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Aerobic dive limits of seals with mutant myoglobin using combined thermochemical and physiological data

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ABSTRACT

This paper presents an integrated model of convective O_2-transport, aerobic dive limits (ADL), and thermochemical data for oxygen binding to mutant myoglobin (Mb), used to quantify the impact of mutations in Mb on the dive limits of Weddell seals (*Leptonychotes weddellii*). We find that wild-type Mb traits are only superior under specific behavioral and physiological conditions that critically prolong the ADL, action radius, and fitness of the seals. As an extreme example, the mutations in the conserved His-64 reduce ADL up to 14 ± 2 min for routine aerobic dives, whereas many other mutations are nearly neutral in terms of ADL and the inferred fitness. We also find that the cardiac system, the muscle O_2-store, animal behavior (i.e. pre-dive ventilation), and the oxygen binding affinity of Mb, K_{O_2}, have co-evolved to optimize dive duration at routine aerobic diving conditions, suggesting that such conditions are mostly selected upon in seals. The model is capable of roughly quantifying the physiological impact of single-protein mutations and thus bridges an important gap between animal physiology and molecular (protein) evolution.

1. Introduction

A good example of physiological adaptation in vertebrates is the ~10 times higher myoglobin (Mb) concentration in the skeletal muscles of some diving marine mammals compared to terrestrial mammals (Polasek et al., 2006). For aquatic, air-breathing animals that spend much of their lives submerged, oxy-Mb contributes significantly to total body oxygen stores. This adaptation, which increases aerobic breath-hold duration, is critical for the survival of diving mammals, but apparently not for terrestrial mammals where the role of Mb has been extensively studied (Weber et al., 1974; Noren and Williams, 2000; Lin et al., 2007; Endeward et al., 2010; Gros et al., 2010; Helbo and Fago, 2012), despite having similar properties such as P_{50} (i.e. P_{O_2} at half saturation of Mb) values and diffusion constants (Ponganis et al., 2008). Indeed, Mb knock-out mice with essentially unaffected phenotypes (Garry et al., 1998) stirred debate about other possible roles of Mb in terrestrial animals, which have later been confirmed (Cossins and Berenbrink, 2008; Hendgen-Cotta et al., 2008). These differences indicate that Mb plays a role for the fitness of diving mammals that is not shared by terrestrial animals.

Mb is a 17 kDa protein that binds O_2 with a 1:1 stoichiometry at its heme group, which is buried in a hydrophobic cavity within the protein (Phillips, 1980; see Fig. 1). The most important amino acid residues for this function are the distal and proximal His on each side of heme. O_2-binding involves forming a hydrogen bond to the $N_\varepsilon H$ of imidazole in His-64 (Perutz and Mathews, 1966). Studies of site-directed mutants have shown a 100-fold decrease in oxygen affinity by replacing His with apolar amino acids (Olson, 2008).

While most terrestrial animals have unlimited access to O_2, diving mammals must store oxygen in their blood, muscle and lungs to maintain aerobic metabolism while submerged. The observation that most diving mammals stay within their aerobic dive limit (ADL) during routine dives involving foraging, mating and migration is well-established (Kooyman et al., 1980; Ponganis et al., 1993; Costa et al., 2001). For Weddell seals, the ADL during routine dives has been calculated to be about 20 min but theoretically may range from ~5 to 30 min depending on the level of muscle metabolism, individual variation, and changes in convective oxygen transport associated with the dive response (Davis and Kanatous, 1999).

The role of Mb in prolonging the ADL can be understood from physiological models of the relationship between dive response and Mb concentration in seals (Wright and Davis, 2006). The oxy-Mb represents approximately one-third of the total O_2 store and half of the total O_2 used during aerobic dives (Wright and Davis, 2006) and thus contributes significantly to the ADL. Other adaptations such as increased blood volume and hematocrit and efficient modes of locomotion (stroke-and-glide swimming) (Costa et al., 1998; Williams et al., 2000; Costa et al., 2001; Williams, 2001) also enhance the ADL.

By applying thermochemical data to an integrated model of oxygen storage and transport, we recently showed that wild type (WT) Mb is more efficient in storing and transporting oxygen under severely hypoxic conditions in sperm whales (Dasmeh and Kepp, 2012). However and somewhat surprisingly, WT Mb is only more proficient

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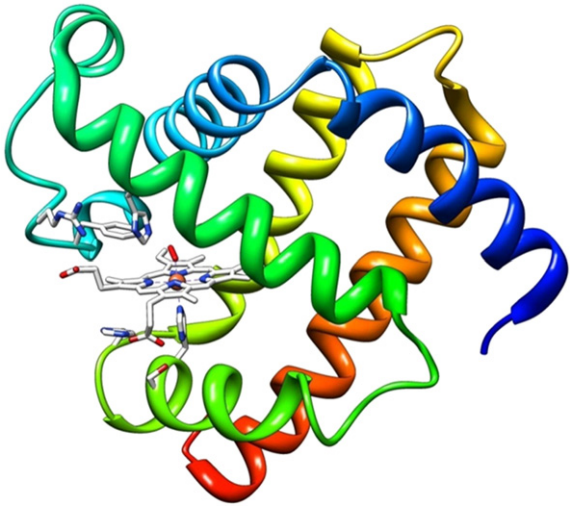

Fig. 1. Structure of oxy-Mb showing the heme and proximal and distal histidines. The image was produced using *Chimera*, University of California, San Francisco (Pettersen et al., 2004).

compared to low-affinity mutants under very hypoxic conditions, whereas low-affinity mutants are in fact *better* O_2-transporters under normoxic conditions, due to a steeper gradient of the saturation curve in this regime, affecting the diffusion rate (Dasmeh and Kepp, 2012). That WT Mb only shows its full importance at low oxygen partial pressure is consistent with conditions occurring at the end of routine dives, where hypoxia has been found necessary to efficiently release O_2 from Mb (Davis et al., 2004; Lin et al., 2007). This optimal use of oxy-Mb (i.e., full desaturation during dives) has also been confirmed in penguins (Williams et al., 2011).

To understand the potential evolutionary significance of Mb in marine mammals, we integrated thermochemical data of O_2-binding to Mb in sperm whales (Scott et al., 2001) with recently developed oxygen transport models (Dasmeh and Kepp, 2012) and ADL models for the Weddell seal (Davis and Kanatous, 1999). We found that the O_2-affinity of WT Mb contributes to a significantly higher ADL than e.g. His-64 impaired mutants (up to 14 min longer dives, depending on cardiac adjustments associated with the dive response and animal behavior), whereas many other mutations affect ADL only marginally. The model thus directly demonstrates and quantifies the effect of mutations in this single O_2-binding protein on the diving capacity, and by inference, the evolutionary fitness, of these animals.

2. Materials and methods

2.1. Experimental data

The values of K_{O_2} for single point mutants of sperm whale (*Physeter macrocephalus*) Mb (Scott et al., 2001) were used to calculate the seal Mb mutant ADLs (see theory appendix and Supplementary Information, Table S1). From a Blast ClustalW alignment from the UniProt interface (UniProt Consortium, 2011) of harbor seal (*Phoca vitulina*), gray seal (*Halichoerus grypus*) and sperm whale Mb, all have identical sequence lengths (154), including 128 identical positions and 22 similar positions (Bradshaw and Gurd, 1969). Notably, all the sites studied in this work are identical in WT whales and seals.

The sperm whale K_{O_2} values were first corrected from 20 °C to 37 °C by a factor of 0.2457 based on the temperature dependence of P_{50} (Schenkman et al., 1997) and the oxygen solubility in the muscle tissue, α_{O_2} (Mahler et al., 1985). Then, using the P_{50} values of 3.75 mm Hg and 3.45 mm Hg for sperm whale and Weddell seal WT Mb, all mutant seal Mb K_{O_2}-values were calculated by multiplying the sperm whale mutant K_{O_2} at 37 °C by 1.085 (the ratio of WT seal K_{O_2} to WT whale K_{O_2}) (see supporting information for uncorrected mutant ADLs, Figs. S1–S9). The applied, temperature-corrected K_{O_2} values are found in Table S2 (Supporting Information). Given the small difference between P_{50} for whale and seal, which is within the experimental uncertainty of thermochemically measured K_{O_2}, the use of whale mutant data is a good approximation and does not affect the significance of the conclusions, although the quantitative uncertainties in animal-specific mutant ADL are up to ~2 min (vide infra).

2.2. Computing the myoglobin saturation

The saturation S of Mb by O_2 is computed with the model using the experimentally verified parameters and variables shown in Table 1. S can be expressed in terms of the Hill equation (Hill, 1936):

$$S = \frac{\left(P_{O_2}\right)^n}{(P_{50})^n + \left(P_{O_2}\right)^n} \tag{1}$$

Table 1
List of parameters and variables used in this work.

Parameter	Symbol	Values used in the present work
Cardiac output	$\dot{V}_b$	42.00 L min^{-1} at rest
Brain blood flow rate	$\dot{Q}_B$	0.36 L min^{-1} at rest
Heart blood flow rate	$\dot{Q}_H$	1.84 L min^{-1} at rest
Skeletal muscle blood flow rate	$\dot{Q}_M$	7.90 L min^{-1} at rest
Blood flow rate for splanchnic, renal, cutaneous, and other peripheral tissues	$\dot{Q}_{SRC}$	32.63 L min^{-1} at rest
Brain oxygen consumption rate	$\dot{V}_{BO_2}$	13.3 mL O_2 min^{-1} at rest
Heart oxygen consumption rate	$\dot{V}_{HO_2}$	112.5 mL O_2 min^{-1} at rest
Skeletal muscle oxygen consumption rate	$\dot{V}_{MO_2}$	216.6 mL O_2 min^{-1} at rest
O_2-consumption rate for splanchnic, renal, cutaneous, and other peripheral tissues	$\dot{V}_{SRCO_2}$	555 mL O_2 min^{-1} at rest
Heart beat rate	f_h	51.5 beats min^{-1} at rest
Arterial blood oxygen saturation	S_a	100–38%
Venous blood oxygen saturation	S_v	86–36%
Arterial blood P_{O_2}	P_a	119–22 mm Hg
Venous blood P_{O_2}	P_v	55–21 mm Hg
P_{O_2} at mitochondria	P_{mit}	~0 mm Hg
P_{O_2} at capillary	P_c	87–21 mm Hg
Average Mb saturation for mutants	$<S>$	99–27%
Average oxygen pressure in cell	P_{O_2}	$P_{mit}<P_{O_2}<P_c$
P_{O_2} at which $S=0.5$	P_{50}	~1–3 mm Hg for wild type Mb
Bimolecular Mb oxygenation constant	K_{O_2}	~1 μM^{-1}
Oxygen solubility in the muscle tissue	α_{O_2}	9.4×10^{-7} mol L^{-1} mm Hg^{-1}
Mb concentration in Weddell seal muscle tissue	C_{Mb}	54 g kg^{-1} muscle