groups of pyrrole rings A and C respectively of the heme moiety (Furtmuller et al., 2006) (Fig. 2A). A third covalent bond as sulfonium ion linkage is present in MPO between the sulfur atom of a methionine residue and the terminal β-carbon of the vinyl group of pyrrole ring A (Kooter et al., 1999) (Fig. 2B). The covalent linkages are known to have been formed through selfprocessing mechanism in the native mammalian heme peroxidases (Zederbauer et al., 2007; Sievers, 1981; Cals et al., 1991; Suriano et al., 2001). However, recent biochemical (Colas et al., 2002) and structural (Singh et al., 2016a,b) studies have indicated that the ester linkage involving Glu258 (LPO numbering scheme) is observed only in a fraction of protein molecules (Carpena et al., 2009) (PDB IDs: 5B72, 5GLS, 5WV3, 3F9P, 5FIW). It has been stated that the full cross-linking is essential for constructing a wellordered substrate binding site on the distal heme side which may also improve the value of redox potential for an efficient catalytic action (Zederbauer, 2005). In this context, the biochemical studies using a compound, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) have shown that the oxidizing activity of these enzymes improved concomitantly with the increase in the degree of heme-covalent binding (DePillis et al., 1997).

The pairwise sequence identities of amino acid residues of LPO, MPO, EPO and TPO are in the range of 46% to 57%. They have highly conserved amino acid sequences for the substrate binding sites. Structures of LPO and MPO showed that their substrate binding sites on the distal heme side have an overall similar structural architecture indicating similar modes of binding of substrates. The glutamate residue is an integral part of the distal heme cavity. Since these enzymes have similar catalytic actions, the role of cross-linking involving glutamate residue may also have similar effects.

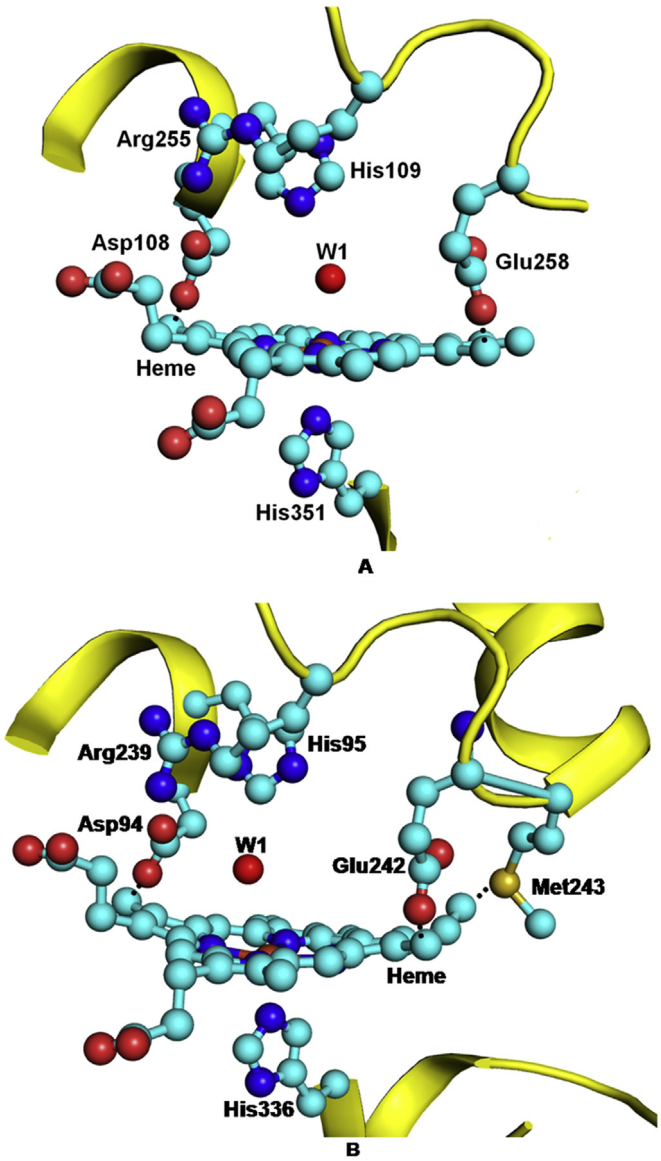

Fig. 2. (A) The prosthetic heme moiety is cross-linked through two ester covalent bonds involving Glu258 and Asp108 with 1-methyl and 5-methyl groups of pyrrole rings A and C respectively in LPO. **(B)** In MPO, the cross-linked ester covalent bond involve Glu242 and Asp94 respectively. It has an additional sulfonium ion linkage involving Met243 with vinyl group of pyrrole ring A.

In this context, there have been multiple questions about the nature and extent of cross-linking of the prosthetic heme group to proteins of the mammalian heme peroxidase superfamily. Initially, it was believed that the heme moieties were fully cross-linked to these enzymes. Later on, some biochemical studies reported that the native proteins were not fully cross-linked (Schuller et al., 1996) and that the cross-linking occurred through a self processing mechanism (Schuller et al., 1996). It is well known that H_2O_2 is an essential co-substrate for the catalytic action of heme peroxidases. It was reported that an exposure to H_2O_2 enhanced the cross-linking from a partially cross-linked state to a fully cross-linked state (Colas et al., 2002). The question also arose as to why the prosthetic moiety was not already fully cross-linked to the protein? Did it mean that the partially linked enzyme was partially active or it was simply an efficient catalytic action? In the partially cross-linked enzyme which covalent bond was fully formed and which

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