The rate of the deoxygenation reaction limits myoglobin- and hemoglobin-facilitated O₂ diffusion in cells

Volker Endeward

Zentrum Physiologie, Vegetative Physiologie 4220, Medizinische Hochschule Hannover, Hannover, Germany

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Endeward V. The rate of the deoxygenation reaction limits myoglobin- and hemoglobin-facilitated O₂ diffusion in cells. J Appl Physiol 112: 1466–1473, 2012. First published February 23, 2012; doi:10.1152/japplphysiol.00835.2011.—A mathematical model describing facilitation of O₂ diffusion by the diffusion of myoglobin and hemoglobin is presented. The equations are solved numerically by a finite-difference method for the conditions as they prevail in cardiac and skeletal muscle and in red cells without major simplifications. It is demonstrated that, in the range of intracellular diffusion distances, the degree of facilitation is limited by the rate of the chemical reaction between myoglobin or hemoglobin and O₂. The results are presented in the form of relationships between the degree of facilitation and the length of the diffusion path on the basis of the known kinetics of the oxygenation-deoxygenation reactions. It is concluded that the limitation by reaction kinetics reduces the maximally possible facilitated oxygen diffusion in cardiomyocytes by ~50% and in skeletal muscle fibers by ~20%. For human red blood cells, a reduction of facilitated O₂ diffusion by 36% is obtained in agreement with previous reports. This indicates that, especially in cardiomyocytes and red cells, chemical equilibrium between myoglobin or hemoglobin and O₂ is far from being established, an assumption that previously has often been made. Although the “O₂ transport function” of myoglobin in cardiac muscle cells thus is severely limited by the chemical reaction kinetics, and to a lesser extent also in skeletal muscle, it is noteworthy that the speed of release of O₂ from MbO₂, the “storage function,” is not limited by the reaction kinetics under physiological conditions.

myoglobin; hemoglobin

MYOGLOBIN (Mb) and hemoglobin (Hb) can facilitate O₂ diffusion by diffusion of their oxygenated forms that occurs in parallel to the diffusion of dissolved oxygen. This has been demonstrated first for Hb by Scholander (41), Hemmingsen and Scholander (18), and Wittenberg (45, 46) and thereafter by Wittenberg for both Hb and Mb (47) in solutions of these hemoproteins forming layers of 150-μm thickness. In a theoretical study, Wyman (49) showed that chemical reaction in conjunction with rotational protein diffusion could not be the mechanism of this facilitation because the chemical reaction rate is too slow but that the reaction rates are sufficiently fast to allow a facilitation by translational protein diffusion. This mechanism was confirmed later by experimental determinations of the hemoglobin diffusion coefficient in concentrated Hb solutions (31, 39).

As pointed out very early on by Moll (30) and Wyman (49), facilitated O₂ diffusion can only be effective if the relaxation time of the chemical reaction between O₂ and Hb or Mb, respectively, is considerably shorter than the relaxation time of diffusion. Since the latter depends on the length of the diffusion path, or the layer thickness, it follows that hemoprotein-mediated facilitated O₂ diffusion will begin to decrease when the diffusional path length falls below a certain limit, whose value depends on the kinetics of the chemical reaction. This limit has been studied theoretically for the case of Hb-facilitated O₂ diffusion by Kutchai et al. (23), who showed that the process starts to become limited by the speed of the chemical reaction, when the thickness of the layer of hemoglobin solution decreases below ~100 μm. Consequently, they predicted for the average thickness of a red cell (1.6 μm) a strong limitation of facilitation by the reaction kinetics. Although the necessity of establishing also for Mb a relation between diffusion path length and degree of facilitation has been recognized by Wittenberg and Wittenberg (48) and by Gros et al. (17), no such calculation has been published so far. The information is clearly needed since in many estimations in the literature of the role of Mb for O₂ transport in cardiac and skeletal muscle cells, the tacit assumption has been made that the chemical reaction rate is not limiting in this process, and full chemical equilibrium exists within cells or layers of solution (5, 6, 25, 28, 34). In the present paper, therefore, the question is addressed whether Mb-facilitated O₂ diffusion in cardiac and skeletal muscle cells is limited by the speed of the chemical reaction for the diffusion path lengths given by the dimensions of these cells. The mathematical model consists of a complete description of the process of facilitated O₂ diffusion, which is solved numerically in a straight-forward fashion using a finite-difference method. This simple approach was not feasible 40 yr ago due to prohibitive computation times but is now viable as a result of the drastically enhanced computation speed of personal computers, even though the computation of one set of conditions as reported here still requires several days, and in special cases even weeks. The present paper assesses the question of reaction-limitation in muscle cells by treating the problem as a one-dimensional or cylindrical one. The one-dimensional approach has also been applied by Moll (32) and Kutchai et al. (23) to the case of red cells. In contrast, Clark et al. (8) modeled O₂ unloading from red cells as a three-dimensional, time-dependent problem by using an elaborate approximate analytical solution on the basis of a boundary layer concept by matched asymptotic expansions. To compare the present numerical method with that of Kutchai et al. (23) as applied to Hb solutions and red cells, computations of facilitated diffusion by Hb diffusion for the conditions of human red blood cells have been included in this study.

METHODS

The equations. In the simpler case of Mb, the reaction of O₂ with the heme protein is given by:

\[
\frac{d\text{MbO}_2}{dt} = k_{\text{obs}} \text{Mb} \text{O}_2 \text{O}_2
\]

\[
\frac{d\text{Mb}}{dt} = -k_{\text{obs}} \text{Mb} \text{O}_2
\]

\[
\frac{d\text{MbO}_2}{dt} = k_{\text{obs}} \text{Mb} \text{O}_2 - k_{\text{off}} \text{MbO}_2
\]

\[
\frac{d\text{Mb}}{dt} = k_{\text{off}} \text{MbO}_2
\]

\[
\text{Mb} \text{O}_2 + \text{O}_2 \rightarrow \text{Mb} \text{O}_2 \text{O}_2
\]

\[
\text{Mb} \text{O}_2 \text{O}_2 \rightarrow \text{Mb} + 2\text{O}_2
\]

\[
\text{Mb} + \text{O}_2 \rightarrow \text{MbO}_2
\]

\[
\text{MbO}_2 + \text{O}_2 \rightarrow \text{Mb} \text{O}_2 \text{O}_2
\]

\[
\text{Mb} + 2\text{O}_2 \rightarrow \text{Mb} \text{O}_2 \text{O}_2
\]

Address for reprint requests and other correspondence: V. Endeward, Medizinische Hochschule Hannover, Vegetative Physiologie 4220 Carl-Neubergstr. 1, 30625 Hannover, Germany (e-mail: Endeward.Volker@MH-Hannover.de).

The rate of the oxygenation-deoxygenation reaction then is described by:

$$d[\text{MbO}_2]/dt = k_A[\text{Mb}][\text{O}_2] - k_D[\text{MbO}_2]$$

where $[\text{MbO}_2]$ is the concentration of oxymyoglobin, $[\text{Mb}]$ is the concentration of deoxymyoglobin, $t$ is time, $k_A$ is the second-order rate constant of association of Mb and O$_2$, and $k_D$ is the first-order rate constant of dissociation of MbO$_2$.

The diffusion processes of O$_2$ and oxymyoglobin are described by Fick’s second law of diffusion:

$$\frac{\partial [\text{O}_2]}{\partial t} = D_{\text{MbO}_2} \frac{\partial^2 [\text{O}_2]}{\partial x^2}$$

$$\frac{\partial [\text{MbO}_2]}{\partial t} = D_{\text{MbO}_2} \frac{\partial^2 [\text{MbO}_2]}{\partial x^2}$$

where $D_{\text{MbO}_2}$ is the diffusion coefficient of oxymyoglobin (identical to that of myoglobin), and $x$ is the diffusion path.

Since facilitated diffusion is a process of simultaneous diffusion and reaction of free and bound O$_2$, it can be stated that, in a certain infinitesimal volume element, at time, $k_A$ is the second-order rate constant of association of Mb and O$_2$, and $k_D$ is the first-order rate constant of dissociation of MbO$_2$.

The analogous consideration applies to the change of MbO$_2$ concentration with time:

$$\frac{\partial [\text{MbO}_2]}{\partial t} = D_{\text{MbO}_2} \frac{\partial^2 [\text{MbO}_2]}{\partial x^2} - k_A[\text{Mb}][\text{O}_2] + k_D[\text{MbO}_2]$$

A layer of Mb solution of defined thickness $\ell$ (treated as a plane sheet) is considered, and, by numerically solving Eqs. 5 and 6, temporal and spatial courses of $[\text{O}_2]$ and $[\text{MbO}_2]$ within this layer are obtained. After the calculations have been continued for a sufficient time interval, steady state is reached, the concentration profiles of O$_2$ and MbO$_2$ in the layer become time-independent, and the fluxes of free and Mb-bound O$_2$ as well as the total O$_2$ flux have assumed their steady-state average fluxes of O$_2$ (free) and MbO$_2$ (facilitated) were obtained. After the calculations have been continued for a sufficient time interval, steady state is reached, the concentration profiles of O$_2$ and MbO$_2$ in the layer become time-independent, and the fluxes of free and Mb-bound O$_2$ as well as the total O$_2$ flux have assumed their steady-state average fluxes of O$_2$ (free) and MbO$_2$ (facilitated) were obtained.

In the case of hemoglobin, the equations describing facilitated diffusion are identical to those for Mb. However, the correct reaction equation has to be written as

$$\text{Hb} + 4\text{O}_2 \leftrightarrow \text{Hb}_4\text{(O}_2)_4$$

where the equilibrium constant and the kinetic constants differ between each of the single oxygenation steps. The calculation here is simplified by 1) considering the chemical reaction to occur with identical hemoglobin monomers, 2) using the half-saturation pressure of the O$_2$ binding curve in combination with the overall kinetic constants $k_A$ and $k_D$ to approximate the kinetics of the Hb-O$_2$ reaction in a fashion identical to that of Mb (see Eq. 2):

$$d[\text{HbO}_2]/dt = k_A[\text{Hb}][\text{O}_2] - k_D[\text{HbO}_2]$$

This treatment of the oxygenation-deoxygenation kinetics of Hb is identical to that used by previous investigators of the reaction- and path-length-dependence of facilitated O$_2$ diffusion in red cells (23, 32).

**Parameters.** In the case of myoglobin, the reaction rate constant $k_D$ of 11–12 s$^{-1}$ reported for 20°C by Antonini (1) and Gibson et al. (13) is used. With $\Delta H_{\text{int}}$ of 19 kcal/mol, one obtains a value of $k_D$ at 37°C of 60 s$^{-1}$, which is used in the present calculations that all pertain to 37°C. With a half-saturation pressure ($P_{O_2}$) of Mb of 2.4–2.8 Torr (1, 40) and an O$_2$ solubility $\alpha_O_2$ in muscle tissue of 1.5 $\times$ 10$^{-6}$ mol·1$^{-1}$·Torr$^{-1}$ (cf. Ref. 10), one obtains from this $k_D$ value a $k_D$ value of $\leq$ 15.4 $\times$ 10$^{-6}$ M$^{-1}$·s$^{-1}$. These rate constants imply half-times of MbO$_2$ dissociation of 12 ms and, depending on the concentrations of the reaction partners, of ca. 0.5 ms for the association of Mb with O$_2$. These numbers indicate that dissociation is considerably slower than association and thus will be the primary cause when the rate of chemical reaction becomes rate limiting in the facilitated diffusion process. The Mb diffusion coefficient inside cardiac and skeletal muscle cells was taken to be $2 \times 10^{-7}$ cm$^2$/s (20, 35, 36, 37), and an additional calculation was performed with the $D_{\text{Mb}}$ of $8 \times 10^{-7}$ cm$^2$/s derived from NMR measurements for rat cardiac cells by Lin et al. (26, 27). $D_{\text{Mb}}$ in muscle tissue was taken to be $D_{\text{Mb}} = k_D/\alpha_O_2 = 1.3 \times 10^{-5}$ mmol·cm$^{-1}$·min$^{-1}$·mmHg$^{-1}/1.5 \times 10^{-6}$ mol·1$^{-1}$·Torr$^{-1} = 1.4 \times 10^{-2}$ cm$^2$/s, where $k_D$ is Krogh’s diffusion constant describing the gas transport rate per area and partial pressure gradient (cf. 10). Myoglobin concentration used for cardiac muscle tissue was 0.19 mM (42). The boundary PO$_2$ values were set to be 10 Torr at $x = 0$ and 0.1 Torr at $x = \ell$, thus approximating the most favorable conditions for facilitated O$_2$ diffusion conceivable in physiological situations. The thickness of the layer considered in the calculations was the major variable, and ranges used were intended to encompass cardiac and skeletal muscle fiber radii and diameters, respectively.

In the case of hemoglobin, a $k_A$ value of 3.5 $\times$ 10$^6$ M$^{-1}$·s$^{-1}$ has been reported by Gibson et al. (12) for pH 7.1 and 37°C, which is similar to that obtained by Mochizuki et al. (29). For the present calculations, we have used the values reported by Bauer et al. (3) for the in vivo conditions inside red cells (pH 7.2; [2,3-bisphosphoglycerate] 6.5 mM; 37°C). Their $k_D$ is 250 s$^{-1}$ and $k_A$ is 6.1 $\times$ 10$^6$ M$^{-1}$·s$^{-1}$ (with an O$_2$ solubility of 1.5 $\times$ 10$^{-6}$ mol·1$^{-1}$·mmHg$^{-1}$ as mentioned above, and the ratio of $k_D/k_A$ corresponds to an O$_2$ half-saturation pressure for hemoglobin of $P_{O_2} = 27$ Torr). This $k_D$ for Hb is markedly faster than that used in the case of Mb. $D_{\text{Mb}}$ inside the red cell membrane has to be taken as the same as in muscle tissue. $D_{\text{Mb}}$ within the red cell is 6.4 $\times$ 10$^{-8}$ cm$^2$/s (31). Intraerythrocytic hemoglobin concentration is 20 mM Mb monomer. The boundary PO$_2$ values were set to 40 and 100 Torr, respectively, which represents the physiological range experienced by a red cell in the lung (and in the tissue) under resting conditions.

**Numerical solution.** Using the finite difference method as implemented in MATLAB 2008b, Eqs. 5 and 6 were solved numerically. In the case of Mb in a muscle cell, the boundary PO$_2$ values given above were assigned to $x = 0$ (PO$_2$ of 10 Torr) and $x = \ell$ (PO$_2$ of 0.1 Torr), respectively. Throughout the interior of the layer, a homogeneous
initial PO2 of 0.1 Torr was set, with the exception of $x = 0$, which was set to 10 Torr. In the case of hemoglobin, $x_0$ was set to 100 Torr, and the interior of the layer as well as $x = 6$ were set to 40 Torr. The layers were usually divided into $N = 200$ sections, i.e., $\Delta x = \ell/200$. In several cases, $N$ was raised to 500 or 1,000. The time interval inserted into Eqs. 5 and 6 (after conversion of the differential equations into difference equations) was determined in dependence of the inserted value of $\Delta x$ from the following relation (9):

$$\Delta t \approx (\Delta x)^2 / (2D_{O2})$$

(11)

where it was ascertained that $\Delta t$ was sufficiently small by making sure that a further reduction of $\Delta t$ did not affect the results. These values of $\Delta x$ and $\Delta t$, pertaining to a given value of $\ell$, were used in conjunction with the following boundary conditions: 1) the boundary $P_{O2,x=0}$ was set to 10 Torr (as mentioned above, for the case of Mb); 2) the boundary $P_{O2,x-\ell}$ was set to 0.1 Torr (as mentioned above, for the case of Mb); 3) the sum of $[Mb]$ and $[MbO2]$ was set to be $[Mbtot]$ everywhere in the layer, where $[Mbtot]$ is the total Mb concentration and a constant (0.19 mM in the case of Mb).

The boundary concentrations of Mb and MbO2 were obtained from the calculation, but initial estimates were derived from $[Mbtot]$ and the boundary PO2 values on the basis of the assumption of chemical equilibrium. The boundary conditions in the case of Hb were formulated analogously.

For these conditions, Eqs. 5 and 6 were numerically integrated and solved for the profiles of $P_{O2}$, $[Mb]$, and $[MbO2]$ throughout the layer. The calculation cycles were repeated until time-independent concentration profiles of $[O2]$ and $[MbO2]$, or $[HbO2]$, respectively, were obtained in the layer. For each calculation cycle, %Facilitation (at $x = \ell$), defined as ratio of facilitated flux over free flux in the last volume segment adjacent to $x = \ell$, $F_{MbO2}/F_{O2}$, or $F_{HbO2}/F_{O2}$, was calculated in the following way: in the last segment, adjacent to $x = \ell$, the total flux of $O2$ leaving this segment was determined for each point of time from the balance between the $O2$ entering the segment and the $O2$ consumed/produced by chemical reaction. After steady-state conditions have been established, the total flux leaving this element is identical to the total $O2$ flux occurring across the entire layer, because total flux must then be the same everywhere in the layer. Facilitated flux averaged over the entire layer is obtained as the difference between this total $O2$ flux and the free $O2$ flux as determined from the overall $P_{O2}$ difference $P_{O2,x=0} - P_{O2,x-\ell}$ and $D_{O2}$. It follows from this way of calculation that %Facilitation is representative for the entire cell/tissue layer only after steady-state conditions have been reached. Figure 1 shows for the case of $x = 5 \mu m$ that %Facilitation ($x = \ell$) starts with a value of 0, a consequence of the initial conditions described, then increases with time, and reaches a plateau after ~0.2 ms, indicating that steady state is established in the layer after this time.

RESULTS AND DISCUSSION

The kinetics of the reaction between hemoprotein and $O2$ causes a dependence of the degree of facilitation of $O2$ diffusion upon the length of the diffusion path. When the oxygenated hemoprotein and $O2$ diffuse alongside through a cell or layer of solution, albeit at different concentration gradients and diffusivities of protein and $O2$, facilitated diffusion can develop by association reaction between hemoprotein and $O2$ (predominantly in the first part of the entire diffusion path to be overcome) and thereafter by dissociation reaction (predominantly in the later part of the entire diffusion path). In this way, the chemical reactions make possible the carriage of $O2$ by the hemoprotein over a certain diffusion distance, a process called facilitated diffusion, as well as its timely release when and where the free $O2$ is needed, e.g., at the mitochondrion. This mechanism can therefore operate only when the kinetics of the association as well as the dissociation reaction are fast enough to permit reaction times sufficiently short compared with the time required for $O2$ to cross the entire diffusion distance. This condition for a facilitation of $O2$ diffusion has been recognized and formulated early on by Moll (30) and Wyman (49). Wyman showed theoretically that the given reaction kinetics of Mb and $O2$ allows facilitation by translational diffusion of Mb to occur in thicker layers of Mb solution, but clearly not by rotational diffusion, where the effective “diffusion distance” is equal to the diameter of the carrier protein, ~42 Å in the case of Mb and 62 Å in the case of Hb (43, 17). This general concept was later confirmed by Gros et al. (15, 16). They showed that facilitated diffusion can occur by rotational carrier diffusion in addition to translational diffusion in the case of the protonation reaction, which is
extremely fast with half-times in the nanosecond range. Interestingly, even with such fast reaction kinetics, facilitated proton diffusion by rotational protein diffusion was demonstrable (15, 16) only with two very large proteins, earthworm hemoglobin (d = 276 Å, molecular weight of \(3.7 \times 10^6\) Da) and apoferritin (d = 146 Å, molecular weight of 450,000 Da), but was absent in the two significantly smaller proteins myoglobin (d = 42 Å, molecular weight of 17,000) and serum albumin (d = 72 Å, molecular weight of 67,000 Da). These experimental observations clearly demonstrate the positive correlation between “diffusion distance” and the effectiveness of facilitated diffusion at a given reaction kinetics. The results of the calculations presented in the following show that this relationship is also an important parameter when one considers facilitated O2 diffusion at a given reaction kinetics. The results of the observations clearly demonstrate the positive correlation at shorter diffusion distances for the case of Mb-facilitated O2 diffusion in muscle. The example was calculated for a diffusion path length of 3.5 \(\mu\)m, the constants as defined above for facilitated O2 diffusion in muscle cells, and the boundary conditions listed above. As discussed below, 3.5 \(\mu\)m represents a reasonable estimate of the effective diffusion path in cardiomyocytes. Figure 2 shows the gradients of [O2], [MbO2], and [Mb] established under steady-state conditions for a case in which facilitation is markedly limited by the rate of MbO2 deoxygenation. The boundary concentrations of O2 of course reflect the inserted boundary PO2 values mentioned above. In the curve representing the [O2] gradient calculated with \(k_D\) = 60 s\(^{-1}\) (continuous line), there is a slight bend in the middle part, the curve being somewhat flatter in the second half compared with the first half. The O2 gradient in the second half is less steep because there, as is apparent from the middle curve of Fig. 2, the oxymyoglobin gradient (and thus facilitated diffusion) is greater than in the first half. This behavior reflects the fact that the total flux of O2 under steady-state conditions must be identical everywhere along the diffusion path. The dashed curve shows the [O2] gradient calculated for values of \(k_D\) and \(k_A\) that are both 1,000-fold greater than those employed for the continuous curve. It is apparent that this results in a more pronounced bend in the course of the [O2] gradient, which is due to the increase in the oxymyoglobin gradient in the second half of the (dashed) curve in Fig. 2, middle. The oxymyoglobin gradient shown in the middle panel directly illustrates the mechanism of reaction limitation: with the factual (low) reaction velocity, [MbO2] at the right-hand boundary does not fall to the low level of 0.007 mM predicted for chemical equilibrium but remains at 0.061 mM, \(-10\) times higher. This indicates a build-up of undissociated MbO2 at the right-hand boundary due to a limitation of MbO2 deoxygenation by the slow deoxygenation kinetics. When the reaction is accelerated by a factor of 1,000, this build-up almost disappears (dashed curve). It is noted that facilitation, when calculated with the true kinetic constants, in this example is reduced to a little over 50% of its maximum at chemical equilibrium. In conjunction with this, the MbO2 concentration difference (continuous line) between the boundaries of the layer is reduced to the same percentage. Thus facilitation is reduced because, and to the extent that, the oxymyoglobin concentration difference is reduced due to slow deoxygenation kinetics.

**Dependence of myoglobin-facilitated O2 diffusion on path length: comparison with effective diffusion paths in cardiac and skeletal muscle cells.** Figure 3 shows the results of calculations determining the degree of Mb-facilitated diffusion for various layer thicknesses, i.e., diffusion distances \(\ell\). The intracellular Mb diffusion coefficient employed is \(2 \times 10^{-7}\) cm\(^2\)/s. All data points represent steady-state conditions and are taken from the plateaus of figures of the type of Fig. 1. It is apparent that \%Facilitation depends strongly on the length of the diffusion path. Maximum facilitation for the conditions representing the situation in a muscle cell amounts to \(-14\%\), a figure that is reached only when the reaction kinetics is infinitely fast and does not limit the diffusion-reaction process at all (horizontal line in Fig. 3). At a path length of 1 \(\mu\)m, \%Facilitation is \(<2\%\), and half-maximal facilitation is attained at an \(\ell\) slightly below 3 \(\mu\)m. Figure 3 also shows that full maximal facilitation is not yet reached at \(\ell = 25\) \(\mu\)m, where it amounts to 12\%, i.e., 6/7 of the maximum. Figure 4 shows that the maximum is not even completely established at \(\ell = 100\) \(\mu\)m.

**Facilitation in cardiomyocytes.** What is the implication of these results for Mb-facilitated O2 diffusion in cardiac muscle...
cells? In the case of human heart tissue, Armstrong et al. (2) determined an average radius of cardiomyocytes in intact cardiac tissue of 7 μm. From published capillary densities, Endeward et al. (10) derived the radius of the Krogh cylinder used to describe O2 supply to cardiac tissue to be 10 μm, i.e., of similar magnitude. In applying the results of Figs. 1, 3, and 4, the simplification is made that the muscle fibers are “plane sheets,” and they are considered as cuboids rather than cylinders. This implies that the diffusion process is taken to occur across two opposite sides of the cuboid into its interior. The maximal diffusion distance that has to be overcome by O2 within the cardiomyocyte is expected to be of the order of 7 μm, and often, most pronounced in the case of subsarcolemmal mitochondria, considerably less. Using a path length of 7 μm, a %Facilitation of 10% is read from Fig. 3, i.e., only ~2/3 of the facilitation expected when no reaction limitation occurs. If one assumes the average diffusion path length of O2 in the cardiomyocyte to be half the fiber radius, 3.5 μm, %Facilitation is only 7.6% or slightly more than one-half of the facilitation in the absence of reaction limitation. In conclusion, facilitated O2 diffusion in heart tissue is markedly limited by the speed of the Mb deoxygenation reaction and will amount to approximately one-half of the value predicted from PO2 gradients and O2 and Mb diffusivities ignoring the finite speed of the O2-Mb kinetics.

The cardiomyocyte as a cylinder. Considering the cardiomyocyte as a cuboid with two rather than four sides accessible to O2 diffusion is of course a major simplification. The calculation was therefore repeated assuming the cardiac muscle fiber to be represented by a cylinder of radius a = 7 μm. In this case, it had to be assumed that O2 has access to the cell on the entire cylinder surface, i.e., PO2 was set to 10 Torr every-where at the cylinder surface and O2 diffusion occurred from the surface toward the interior of the cylinder. This is of course also a major simplification since, physiologically, O2 supply is not present equally at the entire circumference of the cylinder. Two cases were considered. First, the “O2 sink” was assumed to be in the center of the cylinder, at r = 0. This results in an extremely small degree of facilitation of O2 diffusion by 2.2%. This is due to the cylindrical geometry, which implies the available “effective diffusion area” to decrease drastically toward the center of the cylinder. Therefore, the gradient of O2 is very shallow in the outer regions of the cylinder, and Mb there remains almost fully saturated. The gradients of PO2 and MBO2 begin to become steeper just before the center of the cylinder where PO2 = 0.1 Torr is reached. Thus facilitation can only occur on the last fractions of a micrometer from the center, and on this short diffusion distance reaction limitation is severe. Of course, it is entirely unrealistic to base this calculation on a localization of all mitochondria in the center of the cylinder. The second approach was therefore based on the more reasonable assumption of the O2 sink being localized in the cylinder halfway between r = 0 and r = a, i.e., at r = a/2. This yields a facilitation of intracellular O2 diffusion of 7.4%, i.e., a %Facilitation almost identical to what has been derived above for the cuboid model. It may be noted that %Facilitation will decrease again as the O2 sink moves toward the cylinder surface because of the decreasing diffusion path length. As can be appreciated from Fig. 3, O2 transport to subsarcolemmal mitochondria, ~1 μm below the surface membrane, will hardly be supported by facilitated diffusion.

Facilitation in skeletal muscle. The dimensions of most skeletal muscle fibers are considerably greater than those of cardiomyocytes, often with radii between 25 and 50 μm. O2 supply occurs essentially in a radial direction (19). Jürgens et al. (19) derived a Krogh cylinder radius of 24 μm for the human quadriceps femoris muscle. The diffusion distance to be overcome by O2 then will be ~25 μm maximally, corresponding to 12% facilitation (6/7 of the maximal 14%) according to Fig. 3. The figure implies, as in the case of cardiac muscle cells considered above, the assumption of diffusion into a plane sheet. If the average diffusion path is again taken to be half the fiber radius, 12 μm, %Facilitation becomes 11% or 4/5 of the maximum predicted for infinite reaction speed. With the
same Mb diffusion coefficient of $2 \times 10^{-7} \text{cm}^2/\text{s}$, maximal facilitation is not even reached with the radius of a very thick skeletal muscle fiber of 50 $\mu$m, as is apparent from the lower half of Fig. 4. The cylinder approach described above, now applied to skeletal muscle, again yields a very similar facilitation, 10.7% for $r = a/2$. In conclusion, even in the much larger skeletal muscle fibers, some reaction-limitation of facilitated $O_2$ diffusion remains, reducing %Facilitation to 4/5 to 6/7 of the maximal value. Nevertheless, it is clear that the dimensions of skeletal muscle fibers are considerably more favorable for Mb-facilitated $O_2$ diffusion than those of cardiomyocytes.

Effect of $D_{Mb}$ on facilitation. Figure 4 illustrates the effect of $D_{Mb}$ on these calculations. Lin et al. (26, 27) reported a $D_{Mb}$ in cardiac tissue of $8 \times 10^{-7} \text{cm}^2/\text{s}$ (37°C) from NMR measurements, whereas Baylor and Pape (4), Jürgens et al. (20), and Papadopoulos et al. (35–37), using classical techniques, consistently found values of $\sim 2 \times 10^{-7} \text{cm}^2/\text{s}$ for 37°C. As recently discussed in a joint review (17), the higher $D_{Mb}$ obtained by NMR might be due to much shorter (but yet to be determined precisely) effective diffusion distances of the Mb inside the muscle cell when observed by NMR, which might prevent some of the more widely spaced intracellular obstacles hindering Mb diffusion over larger distances to become effective in the NMR measurement. The upper half of Fig. 4 has been obtained with $D_{Mb} = 8 \times 10^{-7} \text{cm}^2/\text{s}$ and shows, first, that maximal facilitation of course is fourfold greater than with $D_{Mb} = 2 \times 10^{-7} \text{cm}^2/\text{s}$. Second, it is apparent that, with increasing speed of Mb diffusion, the limitation by the speed of the chemical reaction becomes more pronounced. For an assumed average diffusion distance of 3.5 $\mu$m, as used above for the heart, one finds 7.6% facilitation with $D_{Mb} = 2 \times 10^{-7} \text{cm}^2/\text{s}$, or 54% of the maximum, whereas with $D_{Mb} = 8 \times 10^{-7} \text{cm}^2/\text{s}$ one reads a %Facilitation of 26% from the upper curve of Fig. 4, corresponding to 46% of the maximal value given by the horizontal line. This reflects the fact that the relation between the speed of diffusion and the speed of the chemical reaction is the critical parameter determining reaction limitation of facilitation: the faster the diffusion process, the greater the requirement for rapid chemical reaction rates. For either $D_{Mb}$ value, the conclusion remains that for $\ell = 3.5 \mu$m, roughly only about one-half of the maximal facilitation is achieved. It is concluded from these considerations that Mb-facilitated diffusion in the heart is expected to be severely limited by reaction velocity, by $\sim 50\%$, whereas in skeletal muscle this limitation is also present but of lesser importance.

Effect of $O_2$ consumption on facilitation. The presented computational approach does not take into account the fact that, in muscle tissue, intracellular $O_2$ transport is combined with intracellular $O_2$ consumption. Can $O_2$ consumption affect the role of Mb-facilitated diffusion? We have incorporated into the above equations an $O_2$ consumption that is homogeneously distributed across the sarcoplasm. To estimate the effect of $O_2$ consumption on facilitation, some calculations were performed for the following altered conditions: 1) $P_{O_2}$ at $x = 0$ was held at 10 Torr, as above; 2) a maximal specific cardiac $O_2$ consumption of 600 ml $O_2\text{-min}^{-1}\text{kg}^{-1}$ (10) was inserted; 3) the total flux of $O_2$ at $x = \ell$ was set to zero. The latter condition reflects the fact that, in a plane sheet (i.e., the cuboid model), the entire half-thickness of the sheet is supplied with $O_2$ from one side of the sheet, and no $O_2$ diffuses into the other half of the sheet, i.e., all $O_2$ entering the sheet under steady-state conditions at $x = 0$ is consumed within this half of the sheet. The path length (equal to half-thickness) in these calculations was 7 $\mu$m. This yielded the gradients of $[O_2]$ and $[\text{MbO}_2]$ under conditions of maximal $O_2$ consumption of cardiomyocytes, which were used to calculate free and facilitated fluxes. Accordingly, in the maximally respiring cardiomyocyte at capillary $P_{O_2} = 10$ Torr, %Facilitation amounts to only 4.7% (1/3 of the maximum) when the cuboid model is applied and to 3.3% (1/4 of the maximum) when the cylinder model is used. Thus facilitated $O_2$ diffusion in this case is of even lesser significance than when we consider merely $O_2$ diffusion without $O_2$ consumption. Besides by the reaction limitation of facilitation, this is caused by boundary $P_{O_2}$ values at $x = \ell$ that turn out to be $\sim 4$ Torr (cuboid) and $\sim 7$ Torr (cylinder), respectively. This implies that, even at maximal $O_2$ consumption, in conjunction with $P_{O_2} = 10$ Torr, a $P_{O_2} = \ell$ substantially above 0.1 Torr is maintained in the cardiomyocyte, thus further reducing the contribution of facilitated $O_2$ diffusion.

Dependence of hemoglobin-facilitated $O_2$ diffusion on path length: comparison with the effective diffusion path in red cells. Only in the case of red blood cells, efforts have been made previously to determine to what extent facilitated $O_2$ diffusion inside cells is limited by the speed of the Hb-$O_2$ kinetics. The present computational approach was applied to this problem to compare the results with these previous reports. For a red cell in its resting discoid shape, the effective thickness for considerations of gas uptake is 1.6 $\mu$m, and, since gas is taken up from both sides of the disc, the relevant diffusion path for $O_2$ is half of this value, 0.8 $\mu$m (11). For this diffusion path, Fig. 5 predicts a facilitation of $\sim 13\%$ for the conditions given above including the boundary $P_{O_2}$ values of 40 and 100 Torr. The maximum %Facilitation under these conditions is calculated to be 20.3%. Thus the limitation by the speed of the Hb-$O_2$ reaction decreases facilitation to 64% of the maximum. Similar calculations for the facilitation of steady-state $O_2$ diffusion through layers of 33 g% hemoglobin solution have been reported by Kutchai et al. (23), who obtained for a layer thickness of 0.75 $\mu$m and similar boundary $P_{O_2}$ of 50 and 125

![Fig. 5. Steady-state %Facilitation in a 20 mM hemoglobin solution (as it exists inside red cells) as a function of layer thickness. Boundary $P_{O_2}$ values of 40 and 100 Torr, $D_{Mb} = 6.4 \times 10^{-7} \text{cm}^2/\text{s}$. For 0.8 $\mu$m, the half-thickness of the human red cell, a facilitation of $\sim 13\%$ is calculated, which represents $\sim 64\%$ of the maximal facilitation of 20.3%.

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Torr, a %Facilitation of 16.8%, a figure that represented 59% of the maximum facilitation possible in the absence of reaction limitation. Thus the present results on red cells agree quite well with those of Kutchai et al. (23).

The measurements of steady-state O₂ fluxes across layers of packed red cells by Moll (33) and Kutchai and Staub (24) touch on the question of reaction limitation inside red cells but seem to be at variance with both theoretical results just mentioned. Both groups performed these measurements in the absence and presence of sufficient CO to completely block the hemoglobin and found a major reduction of O₂ flux in the presence of CO. Moll (33) estimated that the CO-dependent O₂ flux corresponds roughly to what is expected from measured intracellular Hb diffusion coefficients, and Kutchai and Staub (24) observed very similar facilitated fluxes in packed red cells and in hemoglobin solution. Thus both of these experimental studies did not obtain evidence for a reaction limitation of facilitated O₂ diffusion, which should have been apparent in 165- to 300-μm-thick layers of packed intact red cells but not in layers of Hb solution of identical thickness. This seems to disagree with the theoretical results by Kutchai et al. (23) and those reported in the present study. It should be noted, however, that in layers of packed red cells with O₂ diffusion occurring from one side of the layer to the other, the effective intracellular diffusion distance is 1.6 μm in red cells oriented in parallel to the plane of layer rather than the half-thickness of 0.8 μm and may be as great as 8 μm in red cells oriented perpendicularly to the plane of the layer. Other experimental and theoretical evidence suggests, however, that some reaction limitation should be expected in these experiments. Wittenberg (47) reported measurements of the dependence of Hb-facilitated O₂ fluxes on the thickness of the layer of Hb solution. Kutchai et al. (23) replotted Wittenberg’s data to show that facilitation in % of the maximum is independent of layer thickness between 300 and 60 μm but is markedly decreased at a thickness of ~25 μm. The theoretical results of Kutchai et al. (23) are in good agreement with these data and show that some reaction limitation begins to appear below a layer thickness of 100 μm and causes a decrease in %Facilitation to 45% of the maximum at a layer thickness of 5 μm. (It may be noted that these data and calculations pertain to boundary values of 100 and 0 Torr, which are quite different from the values considered in the present study.) Overall, one can conclude that a majority of studies demonstrates that facilitated O₂ diffusion in Hb solutions starts to become reaction limited at layer thicknesses of <100 μm and that, under physiological conditions, this limitation cuts down facilitation inside red cells by roughly 40%.

Implications of the results for the potential roles of myoglobin as a cellular O₂ store vs. a cellular O₂ transporter. Applying a modified Krogh cylinder model, we previously have shown that, in the heart, myoglobin-facilitated O₂ diffusion plays no role during diastole when coronary perfusion is high and quasi-steady-state conditions exist in the tissue (10). This is due to the absence of major gradients of oxygenated Mb during diastole, since almost all of the Mb in the heart are fully oxygenated even under a significantly elevated work state (21, 22, 50). An analogous observation of full saturation of Mb has been made for resting skeletal muscle (7, 44), again implying the absence of a role of Mb for intracellular O₂ transport. During systole, when in major parts of the left ventricle wall, coronary perfusion almost ceases, and the amount of Hb present in the capillaries is greatly reduced, myocardial PO₂ drastically falls, and substantial intracellular gradients of MBO₂ develop (10). In this phase of the cardiac cycle, MBO₂ plays a role both as an O₂ store and as an intracellular O₂ transporter. Endeward et al. (10) estimated that systolic O₂ supply of the left ventricular myocardium is supported to the extent of ~20% by Mb-facilitated O₂ diffusion and of 10% by the storage function of Mb, whereas the remaining 70% is provided by O₂ bound to capillary Hb and by O₂ dissolved in the tissue. It should be noted that these calculations were based on the assumption that Mb-facilitated O₂ diffusion in cardiomyocytes is not limited by MBO₂ reaction kinetics. Therefore, the numbers reported by Endeward et al. (10) for the contribution of facilitated diffusion have to be revised downward, approximately cutting the number of 20% down to 10%. This would mean that the relative roles of the storage and transport function of Mb might actually be of about equal size, and both of them together would account for roughly only one-fifth of systolic O₂ supply. Thus O₂ sources in the tissue other than MBO₂ would be even more dominating in this phase. A noteworthy aspect of these findings is that facilitated O₂ diffusion in the heart occurs only in conjunction with a depletion of the MBO₂ store and then presumably serves to support the delivery of the released O₂ to the mitochondria. These combined and about equal effects of Mb as an O₂ store and as an O₂ transporter should then be responsible for the compensatory adaptations observed in Mbo2 null mice by Gödecke et al. (14). The situation is likely somewhat different during prolonged contractile activity of skeletal muscle, where, after an initial fall in tissue PO₂ and MBO₂ resembling the systolic phase in the heart, a new steady state is reached, which is characterized by a time-independent partial desaturation of Mb (7, 38, 44). This latter situation appears to represent an example in which some facilitation can occur under quasi-steady-state conditions, although calculations on the basis of a Krogh cylinder model predict that this facilitation is of minor importance for sarcoplasmic O₂ transport under conditions of heavy exercise (19).

It has been shown here that facilitated O₂ diffusion is clearly limited by the rate of the (deoxygenation) kinetics of Hb or Mb when the cells considered are not exceptionally large. This concerns the transport function of Hb and Mb. Does it also compromise the O₂ storage function of Mb in the heart? Above, estimates are given of the half-times of MBO₂ dissociation (12 ms) and of Mb association with O₂ (~0.5 ms). Can the rate of MBO₂ dissociation limit the rate at which O₂ is released from MBO₂ during systole? The shortest possible duration of the systole in the maximally working human heart is 150 ms (10). This is >10-fold the half-time of the dissociation reaction, and thus any significant reaction limitation of the release of O₂ from the MBO₂ store is not to be expected. It appears that the kinetics of the Mb oxygenation-deoxygenation reaction is much better adapted to the storage function than to the transport function of Mb, at least in the heart.

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AUTHOR CONTRIBUTIONS

Author contributions: V.E. conception and design of research; V.E. analyzed data; V.E. interpreted results of experiments; V.E. prepared figures; V.E. drafted the manuscript; V.E. edited and revised the manuscript; V.E. approved the final version of the manuscript.

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