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Hypothesis

Catalytic Peroxidation of Nitrogen Monoxide and Peroxynitrite by Globins

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Summary

Globins are generally considered as carriers of diatomic gaseous ligands (*e.g.*, O_2 and NO) in metazoa. Recently, the (pseudo-)enzymatic activity of globins towards reactive nitrogen and oxygen species has been elucidated. In particular, some globins (*e.g.*, hemoglobin and myoglobin) catalyze the enzymatic scavenging of NO and peroxynitrite in the presence of H_2O_2. Indeed, H_2O_2 oxidizes some globins leading to the formation of water and of the heme-protein ferryl derivative, which, in turn, oxidizes NO and peroxynitrite leading to the formation of the globin ferric species, NO_2^-, and NO_3^-. Here, we hypothesize that NO, peroxynitrite, and H_2O_2 are co-substrates for the peroxidase activity of some globins, this catalytic activity was reported in 1900 for the first time, even though the substrates have never been identified firmly up to now.

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Abbreviations EPO, eosinophil peroxidase; heme-Fe(III), ferric heme-protein; heme-Fe(IV)=O, ferryl heme-protein; heme-Fe(III)-ONO, *O*-nitrito ferric heme-protein; Hb, hemoglobin; Lb, leghemoglobin; Mb, myoglobin; MPO, myeloperoxidase; Ngb, neuroglobin; trHbN, truncated Hb N; trHbO, truncated Hb O.

The hemoglobin (Hb) superfamily includes several heme-proteins, generally referred to as globins, which are found in all kingdoms of living organisms (*1, 2*). Globin functions have been the subject of active debate, in addition to dioxygen transport and storage. Several functions have been proposed recently, including control of nitrogen monoxide levels, O_2 sensing, and dehaloperoxidase activity (*3–15*).

Globins share physical, spectroscopic, and chemical similarities with peroxidases (*16, 17*). In fact, as demonstrated first in 1900 (*18*), Hb reacts readily with hydrogen peroxide (H_2O_2). In 1923, the peroxidase activity of Hb has been reported (*19*), and in 1938, the modulation of the peroxidase activity of Hb by haptoglobin has been demonstrated (*20*). The reaction of myoglobin (Mb) with H_2O_2, on the other hand, apparently was not considered until 1952 (*21*), and the ability of Mb to catalyze peroxide oxidation of substrates was not reported until 1955 (*22*). Upon reaction with H_2O_2, Mb and Hb form the cytotoxic ferryl derivative (heme-Fe(IV)=O), which is similar to compound II formed by peroxidases (*23, 24*). Heme-Fe(IV)=O is able to oxidize a wide range of reducing substrates, such as phenols and aromatic amines, even though substrate peroxidation by Hb and Mb is far less efficient than that of peroxidases (*24, 25*), ruling out the possibility that the potential peroxidase activity of Hb and Mb is exerted on this class of substrates under normal conditions.

Here, we hypothesize that the capability of some globins (*e.g.*, Hb and Mb) to form a compound II-like species under oxidative stress may be actually exploited to avoid the building up of NO and peroxynitrite,[1] which can be then identified as the 'true' substrates for the peroxidase activity of Hb and Mb.

Heme-proteins share the ability of detoxifying nitrogen reactive species, for example, NO. Even though leukocyte peroxi-

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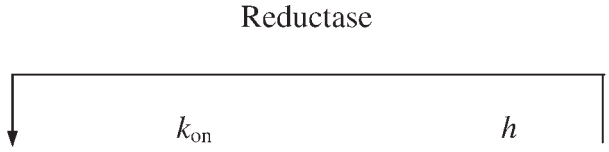
[1]The term peroxynitrite is used in the text to refer generically to both $ONOO^-$ and its conjugated acid HOONO (*38*).

Table 1
NO scavenging by heme-Fe(II)-O_2



Heme-protein	k_{on} (M^{-1} s^{-1})	h (s^{-1})
M. tuberculosis trHbN[a]	7.5×10^8	Fast
M. tuberculosis trHbO[b]	6.0×10^5	Fast
M. leprae trHbO[c]	2.1×10^6	3.4
E. coli flavoHb[d]	$\geq 6.0 \times 10^8$	$\sim 2.0 \times 10^2$
Glycine max Lb[e]	8.2×10^7	Fast
Horse heart Mb[f]	4.4×10^7	$>3.4 \times 10^2$
Murine Ngb[g]	$>7.0 \times 10^7$	$\sim 3.0 \times 10^2$
Human Hb	8.9×10^{7f}	$>5.8 \times 10^{1h}$
		$>3.3 \times 10^{1h}$

[a]pH = 7.5 and 23.0°C. From (*87*).
[b]pH = 7.5 and 23.0°C. From (*88*).
[c]pH = 7.3 and 20.0°C. From (*82*).
[d]pH = 7.0 and 20.0°C. From (*36*).
[e]pH = 7.3 and 20.0°C. From (*89*).
[f]pH = 7.0 and 20.0°C. From (*90*).
[g]pH = 7.0 and 20.0°C. From (*91*).
[h]The two values represent the decay rates for Fe(III)OONO α- and β-Hb subunits. pH = 7.5 and 20.0°C. From (*27*).

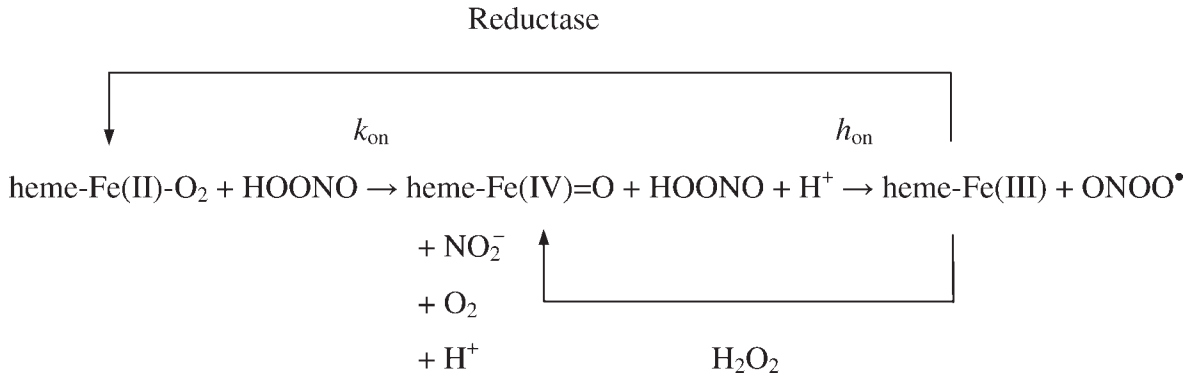
dases are believed to play a dominant role in the consumption of NO-derived oxidants at sites of inflammation (as a part of host defenses against oxidative tissue injury), ferrous oxygenated Hb and Mb (HbO_2 and MbO_2, respectively) indeed are involved in the major pathway for NO removal from the vascular compartment and in the protection of mitochondrial respiration (*4, 6, 10, 12, 14, 26*), respectively. Hereafter, we deal with these reactions under aerobic and anaerobic conditions, discriminating between reducing environmental conditions and oxidative conditions.

Under aerobic and reducing conditions, the rapid and irreversible reaction of the ferrous oxygenated derivative of heme-proteins (heme-Fe(II)-O_2) with NO and peroxynitrite occurs. This reaction gives rise to the ferric species (heme-Fe(III)) and nitrate (NO_3^-) as the final reaction products, displaying as a reaction intermediate the heme-Fe(III)-peroxynitrite complex (*27, 28*) (see Table 1). On the other hand, the reaction of heme-Fe(II)-O_2 with peroxynitrite gives rise to heme-Fe(IV)=O, nitrite (NO_2^-), O_2, and H^+; then, heme-Fe(IV)=O may react with a second peroxynitrite molecule, leading to the formation of the heme-Fe(III) species and the peroxynitrite radical ($ONOO^\bullet$) as the final reaction products (*28*) (see Table 2).

NO scavenging is also facilitated by the direct interaction of ferrous nitrosylated heme-Fe(II) (heme-Fe(II)-NO) with O_2, giving rise to heme-Fe(III) and NO_3^- as the final reaction products. However, the intermediate(s) are different for reaction(s) catalyzed by the hexa-coordinated human neuroglobin (Ngb) on one side and by penta-coordinated globins, such as Mb (*29–31*) (see Table 3). O_2-mediated NO scavenging by ferrous nitrosylated horse heart Mb and human Hb (Mb(II)-NO and Hb(II)-NO, respectively) appears to occur with a reaction mechanism, in which NO that is initially bound to heme-Fe(II) is displaced by O_2 but may stay trapped in a protein cavity(ies) close to the heme. In the second step, ferrous oxygenated horse heart Mb and human Hb (Mb(II)-O_2 and Hb(II)-O_2, respectively) react with NO giving the transient heme-Fe(III)-peroxynitrite species preceding the formation of the final products. The rate-limiting step in catalysis appears to be NO dissociation from heme-Fe(II)-NO (*29*). A slight rearrangement within the protein structure, taking place after formation of ferric human Ngb [Ngb(III)] and possibly reflecting the penta-to-hexa-coordination transition of the heme-Fe-atom, has been postulated to be the rate- limiting step in O_2-mediated NO scavenging (*30*).

NO and peroxynitrite detoxification by heme-Fe(II)-O_2 and O_2-mediated NO scavenging by heme-Fe(II)-NO, indeed, all reflect the superoxide character of the initial or transient heme-Fe(II)-O_2 species (*i.e.*, heme-Fe(III)-O_2^-) (*32*) (see Tables 1, 2, and 3).

Table 2
Peroxynitrite scavenging by heme-Fe(II)-O_2 and heme-Fe(IV)=O



Heme-protein	k_{on} (M^{-1} s^{-1})	h_{on} (M^{-1} s^{-1})
M. leprae trHbO[a]	4.8×10^4	1.3×10^4
Glycine max Lb[b]	5.5×10^4	2.1×10^4
Horse heart Mb[c]	5.4×10^4	2.2×10^4
Human Hb[d]	3.3×10^4	3.3×10^4

[a]pH 7.3 and 20.0°C. From (*83*).
[b]pH 7.3 and 20.0°C. From (*89*).
[c]pH 7.5 and 20.0°C. From (*92*).
[d]pH 7.4 and 20.0°C. From (*93*).

Heme-Fe(II)-NO also facilitates peroxynitrite scavenging; this reaction proceeds in two steps, a rapid conversion from heme-Fe(II)-NO to the heme-Fe(III)-NO intermediate, which then dissociates into NO and heme-Fe(III) (*28*) (see Table 4). Preliminary results (*33, 34*) indicate that deoxygenated and ferrous carbonylated globins may also facilitate peroxynitrite detoxification, giving rise to the heme-Fe(III) species.

All reactions depicted in Tables 1–4 are considered as 'pseudo-enzymatic processes' because they need a reductase partner(s) to restore heme-Fe(II), which is absolutely necessary for a new catalytic cycle. In particular, NADH-metHb and -metMb reductases catalyze the conversion of heme-Fe(III) to heme-Fe(II) *in vivo*. As a matter of fact, the enzymatic heme-Fe(III) reduction is the rate-limiting step of the whole process, this representing a severe limitation for the efficiency of these mechanisms *in vivo* (*4, 28, 35–43*).

Under highly oxidative conditions, the redox equilibrium of globins is shifted in favor of the heme-Fe(III) form, impairing their role as O_2 carriers. However, under these conditions, usually the high H_2O_2 concentration facilitates the oxidation of the heme-Fe(III) of some globins (*e.g.*, Hb and Mb), giving rise to the formation of the compound II-like species heme-Fe(IV)=O. This highly oxidative form facilitates NO, peroxynitrite and NO_2^- scavenging (see Tables 2, 5, and 6), because NO detoxification by heme-Fe(IV)=O leads to the formation of heme-Fe(III) and NO_2^- (*44–47*) (see Table 5). The reactions of heme-Fe(IV)=O with peroxynitrite and NO_2^- generate $ONOO^•$ and the nitrogen dioxide radical ($^•NO_2$), respectively, which could contribute to tyrosine nitration and thus to the inactivation of proteins (*28, 48–51*) (see Tables 2 and 6). The reaction of heme-Fe(IV)=O with NO (see Table 5) is significantly faster than those of heme-Fe(IV)=O with peroxynitrite and NO_2^- (see Tables 2 and 6). These reactions, depicted in Tables 2, 5, and 6, do not require partner oxido-reductive enzyme(s), because the system oscillates between the oxidation of the heme-Fe(III) species to heme-Fe(IV)=O by H_2O_2, and the heme-Fe(IV)=O reduction back to heme-Fe(III) by NO, peroxynitrite, and NO_2^- (*28, 44–47*). Interestingly, catalytic parameters for NO scavenging by heme-Fe(II)-O_2 (*43*) and heme-Fe(IV)=O (*47*) are closely similar (see Tables 1 and 5) and high enough to indicate that both reactions could occur efficiently *in vivo*.

In contrast with penta-coordinated globins (*e.g.*, Hb and Mb) (*28, 44–51*), heme-Fe(III) human Ngb apparently does not generate the heme-Fe(IV)=O form when exposed to H_2O_2 and peroxynitrite, another feature of Ngb that may contribute to neuronal survival after hypoxia and that may be related to heme-Fe-atom hexa-coordination (*28, 30, 31*).

Beside globins, heme-Fe(IV)=O peroxidases may facilitate NO and NO_2^- detoxification (see Tables 5, 6, and 7). However, in the case of mammalian peroxidases, such as myeloperoxidase (MPO) and eosinophil peroxidase (EPO), the rate constants for NO oxidation to NO_2^- are 2–3 orders of magnitude lower than

Table 3
O_2-mediated NO scavenging by heme-Fe(II)-NO

Human Ngb[a]

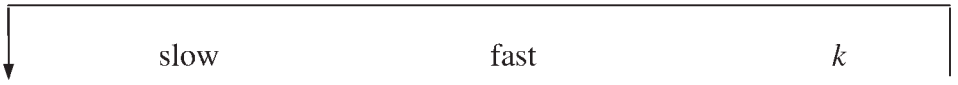
Reductase

heme-Fe(II)-NO + O_2 → heme-Fe(III)* → heme-Fe(III) + NO_3^-

$k = 5.0\times10^{-4}$ s^{-1}

Horse heart Mb[b]

Reductase



slow fast k

heme-Fe(II)-NO + O_2 ↔ heme-Fe(II)-O_2 + NO → heme-Fe(III)-OONO → heme-Fe(III) + NO_3^-

$k = 2.6\times10^{-4}$ s^{-1}

Human Hb[c]

Reductase

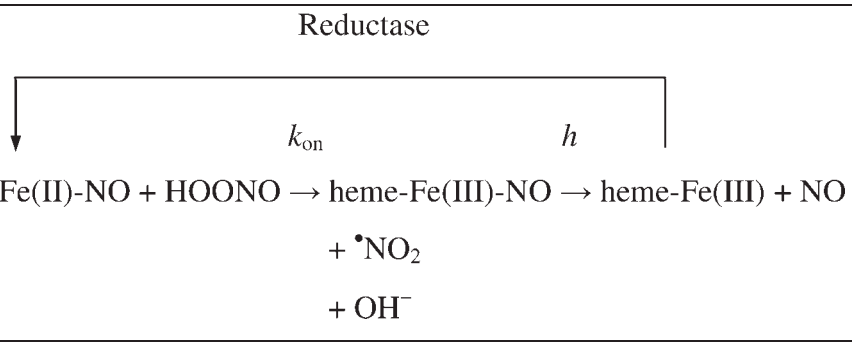
slow fast

heme-Fe(II)-NO + O_2 ↔ heme-Fe(II)-O_2 + NO → heme-Fe(III) + NO_3^-

[a]pH 7.5 and 25.0°C. From (*30*).
[b]pH 7.0 and 20.0°C. From (*94*).
[c]pH 7.2 and room temperature. From (*95*).

those reported for the heme-Fe (IV)=O derivative of globins, whereas in the case of plant peroxidases, such as horseradish peroxidase (HRP), the rate constant is only 10-fold slower than for globins (*47, 48, 52–59*) (see Table 5). Further, the formation of the heme-Fe(III)-ONO species is significantly faster in globins than in peroxidases (and possibly in catalase), where the formation of the heme-Fe(III)-ONO species is the rate-limiting step. Conversely, the dissociation of the heme-Fe(III)-ONO species and the *O*-nitrito isomerization is significantly faster in peroxidases than in the heme-Fe(III) species of globins where it represents instead the rate-limiting step (*44–47, 52–55*) (see Table 5). On the other hand, the rate constant for NO_2^- scavenging by heme-Fe(IV)=O MPO is similar to that observed for heme-Fe(IV)=O globin action (*44–47, 53*) (see Table 6).

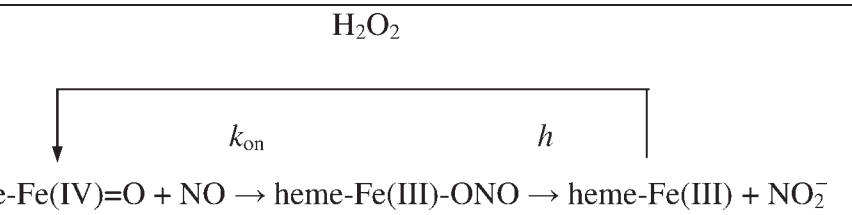
Table 4
Peroxynitrite scavenging by heme-Fe(II)-NO



Heme-protein	k_{on} (M^{-1} s^{-1})	h (s^{-1})
M. leprae trHbO[a]	$>1.0 \times 10^8$	2.6×10^1
Glycine max Lb[b]	8.8×10^3	2.0
Horse heart Mb[c]	3.1×10^4	$\sim 1.2 \times 10^1$
Human Ngb[d]	$>1.3 \times 10^5$	1.2×10^{-1}
Human Hb[e]	6.1×10^3	~1.0

[a]pH 7.3 and 20.0°C. From (*83*).
[b]pH 7.3 and 20.0°C. From (*46*).
[c]pH 7.5 and 20.0°C. From (*96*).
[d]pH 7.2 and 25.0°C. From (*30*).
[e]pH 7.2 and 20.0°C. From (*97*).

Table 5
NO scavenging by heme-Fe(IV)=O



Heme-protein	k_{on} (M^{-1} s^{-1})	h (s^{-1})
M. leprae trHbO[a]	7.8×10^6	2.1×10^1
Glycine max Lb[b]	1.8×10^6	$>5.0 \times 10^1$
Horse heart Mb[c]	1.7×10^7	6.0
Human Hb[d]	2.4×10^7	4.8×10^{-1}
		1.2×10^{-1}
Horseradish peroxidase[e]	1.0×10^6	Fast
Porcine eosinophyl peroxidase[f]	1.7×10^4	Fast
Bovine lactoperoxidase[f]	8.7×10^4	Fast
Human myeloperoxidase[g]	8.0×10^3	Fast

[a]pH = 7.2 and 20.0°C. From (*47*)
[b]pH = 7.0 and 20.0°C. From (*46*).
[c]pH = 7.0 and 20.0°C. From (*44*).
[d]pH = 7.0 and 20.0°C. Biphasic kinetics of heme-Fe(III)-ONO decay (represented by values of h) has been attributed to α- and β-chains. From (*45*).
[e]pH = 7.4 and 20.0°C. From (*52*).
[f]pH = 7.0 and 25.0°C. From (*54*).
[g]pH = 7.0 and 25.0°C. From (*53*).

Table 6
NO_2^- scavenging by heme-Fe(IV)=O

H_2O_2

k_{on}

heme-Fe(IV)=O + NO_2^- → heme-Fe(III) + $^{\bullet}NO_2$

Heme-protein	k_{on} (M^{-1} s^{-1})
M. leprae trHbO[a]	3.1×10^3
Glycine max Lb[b]	2.1×10^2
Horse heart Mb[c]	1.6×10^1
Human Hb[d]	7.5×10^2
Human myeloperoxidase[e]	5.5×10^2

[a]pH = 7.2 and 20.0°C. From (*47*).
[b]pH = 7.0 and 20.0°C. From (*46*).
[c]pH = 7.5 and 20.0°C. From (*44*).
[d]pH = 7.0 and 20.0°C. From (*45*).
[e]pH = 7.0 and 15.0°C. From (*53*).

Both peroxidases and globins are able to perform the peroxynitrite detoxification under oxidative stress conditions. In the case of peroxidases, the reaction with peroxynitrite brings about the fast formation of the compound II-like heme-Fe(IV)=O species (*60*), likely through the formation of a transient Fe(III)-peroxynitrite complex, followed by its conversion to heme-Fe(IV)=O and $^{\bullet}NO_2$ (*61*). This fast event is then followed by a very slow reduction of heme-Fe(IV)=O back to Fe(III), which is driven by the oxidation of NO_2^- (in a redox equilibrium with $^{\bullet}NO_2$) to NO_3^- (*61*) (see Table 7). Although no kinetic parameters are instead available for these reactions in catalase, a role played by catalase in the detoxification of NO has been reported (*55*).

In the case of globins, horse heart heme-Fe(III) Mb and human heme-Fe(III) Hb catalyze the isomerization of peroxynitrite to NO_3^- (*27, 62*) (see Tables 1, 3, 5, and 7); in contrast, peroxynitrite does not react with hexa-coordinated heme-Fe(III) human Ngb, as reported for H_2O_2 (*28, 30, 31*).

Heme-Fe(III) species also facilitate NO scavenging through the formation of the Fe(III)-NO complex, giving rise to heme-Fe(II)-NO as the final reaction product. This reaction proceeds in three steps: (*i*) reversible heme-Fe(III) nitrosylation (*i.e.*, heme-Fe(III)-NO formation) followed by fast conversion to heme-Fe(II)-NO^+; (*ii*) H_2O/OH^- catalyzed conversion of heme-Fe(II)-NO^+ to heme-Fe(II); and (*iii*) reversible heme-Fe(II) nitrosylation by a second NO molecule (*i.e.*, heme-Fe(II)-NO formation). NO binding to heme-Fe(III) *S. inaequivalvis* HbI and human Ngb(III) (*30, 63*) appears to be rate limiting, whereas the conversion of heme-Fe(II)-NO^+ to heme-Fe(II) is the rate-limiting step for the reductive nitrosylation of heme-Fe(III) *Glycine max* leghemoglobin (Lb(III)), sperm whale Mb(III), human Hb(III), and human myeloperoxidase (*46, 53, 64*) (see Table 8).

Lastly, heme-based reactions involving peroxynitrite appear to be facilitated by carbon dioxide (CO_2). Indeed, peroxynitrite may rapidly react with CO_2 forming an adduct, believed to be 1-carboxylato-2-nitrosodioxidane ($ONOOC(O)O^-$). This transient intermediate decays by homolysis of the O—O bond giving rise to NO_3^- and CO_2 as final products, trioxocarbonate($^{\bullet}1-$) ($CO_3^{\bullet -}$) and $^{\bullet}NO_2$ being the reaction intermediates. Note that $CO_3^{\bullet -}$ and $^{\bullet}NO_2$ are stronger oxidant species than peroxynitrite (*50, 65*).

The comparison of globin and peroxidase action (see Tables 5, 6, 7, and 8) allows the following considerations. (*i*) The detoxification activity of NO and peroxynitrite by the heme-Fe(IV)=O species of globins (occurring under oxidative conditions) is higher than that of peroxidases. (*ii*) The NO_2^- detoxification activity of mammalian peroxidases is higher than that reported for plant peroxidases and globins. (*iii*) The heme-Fe(III) derivative of peroxidases detoxifies peroxynitrite more efficiently than the heme-Fe(IV)=O species of globins.

Peroxidation of classical peroxidase substrates (*e.g.*, phenols) by globins occurs at a much slower rate, with respect to the heme-enzymes (*24, 48, 58*). The different catalytic behavior of globins and peroxidases for different substrates might be due to the strong hydrogen bond present in peroxidases between the proximal histidyl residue and a conserved aspartate residue (*45*). Moreover, it may be also referred to the highly positive charge present in the heme distal side of peroxidases (see Fig. 1), which significantly lowers the pK_a values of catalytic His and Arg distal residues (*66, 67*). This idea is further strengthened by the following: (*i*) the evidence that site-directed mutants of horse heart Mb (Thr39Ile, Lys45Asp, Phe46Leu, and Ile107Phe) and sperm whale Mb (Thr67Arg and Thr67Arg/Ser92Asp) display a significant increase of the peroxidase activity (*25, 68, 69*), and (*ii*) site-directed mutants of cytochrome *c* peroxidase (His175Gln, His175Glu, and His175Cys) and horseradish peroxidase (Arg38Leu, His42Glu, His42Gln) show a substantial decrease of the peroxidase activity (*70–72*).

The peroxidase activity of globins appears to be at the root of the *Mycobacterium leprae* ability to persist *in vivo* in the presence of reactive nitrogen and oxygen species. Indeed, during infection, *M. leprae* is faced with the host macrophagic environment, where low pH, low pO_2, high pCO_2, combined with the toxic activity of reactive nitrogen and oxygen species (including NO, superoxide ($O_2^{\bullet -}$), and H_2O_2) contribute to limit the growth of the bacilli *in vivo* (*43, 47, 73–78*).

The ability of *M. leprae* to persist *in vivo* in the presence of reactive nitrogen and oxygen species implies the presence in this elusive mycobacterium of (pseudo-)enzymatic detoxification systems, including truncated hemoglobin O (trHbO) (*43, 77–84*). *M. leprae* trHbO has been reported to facilitate NO and peroxynitrite scavenging using O_2, NO, and H_2O_2 as co-factors (*43, 47, 78, 82–84*) (see Tables 1, 2, 4, 5, and 6). Interestingly, kinetics of NO detoxification by the heme-Fe(IV)=O derivative

Table 7
Peroxynitrite scavenging by heme-Fe(III)

Globins

$$\text{heme-Fe(III)} + \text{HOONO} \underset{}{\overset{k_{on}}{\leftrightarrow}} \text{heme-Fe(III)-OONO} \xrightarrow{h} \text{heme-Fe(III)} + \text{NO}_3^- + \text{H}^+$$

	k_{on} (M^{-1} s^{-1})	h (s^{-1})
Horse heart Mb	2.9×10^4[a]	$>3.4\times10^2$[b]
Human Hb	1.2×10^4[a]	$>5.8\times10^1$[c]
		$>3.3\times10^1$[c]

Peroxidases

$$\text{heme-Fe(III)} + \text{HOONO} \underset{}{\overset{K_1}{\leftrightarrow}} \text{heme-Fe(III)-OONO} \xrightarrow{k_2} \text{heme-Fe(IV)=O} \xrightarrow{h} \text{heme-Fe(III)} + \text{NO}_3^- + \text{H}^+ + {}^{\bullet}\text{NO}_2$$

	k_{on} ($= K_1 \times k_2$; M^{-1} s^{-1})	h (s^{-1})
Human myeloperoxidase[d]	6.8×10^6	≤ 0.1
Bovine lactoperoxidase[e]	3.3×10^5	nd
Horse radish peroxidase[f]	3.2×10^6	nd

[a]pH = 7.0 and 20.0°C. From (*98*)
[b]pH = 7.0 and 20.0°C. From (*90*).
[c]The two values represent the decay rates for Fe(III)OONO α- and β-Hb subunits. pH = 7.5 and 20.0°C. From (*27*).
[d]pH = 7.0 and 25°C. From (*61*).
[e]pH = 7.4 and 12°C. From (*60*).
[f]pH = 6.8 and 25°C. From (*60*).
nd, not determined.

of *M. leprae*, induced by H_2O_2, is faster than any other mycobacterial reactions involved in scavenging of reactive nitrogen and oxygen species (*47*) (see Tables 1, 2, 4, 5, and 6). This appears to be in agreement with the absence in *M. leprae* of a specific reductase(s) converting heme-Fe(III) (obtained from the reaction of heme-Fe(II)-O_2 and heme-Fe(II)-NO with NO and peroxynitrite) to heme-Fe(II), this enzymatic process being pivotal to start a new catalytic cycle (*43, 47, 77, 78, 82–84*).

Paradoxically, NO, peroxynitrite, and NO_2^- can serve as antioxidants of the highly oxidizing heme-Fe(IV)=O derivative of globins, which could be responsible for the oxidative damage of biological membranes (*85*) and inactivation of heme-based enzymes (*e.g.*, cytochrome *c* peroxidase) (*86*).

As a whole, peroxidases appear to be able to detoxify from oxidative compounds (such as peroxynitrite) through the oxidation of the resting heme-Fe(III) state to heme-Fe(IV)=O under normal oxidizing conditions. Whenever the environment becomes highly oxidative massive oxidation of globins to heme-Fe(IV)=O takes place; this facilitates NO, peroxynitrite, and NO_2^- detoxification, boosting the detoxification mechanism, because NO, peroxynitrite, and NO_2^- can serve as antioxidants of the highly oxidizing heme-Fe(IV)=O species. Therefore, under these highly oxidative conditions globins appear to facilitate NO, peroxynitrite, NO_2^-, and H_2O_2 scavenging without needing a reductase partner(s), which in such condition is potentially devoid of reducing co-factors (*e.g.*, NADH and $FADH_2$). Although the *in vivo* role of heme-Fe(IV)=O globins in scavenging reactive nitrogen species is still uncertain, NO, peroxynitrite, and NO_2^- could be the 'true' substrates of globins when acting as peroxidases, H_2O_2 being the co-substrate.

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Table 8
NO scavenging by heme-Fe(III)

$$\text{heme-Fe(III)} + \text{NO} \underset{k_{off}}{\overset{k_{on}}{\leftrightarrow}} \text{heme-Fe(III)-NO} \xrightarrow{\text{fast}} \text{heme-Fe(II)-NO}^{+} + \text{H}_2\text{O/OH}^{-} \xrightarrow{h} \text{heme-Fe(II)} + \text{NO}_2^{-} + (2)\text{H}^{+} \underset{l_{off}}{\overset{l_{on}}{\rightarrow}} \text{heme-Fe(II)-NO}$$

Heme-protein	k_{on} (M^{-1} s^{-1})	k_{off} (s^{-1})	h (s^{-1})	l_{on} (M^{-1} s^{-1})	l_{off} (s^{-1})
Glycine max Lb	1.4×10^{5}[a]	3.0[a]	4.8×10^{-4}[a]	1.2×10^{8}[b]	2.4×10^{-5}[b]
S. inaequivalvis HbI	3.2×10^{1}[c]	$<1.0 \times 10^{3}$[c]	$>6.0 \times 10^{-1}$[c]	1.6×10^{7}[d]	nd
Sperm whale Mb	1.9×10^{5}[e]	1.4×10^{1}[e]	$<8.8 \times 10^{-4}$[f]	1.7×10^{7}[g]	1.2×10^{-4}[g]
Mouse Ngb[h]	nd	nd	nd	2.0×10^{5}	2.0×10^{-4}
Human Ngb[i]	2.1×10^{1}	2.5×10^{-3}	$>2.0 \times 10^{-1}$	nd	nd
	2.9	2.5×10^{-3}	$>5.0 \times 10^{-2}$	nd	nd
Human Hb[j]	1.7×10^{3}[k]	6.5×10^{-1}[k]	1.3×10^{-3}[l]	2.6×10^{7}[m]	4.6×10^{-5}[b]
	6.4×10^{3}[k]	1.5[k]	1.3×10^{-3}[l]	2.6×10^{7}[m]	2.2×10^{-5}[b]
Human myeloperoxidase[n]	1.1×10^{6}	1.1×10^{1}	Slow	1.0×10^{5}	4.6

[a]pH = 7.0 and 20.0°C. From (*46*).
[b]pH = 7.0 and 20.0°C. From (*99*).
[c]pH = 7.5 and 20.0°C. From (*63*).
[d]pH = 7.0 and 20.0°C. From (*100*).
[e]pH = 6.5 and 20.0°C. From (*101*).
[f]pH < 8.3 and 20.0°C. From (*64*).
[g]pH = 7.0 and 20.0°C. From (*102*).
[h]pH = 7.0 and 25.0°C. From (*103*).
[i]pH = 7.0 and room temperature. Biphasic kinetics has been attributed to fast and slow reacting form. From (*30*).
[j]Biphasic kinetics has been attributed to α- and β-chains.
[k]pH = 7.0 and 20.0°C. From (*104*).
[l]pH = 7.0 and 20.0°C. From (*64*).
[m]pH = 7.0 and 20.0°C. From (*105*).
[n]pH = 7.0 and 10.0°C. From (*53*).
nd, not determined.

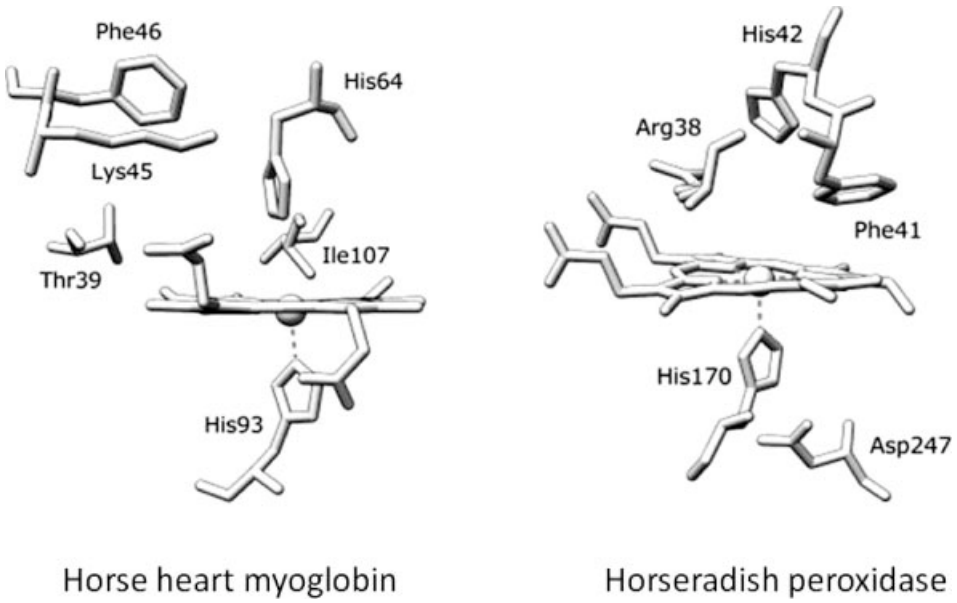

Figure 1. Key residues of heme surroundings of horse heart Mb (PDB code: 2v1k) and horseradish peroxidase (PDB code: 1h58).

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