Effects of the arrangement of a distal histidine on regioselectivity of the coupled oxidation of sperm whale myoglobin mutants

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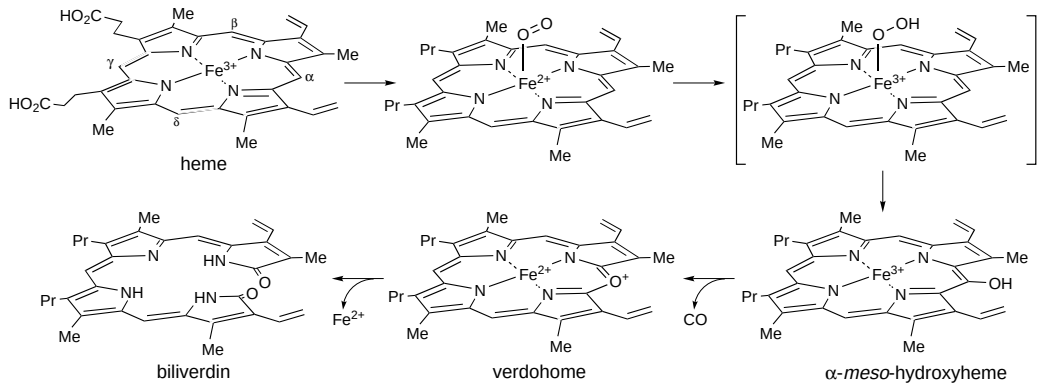
The arrangement of a distal histidine of sperm whale myoglobin alters the regioselectivity of the heme degradation by coupled oxidation.

Heme oxygenase (HO), a monooxygenase of the heme catabolism,[1] utilizes electrons from NADPH and molecular oxygen to transform heme into biliverdin (Scheme 1). The formation of an oxy complex[2] is followed by the hydroxylation of the α-*meso*-carbon presumably *via* a ferric hydroperoxide intermediate.[3] The release of carbon monoxide from hydroxyheme and subsequent ring opening affords biliverdin.[1] Therefore, α-*meso*-hydroxyheme formation from the oxy complex is a key step for the regiospecific opening of the tetrapyrrole macrocycle. Recently, Torpey and co-workers reported that the regiochemistry of the reaction is primarily controlled by the electronic effect of the heme.[4] However, resonance Raman studies suggested that an oxygen molecule of the O_2-bound heme–HO complex had a bent structure and that the terminal oxygen atom was in van der Waals contact with the α-*meso*-carbon of the porphyrin ring.[5] These results might imply that orientation of the bound oxygen also affects the regioselective heme degradation by HO.

Myoglobin (Mb), normally an oxygen storage protein, is also known to transform its prosthetic heme into mainly biliverdin IXα in the presence of ascorbate under aerobic conditions, the so-called coupled oxidation of Mb.[6] Although the coupled oxidation of Mb is much slower than the heme degradation catalyzed by HO, Mb and heme–HO complex both have a histidine residue as a ligand of the heme iron.[7] Furthermore, the crystal structure of oxyMb indicates that the molecular oxygen bound to the heme is restricted by the distal histidine (His-64) toward the α-*meso*-position, and the oxy complex is stabilized by a hydrogen bond with His-64 (Fig. 1). In order to examine whether or not the reorientation of the bound oxygen caused by removal or relocation of the distal histidine affects the regiospecific degradation of heme in Mb, we have constructed H64L, L29H/H64L, F43H/H64L and I107H/H64L Mb and analyzed the biliverdin regioisomers generated by the coupled oxidation of the MBs.§

The high-pressure liquid chromatograph (HPLC) trace of the biliverdin regioisomers prepared from protoheme IX [Fig. 2(*a*)] exhibits four separate peaks. Comparison of the elution profiles of authentic biliverdins allows us to identify the peaks at 8.4, 9.8, 10.4, and 13.6 min as the α-, β-, δ- and γ-isomers, respectively. Wild type Mb affords mainly biliverdin IXα with a trace amount of the β-isomer, as reported previously [Fig. 2(*b*)].[8] Elimination of the distal histidine does not alter the major product [Fig. 2(*c*)]; however, a small amount of the γ-isomer, which is absent in the case of wild type Mb, is observed presumably because the replacement of His-64 with a smaller residue such as Leu allows the bound oxygen to be directed toward the γ-*meso* position (Fig. 1). Interestingly, the His-64 → Leu/Phe-43 → His double mutation significantly alters the product distribution [Fig. 2(*d*)]. Although one of the major degradation products for F43H/H64L Mb is still biliverdin IXα (40%) as observed for the wild type and H64L mutant, a significant amount of the β- (16%) and γ-isomers (44%) are accumulated (Table 1). More importantly, the L29H/H64L mutant is almost regiospecifically oxidized to biliverdin IXγ [Fig. 2(*e*)]. Finally, the substitution of Ile-107 of the H64L mutant with a histidine residue does not change the regioselectivity with respect to H64L Mb [Fig. 2(*f*) and (*d*)].

We have for the first time observed changes in the regioselectivity of the coupled oxidation process of Mb by relocating the distal His.¶ In particular, L29H/H64L Mb is almost regiospecifically oxidized to biliverdin IXγ, although the distal His at position 29 is located too far from the heme iron (6.6 Å)[9] to directly interact with the bound oxygen. The increased amount of the γ-isomer for H64L Mb can be explained by elimination of His-64, which sterically blocked the γ-*meso*-position.[10] Introduction of the His at various positions in H64L Mb tends to further enhance γ-isomer formation. Although the mechanism is not clear at this moment, these observations could imply that the distal His, except for His-64



Scheme 1

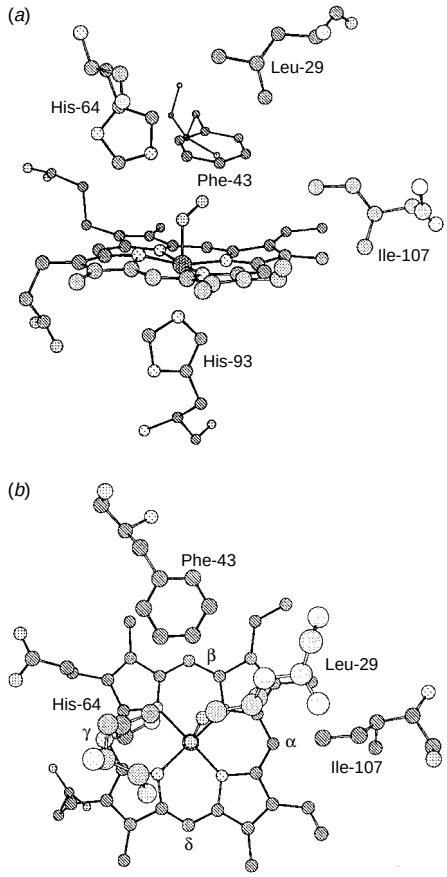


Fig. 1 Heme environmental structure of myoglobin. Heme and some selected residues are shown. (*a*) Side view. (*b*) Top view.

in the wild type, enhances orientation of the bound oxygen toward the γ-*meso*-position by an indirect effect, *e.g.* a biased polarity in the distal site. As discussed before, His-64 in wild type Mb forces the oxygen toward the α-position, thus preventing γ-isomer formation.

Very recently, we have shown that F43H/H64L Mb reacts with H_2O_2 much faster than wild type Mb to form compound I as an observable species.[11] Detailed kinetic studies support the participation of His-43 in F43H/H64L Mb as a general acid/base catalyst, *i.e.* the hydrogen peroxide bound to heme interacts with His-43 *via* a hydrogen bond. In fact, the distance between the terminal oxygen atom and the N_ε atom of His-43 is estimated to be approximately 3 Å, within the range of normal hydrogen bond distances, based on calculations using DISCOVER.[12] Thus, the increase in the ratio of the β-isomer for the oxidation of F43H/H64L Mb would be due to a hydrogen bond between the terminal oxygen atom and the His-43 near the β-*meso*-position in the oxy complex.

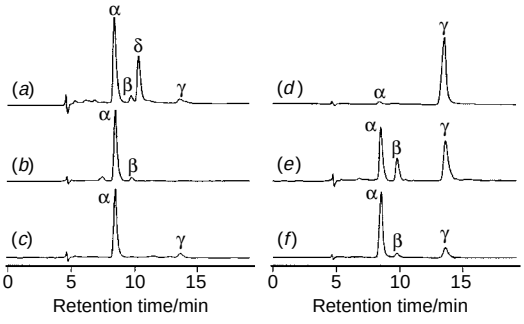


Fig. 2 HPLC analysis of the biliverdin regioisomers isolated from the coupled oxidation of Mbs with ascorbate at 37 °C for 3 h: (*a*) hemin, (*b*) wild type Mb, (*c*) H64L Mb, (*d*) L29H/H64L Mb, (*e*) F43H/H64L Mb and (*f*) I107H/H64L Mb

Table 1 Ratio of four biliverdin isomers produced by the coupled oxidation of Mbs[a]

	Ratio (%)			
Mb	α :	β :	γ :	δ
Wild-type	95 :	5 :	0 :	0
H64L	94 :	0 :	6 :	0
L29H/H64L	3 :	0 :	97 :	0
F43H/H64L	40 :	16 :	44 :	0
I107H/H64L	72 :	6 :	22 :	0

[a] Ratio of the peak area.

In summary, the arrangement of a distal histidine of Mb alters the regioselectivity of the heme degradation even without direct interaction of histidine with the peroxide bound to heme. This type of effect on the regioselectivity in HO reaction has never been considered. Introduction of a more polar or even charged residue in the active site to study the basis for regioselectivity of the coupled oxidation of Mb is now under way.

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Notes and References

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§ To a 250 μl solution of ferric Mb or its mutants (40 μm) was added sodium ascorbate (2 mg) and the resulting solution was incubated at 37 °C for 3 h. Extraction of the biliverdin regioisomers was previously reported by Brown and Docherty.[13] The products dissolved in MeOH were loaded onto a HPLC (Waters 600) apparatus equipped with Waters 741 data module and a TOSOH CO-8020 column oven. A reversed-phase HPLC column (Whatmann Partisil 5 ODS-3) was employed at 40 °C at a flow rate of 0.7 ml min^{-1} with 75 : 25 (v/v) MeOH–25 mM ammonium phosphate buffer (pH 3.5) and monitored at 380 nm. A mixture of four biliverdin regioisomers was prepared from protoheme IX according to the method previously reported [ref. 4(*a*)].

¶ We assumed that the structural framework of the mutant proteins remained unchanged.

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