Virtual cooperativity in myoglobin oxygen saturation curve in skeletal muscle in vivo

Akitoshi Seiyama*

Address: Division of Physiology and Biosignaling, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

Email: Akitoshi Seiyama* - aseiyama@phys1.med.osaka-u.ac.jp

* Corresponding author

Abstract

Background: Myoglobin (Mb) is the simplest monomeric hemoprotein and its physicochemical properties including reversible oxygen (O₂) binding in aqueous solution are well known. Unexpectedly, however, its physiological role in intact muscle has not yet been established in spite of the fact that the role of the more complex tetrameric hemoprotein, hemoglobin (Hb), in red cells is well established. Here, I report my new findings on an overlooked property of skeletal Mb.

Methods: I directly observed the oxygenation of Mb in perfused rat skeletal muscle under various states of tissue respiration. A computer-controlled rapid scanning spectrophotometer was used to measure the oxygenation of Mb in the transmission mode. The light beam was focused on the thigh (quadriceps) through a 5-mm-diameter light guide. The transmitted light was conducted to the spectrophotometer through another 5-mm-diameter light guide. Visible difference spectra in the range of 500–650 nm were recorded when O₂ uptake in the hindlimb muscle reached a constant value after every stepwise change in the O₂ concentration of the buffer.

Results: The O₂ dissociation curve (ODC) of Mb, when the effluent buffer O₂ pressure was used as the abscissa, was of a sigmoid shape under normal and increased respiratory conditions whereas it was of rectangular hyperbolic shape under a suppressed respiratory condition. The dissociation curve was shifted toward the right and became more sigmoid with an increase in tissue respiration activity. These observations indicate that an increase in O₂ demand in tissues makes the O₂ saturation of Mb more sensitive to O₂ pressure change in the capillaries and enhances the Mb-mediated O₂ transfer from Hb to cytochrome oxidase (Cyt. aa₃), especially under heavy O₂ demands.

Conclusion: The virtual cooperativity and O₂ demand-dependent shifts of the ODC may provide a basis for explaining why Mb has been preserved as monomer during molecular evolution.

Background

Mb is a monomeric hemoprotein with a molecular weight of 17 kDa, carrying a single oxygen (O₂)-binding site per molecule. It is located near the contractile elements and cell membranes in the red skeletal and cardiac muscles of vertebrates [1]. Previously, Millikan [2,3] proposed the following three possible physiological functions for Mb: (a) an O₂ store during temporary deficits in O₂ supply, (b) an intracellular O₂ transport agent and (c) an intracellular catalyst. Among them, the first function has traditionally
been accepted. In the muscles of a beating heart and exercising skeletal muscles, Mb acts as a short-term O2 store (i.e., an O2 buffer), tiding the muscles over from one contraction to the next. The rich Mb content in skeletal muscles in aquatic mammals is considered to provide a long-term O2 store during diving. However, this role of Mb, at least in human, is not significant because its oxygen storage capacity is so low that the total oxygen bound to Mb is exhausted within ca. 5.5 s after being cut off from the O2 supply [4]. The second function, called “facilitated O2-diffusion by Mb”, was based on findings in in vitro experiments [5,6]. The conditions required for this facilitated diffusion to occur are [7]: (a) existence of deoxygenated Mb in a certain fraction (or certain low intracellular partial pressure of O2), (b) existence of a spatial gradient of oxygenated Mb concentration as a driving force for translational diffusion of Mb, and (c) sufficient mobility of the oxygenated Mb to permit diffusion. Although this mechanism has been widely accepted, several discrepancies remain unresolved [8-12]. As for the third function, Doeller and Wittenberg [13] proposed the occurrence of Mb-mediated antioxidative phosphorylation in heart myocytes under aerobic conditions. However, Mb concentration is not closely related to the oxidative capacity of muscles, that is, the concentration is higher in skeletal muscles (~0.5 mmole/kg wet wt.) than in heart muscles (~0.25 mmole/kg wet wt.) [7].

Thus, the physiological roles of Mb have not yet been established. Recently, alternative functions of (d) O2 sensing and (e) nitric oxide scavenging were proposed [14]. Another recent paper [15] seemed to have totally scrambled the past long-term disputes about the physiological significance of Mb. It was shown using gene-knockout technology that mice without Mb are fertile, exhibit normal exercise capacity, and have a normal ventilatory response to low O2 levels, suggesting that Mb is not essential for apparently normal cardiovascular and musculoskeletal function in a terrestrial, homoiothermic mammal. However, it has been reported that the disruption of Mb results in the activation of multiple compensatory mechanisms such as increases in Hb concentration, hematocrit, coronary flow, coronary reserve, and capillary density [16]. Further, a Mb-like hemoprotein, neuroglobin, has been found in the vertebrate brain [17] contrary to the long-held belief that Mb is restricted to vertebrate cardiomyocytes and oxidative skeletal myofibers. These studies imply that further investigations are required to reveal the physiological role of Mb in intact organs.

In contrast to Mb, which shows a rectangular hyperbolic ODC, the vertebrate Hb, a tetramer carrying four O2 binding sites, shows a sigmoid ODC that is described in terms of a four-step cooperative O2 binding. It is widely accepted that the sigmoid ODC enables Hb to transport O2 with high efficiency: it is nearly fully saturated with O2 in the lungs and it unloads O2 sensitively depending on decreases in the partial pressure of oxygen (PO2) in peripheral tissues. Here, no convincing explanation has been given for the question: does the hyperbolic ODC of Mb have any physiological adequacy or reasonability? The Bohr effect of Hb (pH dependence of O2 affinity) has physiological significance, in that it enhances O2 unloading from Hb in the capillaries where pH tends to decrease and in that it increases the solubility of CO2 as bicarbonate in the venous blood through deoxygenation-induced uptake of protons by Hb. In contrast, Mb lacks the Bohr effect and it had long been believed that Mb was a totally nonallosteric protein, although recently lactate, a metabolic product, was found to cause a right-shift of the ODC for horse and sperm whale Mbs [18].

It is well established that the O2 affinity of Mb is higher than that of Hb but lower than that of Cyt. aa3, as known from the relative positions of the ODCs for Mb and Hb and the oxidation curve for Cyt. aa3 (Fig. 1). This fact led one to the idea that Mb acts as an intracellular O2 transfer agent from Hb (vascular space) to Cyt. aa3 (mitochondria). Here, one must not overlook an important fact. The three curves in Fig. 1 are drawn with the same PO2 scale. Therefore, they give O2 saturation (Y) or the degree of oxidation for the individual proteins when dissolved in the same solution and are in equilibrium with oxygen at the given PO2 value. However, in vivo, they sense different PO2 values due to the presence of a PO2 gradient along the path from the inside of red cells to the mitochondria in myocytes. Thus, the relative positions of the three curves in Fig. 1 must be considered with this precaution, and direct in vivo observations of Y or the degree of oxidation for these three individual proteins are required to get insight into their ensemble functional roles. Recently, using 1H nuclear magnetic resonance spectroscopy, Mole et al. [19] and Richardson et al. [20] directly observed Y...
for Mb in human skeletal muscles under exercise of different intensities or during breathing of air with different O$_2$ contents. In these studies, Mb was used as an indicator of intracellular PO$_2$, and no attention was paid to the relation between Mb saturation and capillary PO$_2$.

In the present study, we directly measured Y for Mb in isolated rat hindlimb muscles, perfused with a Hb-free medium, under vigorous changes in respiration conditions. We plotted the Y values as a function of buffer PO$_2$ and found that the apparent ODC thus plotted for skeletal muscle Mb was rectangular hyperbolic under a suppressed metabolic activity condition but it became sigmoid under enhanced metabolic activity conditions, realizing virtually cooperative O$_2$ binding by monomeric Mb.

Methods

Muscle perfusion

All experimental procedures were performed according to the institutional guidelines for animal care and use of the Committee for Animal Care of Osaka University and the Japanese Physiological Society. Male Wistar rats (250 to 300 g body weight, N = 12) fed on a commercial diet were used. Rats were anesthetized with sodium pentobarbital (30 mg/kg body wt., intraperitoneal injection). Prepara-

tion of the isolated rat hindlimb and the perfusion apparatus were described previously [21,22]. Surgery was modified from those of Ruderman et al. [23] and Shiota et al. [24]. After a midline abdominal incision, the superficial epigastric vessels were ligated. The abdominal wall was then incised from the pubic symphysis to the xiphoid process. The spermary, testis, and inferior mesenteric arteries and veins were ligated, and the spermaries, the testises, and part of the descending colon were excised, together with contiguous adipose tissue. The caudal artery and internal iliac artery and vein were also ligated. Ligature were placed around the neck of the bladder, the coagulating gland and the prostate gland. While carefully
with the buffer equilibrated with 95% O2 + 5% CO2 for 30 min. Before each measurement, the rat hindlimb was perfused with an oxygen electrode. The rate of O2 uptake was calculated from the flow rate and the difference in O2 concentration between the influent and the effluent buffers.

### Table 1: Values of muscle perfusion parameters and Mb oxygenation parameters in various tissue respiration states

| Respiration state: | Suppressed (0.4 mM KCN) | Control | Enhanced (5 µM DNP) | Enhanced (10 µM DNP) |
|-------------------|-------------------------|---------|---------------------|---------------------|
| Influent PO2      | 700                     | 700     | 700                 | 700                 |
| Effluent PO2      | 652 ± 14                | 579 ± 6 | 512 ± 15            | 418 ± 18            |
| Vmax              | 0.09                    | 0.27    | 0.42                | 0.68                |
| PV50              | 83                      | 160     | 170                 | 180                 |

a. 2,4-dinitrophenol; b. Maximal value of steady-state O2 uptake rate (V) at infinite influent PO2 (in µmol/min/g muscle); c. effluent PO2 at V = half Vmax (in mmHg). Values of Vmax and PV50 were obtained from solid lines shown in Figure 2.

### Analysis of data

Changes in the O2 uptake rate were analyzed using a rectangular hyperbolic curve equation: \( V = \frac{V_{\text{max}}(P_{\text{O2}}/P_{50})}{1 + (P_{\text{O2}}/P_{50})} \). Here, the maximal rate of O2 uptake (Vmax) and effluent buffer PO2 at half maximal O2 uptake (P50) were obtained from the slope (1/Vmax) and the ordinate intercept (P50/Vmax) of the Hanes-Woolf plot (effluent PO2/V vs. effluent PO2). Changes in oxygen saturation of Mb (Y) were analyzed using the Hill equation [25], \( Y = \frac{P_{\text{O2}}^n}{P_{\text{O2}}^n + P_{\text{50}}^n} \), where P50 is PO2 at half saturation of Mb (Y50) and n is the Hill coefficient. In the original Hill equation, n was treated as a constant. This equation expressed the ODC of Mb well but not the ODC of Hb because the Hill plot for Hb deviated from a straight line at both extremes. To make the Hill plot applicable to Hb, Wyman [26] extended the equation by linearizing it in the form: \( \log \{ Y/(1 - Y) \} = n \log P_{\text{O2}} - \log P_{50} \) where n was treated as a variable. This extension allowed cooperativity measured by n to vary depending on Y.

### Results

**Oxygen uptake by perfused muscle in different respiration states**

Figure 2 shows the steady-state O2 uptake rate (V) of a perfused muscle. The respiration rate of the muscle was varied by controlling mitochondrial respiration activity by about 7.5-fold (compare the Vmax values described below) from a suppressed state with an inhibitor (KCN) of mitochondrial respiration to enhanced states with two levels of an uncoupler (2,4-dinitrophenol) of mitochondrial respiration. Three preparations of muscle were used for the experiments in each mitochondrial activity state. The actual PO2 values of the influent and effluent buffers at the maximal O2 inflow rate are listed in Table 1. Changes in the value of V were well expressed by a rectangular hyperbolic curve as a function of effluent buffer PO2 (Fig. 2). Table 1 also gives the estimated Vmax and P50 obtained from these data as described in Materials and Methods. Vmax and P50 became larger by approximately 7.5-fold and 2-fold, respectively, for the maximal increase in respiration activity. With elevation of respiration activity, the critical PO2, at which O2 uptake of perfused hindlimb muscle starts to decrease, increased to higher values. This indicates that, under higher respiration activity, O2 supply
the flow rate of the perfusate and the capillary PO\textsubscript{2} were expressed by means of the Hill plot (log [Y/(1-Y)] vs. activity was enhanced. These oxygenation data were further expressed by means of the Hill plot (log P\textsubscript{Y50} vs. P\textsubscript{V50} value (not shown). The n\text{app} value also increased from 1.10 in the non-respiring state to 2.23 at 7.5-fold enhanced respiration activity. The log P\textsubscript{Y50} value became larger with an increase in muscle respiration activity. The log P\textsubscript{V50} value was nearly linearly related to the log P\textsubscript{Y50} value (not shown). The n\text{app} value also increased from 1.10 in the suppressed respiration activity state to 1.85 in the 7.5-fold enhanced respiration activity state.

Since this virtual cooperativity is of particular interest, its relation to O\textsubscript{2} uptake rate was further examined. Figure 4 shows the dependence of n\text{app} on V at the half O\textsubscript{2} saturation point of Mb (V\textsubscript{Y50}). The n\text{app} value asymptotically increased from unity for the non-respiring state to 2.23 at infinite V\textsubscript{Y50}. These results indicate that the apparent ODC of Mb in the perfused muscle is transformed from a hyperbolic curve to a sigmoid curve depending on the magnitude of tissue respiration. Effect of the Hill coefficient (n) on ratio of substrate (or ligand) concentrations necessary to change enzyme activity from 90% to 10% of maximal can be expressed with a parameter, R (\(= 81^{1/n}\)) [27]. Here, the O\textsubscript{2} transport efficiency (EO\textsubscript{2}) was estimated as ratio of the parameter at n\text{app} = 1 to that at a given value of n\text{app} (Fig. 4 inset). Figure 5 shows the effect of muscle respiration on the O\textsubscript{2} gradient between effluent and the perfused tissue. Assuming the effluent buffer PO\textsubscript{2} approximates the capillary PO\textsubscript{2}, the calculated O\textsubscript{2} gradient from capillary to cytoplasmic space (\(\Delta P\text{O}_2\)) is plotted against V\textsubscript{Y50}. Here, the P\text{50} value of Mb in the perfused muscle was 2.3 mmHg [21]. \(\Delta P\text{O}_2\) increased with the increase in V\textsubscript{Y50}. This result indicates the presence of a large O\textsubscript{2} diffusion barrier between capillary lumen and cytoplasmic space.

### Discussion

In the present study, by using computer-controlled rapid scanning fiber-optic spectrophotometry, I directly measured Y for Mb in isolated rat hindlimb muscles under extensive changes in respiration rate caused by mitochondrial activity control or perfusate PO\textsubscript{2} control. It is assumed that capillary PO\textsubscript{2} may be approximated by effluent PO\textsubscript{2} in the present experiment, and I plotted the Y values as a function of effluent buffer PO\textsubscript{2}. Thereby, I expected that this treatment enabled a meaningful comparison of the ODCs for Mb and Hb. I found that thus plotted apparent ODC for skeletal muscle Mb was hyperbolic under a suppressed metabolic activity condition whereas it became sigmoid under enhanced metabolic activity conditions, realizing virtually cooperative oxygenation of the monomeric Mb.

It is generally accepted that cooperative O\textsubscript{2} binding by Hb is advantageous for efficient O\textsubscript{2} transfer from the alveolar gas to red cells and from red cells to peripheral tissues. Based on the Hill equation, Graby and Meldon [28] showed that an n value (here, n is a constant) of 1.5 to 2.0 is more favourable for minimizing the change in blood flow under resting conditions than the normal n value of 2.5 to 3.0, whereas an n value as large as 3 is beneficial for a large amount of O\textsubscript{2} extraction under vigorous exercise. Kobayashi et al. [29] showed that, under resting conditions, O\textsubscript{2} release from Hb becomes most sensitive to PO\textsubscript{2} change at Y = 38% where cooperativity measured by n (here, n is a variable of PO\textsubscript{2}) is not maximal, whereas it becomes less sensitive at the mixed venous blood PO\textsubscript{2} where Y is around 70% and cooperativity is nearly maximal. These reports indicate that, under resting conditions, the blood reserves an O\textsubscript{2} transport capacity to meet possible increases in O\textsubscript{2} demand, e.g. under exercise conditions, and the sigmoid character of ODC becomes more important under such conditions. This situation is real-
ized by maintaining \( Y \) at a rather high level (70%) below which the \( Y \) value drops sharply upon \( \text{PO}_2 \) decrease within the very steep middle portion of ODC.

The present study has clearly shown that the apparent ODC for Mb in intact skeletal muscle is sigmoid, the \( n_{app} \) value being 1.46 under the control condition (Table 2) and 2.23 under the maximal respiratory condition (Fig. 4). These \( n_{app} \) values greater than unity imply that the muscle Mb binds \( \text{O}_2 \) in a virtually cooperative manner with variation of effluent buffer \( \text{PO}_2 \). This phenomenon implies that the sensitivity of \( Y \) for Mb to vessel \( \text{PO}_2 \) change becomes higher for increased \( \text{O}_2 \) demands than for normal \( \text{O}_2 \) demand. In addition to this effect, the rightward shift of the ODC upon increases in oxygen demand will undoubtedly enhance \( \text{O}_2 \) unloading from Mb. These effects will facilitate Mb-mediated \( \text{O}_2 \) transfer from Hb to Cyt. aa₃, especially for heavy \( \text{O}_2 \) demands. Based on the Hill equation, the \( \text{O}_2 \) transport efficiency of Mb in the perfused muscle is estimated to increase ca. 4-fold under the control condition and ca. 11-fold under maximally respiring condition (Fig. 4 inset).

The \( \text{Mb}s \) isolated from body wall or radular muscle of a limited number of annelidan and molluscan species are dimers and show some cooperativity in oxygen binding (1 < \( n < 2 \) but no Bohr effect [30]. The physiological signif-

icance of these dimeric \( \text{Mb}s \) is unknown. As shown in the present study, the ODC of monomeric Mb can exhibit virtual cooperativity and \( \text{O}_2 \) demand-dependent shifts. The virtually cooperative and \( \text{O}_2 \) demand-dependent shifts of Mb oxygenation in vivo are probably common features at least for vertebrate Mbs, and this may provide a basis for explaining why the vertebrate Mb has been preserved as a monomer during molecular evolution.

The virtual cooperativity in Mb oxygenation observed in the present study is explained in terms of the \( \text{PO}_2 \) gradient along the \( \text{O}_2 \) diffusion path. If the tissue \( \text{O}_2 \) demand was null, then the \( \text{PO}_2 \) gradient would be absent and the apparent ODC for Mb would be identical with the real ODC for Mb in solution. At a steady state with a certain level of \( \text{O}_2 \) demand a \( \text{PO}_2 \) gradient develops across red cell membrane, blood plasma, capillary wall, sarcolemma and sarcoplasm, making the \( \text{PO}_2 \) sensed by Mb lower than the capillary \( \text{PO}_2 \). Then, the apparent ODC will be shifted toward the right because a capillary \( \text{PO}_2 \) value higher than the \( \text{PO}_2 \) value sensed by Mb is needed to maintain the same \( Y \) value as that which occurs in the absence of a \( \text{PO}_2 \) gradient. When the tissue \( \text{O}_2 \) demand is kept constant, the ratio of capillary \( \text{PO}_2 \) to sarcoplasm \( \text{PO}_2 \) will become larger at low capillary \( \text{PO}_2 \) than at high capillary \( \text{PO}_2 \). This will cause a more extensive rightward shift of the apparent ODC in the low saturation range than in the high saturation range, making the curve steeper than the real one. Increase in tissue oxygen demand will enhance this mechanism and make the curve more right-shifted and sigmoid. All the apparent ODCs observed in the present study are shifted toward the right compared to the real one measured for Mb in solution (Fig. 3).

At present, detailed explanations for this cooperative mechanism is difficult. However, it could be argued that heterogeneous oxygenation in tissue [31] and in single myocytes [32] might be responsible in part for the shift and the shape change of the Mb ODC, and might also enhance intercellular \( \text{O}_2 \) transfer, i.e., re-distribution of \( \text{O}_2 \) among adjacent myocytes, although we adopted high and constant flow rate perfusion conditions (i.e., about 50 times higher than normal blood flow) and, thus, the perfused vessels of muscle were always passively dilated.

Unfortunately, it is not practical to use a Hb solution or a red cell suspension as the perfusate in our experiments because the absorption spectra for Hb and Mb are too similar and independent observations of Mb oxygenation are not feasible, especially when the concentration of Hb is much higher than that of Mb. To overcome the problem that the \( \text{O}_2 \) solubility of the buffer is much smaller than that of a Hb solution or a red cell suspension two strategies were employed: one was to make the \( \text{PO}_2 \) of the influent buffer as high as that of water vapor-saturated \( \text{O}_2 \) (ca.
the control metabolic rate. The large O₂ diffusion barrier plasmic space in perfused hindlimb muscle (illary bed is not constant and frequently only plasma flow. However, it is well known that the blood flow in the capillary is not right-shifted compared to the whole blood ODC (Fig. 1). One may suppose that Mb cannot work when the capillary PO₂ is in the physiological range (40 to 100 mmHg) because its O₂ saturation is too low to function, as judged from Fig. 3A. However, the actual apparent ODCs for Mb in intact muscles with blood circulation will be shifted much more toward the left compared to those shown in Fig. 3A, and Mb can be saturated with O₂ to practical levels. The important point is that the difference in in vivo O₂ saturation between Hb and Mb is not so large as that expected from the ODCs in Fig. 1. In fact, Y for Mb in working muscles is less than around 50% [19,20,31-33]. Red blood cell (RBC) in perfusion buffer appears to exert considerable effects on intracellular oxygenation in the beating heart [34], probably due to the facilitated O₂ transfer by RBC motion within capillary lumen [35]. Therefore, the virtually cooperative oxygenation of Mb might be only demonstrated in organs perfused with RBC-free medium. However, it is well known that the blood flow in the capillary bed is not constant and frequently only plasma flow is observed. In this case, the virtually cooperative oxygenation of Mb may play a significant role for O₂ transfer from capillary to mitochondria.

In summary, I found that the ODC for Mb in intact skeletal muscle is sigmoid and right-shifted. This virtually cooperative O₂ binding by Mb and the right-shift of ODC become more marked as tissue respiration activity is increased. Hence, increase in O₂ demand in tissues makes the O₂ saturation of Mb more sensitive to capillary PO₂ change and enhances Mb-mediated O₂ transfer from red cell to mitochondria. The virtual cooperativity and O₂ demand-dependent shifts of ODC may give a basis for explaining why Mb has been preserved as a monomer during molecular evolution. Preservation of a monomeric structure may be required to retain multi-functional role of Mb in vivo.

Acknowledgements
This work was supported in part by a research grant from the Ministry of Education, Science and Culture of Japan.

References
1. Kagen LJ: Myoglobin New York, London: Columbia University Press; 1973.
2. Millikan GA: Experiments on muscle haemoglobin in vivo; the instantaneous measurement of muscle metabolism. Proc R Soc Lond B Biol Sci 1937, 123:218-241.
3. Millikan GA: Muscle hemoglobin. Physiol Rev 1939, 19:503-523.
4. Wyman J: Facilitated diffusion and the possible role of myoglobin as a transport mechanism. J Biol Chem 1966, 241:115-121.
5. Wittenberg JB: Oxygen transport: a new function proposed for myoglobin. Biol Bull 1959, 117:402-403.
6. Scholander PF: Oxygen transport through hemoglobin solutions, Science 1960, 131:585-590.
7. Wittenberg JB: Myoglobin-facilitated oxygen diffusion: Role of myoglobin in oxygen entry into muscle. Physiol Rev 1970, 50:559-636.
8. Jones DP, Kennedy FG: Intracellular O₂ gradients in cardiac myocytes. Lack of a role for myoglobin in facilitation of intracellular diffusion. Biochem Biophys Res Commun 1982, 105:419-424.
9. Kennedy FG, Jones DP: Oxygen dependence of Mitochondrial function in isolated rat cardiac myocytes. Am J Physiol 1986, 250:C374-C383.
10. Livingston DJ, LaMar GN, Brown WD: Myoglobin diffusion in bovine heart muscle. Science 1983, 220:71-73.
11. Jurgens KD, T, Gros G: Diffusivity of myoglobin in intact skeletal muscle cells. Proc Natl Acad Sci USA 1994, 91:3829-3833.
12. Paradopoulos S, Jurgens KD, Gros G: Diffusion of myoglobin in skeletal muscle cells-dependence on fiber type, contraction and temperature. Pflugers Arch 1995, 430:519-525.
13. Doeller J, Wittenberg BA: Myoglobin function and energy metabolism of isolated cardiac myocytes: effect of sodium nitrite. Am J Physiol 1991, 261:H53-H62.
14. Garry DJ, Meeson A, Yan Z, Williams RS: Life without myoglobin. Cell Mol Life Sci 2000, 57:896-898.
15. Garry DJ, Ordway GA, Lorenz JN, Radford NB, Chin ER, Grange RW, Basell-Duby R, Williams RS: Mice without myoglobin. Nature 1998, 395:905-908.
16. Godecke A, Flugel U, Zanger K, Ding Z, Hirchenhain J, Decking UKM, Schrader J: Disruption of myoglobin in mice induces multiple compensatory mechanisms. Proc Natl Acad Sci USA 1999, 96:10495-10500.
17. Burbester T, Weich B, Reinhardt S, Hankeln T: A vertebrate globin expressed in the brain. Nature 2000, 407:520-522.
18. Giardina B, Ascenzi P, Clementi ME, De Sanctis G, Rizzi M, Coletta M: Functional modulation by lactate of myoglobin. J Biol Chem 1996, 271:16999-17001.
19. Mole PA, Chung Y, Tran T-K, Sailleuta N, Hurd R, Jue T: Myoglobin desaturation with exercise intensity in human gastrocnemius muscle. Am J Physiol 1999, 277:R173-R180.
20. Richardson RS, Noyzewski RA, Kendrick KF, Leigh JS, Wagner PD: Myoglobin O2 desaturation during exercise. Evidence of limited O2 transport. J Clin Invest 1995, 96:1916-1926.
21. Seiyama A, Maeda N, Shiga T: Optical measurement of perfused rat hindlimb muscle with relation of oxygen metabolism. Jpn J Physiol 1991, 41:49-61.
22. Seiyama A, Kosaka H, Maeda N, Shiga T: Effect of hypothermia on skeletal muscle metabolism in perfused rat hindlimb. Cryobiology 1996, 33:338-346.
23. Ruderman NB, Houghton CRS, Hems R: Evaluation of the isolated perfused rat hindquarter for the study of muscle metabolism. Biochem J 1971, 124:639-651.
24. Shiota T, Sugano M: Characteristics of rat hindlimbs perfused with erythrocyte- and albumin-free medium. Am J Physiol 1986, 251:C78-C84.
25. Hill AV: The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curves. J Physiol 1910, 40:4-7.
26. Wyman J: Linked functions and reciprocal effects in hemoglobin: A second look. Adv Protein Chem 1964, 19:223-286.
27. Taketa K, Fogell BM: Allosteric inhibition of rat liver fructose 1,6-diphosphatase by adenosine 5'-monophosphate. J Biol Chem 1965, 240:651-662.
28. Garbyb L, Meldon J: The Respiratory Functions of Blood New York, London: Plenum Medical Book; 1977.
29. Kobayashi M, Kimura S, Ishigaki KI, Makino N, Imai K: Significance of oxygen affinity of fetal and adult human hemoglobins. Zool Sci 1996, 13:661-664.
30. Suzuki T, Imai K: Evolution of myoglobin. Cell Mol Life Sci 1998, 54:979-1004.
31. Gayecki TEJ, Honig CR: O2 gradients from sarcolemma to cell interior in red muscle at maximal VO2. Am J Physiol 1986, 251:H789-H799.
32. Takahashi E, Doi K: Regulation of oxygen diffusion in hypoxic isolated cardiac myocytes. Am J Physiol 1996, 271:H1734-H1738.
33. Wittenberg BA, Wittenberg JB: Transport of oxygen in muscle. Annu Rev Physiol 1989, 51:857-878.
34. Schenkmman KA, Beard DA, Ciesielski WA, Feigl EO: Comparison of buffer and red blood cell perfusion of guinea pig heart oxygenation. Am J Physiol 2003, 285:H1819-H1825.
35. Seiyama A, Shiga T: Oxygen transfer in peripheral organs: Researches on intact organs with optical techniques. Adv Exerci Sports Physiol 1998, 4:37-49.
36. Imai K: Oxygen transport and its regulation. Rinsha Kensa (in Japanese) 1986, 30:363-371.