Oxidation of Oxymyoglobin to Metmyoglobin with Hydrogen Peroxide: Involvement of Ferryl Intermediate†

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ABSTRACT: Hydrogen peroxide, one of the potent oxidants in muscle tissues, can induce very rapid oxidation of oxymyoglobin (MbO_2) to metmyoglobin (metMb) with an apparent rate constant of 7.5×10^4 h^{-1} M^{-1} (i.e., 20.8 s^{-1} M^{-1}) over the wide pH range of 5.5–10.2 in 0.1 M buffer at 25 °C. Its molecular mechanism, however, is quite different from that of the autoxidation of MbO_2 to metMb. Kinetic analysis has revealed that the hydrogen peroxide oxidation proceeds through the formation of ferryl-Mb(IV) from deoxy-Mb(II), which is in equilibrium with MbO_2, by a two-equivalent oxidation with H_2O_2. Once the ferryl species is formed, it reacts rapidly with another deoxy-Mb(II) in a bimolecular fashion so as to yield 2 mol of metMb(III). Under physiological conditions, the rate-determining step was the oxidation of the deoxy species by H_2O_2, its rate constant being estimated to be on the order of 3.6×10^3 s^{-1} M^{-1} at 25 °C. These findings lead us to the view that a good supply of dioxygen provides rather an important defense against the oxidation of myoglobin with hydrogen peroxide in cardiac and skeletal muscle tissues.

In red muscles, such as cardiac and skeletal, myoglobin plays an essential role in maintaining aerobic metabolism, both as an oxygen store and as an entity facilitating oxygen diffusion (Theorell, 1934; Millikan, 1937; Wittenberg, 1970). During reversible oxygen binding, however, the oxygenated myoglobin (MbO_2)[1] is known to be oxidized easily to the ferric met form (metMb), which cannot be oxygenated and is therefore physiologically inactive, with generation of the superoxide anion (Gotoh & Shikama, 1976).

Recently, it has been shown that the autoxidation reaction of oxymyoglobin is not a simple, dissociative loss of O_2^- from MbO_2 but is a nucleophilic displacement of the bound dioxygen from MbO_2 in the form of O_2^- by the water molecule or hydroxyl ion, which can enter the heme pocket from the surrounding solvent, so that the iron is converted to the ferric met form (Satoh & Shikama, 1981; Shikama, 1984, 1985; Shikama & Matsuoka, 1986).

On the other hand, hydrogen peroxide also induces very rapid oxidation of MbO_2 to metMb. Moreover, even in vivo this reagent must act as at least one of the most potent oxidants of myoglobin, since H_2O_2 can be produced, presumably in a considerable amount for lack of catalase in muscle tissues, by dismutation of the superoxide anion (O_2^-) generated from a large number of biochemical reactions (Fridovich, 1978). In cardiac and skeletal muscles in particular, substantial amounts of O_2^- may also be generated from electron-transport systems in mitochondria (Forman & Kennedy, 1975) as well as in microsomes (Aust et al., 1972), both of these subcellular organelles occupying a fairly large volume of those tissues.

Thus, the reaction of hydrogen peroxide on MbO_2 has been written as (Winterbourn et al., 1976; Tomoda et al., 1981)

$$2MbO_2 + H_2O_2 + 2H^+ \rightarrow 2metMb + 2O_2 + 2H_2O \quad (1)$$

but the mechanistic details of this oxidation reaction, which are of clinical, as well as chemical, interest, have been quite unclear.

In this paper, we have first examined both the rate and the stoichiometry of oxidation of MbO_2 with H_2O_2 over the wide pH range of 5.5–10.2 at 25 °C. We have then demonstrated that H_2O_2 can oxidize deoxy-Mb more than 100 times more easily than can oxy-Mb and that the hydrogen peroxide oxidation of myoglobin proceeds actually through a two-equivalent oxidation of the deoxy form, which is in equilibrium with MbO_2. These results seemed to be of clinical importance, too, since ischemia is known to cause abrupt cell destruction in cardiac and skeletal muscle tissues (Levine et al., 1971; Kagen et al., 1975; Suzuki et al., 1980). In vivo, therefore, a good supply of oxygen can provide a strong defense against the danger of hydrogen peroxide oxidation of myoglobin.

Materials and Methods

Chemicals. A reagent-grade 30% hydrogen peroxide (Mitsubishi Gas Chemical Ind.) was used without further purification. For the stock solution, ca. 0.15 M H_2O_2 was prepared by dilution of 1.5 mL of the reagent with distilled water up to 100 mL. The concentration was determined spectrophotometrically with an absorption coefficient of 43.6 M^{-1} cm^{-1} at 240 nm (Beers & Sizer, 1952; Hildebrandt et al., 1978). Tris and Caps for buffer systems and all other chemicals were of reagent grade from Wako Pure Chemical, Osaka; solutions were made with deionized and glass-distilled water.

Preparation of Oxymyoglobin. Native oxymyoglobin was isolated directly from sperm whale skeletal muscle according to our standard procedure (Suzuki & Shikama, 1983). After being separated completely from hemoglobin by gel filtration on a Sephadex G-50 column (Pharmacia, Uppsala, Sweden), the myoglobin was applied to a DEAE-Sephadex column (Pharmacia, A-25) and developed into its polymorphic forms with 15 mM Tris-HCl buffer (pH 9.0). The major fraction, which was first eluted with 50 mM Tris-HCl buffer (pH 9.0), was dialyzed against 5 mM Tris-HCl (pH 7.0) and applied to a CM-cellulose column (Whatman, Clifton, NJ, CM-32) equilibrated with the same buffer, to separate the MbO_2 completely from the metMb with 15 Tris-HCl buffer (pH 7.5).

† This paper is dedicated to Professor I. Yamazaki of the Research Institute of Applied Electricity, Hokkaido University, Sapporo, on the occasion of his 63rd birthday.

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[1] Abbreviations: Mb, myoglobin; MbO_2, oxymyoglobin; metMb, metmyoglobin; Tris, tris(hydroxymethyl)aminomethane; Caps, 3-(cyclohexylamino)propanesulfonic acid.

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The major MbO_2 thus obtained was placed in 10 mM Tris-HCl buffer (pH 9.0) and kept at low temperature (0–4 °C) before use. The concentration of myoglobin was determined, after conversion into cyanometmyoglobin, with an extinction coefficient of 10.7 mM^{-1} cm^{-1} at 540 nm (Hanania et al., 1966).

Here it should be noted that we have always used fresh MbO_2 samples with an absorbance ratio of α to β maximum (α/β) of higher than 1.05, in order to check the extent of contamination of metMb to less than 5% (Suzuki & Shikama, 1983). This was essential for our kinetic and stoichiometric study, since metMb can also react with H_2O_2 so as to reduce its effective concentration.

Oxidation Rate Measurements. In the presence of H_2O_2 from 0.5 to 10 μM, the rate of oxidation of MbO_2 (50 μM) was measured in 0.1 M buffer at 25 °C over the pH range of 5.5–10.2, according to the following specifications: A 4-mL solution containing 50 μM fresh MbO_2 in 0.1 M appropriate buffer was placed in a test tube and incubated in a water bath (Lauda circulator) maintained at 25 (±0.1) °C. The reaction was started by the addition of 40 μL of H_2O_2 solution (50 μM to 1 mM) with a micropipet (Gilson), and the tube was then sealed with a ground-glass stopper. For spectrophotometry, the reaction mixture was quickly transferred to a quartz cell held at 25 (±0.1) °C, and the changes in the absorption spectrum from 460 to 700 nm were recorded on the same chart at measured intervals of time. For the final state of the runs, the myoglobin was completely converted to metMb by the addition of potassium ferricyanide.

The buffers used were acetate, phosphate, Tris, and Caps. The pH of the reaction mixture was carefully checked with a Hitachi-Horiba pH meter (Model F-7SSII).

Preparation of Deoxymyoglobin. Since the complete removal of oxygen from a MbO_2 solution by exposure to inert gas was rather difficult, deoxy-Mb was prepared from MbO_2 by careful use of sodium dithionite according to the specifications by Waterman (1978) and by Di Iorio (1981): A 3-mL sample of the MbO_2 solution (50 μM), which was buffered with 0.1 M phosphate (pH 7.0) and deaerated as far as possible by passage through nitrogen, was placed in a quartz cell. The top of the solution was then covered with a 5-mm layer of liquid paraffin, through which a minimum volume (ca. 30 μL) of 30 mM $Na_2S_2O_4$ solution was syringed little by little until the complete spectrum of the deoxygenated form was obtained for an extended time. To examine the reactivity, a given quantity of H_2O_2 was then added to this deoxy-Mb solution with a microsyringe through the layer of liquid paraffin.

Spectrophotometric Measurements. Spectrophotometric measurements were carried out in a Hitachi two-wavelength double-beam spectrophotometer (Model 557) equipped with a thermostatically controlled cell holder. Temperature was controlled by a water bath (Lauda thermostat) maintained to within ±0.1 deg.

Results

Biphasic First-Order Plots for the Oxidation of MbO_2 to MetMb. In the presence of H_2O_2, oxymyoglobin was oxidized easily to metmyoglobin under air-saturated conditions:

$$MbO_2 \xrightarrow{k_\phi^{obsd}} \text{metMb} \qquad (2)$$

where k_ϕ^{obsd} represents the first-order rate constant observed at a given pH and in a given concentration of the added H_2O_2. This process of oxidation was followed by a plot of $-\ln([MbO_2]_t/[MbO_2]_0)$ versus time t, where the ratio of MbO_2 concentration after time t to that at time $t = 0$ can be monitored by the absorbance changes at 581 nm (α peak of MbO_2).

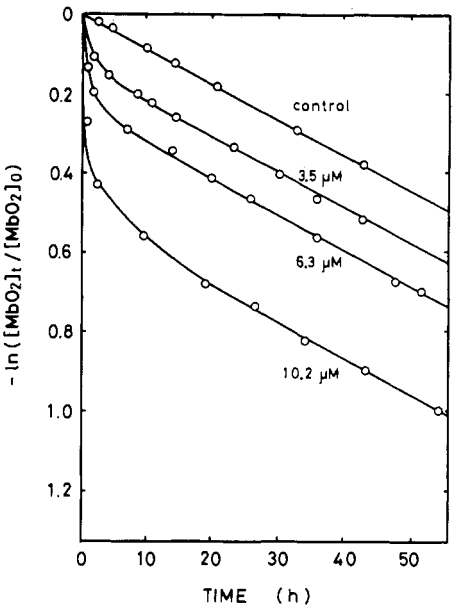

FIGURE 1: First-order plots for the oxidation of MbO_2 to metMb in the presence of H_2O_2. The reactions were started by addition of H_2O_2 at the indicated concentrations to 50 μM fresh MbO_2 solution in 0.1 M phosphate buffer, pH 7.0, at 25 °C. For control, the autoxidation of MbO_2 in buffer alone is also plotted, with the rate constant of $k_A = 0.78 \times 10^{-2}$ h^{-1} being obtained from the slope of the straight line.

Figure 1 shows such a first-order plot for the oxidation of MbO_2 to metMb in the presence of H_2O_2, in less than half the moles of MbO_2, in 0.1 M phosphate buffer, pH 7.0, at 25 °C. Just after the addition of H_2O_2 there appeared very quick oxidation, the rate of which increases with increasing concentrations of the added H_2O_2. This abrupt, initial phase was then followed by a slow and constant oxidation, the rate of which is independent of the concentrations of the added H_2O_2 and rather corresponds exactly to that for the normal autoxidation of MbO_2 in buffer alone at a given pH. This clearly indicates that the oxidation of MbO_2 with H_2O_2 has been completed in the initial phase of the reaction.

Stoichiometry and Rate Constant for the Oxidation of MbO_2 with H_2O_2. In Figure 1, the latter process of oxidation of MbO_2 may be written simply as

$$[MbO_2]_t = [MbO_2]_0(1 - f_\phi)\exp(-k_A t) \qquad (3)$$

where f_ϕ represents the molar fraction of MbO_2 that had been oxidized with the added H_2O_2 in the initial phase and k_A is the first-order rate constant for autoxidation of MbO_2 in buffer alone at a given pH. By transforming eq 3 for the first-order plot we obtain

$$-\ln([MbO_2]_t/[MbO_2]_0) = -\ln(1 - f_\phi) + k_A t \qquad (4)$$

If we extrapolate each straight line of slope k_A from the latter phase to time $t = 0$, the intercept on the ordinate would be expected to give each corresponding quantity of $-\ln(1 - f_\phi)$.

With the value of f_ϕ thus obtained, the moles of MbO_2 oxidized with the added moles of H_2O_2, $[MbO_2]_\phi$, and hence their stoichiometric ratio, n_ϕ, which we are primarily concerned with, can be calculated as

$$n_\phi \equiv \frac{[MbO_2]_\phi}{[H_2O_2]_0} = \frac{f_\phi[MbO_2]_0}{[H_2O_2]_0} \qquad (5)$$

Figure 2 summarizes such stoichiometric plots at three different values of pH, and it was thus concluded that 2 mol of MbO_2 can be oxidized to metMb with 1 mol of H_2O_2, at any given pH from 5.5 to 10.2.

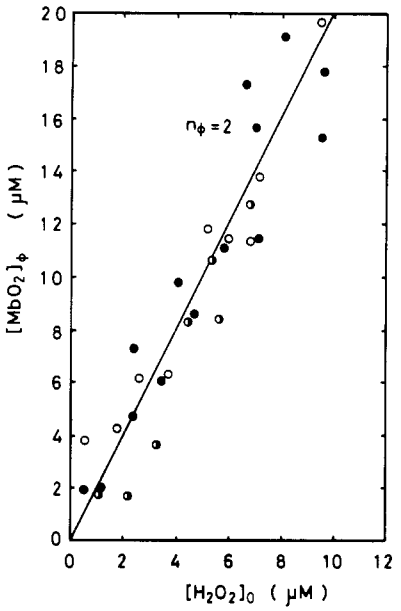

FIGURE 2: Stoichiometric plot for the amounts of MbO_2 oxidized with various amounts of added H_2O_2. The theoretical line (—) having a slope of $n_\phi = 2$ is in good agreement with the experimental data over the whole range of pH 5.5 (○) to pH 7.0 (●) to pH 10.0 (◑) in 0.1 M buffer at 25 °C.

Our next step was to analyze initial rates for the oxidation of MbO_2 by varying the concentrations of the added H_2O_2 from 0.6 to 10.8 μM and at various values of pH from 5.5 to 10.2. Figure 3 gives such examples, for instance, at pH 7.0 and pH 5.5. The observed first-order rate constant, k_ϕ^{obsd}, was linearly dependent upon the concentrations of the added H_2O_2, while the intercept on the ordinate corresponds to k_A, the rate constant for the autoxidation of MbO_2 in buffer alone. At any given pH, therefore, the observed rate constant for the oxidation of MbO_2 in the presence of added H_2O_2 may be written as

$$k_\phi^{obsd} = k_A + k_\phi'[H_2O_2]_0 \quad (6)$$

where k_ϕ' represents the rate of the H_2O_2-induced oxidation of MbO_2, its value being determined to be 7.5×10^4 h^{-1} M^{-1}, from the slope of the graph, in 0.1 M buffer, pH 7.0, at 25 °C. Here it should be noted that the same order of magnitude of $k_\phi' = 6.2 \times 10^4$ h^{-1} M^{-1} was also obtained for bovine heart MbO_2 at pH 7.2 and 25 °C (Y. Sugawara and K. Shikama, unpublished data).

In so far as the pH was examined from 5.5 to 10.2, the values of k_ϕ' were essentially the same, this clearly indicating that the oxidation of MbO_2 with H_2O_2 can proceed without any proton assistance over the whole range of pH studied. This is in sharp contrast to the rate of autoxidation of MbO_2, which depends strongly upon the pH of the solution (Shikama, 1985; Shikama & Matsuoka, 1986).

True Reactant and Product in the Oxidation of MbO_2 with H_2O_2. As mentioned above, each 1 mol of H_2O_2 was found to convert 2 mol of MbO_2 into metMb with the rate constant of $k_\phi' = 7.5 \times 10^4$ h^{-1} M^{-1} (i.e., 20.8 s^{-1} M^{-1}). As has been suggested in analogous cases such as the oxidation of MbO_2 with ferricyanide (Antonini et al., 1965) or with ferricytochrome *c* (Yamazaki et al., 1964) and the oxidation of HbO_2 with ferricytochrome b_5 (Mauk et al., 1984), however, it was also possible to assume that the reaction of MbO_2 with H_2O_2 proceeds actually through the oxidation of the unliganded deoxy form, which is in equilibrium with MbO_2, by

$$MbO_2 \underset{}{\overset{K_d}{\rightleftharpoons}} O_2 + Mb \xrightarrow[k_\phi]{+H_2O_2} metMb \quad (7)$$

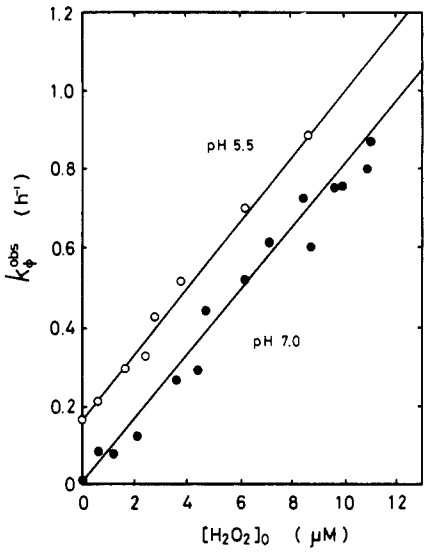

FIGURE 3: Plots of the observed first-order rate constant, k_ϕ^{obsd}, as a function of the concentrations of the added H_2O_2 for the oxidation of MbO_2 in 0.1 M buffer at 25 °C. The slope of each graph at the indicated pH value gives k_ϕ' (h^{-1} M^{-1}), the rate constant for the H_2O_2-induced oxidation of MbO_2. The intercept on the ordinate corresponds to k_A, the rate constant for the autoxidation of MbO_2 in buffer alone. The hydrogen peroxide oxidation was independent of pH from 5.5 to 10.2, with a constant value of $k_\phi' = 7.5$ (±1.0) × 10^4 h^{-1} M^{-1}.

where K_d represents the oxygen dissociation constant, and k_ϕ is the rate constant for the oxidation of deoxy-Mb with H_2O_2.

If it is the case, the rate of the oxidation of MbO_2 with H_2O_2 should be rewritten in terms of the oxidation of the deoxy form:

$$\begin{aligned} \text{rate} &= k_\phi'[MbO_2][H_2O_2]_0 \\ &\equiv k_\phi[Mb][H_2O_2]_0 \end{aligned} \quad (8)$$

By the use of $K_d = [Mb][O_2]/[MbO_2]$, eq 8 leads us to the conversion

$$k_\phi = k_\phi'\frac{[O_2]}{K_d} \quad (9)$$

Here, taking a value of $[O_2] = 2.5 \times 10^{-4}$ M and also a value of $K_d = 1.45 \times 10^{-6}$ M, which was calculated from the literature value at 20 °C with the corresponding ΔH for sperm whale myoglobin (Antonini & Brunori, 1971), eq 9 gives the value of $k_\phi = 3.6 \times 10^3$ s^{-1} M^{-1} for the oxidation of deoxy-Mb with H_2O_2 at pH 7.0 and 25 °C.

As a matter of fact, it is true that a given quantity of H_2O_2 can induce the oxidation of deoxy-Mb more than 100 times more easily than that of MbO_2. Figure 4 shows an example of the spectral changes with time when deoxy-Mb reacted with H_2O_2 in 0.1 M phosphate buffer, pH 7.0, at 25 °C. The final spectrum was identified to be the metMb with the isosbestic points occurring at 524 and 605 nm. In spite of some experimental limitations due to a small remnant of $Na_2S_2O_4$ in the solution, it was unequivocally confirmed that 1 mol of the added H_2O_2 can also convert 2 mol of deoxy-Mb to metMb within the experimental errors. The rate constant of ~3.5 × 10^3 s^{-1} M^{-1} was also obtained more directly for the oxidation of deoxy-Mb with H_2O_2 at pH 7.0 and 25 °C. These findings would appear to imply that eq 7 represents a reasonably good approximation, although we cannot eliminate completely the possibility that H_2O_2 oxidizes MbO_2 directly, but at an undetectably low rate.

Our next step was to disclose what is the true product of this oxidation reaction. In Figure 5, therefore, it would be appropriate to present the reference spectra of some myoglobin

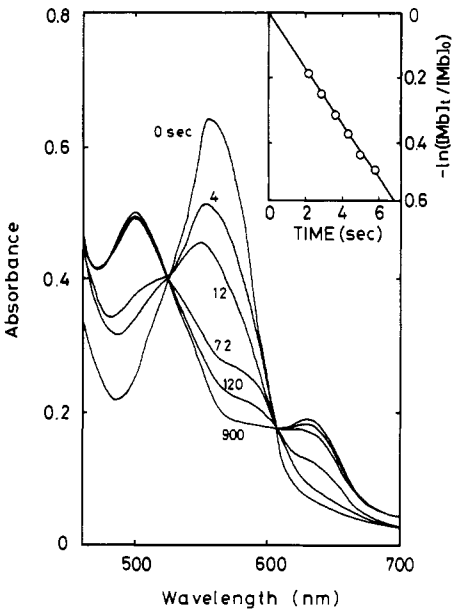

FIGURE 4: Spectral changes with time for the oxidation of deoxy-Mb to metMb with H_2O_2 at 25 °C. Scans were made at the indicated times after mixing 25 μM H_2O_2 with 50 μM deoxy-Mb in 0.1 M phosphate buffer, pH 7.0. The final spectrum was identified to be metMb with the isosbestic points at 524 and 605 nm. The detailed first-order plot monitored at 557 nm is inserted to obtain the rate constant of $k_\phi = 3.5 \times 10^3\ s^{-1}\ M^{-1}$.

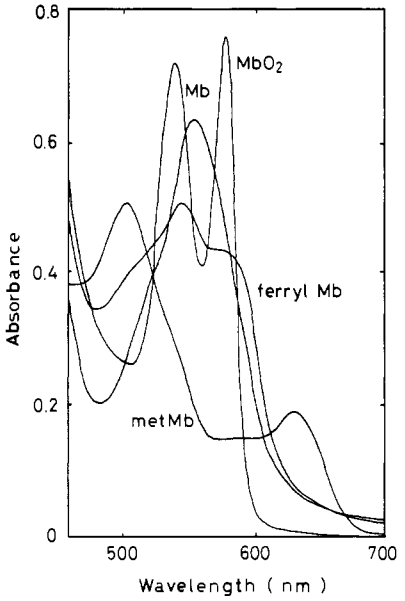

FIGURE 5: Spectral characterization of myoglobin derivatives. The concentration was 50 μM each in 0.1 M phosphate buffer, pH 7.0, except that in 0.1 M Tris-HCl buffer, pH 8.4, the ferryl-Mb was prepared from 50 μM metMb by the addition of 100 μM H_2O_2.

derivatives that may be needed for identification of the reaction products. To separate and reveal such a possible intermediate, if any, involved, we have made various attempts. Finally, by lowering the reaction temperature down to 2 °C, we have found spectral evidence for the formation of ferryl-Mb(IV) during the course of the oxidation of deoxy-Mb with H_2O_2 to metMb. As clearly demonstrated in Figure 6, the spectra with a peak at 545 nm and a shoulder over 570–580 nm, characteristic of the ferryl-Mb as shown in Figure 5, appeared transiently at an early stage of the reaction at 2 °C. This spectrum, however, immediately mixed with that of the met species and ended up as the complete spectrum of metMb with recovering of the isosbestic point deviations.

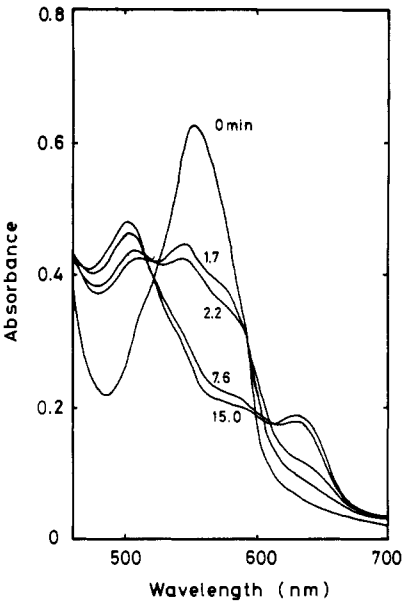

FIGURE 6: Spectral evidence for the formation of ferryl-Mb during the course of oxidation of deoxy-Mb to metMb with H_2O_2. The experimental conditions were the same as in Figure 4, except at 2 °C. The spectra with a peak at 545 nm and a shoulder over 570–580 nm, characteristic of the ferryl-Mb, appeared transiently at an early stage of the reaction. Once the ferryl species is formed, it reacts rapidly with another deoxy-Mb, with the spectra ending up as that of metMb.

On the basis of these results and mechanistic considerations, the overall reaction scheme can be written, therefore, as

$$2MbO_2 \rightleftharpoons 2Mb(II) + 2O_2 \quad (10)$$

$$Mb(II) + H_2O_2 \rightarrow \text{ferryl-Mb(IV)} + 2OH^- \quad (11)$$

$$\text{ferryl-Mb(IV)} + Mb(II) \rightarrow 2\text{metMb(III)} \quad (12)$$

$$2OH^- + 2H^+ \rightarrow 2H_2O \quad (13)$$

$$2MbO_2 + H_2O_2 + 2H^+ \rightarrow 2\text{metMb} + 2O_2 + 2H_2O \quad (1)$$

where reactions 10 and 11 represent the primary formation of ferryl-Mb(IV) from deoxy-Mb(II), which is in equilibrium with MbO_2, by a two-equivalent oxidation with H_2O_2. Once the ferryl species is formed, it reacts rapidly with another deoxy-Mb so as to yield 2 mol of metMb. Equation 12, therefore, involves a very rapid bimolecular reaction of the protein molecules, so that it can be disclosed only by lowering the rate by lowering the temperature. Reaction 12 also provides a reasonable basis for the overall stoichiometry that 1 mol of H_2O_2 oxidizes 2 mol of MbO_2 to metMb. Reaction 13 agrees well with our findings that the hydrogen peroxide oxidation of myoglobin is independent of pH and can proceed without any proton catalysis.

From the mechanism proposed above, we can rule out the following alternative pathways of metMb formation:

$$Mb(II) + H_2O_2 \rightarrow \text{metMb(III)} + OH^- + HO^\bullet$$

$$Mb(II) + HO^\bullet \rightarrow \text{metMb(III)} + OH^-$$

since both the rate and the stoichiometry for the oxidation of MbO_2 with H_2O_2 were not affected at all by the presence of mannitol, a good scavenger for $HO^\bullet$ radical, in so far as examined from 10 mM to 0.2 M.

Discussion

In the overall reaction of eq 10–13, the formation of deoxy-Mb can be a critical step for the oxidation of MbO_2, since the deoxy form is the preferred target for H_2O_2. Judging from the oxygen dissociation constant (K_d), the amount of deoxy-Mb that is in equilibrium with MbO_2 is only about 0.5%, so that

its spectrophotometric appearance should be negligibly small in the MbO_2 solution. However, once the deoxy form reacts with H_2O_2, MbO_2 goes to dissociate another deoxy-Mb very quickly to an equilibrium extent. Its dissociation rate (k_{off}) is known to be on the order of $\simeq 10\ s^{-1}$ (Antonini & Brunori, 1971). This value is indeed much higher than the actual rate of hydrogen peroxide oxidation of deoxy-Mb in eq 11, even if its rate constant is $k_\phi = 3.6 \times 10^3\ s^{-1}\ M^{-1}$ at 25 °C, when the concentration of H_2O_2 is on the order of micromolar. From these considerations, it is reasonably concluded that eq 11 must be a rate-determining step for the overall oxidation reaction of MbO_2 with H_2O_2 under physiological conditions. As for the rate of ferryl-Mb–deoxy-Mb reaction, which is of interest, on the other hand, our present study does not give the direct measure but gives a lower limit of being not less than the value of k_ϕ.

In our experiments, the concentration of the added H_2O_2 was mostly less than half the moles (50 μM) of myoglobin. This was essential, since in the presence of a large excess peroxide the metMb formed by eq 12 reacted again with excess H_2O_2 in a very complicated way (George & Irvine, 1952; Uyeda & Peisach, 1981). In fact, the formation and accumulation of this secondary ferryl species originating, not from deoxy-Mb but from metMb, used to veil the elementary processes involved in the hydrogen peroxide oxidation of myoglobin (Whitburn, 1987).

Even in buffer alone, as mentioned already, MbO_2 is oxidized to metMb with the generation of the superoxide anion. For this autoxidation reaction, it has long been observed that the rate increases with increasing hydrogen ion concentration and increases with decreasing partial pressure of O_2 (Brown & Mebine, 1969; Wallace et al., 1982). Although several proposals have therefore been made concerning the mechanism of this reaction (Shikama, 1984), it seems that there is no provision in their scheme for the inverse dependence of the autoxidation rate upon oxygen pressure. Our novel results, however, can provide a most reasonable explanation to this respect, since H_2O_2 can be produced by dismutation of the superoxide anion generated from the normal autoxidation of MbO_2 (Tajima & Shikama, 1987). Accordingly, it must act as at least one of the potent oxidants of the deoxy-Mb that increases with decreasing O_2 pressure.

In conclusion, we cannot eliminate completely the possibility that H_2O_2 oxidizes MbO_2 directly. However, it is true that H_2O_2 can oxidize deoxy-Mb more than 100 times more easily than can oxy-Mb, through the formation of ferryl species, to metMb. These results lead us to a new view that a good supply of oxygen provides rather an important defense against the oxidation of myoglobin with hydrogen peroxide, one of the potent oxidants in muscle tissues. These findings seem to be of primary importance in view of clinical biochemistry of "oxidant stress", as well as of pathophysiology of ischemia in cardiac and skeletal muscle tissues.

ACKNOWLEDGMENTS

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Registry No. H_2O_2, 7722-84-1; O_2, 7782-44-7.

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