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Living in an Oxygen Atmosphere – NO• Problem?

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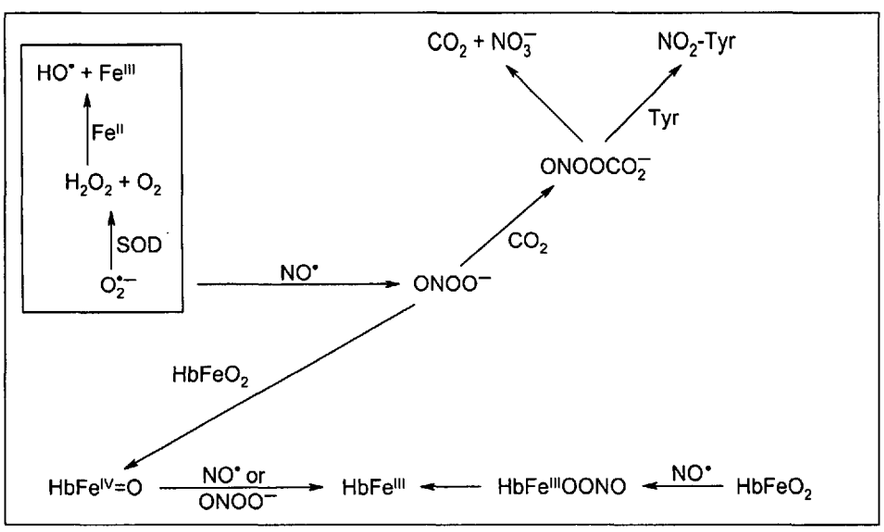
Abstract: Formation of nitrogen monoxide *in vivo* as a signal molecule and as part of the immunological response leads to the formation of oxidized haemoproteins and a powerful oxidant, peroxynitrite. The latter is an unstable isomer of nitrate that, in the protonated form, oxidizes and nitrates biomolecules. Peroxynitrite is formed from the diffusion-controlled reaction of superoxide with nitrogen monoxide. The hypothesis that peroxynitrous acid undergoes homolysis to nitrogen dioxide and hydroxyl radicals is not substantiated by analyses of the products of peroxynitrite decay. Peroxynitrite has been stabilized as a ligand of cobalt(III). Nitrogen monoxide reacts very rapidly with oxyhaemoglobin and oxymyoglobin to generate intermediate iron(III)peroxynitrito complexes that do not nitrate the tyrosine residues in the globin. Peroxynitrite oxidizes oxymyoglobin to the iron(III) form of the protein *via* the high valent oxoiron(IV)myoglobin. The two tyrosine residues of myoglobin are nitrated in only very low yield. Peroxynitrite can be efficiently scavenged by an iron(III)myoglobin mutant in which the distal histidine has been replaced with an alanine. We propose that the reactions of myoglobin with nitrogen monoxide and peroxynitrite may be essential to preserve respiration in the skeletal muscle.

Keywords: Kinetics · Myoglobin · Nitrogen monoxide · Oxygen toxicity · Peroxynitrite

1. Introduction

On earth, the reduction of oxygen, as compared to that of other elements, is the most convenient, and energetically the most favorable process to drive processes essential to support life [1]. The product of the complete reduction is harmless, however, partial reduction leads to superoxide [dioxide(•1-)], hydrogen peroxide, and/or the hydroxyl radical *via* Fenton chemistry [2]. Defense systems include enzymes such as superoxide dismutase, catalase, and glutathione peroxidase to intercept or prevent formation of such species. Excess production of partially reduced oxygen species has already during the seventies and eighties been linked to various diseases [3]. This simple picture of oxygen toxicity (Scheme) became far more complicated when it became clear that nitrogen monoxide is formed *in vivo*. The discovery of a physiological role for this simple molecule was honored by the Nobel prize to R.F. Furchgott, L.J. Ignarro, and F. Murad. At a concentration in the nanomolar range, nitrogen monoxide acts as a messenger that is involved in the relaxation of blood vessels. Its lifetime of *ca.* 100 ms is determined by reaction with haemoglobin and myoglobin. The oxy-forms of these proteins react avidly with nitrogen monoxide to produce the met-forms and peroxynitrite. Remarkably, these proteins can be recycled after enzymatic reduction to bind dioxygen again, as discussed below.

Much higher concentrations – in the micromolar range – of both nitrogen monoxide and superoxide are produced by activated macrophages during the immune response. In 1990 it was proposed that these radicals react to form peroxynitrite [oxoperoxonitrate(1-)] [4]. Peroxynitrite is a potent oxidant that initiates lipid peroxidation and also nitrates some tyrosine residues. It is interesting to note



Scheme

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that nature relies on two inorganic compounds to attack invading organisms: peroxynitrite is produced by macrophages and hypochlorite [oxochlorate(1-)] by leucocytes [5]. However, both species are responsible for 'collateral' damage to nearby healthy tissues. Thus, the nitrogen monoxide molecule has a Janus face: essential for signal transduction, but indirectly damaging to proteins and membranes.

The formation of peroxynitrite can be prevented by superoxide dismutase, which diverts superoxide to produce hydrogen peroxide and dioxygen [6]. However, given the rate constants and estimated concentrations involved, this is not possible in the vicinity of activated macrophages, which accounts for the immunological detection of nitrated tyrosine residues associated with infected tissues [7]. A membrane-permeable, recyclable scavenger of peroxynitrite would be useful in medicine.

Peroxynitrite was first postulated to be formed from the reaction of nitrite with hydrogen peroxide by Baeyer and Villiger one hundred years ago [8]. The anion is relatively stable at low temperature, but the peroxynitrous acid (the p*K*a is 6.8) isomerizes to nitrate at a rate of 1.2 s^{-1} [9]. At a pH near and above the p*K*a of peroxynitrous acid, one finds that also nitrite and dioxygen are formed.

Peroxynitrous acid nitrates phenolic compounds at a low yield of *ca.* 8% relative to total peroxynitrite. However, this yield is more than doubled in the presence of carbon dioxide. The reaction of the peroxynitrite anion with carbon dioxide has a rate constant of 3.4×10^4 $M^{-1}s^{-1}$ [10]. Given a physiological concentration of carbon dioxide of *ca.* 1 mM, most peroxynitrite forms an adduct with this oxide, which rapidly decomposes to nitrate and carbon dioxide. While it would seem that nature has found a convenient way to dispose of a powerful oxidant, the doubling of the yield of nitration of tyrosine indicates that reactive intermediates are formed that are more effective than peroxynitrite alone.

2. $NO^{\bullet}$ and $O_2^{\bullet-}$

2.1. *Formation and Properties of Peroxynitrite*

We have reinvestigated the equilibrium reaction of superoxide with nitrogen monoxide, Eqn. 1:

$$O_2^{\bullet-} + NO^{\bullet} \leftrightarrows ONOO^- \quad (1)$$

The forward reaction is arguably the fastest bimolecular reaction in biology. Published rate constants span the range from $(0.4 - 1.9) \times 10^{10}$ $M^{-1}s^{-1}$ [11–14]. Photolysis of an anaerobic alkaline peroxynitrite solution produces superoxide and nitrogen monoxide that recombine at a rate of $(1.9 \pm 0.1) \times 10^{10}$ $M^{-1}s^{-1}$. Alternatively, when hydrogen peroxide is photolyzed in the presence of nitrogen monoxide, hydroxyl radicals are formed that react with hydrogen peroxide to form superoxide; from these experiments a rate constant of $(2.2 \pm 0.6) \times 10^{10}$ $M^{-1}s^{-1}$ is derived. These results are in agreement with our earlier investigations [14].

Whether or not peroxynitrous acid can undergo homolysis and form nitrogen dioxide and hydroxyl radicals, is presently hotly debated in the literature. The extent of homolysis is estimated to be *ca.* 30%, while the remaining 70% undergoes isomerization to nitrate. One approach to resolve this question is to determine whether homolysis is thermodynamically feasible. Standard Gibbs energies of formation for superoxide and nitrogen monoxide are known, and recently a value of 16.6 kcal mol^{-1} has been reported for peroxynitrite [15]. This value, which allows homolysis, is based on the rate constants for the forward and backward rate constants of Eqn. 1. A rate constant of 0.017 s^{-1} for the backward rate constant was determined from the rate of formation of trinitromethanide, $C(NO_2)_3^-$ when peroxynitrite was mixed with $C(NO_2)_4$, a scavenger of superoxide. If the interpretation of this experiment were correct, then a much slower reduction would be observed in the presence of nitrogen monoxide. However, we found that the presence of nitrogen monoxide did not influence the rate at which trinitromethanide was formed, which indicates that its formation is due to a process other than the reduction by superoxide. The preferred thermodynamic Gibbs energy of formation of peroxynitrite is therefore 14 kcal mol^{-1}, as derived before by us [16]. The implication of this Gibbs energy of formation is that homolysis of the O-O bond in peroxynitrous acid is not impossible, but very unlikely.

Another implication of homolysis would be that the decomposition of peroxynitrite into nitrite and dioxygen would not be dependent on the peroxynitrite concentration. We examined the decay of peroxynitrite as a function of concentration, temperature and pH and found that, below 5 °C and pH 7, very little nitrite and dioxygen are formed, even when the peroxynitrite concentration is high (2.5 mM). Instead, approximately ≥90% isomerizes to nitrate. At higher pH decomposition increases at the expense of isomerization, up to nearly 80% at pH 10.0 at 5 °C, and 90% at 45 °C. Much less nitrite and dioxygen per peroxynitrite are formed when the peroxynitrite concentration is lower: at 50 μM and pH 10.2 less than 40% decomposes. Thus, we find that the extent of decomposition is dependent on the peroxynitrite concentration, in contrast to two other reports [17][18]. A radical-free mechanism, rather than a free radical mechanism, seems more appropriate. A suitable mechanism could be analogous to the mechanism accepted for other peracids [19], requiring the reaction of the peroxynitrite anion with peroxynitrous acid, for which we have kinetic evidence [14].

Although it is possible to synthesize pure peroxynitrite [20], experiments are often carried out with more convenient, but impure, preparations that contain nitrite and nitrate and sometimes hydrogen peroxide [21]. Experiments with such a preparation led to a report that peroxynitrous acid decomposes to form singlet oxonitrate(1–) ($^1NO^-$) and dioxygen ($^1\Delta_gO_2$)[22]; however, it is more likely that contaminating hydrogen peroxide, which is known to react with peroxynitrite to form singlet oxygen in small yield [23] gave rise to this observation. Thus, all observations of Khan *et al.* [22] could be explained by the presence of hydrogen peroxide; furthermore, quantum mechanical and thermodynamic calculations show that formation of the postulated intermediate, a cyclic form of peroxynitrous acid (trioxazetidine), and the products $^1NO^-$ and $^1\Delta_gO_2$, requires Gibbs energies of *ca.* +415 kJ $mol^{-1}$and *ca.* +180 kJ mol^{-1}, respectively [24].

2.2. *Peroxynitrite and Carbon Dioxide*

The biochemically most relevant reaction of peroxynitrite is the reaction with carbon dioxide. It catalyses the isomerization of peroxynitrite to nitrate *via* an intermediate, presumably $ONOOCO_2^-$, which has an absorption maximum near 650 nm [25]. The reflection spectrum of solid $[(CH_3)_4N][ONOO]$ exposed to carbon dioxide shows a similar band near 650 nm; this absorption decays over minutes.

Stopped-flow experiments, in which carbon dioxide solutions were mixed with alkaline peroxynitrite solutions, indicate the formation of at least one intermediate: The initial absorption at 302 nm is less than that due to the known initial

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amount of peroxynitrite, which indicates that reactions take place within the mixing time, and this absorption is dependent (but not linearly) on the concentrations of peroxynitrite and carbon dioxide.

We found that reaction of peroxynitrite with carbon dioxide forms some trioxocarbonate (•1–) ($CO_3^{\bullet-}$) and nitrogen dioxide radicals *via* homolysis of the O-O bond in $ONOOCO_2^-$. We determined the extent of radical formation by mixing peroxynitrite, carbon dioxide, and nitrogen monoxide. The latter reacts with trioxocarbonate(•1-) and nitrogen dioxide radicals to form, effectively, three nitrite per homolysis, while $ONOOCO_2^-$, which does not undergo homolysis, yields nitrate and carbon dioxide. Based on the independent nitrate and nitrite analyses, there is (96±1)% conversion to nitrate, while only (3±1)% of all $ONOOCO_2^-$ undergoes homolysis [26], significantly less than that suggested in the literature [27-30].

2.3. *Scavenging of Peroxynitrite*

Attempts to find scavengers of peroxynitrite that can compete with carbon dioxide have been unsuccessful so far. Although flavonoids and similar phenolic compounds have been regarded as scavengers [31][32], they do not increase the rate of decay of peroxynitrite. Alkanones catalyze the formation of nitrate by forming a dioxirane and nitrite; these react to reform the original alkanone and nitrate; however, the rate constants are too small to be significant [33]. At present, manganese-containing porphyrins hold the record for the fastest reaction of peroxynitrite, 2×10^7 $M^{-1}s^{-1}$[34]. Methionine also reacts with peroxynitrite, but here too the reaction is too slow. Interestingly, we found that, of every three methionines that react with peroxynitrite, two are oxidized to methionine sulfoxides and one is unchanged. The N-containing products are two nitrites and a nitrate. It would seem that one out of every three methionines catalyses the isomerization of peroxynitrite to nitrate [35].

2.4. *Stabilization of Peroxynitrite*

When nitrogen monoxide is added to a solution of pentacyanosuperoxocobaltate(III), a new compound, pentacyanoperoxynitritocobaltate(III), is formed (Fig. 1) [36] that at pH 2 releases peroxynitrite over several days. The release is accelerated by light. When left in the dark, a slow isomerization within the coordination sphere takes place and pentacyano-nitratocobaltate(III) is formed, which has not been synthesized before. At pH 6 the compound is stable [37].

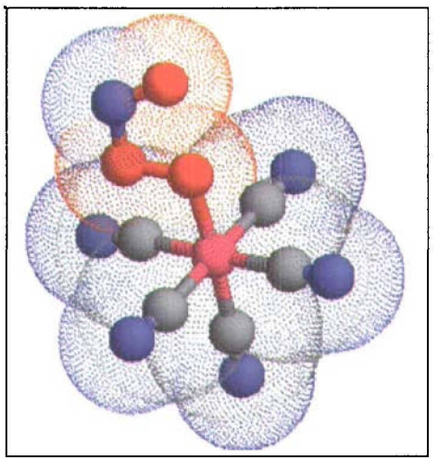

Fig. 1. Possible structure for pentacyanoperoxynitritocobaltate(III).

Experiments with oxotitanium(2+), which forms a stable complex with hydrogen peroxide, have not yielded a complex that could be isolated.

3. NO• and haemoproteins

Probably one of the most significant aspects of nitrogen monoxide chemistry is its ability to react in a unique way with the haem centers of different proteins such as nitric oxide synthase, guanylate cyclase, haemoglobin (Hb), and myoglobin (Mb) [38]. Of particular interest is the rapid reaction of nitrogen monoxide with oxyhaemoglobin, which significantly reduces the half-life of nitrogen monoxide *in vivo* and is the cause for an increase in blood pressure observed when extracellular haemoglobin-based blood substitutes are administered [39].

3.1. *Reactions of Nitrogen Monoxide with Oxyhaemoglobin and Oxymyoglobin*

We have recently shown that, in analogy to the reaction between nitrogen monoxide and superoxide, the reactions of oxyhaemoglobin and oxymyoglobin with NO• generate intermediate iron (III)peroxynitrito complexes that were characterized by rapid-scan UV/VIS spectroscopy [39][40]. The intermediate peroxynitrite complexes $MbFe^{III}OONO$ and $HbFe^{III}OONO$ can be observed at alkaline pH, but rapidly decay to nitrate and the aquoiron(III) form of the proteins under neutral or acidic conditions. The rate of decay of the peroxynitrite complex of the two haemoglobin subunits (36 s^{-1} and 7 s^{-1} at pH 9.5) is lower than that measured for $MbFe^{III}OONO$ (205 s^{-1} at pH 9.5). However, these rates are significantly larger than that of the decay of free peroxynitrite under the same conditions (0.11 s^{-1} at pH 9.5) [14]. Our mechanistic studies showed that no free peroxynitrite is formed during the reactions of NO• with these oxyproteins, and that nitrate is formed quantitatively, both at pH 7.0 and 9.0. Analysis of the proteins after cycling the oxidation by NO• and the reduction with ascorbic acid for 10 times indicates that less than 1% of the tyrosine residues are nitrated. These results show that, when peroxynitrite is coordinated to the haem of myoglobin or haemoglobin, it rapidly isomerizes to nitrate which prevents nitration of tyrosine residues of the globin.

3.2. *Reactions of Nitrite and Nitrogen Monoxide with Oxoiron(IV)myoglobin*

Recent studies suggest that NO• can also serve as an antioxidant, for instance by inhibiting lipid oxidation [41]. Oxo(IV) myoglobin (ferryl myoglobin, $MbFe^{IV}=O$) is a highly oxidizing species proposed to be at least in part responsible for the oxidative damage caused by the reperfusion of ischaemic tissues. We have recently determined the rate constant for the reaction between $MbFe^{IV}=O$ and NO• [$(17.9 \pm 0.5) \times 10^6$ M^{-1} s^{-1} at pH 7.5 and 20 °C] [42]. The large value of this rate constant implies that this reaction is very likely to take place *in vivo* and might represent a detoxifying pathway for $MbFe^{IV}=O$ and, thus, an additional antioxidant function of NO•.

Nitrite is one of the major end products of NO• metabolism and its local concentration reflects that of NO•. For instance, increased nitrite levels are found under pathophysiological conditions such as inflammation, when NO• production is elevated. We have found that the rate of reaction of $MbFe^{IV}=O$ with nitrite is significantly lower (16 ± 1 M^{-1} s^{-1} at pH 7.5 and 20 °C) than that with NO•. Thus, the reaction with nitrite probably plays a role only when NO• has been consumed completely and large concentrations of nitrite are still present. In contrast to the protecting role of NO•, the reaction with nitrite generates nitrogen dioxide that can contribute to tyrosine nitration. Indeed, we have demonstrated that nitrite can cause nitration of added tyrosine in the presence of iron(III)myoglobin and hydrogen peroxide [42].

3.3. *Reaction of Peroxynitrite with Oxymyoglobin and Oxyhaemoglobin*

If one considers the typical concentrations of different biological targets and their relative rates of reaction with peroxy-

nitrite, it becomes evident that *in vivo* peroxynitrite disappears mainly by reacting with carbon dioxide, glutathione, selenium-containing proteins or metalloproteins, in particular haem proteins [43]. Thus, haemoglobin and myoglobin are potential targets for peroxynitrite in the blood vessels and the red muscle, respectively. It has been reported that, despite the presence of a large concentration of carbon dioxide in the blood (*ca.* 1 mM), peroxynitrite is able to diffuse across the red-blood-cell membrane and react with oxyhaemoglobin [44].

We have recently shown that the peroxynitrite-mediated oxidation of oxymyoglobin proceeds *via* the intermediate oxoiron(IV) complex, which, in a second step, reacts with a further equivalent of peroxynitrite to yield the iron(III)myoglobin [45]. The pH- and oxygen-concentration-dependence of the observed rate constants for the two steps indicate that the protonated form of peroxynitrite (HOONO) and deoxymyoglobin ($MbFe^{II}$), which is in equilibrium with oxymyoglobin, are the reactive species [45]. The second-order rate constant values obtained at pH 7.3 and 20°C for the two steps of the reaction are $(5.4 \pm 0.2) \times 10^4$ M^{-1} s^{-1} and $(2.2 \pm 0.1) \times 10^4$ M^{-1} s^{-1}, respectively. Analogous studies with haemoglobin suggest that its reaction with peroxynitrite follows the same mechanism. In this case, the second-order rate-constant values measured at pH 7.0 and 20 °C for the two steps are $(8.8 \pm 0.4) \times 10^4$ M^{-1} s^{-1} and $(9.4 \pm 0.7) \times 10^4$ M^{-1} s^{-1}, respectively.

In addition, oxymyoglobin effectively protects against peroxynitrite-mediated nitration of free tyrosine. About 0.1 equiv. of oxymyoglobin (relative to the free tyrosine) is required to inhibit tyrosine nitration (IC_{50}) (M. Exner and S. Herold, unpublished results). A variety of different analytical techniques (MALDI-TOF mass spectroscopy, HPLC-analysis and immunohistochemical analysis with nitrotyrosine antibodies) were employed to disclose whether the two tyrosine residues of myoglobin (Fig. 2) are nitrated in the course of the reaction with peroxynitrite. Our results show that peroxynitrite is able to nitrate tyrosine residues of oxymyoglobin in a concentration dependent way, but in extremely low yields (M. Mehl, M. Exner and S. Herold, unpublished results). When added in equimolar amounts, peroxynitrite nitrates less then 1% of the available tyrosine residues of oxymyoglobin. Significantly larger nitrotyrosine amounts are detected when peroxynitrite is allowed to react with apoMb, that is myoglobin without the haem. These results show that peroxynitrite is scavenged by the direct reaction with the haem and not by an unspecific reaction with the globin.

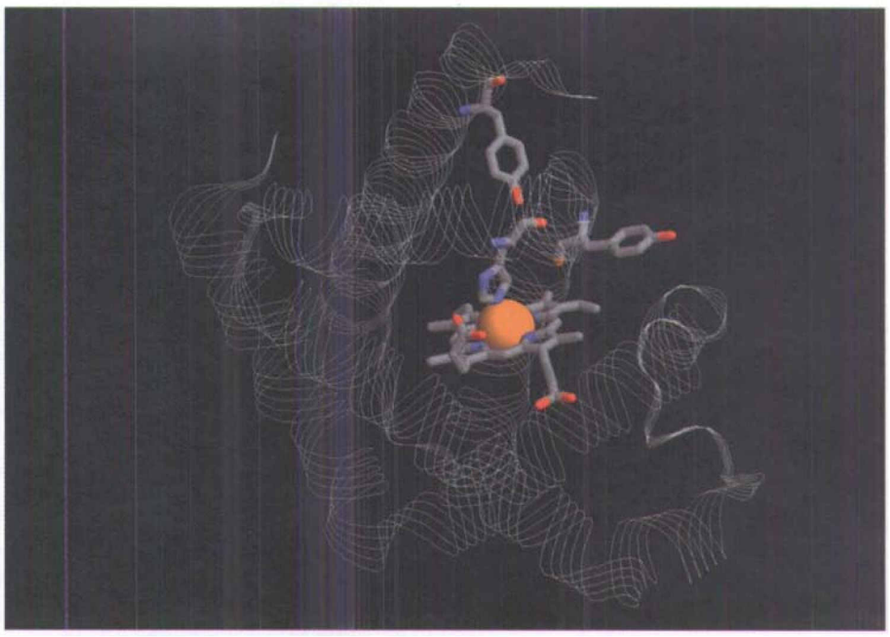

Fig. 2. Structure of myoglobin with the two tyrosine residues highlighted.

3.4. *Myoglobin Mutants as Scavengers of Peroxynitrite*

The reactivity of iron(III)myoglobin towards peroxynitrite is regulated by the presence of the distal histidine (His64), which partly blocks the active site and stabilizes, *via* a strong hydrogen bond, the water ligand coordinated to the iron [46]. In the presence of wild-type metMb the lifetime of peroxynitrite is almost unchanged ($k_{cat} = (1.4 \pm 0.1) \times 10^4$ M^{-1} s^{-1}). In contrast, the myoglobin mutant in which His64 is replaced by an alanine (H64A) is an efficient catalyst for the isomerization of peroxynitrite ($k_{cat} = (6.0 \pm 0.1) \times 10^6$ M^{-1} s^{-1} at pH 7.4 and 20 °C). Interestingly, H64A is an efficient scavenger also in the presence of 1.2 mM CO_2 ($k_{cat} = (9.6 \pm 0.2) \times 10^6$ M^{-1} s^{-1} at pH 7.3 and 20 °C). Ion chromatographic analysis of the nitrogen-containing products shows that, in the presence of 0.01 equiv. of H64A, nitrate is formed quantitatively. HPLC analysis reveals that 0.05 equiv. of H64A prevent nitration of free tyrosine by peroxynitrite almost completely both in the absence as well as in the presence of physiological amounts of carbon dioxide (1.2 mM).

3.5. *Biological Significance: a New Function of Myoglobin?*

Haemoglobin, the constituent of red blood cells responsible for the transport of dioxygen, and myoglobin, present in skeletal muscles to store dioxygen, are probably the most thoroughly studied proteins. Paradoxically, it has recently been proposed that these proteins may display additional biological functions that remain a matter of continuing investigations. The original role attributed to myoglobin, that is storage of dioxygen and facilitation of its transport through muscle tissue to mitochondria, has recently been challenged. The discovery that a mutant mouse devoid of myoglobin is capable of apparently normal muscle function raised questions about the real significance of this protein [47]. Our research in this field has shown that the reactions of nitrogen monoxide and peroxynitrite with oxymyoglobin are fast and are thus very likely to take place *in vivo*. NO• can inhibit mitochondrial respiration by reversibly binding to cytochrome *c* oxidase [48]. Intracellular scavenging of NO• would therefore contribute to preserve respiration in the skeletal muscle and in the heart and, consequently, protect the energy-producing machinery. It may thus be possible that one of the essential functions of myoglobin is to scavenge nitrogen monoxide and peroxynitrite. This hypothesis is supported by

the observation that in transgenic mice lacking myoglobin, physiological parameters of the heart are more severely affected by endogenously formed and exogenously applied nitrogen monoxide [49].

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[1] P. George, in 'Oxidases and Related Redox Systems', Eds. T.E. King, H.S. Mason and M. Morrison Wiley, New York, **1965**, pp. 3–36.
[2] W.H. Koppenol, in 'Iron Chelators. New Development Strategies', Eds. D.G. Badman, R.J. Bergeron, G.M. Brittenham, The Saratoga Group, Ponte Vedra Beach, FL, **2000**, pp. 3–10.
[3] J.M.C. Gutteridge, *Free Radical Res. Commun.* **1993**, *19*, 141.
[4] J.S. Beckman, T.W. Beckman, J. Chen, P.A. Marshall, B.A. Freeman, *Proc. Natl. Acad. Sci.USA* **1990**, *87*, 1620.
[5] J.K. Hurst, S.V. Lymar, *Acc. Chem. Res.* **1999**, *32*, 520.
[6] J.M. McCord, I. Fridovich, *J. Biol. Chem.* **1969**, *244*, 6049.
[7] Y.Z. Ye, M. Strong, Z.Q. Huang, J.S. Beckman, *Methods Enzymol.* **1996**, *269*, 201.
[8] A. Baeyer, V. Villiger, *Ber. deutschen chem.Ges.* **1901**, *34*, 755.
[9] W.H. Koppenol, J.J. Moreno, W.A. Pryor, H. Ischiropoulos, J.S. Beckman, *Chem. Res. Toxicol.* **1992**, *5*, 834 .
[10] S.V. Lymar, J.K. Hurst, *J. Am. Chem. Soc.* **1995**, *117*, 8867.
[11] R.E. Huie, S. Padmaja, *Free Radical Res. Commun.* **1993**, *18*, 195.
[12] S. Goldstein, G. Czapski, *Free Radical Biol. Med.* **1995**, *19*, 505.
[13] K. Kobayashi, M. Miki, S. Tagawa, *J. Chem. Soc. Dalton Trans.* **1995**, 2885.
[14] R. Kissner, T. Nauser, P. Bugnon, P.G. Lye, W.H. Koppenol, *Chem. Res. Toxicol.* **1997**, *10*, 1285.
[15] G. Merényi, J. Lind, *Chem. Res. Toxicol.* **1998**, *11*, 243.
[16] W.H. Koppenol, R. Kissner, *Chem. Res. Toxicol.* **1998**, *11*, 87.
[17] S. Pfeiffer, A.C.F. Gorren, K. Schmidt, E.R. Werner, B. Hansert, D.S. Bohle, B. Mayer, *J. Biol. Chem.* **1997**, *272*, 3465.
[18] J.W. Coddington, J.K. Hurst, S.V. Lymar, *J. Am. Chem. Soc.* **1999**, *121*, 2438.
[19] J.F. Goodman, P. Robson, *Trans. Faraday Soc.* **1963**, *59*, 2871, R. Kissner, W.H. Koppenol, *J. Am. Chem. Soc.* **2001**, in press.
[20] D.S. Bohle, B. Hansert, S.C. Paulson, B.D. Smith, *J. Am. Chem. Soc.* **1994**, *116*, 7423.
[21] R.M. Uppu, G.L. Squadrito, R. Cueto, W.A. Pryor, *Methods Enzymol.* **1996**, *269*, 285.
[22] A.U. Khan, D. Kovacic, A. Kolbanovskiy, M. Desai, K. Frenkel, N.E. Geacintov, *Proc. Natl. Acad. Sci.USA* **2000**, *97*, 2984.
[23] P. Di Mascio, E.J.H. Bechara, M.H.G. Medeiros, K. Briviba, H. Sies, *FEBS Lett.* **1994**, *355*, 287.
[24] G.R. Martinez, P. Di Mascio, M.G. Bonini, O. Augusto, K. Briviba, H. Sies, P. Maurer, U. Röthlisberger, S. Herold, W.H. Koppenol, *Proc. Natl. Acad. Sci. USA* **2000**, *79*, 10307.
[25] R. Meli, T. Nauser, W.H. Koppenol, *Helv. Chim. Acta* **1999**, *82*, 722.
[26] R. Meli, T. Nauser, P. Latal, W.H. Koppenol, *J. Biol. Inorg.Chem.* **2001**, in press.
[27] M.G. Bonini, R. Radi, G. Ferrer-Sueta, A.M.D. Ferreira, O. Augusto, *J. Biol. Chem.* **1999**, *274*, 10802.
[28] S. Goldstein, G. Czapski, *Inorg. Chem.* **1997**, *36*, 5113.
[29] G.R. Hodges, K.U. Ingold, *J. Am. Chem. Soc.* **1999**, *121*, 10695.
[30] J.W. Coddington, S. Wherland, J.K. Hurst, *Inorg. Chem.* **2001**, *40*, 528.
[31] G.R.M.M. Haenen, J.B.G. Paquay, R.E.M. Korthouwer, A. Bast, *Biochem. Biophys. Res. Commun.* **1997**, *236*, 591.
[32] A.S. Pannala, S. Singh, C. Rice-Evans, *Methods Enzymol.* **1999**, *299*, 207.
[33] S. Tibi, W.H. Koppenol, *Helv. Chim. Acta* **2000**, *83*, 2412.
[34] G. Ferrer-Sueta, I. Batinic-Haberle, I. Spasojevic, I. Fridovich, R. Radi, *Chem. Res. Toxicol.* **1999**, *12*, 442.
[35] D. Perrin, W.H. Koppenol, *Arch. Biochem. Biophys.* **2000**, *377*, 266.
[36] P.K. Wick, R. Kissner, W.H. Koppenol, *Helv. Chim. Acta* **2000**, *83*, 748.
[37] P.K. Wick, R. Kissner, W.H. Koppenol, *Helv. Chim. Acta* **2001**, in press.
[38] J.M. Fukuto, D.A. Wink, in 'Metals in Biology', Ed. H. Siegel, Marcel Dekker, New York, 1999, Vol. 36, p. 547.
[39] D.H. Doherty, M.P. Doyle, S.R. Curry, R.J. Vali, T.J. Fattor, J.S. Olson, D.D. Lemon, *Nature Biotechnol.* **1998**, *16*, 672.
[40] S. Herold, *FEBS Lett.* **1999**, *443*, 81.
[41] S. Herold, M. Exner, T. Nauser, *Biochemistry* **2001**, *40*, 3385.
[42] H. Rubbo, R. Radi, D. Anselmi, M. Kirk, S. Barnes, J. Butler, J.P. Eiserich, B.A. Freeman, *J. Biol. Chem.* **2000**, *275*, 10812.
[42] S. Herold, F.-J. Rehmann, *J. Biol. Inorg. Chem.* **2001**, in press.
[43] W.H. Koppenol, in 'Metals in Biology', Ed. H. Siegel, Marcel Dekker, New York, **1999**, Vol. 36, p. 597.
[44] A. Denicola, J.M. Souza, R. Radi, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 3566.
[45] M. Exner, S. Herold, *Chem. Res. Toxicol.* **2000**, *13*, 287.
[46] S. Herold, T. Matsui, Y. Watanabe, *J. Am. Chem. Soc.* **2001**, *123*, 4085.
[47] D.J. Garry, G.A. Ordway, J.N. Lorenz, N.B. Radford, E.R. Chin, R.W. Grange, R. Bassel-Duby, R.S. Williams, *Nature* **1998**, *395*, 905.
[48] G.C. Brown, *Biochim. Biophys. Acta* **1999**, *1411*, 351.
[49] U. Flögel, M.W. Merx, A. Gödecke, U.K.M. Decking, J. Schrader, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 735.