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Review

Unmasking the Janus face of myoglobin in health and disease

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Summary

For more than 100 years, myoglobin has been among the most extensively studied proteins. Since the first comprehensive review on myoglobin function as a dioxygen store by Millikan in 1939 and the discovery of its structure 50 years ago, multiple studies have extended our understanding of its occurrence, properties and functions. Beyond the two major roles, the storage and the facilitation of dioxygen diffusion, recent physiological studies have revealed that myoglobin acts as a potent scavenger of nitric oxide ($NO^{\bullet}$) representing a control system that preserves mitochondrial respiration. In addition, myoglobin may also protect the heart against reactive oxygen species (ROS), and, under hypoxic conditions, deoxygenated myoglobin is able to reduce nitrite to $NO^{\bullet}$ leading to a downregulation of the cardiac energy status and to a decreased heart injury after reoxygenation. Thus, by controlling the $NO^{\bullet}$ bioavailability *via* scavenging or formation, myoglobin serves as part of a sensitive dioxygen sensory system. In this review, the physiological relevance of these recent findings are delineated for pathological states where $NO^{\bullet}$ and ROS bioavailability are known to be critical determinants for the outcome of the disease, e.g. ischemia/reperfusion injury. Detrimental and beneficial effects of the presence of myoglobin are discussed for various states of tissue oxygen tension within the heart and skeletal muscle. Furthermore, the impact of myoglobin on parasite infection, rhabdomyolysis, hindlimb and liver ischemia, angiogenesis and tumor growth are considered.

Key words: myoglobin, nitric oxide, nitrite, normoxia, hypoxia, ischemia/reperfusion.

Introduction

The research on myoglobin covers the diversity of scientific fields ranging from biology, chemistry and physics to medicine. The body of acquired knowledge leads to a better understanding of the complexity of myoglobin and its essential role in living systems. *In vitro* studies are concerned with the structural and genomic organization of myoglobin, its kinetic and dynamic behavior, and its binding, electrocatalytic and reduction properties. Further studies including *ex vivo* and *in vivo* animal models scrutinize its functional role including medical aspects. In this review, we examine the functions of myoglobin for a variety of diseases depending on the oxygen tension of the tissue with regard to its beneficial as well as potential deleterious effects.

Myoglobin – the basics

Myoglobin was discovered spectroscopically by Mörner in 1897 (Mörner, 1897). In order to distinguish this muscle pigment – noted as muscle hemoglobin – from blood hemoglobin, Mörner first suggested the name myochrome. The notation myoglobin was introduced by Günther, who confirmed the results of Mörner in 1921 (Günther, 1921). For a long time, the general consensus persisted that in vertebrates the expression of myoglobin is restricted to muscle tissue like cardiomyocytes, oxidative skeletal myofibers (Millikan, 1939; Wittenberg and Wittenberg, 1989) and vascular smooth muscle cells (Qiu et al., 1998; Rayner et al., 2009). However, recent studies in fish surprisingly revealed a widespread non-muscle expression under normoxia and in response to hypoxia, including liver, kidney, gills and brain (Cossins et al., 2009; Fraser et al., 2006; van der Meer et al., 2005). Furthermore, it has been discovered that some human tumors of non-muscle origin, including breast, lung, ovary and colon carcinomas, express myoglobin at significant levels (Flonta et al., 2009).

Due to its structure, which was first resolved in atomic detail in the pioneering X-ray diffraction experiment of John Kendrew and co-workers (Kendrew et al., 1960), myoglobin is capable of binding a wide variety of small ligands such as dioxygen, carbon monoxide and nitric oxide ($NO^{\bullet}$) (Antonini and Brunori, 1971). The ligand binding occurs at the iron atom, the center of the prosthetic heme group, which is embedded in the protein by non-covalent bonds. The fifth coordination site of the iron atom is attached to a ring nitrogen atom of a histidine (His) of the polypeptide chain termed as proximal His or His93. On the distal side of the iron atom, the sixth coordination position can be occupied by ligands (Kendrew, 1963). Another histidine localized distal but not attached to the heme (His64) has been evolutionarily conserved in a position suitable for hydrogen-bond formation and is thought to play a role in fine adjusting the ligand affinities of myoglobin (Kendrew, 1963; Phillips, 1980). Recently, it was discovered that in the heart myoglobin represents dioxygenase and nitrite reductase properties. Oxygenated myoglobin is capable of reacting irreversibly with $NO^{\bullet}$, yielding nitrate by dioxygenation (Flögel et al., 2001). Deoxygenated myoglobin acts as a functional nitrite reductase. Under hypoxic conditions, it regulates the generation of bioactive $NO^{\bullet}$ by reducing nitrite (Rassaf et al., 2007; Shiva et al., 2007a).

The backbone of vertebrate myoglobin consists of about 153 amino acids forming eight right-handed α-helices folded into a

highly conserved geometry (Kendrew et al., 1960; Shoenborn et al., 1965; Tilton et al., 1984). As common among cytosolic proteins the folding occurs in a way that almost all of the polar residues are on the outside of the protein facing the aqueous environment. Its hydrophobic groups extend almost entirely into the protein representing an important part in maintaining the stability of the folded protein by a large number of van der Waals interactions (Kendrew, 1963). The heme prosthetic group is inserted into a hydrophobic cleft of the protein, and the heme group itself is surrounded by the distal pocket. Within the protein are four cavities termed Xe1 to Xe4, which were investigated by xenon-binding studies using X-ray diffraction (Tilton et al., 1984). These interior cavities are no packing defects; they represent transient binding sites for ligands on their migration pathway inside the protein (Frauenfelder et al., 2001; Nienhaus et al., 2003a; Ostermann et al., 2000; Scott et al., 2001). Surprisingly, the X-ray structure of myoglobin lacks channels that enable ligands to enter the protein and migrate to the heme group. Accordingly, conformational relaxations of myoglobin to open transient channels are essential for the entrance of the ligands. In this context, an involvement of a rotation of the distal histidine (His64) by opening the so-called histidine gate was proposed (Perutz and Matthews, 1966).

The required structural heterogeneity of myoglobin and the presence of a variety of conformational substates due to the migration pathways and interactions with the ligands have been demonstrated by Austin and co-workers (Austin et al., 1975). Further detailed spectroscopic and crystallographic studies substantiated the important role of protein conformational dynamics and ligand passage among internal cavities in ligand binding (Brunori et al., 2000; Dantsker et al., 2002; Nienhaus et al., 2003a; Ostermann et al., 2000; Scott et al., 2001). The migration of the ligand into the cavities induces structural changes of the amino acid residues around the cavities with a 'breathing' motion (Tomita et al., 2009). These sequential motions of the ligand and the cavity suggest a self-opening mechanism of the migration channel arising by induced fit (Tomita et al., 2009). Moreover, a transient sequestration of the ligands in the interior cavities of myoglobin could enhance the binding reaction to the sixth coordination site of the heme iron atom by the spatial proximity, which facilitates multiple collisions of the ligands with the iron atom (Brunori, 2001; Nienhaus et al., 2003b). This is illustrated by the different interactions of $NO^{\bullet}$ within the myoglobin molecule which contribute to the extreme high reactivity of the heme iron towards $NO^{\bullet}$ as described by Nienhaus and co-workers in detail (Nienhaus et al., 2008). They observed conformational heterogeneity of myoglobin in NO-bound states, the migration of $NO^{\bullet}$ to an internal cavity (Xe4) and the interaction of $NO^{\bullet}$ with the His64 imidazole side chain depending on the particular protein conformation.

Furthermore, the electronic state of myoglobin plays an essential role for its functional properties. Under physiological as well as pathophysiological conditions, the iron atom of the heme group is capable of three different oxidation states representing the ferrous, ferric and ferryl (Fe^{II}, Fe^{III}, Fe^{IV}, respectively) forms of myoglobin; the globin itself may also turn into a radical. Throughout the protein are redox-active amino acids as well as residues, which can exist in different protonation states. The energy landscape of myoglobin comprises a large number of energy minima, which represents conformational substates. The occupation of internal cavities by a ligand influences the energy landscape through stabilizing certain conformational substates, thereby causing additional local energy minima (Frauenfelder et al., 2001; Frauenfelder and McMahon, 2001).

The role of myoglobin during parasite infection under normoxic conditions

Beyond the initial finding of the role of myoglobin in intracellular oxygen supply by serving as a dioxygen reservoir and facilitated transporter of dioxygen (Merx et al., 2001; Wittenberg, 1970; Wittenberg and Wittenberg, 1989), the determination of myoglobin's functions *in vivo* was particularly invigorated by the generation of myoglobin-deficient mice (Garry et al., 1998; Gödecke et al., 1999). Based on the setting of these mice, Flögel et al. could present experimental evidence for a further important function of oxygenated myoglobin – the scavenging of intracellular $NO^{\bullet}$ by dioxygenation, yielding nitrate (Flögel et al., 2001). Further studies also revealed the physiological relevance of the $NO^{\bullet}$ scavenger function of myoglobin, particularly with regard to the protection against the deleterious cardiophysiological effects of excessive $NO^{\bullet}$ (Flögel et al., 2001; Gödecke et al., 2003; Wegener et al., 2002; Wunderlich et al., 2003), supporting an essential role of myoglobin in maintaining cardiac $NO^{\bullet}$ homeostasis [see Flögel et al. for a review on p. 2726 (Flögel et al., 2010)].

In mammals, the $NO^{\bullet}$ generation is upregulated in response to cytokine stimulation due to infection by a variety of protozoan and helminth parasites (Brunet, 2001; James, 1995). Increased $NO^{\bullet}$ production is most likely due to the elevated activity of inducible nitric oxide synthase (iNOS or NOS2). Stimulating agents are generally regarded to be pro-inflammatory cytokines released during the systemic inflammatory response. During infections involving parasites, cytokines such as IFN-γ, TNF-α and IL-1β and parasite membrane/cytosol parts can stimulate iNOS activity in most cells (Almeida et al., 2000; Forstermann et al., 1995; Tachado et al., 1996). It has been shown that $NO^{\bullet}$ could protect against infections with *Trypanosoma cruzi* (Vespa et al., 1994), *Toxoplasma gondii* (Adams et al., 1990), *Leishamania donovani* (Murray and Nathan, 1999), *Leishmania major* (Stenger et al., 1996) and *Schistosoma mansoni* (Wynn et al., 1994). Recently, a $NO^{\bullet}$-mediated inhibition of the catalytic activity of cruzipain, the major papain-like cysteine (Cys) proteinase in *T. cruzi*, has been demonstrated. This dose-dependent effect has been assigned to the nitrosylation of the Cys catalytic residue (Venturini et al., 2000a; Venturini et al., 2000b). Considering these antiparasitic effects of $NO^{\bullet}$ by mediating chemical modifications of proteins, which is directly killing or decreasing parasite growth (Brunet, 2001; Venturini et al., 2000a; Venturini et al., 2000b), Ascenzi et al. proposed that in this case the degradation of $NO^{\bullet}$ may represent a detrimental function of myoglobin (Ascenzi et al., 2005) (Fig. 1A). They assumed an inter-relationship between the preferred colonization of myocytes by parasites and the $NO^{\bullet}$ scavenging of myoglobin. This tissue tropism of parasites could be observed in patients with Chagas disease, which is characterized by the hemoflagellate protozoan parasite *T. cruzi* as the etiological agent. The mortality and morbidity of this disease are mainly caused by chronic processes, which result particularly in progressive fatal cardiomyopathy (Rassi et al., 2009). The preferential colonization of cardiomyocytes by *T. cruzi* may be caused by the efficient myoglobin-mediated $NO^{\bullet}$ inactivation within the heart protecting the parasite from the trypanocidal effects of $NO^{\bullet}$ (Ascenzi et al., 2001). In analogy, this phenomenon could also provide the base for the colonization of heart and skeletal muscles by *T. gondii*, an intracellular parasite, which is found worldwide (Ascenzi et al., 2005). Most cases of toxoplasmosis in humans are asymptomatic. However, this pathogen can cause fever, myalgias, headache, lymphadenopathy and transaminase elevation. Immunocompromised patients may experience severe manifestations, including encephalitis, and multi-system organ

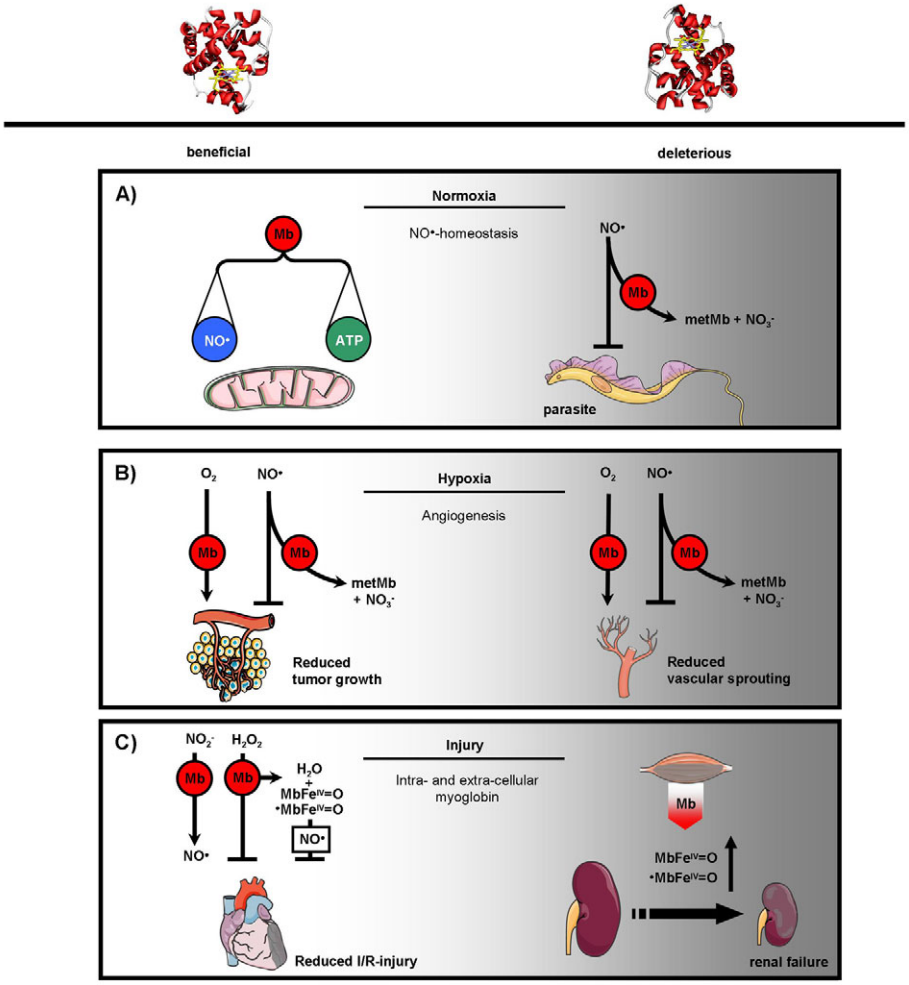

Fig. 1. Schematic diagram displaying the possible effects of myoglobin (Mb), ultimately modulating physiological functions and diseases. (A) The regulation of nitric oxide ($NO^{\bullet}$) homeostasis by Mb could prevent or diminish the $NO^{\bullet}$-mediated inhibition of cytochrome *c* oxidase resulting in the protection of the mitochondrial respiratory chain. By contrast, the $NO^{\bullet}$ scavenging might avoid the protective effect of $NO^{\bullet}$ against infections with parasites. (B) An enhanced dioxygen (O_2) supply and elimination of $NO^{\bullet}$ by Mb could impair the angiogenesis, which would be beneficial in cancer and detrimental in the setting of hindlimb ischemia. (C) The highly oxidizing species ferryl Mb ($MbFe^{IV}=O$, $^{\bullet}MbFe^{IV}=O$) might be less harmful in myocardial ischemia/reperfusion (I/R) injury in comparison with renal failure depending on the occurrence of Mb nitrite reductase and peroxidase activity, which compensate for the deleterious effects of ferryl Mb. This would be enhanced by the reductive properties of $NO^{\bullet}$ and nitrite (ATP, Adenosine-59-triphosphate; H_2O_2, hydrogen peroxide; metMb, ferric myoglobin; NO_2^-, nitrite; NO_3^-, nitrate).

failure, including myocarditis (Gagne, 2001). Does this apparent parasite protection stand for an undesirable outcome of myoglobins' $NO^{\bullet}$ scavenger property? It will be intriguing to follow how experimental studies using myoglobin-deficient mice as adequate controls will contribute to resolve this issue.

The role of myoglobin in hypoxic-induced angiogenesis

The major functions of myoglobin under normoxic conditions, the dioxygen supply and the scavenging of $NO^{\bullet}$ to preserve mitochondrial respiration, raised the question about the role of these interactions under hypoxic conditions. Interestingly, a hypoxia-induced cardiac dysfunction in myoglobin-deficient mice could be suppressed by the inhibition of NO synthases, which implicates a $NO^{\bullet}$-mediated mechanism (Mammen et al., 2003).

Low oxygen environments in natural habitats demand adaptations in their residents relating to the dioxygen supply. Skeletal muscles of humans and small animals living at high altitude exhibit a more than tenfold higher concentration of myoglobin as also observed for diving mammals and birds compared with non-diving species at sea level (Kanatous et al., 2008; Kooyman and Ponganis, 1998). The muscle myoglobin content of diving mammals is regulated by their diving behavior and directly correlates with the dive type and duration (Kanatous et al., 2008; Wright and Davis, 2006). Additional adaptations like promotion of angiogenesis, vascular remodeling and mitochondrial volume density are coordinated by increased hypoxia-inducible factor (HIF-1α) activation. Surprisingly, in mice exposed to chronic hypoxic conditions, an increase of myoglobin expression could not be observed as a consequence of oxygen deprivation as the sole stimulus, and no link to an involvement of HIF-1 has been recognized (Kanatous et al., 2009). However, myoglobin levels were elevated during hypoxia in the working heart, and in skeletal muscle this phenomenon was similarly found only in combination with exercise induced by electrical stimulation (Kanatous et al., 2009). In this context, it has been proposed that the induction of myoglobin gene expression depends on the release of calcium from the sarcoplasmic reticulum of the skeletal myofibers due to motor nerve stimulation in association with hypoxia, which activates the calcineurin/NFAT (nuclear factor of activated T-cells) pathway. Thereby, NFAT represents a key transcription factor in the expression of myoglobin (Kanatous et al., 2009).

Noteworthy, it has recently been discovered that tumor cells originating from non-muscle tissue like human epithelial tumors, including breast, colon, lung and ovary carcinomas, also express myoglobin significantly at early stages of tumor development (Flonta et al., 2009). In human breast cancer cell lines, the content of myoglobin was increased after exposure to hypoxia and oxidative stress. Furthermore, the expression of myoglobin was also induced upon stimulation with $NO^{\bullet}$ (Flonta et al., 2009), analogous to the observations in smooth muscle cells (Rayner et al., 2009). Similarly as described above, the tumor cell adaptations to hypoxic stress are primarily mediated by the transcription factor HIF-1α (Harris, 2002; Kallergi et al., 2009), which, in cancer, is also a key regulator of

angiogenesis, i.e. the growth and development of new capillary blood vessels from pre-existing vascular structures. Tumor hypoxia could be the result of an increased metabolic activity and oxygen consumption by rapidly proliferating tumor cells (North et al., 2005) and an inadequate supply of oxygen as a consequence of structurally and functionally disturbed microcirculation and the deterioration of diffusion conditions, respectively (Vaupel et al., 1989).

Beyond the surprising finding that tumor cells exhibit detectable amounts of myoglobin, the functional effect of this expression was investigated *in vivo* by Galluzzo et al. (Galluzzo et al., 2009). Using lentiviral vector technology the myoglobin gene was introduced into human lung carcinoma cells and afterwards the transduced cells were injected into mice. The results demonstrated that the ectopic expression of myoglobin promoted tumor oxygenation, as reflected by an enhanced tissue dioxygen partial pressure, reduced the tumor growth and suppressed its invasion (Fig. 1B). The expression of mutant forms of myoglobin, unable to bind dioxygen but capable of inactivating reactive oxygen species (ROS), led to tumor expansion, promotion and metastasis (Galluzzo et al., 2009). The role of $NO^{\bullet}$ in cancer is not unambiguous but it has been reported that $NO^{\bullet}$ as well as ROS may stabilize HIF-1α, a key player in the tumor progression (Quintero et al., 2006; Simon, 2006), thereby mimicking hypoxia and thus initiating a genetic programme that helps the tumor to survive and grow. As pointed out above myoglobin is able to reduce oxidative and nitrosative stress (Flögel et al., 2001; Flögel et al., 2004). Thus, in cancer cells these functions may preserve the aerobic mitochondrial respiration and attenuate nitrosative as well as oxidative stress resulting in an efficient degradation of HIF-1α, which prevents the activation of a signal cascade that would prepare the ground for tumor expansion. This raises the interesting question whether oxygen supply and $NO^{\bullet}$ scavenging functions of myoglobin are also involved in the attenuation of angiogenesis after hindlimb ischemia, which should result in detrimental effects.

Angiogenesis is strongly stimulated in response to tissue hypoxia or ischemic injury. One of the most potent angiogenic growth factors is represented by the vascular endothelial growth factor (VEGF) that induces proliferation, migration, survival and permeability of endothelial cells (Ferrara et al., 2003; Holmes et al., 2007). Besides VEGF as a key player, $NO^{\bullet}$ has also been reported to play a pivotal role in angiogenesis (Murohara et al., 1998; Namba et al., 2003). Using mouse models of operatively induced hindlimb ischemia in the setting of a healthy endothelium, it was independently shown by two research groups that the overexpression of myoglobin attenuated perfusion recovery and capillary density as well as increased limb necrosis and apoptosis in comparison with wild-type mice. In both types of mice, no differences in VEGF production and endothelial nitric oxide synthase (eNOS) activation could be observed (Hazarika et al., 2008; Yang et al., 2009). Hazarika et al. determined a decrease in nitrite as well as nitrate concentrations and an increase in protein-bound NO, and suggested that myoglobin levels can serve as significant regulators of bioavailable $NO^{\bullet}$ in skeletal muscle and can modulate the angiogenic response in an endothelium-independent manner (Fig. 1B). However, Yang et al. proposed that another mechanism other than the elimination of $NO^{\bullet}$ by myoglobin leading to reduced free $NO^{\bullet}$ levels might be involved in this phenomenon, based on their findings that the cell proliferation does not differ between the myoglobin-overexpressing and wild-type mice, and taking into consideration that $NO^{\bullet}$ might be a general physiological regulator of cell growth (Luczak et al., 2004; Ulibarri et al., 1999). Clearly, additional research on the exact involvement of myoglobin overexpression impairing the angiogenesis needs to be performed.

The role of myoglobin in renal failure and myocardial ischemia/reperfusion injury

It has been suggested that myoglobin might be a key determinant of damage in the ischemic and then reoxygenated heart caused by its ability to exhibit a peroxidatic activity leading to higher oxidation states of the heme (Detweiler et al., 2002; Galaris et al., 1989; Witting et al., 2006). *In vitro* studies revealed that hydrogen peroxide (H_2O_2) reacts with ferrous and ferric myoglobin to generate the highly oxidizing species ferryl myoglobin (George and Irvine, 1951; Harel and Kanner, 1988; Witting and Mauk, 2001). One effect of the formation of ferryl heme represents the heme to protein cross-linked form of myoglobin (Mb-X), a specific interaction of the ferryl heme with a protein-based radical (Reeder et al., 2002). The ferryl oxidation state of myoglobin promotes the oxidation of lipids by the abstraction of a hydrogen atom from the lipid, forming a lipid radical (Patel et al., 1996; Rodriguez-Malaver et al., 1997). Thereby, Mb-X represents the more cytotoxic form than the ferryl myoglobin and oxidizes free and membrane-bound lipids (Osawa and Williams, 1996; Reeder et al., 2002; Vuletich et al., 2000), which may give rise to the initiation of deleterious chain reactions.

In the urine of patients with rhabdomyolytic-associated acute renal failure this cytotoxic derivative of myoglobin has been identified (Holt et al., 1999), implying a pathological involvement of myoglobin in this disease (Holt et al., 1999; Moore et al., 1998). Rhabdomyolysis is the breakdown of muscle fibers resulting in the release of intracellular components into the circulation. Some of these are harmful to the kidney and frequently result in renal diseases. The released myoglobin is filtered by the kidneys, where it precipitates and causes obstructive cast formation. Besides the formation of casts, myoglobin can exert a direct cytotoxic effect through the enhancement of local oxidative stress in the tubular cells (Fig. 1C). It is a matter of common knowledge that within the rhabdomyolytic kidney, free and membrane-bound lipids are oxidized by a non-cyclooxygenase mechanism yielding isoprostanes (Awad et al., 1993), which is likely to be caused by the precipitated myoglobin.

It is important to note that in this case myoglobin is released from the cells and is thereby withdrawn from the reductant/antioxidant-rich system. Obviously, a differentiated point of view is required for assessing the role of intracellular myoglobin in myocardial ischemia/reperfusion. One of the well-known antioxidant defense mechanisms against ferryl myoglobin is represented by ascorbate. Investigations have shown that ascorbate directly suppressed the accumulation of ferryl myoglobin in isolated ischemic rat hearts (Arduini et al., 1990). Another important cellular reductant for the generated ferric myoglobin is represented by an enzyme named metMb reductase, which rapidly and directly reduces the ferric form to the ferrous state (Hagler et al., 1979). Interestingly, $NO^{\bullet}$ is also able to reduce ferryl myoglobin to the ferric form *via* a rapid formation of an intermediate limiting these highly oxidizing species and yielding nitrite (Dee et al., 1991). In comparison with the rate constant for the reaction with ascorbate ($2.7\pm0.8\,l\,mol^{-1}\,s^{-1}$ at pH 7.1 and 25°C) the rate constant determined for the $NO^{\bullet}$-mediated reduction of ferryl myoglobin is significantly higher ($17.9\pm0.5\times10^{6}\,l\,mol^{-1}\,s^{-1}$ at pH 7.5 and 20°C) (Herold and Rehmann, 2001). However, it has to be taken into account that during ischemia/reperfusion the bioavailability of $NO^{\bullet}$ is reduced because the enzymatic generation by the $NO^{\bullet}$ synthase requires dioxygen. Remarkably, along the physiological oxygen gradient nitrite represents an endocrine storage pool of $NO^{\bullet}$ (Bryan et al., 2005; Gladwin, 2005). Experiments carried out by our groups using wild-type and myoglobin-deficient mice under moderate hypoxia due to

acute coronary artery inflow reduction established a novel homeostatic mechanism mediated by myoglobin during oxygen deprivation (Rassaf et al., 2007). The imbalance of oxygen supply and demand, a consequence of acute hypoxia, results in increased levels of deoxygenated myoglobin, which is able to reduce nitrite to bioactive NO•. Thus, the decrease in tissue oxygen tension switches the activity of myoglobin from being a NO• scavenger under normoxic conditions to a NO• producer in hypoxia (Fig. 2). This reflects an important oxygen sensoring by deoxygenated myoglobin through which NO• can regulate muscle function and energetics. The mechanism strongly resembles the characteristics described for acute hibernation (Rassaf et al., 2007). Myocardial short-term hibernation implies an adaptive reduction of energy expenditure through reduced contractile function in response to acute coronary artery inflow reduction. This restores myocardial energy balance over time and maintains myocardial integrity and viability (Heusch, 1998). Under ischemic conditions nitrite is also reduced to NO• in rat hearts (Tiravanti et al., 2004), and it has been reported that nitrite modulates the mitochondrial resilience to reperfusion injury by the reversible inhibition of complex I *via S*-nitrosation (Shiva et al., 2007b). This mechanism attenuates the formation of ROS by mitochondria consistent with a decrease in oxidative protein damage (Burwell et al., 2006; Chen et al., 2006; Taylor et al., 2003). Nitrite itself might also be able to reduce ferryl myoglobin to the ferric form (Herold and Rehmann, 2001); in addition, the generated nitrogen dioxide could oxidize the thiols at complex I.

The protective effects of NO• during myocardial ischemia/reperfusion are generally accepted (Bolli, 2001; Jones and Bolli, 2006). Our group could provide experimental evidence that the reduction of exogenous nitrite to NO• during myocardial ischemia/reperfusion leads to a marked decrease of myocardial infarct size, which is critically dependent on the presence of myoglobin (Hendgen-Cotta et al., 2008). Concomitantly, ROS formation was attenuated accompanied by lower protein oxidation damage in wild-type mice. However, in myoglobin-deficient mice, no cytoprotective effects of nitrite could be observed. By contrast, these mice showed increased ROS levels and a higher inactivation of aconitase activity (Hendgen-Cotta et al., 2008). These data established a pivotal role of myoglobin in myocardial ischemia/reperfusion injury indicating much more beneficial effects mediated by its nitrite reductase and peroxidase activity (Fig. 1C) than a detrimental impact due to a formation of ferryl myoglobin.

Of note, myoglobin gene expression has been demonstrated to attenuate hepatic ischemia/reperfusion injury in an *in vivo* rat model (Nitta et al., 2003). The results clearly showed that the expression of human myoglobin in the liver by adenovirus-mediated gene transfer prior to ischemia increased the ATP levels, diminshed oxidatively modified protein levels and maintained the viability of the liver tissue as compared with wild-type rats. Analysis of histological sections of the liver obtained 180 min after reperfusion revealed that the observed necrotic change and vacuolation in hepatocytes of wild-type rats were not present in liver cells of myoglobin transfected mice (Nitta et al., 2003).

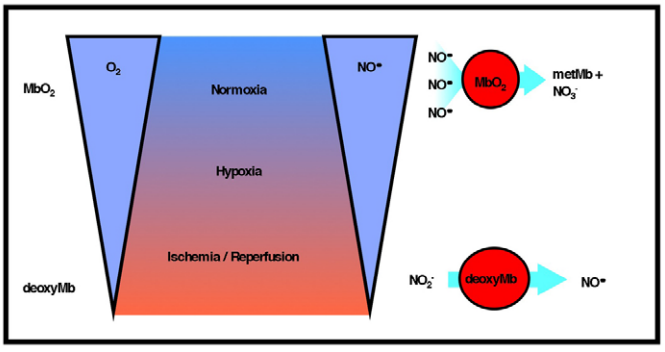

Fig. 2. Schematic diagram showing the functional roles of myoglobin (Mb) with regards to the dioxygen (O_2) and nitric oxide (NO•) levels along the oxygen gradient. Recent studies support the conversion of Mb from a NO• scavenger under normoxia to a NO• producer under hypoxic conditions. The dioxygenation of NO• yields ferric myoglobin (metMb) and nitrate (NO_3^-). The reduction of nitrite (NO_2^-) by deoxygenated Mb (deoxyMb) leads to the generation of NO•.

Conclusion

Taken together the previously discovered functions of myoglobin including the handling of biologically important NO• seem to have pathophysiological relevance for various disease states, where in most cases the beneficial effects are likely to outweigh the detrimental effects. Surprisingly, this is not only true for tissues that are well known to express substantial amounts of this protein, like the heart and skeletal muscles but also for liver, tumor cells and angiogenesis, when the target structures are genetically transfected with myoglobin. Although the underlying mechanisms of these effects have yet to be scrutinized, it will be absorbing to follow how these relationships may be transferred into a clinical context or utilized for future drug design or therapy. This indicates impressively that the research on this well-known protein still turns out to be an important task.

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