

On optima: The case of myoglobin-facilitated oxygen diffusion

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Abstract

The process of myoglobin/leghemoglobin-facilitated oxygen diffusion is adapted to function in different environments in diverse organisms. We enquire how the functional parameters of the process are optimized in particular organisms. The ligand-binding properties of the proteins, myoglobin and plant symbiotic hemoglobins, we discover, suggest that they have been adapted under genetic selection pressure for optimal performance. Since carrier-mediated oxygen transport has probably evolved independently many times, adaptation of diverse proteins for a common functionality exemplifies the process of convergent evolution. The progenitor proteins may be built on the myoglobin scaffold or may be very different.

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1. Introduction

The rates of oxygen consumption and the dimensions of the nitrogen-fixing legume root nodule and of the vertebrate heart and red skeletal muscles are such that diffusion of free oxygen cannot support the oxygen required by the bacteroids or mitochondria for oxidative phosphorylation. Translational diffusion of cytoplasmic leghemoglobin or myoglobin molecules carrying oxygen molecules pick-a-back provides the required additional flux. This latter process has been termed “myoglobin-facilitated oxygen diffusion.” It must not be supposed that a protein molecule, bearing its load of oxygen traverses the entire distance. Rather, oxygen, in near-equilibrium with the heme protein, continuously dissociates and recombines. At first glance, it would seem that the oxy-protein molecules diffuse too slowly to support the required flux, but, in fact, the diffusion coefficient of diatomic oxygen is only twenty times that of myoglobin, and myoglobin and leghemoglobin

occur in their respective tissues at enormous concentration. In working heart and muscle, the concentration of myoglobin-bound oxygen exceeds thirty-fold that of free oxygen; in the root nodule, leghemoglobin-bound oxygen exceeds free oxygen by a factor of 10^5 . The largest part of the oxygen flux to the bacteroids or mitochondria is supported by carrier-mediated transport.

The dramatic constancy of myoglobin or leghemoglobin fractional saturation with oxygen in every working tissue (Millikan, 1939; Wittenberg and Wittenberg, 2003), indeed in each individual cell of working heart or muscle (Gayeski and Honig, 1991), commands attention and demands explanation. That myoglobin/leghemoglobin must be partially desaturated somewhere in the system in order to transport oxygen is obvious but not immediately apparent. Consider that: in the absence of oxymyoglobin, myoglobin-facilitated oxygen diffusion will vanish. If, on the other hand, myoglobin is fully saturated with oxygen everywhere in the system, there will be no gradient of myoglobin oxygenation, and, in the absence of a gradient, net myoglobin-facilitated diffusion must vanish (Wyman, 1966). In this essay we ask: How is the fractional saturation of myoglobin and leghemoglobin optimized in particular tissues? We go on to ask: How are the genetically-determined properties of the oxygen-binding proteins optimized?

Abbreviations: K_m , Michaelis constant; NMR, Nuclear magnetic resonance; P_{50} , Oxygen pressure (mm Hg) at half-saturation with oxygen; s, second; μmol , micromole.

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2. The facts

2.1. Symbiotic hemoglobins in plant/symbiont associations

Leghemoglobin, a protein similar to myoglobin but with 10-fold greater oxygen affinity, transports oxygen from the cell membrane of the central cells of the legume root nodule to the symbiosomes, membrane-bound intracellular organelles housing the bacteroids, the intracellular nitrogen-fixing form of the bacteria *Rhizobium* or *Bradyrhizobium*. Thus leghemoglobin enables the bacteroids to sustain the vigorous oxygen uptake required for active nitrogen fixation (Wittenberg et al., 1974). Leghemoglobin, within the active nodule is always partially oxygenated (Appleby, 1969; Becana and Klucas, 1992). Leghemoglobin oxygen saturation, perturbed experimentally by changing the ambient oxygen concentration of nodules attached to the undisturbed living plant, soon returns to its prior value (Klucas et al., 1985). As a system of air passages makes the oxygen pressure in the nodule everywhere the same (Goodchild, 1977), a variable barrier to protect against the outside air is required (Criswell et al., 1976) and is found in the inner layer of the nodule cortex (Becana and Klucas, 1992). Indeed in the Australian tree *Casuarina*, nodulated by the actinomycete *Frankia*, each individual symbiont-harboring, hemoglobin-containing cell is surrounded by a lignified, presumably oxygen impermeable capsule (Berg and McDowell, 1988).

2.2. Myoglobin in vertebrate tissues

Myoglobin in vertebrate tissues likewise always functions in states of partial oxygenation, ranging in mammalian heart or muscle working in sustained steady-states, from 50 to 70% saturation.¹ The oxygen partial pressure is always not far from the P_{50} of myoglobin, 2.5 mm Hg at 37 °C. Myoglobin oxygen saturation resists change over wide ranges of work load and oxygen availability, reviewed by Wittenberg and Wittenberg (2003). Sarcoplasmic oxygen pressure is small compared to the oxygen pressure available in capillary blood, say 100 to 20 mm Hg, and two barriers impede oxygen flow from the capillary blood to the muscle cell. The first, imposed by diffusion across the capillary wall and intervening interstitial tissue, contributes the largest part of the oxygen pressure drop from capillary to mitochondrion, about 20 mm Hg (Landis and Pappenheimer, 1963; Honig et al., 1984; Katz et al., 1984; Wittenberg and Wittenberg, 1985). The second limitation is found in the active control of fraction of the capillaries open at any one time, which is kept just sufficient to supply the oxygen needs of the tissue (Krogh, 1919). The capillaries open in response to local low oxygen pressure (Hilton and Eichholtz, 1925) and to nitric oxide

¹ The assertion in the recent literature that myoglobin is largely saturated with oxygen in the mammalian heart arises from misinterpretation of experiments using NMR to detect deoxymyoglobin in the in situ, blood-perfused, beating dog heart. These measurements are limited by small signal size and by signal to noise ratio, so that values near full myoglobin oxygen saturation are inaccessible (Kreutzer and Jue, 1991; Chen et al., 1997; Schenkman, 2001). No exact fractional saturation can be assigned to the cardiac myoglobin in these experiments, nor can the intra-sarcoplasmic oxygen pressure be calculated.

generated in the muscle cell and elsewhere and destroyed by reaction with oxy- or deoxymyoglobin. For an overview of the mechanisms by which the capillary responds to oxygen or nitric oxide are mediated see Crawford et al. (2006).

The concentration of myoglobin in muscle increases in response to mitochondrial demand for oxygen and can vary widely, frequently reaching about 300 $\mu\text{mol kg}^{-1}$ wet mass tissue in active muscles, probably making it the most abundant soluble protein in the cell (Millikan, 1939; Wittenberg, 1970). Myoglobin concentration in those hearts that have been examined largely falls in a narrower range, near 200 $\mu\text{mol kg}^{-1}$ wet mass tissue (Wittenberg, 1970; Schuder et al., 1979). Myoglobin expression is under genetic control, mediated in part through oxygen availability (Williams and Neuffer, 1996; Yan et al., 2001).

3. Discussion

3.1. The nitrogen-fixing plant root nodule

We address leghemoglobin first because, within the nodule, leghemoglobin serves the singular function of oxygen transport (Appleby, 1984); the oxygen partial pressure is very small; free dissolved oxygen (about 10–30 nM) virtually vanishes (Appleby, 1969; Becana and Klucas, 1992), and essentially the total oxygen flux is leghemoglobin-mediated, simplifying analysis. The nitrogenase enzyme system is highly intolerant of oxygen, imposing the severe constraint that oxygen must not penetrate to the site of nitrogen fixation within the bacteroids but, simultaneously, must be supplied at a goodly rate to the terminal oxidases situated in the bacteroid cell membrane.

Gibson et al. (1989) have examined the properties of eleven plant symbiotic hemoglobins, including two from non-legumes, nodulated, respectively, by the bacterial symbiont *Rhizobium* and by the actinomycete symbiont *Frankia*, Table 1. All have rate constants in their reactions with oxygen very similar to those of soybean. Frequently nodules contain multiple leghemoglobin components, and these display very little scatter in the rate constants for their reactions with oxygen (Gibson et al., 1989; Martin et al., 1990). The rates of oxygen combination are very fast, within an order of magnitude of that expected for diffusion of oxygen into a sink, and Gibson et al. speculate that these rates and

Table 1
Kinetic and equilibrium constants for the reactions of plant symbiotic hemoglobins with oxygen

Protein	Combination, $\mu\text{M}^{-1} \text{s}^{-1}$	Dissociation, s^{-1}	Equilibrium, nM
Soy	120	5.6	48
Kidney bean	130	6.2	48
Cowpea II	140	5.5	39
<i>Sesbania</i> II	210	7.5	36
Green pea I	250	16	65
Green pea IV	260	16	61
Broad bean V	260	19	78
Lupin I	{540}	20	36
Lupin II	{320}	25	78
<i>Parasponia</i> I	165	15	89
<i>Casuarina</i> II	41	6	135

From Gibson et al. (1989).

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