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Peroxynitrite scavenging by ferryl sperm whale myoglobin and human hemoglobin

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ABSTRACT

Globins protect from the oxidative and nitrosative cell damage. Here, kinetics of peroxynitrite scavenging by ferryl sperm whale myoglobin (Mb—Fe(IV)=O) and human hemoglobin (Hb—Fe(IV)=O), between pH 5.8 and 8.3 at 20.0 °C, are reported. In the absence of CO_2, values of the second-order rate constant for peroxynitrite scavenging by Mb—Fe(IV)=O and Hb—Fe(IV)=O (i.e., for Mb—Fe(III) and Hb—Fe(III) formation; k_{on}) are $4.6 \times 10^4\ M^{-1}\ s^{-1}$ and $3.3 \times 10^4\ M^{-1}\ s^{-1}$, respectively, at pH 7.1. Values of k_{on} increase on decreasing pH with pK_a values of 6.9 and 6.7, this suggests that the ONOOH species reacts preferentially with Mb—Fe(IV)=O and Hb—Fe(IV)=O. In the presence of CO_2 (=1.2×10^{-3} M), values of k_{on} for peroxynitrite scavenging by Mb—Fe(IV)=O and Hb—Fe(IV)=O are essentially pH-independent, the average k_{on} values are $7.1 \times 10^4\ M^{-1}\ s^{-1}$ and $1.2 \times 10^5\ M^{-1}\ s^{-1}$, respectively. As a whole, Mb—Fe(IV)=O and Hb—Fe(IV)=O, obtained by treatment with H_2O_2, undertake within the same cycle H_2O_2 and peroxynitrite detoxification.

Peroxynitrite is implicated in several physiological and pathological events, including cell signaling, drug metabolism, microbial pathogenesis, atherosclerosis, inflammation, and neurodegenerative disorders. It reacts with various bio-molecules including proteins, lipids, and DNA by either direct reaction with a target molecule or immediately after homolysis to $^{\cdot}NO_2$ and hydroxyl radical ($^{\cdot}OH$) or after reaction with CO_2 and homolysis to $CO_3^{\cdot-}$ and $^{\cdot}NO_2$ [1–11].

Besides their role in O_2 transport and storage, globins also catalyze several reactions aimed to scavenge toxic reactive nitrogen and oxygen species. These reactions play an important physiological role in the defense against nitrosative and oxidative stress [7,12–16]. Peroxynitrite scavenging has been reported to be facilitated by the ferrous oxygenated (heme–Fe(II)–O_2), ferrous nitrosylated (heme–Fe(II)–NO), and ferric (heme–Fe(III)) derivatives of heme-proteins [7,15,17–29].

Here, a detailed kinetic study of peroxynitrite scavenging by the ferryl derivative of sperm whale Mb (Mb—Fe(IV)=O) and human Hb (Hb—Fe(IV)=O) is reported. Mb—Fe(IV)=O and Hb—Fe(IV)=O, obtained by treatment with hydrogen peroxide (H_2O_2), catalyze peroxynitrite scavenging. In turn, peroxynitrite acts as an antioxidant of Mb—Fe(IV)=O and Hb—Fe(IV)=O and could prevent cell damage. Therefore, Mb and Hb appear to be involved in both H_2O_2 and peroxynitrite scavenging.

Materials

Ferric sperm whale Mb (Mb—Fe(III)) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Ferrous oxygenated sperm whale Mb (Mb—Fe(II)—O_2) was prepared by adding few grains of sodium dithionite to the Mb—Fe(III) solution, then the solution was desalted by passing it throughout a G25 Sephadex gel filtration column equilibrated in air with 1.0×10^{-1} M phosphate buffer, at pH 7.2 and 20 °C [30]. Ferrous oxygenated human Hb (Hb—Fe(II)—O_2) was prepared from blood samples according to literature [30]. Ferric human Hb (Hb—Fe(III)) was prepared by adding a few grains of sodium ferricyanide to the Hb—Fe(II)—O_2 solution [30]. Sperm whale Mb—Fe(IV)=O and human Hb—Fe(IV)=O were prepared by adding 7–15 equivalents of H_2O_2 to the Mb—Fe(III) and Hb—Fe(III) solutions (5.0×10^{-2} M phosphate buffer, pH 7.2) at 20.0 °C. After a reaction time of few minutes, the Mb—Fe(IV)=O and Hb—Fe(IV)=O

Abbreviations: Fe(III), ferric heme-protein; Fe(IV)=O, ferryl [oxo-Fe(IV)] heme-protein; Fe(II)—NO, ferrous nitrosylated heme-protein; Fe(II)—O_2, ferrous oxygenated heme-protein; Hb, hemoglobin; Lb, leghemoglobin; Mb, myoglobin; trHbO, truncated HbO

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$$\text{heme-Fe(IV)=O} + \text{peroxynitrite} \xrightarrow{k_{on}} \text{heme-Fe(III)}$$

Scheme 1.

solutions were stored on ice and used within 1 h. The heme-protein concentration was determined spectrophotometrically with ε values listed in Supplementary Table 1.

The solutions of the experiments in the presence of CO_2 were prepared by adding the required amount of a 5.0×10^{-1} M $NaHCO_3$ solution [15,19,21,22,24,25,29].

H_2O_2 (from Fluka GmbH, Buchs, Switzerland) was diluted with the 5.0×10^{-2} M phosphate buffer solution (pH 7.2); the H_2O_2 concentration was determined spectrophotometrically at 240 nm ($\varepsilon_{240nm} = 3.94 \times 10^{1}$ M^{-1} cm^{-1}) [31].

Peroxynitrite was prepared from potassium superoxide (KO_2) and ·NO and from nitrous acid (HNO_2) and H_2O_2 [32,33]. The peroxynitrite stock solution was diluted with degassed 1.0×10^{-2} M sodium hydroxide (NaOH) to reach the desired concentration. The peroxynitrite concentration was determined spectrophotometrically at 302 nm ($\varepsilon_{302nm} = 1.67 \times 10^{3}$ M^{-1} cm^{-1}) [34].

All the other products (from Merck AG, Darmstadt, Germany, or Sigma–Aldrich, St. Louis, MO, USA) were of analytical grade and used without purification.

Methods

Kinetics of peroxynitrite scavenging by sperm whale Mb—Fe(IV)=O and human Hb—Fe(IV)=O were determined, in the absence and presence of CO_2, by rapid mixing the Mb—Fe(IV)=O and Hb—Fe(IV)=O solutions (final concentration, 3.2×10^{-6} and 2.9×10^{-6} M, respectively) with the peroxynitrite solution (final concentration, 2.0×10^{-5} to 4.0×10^{-4} M), at pH values ranging between 5.8 and 8.3 (final concentration, 2.0×10^{-1} M phosphate buffer) and 20.0 °C; no gaseous phase was present. Kinetics was monitored between 360 and 460 nm [15,19,21,23–29].

The time course of peroxynitrite scavenging by Mb—Fe(IV)=O and Hb—Fe(IV)=O, in the absence and presence of CO_2, was fitted to a single exponential process according to the minimum reaction mechanism represented by Scheme 1 [29].

Values of the pseudo-first-order rate constant k for peroxynitrite scavenging by Mb—Fe(IV)=O and Hb—Fe(IV)=O, in the absence and presence of CO_2, were determined according to Eq. (1) [29]:

$$[\text{Fe(IV)=O}]_t = [\text{Fe(IV)=O}]_i \times e^{-k \times t} \quad (1)$$

Values of k_{on}, in the absence and presence of CO_2, were determined according to Eq. (2) [29]:

$$k = k_{on} \times [\text{peroxynitrite}] + a \quad (2)$$

where a is the value of k in the absence of peroxynitrite.

The pK_a values describing the pH-dependence of k_{on} for peroxynitrite scavenging by Mb—Fe(IV)=O and Hb—Fe(IV)=O, in the absence of CO_2, were obtained, at 20.0 °C, according to Eq. (3) [29]:

$$k_{on} = ((k_{lim(top)} - k_{lim(bottom)}) \times 10^{-pH})/(10^{-pH} + 10^{-pKa}) + k_{lim(bottom)} \quad (3)$$

where $k_{lim(top)}$ and $k_{lim(bottom)}$ represent the asymptotic values of k_{on} under conditions where pH $\ll$pK_a and pH $\gg$pK_a, respectively.

In some cases, catalase was added to the Mb—Fe(IV)=O and Hb—Fe(IV)=O solutions prior to reaction with peroxynitrite to destroy excess H_2O_2. According to literature [29,35,36], catalase does not affect peroxynitrite scavenging by Mb—Fe(IV)=O and Hb—Fe(IV)=O, in the absence and presence of CO_2.

Kinetics of peroxynitrite scavenging by sperm whale Mb—Fe(II)—O_2 and human Hb—Fe(II)—O_2 were determined, in the absence and presence of CO_2, by rapid mixing the Mb—Fe(II)—O_2 and Hb—Fe(II)—O_2 solutions (final concentration, 3.4×10^{-6} and 3.3×10^{-6} M, respectively) with the peroxynitrite solution (final concentration, 2.0×10^{-5} to 4.0×10^{-4} M), at pH 7.1 (final concentration, 2.0×10^{-1} M phosphate buffer) and 20.0 °C; no gaseous phase was present. Kinetics was monitored between 360 and 460 nm [19,22,24,25].

The time course of peroxynitrite scavenging by sperm whale Mb—Fe(II)—O_2 and human Hb—Fe(II)—O_2, in the absence and presence of CO_2, was fitted to two consecutive mono-exponential processes according to the minimum reaction mechanism represented by Scheme 2 [19,22,24,25].

Values of the pseudo-first-order rate constants h and k for peroxynitrite scavenging by Mb—Fe(II)—O_2 and Hb—Fe(II)—O_2, in the absence and presence of CO_2, were determined according to Eqs. (4a–c) [37]:

$$[\text{Fe(II)—O}_2]_t = [\text{Fe(II)—O}_2]_i \times e^{-h \times t} \quad (4a)$$

$$[\text{Fe(IV)=O}]_t = [\text{Fe(II)—O}_2]_i \times (h \times ((e^{-h \times t}/(k-h)) + (e^{-k \times t}/(h-k)))) \quad (4b)$$

$$[\text{Fe(III)}]_t = [\text{Fe(II)—O}_2]_i - [\text{Fe(II)—O}_2]_t + [\text{Fe(IV)=O}]_t \quad (4c)$$

Values of h_{on} and k_{on}, in the absence and presence of CO_2, were determined according to Eqs. (5a) and (5b) [29]:

$$h = h_{on} \times [\text{peroxynitrite}] + a \quad (5a)$$

$$k = k_{on} \times [\text{peroxynitrite}] + a \quad (5b)$$

where a is the value of h or k in the absence of peroxynitrite.

The results are given as mean values of at least four experiments plus or minus the corresponding standard deviation. All data were analyzed using the MatLab program (The Math Works Inc., Natick, MA, USA).

Results and discussion

Mixing of sperm whale Mb—Fe(IV)=O or human Hb—Fe(IV)=O with peroxynitrite solutions, in the absence and presence of CO_2, leads to the formation of Mb—Fe(III) and Hb—Fe(III), respectively. Under all the experimental conditions, the time course of peroxynitrite scavenging by Mb—Fe(IV)=O and Hb—Fe(IV)=O corresponds to a monophasic process (Scheme 1). Moreover, values of k for peroxynitrite scavenging by Mb—Fe(IV)=O and Hb—Fe(IV)=O are wavelength-independent under pseudo-first order conditions at fixed peroxynitrite concentration and pH (data not shown).

Plots of k versus [peroxynitrite] are linear, the slope corresponds to k_{on} (Figs. 1 and 2). In the absence of CO_2, the y-axis intercept of plots of k versus [peroxynitrite] (i.e., a; see Eq. (2)) corresponds to $a \cong 0$ s^{-1} (Figs. 1 and 2 and Supplementary Tables 2 and 3). On the other hand, in the presence of CO_2 (Figs. 1 and 2 and Supplementary Tables 2 and 3), the y-axis intercept of plots

$$\text{heme-Fe(II)-O}_2 + \text{peroxynitrite} \xrightarrow{h_{on}} \text{heme-Fe(IV)=O} + \text{peroxynitrite} \xrightarrow{k_{on}} \text{heme-Fe(III)}$$

Scheme 2.

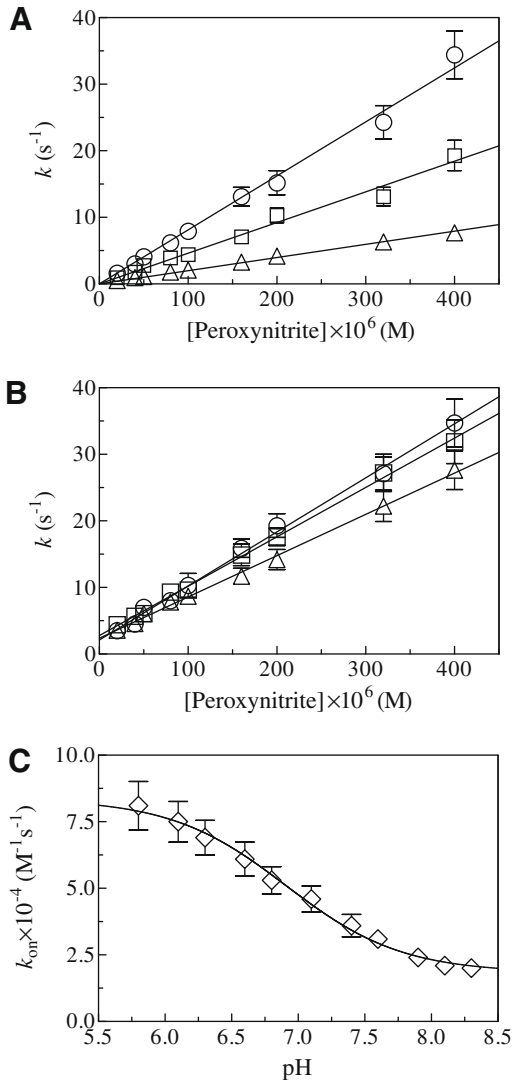

Fig. 1. Kinetics of peroxynitrite scavenging by sperm whale Mb—Fe(IV)═O, at 20.0 °C. (A) Dependence of k on the peroxynitrite concentration, in the absence of CO_2, at pH 5.8, 7.1, and 8.3 (circles, squares, and triangles, respectively). The analysis of data according to Eq. (2) allowed to determine $k_{on} = 8.1 \times 10^4\ M^{-1}\ s^{-1}$ (circles), $4.6 \times 10^4\ M^{-1}\ s^{-1}$ (squares), and $2.0 \times 10^4\ M^{-1}\ s^{-1}$ (triangles). (B) Dependence of k on the peroxynitrite concentration, in the presence of CO_2, at pH 6.1, 7.1, and 7.9 (circles, squares, and triangles, respectively). The analysis of data according to Eq. (2) allowed to determine $k_{on} = 8.1 \times 10^4\ M^{-1}\ s^{-1}$ and $a = 2.1\ s^{-1}$ (circles), $k_{on} = 7.4 \times 10^4\ M^{-1}\ s^{-1}$ and $a = 2.8\ s^{-1}$ (squares), and $k_{on} = 6.2 \times 10^4\ M^{-1}\ s^{-1}$ and $a = 2.4\ s^{-1}$ (triangles). (C) pH-dependence of k_{on} in the absence of CO_2. The analysis of data according to Eq. (3) allowed to determine $pK_a = 6.9 \pm 0.1$, $k_{lim(top)} = (8.4 \pm 0.2) \times 10^4\ M^{-1}\ s^{-1}$, and $k_{lim(bottom)} = (1.8 \pm 0.1) \times 10^4\ M^{-1}\ s^{-1}$. Where not shown, standard deviation is smaller than the symbol. The Mb—Fe(IV)═O concentration was 3.2×10^{-6} M. The CO_2 concentration was 1.2×10^{-3} M. For details, see text.

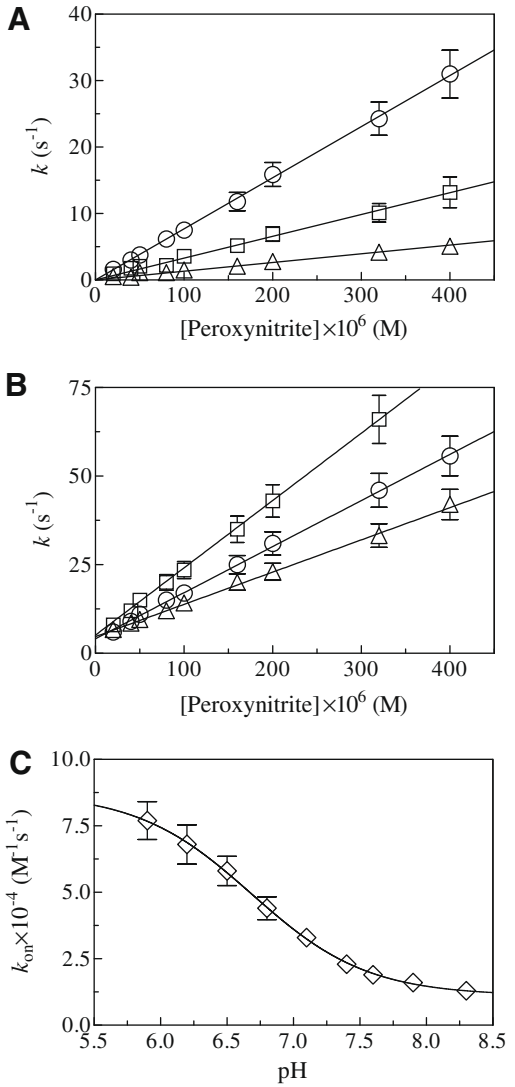

Fig. 2. Kinetics of peroxynitrite scavenging by human Hb—Fe(IV)═O, at 20.0 °C. (A) Dependence of k on the peroxynitrite concentration, in the absence of CO_2, at pH 5.9, 7.1, and 8.2 (circles, squares, and triangles, respectively). The analysis of data according to Eq. (2) allowed to determine $k_{on} = 7.7 \times 10^4\ M^{-1}\ s^{-1}$ (circles), $3.3 \times 10^4\ M^{-1}\ s^{-1}$ (squares), and $1.3 \times 10^4\ M^{-1}\ s^{-1}$ (triangles). (B) Dependence of k on the peroxynitrite concentration, in the presence of CO_2, at pH 5.9, 7.1, and 8.2 (circles, squares, and triangles, respectively). The analysis of data according to Eq. (2) allowed to determine $k_{on} = 1.3 \times 10^5\ M^{-1}\ s^{-1}$ and $a = 4.1\ s^{-1}$ (circles), $k_{on} = 1.9 \times 10^5\ M^{-1}\ s^{-1}$ and $a = 5.1\ s^{-1}$ (squares), and $k_{on} = 9.1 \times 10^4\ M^{-1}\ s^{-1}$ and $a = 4.7\ s^{-1}$ (triangles). (C) pH-dependence of k_{on} in the absence of CO_2. The analysis of data according to Eq. (3) allowed to determine $pK_a = 6.7 \pm 0.2$, $k_{lim(top)} = (8.7 \pm 0.9) \times 10^4\ M^{-1}\ s^{-1}$, and $k_{lim(bottom)} = (1.1 \pm 0.1) \times 10^4\ M^{-1}\ s^{-1}$. The Hb—Fe(IV)═O concentration was 2.9×10^{-6} M. The Co_2 concentration was 1.2×10^{-3} M. For details, see text and Fig. 1.

of k versus [peroxynitrite] shows values of a ranging between 2.1 and 6.2 s^{-1} at different pH values. Since peroxynitrite scavenging by Mb—Fe(IV)═O and Hb—Fe(IV)═O is not likely to be a reversible process, $2.1 \geqslant a \geqslant 6.2\ s^{-1}$ may be indicative of a reaction mechanism more complex than that reported in Scheme 1 [19,22,24,25].

As shown in Figs. 1 and 2 and Supplementary Tables 2 and 3, values of k_{on} for peroxynitrite scavenging by Mb—Fe(IV)═O and Hb—Fe(IV)═O increase on decreasing pH from 8.3 to 5.8, in the absence of CO_2; the analysis of data according to Eq. (3) allowed to determine values of $pK_a = 6.9$ and 6.7, respectively. The pK_a values for peroxynitrite scavenging by Mb—Fe(IV)═O (=6.9) and Hb—Fe(IV)═O (=6.7), in the absence of CO_2, are similar to those reported for: (i) peroxynitrite detoxification by ferryl *Mycobacterium leprae* truncated HbO (=6.7; trHbO—Fe(IV)═O) [29], and (ii) the peroxynitrous acid/peroxynitrite (i.e., ONOOH/ONOO) equilibrium (=6.5–6.8) [10,34]. This suggests that peroxynitrous acid is the species that reacts preferentially with heme–Fe(IV)═O. According to Eq. (3), $k_{lim(top)}$ and $k_{lim(bottom)}$ could represent the second-order rate constants for Mb—Fe(IV)═O- and Hb—Fe(IV)═O-mediated scavenging of peroxynitrous acid at $pH \ll pK_a$ and of peroxynitrite at $pH \gg pK_a$, respectively. In agreement with: (i) kinetics of peroxynitrite scavenging by *M. leprae* trHbO—Fe(IV)═O [29], and (ii) kinetic simulations concerning peroxynitrite scavenging by horse heart Mb—Fe(IV)═O [19], $k_{lim(top)}$ values for peroxynitrite scavenging by Mb—Fe(IV)═O and Hb—Fe(IV)═O exceed those of $k_{lim(bottom)}$ (i.e., $k_{lim(top)}/k_{lim(bottom)} = 4.7$ and 7.9, respectively). Accordingly, the reaction of Hb—Fe(IV)═O with ONOOH shows a lower activa-

tion barrier (by about 5.1 kJ mol^{-1}) with respect to that with $ONOO^-$. In the case of Mb—Fe(IV)=O, the reactivity difference between ONOOH and $ONOO^-$ is much lower (amounting to about 3.7 kJ mol^{-1}).

In the presence of CO_2, values of k_{on} for peroxynitrite scavenging by Mb—Fe(IV)=O and Hb—Fe(IV)=O are pH-independent (the average k_{on} values are 7.1×10^4 M^{-1} s^{-1} and 1.2×10^5 M^{-1} s^{-1}, respectively; Figs. 1 and 2, and Supplementary Tables 2 and 3), as reported for *M. leprae* trHbO–Fe(IV)=O, *Glycine max* leghemoglobin–Fe(IV)=O (Lb–Fe(IV)=O), horse heart Mb—Fe(IV)=O, and human Hb—Fe(IV)=O [19,22,24,29]. This agrees with the reaction mechanism proposed for peroxynitrite scavenging by heme–Fe(IV)=O in the presence of CO_2 involving the transient highly reactive species $^{\cdot}NO_2$. The formation of $^{\cdot}NO_2$, possibly representing the rate-limiting step of the whole process, does not depend on the $ONOOH \leftrightarrow ONOO^- + H^+$ equilibrium (and thus on pH), but instead on the CO_2 concentration [19,22,24,29]. Also values of a for peroxynitrite scavenging by Mb—Fe(IV)=O and Hb—Fe(IV)=O in the presence of CO_2 are pH-independent (the average a values are 2.9 and 4.9 s^{-1}, respectively) (see Supplementary Tables 2 and 3).

To support the kinetic mechanism of peroxynitrite scavenging by Mb—Fe(IV)=O and Hb—Fe(IV)=O (Scheme 1), kinetics of peroxynitrite detoxification by sperm whale Mb—Fe(II)—O_2 and human Hb—Fe(II)—O_2 were investigated. Mixing of Mb—Fe(II)—O_2 or Hb—Fe(II)—O_2 with peroxynitrite solutions, in the absence and presence of CO_2, leads to the formation of Mb—Fe(III) and Hb—Fe(III), respectively, via the transient formation of Mb—Fe(IV)=O and Hb—Fe(IV)=O, respectively. Under all the experimental conditions, the time course for peroxynitrite scavenging by Mb—Fe(II)—O_2 and Hb—Fe(II)—O_2 corresponds to a biphasic process (Scheme 2). Moreover, values of h and k for peroxynitrite scavenging by Mb—Fe(III) and Hb—Fe(III) are wavelength-independent under pseudo-first order conditions at fixed peroxynitrite concentration (data not shown).

Plots of h and k versus [peroxynitrite] are linear, the slope corresponds to h_{on} and k_{on} (see Eqs. (5a) and (5b)) (Supplementary Figs. 1 and 2). In the absence of CO_2, the y-axis intercept of plots of h and k versus [peroxynitrite] corresponds to $a \cong 0$ s^{-1} (Supplementary Figs. 1 and 2). On the other hand, in the presence of CO_2 (Supplementary Figs. 1 and 2), the y-axis intercept of plots of h and k versus [peroxynitrite] display values of a ranging between 4.7 s^{-1} and 1.2×10^1 s^{-1}. Since peroxynitrite scavenging by Mb—Fe(II)—O_2 and Hb—Fe(II)—O_2 is not likely to be a reversible process, 4.7 s^{-1} $\geqslant a \geqslant 1.2 \times 10^1$ s^{-1} may be indicative of a reaction mechanism more complex than that reported in Scheme 2 [19,22,24,25].

Values of k_{on} for peroxynitrite scavenging by Mb—Fe(IV)=O and Mb—Fe(II)—O_2, and by Hb—Fe(IV)=O and Hb—Fe(II)—O_2 match each other (Table 1), according to Schemes 1 and 2. Moreover, values of h_{on} and k_{on} for the peroxynitrite scavenging by Hb—Fe(IV)=O and Hb—Fe(II)—O_2 are in agreement with those reported previously, in the absence and presence of CO_2 [22] (see Table 1).

Values of k_{on} for the peroxynitrite scavenging by sperm whale Mb and human Hb derivatives are grossly similar to those reported for *M. leprae* trHbO, *Glycine max* Lb, and horse heart Mb action (Table 1) [19,22,24,29], indicating that the reactions depicted in Schemes 1 and 2 do not appear to reflect the different geometry of the heme-distal pocket. In fact, sperm whale Mb, horse heart Mb, and human Hb display the classical histidyl-based heme-distal pocket; the ligand entry to and exit from the heme-distal site occurs via the so-called 'E7-gate' [38–40]. On the other hand, the heme-distal region of *M. leprae* trHbO is completely different, indeed the HisE7 residue present in sperm whale Mb and human Hb chains is replaced by Ala [15,41]. Moreover, cavity systems present in the protein matrix appear to facilitate ligand entry to and exit from the *M. leprae* trHbO heme-distal pocket, the so-called 'E7-gate' being inoperative [15,41].

Table 1
Values of kinetic parameters for peroxynitrite scavenging by ferryl and ferrous oxygenated heme-proteins (in italics and bold, respectively; see Schemes 1 and 2, respectively).

Heme-protein	[CO_2] (M)	h_{on} (M^{-1} s^{-1})	k_{on} (M^{-1} s^{-1})
Mycobacterium leprae trHbO	0[a]	–	*1.5×10^4*[a]
	1.2×10^{-3}[a]	–	*2.2×10^4*[a]
	0	**4.8×10^4**[b]	**1.3×10^4**[b]
	1.2×10^{-3}[b]	**6.3×10^5**[b]	**1.7×10^4**[b]
Glycine max Lb[c]	0	–	*3.4×10^4*
	1.2×10^{-3}	–	*2.3×10^5*
	0	**5.5×10^4**	**2.1×10^4**
	1.2×10^{-3}	**8.8×10^5**	**3.6×10^5**
Sperm whale Mb[d]	0	–	*4.6×10^4*
	1.2×10^{-3}	–	*7.4×10^4*
	0	**7.3×10^4**	**3.8×10^4**
	1.2×10^{-3}	**6.8×10^4**	**4.6×10^4**
Horse heart Mb	0[e]	–	*1.9×10^4*[e]
	1.2×10^{-3}[e]	–	*2.6×10^4*[e]
	0[f]	**5.4×10^4**[f]	**2.2×10^4**[f]
	1.2×10^{-3}[f]	**4.1×10^5**[f]	**3.2×10^4**[f]
Human Hb[d]	0	–	*3.3×10^4*
	1.2×10^{-3}	–	*1.9×10^5*
	0	**2.9×10^4**	**1.7×104**
	1.2×10^{-3}	**2.1×10^5**	**1.6×10^5**

[a] pH 7.2 and 20.0 °C. From [29].
[b] pH 7.3 and 20.0 °C. From [25].
[c] pH 7.3 and 20.0 °C. From [24].
[d] pH 7.1 and 20.0 °C. Present study.
[e] pH 7.5 and 20.0 °C. From [19].
[f] pH 7.3 and 20.0 °C. From [19].

Conclusions

The catalytic parameters for peroxynitrite-mediated reduction of heme–Fe(IV)=O (present study) and heme–Fe(II)—O_2 are similar (Table 1) and high enough to indicate that both reactions could occur in vivo [7,15]. Peroxynitrite scavenging by heme–Fe(IV)=O, obtained by treatment with H_2O_2, could be relevant under anaerobic and oxidative conditions, as occurs in ischemia-reperfusion injury and other cardiovascular pathological situations [3,7,10]. In turn, peroxynitrite can act as a scavenger of the highly oxidizing heme-Fe(IV)=O species, which could be responsible for the oxidative cell damage [42]. Therefore, heme-globins can undertake within the same cycle H_2O_2 and peroxynitrite detoxification.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.09.050.

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