**Editorial Focus:** *Myoglobin-2, an adaptation for life at low oxygen pressure.*

Focus on: “Functional differentiation of myoglobin isoforms in the hypoxia-tolerant carp”

Beatrice A. Wittenberg and Jonathan B. Wittenberg

*Department of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, New York*

Submitted 19 December 2011; accepted in final form 6 January 2012

The common carp lives in stagnant ponds where it must weather long periods of low environmental oxygen pressure. In a dramatic adaptation to life in this environment, carp and a few related fishes develop a novel myoglobin-like protein, myoglobin-2, found only in the brain (3), where it may assure the oxygen supply and protect the neurons during periods of hypoxia.

In this issue, Helbo et al. (5) build on the earlier work of Fraser et al. (3) who discovered that the hypoxia-tolerant carp *Cyprinus carpio* simultaneously expresses two different myoglobin-like proteins. Myoglobin-1 (Mb-1), the myoglobin familiar from other animals, is expressed in the muscle and several other tissues including the liver, where its estimated concentration is about 60 μM, commensurate with that found in the hearts of several fishes. Myoglobin-2 (Mb-2), expressed solely in the brain, is a novel protein that shares 73% sequence identity with Mb-1. The residues that differ between the two proteins form two groups on the surface of the protein, remote from the heme. The occurrence of two myoglobin-like proteins in a single vertebrate organism is very rare. The authors proposed that it may be linked to a whole genome duplication event in carp within the last 12–15 million years.

The present paper of Helbo et al. (5) shows that Mb-1 and Mb-2 differ widely in the kinetics and equilibria of their reactions with oxygen and other ligands. The kinetics of reactions of Mb-2 diverge from those of classical myoglobin and Mb-1 mainly in the much larger rate of dissociation of bound oxygen $k_{O_2, off} = 75 s^{-1}$. The authors also extend the amino acid differences between the two proteins, noting an unique, nonconservative Lys to Gln substitution in position 82 (F3) in the heme-linked F-helix of Mb-2 that may be linked to the increased oxygen dissociation rate of Mb-2 compared with that of Mb-1. Mb-1 exhibits kinetics similar to those of classical myoglobin. The rapid rate of dissociation of oxygen from Mb-2 falls outside the bounds of the cluster of values defined by most myoglobins and leghemoglobins (2, 4, 9) but may be compared with that found for the myoglobins of some scombrid fishes (7) including the yellowfin tuna, cited by the authors. Accordingly, the author’s findings allow two interpretations: 1) That Mb-2 performs a novel function(s) and 2) That Mb-2 performs its familiar functions of oxygen storage and transport, reaction with diverse ligands, but in a novel environment. In either event, the 12–15 million years since the proposed whole genome duplication event leaves abundant time for the properties of Mb-2 to have become optimized for new function(s) or a new environment (10).

Mb-2 finds an immediate analogy in globin E (GlbE) found in the photoreceptors of the bird eye, where it probably serves to sustain the oxygen supply to the retina (2). GlbE shares rapid dissociation of bound oxygen ($k_{O_2, off} = 85 s^{-1}$ at 25°C) (2) with Mb-2 and may also be the product of a recent duplication of the myoglobin gene (6). A striking functional analogy is offered by neuroglobin that assists the oxygen supply to another neural tissue, the photoreceptors of the mammalian retina (1, 8).

Myoglobin-2 falls within a large class of cytoplasmic, frequently monomeric, oxygen-binding proteins that transport oxygen to meet the large demands imposed by chemical or mechanical work. Such proteins occur sporadically throughout the invertebrate, possibly protostome, animal, and plant worlds and are often recognized by their large tissue concentration imposed by the requirement that the capacity for oxygen transport is proportional to the concentration of the carrier protein (9, 11). Myoglobin-2 is one of the few such proteins found in the brain where, in all probability, it serves to meet the oxygen demand of neurons operating at very low oxygen pressure. Myoglobin-2 is of particular interest because its phylogenetic origin can be traced to a single whole genome duplication event, providing an opportunity to discover the rate of evolution of a protein under the batting of Darwinian natural selection.

Because of its chemical reactivities, myoglobin inevitably must assume functions other than oxygen transport or storage. Scavenging nitric oxide and thereby maintaining nitric oxide homeostasis, for instance, is a well-established role for mammalian skeletal muscle and cardiac myoglobin. In this regard, Mb-2 is here shown to have a smaller rate constant for nitrite reduction, generating nitric oxide from nitrite during hypoxia than Mb-1. Helbo et al. (5) also focus on hydrogen peroxide scavenging and find that, in vitro, Mb-2 removes hydrogen peroxide almost twice as fast as Mb-1. They suggest that this property may be of significance in protecting the brain during extended periods of hypoxia.

The present study describes the chemical activities of Mb-2 in vitro. One can imagine that future studies with advanced technology may permit a defined description of the actual function of intracellular Mb-2, surrounded as it is by complex chemical and enzymatic interactions in addition to structural and geometric barriers. To understand what is the advantage of Mb-2 to the carp brain living in low oxygen environments we want to learn in which cell it occurs, where in the cell it occurs, and, what is the local concentration in whatever cytoplasmic compartment Mb-2 is confined to.
DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: B.A.W. drafted manuscript; B.A.W. and J.B.W. edited and revised manuscript; B.A.W. and J.B.W. approved final version of manuscript.

REFERENCES