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Review

Life without myoglobin

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Abstract. Hemoproteins are widely distributed among prokaryotes, unicellular eukaryotes, plants and animals [1]. Myoglobin, a cytoplasmic hemoprotein that is restricted to cardiomyocytes and oxidative skeletal myofibers in vertebrates, has been proposed to facilitate oxygen transport to the mitochondria [1–3]. This cytoplasmic hemoprotein was the first protein to be subjected to definitive structural analysis and has been a subject of long-standing and ongoing interest to biologists [1–3]. Recently, we utilized gene disruption technology to generate mice that are viable and fertile despite a complete absence of myoglobin [4]. This unexpected result led us to reexamine existing paradigms regarding the function of myoglobin in striated muscle.

Key words. Myoglobin; oxygen transport; heart; skeletal muscle; transgenic mice.

Transcriptional regulation

Evidence suggests that myoglobin and hemoglobin proteins of modern organisms have evolved from a common ancestral gene [1]. Like the α- and β-globin genes, the myoglobin gene of vertebrates consists of three exons and two introns. Exon 2 of the myoglobin gene encodes amino acids 31–105, including the heme-binding domain of the 154-residue monomeric cytoplasmic protein [5]. Previous studies using a transgenic strategy determined that transcriptional control motifs sufficient to confer expression selectively in cardiac and skeletal myocytes are contained within the proximal 400 nucleotides upstream from the transcriptional start site of the human myoglobin gene [6]. A detailed mutational analysis of the 0.4-kb upstream fragment of the human myoglobin gene revealed three essential activation elements that are required for transcriptional activity in myocytes and include a TATA box, CCAC box and an A/T motif [7]. These motifs are conserved in myoglobin genes from other mammalian species including the seal and mouse [5, 7]. Several candidate cognate binding factors that recognize the CCAC and A/T motifs of the myoglobin gene and regulate transcriptional activity include Sp1 and muscle enhancer factor-2 (MEF2 factors of the MADS box family), respectively. MEF2 also is implicated in the selective expression of myoglobin within specialized subtypes of skeletal myofibers [8].

Role in oxygen transport

A number of studies support a role for myoglobin in facilitated oxygen transport from the erythrocyte to the mitochondria in cardiomyocytes and oxidative skeletal myofibers [1]. Elevated levels of myoglobin have been observed in skeletal muscle of mammals and birds adapted to hypoxic environments (e.g. prolonged underwater diving or high altitude) [9–12]. There is also a correlation between increased myoglobin content and endurance training of rodents. Moreover, clinical studies suggest that myoglobin desaturates in proportion to exercise intensity and thus supports a role for myoglobin in the transport of oxygen [13–15]. Myoglobin is

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a cytoplasmic hemoprotein that does not cross mitochondrial membranes. Calculations of oxygen flux by simple diffusion suggests that in the absence of myoglobin, oxygen pressure at the center of the working myocytes, under normoxic conditions, would fall to insufficient levels to maintain oxidative phosphorylation [16]. Oxygen binds reversibly to the heme domain of the myoglobin protein, and gradients of myoglobin saturation and oxygen pressure within the myocytes are shallow (2–3 torr) [1, 17–20]. Mitochondria in the normally perfused working heart are not limited by the supply of oxygen, as myoglobin is approximately 50% saturated with oxygen and any change in oxygen pressure is maximally buffered by equilibrium with myoglobin. Oxygen limitation of the mitochondria is observed only when myoglobin is approximately less than 30% saturated with oxygen [1, 3]. Notably, myoglobin saturation in individual myocytes of normally perfused working heart or skeletal muscles do not fall below this level, even during intense contractile work.

A number of pharmacological and biochemical studies support a role for myoglobin in the transport of oxygen to the mitochondria [2, 3]. These studies utilized chemical agents such as carbon monoxide or sodium nitrite to inactivate myoglobin. These agents function either in the binding of the heme domain of myoglobin (i.e. carbon monoxide) or in the oxidation of myoglobin (i.e. sodium nitrite) that inhibits the binding to oxygen. Use of these chemical agents to inhibit myoglobin function results in a reduction of oxygen consumption, adenosine triphosphate synthesis and muscle contractility.

Gene knockout results

Due to the lack of specificity of the chemical agents used in the pharmacological studies, we undertook a gene disruption strategy to produce transgenic mice carrying mutant (null) alleles at the myoglobin locus [4]. Animals homozygous for the myoglobin null allele produce no myoglobin protein whatsoever. Remarkably, we found that mice without myoglobin were healthy and displayed no evidence of congestive heart failure. Both males and females without myoglobin are fertile, and females survive the hemodynamic stress of pregnancy without apparent difficulty. Using a standard exercise treadmill protocol, we observed a comparable exercise capacity between the myoglobin mutant mice and wild-type mice. When examined as an isolated working heart preparation or isolated skeletal muscle preparations, muscles from myoglobin mutant mice exhibited no differences from wild-type muscles with respect to contractile performance across a range of work conditions and oxygen availability. Hearts and skeletal muscles from mice lacking myoglobin were strikingly depigmented, but otherwise were without evidence of gross or microscopic pathology (fig. 1).

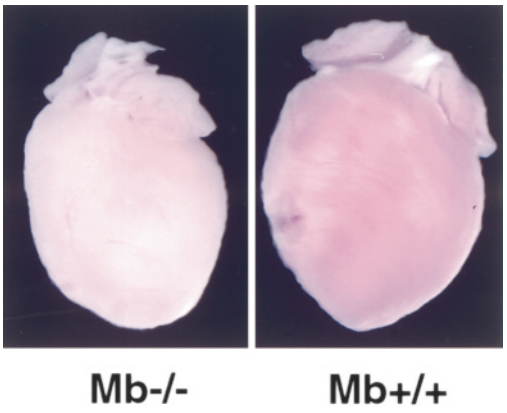

Figure 1. Gross appearance of myoglobin mutant and wild-type adult hearts. In the absence of myoglobin (Mb $-/-$) the heart is depigmented compared with the wild-type (Mb $+/+$) heart.

These results suggested either that previous concepts of the important functional role for myoglobin in oxygen transport are incorrect, or that transgenic mice surviving in the absence of myoglobin developed powerful cellular or molecular adaptations that maintain oxygen transport when myoglobin is lacking. Preliminary studies in our laboratory support the presence of an adaptive molecular process in skeletal and cardiac muscles of myoglobin null animals that preserves muscle performance in the absence of myoglobin. Furthermore, a recent paper by Godecke et al. [21] both confirmed our initial observations and provided evidence for specific adaptations that serve to rescue the null phenotype. These include an increase in hemoglobin concentrations, in coronary blood flow and in vascular density of adult myoglobin mutant mice. Our own studies (to be reported elsewhere) confirm the presence of these adaptations, and point to additional compensatory mechanisms acting at a molecular level to maintain metabolic homeostasis in cardiac and skeletal muscle of animals without myoglobin.

Alternative functions for myoglobin

Although a majority of the studies on myoglobin have focussed on its role in oxygen transport, alternative functions for this protein have been proposed (fig. 2). Although myoglobin does not cross the mitochondrial membrane, Doeller and Wittenberg have proposed a process termed myoglobin-mediated oxidative phosphorylation [2]. This is distinguished from a role for myo-

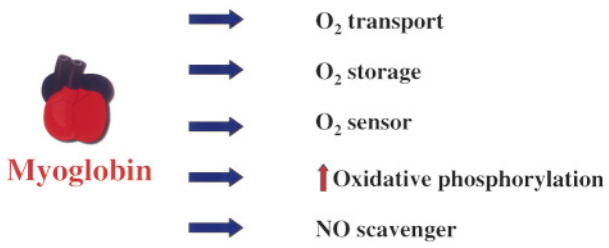

Figure 2. Alternative functions for myoglobin in cardiomyocytes and oxidative skeletal myofibers.

globin bulk transport of oxygen or as a reservoir for oxygen on the premise that reducing or oxidizing equivalents transduce the effect of oxymyoglobin across the mitochondrial membrane and enhance mitochondrial oxidative phosphorylation. Myoglobin may also function as a nitric oxide (NO) scavenger [22, 23]. For example, myoglobin may scavenge NO and thus decrease the respiratory inhibition of nitric oxide (i.e. binding of NO to cytochrome oxidase) [23, 24]. It has been proposed that tissue hemoproteins in bacteria and microbes may have arisen initially to detoxify nitric oxide and only later evolved toward a molecule that is optimized for oxygen delivery, so as to support the evolution of larger multicellular life forms [16, 22].

Summary

Myoglobin is an evolutionarily conserved hemoprotein that is restricted in vertebrates to heart and oxidative skeletal myofibers. Pharmacological and biochemical studies demonstrated that myoglobin facilitates oxygen transport from the erythrocyte to the mitochondria and predicted that myoglobin should be an essential protein. Gene knockout studies, however, reveal that myoglobin mutant mice have preserved cardiac and skeletal muscle function. A number of cellular and molecular adaptations that preserve muscle function in the absence of myoglobin have been identified. However, additional studies of myoglobin mutant mice should yield new insights into the complete repertoire of functions of this ancient protein, and of the adaptive responses that preserve oxidative metabolism when myoglobin is absent.

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