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Review Article

Structure, function, and environmental adaptation of myoglobin across various species

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Abstract

As an oxygen carrying globulin, in addition to hemoglobin, myoglobin (Mb) has also attracted more attention in recent years. Mb is one of the simplest and typical monomeric globular proteins. It harbors the heme group that contains a central iron atom embedded in heme binding pocket. Mb is oxygen storage and transport protein present in skeletal and cardiac muscle. Since the first determination of Mb structure in sperm whales, this protein has been extensively evaluated by scientist world over. This paper discusses Mb in terms of its distribution in animals, physiological function, use in disease diagnosis, and role in adaptation to hypoxia. In particular, Mb plays an important role in hypoxic adaptation in animals and should deserve more attention.

Key words: Adaptation to hypoxia, Disease diagnosis, Distribution, Myoglobin, Physiological function

Highlights

- We reviewed the distribution and modification of myoglobin in animals.
- The biological functions of myoglobin, such as carrying oxygen and scavenging NO, were summarized.
- The role of myoglobin in disease treatment was expounded.
- The adaptive changes of myoglobin as an oxygen carrying globulin in environmental adaptation were reviewed

INTRODUCTION

Globins are small, globular proteins that are mainly responsible for oxygen transport and storage, but may perform other functions as well. Some of the prominent globins are hemoglobin (Hb), Mb, neuroglobin (Ngb), cytoglobin (Cygb), globin X (GbX), globin Y (GbY), globin E (GbE) and androglobin (Ludemann *et al*., 2020). The homology and phylogenetic relationship between globin are also constantly updated and revised. Through the phylogenetic analysis of all known globin family members, it is considered that the globin family originated from a common ancestor (Burmester and Hankeln, 2014). Hb and Mb appear to play critical roles in the maintenance of cellular oxygen supply in support of aerobic metabolism in gnathostomes, due to its special function and small molecular weight, they become a classic model for studying the evolution and function to adapt the hypoxic environment (Biscotti *et al*., 2016). The rest of the globin functions are not oxygen-carrying, and their specific roles have not been thoroughly studied.

Myoglobin (Mb) belongs to the globin superfamily and its main function is the binding of oxygen in vertebrate muscles and heart ventricles. It is the first protein whose three-dimensional (3D) structure was visualized (Kendrew *et al*., 1960). The cytoplastic hemoprotein Mb is found in mammals, reptiles, amphibians, teleost and chondrichthyes fishes, prokaryotes and fungi, etc. However, not all species contain Mb, it was reported that Notothenioid fish lossed express Mb in some

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cases (Sidell and O'Brien, 2006). The Mb gene also appears to have been deleted in some amphibians (Hoffmann *et al*., 2011). Hb absorbs and binds oxygen molecules as much as possible in the lungs with high oxygen concentration. However, in contrast to globin chains present in Hb, Mb lacks allosteric and cooperative functions and has higher affinity for oxygen than Hb, when Hb circulates to the environment with low oxygen concentration such as muscles, Mb can bind to oxygen near the capillary and transport oxygen into the cell and finally reaches to the mitochondria for life activities. Unlike Hb, which forms a heterotetramer with four symmetrically arranged dimers (α1β1 and α2β2) (Shibayama *et al*., 2014), Mb is a typical monomeric globular protein, however, its 3D structure is very similar to that of Hb subunit. Therefore, it can bind oxygen near capillaries and transport it to mitochondria. However, in contrast to the globin chains present in hemoglobin, Mb lacks allosteric and synergistic functions due to being a monomer. Mb has a molecular weight of 16–17 kDa and consists of a single polypeptide chain of approximately 153 amino acids with 8 α-helices (Suzuki and Imai, 1998), named α1-α8 or A-H (Fig. 1). The deep hydrophobic "pocket" is composed of α5(E) and α6(F) helices on the side and α7(G) and α8(H) helices on the bottom, in which heme exists.

The primary structure of Mb has been studied in various species, such as chicken (Deconinck *et al*., 1975), rabbit (Romero-Herrera *et al*., 1976), otter (Sukhomlinov and Sergienko, 1985), yellowfin tuna (Watts *et al*., 1980), goat (Suman *et al*., 2009) and red squirrel (Di Giuseppe *et al*., 2017), and 3D structures of Mb in various species have been published in the Protein Data Bank (PDB). All Mbs are a conserved structure and comprise eight classic α-helices. The structure of Mbs expressed in various species is shown in Fig. 1 and Fig. 2 and their structures are very similar.

However, some archaeo gastropod molluscs, including *Sulculus* and *Turbo*, express an unusual Mb in their buccal masses, with a molecular weight of approximately 40 kDa. This Mb is not expressed from the globin encoding gene but from the gene encoding indoleamine dioxygenase. Incidentally, this Mb has a lower affinity to oxygen compared with that of vertebrate Mbs (Suzuki *et al*., 2003).

Mb plays a key role in muscle metabolism by increasing oxygen availability in muscle cells by absorbing the oxygen released by Hb in the blood (Wittenberg, 1970). In aerobic muscles, Mb facilitates diffusion of intracellular oxygen

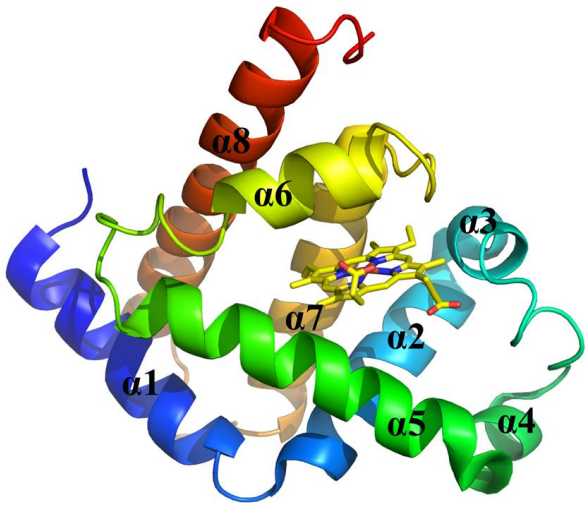

Fig. 1. Three-dimensional (3D) structure of sperm whale (*Physeter catodon*) myoglobin (Mb, PDB ID: 1MBN)

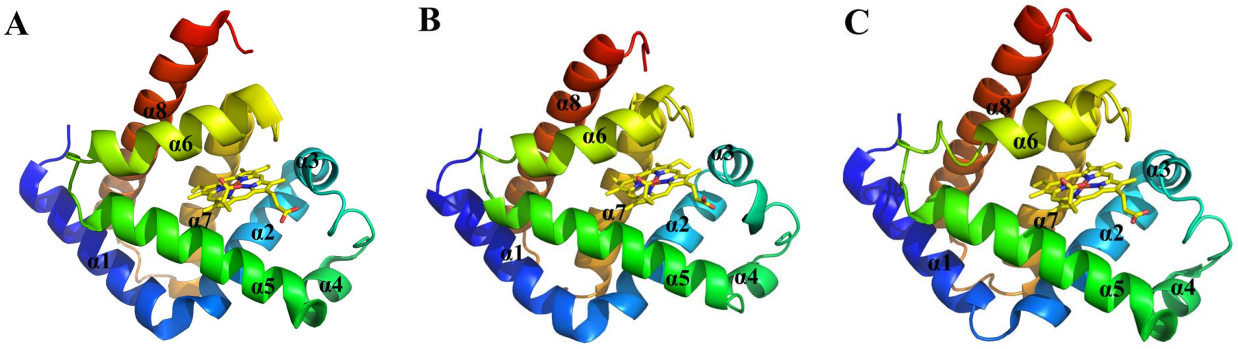

Fig. 2. 3D structure of Mbs expressed in various species (A: *Equus caballus*, PDB ID: 5d5r; B: *Caretta caretta*, PDB ID: 1lhs; C: *Sus scrofa*, PDB ID: 1pmb)

to the mitochondria (Ordway and Garry, 2004). Additionally, Mb can store oxygen for metabolic respiration during the hypoxic period or high oxygen demand, such as vigorous muscle contraction during exercise (Wittenberg and Wittenberg, 1989). The elevated concentration of Mb in diving mammals (Ponganis *et al*., 2002) and at higher altitudes (Gimenez *et al*., 1977; Terrados *et al*., 1990; Dasmeh and Kepp, 2012) provides direct evidence that direct storage of oxygen is the main function of Mb. Owing to its pivotal role, Mb is often termed the "hydrogen atom of biology" (Frauenfelder *et al*., 2003). As a result, biologists have conducted extensive studies to elucidate the relationship between its structure and functions (Garry *et al*., 2000; Ordway and Garry, 2004; Hasan *et al*., 2018). Our goal in this review is to describe on Mb which could also express in other tissues except myocardium and skeletal muscle in some animals, and some species have different subtypes, then to state other function of Mb except for the function of transporting and storing oxygen and its role in diseases. Finally, we focus specifically on the evolution of Mb in hypoxic adaptation. We hope that scientists, especially zoologists or structural scholars, will pay attention to Mb, especially its hypoxic adaptive evolution.

Distribution and modification

Almost all vertebrates carry single Mb encoding gene and the only know exception are some cyprinid fishes, which harbor two copies of the Mb encoding gene. The distribution of Mb was believed to be restricted to muscle and heart cells in vertebrates. However, some studies have reported that in some vertebrates, particularly fishes, Mb distribution is different because it is present in other tissues as well, some of them even exhibiting different subtypes (Huang *et al*., 2006). For example, the West African lungfish *Protopterus annectens* harbors at least seven specific Mb encoding genes (*PanMb1-7*). In these fishes, although Mbs are differentially expressed in various lung tissues, they are mainly expressed in brain. Interestingly, the typical Mb-expressing tissues have much lower levels of Mb (*PanMb1* and *PanMb3*) mRNA. Some Mbs may have evolved to adapt to the oxygen requirements of specific tissues and help lungfishes to survive periods of hypoxic or take over the roles of Ngb and Cygb, which are absent in *P. annectens* (Koch *et al*., 2016; Ludemann *et al*., 2020).

With a few exceptions, Mb mRNA and protein is uniformly expressed in a wide range of muscular and non-muscular tissues, indicating that the tissue-specific distribution of Mb might be a universal phenomenon in teleosts, irrespective of their habitat and lifestyle (Hasan *et al*., 2018). In green sea turtle *Chelonia mydas*, Mb mRNA is expressed in the brain, heart and liver tissues, and is linked to the hypoxia-tolerant trait (Xin *et al*., 2015).

Fraser *et al*. reported that the common carp (*Cyprinus carpio*) expresses two unique Mbs

(Fraser *et al*., 2006). Myg-1 is expressed in the liver, kidney, muscles and gill tissues, and the expression of Myg-1 encoding gene is strongly upregulated under hypoxia. In contrast, Myg-2 expression was reported only in the brain tissue, independent of changes in oxygen pressure (Riggs and Gorr, 2006).

S-nitrosylation, the covalent addition of nitric oxide (NO) to the thiol side chain of cysteine, is an important post-transitional modification that can alter the function of various proteins (Turan and Meuwly, 2021). In blackfin tuna, *S*-nitrosylation and formation of the *S*-nitrosothiol group induces conformational changes in Mb (Schreiter *et al*., 2007), which confers protection against oxidative stress during hypoxia and reoxygenation, and enables allosteric regulation of its function. It is believed that this modification also exists in other species and regulates the function of Mb.

Function

Mb performs the crucial function of oxygen storage, especially during systolic compression. Mb performs additional protective functions, such as maintaining NO homeostasis by scavenging or generating NO during normoxia and hypoxia. In this way, it modulates mitochondrial respiration under physiological and pathological conditions, protecting the heart from the deleterious effects of excessive NO. However, under hypoxia, deoxygenated Mb produces NO, which protects cells from short phases of hypoxia and myocardial ischemia/ reperfusion injury (Hendgen-Cotta *et al*., 2014b). In vascular smooth muscle cells experiencing hypoxia, Mb acts as a major source of NO by reducing endogenous nitrite. This leads to vasodilation without direct involvement of endothelial nitric oxide synthase and is independent of effects on cardiac function (Hendgen-Cotta *et al*., 2014a). Additionally, it can neutralize reactive oxygen species (ROS) (Huang *et al*., 2006).

Except scavenging or generating NO, Mb also can bind to fatty acids, such as palmitate (Shih *et al*., 2014), and may facilitate fatty acid transport in cells under certain physiological conditions (Sriram *et al*., 2008; Shih *et al*., 2015). In oxygenated state, Mb acts as a transporter for fatty acids in muscles, and releases fatty acids in the deoxygenated state (Chintapalli *et al*., 2015). Furthermore, Mb plays a pivotal role in controlling the lipid-droplet equilibrium in interscapular brown adipose tissues by promoting a more plurilocular ("browning") phenotype in conjunction with maintaining elevated levels of certain saturated fatty acids (e.g., palmitate) in brown adipocytes (Aboouf *et al*., 2021). As Mb is abundantly expressed in type 1 oxidative muscle fibers and cardiomyocytes that rely heavily on fat oxidation and trafficking, its binding to palmitate and palmitoylcarnitine may have important physiological relevance to support normal muscle trafficking and sequestration of these bioactive lipids (Chintapalli *et al*., 2018).

Mb is a significantly more efficient nitrite reductase than isolated mitochondria. Further, deoxygenated myoglobin catalyzes the nitrite-dependent S-nitrosation of mitochondrial proteins (Quesnelle *et al*., 2020). Under physiological conditions, Mb constitutes an important barrier that efficiently protects the heart from nitrosative stress (Godecke *et al*., 2003). Additionally, Mb is implicated in antioxidation defense in cells. In vertebrates, the peroxidase activity of Mb might be an important antioxidant mechanism in the cardiac and skeletal muscles under various physiological conditions, such as skeletal muscle contraction or hypoxia (Mannino *et al*., 2019).

Disease diagnosis

Mb may contribute substantially to the oxidative stress associated with ischemia/ reperfusion injury in myocardial tissues (Gunther *et al*., 1999). It has been shown that the serum Mb/arbonic anhydrase III ratio is a reliable marker of acute myocardial infarction in patients with renal failure; therefore, it might be a diagnostic tool for detecting acute

myocardial infarction in this patient group (Vuori *et al*., 1997; de Winter *et al*., 2000). Measuring the Mb/carbonic anhydrase III ratio during the first hours of thrombolysis initiation may be useful in evaluating the success of reperfusion after acute myocardial infarction (Vuotikka *et al*., 2003). Cardiac biomarkers, such as Mb could be utilized in prediction algorithms for deciphering the clinical outcome in COVID-19 patients with myocardial injury (Parvu *et al*., 2022).

Transfer of Mb encoding gene to the liver by researcher enhances ATP levels *in vitro* and *in vivo*, and might be a novel strategy to reduce ischemia-reperfusion injury (Nitta *et al*., 2003). Moreover, Mb plays an important role in the pathophysiology of Dupuytren's disease (Forsman *et al*., 2008). Upon admission, serum Mb levels are found to be elevated in approximately 50% patients with major acute pulmonary embolism. Moreover, elevated Mb level, a marker of myocardial injury, is a powerful predictor of an increased risk of fatal outcomes in major pulmonary embolism (Pruszczyk *et al*., 2003). Diagnostic and prognostic role of Mb was indicated in a study on patients with suspected acute coronary syndrome who had inconclusive electrocardiograms on admission (Stork *et al*., 2000).

Human epithelial tumors, including breast, lung, ovary, and colon carcinomas, overexpress Mb from the earliest stages of the disease. Mb expression is induced by various signals associated with tumor progression, including mitogenic stimuli, oxidative stress and hypoxia. Mb expression is a part of a cellular program aimed at coping with changes in metabolic and environmental conditions associated with neoplastic growth (Flonta *et al*., 2009). Mb was reported to be robustly induced under hypoxia and recently been identified as a hallmark in luminal breast cancer (Quinting *et al*., 2021).

Mb can not only carry oxygen, but also could be a diagnostic tool or powerful predictor of some diseases.

Environmental adaptation

Extended breath-hold diving by birds and mammals for prolonged durations in search for food is one of the most remarkable features of physiological endurance, reflecting the widely accepted function of Mb in cellular oxygen transport and oxygen buffering. This function supports high levels of aerobic muscular activity and use of stored oxygen during extended periods of hypoxia or demanding physiological conditions (Cossins and Berenbrink, 2008).

Elevated Mb levels in skeletal muscles enable aerobic metabolism to be maintained during apnea. Mb expression level is 10- to 30-fold higher in the locomotor muscles of aquatic birds and mammals than in the muscles of their aerial or terrestrial counterparts. Mb levels and body mass are both significantly positively correlated with the maximum dive duration in odontocetes (Noren and Williams, 2000).

In addition to increased Mb levels due to hypoxia, changes in living habits or the environment also alters Mb levels. For instance, Mb levels are higher in the gizzards of herbivorous birds than in those of carnivorous birds, which suggests an adaptation to the increased mechanical work required to grind hard and fibrous food under limited circulatory supply (Enoki and Morimoto, 2000). Similarly, the Mb levels in skeletal muscles of Arctic Yakutian ground squirrel (*Citellus undulatus Pallas*) were approximately 2- to 3-fold higher in winter and autumn than in those in summer, suggesting that high Mb levels may be necessary for hibernation (Postnikova *et al*., 1999). In the Atlantic cod (*Gadus morhua*) growing at different temperatures (4ºC and 10ºC), the mRNA and protein levels of Mb are significantly increased in the heart as a result of acclimation to 4ºC to meet cold-induced metabolic demands; this may have additional functions during cold acclimation, such as protecting cells from ROS and scavenging NO, thereby contributing to the regulation of mitochondrial volume density (Lurman *et al*., 2007).

Generally, teleosts express Mb at a lower

level than expressed in mammals. Although their oxygen binding affinity is comparable to that of mammalian Mb, the oxygen dissociation rates and carbon monoxide combination rates of the teleost Mbs are significantly higher. Fish Mbs are structurally distinct from the mammalian Mbs. Since Mbs in Antarctic teleosts function at extreme environmental temperatures (Cashon *et al*., 1997), their oxygen affinity is greater at colder temperatures than at the physiological temperatures (37–39°C) as observed in birds and mammals (Wright and Davis, 2015).

During evolution of environmental adaptation, adaptation to diving led to not only quantitative but also qualitative changes in Mb (Huang *et al*., 2006), such as the change in the surface charge and stability of Mb. The evolution of cetaceans (whales, dolphins and porpoises) from land to water is one of the most spectacular events in mammalian evolution. Since their divergence approximately 50 million age ago from their terrestrial ancestors, cetaceans underwent a series of adaptations such as 10- to 20-fold increase in Mb levels in skeletal muscles, which was critical for increasing oxygen storage capacity and prolonging dive time. Although the oxygen-binding affinity of Mbs does not differ significantly among mammals, folding stabilities of cetacean Mbs are 2–4 kcal/mol higher than that of terrestrial Mbs (Dasmeh *et al*., 2013). Reportedly, higher Mb folding stability of folded proteins allows whales to conquer the deep niche (Holm *et al*., 2016). The folding stability of sperm whale Mb is more than horse Mb, and it aggregates less at high concentrations as measured by gel filtration and analytical experiments (Regis *et al*., 2005). With evolution, the number of histidine residues and overall net positive charge has increased on Mb in whales and seals. These adaptations account for buffering action of Mb against lactic acid that accumulates during long dives, and its increased solubility; convergent increases in net positive charge in Mb are observed across all diving mammals ranging from water shrew to sperm whale (Mirceta *et al*., 2009). The pinniped Mbs exploited the shielding of hydrophobic surfaces more effectively than the whale Mbs for improving the folding stability (Isogai *et al*., 2021).

In addition to diving animals, the environmental adaptation strategies of Mb have been evaluated in some high-altitude species. Mb concentrations in cardiac and skeletal muscles of humans and terrestrial mammals at different altitudes were related to the altitudes where they lived, and some animals at high altitudes have higher Mb concentrations. Humans can stably enhance Mb expression in skeletal muscle through hypoxia-inducible factor (HIF-1α) in a high-altitude hypoxic environment (Vogt *et al*., 2001). Comparison of Mb sequence between hypoxia-tolerant Tibetan antelope (*Pantholops hodgsonii*) and *Ovisaries* revealed variations at positions G21D and E78K; moreover, significantly higher Mb expression is observed in the cardiac and skeletal muscles of Tibetan antelope (Ma *et al*., 2014). The red tail toad–headed lizard *Phrynocephalus erythrurus* inhabiting the Qinghai–Tibetan Plateau expressed an Mb with three different amino acids compared with the Mb of low-altitude lizard *P. przewalskii* (T13I, K87T and H118Q). Homologous simulation revealed a greater heme pocket in *P. Erythrurus* Mb, which may be more favorable for oxygen binding and unloading. Moreover, Mb levels are notably increased in the skeletal muscles of *P. erythrurus* (Xin *et al*., 2015).

Conclusion

Mb, as the second important oxygen-carrying protein after Hb, has garnered significant attention from scientists world over. Mb, expressed from a conserved gene, is small monomer and is rarely modified (except for rainbow trout). It is found in other tissues of some species, especially in fish, Mb distribution is different because it is present in other tissues as well except the typical muscle and heart cell, and some have different subtypes. Besides transporting and storing oxygen, Mb also has other functions, such as the elimination of NO.

Due to the special function of Mb, it is currently used in the diagnosis of some diseases, such as myocarditis. Since Mb is an oxygen-carrying protein, it has survived the evolution process; especially in a low-oxygen environment. Mb shows several adaptations, such as increased expression levels, altered surface charge, and differential protein stability, in different species with different evolutionary strategies to help them adapt to the changing environment. We believe that with in-depth research studies in the future, the mechanism underlying environmental adaptability of Mb in different species will be clarified further. For instance, birds are adapted to flying life and have more efficient lung gas exchange and blood oxygen transport capacity, as well as a more vigorous metabolic rate and stronger hypoxia tolerance. Therefore, it is more important to systematically explore the adaptation of bird Mb to hypoxia, Mb adaptability to hypoxia has been scarcely studied in them. There is a scope for future research on adaptation of bird Mb to hypoxia.

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